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Monographs on Medicinal Plants along Ganga River
Cynodon dactylon (L.) Pers.



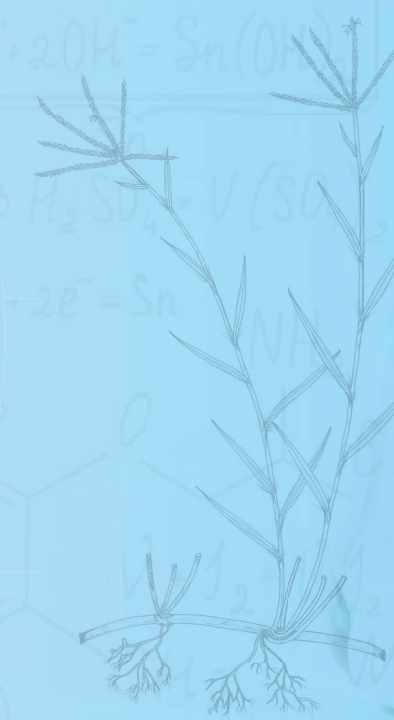
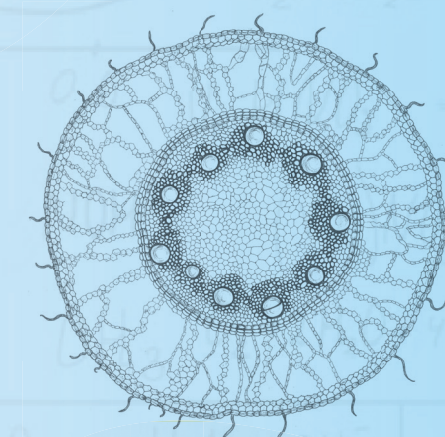
Monographs on Medicinal Plants
along Ganga River

Cynodon dactylon (L.) Pers.

Focusing on

Botanical, Phytochemical, Scientific Validation and
Insilico Analysis Including Medicinal Importance
and Soil Properties

Volume 2



Monographs on Medicinal Plants along Ganga River

Cynodon dactylon (L.) Pers.

Focusing on
Botanical, Phytochemical, Scientific validation and Insilico
analysis including Medicinal importance and Soil properties

Volume 02

Sponsored by



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This monograph is an outcome of the project entitled
"Scientific Exploration of Floral Diversity Near the Ganga Riverbanks for Ethnobotanical Purposes along
with their Conservation and Economic Development of the Region via Skill Development Programs" of The
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ABOUT THE EDITOR

Dr. Acharya Balkrishna, Co-founder of Patanjali Organic Research Institute, is a highly ascetic entrepreneur with a diverse personality who is a specialist in Yoga, Ayurveda, Sanskrit language, Indian sacred books, and the Vedas. Dr. Balkrishna has become a significant source of inspiration for Traditional Medicinal Practitioners and a globally recognized celebrity after dedicating his life to the resurrection of ancient healing and living practices. His maverick leadership as Co-Founder & Managing Director of Patanjali Ayurved Limited, along with overseeing Patanjali Food and Herbal Park and Divya Pharmacy, has propelled Ayurveda into a global business phenomenon with a massive following. Additionally, he has been decorated with prestigious awards such as 'Ayurveda Expert', 'Manav Ratan', 'Bharat Gaurav', 'Indian of the Year', 'Ten Versatile and Dynamic Young Men of India', 'Bheeshma Pusaka', 'Lokmanya Tilak', and 'Transformational Business Leader' for his exceptional knowledge, passion, and service to mankind. According to a study published by Stanford University in the USA and Elsevier in Europe, Dr. Balkrishna has been recognized among the top 2% of scientists worldwide for his research on Ayurveda and Yoga.

With his early age passion for plants, he has become the most renowned & respected herbal specialist for health and nutrition. For the research of novel herbal medication formulations, he explored four rare plants: 'Sanjeevani', 'Somlata', 'Swarnakshiri', 'Swarnadraka', and 'Astavavarga plants'. He has taken many initiatives for Biodiversity conservation, presently working for the establishment of Patanjali Herbal Garden & herbarium, working on the compilation of a unique multivoluminous project i.e., 'World Herbal Encyclopaedia', containing the descriptions of ~50,000 medicinal plant species with the largest collection of plant paintings and drawings. This is being done to strengthen traditional medicine systems of the world. Additionally, Dr. Balkrishna has made significant contributions to the socio-economic development of tribal communities and the upliftment of rural communities with FPOs, CLFs, and SHGs. Furthermore, his endeavors have been directed towards exploring and conserving floral diversity, phytochemical and insilico analysis, investigating soil geochemistry, understanding complex plant-microbe relationships, and monitoring water quality from Gomukh to Gangasagar.

He has published more than 400 research articles in national and international journals received around 20 patents and authored more than 200 books on Yoga, Ayurveda, Agriculture, Herbal Medicine,

and Information Technology and edited more than 40 unpublished ancient Ayurveda manuscripts. With the vision of ensuring universal health for the last two decades, more than 1.5 million patients with several persistent, chronic, and non-communicable diseases have been effectively treated. He established Patanjali Ayurved College and the University of Patanjali with the humanitarian goal of assisting youngsters in achieving their goals and serving the country. Additionally, he has been involved in various government and non-government initiatives. He is also a key proponent of agricultural transformation through organic practices, working to boost agricultural productivity, increase farmer income, and ensure equitable access to a safe, affordable, and nutritious diet year-round. Apart from this, Dr. Balkrishna is actively participating in driving agricultural transformation through the 'Patanjali Farmer Samridhi Programme' by well-trained staff and around one lakh trainers. His generous personality is also reflected through his actions like helping the nation with emergency needs like post-disaster needs assessment, providing free shelter, food, and education to orphan children, and free OPD services at Patanjali Yogpeeth. With his humanitarian attitude, Dr. Balkrishna continues his phenomenal journey of making world records, uplifting mankind through medicine & lifestyle improvement, reviving sustainable agriculture, and preserving nature's gifts in the form of literature and a sustainable living approach.





Vision of Honourable Prime Minister

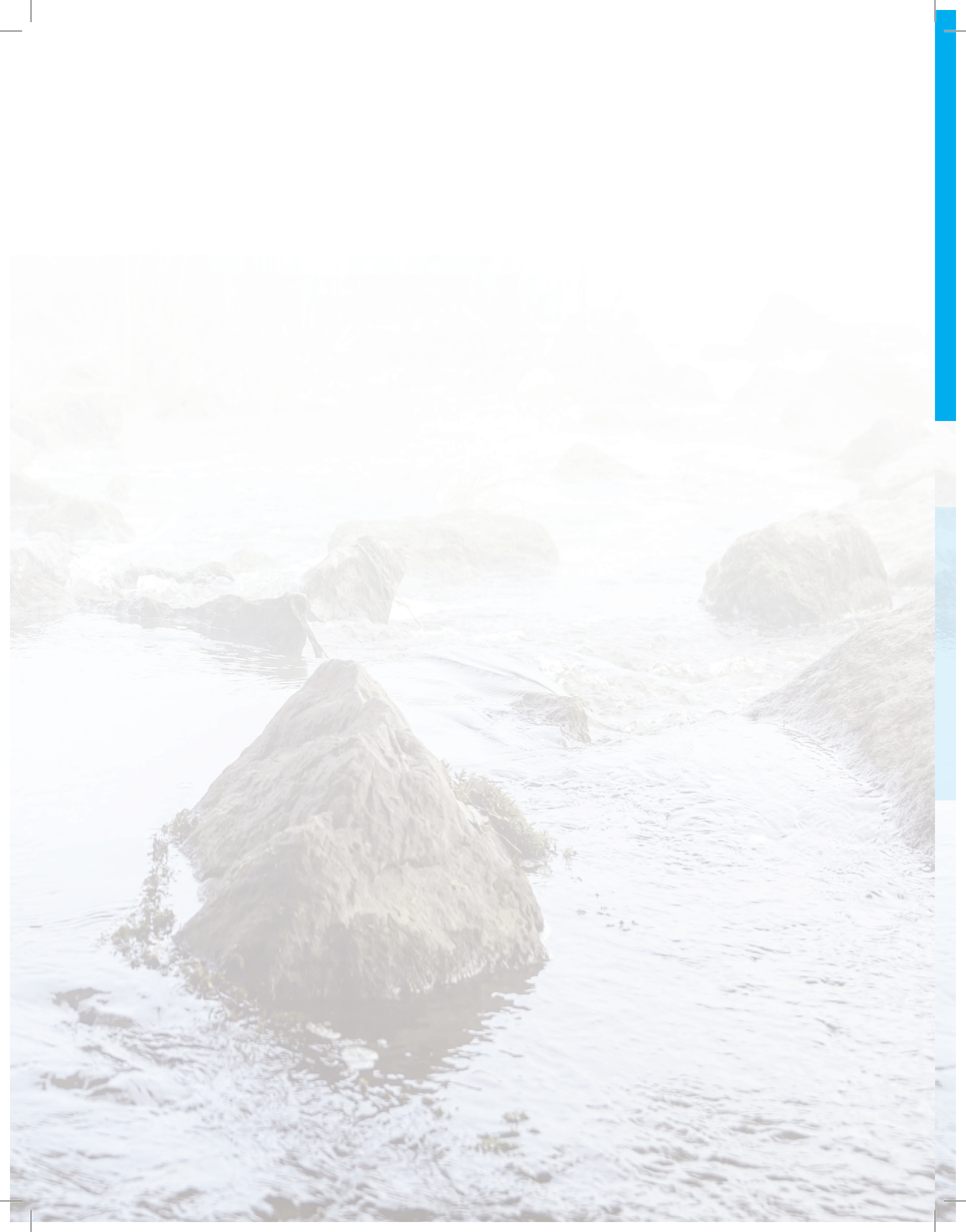
Shri Narendra Modi on Medicinal Plants



1. India's rich biodiversity of medicinal plants can provide affordable healthcare solutions.
2. Medicinal plants are nature's gift to humanity; we must protect and utilize them wisely.
3. Let us revive and promote the use of medicinal plants in everyday life.
4. India's biodiversity of medicinal plants is a treasure trove waiting to be explored.
5. Medicinal plants have been an integral part of our cultural heritage, offering natural remedies for various ailments.
6. The knowledge embedded in our traditional systems like Ayurveda and the use of medicinal plants can address global health challenges sustainably.
7. We must promote research and innovation in harnessing the potential of medicinal plants for healthcare and economic development.



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Vision of Union Minister for Jal Shakti

Shri Chandrakant Raghunath Patil

“

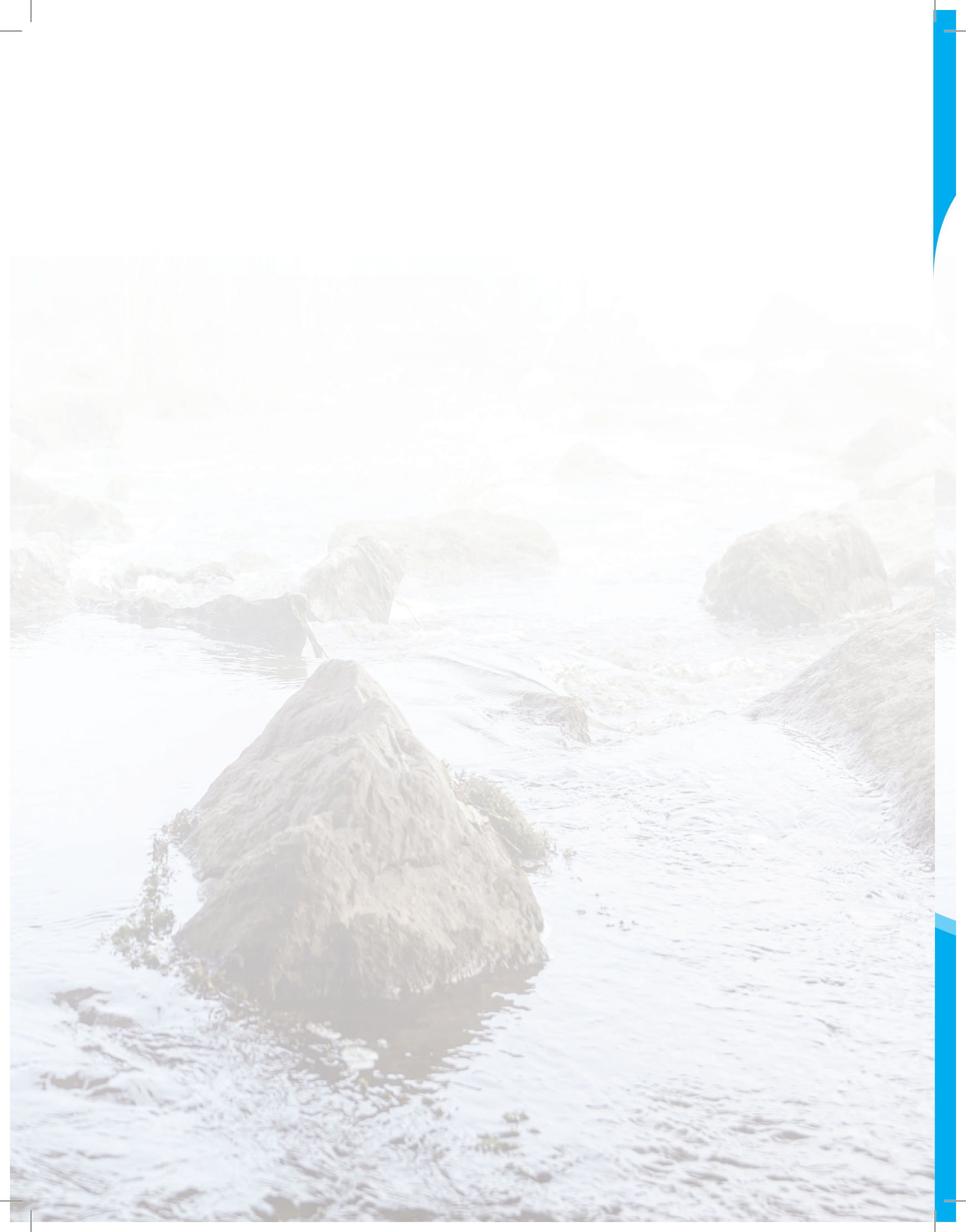
1. We will understand the importance of water, conserve it and ensure rich water resources for future generations.
2. Conservation and enhancement of water resources of our country is a sacred goal. I will work with my dedication and devotion to achieve this.
3. Proper access to water is a strong step towards women's empowerment.
4. Jal Shakti is Nari Shakti, as women are the most affected by water scarcity due to their household responsibilities in rural India.

”

<https://pib.gov.in/PressReleaseDetail.aspx?PRID=2024324>

<https://www.newsonair.gov.in/c-r-patil-emphasizes-water-access-as-essential-for-womens-empowerment-at-catch-the-rain-2024-workshop-in-delhi/>

<https://www.indiatvnews.com/gujarat/will-transform-jal-shakti-into-rashtra-shakti-cabinet-jal-shakti-minister-c-r-patil-after-portfolio-appointment-pm-modi-2024-06-10-936272>



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Uttar Pradesh Forest Department & Biodiversity Board

Bihar Forest Department & Biodiversity Board

Jharkhand Forest Department & Biodiversity Board

West Bengal Forest Department & Biodiversity Board

Ganga Task Force (GTF)

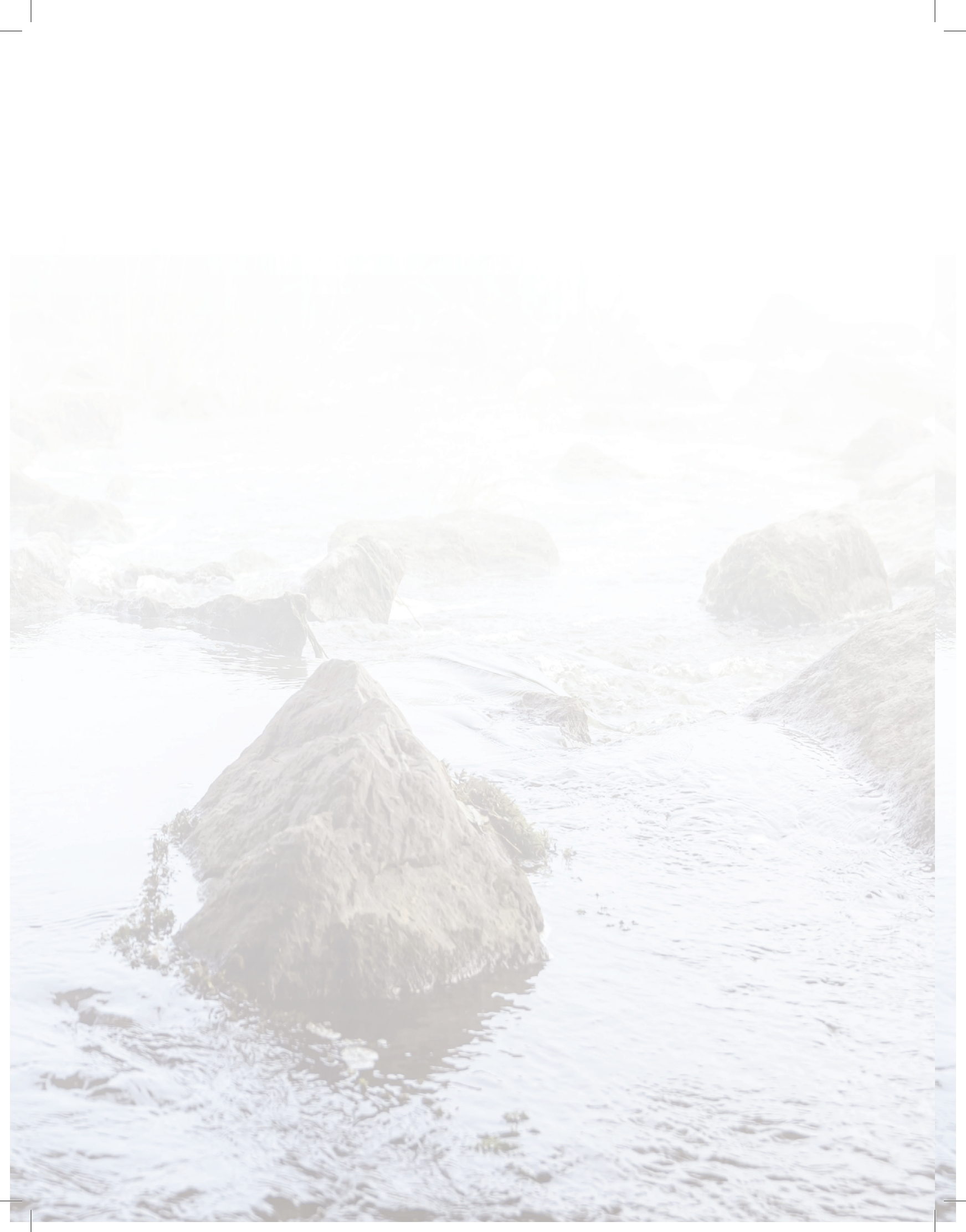


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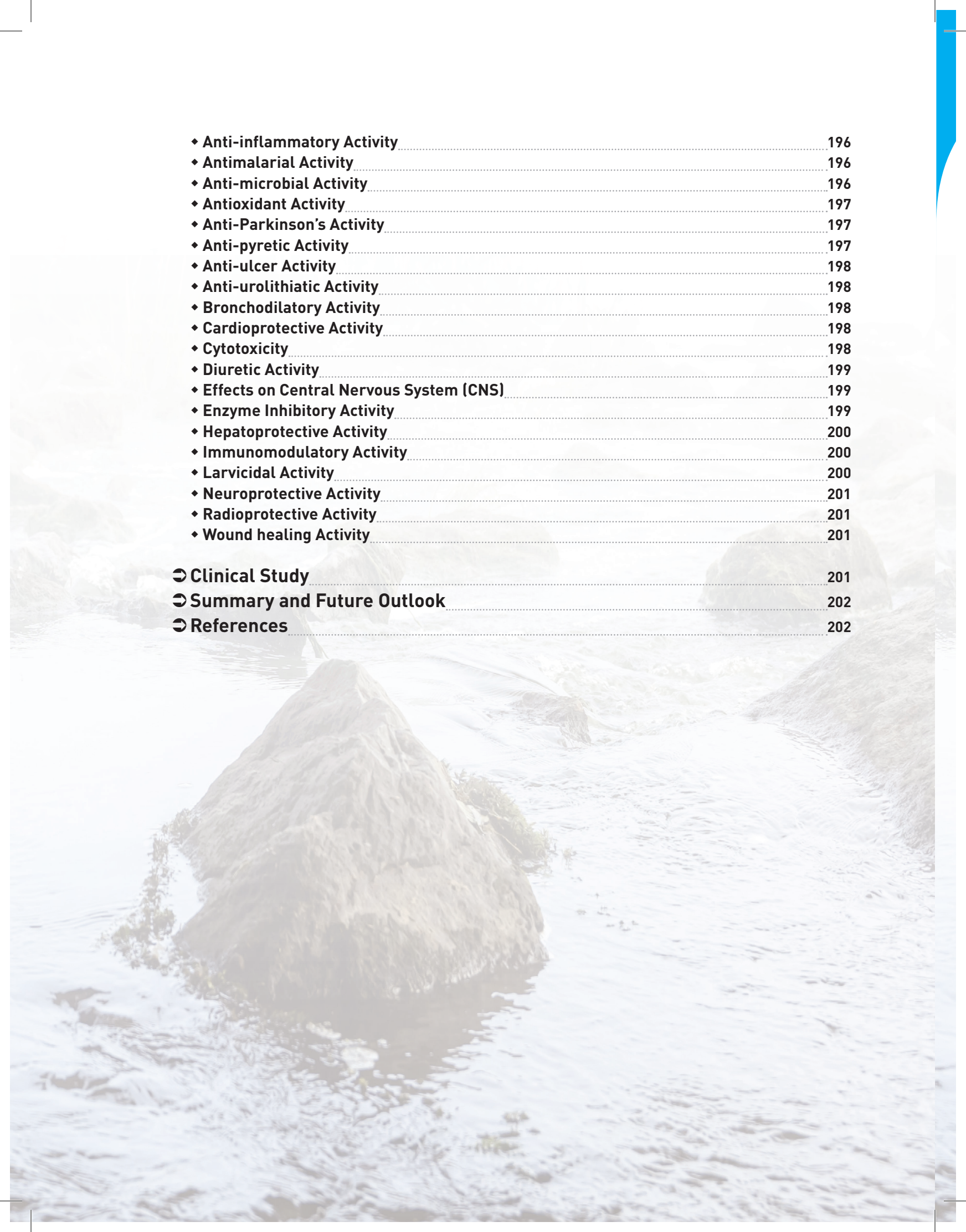
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सत्यमेव जयते
75
आज़ादी का
अमृत महोत्सव

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जल शक्ति मंत्रालय
जल संसाधन,
नदी विकास और गंगा संरक्षण विभाग
GOVERNMENT OF INDIA
MINISTRY OF JAL SHAKTI
DEPARTMENT OF WATER RESOURCES,
RIVER DEVELOPMENT & GANGA REJUVENATION



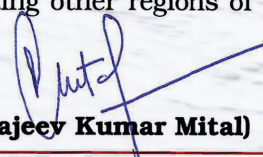
MESSAGE

The Ganga, a river deeply revered in our culture, is both a source of spiritual inspiration and home to diverse ecosystems, including riparian buffers along its banks that connect terrestrial and aquatic systems. These buffers play a crucial role in supporting amphibious life, stabilizing the riverbanks, and maintaining ecosystem services. Therefore, under the "Aviral Ganga" approach for biodiversity conservation and "Arth Ganga" for enhancing livelihoods, a project on floral diversity has been initiated through Namami Gange Mission – II, in collaboration with Patanjali Organic Research Institute (PORI), Haridwar, Uttarakhand.

The project "Scientific Exploration of Floral Diversity Near the Ganga Riverbanks for Ethnobotanical Purposes along with their Conservation and Economic Development of the Region via Skill Development Programs" aims to provide valuable scientific insights into the region's ethnobotanical wealth while promoting sustainable conservation practices. The 18-month study, conducted from Gaumukh to Gangasagar along the Ganga banks, reveals the floral profile, ethno medicinal aspects, applications, livelihood perspectives, and the role of biodiversity in ecosystem resilience, highlighting the symbiotic relationship between conservation and socio-economic development. Findings presented through publications, including project reports, monographs, exploration of ethno medicinal plants, and plant wealth along river Ganga India, will benefit stakeholders, including local communities' well-being.

It gives me immense pleasure to extend my appreciation to the entire team for successfully executing the project, which advances our understanding of the unique floral diversity along the Ganga and its role in ethnobotanical purposes, conservation, and integrating science with local knowledge. The project's focus on environmental stewardship and skill enhancement for sustainable livelihoods is commendable.

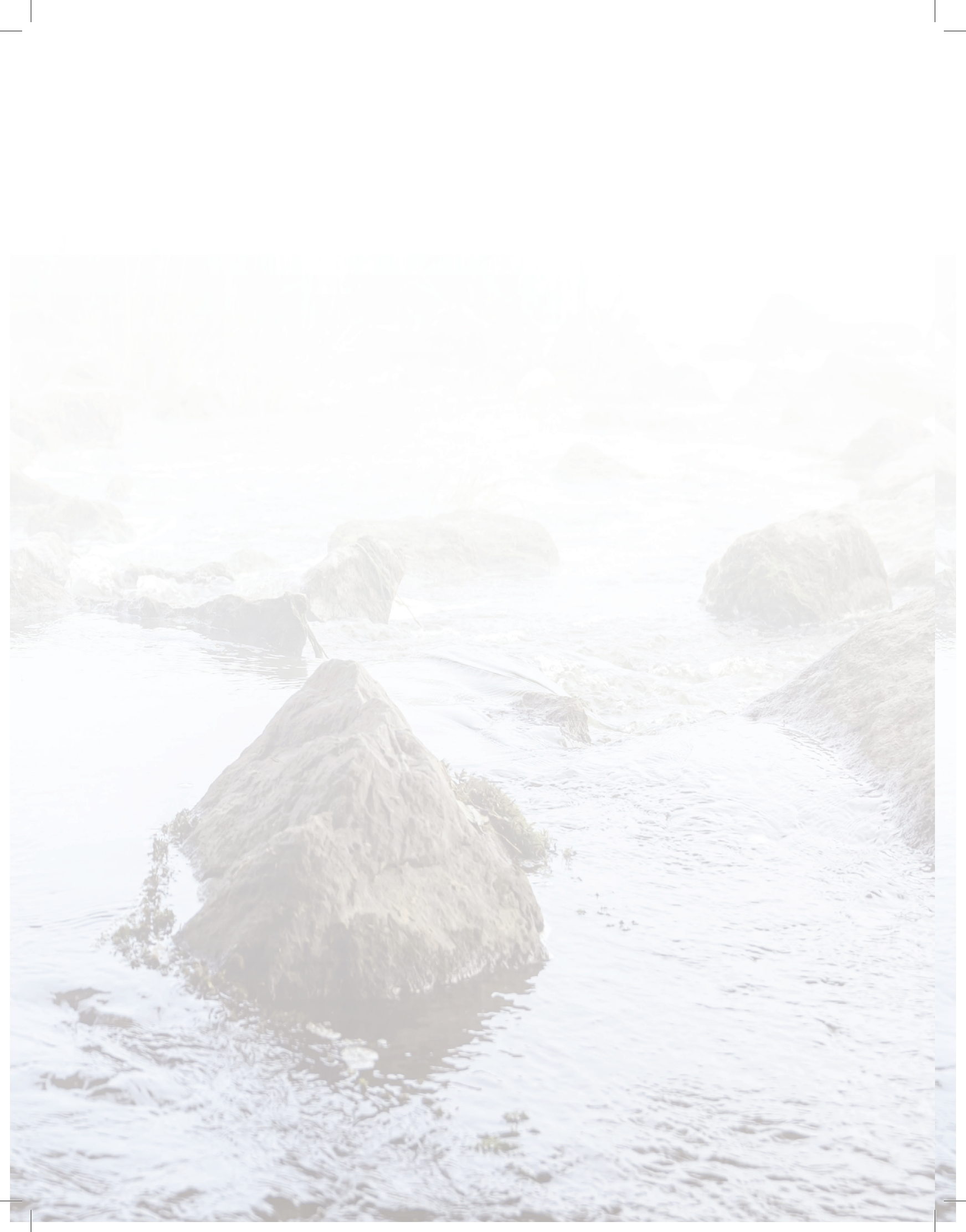
This excellent work documenting the floral diversity along the banks may pave the way for the rejuvenation of the Ganga and set a precedent for biodiversity conservation and socio-economic improvement, potentially benefiting other regions of India as well.


(Rajeev Kumar Mital)



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FOREWORD

*I*n the timeless flow of the sacred Ganga River, lies a profound treasure trove of nature's healing bounty. As we embark on this journey through the pages of this book on Medicinal Plants along the Ganga River, we are reminded of the ancient wisdom that has sustained our civilization for millennia. The Ganga, revered not only for its spiritual significance but also for its ecological richness, nurtures a diverse array of medicinal plants. The plant, meticulously documented in this comprehensive work, are not merely botanical specimen but living reservoir of phytochemicals that hold immense therapeutic potential.

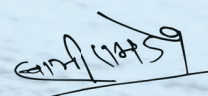
The authors have meticulously documented the plant exploration and botanical study, soil properties analysis, phytochemical analysis, and insilico analysis of the plants found in this region. Their dedication and deep respect for nature's wisdom are evident throughout the pages, making this monograph an invaluable resource for all who seek to deepen their understanding of the healing potential found in the lap of the Himalayas.

Moreover, the inclusion of phytochemical and bioinformatic aspects adds a modern scientific perspective to this ancient knowledge. By unraveling the chemical constituents and molecular mechanisms behind the therapeutic effects, this monograph bridges the gap between traditional wisdom and contemporary scientific advancements, paving the way for evidence-based herbal medicine.

I extend my heartfelt appreciation to Acharya Balkrishna Ji and his devoted team of expert scientists for their unwavering commitment for preserving and disseminating the wisdom of the Medicinal Plants along Ganga River. Their work not only contributes to the scientific community but also instills a deep reverence for nature and its intricate web of life.

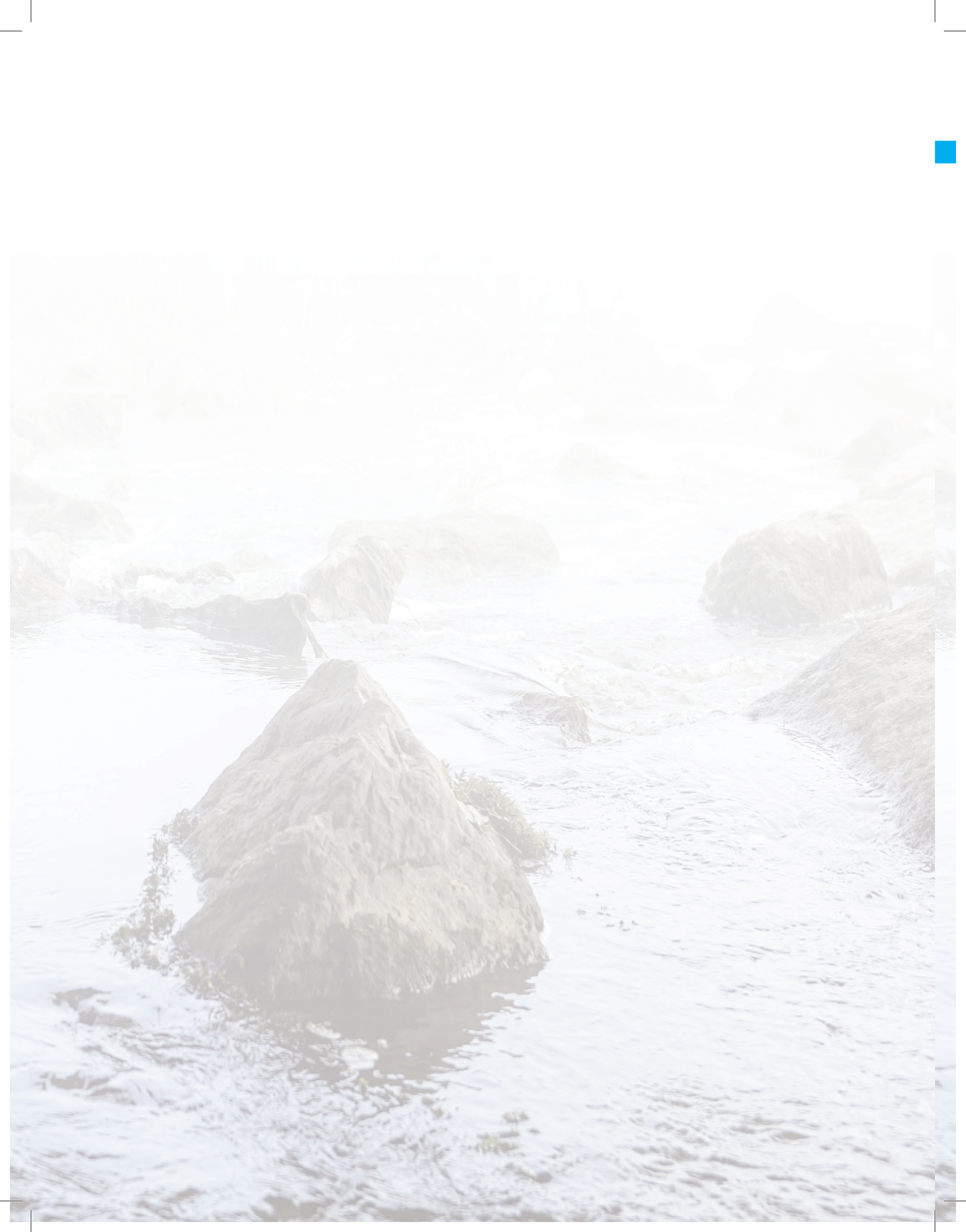
May this monograph serve as a guiding light for researchers, practitioners, and enthusiasts alike, illuminating the path towards a deeper appreciation of nature's pharmacy along the revered Ganga River.

With heartfelt gratitude and blessings,



- Swami Ramdev





PREFACE

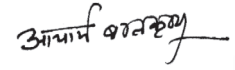
The Ganga River, revered as the lifeblood of India, flows through the heart of the country, nourishing not only the land but also the spirit of the people. It is not just a geographical entity; it is a symbol of life, purity, and sustenance. From its origin in the pristine glaciers of Uttarakhand to its expansive delta in West Bengal, the Ganga traverses diverse landscapes, each rich in its own unique flora and fauna. For centuries, it has supported a wide variety of ecosystems, each harbouring plant species that are integral to the ecological balance, cultural heritage, and traditional practices of the region. This monograph series is dedicated to exploring and documenting the indigenous plants that thrive along this sacred river, from the high-altitude regions of Uttarakhand to the fertile plains of West Bengal. These plants are not merely biological entities; they are part of the cultural and spiritual fabric of the communities that have lived along the Ganga for generations. They are used in traditional medicine, rituals, and daily life, and they play a crucial role in the local economies.

The journey from Uttarakhand to West Bengal covers a vast array of ecosystems, from the alpine forests of the Himalayas to the mangroves of the Sundarbans. At each of the 26 sites we studied, the selected plant species reflect the unique environmental conditions and cultural practices of the region. This series of monographs is a culmination of an extensive research initiative aimed at understanding and preserving the botanical wealth of the Ganga basin. A total of 26 key sites along the river, spanning the length from its source in Uttarakhand to its mouth in the Bay of Bengal, were explored, analysed, and documented. At each of these sites, the indigenous plant species were studied which are adapted to the unique environmental conditions present. The exploration focuses on ten plant species that are not only emblematic of the region but also hold significant ecological, medicinal, and cultural value.

Each monograph in this series provides a comprehensive overview of the plant species, detailing its botanical characteristics, ecological role, traditional uses, and cultural significance. The data for distribution of these plants along the Ganga, highlighting the environmental factors that influence their growth and survival is primarily focussed. To achieve a comprehensive understanding, the selected sites were explored for their botanical diversity, phytochemical properties of the plants, and soil properties examination of each area. Additionally, *in silico* analysis was conducted to assess the plants' potential in combating several diseases. The study also investigated the ethnomedicinal and pharmacological uses and applications of these plants, complemented by a bibliometric analysis to evaluate existing research and knowledge. This exploration also presents as a fresh insight to classical literature of Ayurveda in the form of "Shlokas" and therefore extending beyond the identification of individual species; it adds a fresh perspective to the existing literature and delves into uncovering the intricate web of life that sustains these plants and the human communities that rely on them.

Patanjali Group recognized the critical need to explore the diverse botanical landscape of the Ganga River basin and to conduct scientific, evidence-based research to highlight its ecological and medicinal significance. With this vision, Patanjali Organic Research Institute embarked on an initiative to systematically document and analyse the indigenous plant species found across this region. The goal is to develop scientifically validated insights into the botanical diversity, phytochemical properties, and ethnomedicinal uses of these plants, thereby creating a comprehensive understanding of the current status and challenges associated with preserving this rich natural heritage.

We express our deepest gratitude to Param Pujya Swami Ramdev Ji for his unwavering leadership and support throughout this endeavour, as well as for his invaluable guidance in every aspect of our work. My best wishes to the dedicated and highly skilled experts, who have meticulously gathered data and conducted extensive research on the diverse botanical heritage of the Ganga River basin, utilizing cutting-edge scientific techniques and methodologies. Nature holds immense potential for sustainable practices and this project is a significant contribution to the preservation of these indigenous plant species, ultimately benefiting both ecological balance and the well-being of the communities that rely on them.



Dr. Acharya Balkrishna



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Classical Ayurvedic Insight

Cynodon dactylon (L.) Pers.

Vedic name : (Dūrvikā śataparvā) (दूर्विका शतपर्वा)

Botanical name : *Cynodon dactylon* (L.) Pers., Syn. *Panicum dactylon* L.

Utpatti sthānam

Āṣṭreliyābhidhe deśe śrīlāṅkābrahmadeśayo.

Amerikākhyadeśe'pi bhārate himaparvate.

Aṣṭādaśaśatavyāmatuṅgitaprāyakasthale.

Paścime ghaṭṭake cāpi sahasravāmakocchrite.

Bhuvo bhāge vilokyeta dūrvāvaṃśīyapādapaḥ. (Saumitreya Mahodadhi: 1-3)

Distribution

Around the world it is found in Myanmar, Sri Lanka, Australia, America and other tropical regions. Throughout India it found to an altitude of 2500 m in the Himalayas and 2100 m in the Western Ghats. ^[1-8]

Vānaspatika vivaraṇam

1. Kāyika lakṣaṇam

Visarpī khaḍganikāśapatrayug bahuvarṣikaḥ.

Trṇarūpo dr̥ḍhaścāpi prakandī ca tathā'ṅkurai.

Samyuto vartate kāṇḍaṃ śayānaṃ tanuvigrahaṃ.

Visapiśīlakaṃ sāndrakaṭābharaṇanāmaho.

Kurvad muṣṭisamucchrāyād ratnituṅgikalevaram.

Ārohi tanukañcāpi puṣpitaśākhayā yutam.

Patraṃ dvipaṅktikaṃ nīlaṃ saṅkucadrekhitantathā.

Bhallābhamaṅgulottuṅgāt pañcāṅgulasamucchritam.

Yavāyāmāt samārabhya dviyavāyatakāyakam.

Bhāsvaraṃ dr̥ḍharūpañca sūnyaromā'thavā kila.

Romaśaṃ bhāti picchākṣastanuko jihvikā punaḥ.

Śvetaromayutā bhāti prasūnantvaṅgulocchritāt. (Saumitreya Mahodadhi: 3-9)

Botanical Description

1. Vegetative Characters

An extensively creeping, sward-forming, perennial grass with strong, flat, rhizomes and stolons. Stem slender, prostrate, widely creeping, forming matted tufts, with slender erect or ascending flowering branches 7.5-30 cm high. Leaf sheaths bearded at mouth, otherwise glabrous or thinly pilose. Leaves

distichous, narrowly linear or lanceolate, glaucous, 2-10 cm long and 1.2-3 mm wide, smooth, tight, glabrous or hairy. Ligule a ring of white hairs. Rachis slender. ^[1-8]

2. Puṣpīya lakṣaṇam

Sārdhadvyaṅguluṭuṅge ca dvicatu pañcasamkhyake.
Kaṇiṣe harite cā''ho vṛntakavarṇasaṃyute.
Jāyate tatprasūnālivṛntamaṅguluṭuṅgitāt.
Sārdhadvyaṅguluṭuṅgañca tanukaṃ kaṇiṣaṃ punaḥ.
Ekapuṣpi yavottuṅgād dviyavottuṅgata sthitam.
Paricakrakasaṃkhyātatuṣastu bhallakākṛtiḥ.
Tīkṣṇaśca komalo bhāti tuṣo'dhogo yavocchritaḥ.
Ūrdhvagaśca tuṣaḥ prāyaḥ pratibhāti vilambitaḥ.
Puṣpīyaśca tuṣastiryagdīrghāyatakalevarāt.
Ardhāṇḍasammitākāro yavocchrāyivapurdharaḥ.
Tatpakṣasadrśākārastīkṣṇāgrī nautalābhataḥ.
Yavottuṅgāt samārabhya dviyavocchrāyivigrahaḥ.
Pakṣmābhikāmayenā'sau saṃyukto nautalaṃ prati.
Vandhyapicchākṣajātantu sarṣapākārataḥ khalu.
Yavalambī kadācittu śīrṣe caivā'vaśeṣiṇā.
Kaṇiṣena samāyuktastadīyāṇḍāśayasya tu.
Adhobhāgau sadā hrasvau kośāste ca parāgagāḥ.
Yavārdhalambino bhānti dīrghāyatakalevarāḥ.
Vartikā spaṣṭarūpā'tra tadagraṃ pakṣasannibham.
Phalaṃ yavonnataṃ prāyo dīrghāyāmayutantathā.
Pārśve sampīḍitañcāpi cipiṭaṃ pratibhāsate.
Bījaṃ dīrghāyataṃ sūkṣmaṃ babhruvarṇaṃ sumodayaḥ.
Varṣāhemantayormadhye jāyate ca phalodayaḥ. (Saumitreya Mahodadhi: 9-20)

Floral Characters

Flowers borne on 2-6 green or purplish spike 2.5-5 cm long. Peduncle slender, 2-5 cm long. Spikelets 1-flowered, 2-2.8 mm long. Involucral glumes lanceolate, acute, smooth. The lower 1-1.6 mm long, the upper slightly longer. Floral glume obliquely oblong to semi-ovate, 2 mm long. Lemma keeled, mucronate, 1.5-3 mm long, ciliolate along the keel. Sterile rachilla extension 0.5–1.5 mm long, sometimes topped with a vestigial spikelet. Lodicules 2, short. Anthers oblong, 1 mm long. Style distinct. Stigma feathery. Caryopsis 2.2-2.7 mm long, oblong, laterally compressed. Seeds minute, oblong, brown in colour. Flowering/Fruiting July-January. ^[1-8]

Plant Anatomy

Prayojyāṅgam

Dūrvikāśatapatrāyāḥ prayojyāṅgaṃ bhiṣagvaraiḥ.

Mūlamudāhṛtaṃ tasyāntāracanopavarṇyate. (Saumitreya Mahodadhi:1)

Antaḥsaṃracaneḥṣaṇe

(a) Mūlam

Ekastarīyakā mūlatvacātra tanubhittikaiḥ.

Arīyalambato dṛśyairghanākārasvarūpakaiḥ.

Kośikāvisaraiḥ sṛṣṭā hyadhastvacābhiddṛśyate.

Dvistarakalpataḥ sā tu tanubhittīyalambakaiḥ.

Sparśarekhīyakaiḥ sṛṣṭā'niyatakośikācayaiḥ.

Tanmajjakośikā bhāgadvaye'tra pravibhājītā.

Utaikastarikāścātra tanubhittīyakāḥ punaḥ.

Bahubhujīyakā yuktāḥ kośikāvaraṇairapi.

Staraṣaṭkayutā lambāstanubhittīyakāstathā.

Mṛdulāḥ kośikā dṛśyā antastvaceha vā punaḥ.

Ekastarīyakā sthūlabhittīyakā pralambikāḥ.

Sparśarekhīyakāścāpi vartanta iha kośikāḥ.

Dvistarīya iha prāyaḥ parirambho vinirmitaḥ.

Dṛḍha otakīyakaiḥ sthūlabhittīyakośikāvrajaiḥ.

Samvahanakapūleṣu nyagvāhapoṣavāhakāḥ.

Paridhau samsthitā dṛśyā bhinnāntarālaniṣṭhitāḥ.

Nyagvāho bahirādiḥ saḥ sāmānyatattvasamṇyutaḥ.

Majjabhāgastataḥ kendras hyaṇḍākārāt pragolakāḥ.

Sthūlabhittīyamṛdvībhiḥ kośikābhirvinirmitaḥ.

Naike maṇḍakaṇā dṛśyāḥ saralā golarūpataḥ.

Aṇḍākārāḥ sakoṇā vā te punaryuktarūpiṇaḥ.

Catubhirghaṭaṭakairyuktā dṛśyante sūkṣmikāyane. (Saumitreya Mahodadhi: 2-12)

Plant Parts

Root

Microscopic Characters

Root

Epiblema is composed of single layered, thin walled and radially elongated to cubical cells. Hypodermis is composed of 1-2 layered, thin walled, tangentially elongated to irregular shaped cells. Cortex is differentiated into two zones, 1 or 2 layers of smaller, thin-walled, polygonal, lignified sclerenchymatous and 4-6 layers of thin-walled, elongated parenchymatous cells. Endodermis is single layered, thick-walled, tangentially elongated cells. Pericycle is 1-2 layers composed of thin-walled sclerenchymatous cells. Vascular bundles consist of xylem and phloem, arranged in a ring on different radials. Xylem exarch, having usual elements. Pith is wide occupied at centre and composed of oval to rounded, thick-walled, parenchymatous cells. Starch grains are numerous, simple, round to oval or angular. Compound starch grains having 2-4 components. ^[1, 9-11]

(b) Cūrṇam

Īṣacchvetābhamālokyam cūrṇam nyagvāhavāhikāḥ.

Sakaṇagartabhittīyāḥ kośikāvaraṇairyutāḥ.

Dṛḍhotakīyakā dṛśyāḥ kośikāḥ golarūpataḥ.

Ekalā yutamaṇḍāṃśāstadghaṭakacatuṣṭaye. (Saumitreya Mahodadhi: 13-14)

Powder

Cream coloured. It contains fragments of xylem vessels with pitted walls, thick-walled lignified sclerenchymatous cells and numerous simple round to oval or angular starch grains and compound starch grains having 2-4 components. ^[1, 9-11]

Medicinal Uses

Nasal disorders

Harītakīdāḍimapuṣpadūrvālākṣārasonasyavidhānayoḡāt.

Nivārayatyevacirapraṣṭtamathāṣunāsāntaraṣoṇitaugam. (Rā.Mā. 4:1)

Epistaxis-Instillation of 2-3 nasal drops prepared from *Terminalia chebula* (Black myrobalan), *Punica granatum* (Pomegranate) flower, *Cynodon dactylon* (Devil grass) juice and *Lākṣā* (Lac) juice is useful in epistaxis. ^[12] (Ra.Ma. 4:1)

Abdominal disorders

Pittacchadirvrajeddūrvātaṇḍulodakapānataḥ. (Ga.Ni.Kā.Ci. 14:30)

Pibeccharditrayeṣītaṃ, dūrvāvātaṇḍulāmbunā. (Ga.Ni.Kā.Ci.14:39)

Vomiting-Intake of *Cynodon dactylon* (Devil grass) juice alongwith rice water alleviates vomiting. ^[13] (Gd. Ni.Ka.Ci. 14:30),(Gd.Ni.Ka.Ci. 14:39)

Anorectal disorders

Dūrvāghṛtapradehaḥ. Vyajanapavanaḥsuśītoraktasrāvaṃjayatyāṣu. (Ca.Ci. 14:219)

Hemorrhoids-Intake of *Ghrita* (Clarified butter) processed with *Cynodon dactylon* (Devil grass) juice or topical application of *Cynodon dactylon* (Devil grass) juice mixed with *Ghrita* (Clarified butter) is useful in hemorrhage caused due to hemorrhoids. ^[14] (Ca.Ci. 14:219)

Reproductive system disorders

Saraktapradarāvā'pisāsaṣksrāvā ca garbhiṇī.

Dūrvāyāḥpiṣṭakamprāṣyanāsaṣksrāveṇapīḍyate. (Ca.Da.Ci. 62:24)

Menorrhagia-Intake of rice pounded with *Cynodon dactylon* (Devil grass) juice is useful in hemorrhage and menorrhagia. ^[15] (Ck.Dt.Ci. 62:24)

Dermatological disorders

Dūrvāsvarasasiddham ca ghṛtaṃsyādvraṇaropaṇam. (Ca.Ci. 21:96)

Erysipelas-Intake of *Ghrita* (Clarified butter) processed with *Cynodon dactylon* (Devil grass) juice is useful in erysipelas. ^[14] (Ca.Ci. 21:96)

Svarasena hi dūrvāyāḥpacettailaṃcaturguṇam.

Kacchūvicarcikāpāmābhyamgādevanāśayet. (Vṛ.Mā. 51:192)

Pruritus-Massage with oil processed from *Cynodon dactylon* (Devil grass) juice is useful in pruritus, eczema, cholera. ^[16] (Vṛ.Md. 51:192)

Dūrvāniṣāyutolepaḥkacchūpāmāvināśanaḥ.

Kṛmidadrūharaṣcaivaśītapittaharaḥsmṛtaḥ.(Vṛ.Mā. 52:7)

Urticaria-Topical application of *Cynodon dactylon* (Devil grass) and *Curcuma longa* (Turmeric) paste is useful in pruritus, eczema, worm infestation, ringworm and urticaria. ^[16] (Vṛ.Md. 52:7)

Dūrvāsvarasasiddhamvātailaṃ. pradhānaṃvraṇaropaṇam. (Ca.Ci. 25:93)

(Vṛ.Mā.45:30)

Wound-Topical application of *Sesamum indicum* (Sesame) oil processed with *Cynodon dactylon* (Devil grass) paste and juice enhances wound healing. ^[16] (Vṛ.Md. 45:30), ^[14] (Ca.Ci. 25:93)

Visarpakagranthiviśādirogānlepādinātailaghṛtāpahārāt.

Vināśayeddugdhayutā''ṣuśubhrādūrvāmasūrānapipittajātān.(Vai.Ma. 11:17)

Pruritus-Topical application of paste prepared from *Cynodon dactylon* (Devil grass), *Terminalia chebula* (Black myrobalan), rock salt, *Cassia tora* (Fetid cassia) seed, *Ocimum basilicum* (Basil) leaf pounded with buttermilk is useful in pruritus and ringworm. ^[17] (Vd.Mn. 11:17)

Dūrvā'bhayāsaindhavaṃ ca cakramardaḥkaṭherakaḥ.

Ebhistakrayutolepaḥkaṇḍūdadrūvināśanaḥ.(Śā.Saṃ.Ma.Kha. 11:54)

Topical application of paste prepared from an equal quantity of *Cynodon dactylon* (Devil grass) and *Berberis aristata* (Indian barberry) pounded with buttermilk is useful in pruritus, eczema, worm infestation, ringworm and urticaria. ^[18] (Sh.Sm.Md.Kh. 11:54)

Dūrvāniṣāyutolepaḥkaṇḍūpāmāvināśanaḥ.

Kṛmidadruharaṣcaivaśītapittāpahaḥsmṛtaḥ.(Śā.Sa.U.Kha. 11:55)

Topical application of paste prepared from arsenic trisulphide, *Cynodon dactylon* (Devil grass), rock salt pounded with cow urine is useful in chronic ringworm and pruritus. ^[18] (Sh.Sm.Ut .Kh. 11:55)

Gomūtrapiṣṭairharitālādūrvāsindhūdbhavairlepitamādareṇa.

Prayātināśaṁniyataṁnarāṇāṁdadruṣcapāmāsucirapararūḍhā.(Rā.Mā. 8:6)

Erysipelas and smallpox-Topical application of *Cynodon dactylon* (Devil grass) paste mixed with oil and *Ghrita* (Clarified butter) is useful in erysipelas, smallpox and cyst or intake of *Cynodon dactylon* (Devil grass) with milk is useful in *Pitta* associated smallpox. ^[12] (Ra.Ma. 8:6)

Generalised body disorders

nasyaṁ.....dūrvāsvarasasyacaiva. (Ca.Ci. 4:100)

Raktapitta-Arrhine of *Cynodon dactylon* (Devil grass) juice is useful in *Raktapitta*. ^[14] (Ca.Ci. 4:100)

Lihyāccadūrvāvaṭajāmṣcapallavānmadhudvitīyān.....(Su.U. 45:20)

Intake of *Cynodon dactylon* (Devil grass) and *Ficus benghalensis* (Banyan tree) leaf juice or paste mixed with honey is useful in *Raktapitta*. ^[19] (Su.Ut. 45:20)

Parts used

Whole part and root.

Dose

Juice 10-20 ml or as directed by the Physician.

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Abbreviation

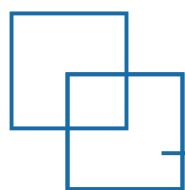
A.Hṛ.Ci.	:	AṣṭāṅgaHṛdayaCikitsā
A.Hṛ.U.	:	AṣṭāṅgaHṛdaya Uttara Tantra
As.Hr.Ut.	:	AṣṭāṅgaHṛdaya Uttara Tantra
Bhā.Pra.Ci.	:	BhāvaprakśaCikitsā
Śā.Sa.Ut.Kha.	:	ŚārṅgadharaSamhitā Uttara Khaṇḍa
Ca.Ci.	:	Caraka SamhitāCikitsāSthāna
Ca.Da.Ci.	:	CakradattaCikitsā
Ca.Si.	:	Caraka Samhitā Siddhi Sthāna
Ca.Sū.	:	Caraka SamhitāSūtraSthāna
Ck.Dt.Ci.	:	CakradattaCikitsā
Ga.Ni.Kā.Ci.	:	GadanigrahaKāyacikitsā
Ga.Ni.Kau.Taṃ.	:	GadanigrahaKaumaryatantra
Ga.Ni.Śā.Ta.	:	GadanigrahaŚālākya Tantra

Ga.Ni.Śa.Taṃ.	:	GadanigrahaŚalyatantra
Hā.Sa.	:	HārāitaSamhitā
Hā.Saṃ.	:	HārāitaSamhitā
Kā.Saṃ.Khilasthāna	:	KāśayapaSamhitāKhilasthāna
Rā.Mā.	:	RājaMārtaṇḍa
Ra.Ra.Sa.	:	Rasa Ratna Samuccaya
Śā.Ni.Guḍūcyādivarga	:	ŚāligrāmaNighaṇṭuGuḍūcyādivarga
Śā.Sa.U.Kha.	:	ŚāraṇadharaSamhitā Uttara Khaṇḍa
Śā.Saṃ.Ma.Kha.	:	ŚāraṇadharaSamhitā Madhyam Khaṇḍa
Sd.Bh.Mm. Ch. Gr.Ci.	:	Siddha BheṣajaMaṇimālāCaturthaGucchaGrahaṇāiCikitsā
Sd.Bh.Mm. Ch. Nd.Vr.Ci.	:	Siddha BheṣajaMaṇimālāCaturthaGuccha Nāḍāivraṇa Cikitsā
Sd.Bh.Mm. Ch.Nt.Rg.	:	Siddha BheṣajaMaṇimālāCaturthaGuccha Netra RogaCikitsā
Sd.Bh.Mm.Ch. Ars.Ci.	:	Siddha BheṣajaMaṇimālāCaturthaGucchaArśaCikitsā
Sd.Bh.Mm.Ch. Vrn.Ci.	:	Siddha BheṣajaMaṇimālāCaturthaGucchaVraṇaCikitsā
Sh.Gr.Ng. Gd.Vg.	:	ŚāligrāmaNighaṇṭuGuḍūcyādivarga
Su.Ci.	:	SuśrutaSamhitāCikitsāSthāna
Su.Ka.	:	SuśrutaSamhitā Kalpa Sthāna
Su.Śā.	:	SuśrutaSamhitāSārīraSthāna
Su.Ut.	:	SuśrutaSamhitā Uttara Tantra
Vai.Jī. Caturthavilāsa	:	Vaidya JāivanamCaturthavilāsa
Vai.Jī. Dvitiyavilāsaḥ	:	Vaidya JāivanamDvitiyavilāsaḥ
Vai.Ma.	:	Vaidya Manoramā
Vaṃ. Se. Āmavātarogādhikāraḥ	:	VaṃgasenaSamhitāĀmavātarogādhikāraḥ
Vaṃ.Se. Vātavyādhidhikāraḥ	:	VaṃgasenaSamhitāVātavyādhidhikāraḥ
Vaṃ.Se.Karṇarogaḥ	:	VaṃgasenaSamhitāKarṇarogādhikāraḥ
Vaṃ.Se.Medarogaḥ	:	VaṃgasenaSamhitāMedarogādhikāraḥ
Vaṃ.Se. Netrarog	:	VaṃgasenaSamhitāNetrarogādhikāra
Vaṃ.Se. Śothādhikāraḥ	:	VaṃgasenaSamhitāŚothādhikāraḥ
Vaṃ.Se. Strīrogaḥ	:	VaṃgasenaSamhitāStrīrogaḥ
Vaṃ.Se.Vātavyādhīḥ	:	VaṃgasenaSamhitāVātavyādhidhikāraḥ
Vṛ.Mā.	:	Vṛndamādhava

CHAPTER

01

Plant Exploration and Botanical Study



INTRODUCTION

Water is the key resource required to sustain life on this planet. It is found in most of the earth as in the ocean, rivers, ponds, streams, ice, etc. The river Ganga is an important source of water in India that makes an important river system from the Himalaya to the Bay of Bengal. The Indian civilization and economy depended on this river system due to its water availability throughout the year (Paul and Sinha, 2013). The river Ganga alone accounts for 25% of India's total water resources (Paul, 2017). Globally more than 300 million people from India, Nepal, and Bangladesh depend on the river Ganga (Gopal, 2000). This is the thirtieth longest river in the world and covers a basin area of 861,404 km² (Rahaman, 2009a). This basin is the most populated area in the world with an average density of 520 persons/km² (Das and Tamminga, 2012). The basin is very rich in heritage, cultural, and religious values. India's about one-fourth area drains by the river Ganga.

The river originates from the Gangotri glacier at Gomukh (30° 36' N; 79° 40' E; 3800 m) in the Uttar Kashi district of Uttarakhand under the name of Bhagirathi; and another tributary *i.e.*, Alaknanda which is originated from the Bhagirath-Kharak (30° 49' N; 79° 17' E) and Satopanth (30° 45' N; 79° 21' E) glaciers. Both the tributaries join at Devprayag from where the name Ganga started. The river has a total length of 2600 km from its main source Gomukh.

Up to Haridwar it flows in the hills after here it enters the plain area. From here it flows southwards, passing through the plains of Uttar Pradesh starting from Bijnor district. Up to Ballia it continuously flows in Uttar Pradesh through different districts after here it enters Bihar near Chausa of Buxar district. It flows up to Katihar district in Bihar and from here it enters the Sahebganj district of Jharkhand. Then it turns southwards and enters the West Bengal from the Murshidabad district. About 40 km from the Farakka, it divides into two streams; the left stream flows eastwards toward Bangladesh while the right stream, known as Bhagirathi, continues to flow south through West Bengal. Its name changed to Hooghly when it flows in the west and south-west of Kolkata. The



Hoogly at Diamond Harbor flows southward and is split into two streams before reaching the Bay of Bengal (Rahaman, 2009b).

The annual water discharge from the river Ganga is about 18700 m³/s which is the fifth highest in the globe (Paul, 2017). The maximum flow of the Ganga exists within the catchment area with a mean maximum flow is 468.7×10^9 m³ which is a total of 25.2 % of the total water resources of India (Sarkar et al., 2012). The main source of the river Ganga is the melting of snow in the Himalayas and monsoon rains. The water of the Ganga is regularly used by the living being for drinking. Outdoor bathing has a spiritual significance for Hindus in which millions of people take a holy dip at least once a year throughout the course of the river, from Gangotri to Ganga Sagar.

The Ganga basin supports biodiversity, species richness, and uniqueness, attracting people from different parts of the earth. Plant communities have been a major magnetism for investigation since time immemorial. Vegetation composition and assemblages of an area form a significant habitat that contributes to the structure and function of such ecosystems. The vegetation pattern along the Ganga varies according to the seasonal changes, flood level, and species composition differs by the function of water supply and different soil types, which has a sharp influence on plant species distribution. Macrophytes such as submerged, emergent, and free-floating aquatic plants are known to accumulate and bioconcentrate heavy metals producing an internal concentration several folds greater than their surroundings (Chen et al., 2008; Allen-Diaz et al., 2008). The river basin has large numbers of medicinal plant species. The natives depend on the river for water and medicinal importance to fulfill their requirements from the beginning.

Cynodon dactylon is one of the medicinal plants that was considered for this study. In Hindi, it is frequently referred to as 'Doob', and in English, 'Bermuda grass'. It is indigenous to southern Europe, Asia, East Africa, and Australia. The weed *Cynodon* has been discovered to have several possible therapeutic uses (Singh et al., 2009). The herb has long been utilized as a diabetic treatment (Kirtikar and Basu, 1996). There have been reports of the antidiabetic, antioxidant, and hypolipidemic efficacious properties of *C. dactylon* leaf extract (Singh et al., 2007, 2008a, b; Rai et al., 2010); it has also been shown to be effective in mending small injuries (Oudhia, 1999; Parihar and Sharma, 2021). On this basis, the current chapter is focused on the morphology, taxonomy, anatomy, and distribution assessment of *C. dactylon* around the Ganga River.

Genus *Cynodon* Rich.

The tiny genus *Cynodon* contains warm-season plants that belong to the tribe Cynodonteae, subfamily Chloridoideae of the family Poaceae. Since the Vedas, it has been the most revered herb and is utilized in holy yogas and remedies. It is a graceful, hardy perennial creeping grass that grows everywhere in the world. Despite having a limited number of species, the genus includes species that are extremely valuable commercially as grass, stabilize soil, and forages to be grazed or stored. Botanically, it contains a few to fifteen bright red racemes that are digitated; each raceme is straight and ranges in length from 4 to 8 cm. Sessile spikelets often grow in two rows on a rachis. Glumes are persistent. Red or purple flowers have lateral compression (Wu, 2011). This genus has 3 types of shoots erect or ascending culms ending in inflorescence, stolons or creeping stems, and rhizome or underground shoots (Jain, 1967).

Several *Cynodon* species are valuable economically because they are used in agriculture, mostly as stored or grazed animal feed. Among the species, *C. dactylon*, which comprises the widely distributed common bermudagrass, is the most significant. These grasses are useful for remediating spoilt soils and reducing soil erosion in addition to providing animal feed. Additionally, they work well as sinks to remove excess nutrients from soils that get a lot of animal waste. Due to their great capacity for biomass generation, they also offer promise as crops for bioenergy feedstock (Casler et al., 2009).

Etymology

The genus belongs to the family “Poaceae” which is based on the genus “*Poa*”. The genus name “*Cynodon*” is derived from the Greek ‘*Kyon*’ which means ‘a dog’ and ‘*Odous*’ which means ‘tooth’; which appears to allude to the rhizomes’ rough, pointed scales that resemble teeth (Jain, 1967).

Historical Background

There have been previous descriptions of several grasses in this genus under the names *Panicum* L., *Capriola* Adanson, *Daetilon* Villars, and *Fibichia* Koel. The term “*Cynodon*” was originally used in 1805 for the grass *Panicum daetilon* in Persoon’s “Synopsis Plantarum.” Since Adanson’s (1763), term *Capriola* was given to this grass earlier and has precedence over *Cynodon*, Kuntze (1891) combined the two names to create *Capriola dactylon* (L.) Kuntze. Holm (1898) talked about both *Cynodon* and the generic name *Capriola*. He attempted to demonstrate that Adanson had not connected the term *Capriola* to any particular grass and that the genus’s boundaries are ambiguous, encompassing several types of grass. Stent (1927) talked about Kuntze and Holm’s perspectives. Adanson had *Panicum daetilon* in mind when he gave the name *Capriola*.

Even though the generic name *Cynodon* is a later homonym, it has been rightfully preserved because the word *Cynodon* is universally used for a globally distributed and commercially

significant grass. Divergent views have also been expressed on who wrote the generic name *Cynodon*. Given that the term was originally used in Persoon's "Synopsis Plantarum," Bentham, Hooker, Stapf, Hackel, Kunth, and several other authors have acknowledged Persoon as the genus's authority. However, Richard ex Persoon is the legitimate source for the generic name *Cynodon* because Persoon linked Richard's name to the word *Cynodon* in his book (Jain, 1967).

Habitat and Distribution

The genus *Cynodon* is a small herb distributed throughout the world. Its native range is Old World. Currently, according to the Plants of the World Online (POWO, 2023) database, it has 15 taxonomically accepted species that are native of Afghanistan, Albania, Aldabra, Algeria, Andaman Is., Angola, Assam, Austria, Azores, Balears, Bangladesh, Benin, Bismarck Archipelago, Borneo, Botswana, Bulgaria, Burkina, Burundi, Cambodia, Cameroon, Canary Is., Cape Provinces, Cape Verde, Caprivi Strip, Central African Repu, Central European Rus, Chad, China North-Central, China South-Central, China Southeast, Congo, Corse, Cyprus, Czechoslovakia, Djibouti, East Aegean Is., East Himalaya, Egypt, Equatorial Guinea, Eritrea, Ethiopia, France, Free State, Gabon, Gambia, Ghana, Great Britain, Greece, Guinea, Guinea-Bissau, Gulf of Guinea Is., Gulf States, Hainan, Hungary, India, Iran, Iraq, Italy, Ivory Coast, Japan, Jawa, Kazakhstan, Kenya, Kirgizstan, Korea, Kriti, Krym, Kuwait, KwaZulu-Natal, Laccadive Is., Laos, Lebanon-

Syria, Lesotho, Lesser Sunda Is., Liberia, Libya, Madagascar, Madeira, Malawi, Malaya, Mali, Maluku, Mauritania, Mauritius, Morocco, Mozambique, Mozambique Channel I, Myanmar, Namibia, Nansei-shoto, Nepal, New Guinea, New South Wales, Nicobar Is., Niger, Nigeria, North Caucasus, Northern Provinces, Northern Territory, Ogasawara-shoto, Oman, Pakistan, Palestine, Philippines, Portugal, Queensland, Rodrigues, Romania, Rwanda, Réunion, Sardegna, Saudi Arabia, Senegal, Seychelles, Sicilia, Sierra Leone, Sinai, Socotra, Solomon Is., Somalia, South Australia, South China Sea, South European Russi, Spain, Sri Lanka, Sudan, Sulawesi, Sumatera, Swaziland, Switzerland, Tadzhikistan, Taiwan, Tanzania, Tasmania, Thailand, Togo, Transcaucasus, Tunisia, Turkey, Turkey-in-Europe, Turkmenistan, Uganda, Ukraine, Uzbekistan, Victoria, Vietnam, West Himalaya, Western Australia, Western Sahara, Yemen, Yugoslavia, Zambia, Zaire, Zimbabwe; and introduced in Alabama, Amsterdam-St. Paul Is, Argentina Northeast, Argentina Northwest, Argentina South, Arizona, Arkansas, Aruba, Ascension, Bahamas, Belgium, Belize, Bermuda, Bolivia, Brazil North, Brazil South, Brazil Southeast, Brazil West-Central, British Columbia, California, Caroline Is., Cayman Is., Chagos Archipelago, Chile Central, Chile North, Christmas Is., Cocos (Keeling) Is., Colombia, Colorado, Connecticut, Cook Is., Costa Rica, Cuba, Delaware, Desventurados Is., District of Columbia, Dominican Republic, Easter Is., Ecuador, El Salvador, Fiji, Florida, French Guiana, Galápagos, Georgia, Germany, Gilbert Is., Guatemala, Guyana, Haiti, Hawaii,

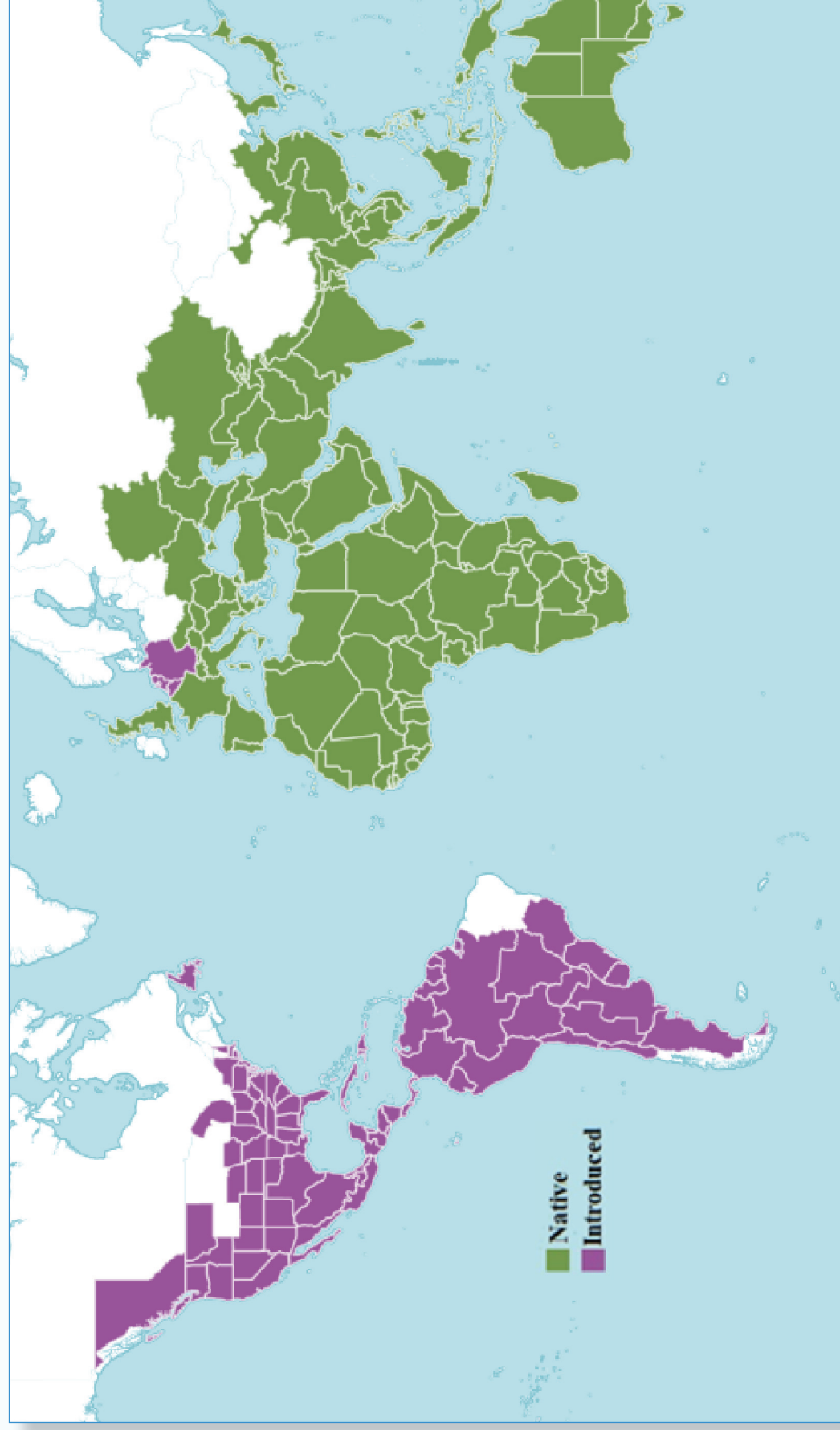


Fig. 1 Global distribution of *Cynodon*

Honduras, Howland-Baker Is., Idaho, Illinois, Indiana, Iowa, Jamaica, Kansas, Kentucky, Kermadec Is., Leeward Is., Line Is., Louisiana, Marianas, Marquesas, Marshall Is., Maryland, Massachusetts, Mexican Pacific Is., Mexico Central, Mexico Gulf, Mexico Northeast, Mexico Northwest, Mexico Southeast, Mexico Southwest, Michigan, Mississippi, Missouri, Montana, Nauru, Nebraska, Netherlands, Netherlands Antilles, Nevada, New Caledonia, New Hampshire, New Jersey, New Mexico, New York, New Zealand North, New Zealand South, Newfoundland, Nicaragua, Niue, Norfolk Is., North Carolina, Ohio, Oklahoma, Oregon, Panamá, Paraguay, Pennsylvania, Peru, Phoenix Is., Pitcairn Is., Puerto Rico, Samoa, Society Is., South Carolina, Southwest Caribbean, St. Helena, Suriname, Tennessee, Texas, Tokelau-Manihiki, Tonga, Trinidad-Tobago, Tristan da Cunha, Tuamotu, Tubuai Is., Turks-Caicos Is., Uruguay, Utah, Vanuatu, Venezuela, Venezuelan Antilles, Virginia, Wake Is., Wallis-Futuna Is., Washington, West Virginia, Windward Is. (Fig. 1).

Botanical Characteristics

Perennials, hermaphrodite, rhizomatous or stoloniferous herbs; stolons with alternating long and short internodes. Culms slender to robust, glabrous, leafy. Ligule a ciliolate membrane. Leaves subopposite, with glabrous or pubescent sheath, blades broadly linear to filiform, flat; ligule membranous or ciliate. Inflorescence solitary, terminal, digitate or sometimes 2 or more closely spaced whorls; racemes unilateral, slender; spikelets

sessile, imbricate, biseriate, 2 rows along the underside of a slender rachis, without awns; rachis flat or semiterete. Spikelets strongly laterally compressed, 1 bisexual floret, with or without rachilla extension, narrowly ovate, awnless, disarticulating above glumes; glumes subequal, shorter or as long as floret, narrow, 1-veined or upper glume 3-veined, apex acuminate, membranous, both or only lower glume persistent; lemma membranous, cartilaginous, keeled, boat-shaped, 3-veined, usually pubescent on keel, apex entire, awnless; palea as long as lemma, (1) 2-carinate; lodicules 2, attached to the margins of the palea; stamens 3; styles 2. Fruit caryopsis ellipsoid, laterally compressed.

Species

Genus *Cynodon* is known to contain a total of fifteen taxonomically accepted species (POWO, 2023) that are as-

1. *C. aethiopicus* Clayton & J.R. Harlan in Kew Bull. 24: 187 (1970)
2. *C. ambiguus* (Ohwi) P.M. Peterson in Taxon 64: 458 (2015)
3. *C. barberi* Rang. & Tadul in J. Bombay Nat. Hist. Soc. 24: 846 (1916)
4. *C. convergens* F. Muell in Fragm. 8: 113 (1873)
5. *C. coursii* A. Camus in Notul. Syst. (Paris) 16: 323 (1961)
6. *C. dactylon* (L.) Pers in Syn. Pl. 1: 85 (1805)
7. *C. incompletus* Nees in Linnaea 7: 301 (1832)

8. *C. × magennisii* Hurcombe in J. S. African Bot. 13: 109 (1947)
9. *C. nlemfuensis* Vanderyst in Bull. Agric. Congo Belge 13: 342 (1922)
10. *C. plectostachyus* (K.Schum.) Pilg in Bot. Jahrb. Syst. 40: 82 (1907)
11. *C. prostratus* (C.A.Gardner & C.E.Hubb.) P.M.Peterson in Taxon 64: 458 (2015)
12. *C. radiatus* Roth in J.J.Roemer & J.A.Schultes, Syst. Veg., ed. 15[bis]. 2: 411 (1817)
13. *C. simonii* P.M.Peterson in Taxon 64: 458 (2015)
14. *C. tenellus* R.Br in Prodr. Fl. Nov. Holland.: 187 (1810)
15. *C. transvaalensis* Burt Davy in Bull. Misc. Inform. Kew 1921: 281 (1921)
4. *C. convergens* F.Muell in Fragm. 8: 113 (1873)
5. *C. coursii* A.Camus in Notul. Syst. (Paris) 16: 323 (1961)
6. *C. dactylon* (L.) Pers in Syn. Pl. [Persoon] 1: 85. 1805 [1 Apr-15 Jun 1805]
7. *C. hirsutus* Stent in Bothalia 2: 227, 286 (1927)
8. *C. incompletus* Nees in Linnaea 7: 301 (1832)
9. *C. magennisii* Hurcombe in J. S. African Bot. 13: 109 (1947)
10. *C. nlemfuensis* Vanderyst in Bull. Agric. Congo Belge 13: 342 (1922)
11. *C. plectostachyus* (K.Schum.) Pilg in Bot. Jahrb. Syst. 40: 82 (1907)
12. *C. prostratus* (C.A.Gardner & C.E.Hubb.) P.M.Peterson in Taxon 64: 458 (2015)
13. *C. radiatus* Roth in Syst. Veg., ed. 15 bis 2: 411 (1817)
14. *C. simonii* P.M.Peterson in Taxon 64: 458 (2015)
15. *C. tenellus* R.Br in Prodr. Fl. Nov. Holland.: 187 (1810)
16. *C. transvaalensis* Burt Davy in Bull. Misc. Inform. Kew 1921: 281 (1921)

The recent database of World Flora Online (WFO, 2023) mentioned it with a total of sixteen accepted species as-

1. *C. aethiopicus* Clayton & J.R.Harlan in Kew Bull. 24: 187 (1970)
2. *C. ambiguus* (Ohwi) P.M.Peterson in Taxon 64: 458 (2015)
3. *C. barberi* Rangachari & Tadul in J. Bombay Nat. Hist. Soc. 24: 846 (1916)

Cynodon dactylon (L.) Pers.

C. dactylon is a perennial creeping herb that is native to eastern Africa and has been found worldwide in tropical and subtropical regions. In different regions of the world, it is widely referred to as *Durva* grass, Bermuda grass, Dog's Tooth grass, Indian Doab, Scutch grass,

Bahama grass, Devil's grass, Couch grass, *Dhub*, *doob*, and *durba*. It grows best in light sandy, medium loam, and heavy clay soils, yet it may be seen growing commonly by roadsides and in lawns. It may grow in shady areas but not in particularly acidic, alkaline,

or saline soils (Bhosale et al., 2013). For its growth, the soil must contain moisture. It may easily take over any uncultivated region and is as common as a weed. It becomes drier and browner in the winter. From the rooted runners, it expanded quite swiftly. Planting in damp weather conditions will ensure a speedy sprout. When sprigged 30-45 cm apart, it provides a full ground cover in 4-8 weeks (Huxley, 1992).

There are two types: seeded and hybrid. Desired qualities are selected for, leading to seeded variety. The majority of the commercial types are hybrids. The genotypes of the seeded varieties are diverse, and they were chosen based on how well they could adapt to various production environments (Ball and Pinkerton, 2002). In 1943, Coastal Bermuda grass, the first hybrid type, was introduced (Stichler and Bade, 1996). Many more have since been produced at Tifton, including Tifton 44, 68, 78, and 85. One of the most widely used types of Bermuda grass is Tifton 85, which was introduced in the 1990s (Heuzé et al., 2015). Additional types, include Zimmerly, Scheffield, Naiser, Luling, Oklan, Guymon, Quickstand, Hardie, Midland, Callie, Coastcross-1, Hill Farm Coastcross-1, Coastcross-2, Brazos, Alicia, Grazer, Russell, Lagrange, and Zimmerly (Ball and Pinkerton, 2002; Hancock et al., 2010). The hybrid type of Coastal Bermuda grass features bigger and longer stems, rhizomes, and leaves. It is immune to the root-knot nematode and has increased resistance to dehydration and leaf spot (Hancock et al., 2010).

It's a competitive, invasive weed. According to the Unani medical system, this plant has a strong, hot taste and pleasant aroma, however, the Ayurvedic Pharmacopoeia describes it as pungent, bitter, and has a distinct smell. It also has cold efficacy (Nagori and Solanki, 2011). It has a lot of phytochemicals. The plant was shown to contain a variety of phytochemicals, including reducing sugars, carbohydrates, proteins, volatile oils, fixed oils, alkaloids, glycosides, terpenoids, triterpenoids, steroids, saponins, tannins, resins, phytosterols (Avvarai, et al., 2011; Annapurna et al., 2013). It has several therapeutic benefits because of its phytochemicals (Singh et al., 2009). It has a great deal of untapped medicinal, ornamental, and other possibilities.

Importance in Hinduism

Indian religion has been using *C. dactylon* from ancient times. is regarded as the second-most holy plant in Indian religious rites, behind Tulsi. Its leaves are employed in the worship of Goddess Saraswati and Lord Ganesh. This Navagraha plant is dedicated to Rahu, the Dragon's Head. It is one of the deities in Hindu rituals that is involved in all "Pujas" (worship rituals) and other celebrations. Offering it to the gods is advantageous because it is a representation of wealth and luck; also, it is excellent for one's health and has healing qualities. Hindu legend states that it originated from Lord Ganesha's tears. Offering Durva grass to Lord Ganesha hence brings wealth and good fortune. It has calcium, protein, vitamins A and C, and other necessary elements.

According to Ayurveda, drinking its juice enhances digestion, boosts immunity, and lessens burning pain in the body. Its effects include relaxing and cooling. This grass is said to have cleansing qualities and the ability to both absorb bad energy and cleanse the surrounding area. It is believed that offering this grass to the gods will purify their auras and foster a happy atmosphere. It is thought that sitting in the Pooja, the grass has a cleansing influence on participants. Giving *Durva* grass to the gods is also regarded as an expression of devotion and submission. According to Indian custom, Brahmans wear it as a ring to ward off demons and evil spirits. Because it has the ability to purify whatever it comes into contact with, the 'pavitram' that has been sanctified by dipping it in holy water and placing it on the right hand's ring finger is effective. It has also been mentioned in Hindu mythology in several epics and other writings (Gupta, 1971).

Classification

Kingdom	-	Plantae
Subkingdom	-	Tracheobinota
Super Division	-	Spermatophyta
Division	-	Mangoliophyta
Class	-	Liliopsida
Subclass	-	Commelinidae
Order	-	Cyperales
Family	-	Poaceae
Genus	-	<i>Cynodon</i>
Species	-	<i>dactylon</i>

Common Names

Bahama grass, Bermuda grass, Dhub grass, Dog's Tooth grass, Hariali grass, Dub, Balikaruka.

Global Distribution

The species *C. dactylon* is widely distributed around the globe. It is native to Australia and the Old World's temperate and subtropical regions. It grows mostly in the subtropical biome and is a perennial or rhizomatous geophyte. It is common in pastures, lawns, and meadows. Currently, according to the Plants of the World Online (POWO, 2023) Database, it is native of Afghanistan, Albania, Aldabra, Algeria, Andaman Is., Angola, Assam, Austria, Azores, Balears, Bangladesh, Benin, Bismarck Archipelago, Borneo, Botswana, Bulgaria, Burkina, Burundi, Cambodia, Cameroon, Canary Is., Cape Provinces, Cape Verde, Caprivi Strip, Central African Repu, Central European Rus, Chad, China North-Central, China South-Central, China Southeast, Congo, Corse, Cyprus, Czechoslovakia, Djibouti, East Aegean Is., East Himalaya, Egypt, Equatorial Guinea, Eritrea, Ethiopia, France, Free State, Gabon, Gambia, Ghana, Great Britain, Greece, Guinea, Guinea-Bissau, Gulf of Guinea Is., Gulf States, Hainan, Hungary, India, Iran, Iraq, Italy, Ivory Coast, Japan, Jawa, Kazakhstan, Kenya, Kirgizstan, Korea, Kriti, Krym, Kuwait, KwaZulu-Natal, Laccadive Is., Laos, Lebanon-Syria, Lesotho, Lesser Sunda

Is., Liberia, Libya, Madagascar, Madeira, Malawi, Malaya, Mali, Maluku, Mauritania, Mauritius, Morocco, Mozambique, Mozambique Channel I, Myanmar, Namibia, Nansei-shoto, Nepal, New Guinea, New South Wales, Nicobar Is., Niger, Nigeria, North Caucasus, Northern Provinces, Northern Territory, Ogasawara-shoto, Oman, Pakistan, Palestine, Philippines, Portugal, Queensland, Rodrigues, Romania, Rwanda, Réunion, Sardegna, Saudi Arabia, Senegal, Seychelles, Sicilia, Sierra Leone, Sinai, Socotra, Solomon Is., Somalia, South Australia, South China Sea, South European Russi, Spain, Sri Lanka, Sudan, Sumatera, Swaziland, Switzerland, Tadzhikistan, Taiwan, Tanzania, Tasmania, Thailand, Togo, Transcaucasus, Tunisia, Turkey, Turkey-in-Europe, Turkmenistan, Uganda, Ukraine, Uzbekistan, Victoria, Vietnam, West Himalaya, Western Australia, Western Sahara, Yemen, Yugoslavia, Zambia, Zaïre, Zimbabwe; and introduced in Alabama, Amsterdam-St.Paul Is, Argentina Northeast, Argentina Northwest, Argentina South, Arizona, Arkansas, Aruba, Ascension, Bahamas, Belgium, Belize, Bermuda, Bolivia, Brazil North, Brazil South, Brazil Southeast, Brazil West-Central, British Columbia, California, Caroline Is., Cayman Is., Chagos Archipelago, Chile Central, Chile North, Christmas Is., Cocos (Keeling) Is.,

Colombia, Colorado, Connecticut, Cook Is., Costa Rica, Cuba, Delaware, Desventurados Is., District of Columbia, Dominican Republic, Easter Is., Ecuador, El Salvador, Fiji, Florida, French Guiana, Galápagos, Georgia, Germany, Gilbert Is., Guatemala, Guyana, Haiti, Hawaii, Honduras, Howland-Baker Is., Idaho, Illinois, Indiana, Iowa, Jamaica, Kansas, Kentucky, Kermadec Is., Leeward Is., Line Is., Louisiana, Marianas, Marquesas, Marshall Is., Maryland, Massachusetts, Mexican Pacific Is., Mexico Central, Mexico Gulf, Mexico Northeast, Mexico Northwest, Mexico Southeast, Mexico Southwest, Michigan, Mississippi, Missouri, Montana, Nauru, Nebraska, Netherlands, Netherlands Antilles, Nevada, New Caledonia, New Hampshire, New Jersey, New Mexico, New York, New Zealand North, New Zealand South, Newfoundland, Nicaragua, Niue, Norfolk Is., North Carolina, Ohio, Oklahoma, Oregon, Panamá, Paraguay, Pennsylvania, Peru, Phoenix Is., Pitcairn Is., Puerto Rico, Samoa, Society Is., South Carolina, Southwest Caribbean, St.Helena, Suriname, Tennessee, Texas, Tokelau-Manihiki, Tonga, Trinidad-Tobago, Tristan da Cunha, Tuamotu, Tubuai Is., Turks-Caicos Is., Uruguay, Utah, Vanuatu, Venezuela, Venezuelan Antilles, Virginia, Wake Is., Wallis-Futuna Is., Washington, West Virginia, Windward Is. (Fig. 2).

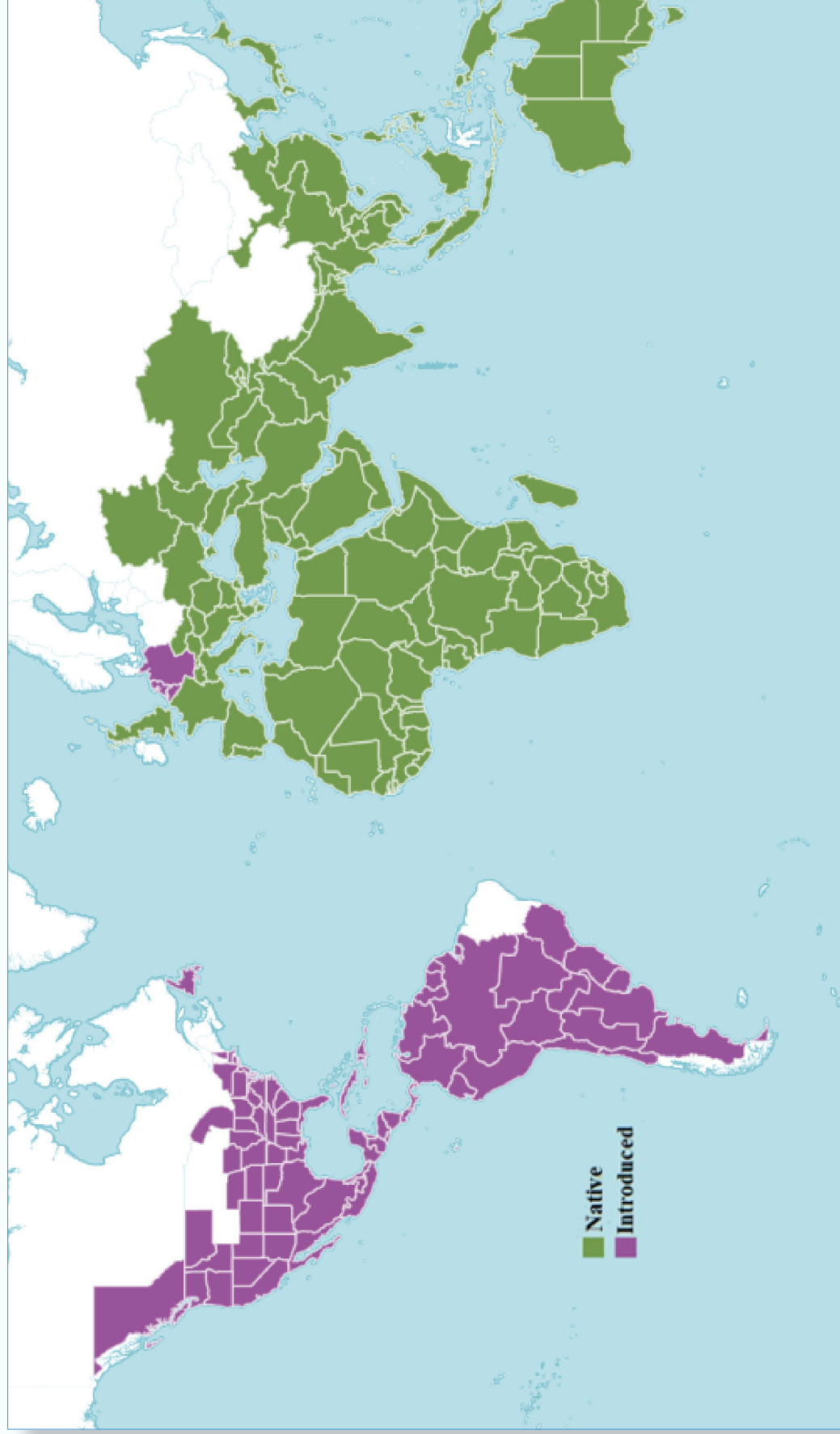


Fig. 2 Global distribution of *C. dactylon*

Distribution in India

It is distributed throughout India.

Ethnopharmacology and Traditional Uses

Worldwide, *C. dactylon* has been utilised extensively in medicine. Its leaf extract has long been used to treat diabetes in India (Kirtikar and Basu, 1996; Singh et al., 2007, 2008b; Rai et al., 2010). It is effective in healing small wounds, to modulate the immune system, and boost hepatic antioxidant levels (Santhi and Annapoorani, 2010; Saradha devi et al., 2011). As a diuretic, anti-emetic, purifying agent, and treatment for dysentery, the rhizome extract is highly beneficial (Ahmed et al., 1994; Shivalinge et al., 2009; Sadki et al., 2010).

The plants' roots and leaves were used by the locals of Pakistan's Changa Manga Forest to treat wounds, diarrhea, hysteria, convulsions, cough, cramps, epilepsy, headache, kidney stones, snake bites, sores, astringent, haemorrhoids, aperient (constipation), used to demulcent, emollient, vulnerary, diuretic, and so on (Qureshi et al., 2010). The same community treated dropsy and secondary syphilis by taking its root juice or decoction orally as a diuretic. To stop piles from bleeding, the root is infused. Fresh cuts and wounds are externally treated with the plant's astringent juice. It is used as an anthelmintic when combined with powdered clove (*Syzygium aromaticum*) (Sharif et al., 2022).

The indigenous people of the upper Himalayan regions - Bhutan, China, India, Nepal, and Pakistan—make extensive use of this plant in their medicinal practices (Heinrich et al., 2021). Indian Himalayan natives treated boils, cuts, wounds, and other skin conditions with a paste made from entire plants, roots, and aerial parts (Kumar et al., 2013; Sharma et al., 2014; Uprety et al., 2016; Singh et al., 2017; Baidya et al., 2020). Higher altitude residents of Nepal apply plant juice and paste to wounds and cuts, and young shoot paste to burns and scabies (Shrestha and Dhillon, 2003; Singh et al., 2012; Uprety et al., 2016). Higher altitude residents of Pakistan use entire plant powder for skin issues and wound healing; they also use paste made from aerial parts for cuts and wounds (Abbasi et al., 2010; Malik et al., 2019; Qaseem et al., 2019). The Algerian Saharan inhabitants utilized the plant's decoction as a purgative and laxative, for renal disorders, skin conditions, haemorrhage, urinary tract infections, urinary stones, gallbladder, and used as anti-virus and fungal treatments (Lakhdari et al., 2016). The indigenous people of KwaZulu-Natal Province in South Africa use this plant to treat wounds, eye infections, skin conditions, allergies, and haemorrhages (Gebashe et al., 2019). This herb is used in cuts, wounds, and bleeding by the indigenous people of Bangladesh's Barisal area (Hossain and Rahman, 2018). The Madhupur, Tangail people treat skin diseases using whole plant paste (Anisuzzaman et al., 2007).

As previously indicated, *C. dactylon* is a great place to obtain its amazing nutraceutical qualities. It benefits diabetic patients and possesses

hypoglycemic qualities (He et al., 2011). Additionally, it demonstrated cardiovascular activity and a highly helpful treatment for heart failure patients (Bagewadi, et al., 2014). It has an anti-arrhythmic effect because it is beneficial to blood flow (Luca, 2012; Das et al., 2021). It showed anti-microbial action and positive examination against a variety of microorganisms, including *Streptococcus pyrogenes*, *Staphylococcus aureus*, and *Escherichia coli* (Pranita et al., 2012; Ashokkumar et al., 2013).

It helps with tasks connected to the central nervous system by changing the brain's amino acid composition and catecholamine levels (Pal, 2008, 2009). In addition to these actions, it exhibits the following: anti-inflammatory (Garg and Paliwal, 2011; Yogesh et al., 2013), anti-pyretic (Garg and Khosa, 2008), anti-diarrheal (Babu et al., 2009), diuretic (Aruna et al., 2013), dermatological (Dande and Khan, 2012; Saroja et al., 2012), antioxidant (Oudhia, 1999; Jananie et al., 2011; Dande, 2012), anti-nephrolithiasis (Albert-Baskar and Ignacimuthu, 2010; Rad et al., 2011), analgesic (Dhande, 2012), and antiviral properties (Babu et al., 2009). Additionally, the plant extract has been shown to have important uses in the treatment of wounds (Oudhia and Pal, 2000), is cardioprotective (Garjani et al., 2009), possess antibacterial activity (Kanimozhi and Ratha, 2012), and treat dropsy and secondary syphilis (Kesari et al., 2006). According to Sivasankari et al. (2014), the residents of Thoppampatti, Dindigul district, Tamilnadu, India, use the whole plant to treat hematuria, epistaxis, inflamed tumors, cuts, wounds, bleeding piles, cystitis, nephritis, scabies, and diarrhea.

Ayurvedic Significance

In various ancient Ayurvedic literatures, it is used in various ailments. Mainly this plant is mentioned for its skin disease, female fertility, and urinary tract infection improvement properties. The Acharyas from the Ayurvedic field have grouped this plant under skin-friendly herbs. It has the property like wound healing and skin color restoration. It is recommended for psoriasis, herpes, unhealed wounds, allergic rashes, and hemorrhoids. It heals the patches of psoriasis and restores the normal color of the skin. Along with these, it is very helpful in other skin problems like itching, healing scaly skin, and imparts normal color to skin (Aikia et al., 2006; Rajakumar and Shivanna, 2009). The female fertility could be improved using this grass. The Ayurvedic practitioners recommend this grass in menorrhagia, irregular menstrual cycle, and habitual abortion. It is very useful in polycystic ovarian syndrome (PCOS), apart from this, it prevents abortion and strengthens the uterus (Aikia et al., 2006; Rajakumar and Shivanna, 2009). This grass has cooling properties hence, it increases the urine output, soothes the inner layers of the bladder, and helps in the improvement of urinary tract infection. It helps to reduce inflammation of the mucosal layer of the bladder in cystitis and urinary tract infections (Aikia et al., 2006; Rajakumar and Shivanna, 2009; Khatun et al., 2020).

Selection of the Sites and Characteristics Studied

The distribution, morphological variations, and association of *C. dactylon* were investigated across the Ganga River. The Ganga began from the Gomukh in Gangotri glacier and ended at Gangasagar in the Bay of Bengal. A total of twenty-six sites were selected with two sites ranging around 100 km in distance

while the whole distance is about 2600 km (Table 1). The plant diversity was assessed upto the 10 km on both sides of each site. The plants were identified and deposited in the Patanjali Research Foundation Herbarium with an acronym of PRFH for future records. A bulk sample of the plant was taken from each site in a large polybag which was deposited in the analytical laboratory for phytochemical profiling.

Table 1. Different studied sites and their GPS coordinates along with *C. dactylon* status

Site	Locality	GPS Coordinates			Status
		Altitude (m)	Latitude (N)	Longitude (E)	
S1	Gomukh	4023	30.80	79.15	Absent
S2	Gangotri	3415	30.98	78.93	Absent
S3	Uttarkashi	1158	30.73	78.44	Absent
S4	Devprayag	830	30.15	78.60	Present
S5	Haridwar	314	29.97	78.17	Present
S6	Bijnor	225	29.37	78.13	Present
S7	Narora	174	28.20	78.38	Present
S8	Budaun	164	28.05	79.12	Present
S9	Farrukhabad	151	27.37	79.63	Present
S10	Bithoor	126	26.61	80.27	Present
S11	Dalmau	115	26.07	81.03	Present
S12	Prayagraj	98	25.45	81.85	Present
S13	Mirzapur	80	25.15	82.58	Present
S14	Varanasi	81	25.32	83.01	Present
S15	Ballia	67	25.76	84.15	Present
S16	Revelganj	52	25.78	84.67	Present
S17	Patna	53	25.61	85.14	Present

Site	Locality	GPS Coordinates			Status
		Altitude (m)	Latitude (N)	Longitude (E)	
S18	Barh	47	25.48	85.72	Present
S19	Bahachouki	55	25.30	86.36	Present
S20	Farka	42	25.23	87.09	Present
S21	Sahebganj	16	25.25	87.65	Present
S22	Farakka Barrage	30	24.82	87.90	Absent
S23	Murshidabad	18	24.18	88.27	Absent
S24	Mayapur	11	23.43	88.39	Present
S25	Hoogli	9	22.91	88.40	Absent
S26	Gangasagar	4	22.19	88.19	Absent

Local Occurrence

The plant was recorded in all the sites, but it was collected for the analytical studies only from sites 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, and 21, due to these sites being the source of its bulk collection. The remaining sites as 1, 2, 3, 22, 23, 25, and 26 were not included as, it was only represented in these sites.

Taxonomic Treatment

Cynodon dactylon (L.) Pers., Syn. Pl. 1: 85. 1805; FBI 7:288. 1896; Prain, Bengal Pl. 2: 1227. 1903. Bot. Bihar & Orissa. 5: 966. 1924; Bor, Grasses India, 469. 1960; Srivastava, FG. 361: 1976. Singh, Fl. Dudhwa National Park, 496. 1996. Paria & Chattopadhyay, Fl. of Hazaribag district, Bihar 2: 1059. 2005; Pusalkar & Singh, Fl. of Gangotri National Park Western Himalaya, India, 360. 2012.

Synonyms

The Plants of the World Online database mentioned it with its 18 homotypic and 80 heterotypic synonyms (Powo, 2023) as-

Homotypic Synonyms

1. *Capriola dactylon* (L.) Kuntze in Revis. Gen. Pl. 2: 764 (1891)
2. *Chloris cynodon* Trin. in Gram. Unifl. Sesquifl.: 229 (1824), nom. superfl.
3. *Cynodon repens* Dulac in Fl. Hautes-Pyrénées: 76 (1867), nom. superfl.
4. *C. sarmentosus* Gray in Nat. Arr. Brit. Pl. 2: 100 (1821 publ. 1822), nom. superfl.
5. *Cynosurus dactylon* (L.) Pers. in Syn. Pl. 1: 85 (1805)
6. *Dactilon officinale* Vill. in Hist. Pl. Dauphiné 2: 69 (1787), nom. superfl.
7. *Digitaria dactylon* (L.) Scop. in Fl. Carniol., ed. 2, 1: 52 (1771)

8. *D. littoralis* Salisb. in Prodr. Stirp. Chap. Allerton: 19 (1796), nom. superfl.
9. *D. stolonifera* Schrad. in Fl. Germ.: 165 (1806), nom. superfl.
10. *Fibichia dactylon* (L.) Beck in Glasn. Zemaljsk. Muz. Bosni Hercegovini 15: 26 (1903)
11. *Fibichia umbellata* Koeler in Descr. Gramin.: 308 (1802), nom. superfl.
12. *Milium dactylon* (L.) Moench in Suppl. Meth.: 67 (1802)
13. *Panicum ambiguum* Le Turq. in Fl. Rouen: 28 (1816), nom. superfl.
14. *P. dactylon* L. in Sp. Pl.: 58 (1753)
15. *P. sanguinale* var. *ambiguum* Lapeyr. in Hist. Pl. Pyrénées: 131 (1813)
16. *Paspalum ambiguum* DC. in J.B.A.M.de Lamarck & A.P.de Candolle, Fl. Franç., éd. 3, 3: 16 (1805), nom. superfl.
17. *P. dactylon* (L.) Lam. in Tabl. Encycl. 1: 176 (1791)
18. *Phleum dactylon* (L.) Georgi in Besch. Nation. Russ. Reich 4: 681 (1800)
6. *Chloris maritima* Trin. in Gram. Unifl. Sesquifl.: 236 (1824)
7. *A. paytensis* Steud. in Syn. Pl. Glumac. 1: 207 (1854)
8. *Cynodon affinis* Caro & E.A.Sánchez in Kurtziana 5: 223 (1969)
9. *A. aristiglumis* Caro & E.A.Sánchez in Kurtziana 5: 236 (1969)
10. *A. aristulatus* Caro & E.A.Sánchez in Darwiniana 17: 514 (1972)
11. *A. barberi* f. *longifolia* Join in Indian Forester 92: 699 (1966)
12. *A. dactylon* var. *affinis* (Caro & E.A.Sánchez) Romero Zarco in Lagasalia 14: 171 (1986)
13. *A. dactylon* var. *aridus* J.R.Harlan & de Wet in Crop Sci.(Madison) 9: 774 (1969)
14. *C. dactylon* var. *biflorus* Merino in Fl. Galicia 3: 310 (1909)
15. *Cynodon dactylon* var. *densus* Hurcombe in J. S. African Bot. 13: 107 (1947)
16. *C. dactylon* var. *elegans* Rendle in W.P.Hiern, Cat. Afr. Pl. 2: 221 (1899)
17. *C. dactylon* subsp. *glabratus* (Steud.) A.Chev. in Rev. Int. Bot. Appl. Agric. Trop. 27: 282 (1947)
18. *C. dactylon* var. *glabratus* (Steud.) Chiov. in Nuovo Giorn. Bot. Ital., n.s., 26: 82 (1919)
19. *C. dactylon* f. *glabrescens* (Beck) Soó in Acta Bot. Acad. Sci. Hung. 17: 123 (1971 publ. 1972), not validly publ.
20. *C. dactylon* subvar. *hirsutissimus* Litard. & Maire in Mém. Soc. Sci. Nat. Maroc 4: 25 (1924)

Heterotypic Synonyms

1. *Agrostis bermudiana* Tussac ex Kunth in Enum. Pl. 1: 259 (1833), pro syn.
2. *A. filiformis* J.Koenig ex Kunth in Enum. Pl. 1: 261 (1833), pro syn.
3. *A. linearis* Retz. in Observ. Bot. 4: 19 (1786)
4. *A. stellata* Willd. in Sp. Pl., ed. 4. 1: 376 (1797)
5. *Capriola dactylon* var. *maritima* (Kunth) Hitchc. in Bull. U.S.D.A. 772: 179 (1920)

21. *C. dactylon* var. *hirsutissimus* (Litard. & Maire) Maire in Bull. Soc. Hist. Nat. Afrique N. 30: 368 (1939)
22. *C. dactylon* var. *longiglumis* Caro & E.A.Sánchez in Kurtziana 5: 210 (1969)
23. *C. dactylon* f. *major* (Beck) Soó in Acta Bot. Acad. Sci. Hung. 17: 123 (1971 publ. 1972)
24. *C. dactylon* var. *maritimus* (Kunth) Hack. in Ark. Bot. 8(8): 40 (1909)
25. *C. dactylon* subsp. *nipponicus* (Ohwi) T.Koyama in Grass. Japan: 498 (1987)
26. *C. dactylon* var. *nipponicus* Ohwi in Acta Phytotax. Geobot. 10: 264 (1941)
27. *C. dactylon* var. *parviglumis* (Ohwi) Fosberg & Sachet in Micronesica 18: 45 (1982 publ. 1984)
28. *C. dactylon* var. *pilosus* Caro & E.A.Sánchez in Kurtziana 5: 210 (1969)
29. *C. dactylon* var. *pulchellus* Benth. in Fl. Austral. 7: 609 (1878)
30. *C. dactylon* var. *sarmentosus* Parodi in Revista Argent. Agron. 23: 185 (1956)
31. *C. dactylon* var. *sarmentosus* Pers. in Syn. Pl. 1: 85 (1805)
32. *C. dactylon* var. *septentrionalis* (Asch. & Graebn.) Ravarut in T.Săvulescu, Fl. Republ. Socialist. România 12: 139 (1972)
33. *C. dactylon* *septentrionalis* Asch. & Graebn. in Syn. Mitteleur. Fl. 2(1): 85 (1899)
34. *C. dactylon* var. *septentrionalis* Asch. & Graebn. in Syn. Mitteleur. Fl. 2(1): 85 (1899)
35. *C. dactylon* var. *stellatus* (Willd.) Steud. in Nomencl. Bot., ed. 2, 1: 463 (1840)
36. *C. dactylon* var. *villosus* Grossh. in Fl. Kavkaza 1: 91 (1928), nom. illeg.
37. *C. dactylon* f. *villosus* (Regel) Roshev. in V.L.Komarov (ed.), Fl. URSS 2: 285 (1934)
38. *C. dactylon* var. *villosus* Regel in Bull. Soc. Imp. Naturalistes Moscou 41(II): 305 (1869)
39. *C. dactylon* f. *viviparus* Beetle in Phytologia 48: 189 (1981)
40. *C. decipiens* Caro & E.A.Sánchez in Kurtziana 5: 262 (1969)
41. *C. distichloides* Caro & E.A.Sánchez in Kurtziana 5: 254 (1969)
42. *C. erectus* J.Presl in C.B.Presl, Reliq. Haenk. 1: 290 (1829)
43. *C. glabratus* Steud. in Syn. Pl. Glumac. 1: 212 (1854)
44. *C. grandispiculus* Caro & E.A.Sánchez in Kurtziana 5: 258 (1969), pro syn.
45. *C. hirsutissimus* (Litard. & Maire) Caro & E.A.Sánchez in Kurtziana 5: 220 (1969)
46. *C. iraquensis* Caro in Dominguezia 6: 5 (1983)
47. *C. laeviglumis* Caro & E.A.Sánchez in Kurtziana 5: 217 (1969)
48. *C. linearis* (Retz.) Willd. in Enum. Pl.: 90 (1809)
49. *C. maritimus* Kunth in F.W.H.von Humboldt, A.J.A.Bonpland & C.S.Kunth, Nov. Gen. Sp. 1: 170 (1816)
50. *C. maritimus* var. *breviglumis* Caro & E.A.Sánchez in Kurtziana 5: 234 (1969)

51. *C. maritimus* var. *grandispiculus* Caro & E.A.Sánchez in Kurtziana 5: 234 (1969)
 52. *C. maritimus* var. *vaginiflorus* Caro in Dominguezia 6: 20 (1983)
 53. *C. mucronatus* Caro & E.A.Sánchez in Kurtziana 5: 239 (1969)
 54. *C. nitidus* Caro & E.A.Sánchez in Darwiniana 17: 510 (1972)
 55. *C. occidentalis* Willd. ex Steud. in Nomencl. Bot., ed. 2, 1: 463 (1840), pro syn.
 56. *C. parviglumis* Ohwi in Bot. Mag. (Tokyo) 55: 538 (1941)
 57. *C. pascuus* Nees in C.F.P.von Martius & auct. suc. (eds.), Fl. Bras. Enum. Pl. 2: 425, 608 (1829)
 58. *C. pedicellatus* Caro in Dominguezia 6: 9 (1983)
 59. *C. polevansii* Stent in Bothalia 2: 278 (1927)
 60. *C. portoricensis* Willd. ex Steud. in Nomencl. Bot., ed. 2, 1: 463 (1840), pro syn.
 61. *C. scabrifolius* Caro in Dominguezia 6: 13 (1983)
 62. *C. stellatus* (Willd.) Willd. in Enum. Pl.: 90 (1809)
 63. *C. tenuis* Trin. in Neue Entdeck. Pflanzenk. 2: 63 (1821)
 64. *C. umbellatus* (Lam.) Caro in Dominguezia 6: 16 (1983)
 65. *Cynosurus uniflorus* Walter in Fl. Carol.: 82 (1788)
 66. *Dactylus officinalis* Asch. in Fl. Brandenburg 1: 810 (1864), pro syn.
 67. *Digitaria glumipatula* (Steud.) Miq. in Fl. Ned. Ind. 3: 439 (1857)
 68. *A. linearis* (Retz.) Spreng. in Syst. Veg., ed. 16. 1: 271 (1824)
 69. *A. linearis* (L.) Pers. in Syn. Pl. 1: 85 (1805)
 70. *D. maritima* (Kunth) Spreng. in Syst. Veg., ed. 16. 1: 272 (1824)
 71. *Fibichia umbellata* var. *biflora* (Merino) Beck in Fl. Nieder-Österreich 1: 76 (1890)
 72. *A. umbellata* f. *glabrescens* Beck in Fl. Nieder-Österreich 1: 76 (1890)
 73. *A. umbellata* var. *glabrescens* Beck in Fl. Nieder-Österreich: 76 (1890)
 74. *A. umbellata* f. *major* Beck in Fl. Nieder-Österreich 1: 76 (1890)
 75. *Panicum glumipatulum* Steud. in Syn. Pl. Glumac. 1: 41 (1853)
 76. *P. lineare* L. in Sp. Pl., ed. 2.: 85 (1762)
 77. *Paspalum umbellatum* Lam. in Tabl. Encycl. 1: 177 (1791)
 78. *Syntherisma linearis* (L.) Nash in Bull. Torrey Bot. Club 22: 420 (1895)
 79. *Vilfa linearis* (Retz.) P.Beauv. in Ess. Agrostogr.: 16 (1812)
 80. *V. stellata* (Willd.) P.Beauv. in Ess. Agrostogr.: 16 (1812)
- While another database i.e., World Flora Online (WFO, 2023) mentioned it with a total of following 95 synonyms-
1. *Agrostis bermudiana* Tuss. ex Kunth in Enum. Pl. 1: 259 (1833)
 2. *A. filiformis* K.D.Koenig ex Kunth in Enum. Pl. 1: 261 (1833)

3. *A. linearis* Retz in Observ. Bot. 4: 19 [1786]
4. *A. stellata* Willd in Sp. Pl., ed. 4, 1: 376 [1797]
5. *Capriola dactylon* (L.) Kuntze in Revis. Gen. Pl. 2: 764 [1891]
6. *A. dactylon* var. *maritima* Hitchc in Bull. U.S.D.A. 772: 179 [1920]
7. *Chloris cynodon* Trin in Gram. Unifl. Sesquifl.: 229 [1824]
8. *A. maritima* Trin in Gram. Unifl. Sesquifl.: 236 [1824]
9. *A. paytensis* Steud in Syn. Pl. Glumac. 1: 207 [1854]
10. *Cynodon affinis* Caro & E.A.Sánchez in Kurtziana 5: 223 [1969]
11. *A. aristiglumis* Caro & E.A.Sánchez in Kurtziana 5: 236 [1969]
12. *A. aristulatus* Caro & E.A.Sánchez in Darwiniana 17: 514 [1972]
13. *A. barberi* f. *longifolia* Join in Indian Forester 92: 699 1966
14. *C. dactylon* f. *glabrescens* (Beck) Soó in Acta Bot. Acad. Sci. Hung. 17: 123 [1971 publ. 1972]
15. *C. dactylon* f. *major* (Beck) Soó in Acta Bot. Acad. Sci. Hung. 17: 123 [1971 publ. 1972]
16. *C. dactylon* f. *villosus* (Grossh.) Regel ex Roshev in Fl. URSS 2: 285 1934
17. *C. dactylon* f. *viviparus* Beetle in Phytologia 48: 189 [1981]
18. *C. dactylon* subsp. *glabratus* (Steud.) A.Chev in Rev. Int. Bot. Appl. Agric. Trop. 27: 282. 1947 [1947]
19. *C. dactylon* subsp. *nipponicus* (Ohwi) T.Koyama in Grass. Japan: 498 [1987]
20. *C. dactylon* var. *affinis* (Caro & Sánchez) Romero Zarco in Lagasalia 14: 171 [1986]
21. *C. dactylon* var. *aridus* J.R.Harlan & de Wet in Crop Sci.(Madison) 9: 774 1969
22. *C. dactylon* var. *biflorus* Merino in Fl. Galicia 3: 310. 1909
23. *C. dactylon* var. *dactylon*
24. *C. dactylon* var. *densus* Hurcombe in J. S. African Bot. 13: 107. 1947
25. *C. dactylon* var. *elegans* Rendle in Cat. Afr. Pl. 2: 221. 1899
26. *C. dactylon* var. *glabratus* (Steud.) Chiov in Nuovo Giorn. Bot. Ital. n.s., 26: 82. 1919
27. *C. dactylon* var. *hirsutissimus* in Bull. Soc. Hist. Nat. Afrique N. 30: 368 [1939]
28. *C. dactylon* var. *longiglumis* Caro & E.A.Sánchez in Kurtziana 5: 210 [1969]
29. *C. dactylon* var. *maritimus* Hack in Ark. Bot. 8(8): 40 [1909]
30. *C. dactylon* var. *nipponicus* Ohwi in Acta Phytotax. Geobot. 10: 264 1941
31. *C. dactylon* var. *parviglumis* (Ohwi) Fosberg & Sachet in Micronesica 18: 45 [1982 publ. 1984]
32. *C. dactylon* var. *pilosus* Caro & E.A.Sánchez in Kurtziana 5: 210 [1969]
33. *C. dactylon* var. *polevansii* (Stent) J.R.Harlan & de Wet in Crop Sci. (Madison) 9: 1969
34. *C. dactylon* var. *pulchellus* Benth in Fl. Austral. 7: 609 [1878]

35. *C. dactylon* var. *sarmentosus* Parodi in Revista Argent. Agron. 23: 185. 1956
36. *C. dactylon* var. *septentrionalis* (Asch. & Graebn.) R'v'rut in Fl. Republ. Socialist. Romania 12: 139. 1972
37. *C. dactylon* var. *stellatus* (Willd.) T.Durand & Schinz in Consp. Fl. Afr. 5: 857 1894
38. *C. dactylon* var. *villosus* Grossh in Fl. Kavkaza 1: 91. 1928
39. *C. dactylon* var. *villosus* Regel in Bull. Soc. Imp. Naturalistes Moscou 41(II): 305. 1869
40. *C. decipiens* Caro & E.A.Sánchez in Kurtziana 5: 262 (1969)
41. *C. distichloides* Caro & E.A.Sánchez in Kurtziana 5: 254 (1969)
42. *C. erectus* J.Presl in Reliq. Haenk. 1(4-5): 290. 1830
43. *C. glabratus* Steud in Syn. Pl. Glumac. 1(3): 212. 1854 [1855 publ. 12-13 Apr 1854]
44. *C. grandispiculus* Caro & E.A.Sánchez in Kurtziana 5: 258 (1969)
45. 45. *C. hirsutissimus* (Litard. & Maire) Caro & E.A.Sánchez in Kurtziana v. 220 (1969)
46. *C. iraquensis* Caro in Dominguezia 6: 5 (1983)
47. *C. laeviglumis* Caro & E.A.Sánchez in Kurtziana 5: 217 (1969)
48. *C. linearis* Willd in Enum. Pl.: 90 (1809)
49. *C. maritimus* Kunth in Nov. Gen. Sp. 1: 170 (1816)
50. *C. maritimus* var. *breviglumis* Caro & E.A.Sánchez in Kurtziana 5: 234 (1969)
51. *C. maritimus* var. *grandispiculus* Caro & E.A.Sánchez in Kurtziana 5: 234 (1969)
52. *C. maritimus* var. *vaginiflorus* Caro in Dominguezia 6: 20 (1983)
53. *C. mucronatus* Caro & E.A.Sánchez in Kurtziana 5: 239 (1969)
54. *C. nitidus* Caro & E.A.Sánchez in Darwiniana 17: 510 (1972)
55. *C. occidentalis* Willd. ex Steud in Nomencl. Bot., ed. 2, 1: 463 (1840)
56. *C. parviglumis* Ohwi in Bot. Mag. (Tokyo) 55: 538 (1941)
57. *C. pascuus* Nees in Fl. Bras. Enum. Pl. 2(1): 425. 1829 [Mar-Jun 1829]; alt. title: Agrost. Bras.
58. *C. pedicellatus* Caro in Dominguezia 6: 9 (1983)
59. *C. polevansii* Stent in Bothalia 2: 278 (1927)
60. *C. portoricensis* Willd. ex Steud in Nomencl. Bot., ed. 2, 1: 463 (1840)
61. *C. repens* Dulac in Fl. Hautes-Pyrénées: 76 (1867)
62. *C. sarmentosus* Gray in Nat. Arr. Brit. Pl. 2: 100 (1821 publ. 1822)
63. *C. scabrifolius* Caro in Dominguezia 6: 13 (1983)
64. *C. stellatus* Willd in Enum. Pl.: 90 (1809)
65. *C. tenuis* Trin in Neue Entdeck. Pflanzenk. 2: 63 (1821)
66. *C. umbellatus* (Lam.) Caro in Dominguezia 6: 16 (1983)
67. *Cynosurus dactylon* (L.) Pers in Syn. Pl. 1: 85. 1805 (1805)

68. *C. uniflorus* Walter in Fl. Carol.: 82 (1788)
69. *Dactilon officinale* Vill in Hist. Pl. Dauphiné 2: 69 (1787)
70. *Dactylus officinalis* Asch in Fl. Brandenburg 1: 810 (1864)
71. *Digitaria ambigua* (Lapeyr. ex DC.) Mérat in Nouv. Fl. Paris (ed. 2) 2: 31 1821
72. *Digitaria dactylon* Scop in Fl. Carniol., ed. 2, 1: 52 (1771)
73. *A. glumaepatula* (Steud.) Miq in Fl. Ned. Ind. 3: 439 1857
74. *A. glumipatula* (Steud.) Miq in Fl. Ned. Ind. 3: 439. 1857 (1857)
75. *D. linearis* Pers in Syn. Pl. 1: 85 (1805)
76. *D. littoralis* Salisb in Prodr. Stirp. Chap. Allerton: 19 (1796)
77. *D. maritima* Spreng in Syst. Veg. 1: 272 (1824)
78. *D. stolonifera* Schrad in Flora Germanica 1 1806
79. *Fibichia dactylon* Beck in Glasn. Zemaljsk. Muz. Bosni Hercegovini 15: 26 (1903)
80. *A. umbellata* Koeler in Descr. Gramin.: 308 (1802)
81. *A. umbellata* f. *glabrescens* Beck in Fl. Nieder-Oesterreich 76. 1890
82. *A. umbellata* f. *major* Beck in Fl. Nieder-Oesterreich 76. 1890
83. *A. umbellata* var. *biflora* (Merr.) Beck in Fl. Nieder-Österreich 1: 76 1890
84. *Milium dactylon* (L.) Moench in Suppl. Meth.: 67 (1802)
85. *Panicum ambiguum* (DC.) Le Turq in Fl. Rouen: 28 (1816)
86. *P. dactylon* L in Sp. Pl.: 58 (1753)
87. *P. glumaepatulum* Steud in Syn. Pl. Glumac. 1(1): 41. 1853 [1855 publ. 10-12 Dec 1853]
88. *P. lineare* L in Sp. Pl. ed. 2: 85 (1762)
89. *Paspalum ambiguum* DC in Fl. Franc. [de Candolle & Lamarck], ed. 3. 3: 16. 1805
90. *P. dactylon* (L.) Lam in Tabl. Encycl. 1: 176 (1791)
91. *P. umbellatum* Lam in Tabl. Encycl. 1: 177. 1791
92. *Phleum dactylon* Georgi in Beschr. Nation. Russ. Reich 4: 681 (1800)
93. *Syntherisma linearis* (L.) Nash in Bull. Torrey Bot. Club 22: 420. 1895 (1895)
94. *Vilfa linearis* P.Beauv in Ess. Agrostogr.: 16 (1812)
95. *V. stellata* P.Beauv in Ess. Agrostogr.: 16 (1812)

Botanical Description

Perennial, stoloniferous, slender scaly rhizomes, sward forming. Culm slender, 1–40 cm sometimes taller, slightly flattened, often tinged purple. Ligules fimbriate, membranous. Leaf sheaths bearded at mouth, otherwise glabrous or thinly pilose; blades linear, short, and narrow, grey-green, 1–14 cm long, usually glabrous, glaucous, apex subacute, acuminate; racemes digitate, 2–6 spikes together at the top of the stem, straight or gently curved, rather stiff, spreading; spikelets sessile, oblong-lanceolate, laterally compressed, 1-flowered. Rachilla extension ca. 1 mm, sometimes with minute rudimentary

floret at apex; glumes linear-lanceolate, chartaceous, 1-nerved, often purplish, usually more than half as long as floret, 1.5–2 mm, 1-veined, keel scabrous, thickened; lemma as long as spikelet, boat-shaped, ovate-oblong, keeled, hairs straight, otherwise glabrous or lateral veins thinly villous, apex subacute;

palea glabrous, keels scaberulous, boat-shaped or oblong, chartaceous. Stamens 3; anthers more than 1 mm long. Ovary c. 0.5 mm long, oblong; stigmas 0.5–1 mm long, pink. Caryopsis subterete, linear, scarcely laterally compressed (Fig. 3A, B).



Fig. 3A Plant habit

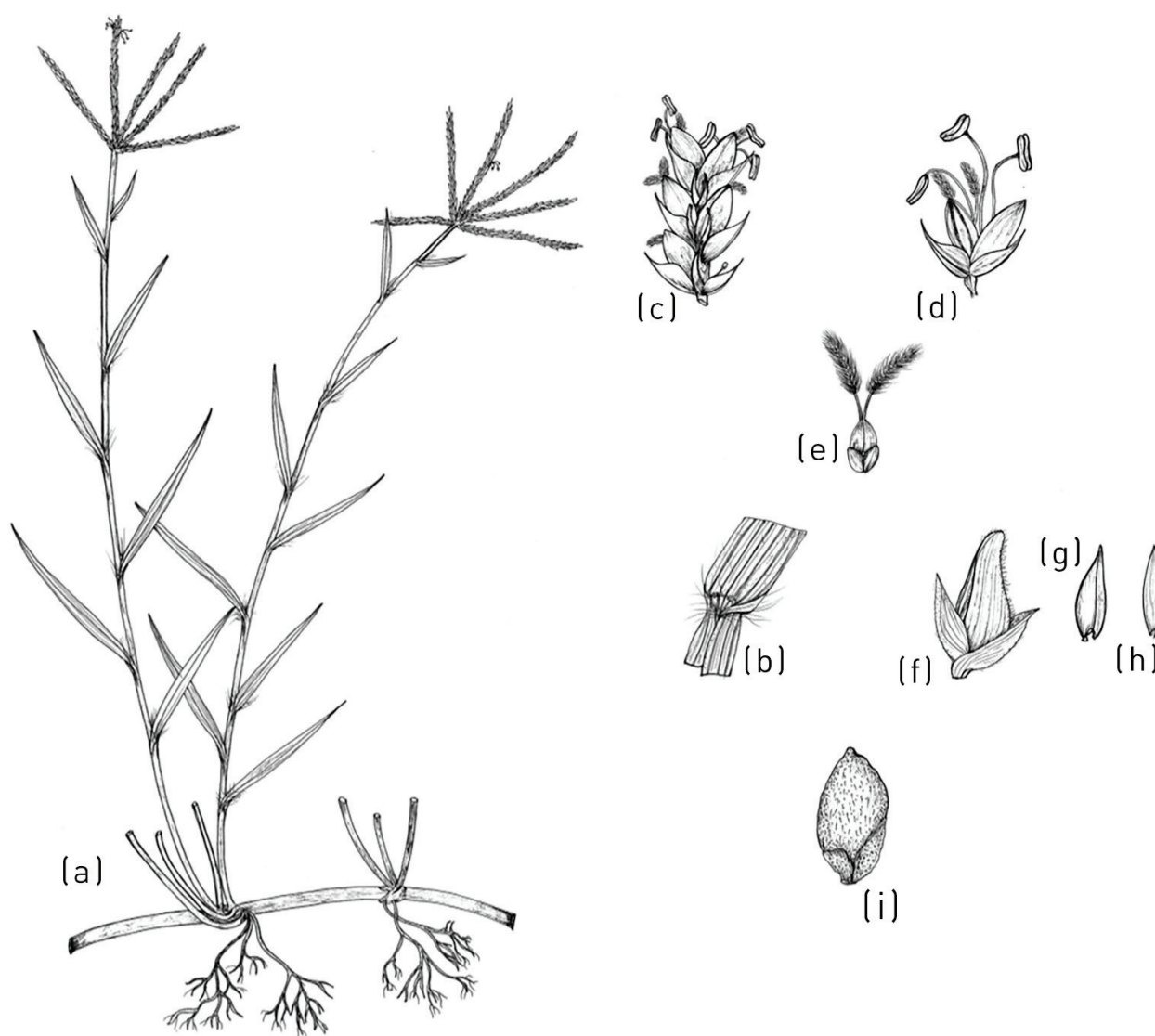


Fig. 3B *Cynodon dactylon* (a) Plant, (b) Leaf sheath, (c) Bisexual spikelet, (d) Flower, (e) Female, part (f), Spikelet (g), Lemma (h), Palea (i) Caryopsis

Chromosome Number

The chromosomal numbers of $2n = 36$ were documented by Avdulow (1931), Burton (1947), Darlington and Wylie (1956), Harlan and de Wet (1969), de Silva and Snaydon (1995), and they believed that the basic chromosome number was 9 ($x = 9$). Several additional research (Forbes and Burton, 1963; Clayton and Harlan, 1970; Chaves et al., 2019)

examined similar basic statistics, although, in these investigations, the diploid number of chromosomes was reported as 18 ($2n = 18$). According to Hunter, 1934; Hurcombe, 1947; Rochecoste, 1962, several other studies found that the basic chromosomes were 10 ($x = 10$), and that the diploid chromosomal count was 40 ($2n = 40$). The majority of the plants in Bogdon's (1977) research were tetraploid, while several plants had deploid

characteristics. Some pentaploid ($5x = 45$) plants with identical basic numbers 9 ($x = 9$) were described by Chaves et al. (2019).

Anatomical Features

Structure of the Leaf Sheath

In the TS of the petiole, the sheath is folded adaxially at the median line, where it is thicker and tapers down to the wings gradually. Both the abaxial and adaxial surfaces are uniformly smooth. The adaxial epidermal cells are tiny,

with thick radial walls and thick cuticle, while the abaxial epidermal layer has oblong cells with dumb-bell-shaped stomata and thin cuticle. The abaxial epidermis also features parallel subsidiary and cubical microscopic silica cells in abundance. The parenchyma cells in the ground tissue have two or more air chambers close to the midrib, and there are numerous collateral vascular strands arranged less evenly throughout the leaf sheath. Of these, the central vascular bundles are bigger than the wings side (Fig. 4).

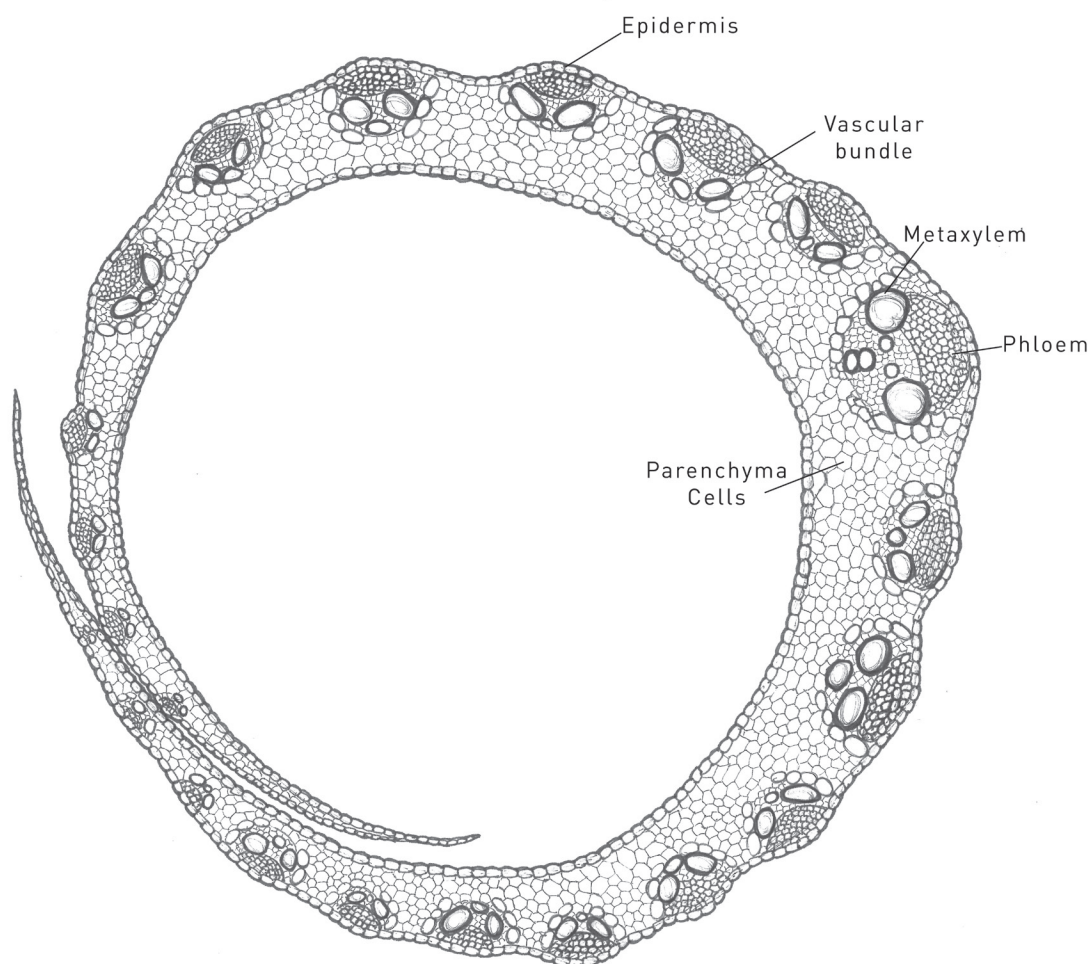


Fig. 4 TS of the leaf sheath

Structure of the Leaf Blade

The lamina exhibits a nearly square to the oval epidermis with an unevenly cutinized outer wall; on the dorsal side, there are bulliform cells that are grouped and lie at the bottom of a well-defined groove between the veins; these cells have thin walls, do not contain chlorophyll, and extend deep into the mesophyll; the mesophyll is not differentiated into palisade and spongy parenchyma; a row

of nearly identical vascular bundles, with the median bundle being larger; Mesophyll tissue is divided by one or two thin-walled, colourless cells that stretch from the bundle sheath to the thin-walled parenchymatous band of stereome near the upper and lower epidermis. The bundle sheath is composed of a single, more or less isodiametric parenchyma cell that contains chloroplast (Fig. 5).

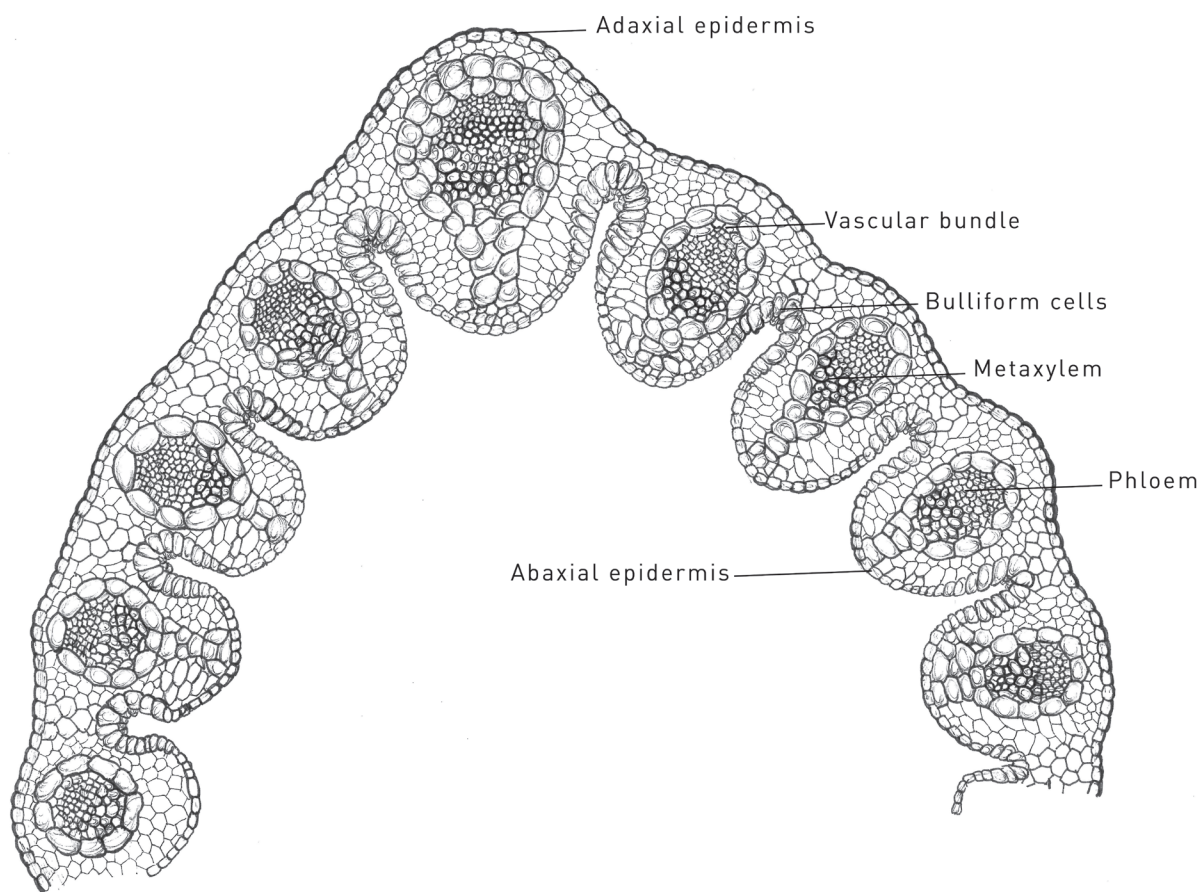


Fig. 5 TS of the leaf blade

Structure of the Stem

The TS of the culm has a single-layered epidermis with walls that are lignified and cutinized. There are one or two layers of sclerenchymatous hypodermis underneath the epidermis. Round to oval parenchymatous cells with thin walls make up the cortex's 3-5 layers. Endodermis is not separate. A continuous ring of 2-5 layers of sclerenchymatous fibres makes up the pericycle. Parachymatous cells

make up the ground tissue. The ground tissue contains collateral, closed vascular bundles that are dispersed. The vessels are annular, scalariform, spiral, and simple. There are no discernible medullary rays; the fibres are short, with thick walls, a limited lumen, and pointy extremities. The ground and cortical tissues contain simple and complicated starch granules (Fig. 6).

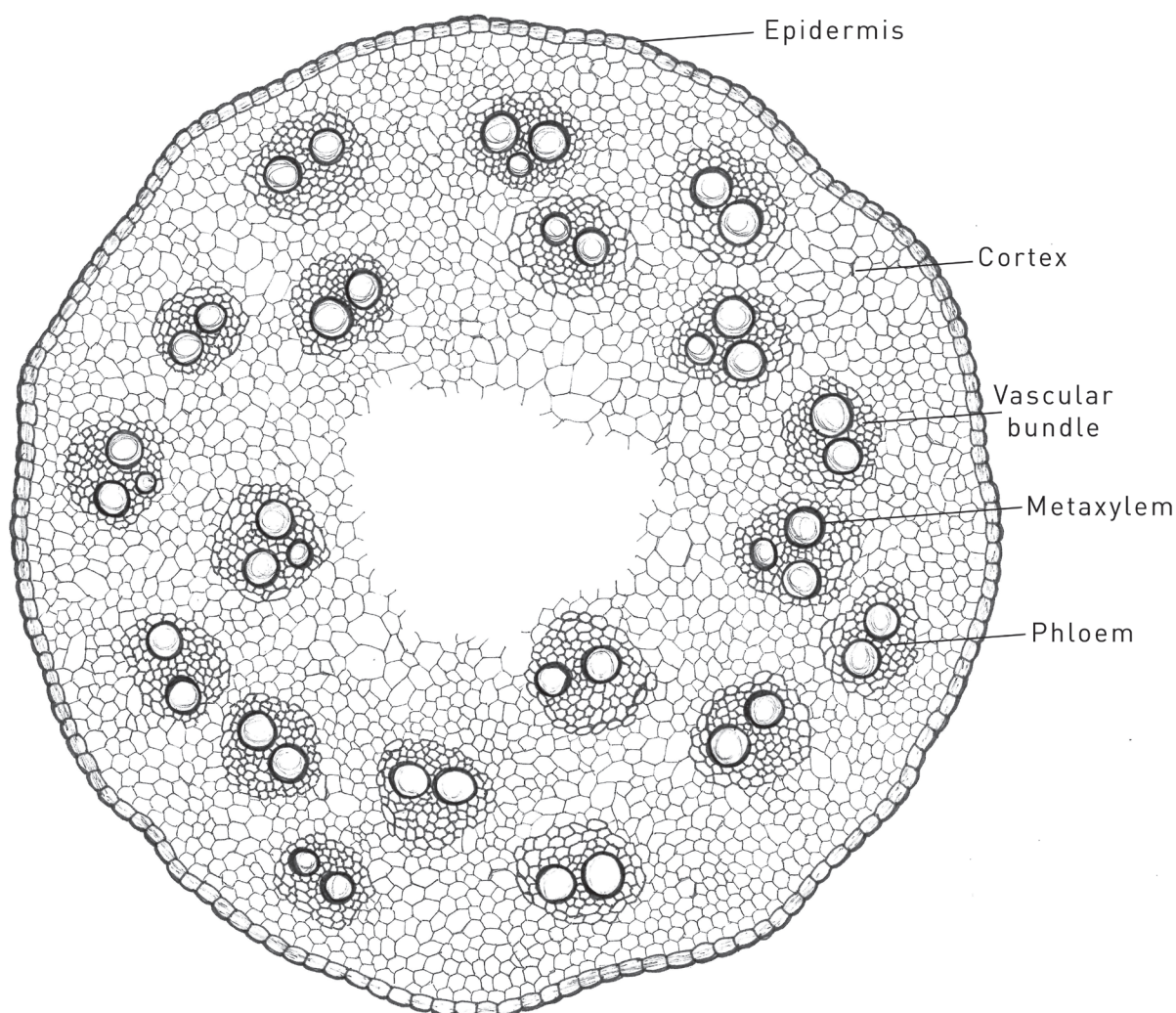


Fig. 6 TS of the stem

Structure of the Root

The epiblema or piliferous layer seen in mature roots is made up of a single layer of radially elongated to cubical cells with thin walls. The root hairs of the epidermis are unicellular. The hypodermis, which consists of 1-2 layered, thin-walled, tangentially elongated to irregular cells, is found underneath the epidermis. The cortex divided into two zones: the inner zone is composed of 4-6 layers of bigger, thin-walled, elongated parenchymatous cells, while the outer zone is composed of 1-2 layers of smaller, thin-walled, polygonal, lignified sclerenchymatous zone.

Endodermis cells are tangentially elongated, single-layered, and have thick walls. The pericycle is made up of one or two layers of thin walled sclerenchymatous cells. The vascular bundles, which are composed of phloem and xylem, are positioned in a ring on several radials. The exarch xylem has typical components, with a broad pith in the core made up of parenchymatous cells with strong walls that range in shape from oval to circular. Several simple starch granules that are angular, circular to oval, or parenchymatous (Fig. 7).

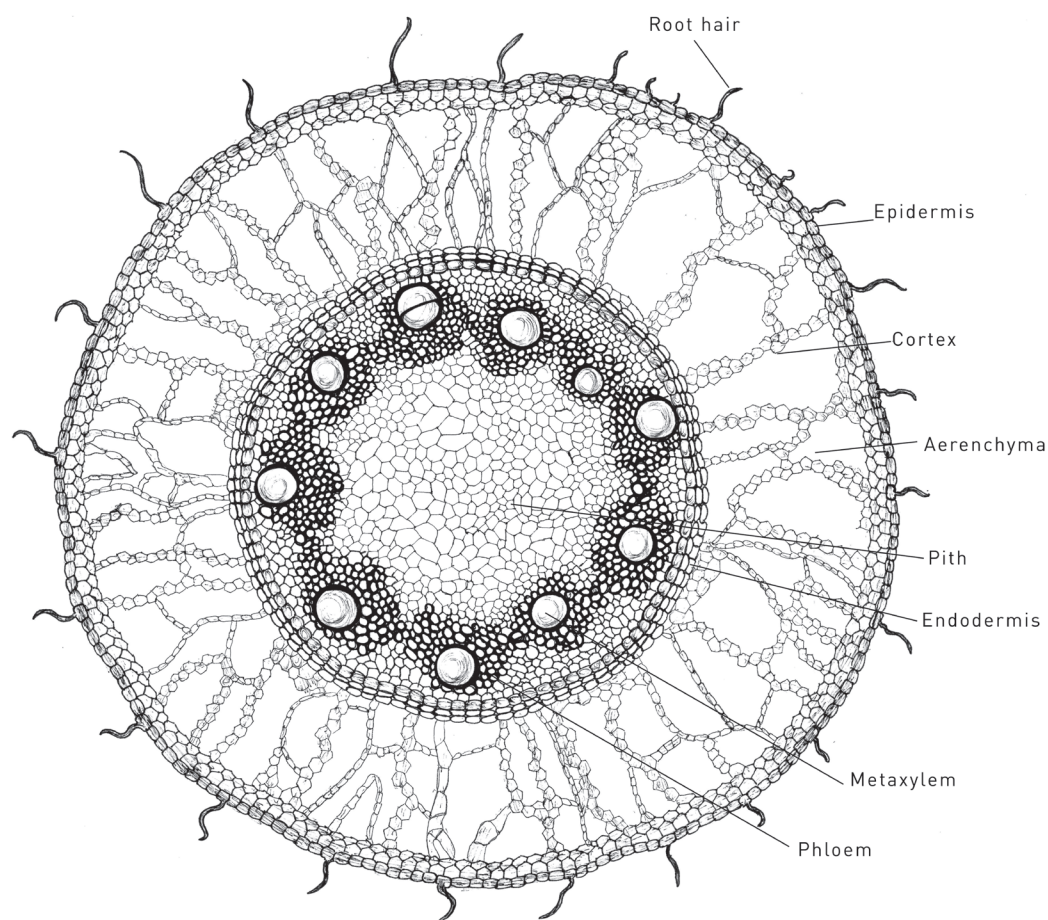


Fig. 7 TS of the roots

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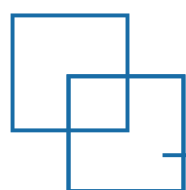
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CHAPTER
02

Bibliometric Analysis



INTRODUCTION

Cynodon dactylon (L.) Pers. is a perennial grass species renowned for its adaptability, resilience, and diverse applications in various fields such as agriculture, environmental conservation, medicine, and landscaping (Gebashe et al., 2019). This species has profound ecological importance, pharmacological profiles, therapeutic uses, and diverse medicinal applications. Its robustness in different climatic conditions, coupled with its medicinal properties, forage potential, soil erosion control, and turfgrass characteristics, has attracted significant attention from researchers across the globe (Iqbal et al., 2022). Apart from its significance in agricultural and ecological implications, *C. dactylon* is esteemed in traditional medicine for its reported medicinal properties. Several studies indicating its potential as an herbal remedy have explored its anti-inflammatory, antioxidant, and antimicrobial characteristics (Savadi et al., 2020; Alex et al., 2023). The grass's phytochemical composition has garnered attention in the scientific community, with efforts focused on identifying and understanding the bioactive compounds responsible for its medicinal potential.

Bibliometric analysis is an indispensable tool in modern scientific research, enabling a comprehensive assessment of the scholarly landscape surrounding a specific subject or topic (Donthu et al., 2021). In the case of *C. dactylon*, bibliometric analysis can offer an insightful exploration into the extensive body of literature, research trends, and knowledge dissemination related to this versatile and widely studied plant species. Bibliometric analysis may explore the quantitative and qualitative aspects of scholarly publications, allowing researchers to examine patterns, trends, and the evolution of scientific literature concerning *C. dactylon*. This analytical approach involves scrutinizing publication metrics, citation patterns, author collaborations, geographical distributions, and thematic emphases within the corpus of scientific literature (Kokol et al., 2021). Furthermore, this analysis can unveil the impact of research through citation networks, indicating influential publications and the dissemination of knowledge within the scientific community.



By systematically analyzing the bibliographic data related to *C. dactylon*, researchers can gain a comprehensive understanding of the breadth and depth of research conducted on this species. Insights gleaned from the bibliometric analysis not only facilitate a holistic view of the existing knowledge but also aid in identifying gaps, directing future research endeavors, and guiding strategic decision-making in areas such as agricultural practices, environmental conservation, pharmaceutical applications, and turfgrass management (Oyenihi et al., 2023). The bibliometric analysis could serve as an invaluable tool to navigate and comprehend the vast and dynamic research landscape surrounding *C. dactylon*, providing researchers with the means to extract meaningful insights, track the progression of knowledge, and drive innovation in diverse fields associated with this multifaceted plant species. At this outset, this analysis aimed to provide thorough insights into the current research trends, significant contributors, and cooperative networks active in this field by methodically examining and measuring the corpus of scholarly literature devoted to *C. dactylon*.

Empowering Insights with Data Source and Tools

The research investigates the research landscape of *Cynodon dactylon* (L.) Pers. through bibliometric analysis sought to provide an extensive understanding of the scope, impact, and evolving trends within academic publications focused on this botanical species. Scholarly articles data for this comprehensive analysis were sourced from Dimensions.ai, employing specific parameters including the exact phrase “*Cynodon dactylon*”, published between 1994 and 2023. This investigation utilized a diverse

set of analytical tools to extract insights including Microsoft Excel facilitated data organization and initial assessments, while the sophisticated capabilities of lens.org and VosViewer (Version 1.6.19) were utilized to explore deeper into bibliometric analysis, examining citation networks, co-authorship patterns, and visual representations of bibliographic data. The focused areas of the study were determined by employing the integrated tools within Lens.org, enabling a thorough exploration of key research domains.

Comprehensive Data-driven Insights

Analyzing the research landscape of *Cynodon dactylon* (L.) Pers. over the last 30 years (1994 to 2023) reveals a comprehensive understanding of scholarly output, its global reach, collaborative networks, and impact. A total of 3227 scholarly articles have been published on this subject by 1268 organizations across 91 countries, involving 10316 authors. A total of 317 authors have demonstrated focused expertise by publishing independently. These scholarly articles were published in 1055 renowned journals, reflecting a diverse dissemination platform. With an average of 14.18 citations per document, the research on *Cynodon dactylon* (L.) Pers. exhibited a soaring impact within the scholarly community. Over time, research trajectories have potentially shifted, possibly indicating a transition from foundational taxonomy and ecological studies to applied research spanning agronomy, pharmacology, ecology, and molecular biology. The global spread of contributions underscores widespread interest and potential collaboration among diverse regions. Collaborative networks among authors and organizations from various countries suggest a transdisciplinary approach to studying different aspects of this plant. Eventually, the bibliometric analysis highlighted the multifaceted understanding and significance of *C. dactylon*, signaling opportunities for innovative applications across agriculture, medicine, pharmacology, and environmental science.

Temporal Analysis and Growth Dynamics

A total of 3227 documents were sourced after extensive searches across bibliographic databases via Dimensions.ai. The present study meticulously delineated the evolutionary trajectory of research about *Cynodon dactylon* from 1994 to 2023. Within this temporal expanse, a discernible augmentation in scholarly publications denoted an escalating scholarly attention towards this subject (Fig. 1). The period spanning from 1994 to 2021, manifested a gradual proliferation in publications, with a conspicuous surge observed in 2021, marked by an impressive count of 243 publications. This surge was succeeded by a sustained trajectory of growth, evidenced by 221 publications in 2020 and 216 in 2022. As of September 2023, the recorded corpus comprised 126 publications, indicating a continued and dynamic exploration of this botanical species, with latent prospects for further data enrichment in subsequent analyses. Across the timeline encompassing 1994 to 2023, an exponential upsurge in scholarly outputs was discerned, as indicated by an R-square value of 0.81. The consistent upward trajectory in publication frequency, coupled with the diverse array of publications, underscored the profound scholarly interest and significance accorded to this scientific domain within the academic community.

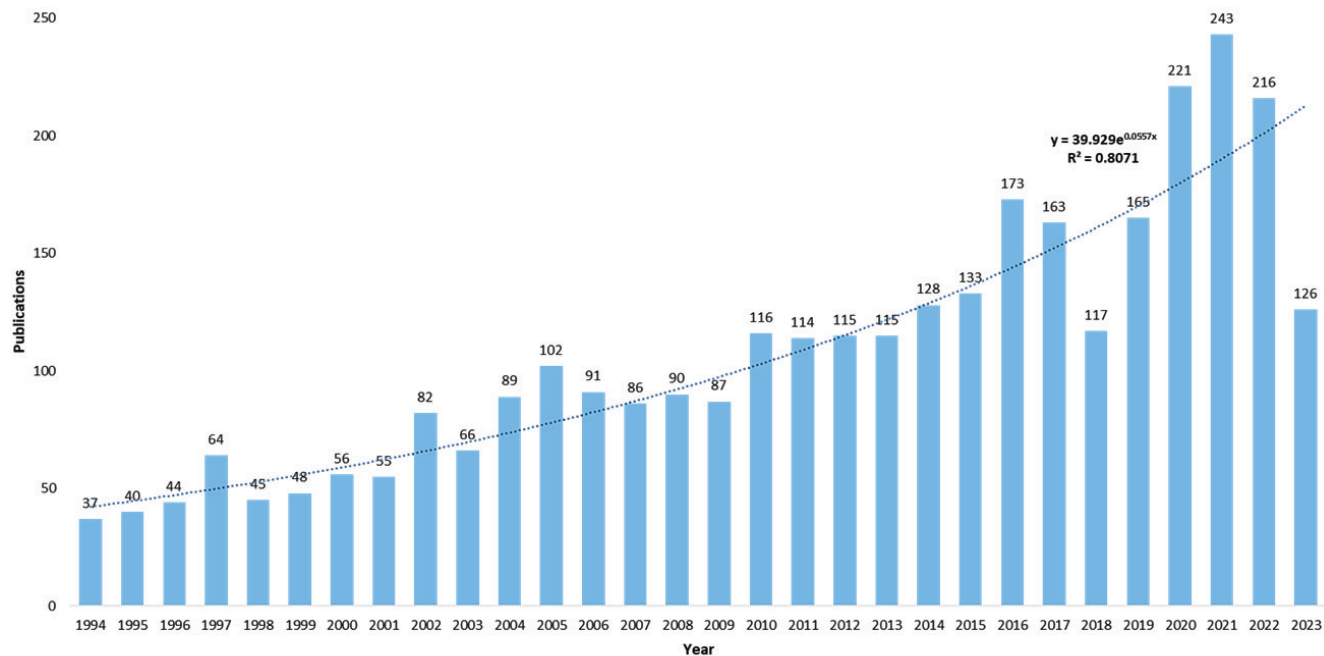


Fig. 1 Publication Trends and Progression Insights in *C. dactylon* Research

Citation Analysis

The citation analysis of research on *C. dactylon* spanning from 1994 to 2023 portrays a dynamic pattern of citation counts over time. Initially, the years from 1994 to 2007 exhibited a consistent growth in citations, albeit with intermittent fluctuations suggesting varying levels of research interest or focus during these periods. Especially, between 2008 and 2014, there were peaks in citation counts, potentially indicating intensified interest or significant breakthroughs within the field, yet accompanied by intermittent declines, indicating potential plateaus in research attention (Fig. 2). The years 2014 and 2015

marked the pinnacle of citations for research on *Cynodon dactylon*, recording 2698 and 2574 citations, respectively. These years signified the highest impact of *C. dactylon* research within the scientific community, showcasing the significant influence and relevance of the studies conducted during that period. However, from 2014 to 2023, a distinct trend emerged, marked by a significant decline in citation counts, particularly evident in the substantial drop observed in 2021, 2022 and 2023. This significant decrease might imply a shift in research priorities, emerging trends, or the need for novel perspectives in *C. dactylon* research.

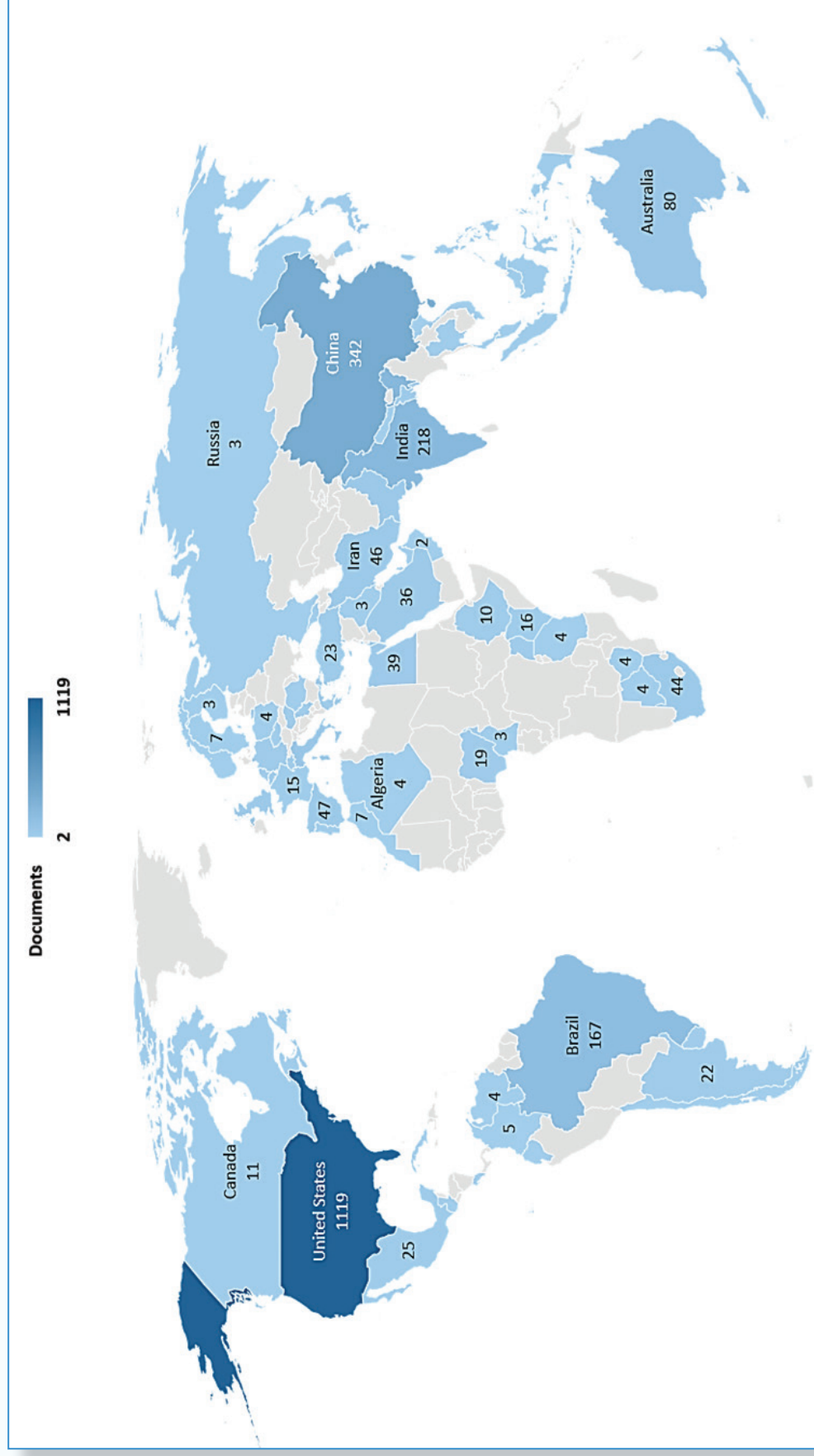


Fig. 3 Global distribution of scholarly production on *C. dactylon*

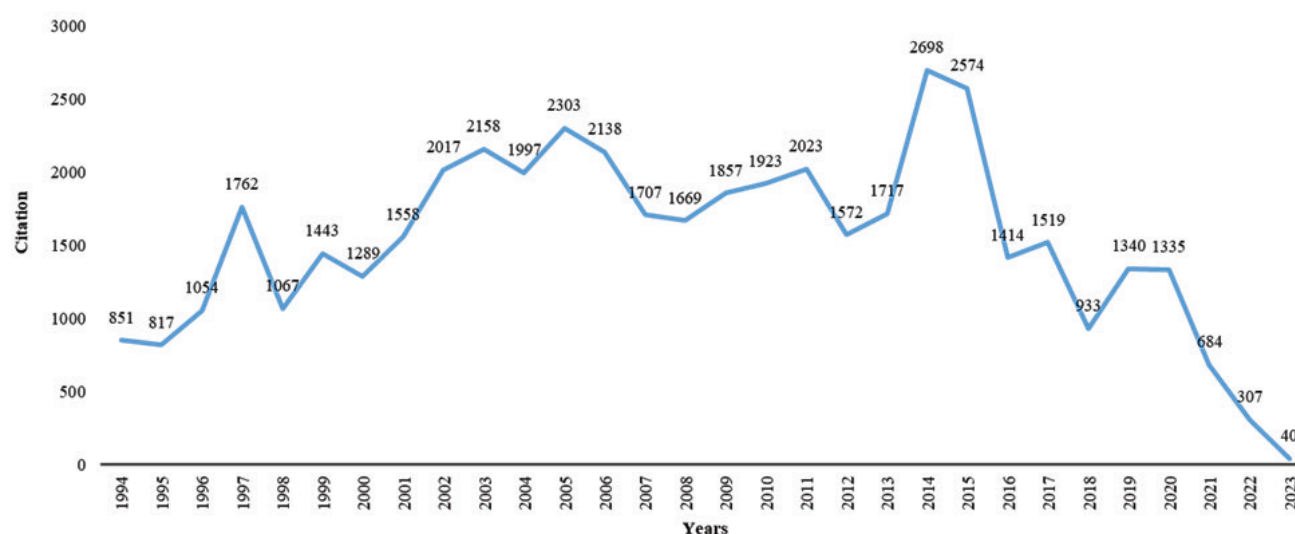


Fig. 2 Citation trends for *C. dactylon* research over time

Country-wise Scholarly Production and Impact Analysis

The analysis of scholarly production related to research on *C. dactylon* across various countries revealed intriguing patterns and insights into global academic contributions and impact. The United States, China, and India led in document contributions with 1119, 342, and 218 documents, respectively, reflecting substantial engagement in *C. dactylon* research (Fig. 3). Specifically, Australia, the United Kingdom, the Netherlands, and Canada demonstrated impressive citation impact per document of 23.58, 31.38, 46.38, and 52.73, respectively, despite producing fewer research articles, highlighting the quality and influence of their research. On the other hand, countries like Brazil, Iran, and Mexico showed moderate document counts with relatively lower citation impact per document of 9.95, 8.78,

and 11.36, respectively, suggesting the possibility for enhanced research visibility or quality. The diverse contributions from countries such as Portugal, Switzerland, New Zealand, and Sweden emphasized quality's significance over quantity with citation per document values of 33.62, 51, 12.4, and 13.43, respectively. These insights emphasize the importance of considering both document volume and citation impact to evaluate scholarly contributions in *C. dactylon* research, highlighting the potential for collaborative efforts among nations to advance understanding in this field.

Most Prominent Authors

The research unveiled the top 10 rankings of authors who contributed to *C. dactylon* studies, representing their research contributions and impact within the scientific community. Jinmin Fu and Erick Amombo prominently led the list, each contributing 30 documents,

reflecting a substantial volume of research output. Especially, Jinmin Fu stood out with an impressive citation count of 922, resulting in an average of 30.73 citations per document, exhibiting the significant impact and recognition of their scholarly contributions within the academic realm. Additionally, Lambert B. McCarty, Grady L. Miller, and Liang Chen also demonstrated commendable scholarly output, each contributing 20 or more documents. Liang Chen, particularly, stood out with an elevated average citation count of 33.58 per document, indicating substantial

influence and recognition in the field. Some authors, like Bernd Leinauer and A. R. Overman, with 19 documents each, garnered only 283 and 61 citations, averaging 14.89 and 3.21 citations per document, respectively, indicating potentially lower impact or visibility in the academic arena. This analysis indicated authors' diverse contributions to *C. dactylon* research, emphasizing both publication quantity and impact through citation metrics, crucial for understanding researchers' recognition and influence in the field.

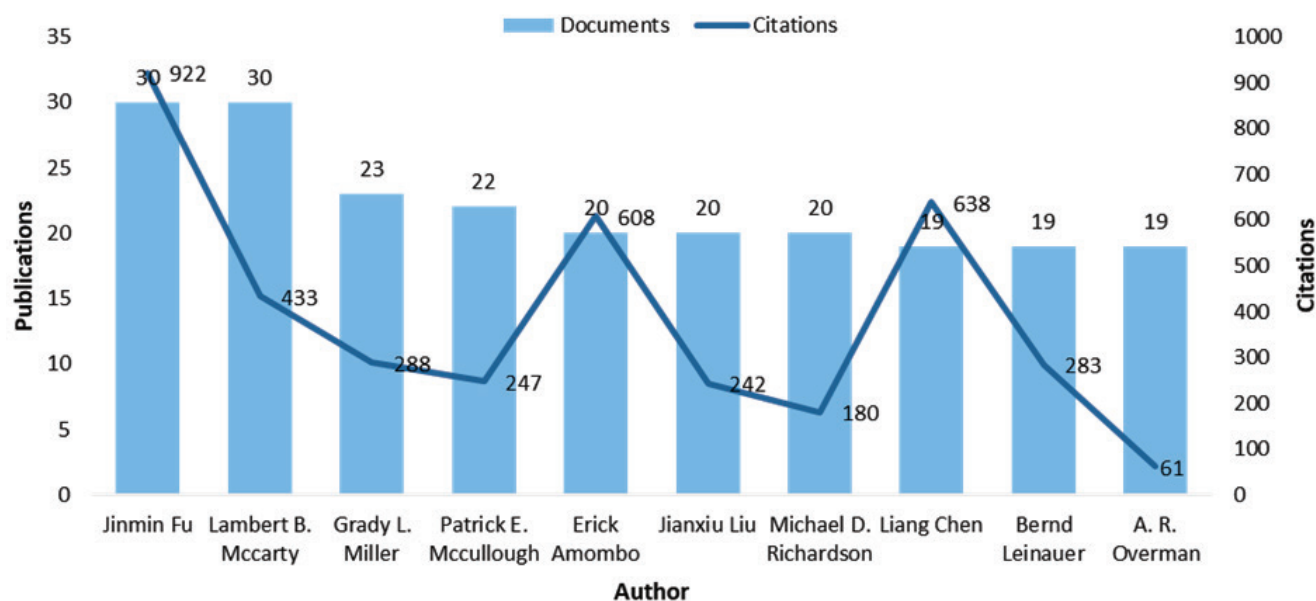


Fig. 4 Most prominent authors based on research contributions to *C. dactylon*

Highly Cited Articles

The top 10 highly cited research articles on *C. dactylon* covered a diverse range of topics, indicating the plant's significance across multiple disciplines. Among these articles, a study by Shi et al. (2014) garnered

the highest number of citations (407) for exploring how exogenous melatonin enhanced Bermudagrass' resistance to abiotic stress. In 2001, Richardson et al. presented a method, cited 342 times, that utilized digital image analysis to quantify turfgrass cover. Similarly, Patrignani et al.'s publication in 2015

introduced 'Canopeo,' a tool to accurately measure fractional green canopy cover, which gathered 280 citations and provided a valuable resource for monitoring vegetation. Another study by Lans et al. (2006) explored ethnobotanical knowledge, documenting traditional medicinal plant usage in Trinidad and Tobago for urinary problems and diabetes mellitus, amassing 261 citations. Pitta et al. (2009) investigated rumen bacterial diversity dynamics during the transition from Bermudagrass hay to winter wheat diets, receiving 257 citations and highlighting the effects of livestock diets. Auddy et al.'s (2003) exploration of the antioxidant properties of Indian medicinal plants used in managing neurodegenerative diseases earned 255 citations. Sponheimer et al.'s (2003) study, cited 232 times, investigated carbon-isotope fractionation in mammalian herbivores,

likely contributing significantly to ecological and dietary research. Reicosky et al. (1997) explored tillage-induced soil carbon dioxide loss in various cropping systems, gathering 218 citations and contributing valuable insights to soil science and agriculture. Guarrera et al.'s research in 2005, cited 212 times, documented traditional phytotherapy practices in Central Italy, offering insights into local medicinal plant usage. Lastly, Crous et al.'s (2014) work, cited 209 times, likely presented descriptive information about various fungal species, contributing significantly to fungal taxonomy and identification (Table 1). Together, these highly cited articles emphasized the interdisciplinary nature and broad applicability of *C. dactylon* research, encompassing fields such as plant physiology, ecology, ethnobotany, and agriculture.

Table 1 Top 10 highly cited research articles published on *C. dactylon*

Rank	Title	Citations	Year	Source	Reference
1	Comparative physiological, metabolomic, and transcriptomic analyses reveal mechanisms of improved abiotic stress resistance in bermudagrass [<i>Cynodon dactylon</i> (L). Pers.] by exogenous melatonin	407	2014	Journal of Experimental Botany	Shi et al., 2014
2	Quantifying Turfgrass Cover Using Digital Image Analysis	342	2001	Crop Science	Richardson et al., 2001
3	Canopeo: A Powerful New Tool for Measuring Fractional Green Canopy Cover	280	2015	Agronomy Journal	Patrignani et al., 2015
4	Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus	261	2006	Journal of Ethnobiology and Ethnomedicine	Lans et al., 2006

Rank	Title	Citations	Year	Source	Reference
5	Rumen Bacterial Diversity Dynamics Associated with Changing from Bermudagrass Hay to Grazed Winter Wheat Diets	257	2009	Microbial Ecology	Pitta et al., 2009
6	Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases	255	2003	Journal of Ethnopharmacology	Auddy et al., 2003
7	An experimental study of carbon-isotope fractionation between diet, hair, and feces of mammalian herbivores	232	2003	Canadian Journal of Zoology	Sponheimer et al., 2003
8	Tillage-induced soil carbon dioxide loss from different cropping systems	218	1997	Soil and Tillage Research	Reicosky et al., 1997
9	Traditional phytotherapy in Central Italy (Marche, Abruzzo, and Latium)	212	2005	Fitoterapia	Guarrera et al., 2005
10	Fungal Planet description sheets	209	2014	Persoonia	Crous et al., 2014

Most Active Journals

The present study showed the top journals for *C. dactylon* research based on their impact factor, publications, and citations. Crop Science by Wiley had emerged as the foremost journal, boasting an impressive impact factor of 4.48 and publishing 167 documents, with 4130 citations on this topic. Subsequently, Agronomy Journal, also from Wiley, maintained a significant presence with an impact factor of 2.65, contributing 128 documents and garnering 2171 citations, marking its significant influence in *C. dactylon* research. The Journal of Animal Science by Oxford Academic primarily focused on animal sciences but had an impact factor of 3.33, along with 104 published documents and 1165

citations related to *C. dactylon*, underscoring its considerable contribution to this area of research. Additionally, Hortscience by the American Society for Horticultural Science presented a significant amount of 98 published documents and 1158 citations, despite a lower impact factor of 1.87, securing a moderate influence within *C. dactylon* research. Weed Technology, published by Cambridge Core, maintained a focus on weed management, including studies involving *C. dactylon*, having an impact factor of 1.80, 94 documents, and 811 citations, indicating a moderate influence in this specific domain of study (Table 2). These journals, among others, contributed diversely to *C. dactylon* research, with Crop Science and Agronomy Journal leading the field in both impact and scholarly influence.

Table 2 Top Journals with the Highest Number of Published Documents and Citations in *Cynodon dactylon* Research

Rank	Source	Publisher	Impact factor	Documents	Citations
1	Crop Science	Wiley	4.48	167	4130
2	Agronomy Journal	Wiley	2.65	128	2171
3	Journal of Animal Science	Oxford Academic	3.33	104	1165
4	Hortscience	American Society for Horticultural Science	1.87	98	1158
5	Weed Technology	Cambridge Core	1.80	94	811
6	Horttechnology	American Society for Horticultural Science	1.38	52	340
7	Journal of Environmental Quality	Wiley	3.86	50	1817
8	Communications In Soil Science and Plant Analysis	Taylor & Francis	1.58	42	262
9	The Professional Animal Scientist	ScienceDirect	1.43	41	239
10	International Turfgrass Society Research Journal	Wiley	-	36	54

Top Productive Organizations

The scholarly articles on *C. dactylon* from diverse organizations have made a significant impact in the scientific arena, as proven by their high publication count, citations, and average citations per document. The University of Florida had dominated *C. dactylon* research, evidenced by 182 documents resulting in 2879 citations, averaging 15.82

citations per document, signifying substantial impact and recognition within the scientific community. Subsequently, Texas A&M University stood as another major contributor, with 123 documents and 1942 citations, demonstrating a comparable impact in this field with an average of 15.79 citations per document. The University of Georgia had shown a prominent outlook in *Cynodon dactylon* studies, boasting 107 documents and

1426 citations, averaging 13.33 citations per document, while the University of Arkansas at Fayetteville, despite a slightly lower document count of 105, had exhibited a high impact with 1726 citations and an average of 16.44 citations per document. Additionally, North Carolina State University, with 96 published documents and 1252 citations, reflected an average of 13.04 citations per document, exhibiting a moderate yet worthy contribution to *C. dactylon* research. Other institutions,

such as Oklahoma State University and the Agricultural Research Service in the United States, had shown remarkable impact with high average citations per document (Table 3). Overall, these organizations collectively contributed significantly to the advancement of knowledge in *C. dactylon* research, each playing a distinctive role in shaping the scientific understanding and impact within this field.

Table 3 Data insights of leading organizations to *Cynodon dactylon* research

Rank	Organization	Country	Documents	Citations	Average Citations per Document
1	University of Florida	United States	182	2879	15.82
2	Texas A&M University	United States	123	1942	15.79
3	University of Georgia	Georgia	107	1426	13.33
4	University of Arkansas at Fayetteville	United States	105	1726	16.44
5	North Carolina State University	United States	96	1252	13.04
6	Oklahoma State University	United States	80	1726	21.58
7	Agricultural Research Service, Southeast Area	United States	64	1255	19.61
8	Auburn University	United States	57	713	12.51
9	Mississippi State University	United States	56	445	7.95
10	University of Tennessee at Knoxville	United States	45	297	6.60

Most Prominent Fields of Study

The assessment of terms co-occurrence within prominent fields of study revealed a total dataset of 4619 scholarly articles from lens.org. Among these articles, the term “*Cynodon dactylon*” appeared with a frequency of 3303, indicating its substantial prevalence and significance within academic literature. Biology accounted for the highest document count with 3023 articles, followed by Agronomy with 2340 articles. This suggests a strong emphasis on biological aspects and agricultural applications concerning *C. dactylon*. Additionally, Botany,

Environmental Science, Chemistry, Animal Science, Horticulture, Weed, and Forage also contributed to the body of knowledge on *C. dactylon*, reflecting a diverse range of disciplines engaged in studying different facets of this grass species. The prevalence of *C. dactylon* in Biology and Agronomy indicated a comprehensive exploration of this species, covering its biological characteristics, ecological impact, agricultural significance, potential uses, and management strategies (Fig. 5). This interdisciplinary interest highlights the importance of understanding *C. dactylon* across various scientific domains, emphasizing its relevance and impact in diverse fields of study.

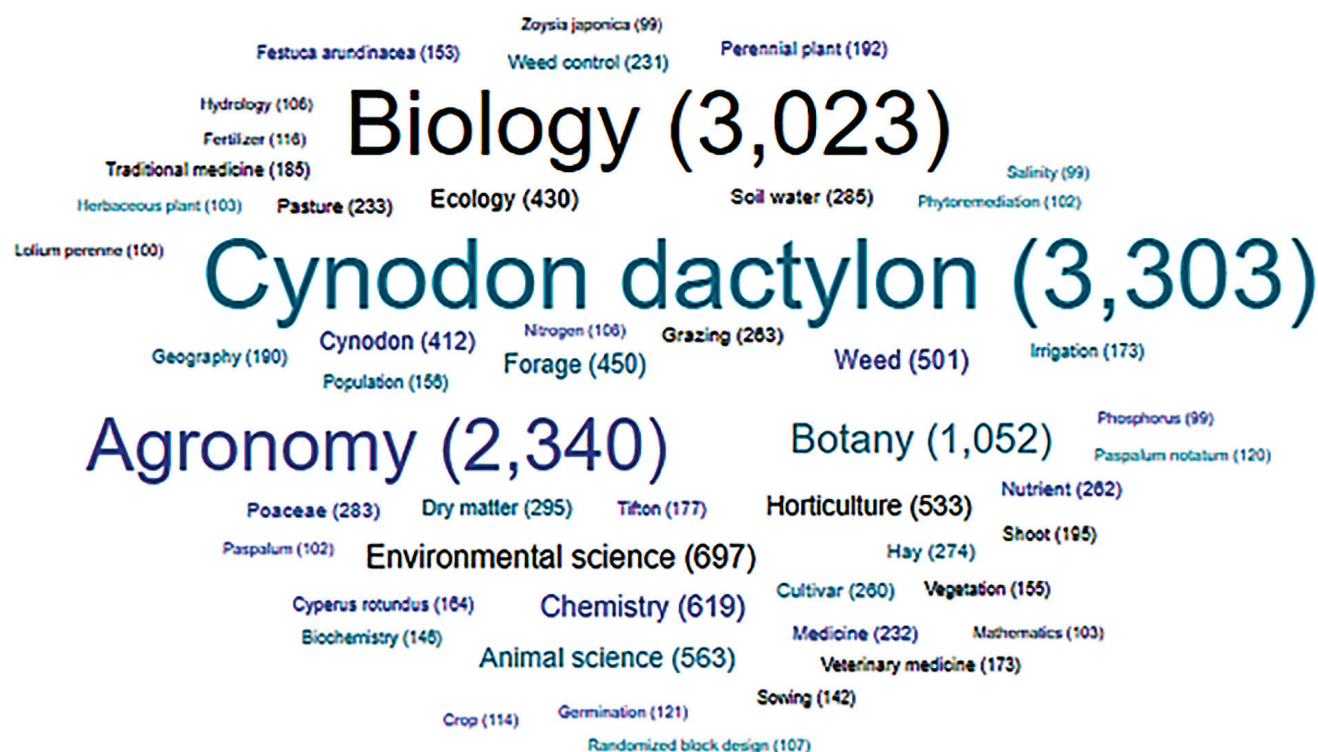


Fig. 5 Terms co-occurrence analysis within prominent areas of study

Research Collaboration Networks

Authors Collaboration Analysis

The comprehensive investigation into authors' collaboration revealed a significant presence of researchers studying *Cynodon dactylon*, as evidenced by co-authorship analysis conducted among a dataset of 10,316 authors. Co-authorship analysis was employed to assess collaboration among authors, specifically targeting those who had published a minimum of 10 documents on *C. dactylon*, each with at least 10 citations per document. The analysis identified 5 clusters of

collaborative authors, with cluster 1 emerging as the largest, comprising 9 authors, followed by cluster 2 with 6 authors. Clusters 3, 4, and 5 consisted of 4 authors each, indicating smaller yet interconnected groups of collaborating authors within the network. Particularly, Jinmin Fu emerged as the most influential author, boasting a total link strength of 117, followed by Erick Amombo and Chen Liang with strengths of 84 and 79, respectively (Fig. 6). These findings illustrated a diverse research community with varying levels of specialization and collaboration within the *C. dactylon* research landscape.

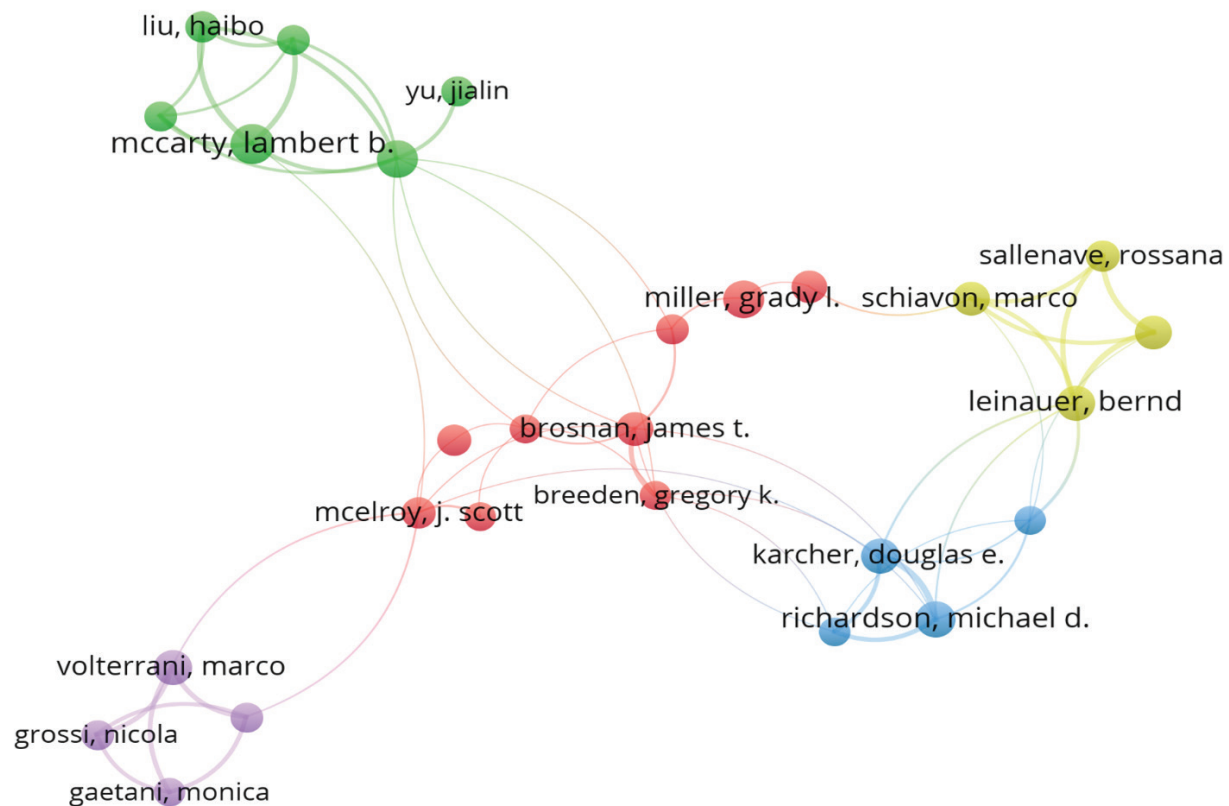


Fig. 6 Collaborative network among the authors on *C. dactylon* research

Journal-wise Collaboration Network

The journal-wise collaboration network, which analyzed 1055 journals studying *Cynodon dactylon*, unveiled 42 interlinks through bibliographic coupling. These connections suggested shared interests and opportunities for collaborative partnerships among journals in the field. The criteria required a minimum of 10 documents on *C. dactylon*, each with at least 10 citations, resulting in the identification of 4 distinct clusters in the journal-wise collaboration network. Cluster 1 was found as the largest cluster, comprised of sixteen journals, followed by cluster 2, cluster 3, and cluster 4, encompassing 12, 9, and 5 journals, respectively. This clustering highlighted

specialized thematic concentrations within *C. dactylon* research. Crop Science exhibited the strongest connection strength (8466), indicating its central role in fostering cooperative projects. Additionally, Agronomy Journal and Horttechnology demonstrated considerable influence, boasting total link strengths of 8055 and 2630, respectively (Fig. 7). The network analysis explored thematic strengths and collaborative potential among journals studying *C. dactylon*. Identification of influential journals like Crop Science, Agronomy Journal, and Horttechnology had provided insights into collaborative dynamics, signaling shared interests, and fostering opportunities for future partnerships and advancements in this domain.

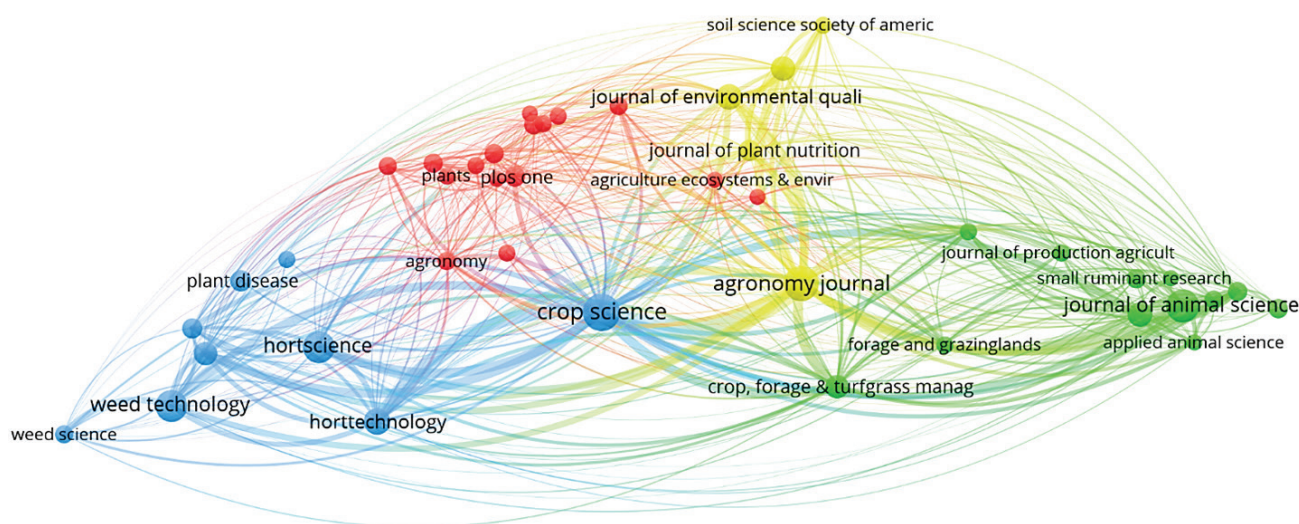


Fig. 7 Journal-wise collaboration network on *C. dactylon* research

Organizational Collaborative Network

The extensive analysis of organizational collaboration in the *Cynodon dactylon* study utilized citation analysis, scrutinizing a vast network involving 1268 organizations and identifying relationships among 66 of them. To be part of this network, organizations had to meet specific criteria, such as publishing a minimum of 10 documents on *C. dactylon*, each garnering at least 10 citations. The resulting network showed 5 distinct clusters, delineating the degree of inter-organizational cooperation. Cluster 1, encompassing twenty organizations, emerged as the largest and demonstrated a significant level of

collaborative engagement. Subsequently, cluster 2 consisted of 18 organizations, while cluster 3, Cluster 4, and cluster 5 contained 12, 9, and 6 organizations, respectively, highlighting closely connected relationships within each cluster. The University of Florida exhibited the highest total link strength, boasting 579 connections, followed closely by the University of Arkansas and North Carolina State University with connection strengths of 522 and 427, respectively (Fig. 8). These insights provided valuable information for researchers, institutions, and policymakers to strategically strengthen collaborations within the field, optimizing efforts, and propelling scientific advancements related to *C. dactylon*.

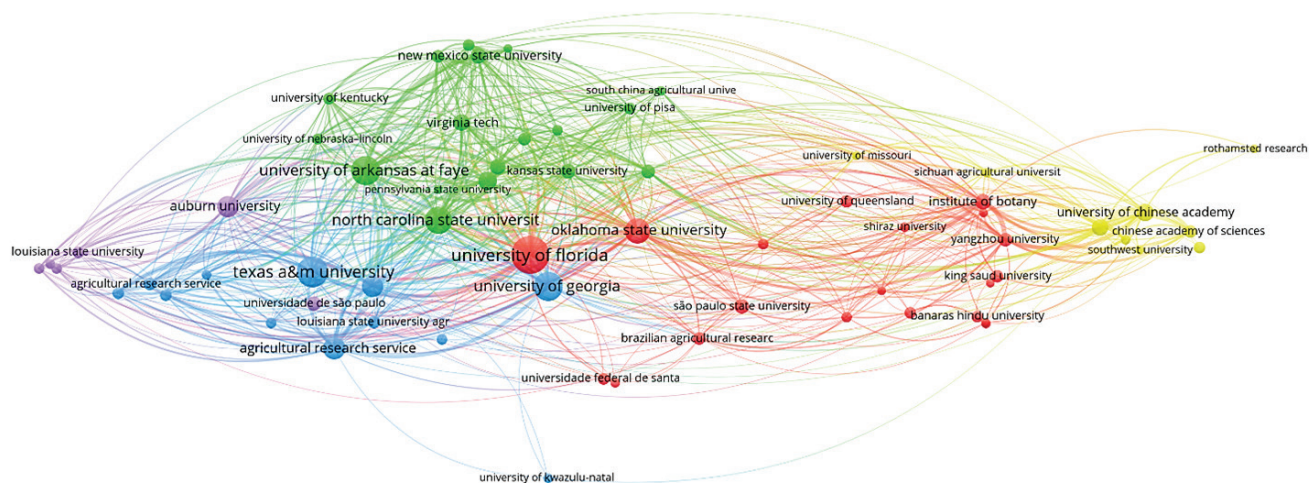


Fig. 8 Clusters of collaborative organizations for *C. dactylon* research

Country-wise Collaboration Network

The study investigated the country-wise collaborative network across 91 countries, uncovering 47 connections within this network. Specific criteria were employed, requiring each country to have a minimum of 5 documents on *C. dactylon* and meet a citation threshold of at least 5 citations per document. Using co-authorship as an analytical method, the study delineated nine distinctive clusters, indicating different levels of collaboration and research emphasis. Cluster 1 encompassed 12 nations, while cluster 2 and 3 comprised 9

and 7 nations, respectively. Similarly, clusters 4, 5, and 6 consisted of 6, 4, and 3 nations, respectively. Additionally, cluster 7 and 8 were composed of 2 nations each, and cluster 9 comprised only one country, revealing more concentrated collaboration networks. The United States emerged as the leading country with a total link strength score of 165, followed by China and Brazil with connection strengths of 94 and 55, respectively (Fig. 9). The analysis highlighted the collaborative patterns among countries, emphasizing the significance of global cooperation in advancing knowledge and understanding of *C. dactylon* research on a broader scale.

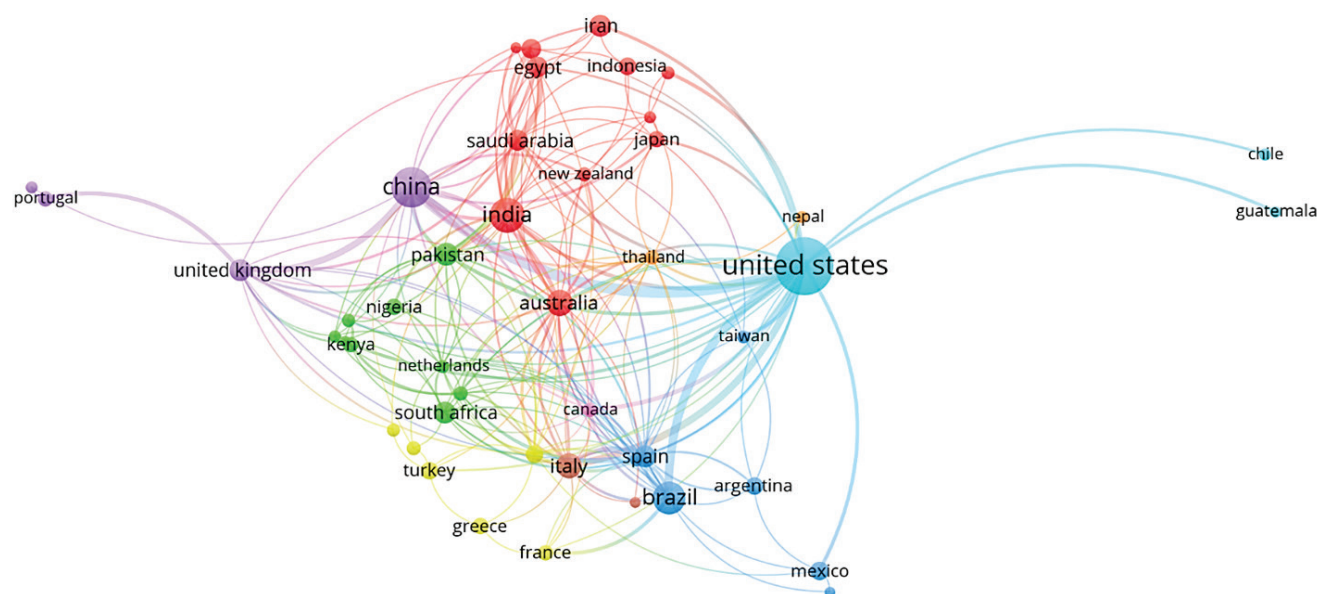


Fig. 9 Country-wise collaboration network of *C. dactylon* research

Conclusion

The bibliometric analysis was conducted on the research landscape of *Cynodon dactylon* (L.) Pers. from 1994 to 2023 presented a comprehensive overview of the temporal trajectory, scholarly contributions, influential authors, journals, organizations, and collaborative networks involved in this field. Temporal analysis revealed a progressive increase in research publications over time, with a significant elevation observed in 2021, showing a growing interest and attention towards *C. dactylon*. Citation analysis highlighted specific years where research on this subject received substantial recognition, especially in 2014 and 2015, signifying the impact of influential works during those periods. Country-wise scholarly production had emphasized the significant involvement of the United States, China, and India in contributing a substantial volume of documents, indicating widespread global engagement in *C. dactylon* research. Remarkably, the University of Florida has emerged as a dominant organization in this domain, demonstrating prolific output and substantial impact within the scientific community. The identification of influential

authors such as Jinmin Fu and Erick Amombo, along with the exploration of their collaborative networks, provided insight into the interconnection and collaborative nature of research endeavors related to *C. dactylon*. The analysis of journals, organizational, and country-wise collaboration networks revealed pivotal platforms and institutions promoting research dissemination and collaboration in this field. Furthermore, the investigation into highly cited articles, productive organizations, and the most active journal emphasized key contributors, impactful studies, and platforms that drove advancements in the understanding of *C. dactylon*. The findings emphasized the growing importance and prevalence of *C. dactylon* within academic literature, with collaborative efforts among researchers, institutions, and countries playing a crucial role in advancing knowledge, disseminating insights, and promoting interdisciplinary perspectives within this domain. As the interest and research on *C. dactylon* continued to evolve, this bibliometric analysis provided a valuable roadmap for future exploration and collaboration in this field.

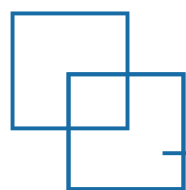
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CHAPTER
03

Soil Properties



INTRODUCTION

Soil is a complex matrix generated by the weathering of rocks that compose the Earth's crust's outer layer. It is generated through the dynamic interplay of minerals, organic matter, air, water, and other living species. It promotes plant growth due to its nature and the availability of nutrients. Plant development and yield are enhanced when the soil is nutrient rich (The State of Queensland, 2013). Soils, in addition to serving as a medium for plant growth, play an important role in gaseous exchange by absorbing and releasing gases such as carbon dioxide, oxygen, and methane. They can also hold water, which is an important resource for plant growth. Soils are host to various living organisms such as animals, fungi, bacteria, and other organisms because of their qualities. As a result of the presence of numerous living creatures, organic matter is constantly recycled through breakdown and accumulation. Soil is also a valuable basis for construction material from a commercial perspective (Soil Science Society of America, 2023).

Soil is made up of biotic and abiotic constituents, which comprise live creatures as biotic factors and minerals, organic matter, gas, and water as abiotic ones. All the components are responsible for plant growth and serve a significant role in preserving soil quality (Needelman, 2013). The percentages of each component vary amongst soil types, such as sandy, clayey, loamy, silty, and so on. The quantities of distinct components in soil are determined by the sites where the soil is available and the parent material from which it forms. The area where the soil is available and the parent material from which it forms determines the respective proportions of individual components in the soil. For instance, there are many different types of soils available in India. The first type is the alluvial soil which covers 46% of the Indian soil area. Rocks of the Himalayas are the parent material, and this kind of soil formation occurs by silt deposition by Indo-Gangetic-Brahmaputra Rivers. The next kind is the black soil which is formed from volcanic rocks in the Deccan Plateau. These soils are rich in clay and cover 16.6% of the total soil cover in India. The other types of soil include red and yellow soil, desert soil, laterite soil, peaty and marshy soil, alkaline soil, and mountain soil (Bhattacharyya et al., 2013).



The percentage composition, quantity, and quality of the components or nutrients in the soil all affect how fertile it is. The fertility of the soil in turn determines the type of crop that can grow in it and its yield and nutritional parameters. Ogundola et al., (2021) found different concentrations of essential oil from the shoots of *Solanum nigrum* L. that were grown in different types of soil. Due to the change in the oil content of the shoot, the free radical scavenging activity varied. It was reported in the study that plants grown on clay loam soil showed the highest radical scavenging activity. Andrade et al., (2011) also reported a similar phenomenon in the case of *Piper dilatatum* Rich. They showed that the essential oil composition of plants collected from different regions of Amazon, Brazil during rainy seasons varied due to environmental variations including variations in soil type. Jimoh et al., (2019) reported that *Amaranthus caudatus* L. exhibited the highest phytochemical yield when grown in clayey loam soil. A similar thing was replicated for the antioxidant activity of the plant extract. Zargoosh et al., (2019) showed that the interaction of site and elevation played a significant effect on the antioxidant potential and total phenol content in *Scrophularia striata* Boiss. The antioxidative capacity was indirectly correlated to elevation, phosphorous, potassium, organic carbon, organic matter, and nitrogen levels for the first site. Similarly, the acidity content had the highest negative correlation with antioxidant capacity. The lime and sand percentage of soil was also negatively related to the phytochemical content.

It has previously been demonstrated that a plant's environment has an impact on the number of secondary metabolites it produces and, ultimately, on the phytochemical composition of that plant (Walker et al., 2001; Srivastava and Shym, 2002; Dorri et al., 2009). The temperature and the humidity of the area in which a plant grows affect the phytochemical profile of the plant (Fox et al., 1999). How the environment regulates phytochemicals composition and quantity of plants is yet to be completely understood. Yet theories state that environmental factors may influence metabolic processes in plants which thereby affects the phytochemical content. At different altitudes, even the same plant shows different growth characteristics due to differences in light intensity, exposure to radiation, wind speed, humidity, water content in soil, and nutrient content in soils. The microbial composition of the soil may also affect the plant phytochemicals. They showed that total phenolic and flavonoid content increased because of *Rhizobium* inoculation in soil as compared to control. This occurred even though plant biomass and nitrogen fixation did not show any increase. Similarly, Egamberdieva et al., (2015) have reviewed in their book that Plant Growth Promoting Rhizobacteria helps plants by improving their salt and heavy metal tolerance, preventing plant diseases, and boosting soil fertility. In the case of medicinal plants specifically, they can enhance the phytochemical

levels by inducing secondary metabolite production. It is thus beneficial to enrich soils with beneficial microbes which can further be utilized for commercial purposes. Thus, it is evident that environmental variations including humidity, temperature, radiations as well and soil microbiomes influence the secondary metabolite production of medicinal plants and hence their phytochemical production. These attributes are beneficial to understand plant physiology and the best possible conditions for the cultivation of medicinal plants. In the current study, emphasize on studying the physicochemical variations in soil quality from different altitudes of sampling sites is given in Table 2. Soil physicochemical parameters like pH, electrical conductivity, organic carbon content, available nitrogen, phosphorous, potassium, sulphur and micronutrients, total moisture content, heavy metal, and bulk density have been studied to understand the quality at different altitudes and how they may influence vegetation in the different sites.

Sampling Sites

The bulk soil and plants (along with the rhizospheric soil) were collected from a total of 26 sites. The numbering of the sites, name of sites along coordinates are given in Table 1.

Table 1 Study sites and coordinates

S.No.	States	Site	Site Code	Latitude	Longitude
1.	Uttarakhand	Gomukh	UK-S1	30.56790	79.03081
2.		Gangotri	UK-S2	31.03920	78.74655
3.		Uttarkashi	UK-S3	30.74619	78.48377
4.		Devprayag	UK-S4	30.1358	81.1218
5.		Haridwar	UK-S5	29.9169	81.5315
6.	Uttar Pradesh	Bijnor	UP-S1	29.2857	82.3243
7.		Narora	UP-S2	28.1465	83.1315
8.		Badaun	UP-S3	27.9394	83.5612
9.		Farrukhabad	UP-S4	27.4104	84.496
10.		Bithoor	UP-S5	26.6159	85.1242
11.		Dalmau	UP-S6	26.3485	85.5979
12.		Prayagraj	UP-S7	25.2537	86.2076
13.		Mirzapur	UP-S8	25.8588	87.0908
14.		Varanasi	UP-S9	25.1518	87.396
15.		Ballia	UP-S10	25.3546	87.9107

16.	Bihar	Revelganj	BH-S1	25.4388	88.2213
17.		Patna	BH-S2	25.3808	88.3809
18.		Barh	BH-S3	25.2311	88.3895
19.		Bahachoki	BH-S4	25.1782	88.1959
20.		Farka	BH-S5	25.2323	78.8535
21.	Jharkhand	Sahibganj	JH-S1	25.1461	79.6282
22.	West Bengal	Farraka	WB-S1	24.8227	80.277
23.		Hazarduari	WB-S2	23.9969	81.1218
24.		Mayapur	WB-S3	23.4129	81.5315
25.		Hoogly	WB-S4	22.8491	82.3243
26.		Gangasagar	WB-S5	22.1774	83.1315

The temperature of a particular site is dependent on its altitude as well as climate conditions. At the time of sample collection, the temperature of the sites as well as altitude

of the sites as shown in live GPS map via GPS map camera app was noted down. The altitude and temperature of different sites for sample collection are shown in the Table 2.

Table 2 Temperature and altitude of different sampling sites

Site	Temperature (°C)	Altitude (m)
UK -S1	09	3794
UK -S2	17	2506
UK -S3	18	1232
UK -S4	23	1014
UK -S5	34	265
UP-S1	29	217
UP-S2	31	179
UP-S3	25	162
UP-S4	27	136
UP-S5	28	113
UP-S6	31	91
UP-S7	33	91
UP-S8	32	91
UP-S9	38	65
UP-S10	34	67

Site	Temperature (°C)	Altitude (m)
BH-S1	25	69
BH-S2	25	36
BH-S3	28	30
BH-S4	30	18
BH-S5	25	23
JH-S1	25	17
WB-S1	28	32
WB-S2	30	14
WB-S3	27	8
WB-S4	30	9
WB-S5	31	3

Soil Analysis

Physicochemical Analysis of Soil

Total Moisture Content (%)

Soil moisture content, often known as water content, is an indication of the quantity of water in the soil. Moisture content is stated as a proportion of the mass of water contained in the pore spaces of soil to the solid mass of particles in that substance. The mass of the sample is determined using a reference temperature of $110 \pm 5^\circ\text{C}$. Almost, all soil tests detect the natural moisture content of the soil, which is critical knowledge for all soil mechanics. The natural moisture content indicates the condition of the soil in the field (Hossain et al., 2022).

Apparatus and equipment required: Non-corrodible vented container, thermostatically

controlled drying oven that maintains temperatures between 105°C to 115°C , Balance of sufficient sensitivity (sensitive to 0.01 g) and container handling apparatus.

Procedure

1. Clean, dry and weigh W_1 the container. The balance needs to be tared before it is used to measure the weight.
2. Weigh W_2 sample of the specimen in the container.
3. Keep the container in the oven for 24 hours. Dry the specimen to a constant weight, maintaining the temperature between 105°C to 115°C . (The time will vary with the type of soil, but 16 to 24 hours is usually sufficient).
4. Record the final constant weight W_3 of the container with the dried soil sample. Peat and other organic soils should be dried

at a lower temperature (approximately 60 °C) for a longer period.

Calculations

1. Weight of the container = W_1 g
2. Weight of the container + Weight of the wet sample = W_2 g
3. Weight of the container + Weight of the dried sample = W_3 g
4. Weight of water in the soil sample = $W_2 - W_3 = M_w$ g
5. Weight of the dry soil = $W_3 - W_1 = M_s$ g
6. Moisture content in the given soil sample = $(M_w \text{ g} / M_s \text{ g}) \times 100\%$

Bulk Density

Bulk density is a commonly measured soil property by agriculturalists and engineers. High bulk density soils are soils with little pore space, so water infiltration is reduced, root penetration is inhibited, and aeration is restricted – reducing agricultural productivity. Low bulk density soils are easily compacted and may settle considerably to the detriment of roads, sidewalks, and building foundations (Bowen, 2016).

Apparatus and equipment required: Top load balance, soil spatula, 100 ml graduated measuring cylinder, 2×50 ml beaker, paper towels and mud bucket.

Procedure

1. Add slightly more than 50 ml of the soil sample to 50 ml beaker.
2. Clean and thoroughly dry a 100 ml graduated cylinder. Weigh and record weight (A).

3. Slowly add soil sample to pre-weighed graduated cylinder to the 10 ml line. Compact the soil by dropping onto a padded surface like a book, notebook, etc. at least ten times from a height of about 2-3 inches.
4. Repeat this process in 10 ml intervals until you reach the 50 ml mark.
5. Use a soil spatula to level the top of the sample in the graduated cylinder and add soil with the spatula until the top of the soil sample is exactly even with the 50 ml line – this is the bulk volume of compacted soil (B) (1 ml = 1 cm³).
6. Weigh and record graduated cylinder plus compact soil weight (C).
7. After drying the beaker, place any soil sample that is still in it back into the sample storage container.
8. Return 50 ml sample in graduated cylinder to 50 ml beaker. Remove all of sample within graduated cylinder.
9. Slowly pour approximately 25 ml of soil sample from beaker into water in the graduated cylinder. Gently stir soil/water mixture to remove any air bubbles. Add the second 25 ml of soil sample and stir again to remove air bubbles.

Calculations

1. Weight of 100 ml graduated cylinder = A
2. Bulk volume of the compacted soil = B
3. Weight of cylinder + compacted soil = C
4. Weight of soil sample = C-A= D
5. Bulk density (g/cm³) = D/B

pH

Principle: The pH of sample is measured with a pH meter, in which the potential of a hydrogen ion indicating electrode (glass electrode) is potentiometrically measured against a calomel saturated reference electrode, which also functions as a salt bridge. Most pH meters now contain a single integrated electrode. The equipment must be calibrated with a standard buffer solution of known pH before measuring the pH of the soil. As temperature affects pH, the pH meter is set to according to the temperature of the solution (Varley, 1972; Jackson, 1973).

Reagents: Buffer solutions (pH 4.0, 7.0, and 9.2)

Equipment required: A balance, 100 ml beaker, measuring cylinder, glass rod, pH meter and ordinary tissue paper.

Procedure

1. 25 g of the soil was weighed in a 100 ml beaker and make it to 50 ml final volume by adding of distilled water. The mixture was stirred well for at least four times within a 30-minute period to allow the soil and water to reach equilibrium.
2. In the meantime, the pH meter was switched on.
3. Initially the instrument was calibrated with buffer solution of known pH 4, 7.0, and 9.2.
4. The electrodes were washed with distilled water and wiped dry with a tissue paper.
5. Then, the electrode was dipped in the sample and the readings were taken.
6. Finally, the electrodes were washed with distilled water and placed back into a beaker containing 4.00-7.00 pH buffer or 3-4M KCl.

Interpretation

pH	Category	Soil Rating/Recommendation
<6.5	Acidic	Requires liming for reclamation
6.5-8.7	Normal	Optimum for most crops
8.8-9.3	Alkaline	Requires application of organic manures
>9.3	Alkali (Sodic)	Requires gypsum for amelioration

Electrical Conductivity

Principle: A conductivity meter known as “Solu Bridge” is used to measure the electrical conductivity of a soil solution. It is based on the Wheat Stone Bridge principle, in which alternating current is utilized instead of direct current to prevent electrode polarization and electrolysis of the solution. In a branched circuit with the conductance cell having resistance R_x , two fixed resistances R_1 and R_2 and a variable resistance (R_v) are linked. The variable resistance (R_v) is adjusted until no current flows through it and the reading is taken. The resistance or conductance (R_v) is measured [Richards, 1954; Chopra and Kanwar, 1976].

Interpretation

EC (1:2 soil water; $\mu\text{S/m}$)	Soil Rating
Below 800	Normal
800 - 1600	Critical for salt sensitive crops
1600 - 2500	Critical for salt tolerant crops
Above 2500	Injurious to all crops

Organic Carbon

Principle: In the presence of concentrated sulphuric acid, a known weight of soil was treated with an excess of standard potassium dichromate solution. The heat of the sulphuric acid gently digests the soil at a low temperature, oxidizing the organic carbon in the soil to CO_2 . The excess potassium dichromate was titrated against a standard solution of ferrous ammonium sulphate in the presence of a diphenylamine indicator and sodium fluoride or phosphoric acid, which distinguishes the

Apparatus and equipment required: Weighing balance, 100 ml beaker, measuring cylinder, glass rod and conductivity meter.

Procedure

1. 25 g of the soil samples was taken in a 100 ml beaker.
2. Added 50 ml distilled water.
3. Intermittent stirring was done with a glass rod for 30 minutes.
4. The samples were left overnight to obtain a clear supernatant.
5. The conductivity of the supernatant liquid was determined with the help of a conductivity meter.

colour due to their flocculating effect. The hue of the suspension varies from violet to blue to vivid green at the terminal point [Walkley and Black, 1934; Jackson, 1973].

Chemicals and reagents used

1. Potassium dichromate solution (1 N $\text{K}_2\text{Cr}_2\text{O}_7$): 49.04 g of analytical grade $\text{K}_2\text{Cr}_2\text{O}_7$ was dissolved in distilled water and the volume was made up to 1 litre.
2. Concentrated sulphuric acid (H_2SO_4).
3. Orthophosphoric acid (85%) or sodium fluoride (NaF).

4. Diphenylamine indicator: 0.5 g diphenylamine indicator was dissolved in a mixture of 100 ml concentrated sulphuric acid and 20 ml distilled water. This was stored in an amber colour bottle.
5. Ferrous ammonium sulphate solution [0.5 N, $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$]: 392 g analytical grade ferrous ammonium sulphate was dissolved in distilled water, to which 15 ml concentrated sulphuric acid was added and volume to made up to 2 litres with distilled water.
5. After cooling, about 0.5 g of NaF or 5 ml orthophosphoric acid, 100 ml of distilled water and 10 drops of diphenylamine indicator solution were added. These were shaken vigorously for complete mixing.
6. This was titrated against N/2 ferrous ammonium sulphate solution till the colour changes from violet to bright green through blue.
7. The volume of ferrous ammonium sulphate solution used for titration was noted down every time.
8. A blank titration was carried out without any soil.

Apparatus required: 250 ml Erlenmeyer (Conical) flask, pipette, burette and measuring cylinder.

Procedure

This process is also known as Walkley and Black's rapid titration method (1934).

1. 2 gm of dried, ground, and sieved soil was taken in a 250 ml conical flask.
2. To it, 10 ml 1 N $\text{K}_2\text{Cr}_2\text{O}_7$ solution was added and shaken gently to mix the contents.
3. Next, 20 ml of concentrated sulphuric acid was added while swirling the flask slowly as the reaction is exothermic and a lot of heat is produced.
4. The flask was kept on a dry tile or asbestos sheet for 30 minutes and left to attain room temperature.

Calculations

1. Weight of soil taken (W) = 2 g
2. Vol. of N/2 ferrous ammonium sulphate used for blank titration = X ml
3. Vol. of N/2 ferrous ammonium sulphate used to titrate excess nascent oxygen = Y ml
4. Vol. of 1N $\text{K}_2\text{Cr}_2\text{O}_7$ used for oxidation of organic carbon in soil = $(X-Y)/2$ ml
5. 1 ml of 1N $\text{K}_2\text{Cr}_2\text{O}_7$ = 0.003 g C
6. % Organic carbon in Soil = $\frac{X - Y/2}{0.003} \times 100/W$

Interpretation

Organic Carbon (%)	Soil Rating
<0.4	Low
0.4-0.75	Medium
>0.75	High

Available Nitrogen

Principle: An excess of alkaline potassium permanganate is applied to a known weight of soil, extracting a relatively easily oxidizable fraction of organic nitrogen. Ammonia is absorbed in excess of boric acid, forming ammonium borate, which is titrated against standard acid to determine the amount of ammonium absorbed (Subbaiah, 1956).

Chemicals and reagents required

1. Potassium permanganate solution (KMnO_4): 3.2 g/l of potassium permanganate was dissolved in distilled water and the volume was made up to 1 litre.
2. Sodium hydroxide solution (NaOH): 25 g/l of sodium hydroxide pellets were dissolved in distilled water and the volume was made up to 1 litre.
3. 0.02 N sulphuric acid (H_2SO_4).
4. Mixed indicator: 0.066 g of methyl red and 0.099 g of bromocresol green were mixed in 100 ml of ethanol.
5. Boric acid: 25 g/l of boric acid was dissolved in one litre of distilled water and to it 40 ml mixed indicator was added. Then, the pH was adjusted to 4.5-5.0 by adding 2.5 % NaOH .

Apparatus and equipment required: Kjeldahl distillation assembly, measuring cylinder, burette, balance, pipettes, and 100 ml conical flask.

Procedure

1. The Kjeldahl assembly was prepared by dipping the respective inlet tubes, one in 0.32% KMnO_4 and second in 2.5% NaOH reagent tanks.

2. 5 g soil sample was weighed and put into distillation tube carefully so that the soil sample does not stick to the sides of the tubes. Soil particles attached to the sides of the tube were washed down with distilled water.
3. The distillation tube was fixed in distillation unit and the sample was moistened with distilled water by pressing the dilution key.
4. 25 ml of 2.5% boric acid was taken in a conical flask and the receiving end of the distillation tube was dipped in it.
5. Next, 25 ml of KMnO_4 and 25 ml NaOH were added in the sample by pressing the respective keys.
6. The heating unit was switched on, and the process ran for 6 minutes.
7. The ammonia gas released from the sample was distilled and collected into the receiver containing acid. After complete digestion, the receiver flasks were removed.
8. The ammonium borate formed in the receiver flask was titrated against 0.02 N H_2SO_4 acid and note the volume of 0.02 N H_2SO_4 utilized. The colour changes from bluish green to wine red.

Calculations

1. Weight of soil taken = 5 g
2. Vol. of 2.5% boric acid taken = 25 ml
3. Vol. of 0.02 N H_2SO_4 used to titrate ammonium borate = X ml
4. 1 ml of 0.02 N H_2SO_4 = 0.00028 g of N
5. Available N (%) = 0.00028 g of N
6. Available N (ppm) = percentage N $\times$ 10,000
7. Available N (Kg/ha) = ppm $\times$ 2.24

Interpretation

Available Nitrogen (kg/ha)	Soil Rating
<272	Low
272-544	Medium
>544	High

Available Phosphorous

Principle: The activity of Ca^{2+} in the soil solution and the pH of the soil regulates the solubility of calcium phosphate in it. The bicarbonate (HCO_3^-) activity in the soil is increased by the 0.5 M NaHCO_3 solution buffered to pH 8.5, which reduces calcium activity. As a result, some phosphate from the surface of calcium phosphate gets dissolved in the soil. Similarly, due to the inactivation of Al and Fe, NaHCO_3 solution removes some phosphorous from Al and Fe phosphates. The precipitation of phosphate released from calcium phosphate is prevented by low Ca^{2+} activity. The soluble phosphate forms heteropoly complexes with molybdate ion freed from ammonium molybdate solution when added to the soil extract. [Bray and Kurtz, 1945; Black, 1965].

Chemicals and reagents required

- Standard phosphorous solution
 - Standard solution of P (100 ppm): 0.4387 g of KH_2PO_4 was dissolved in distilled water and the volume was made up to 1 litre.
 - Standard solution of P (5 ppm): 5 ml of 100 ppm P solution was diluted to 100 ml with distilled water.
- 0.5 M NaHCO_3 : 42 g of NaHCO_3 was dissolved in distilled water and the volume

was made up to one litre after adjusting the pH to 8.5 with sodium hydroxide using a pH meter.

- Sulphuric acid (5N): 139 ml of concentrated H_2SO_4 was diluted to 1 litre.
- Reagent A: Dissolve 12 g of ammonium paramolybdate in 250 ml distilled water. Separately, 0.2908g of potassium antimony tartrate ($\text{KSbO.C}_4\text{H}_4\text{O}_6$) was dissolved in 100 ml of distilled water. Both these dissolved reagents were added to 1 litre of 5 N sulphuric acid. Both were mixed thoroughly and diluted with distilled water to 2 litres.
- Reagent B: 1.056 g of L-ascorbic acid was dissolved in 200 ml of reagent A and mixed. It was prepared freshly before use.

Apparatus and equipment required: 100 ml conical flasks, funnels, pipette, (1 and 5 ml), 25 ml volumetric flasks, 100 ml measuring cylinder, electric shaker, Whatman No. 1 filter paper and spectrophotometer.

Procedure

a) Preparation of a standard curve

The relationship between the intensity of the coloured solution of a substance and the percent transmittance or absorbance of the light rays flowing through the solution was depicted by a standard curve. It was

used to figure out how much of a certain element is present in an unknown sample. 0, 0.5, 1, 2, 3, 4, 5 ml of P solution was taken in seven different 25 ml volumetric flasks to make the standard curve. It will produce a solution with a final concentration of 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 0.1 ppm, respectively. To it, 5 ml of extracting solution and 0.5 ml 5N H_2SO_4 were mixed and shaken well until the evolution of CO_2 stopped. 10ml of distilled water was added while washing the neck of the flask and 4 ml of reagent B and the volume was made up to the mark with distilled water and mixed. All flasks developed a blue colour of variable intensity. The intensity of blue colour was read on spectrophotometer at 880 nm. A standard curve was plotted using P concentration against absorbance value to establish their relationship.

b) Analysis of the test sample

- 1) 1 g of soil sample was taken in a 100 ml flask.
- 2) To it, a pinch of Darco-G 60 and 20 ml of 0.5 N NaHCO_3 solution were added.
- 3) The flask was placed on an electric shaker and mixed at a constant speed

for half an hour. Then the contents were filtered through Whatman No. 1.

- 4) A blank was prepared by following all the steps without addition of soil.
- 5) In case, the filtrate was not clear, a pinch of Darco – G was added.
- 6) 5 ml of the filtrate was taken in a 25 ml volumetric flask and proceed further for colour development as described under preparation of standard curve.

Calculations

1. Weight of soil sample taken = 1 g
2. Vol. of 0.5N NaHCO_3 solution added = 20 ml
3. First dilution = 20 times
4. Vol. of the filter taken for colour development = 5 ml
5. First volume made= 25 ml
6. Second volume = 5 times
7. Total dilution = $20 \times 5 = 100$ times
8. Concentration of P from spectrophotometer = X AU (absorbance units)
9. Available P in soil (ppm) = $X \text{ (AU)} \times 100$
10. Available P (Kg/ ha) = $\text{ppm} \times 2.24$

Interpretation

Available Phosphorus (kg/ha)	Soil Rating
>12.4	Low
12.4-22.4	Medium
22.4-50	High
>50	Very High

Available Potassium

Principle: In a neutral normal ammonium acetate solution, a known weight of soil was shaken. Potassium (K) ions absorbed on soil colloids exchange ammonium ions. The amount of exchangeable and water-soluble potassium in the extract was measured using a flame photometer. The transfer of non-exchangeable K to exchangeable form was hampered during ammonium acetate extraction because ammonium ions, like K^+ , retain strongly charged layers together (Black, 1965; Jackson, 1973).

Chemicals and reagents required

1. Neutral normal ammonium acetate solution (CH_3COONH_4): 77.09 g/l of ammonium acetate was dissolved in distilled water and the volume was made up to 1 litre. The pH of the solution was adjusted to 7 with ammonium solution or acetic acid.
2. Standard solution of K (1000 ppm K): 1.91 g of potassium chloride (KCl) was dissolved in distilled water and the volume was made to 1 litre.
3. Working standard solution of K: The stock solution was diluted 100 times to get 10 ppm K solution.

Apparatus and equipment required: A weighing balance, 150 ml conical flasks, a

shaker, funnels, beaker, Whatman filter paper No 1, pipettes and a flame photometer.

Procedure

1. 5 g of soil was weighed in a 150 ml conical flask.
2. To this, 25 ml of neutral normal ammonium acetate solution was added.
3. The mixture was shaken for 5 minutes on an electric shaker and then filtered through Whatman No. 1 filter paper.
4. 5 ml of the filtered extract was taken in a 25 ml volumetric flask and the volume was made up with distilled water. This solution was fed into the atomizer of the flame photometer and readings were noted down.

The amount of K in the test sample was calculated by using the dilution factor.

Calculations

1. Weight of soil sample taken = 5 g
2. Volume of the neutral normal CH_3COONH_4 solution added = 25 ml
3. Dilution = 5 times
4. Reading of K (ppm) in flame photometer = Y
5. In ppm K = $Y \times \text{total dilution} - A$
6. In kg/ha = $A \times 2.24 - C$

Interpretation

Available Potassium (kg/ha)	Soil Rating
<137	Low
137– 337	Medium
>337	High

Available Sulphur

Principle: Soil was shaken with 0.15 % CaCl_2 solution. During extraction, chloride ions displace adsorbed sulphate while calcium ions decrease soil organic matter extraction and hence eliminate contamination caused by extractable organic sulphur. The turbidity produced by the precipitation of sulphate as barium sulphate is measured on a spectrophotometer at a wavelength of 420 nm. The turbidity is stabilized using gum acacia solution, which prevents the barium sulphate formed from settling (Lisle et al., 1994).

Chemicals and reagents required

1. Extracting solution (0.15% CaCl_2): 1.986 g of calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) was dissolved in distilled water and volume made up to 1 litre.
2. Barium chloride (BaCl_2)
3. Gum acacia solution (0.25%): 0.25 g of gum acacia was dissolved in distilled water and diluted to 100 ml.
4. Standard sulphur solution (100 ppm): 0.5434 g of the reagent grade potassium sulphate (K_2SO_4) was dissolved in distilled water and diluted to 1 litre.

Apparatus and equipment required: 150 ml conical flask, funnel, pipettes, 25 ml volumetric flasks, electric shaker, and spectrophotometer and Whatman No. 42 filter paper.

Procedure

a) Preparation of standard curve

1. 0.25, 0.5, 1.0, 2.5 and 5.0 ml of 100 ppm S solution were taken in different 25 ml

volumetric flasks. It was theoretically calculated to give 0, 1, 2, 4, 7, 10, 20 ppm concentration of sulphur in the final volume.

2. To every flask 10 ml 0.15% CaCl_2 solution and 1 g BaCl_2 were added.
3. Mixed for 1 minute to dissolve all the crystals.
4. Next, 1ml 0.25% solution of gum acacia was added.
5. The volume was made up to the mark for all the flasks and shaken properly for thorough mixing. Within 5-30 minutes after the development of turbidity, the standards were read on a spectrophotometer at 420 nm.
6. 0 absorbance at 0 ppm solution (blank) was adjusted.
7. A standard curve was plotted showing relationship between concentration of S (turbidity) and transmittance/absorbance readings.

b) Analysis of test samples

1. 10g air dried soil was weighed and transferred to a 150 ml conical flask.
2. 50 ml of 0.15% CaCl_2 solution was added, and the mixture was shaken for 30 minutes on an electric shaker.
3. Filter the suspension through Whatman No. 42 filter paper.
4. 20 ml of the filtrate was taken in a 25 ml volumetric flask and the same steps as in case of standard curve were followed.

5. A blank was run with all the chemicals except the soil.
6. The sulphate concentration of unknown samples was determined from the standard curve.
3. First dilution= 5 times
4. Volume of aliquot taken= 20 ml
5. Final volume= 25 ml
6. Second dilution= 1.25 times
7. Total dilution= $5 \times 1.25 = 6.25$ times
8. ppm of S from standard curve= Y
9. ppm of S in soil= $Y \times 6.25$
10. S in kg/ha= ppm of S $\times 2.24$

Calculations

1. Weight of soil taken= 10g
2. Volume of extractant added= 50 ml

Interpretation

Available Sulphur (ppm)	Soil Rating
<10 ppm	Deficient
>10 ppm	Sufficient

Heavy Metal

All the heavy metal i.e. Cr, Ni, As, Sr, Cd, Hg and Pb, are extracted with the help of ICP-MS (Make-Thermo Scientific instrument) (Retka et al., 2010).

Procedure

1. Take the sample and homogenize properly.
2. Take approximately 0.1 to 0.2 g sample in microwave vessels and add 4 ml of HNO_3 (Suprapure grade), add 1 ml of H_2O_2 (Suprapure grade).
3. Kept at room temperature 20-30 minutes for open digestion.
4. Afterwards samples were put in microwave for close digestion.
5. After digestion, samples were transferred into the 50 ml volumetric flasks and volume make up to the mark.
6. Samples were vortexed properly and run of ICP-MS against the linearity.

Calculations

Calculate the concentration of the elements as follows.

Sample Conc.= (Sample reading-reagent blank reading $\times$ dilution factor)/ Sample Wt.

Micronutrient

Principle: All the four micronutrient cations i.e. Zn, Mn, Fe and Cu, are extracted by shaking the soil with DTPA extracting solution containing 0.005M DTPA, 0.1 M TEA (Triethanol amine) and 0.01 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ buffered at pH 7.3. During this extraction, TEA gets protonated as HTEA^+ because of which micronutrient cations from the solid phase comes into solution and are chelated by the DTPA. Buffering of the extractant in the slightly alkaline pH range and inclusion of soluble Ca^{2+} through $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ helps avoiding dissolution of CaCO_3 and thus excludes from the estimation of the occluded micronutrients, which do not form a part of the pool that is

available for absorption by plant roots. After that the contents of the micronutrients cations in the soil extract are estimated on atomic absorption spectrophotometer (Katyal and Sharma, 1991).

Chemicals and reagents required

1. DTPA extraction solution: This solution was prepared to contain 0.005M DTPA, 0.01M CaCl_2 and 0.1M TEA (Triethanolamine) and its pH was adjusted to 7.3. For preparing one litre of this solution, 13.3 ml $(\text{HOCH}_2\text{CH}_2)_3\text{N}$ i.e. TEA, 1.967 g DTPA and 1.47 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ were dissolved in about 500 ml of deionized water. Sufficient time was given for DTPA to dissolve, and the contents are diluted to approximately 900 ml. The pH of the solution was then adjusted to 7.30 ± 0.05 by adding 6N HCL while stirring and finally the contents were diluted to 1 litre. This solution was stable for several months.

Apparatus and equipment required: 1 litre volumetric flask, 100ml narrow mouth polyethylene bottles, pipettes, electric shaker,

Whatman No. 1 filter paper and atomic absorption spectrophotometer.

Procedure

1. 12.5 g of air-dried soil sample was taken and transferred to a 100 ml narrow mouth polyethylene bottle.
2. 50 ml DTPA solution was added, and the bottle was closed with the stopper.
3. The bottle was shaken on an electric shaker for 2 hours at 25°C .
4. The contents were then filtered through Whatman No. 42 filter paper to obtain a clear solution.
5. Also, a blank was run with only DTPA solution without the soil.

Calculations

1. Weight of soil used for extraction = 12.5 g
2. Volume of the extractant used = 50 ml
3. Dilution = 2 times
4. Concentration of the given micronutrient in extract = A mg/kg
5. Available micronutrient in given soil sample = $A \times 2$ mg/kg

Interpretation

Content below which the soil is deficient	
Metal	mg/kg soil
Zn	0.6
Cu	0.2
Fe	4.5
Mn	3.5

Rhizosphere Soil Microbiology

Principle: The principle for the analysis of microbe's different media is used such as nutrient agar, Eosin Methylene blue, MacConkey Agar, Azotobacter, Azospirillum, Rhizobium, zinc solubilising, phosphate solubilising and potash mobilizer. The microbial count is usually expressed in CFU (colony-forming units) per gram or millilitre. The direct count method for enumerating bacteria in natural environments is widely used (Kirchman, 1982).

Procedure

- Different growth medium is used to grow different types of microorganisms is given in Table 3.
- The medium is sterilized in an autoclave.
- Petri dishes are used to hold the growth media.
- A small number of bacteria is needed to inoculate the growth media.
- A 100 µl culture of sample is picked up with the help of pipette and transferred to the growth media and spread with the help of L-shaped spreaders.
- The inoculated growth media is incubated at the optimal temperature and conditions for the bacteria to grow.
- After incubation, the bacterial growth can be observed by looking closely at the colonial growth on the surface of a solid medium.

Table 3 Lists the various growth media that are used to cultivate the various kinds of microorganisms

Serial No.	Target Organism	Synthetic Media Used	Make
1.	Total Bacterial Count	Nutrient Broth	HiMedia (M002-500G)
2.	Total Fecal Count	MacConkey Agar	HiMedia (M008S-500G)
3.	Total Coliform Count	Eosin methylene Agar	HiMedia (M317-500G)
4.	Potash Mobilizer	Aleksandrow	HiMedia (M1997-500G)
5.	Zinc Solubilizer	Zinc sulphate	HiMedia (M2023-500G)
6.	<i>Rhizobium</i>	Rhizobium	HiMedia (M408-500G)
7.	<i>Azotobacter</i>	Azotobacter	HiMedia (M1944-500G)
8.	<i>Azospirillum</i>	Azospirillum + KOH	HiMedia (M1720-500G + M1720-500G)
9.	Phosphate Solubilizes	Pikovskaya	HiMedia (GM1719-500G)

Apparatus and equipment required: Weighing balance, spatula, distilled water, measuring cylinder, and a 1000 ml conical flask.

Calculation

1. Colony forming Units (CFU/ml) = Number of colonies × Dilution factor/Volume of culture

Correlation Studies and Statistical Analysis

The tests have been performed in triplicates and the mean of values along with the standard deviation has been represented graphically. A total of 8 parameters have been studied for 26 sites and each parameter has shown considerable variation. The effect of one parameter on the other may be studied through correlation. A correlation coefficient is an indicator of the relationship between two variables. The correlation coefficient is a statistical measure that indicates the strength and direction of the relationship between

two variables. Its value ranges from -1 to +1, representing different degrees and types of correlation. When the correlation coefficient is closer to +1, it signifies a strong positive relationship between the variables. This means that as one variable increases, the other tends to increase as well. A correlation coefficient around 0 implies no linear relationship between the variables. Changes in one variable do not predict or affect changes in the other. As the correlation coefficient approaches -1, it denotes a strong negative relationship. This suggests that as one variable increases, the other tends to decrease.

Results And Discussion

Physicochemical Characterization of Soil

Moisture content is one of the most essential index qualities for determining the relationship between soil behaviour and index values. Soil moisture content expresses the phase

relationships of water, air, and solids in each volume or weight of material. The consistency of a specific soil, combined with its liquid and plastic limitations, is used to represent its relative consistency in cohesive soil (Hossain et al., 2022). The moisture content of different sampling sites is shown in Fig. 1

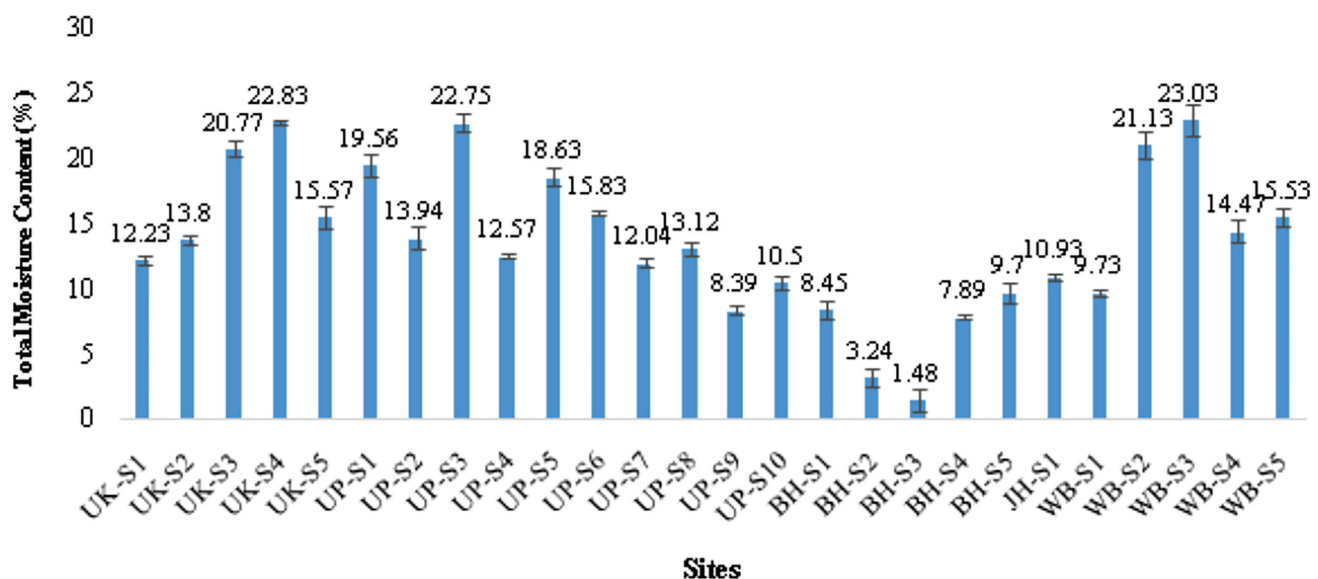


Fig. 1 Total moisture content of different sampling sites

The TMC% was found to be in the range of $1.48 \pm 0.94\%$ (lowest) to $23.03 \pm 1.25\%$ (highest). Soil sample from BH-S3 had very little moisture content i.e., $1.48\% \pm 0.94$. Seven of the soil samples had moisture content below 10%, while in most samples, moisture content was found to be within 20%. Sample from five sites had their TMC% over 20%.

Soil bulk density was another characteristic investigated. It is a measure of how thick or

firmly packed the soil is. The composition of the soil, the structure of the soil peds, the distribution of sand, silt, and clay particles, the volume of pore space, and how densely the particles are packed all influence soil bulk density. Bulk density indicates how easily roots can develop and water can filter through a profile's multiple soil strata (The Globe Program). The soil bulk density of different sampling sites is illustrated in Fig. 2.

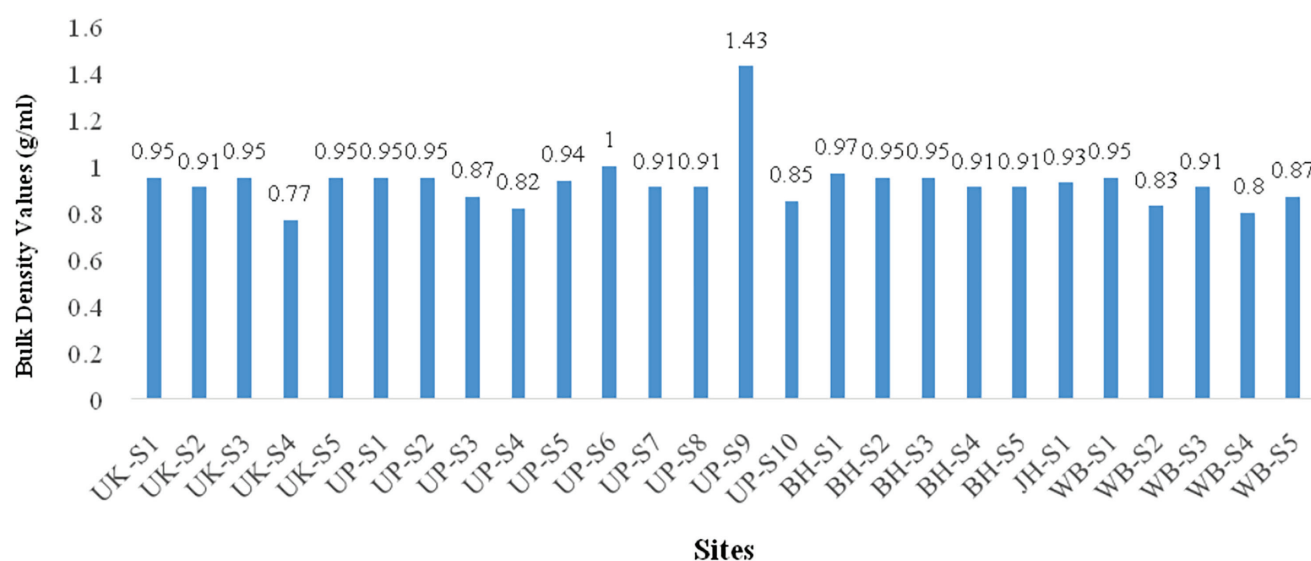


Fig. 2 Soil bulk density of different sampling sites

From the above data, it was deduced that soil bulk density ranged from 0.77 g/ml to 1.43 g/ml. Soil bulk density of UP-S9 was highest i.e. 1.43 g/ml but that of UK-S4 was lowest i.e. 0.77 g/ml. Six of the sites had their bulk density below 0.90 g/ml while the remaining sites had their bulk density values above 0.90 g/ml.

The pH of soil is an important physical attribute that determines species richness

and density (Gough et al., 2000). This happens as soil pH determines the amount of nutrients that are soluble in soil water and their availability to plants. Some nutrients are more available under acidic conditions while some are available under alkaline condition. However, extreme condition of acidity or alkalinity are harmful for the soil as well as plant growth. The pH of the different sampling sites is shown in Fig. 3.

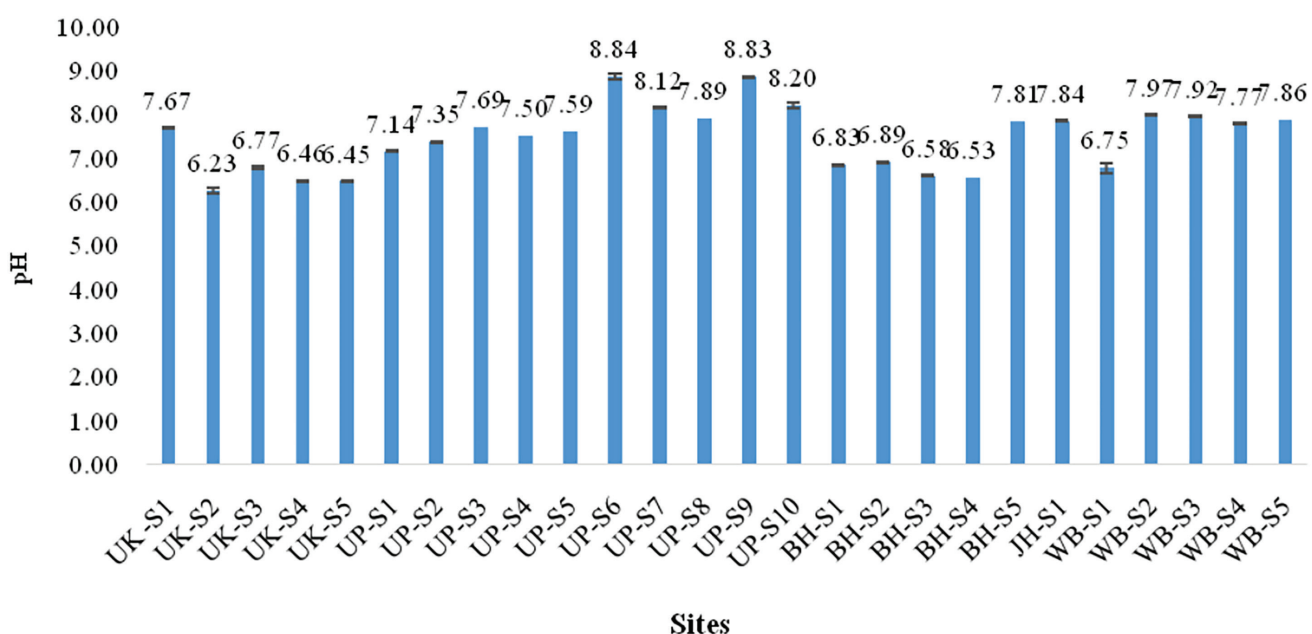


Fig. 3 Soil pH of different sampling sites

The pH of the sample sites ranged from as low as 6.23 ± 0.06 to as high as 8.84 ± 0.01 . Soil samples from UK-S2, UK-S4 and UK-S5 were found to be acidic, with pH levels lower than 6.5. The pH of 9 sites was neutral, whereas the pH of the remaining sites was alkaline.

The next parameter studied, was electrical conductivity of the soil. Soil electrical conductivity is an indicator of soil salinity, clay

content and the nutrient concentration in the field (Heiniger et al., 2003). As shown above, electrical conductivity may be categorized in different divisions and that may be used to determine the salinity of the soil so that which crop could be sown may be decided. The electrical conductivity of soils from different sampling sites was determined and is represented in Fig. 4.

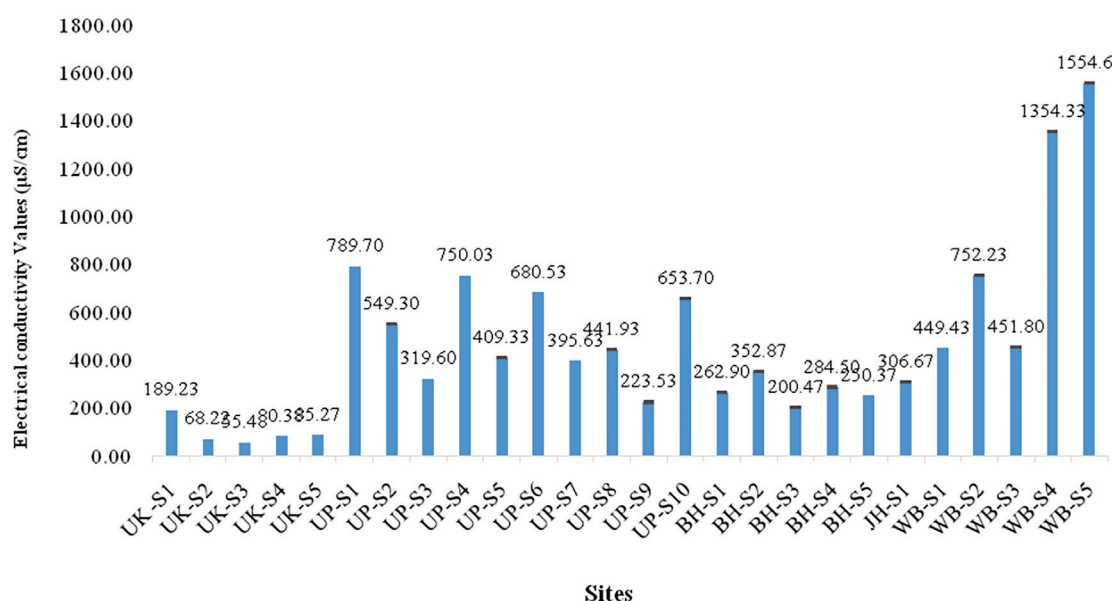


Fig. 4 Soil electrical conductivity at different sampling sites

The electrical conductivity of almost all the soil samples were within the prescribed limits of having EC below 800 μ S/cm. Soil samples from WB-S4 and WB-S5 showed the highest electrical conductivity value of 1354.33 $\pm$ 2.52 μ S/cm and 1554.67 $\pm$ 2.52 μ S/cm respectively. Thus, it may be said that the soil electrical conductivity was within the range at remaining all sampling sites and hence favourable for plant growth.

Soil organic carbon is a measure of the organic content present in soils. It is an important determinant of the hydraulic properties of soil like porosity, water retention and hydraulic conductivity. Thus overall, it is an important parameter determining food content in soil for uptake by plants (Allison, 1965; Rawls et al., 2004). The organic carbon content as measured for the 26 soil samples is shown in Fig. 5.

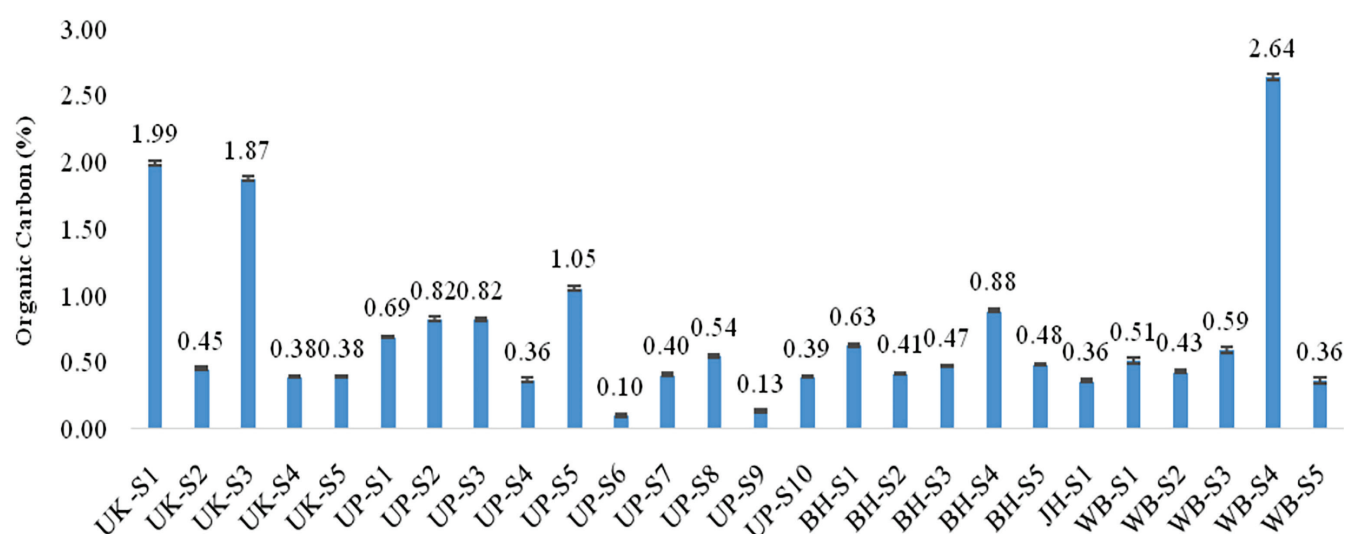


Fig. 5 Soil organic carbon content at different sites

According to the data, the organic carbon content of soil ranged from 0.10 $\pm$ 0.01% to 2.64 $\pm$ 0.03%. UP-S6, which had the highest pH value of 8.84 $\pm$ 0.01, had the lowest organic carbon content of 0.10 $\pm$ 0.01%. The highest quantity of organic carbon content was discovered in WB-S4 (2.64 $\pm$ 0.03%), which had an alkaline pH (7.77 $\pm$ 0.02). Soil samples from 8 different sites showed low organic carbon concentration, whereas soil samples from 11 different sites had medium organic carbon content. A total of 7 sites had high levels of organic carbon.

The next soil parameter studied was available nitrogen content. Nitrogen is the most important plant macronutrient, and its proper availability is a crucial determinant for plant growth and its optimal health. Researchers (Baričević and Zupančič, 2002) showed that increasing concentration of N added to soil resulted in an increased concentration of alkaloids which is a critical component in extracts of medicinal plants. The available nitrogen content in soils from the different sites is shown in Fig. 6.

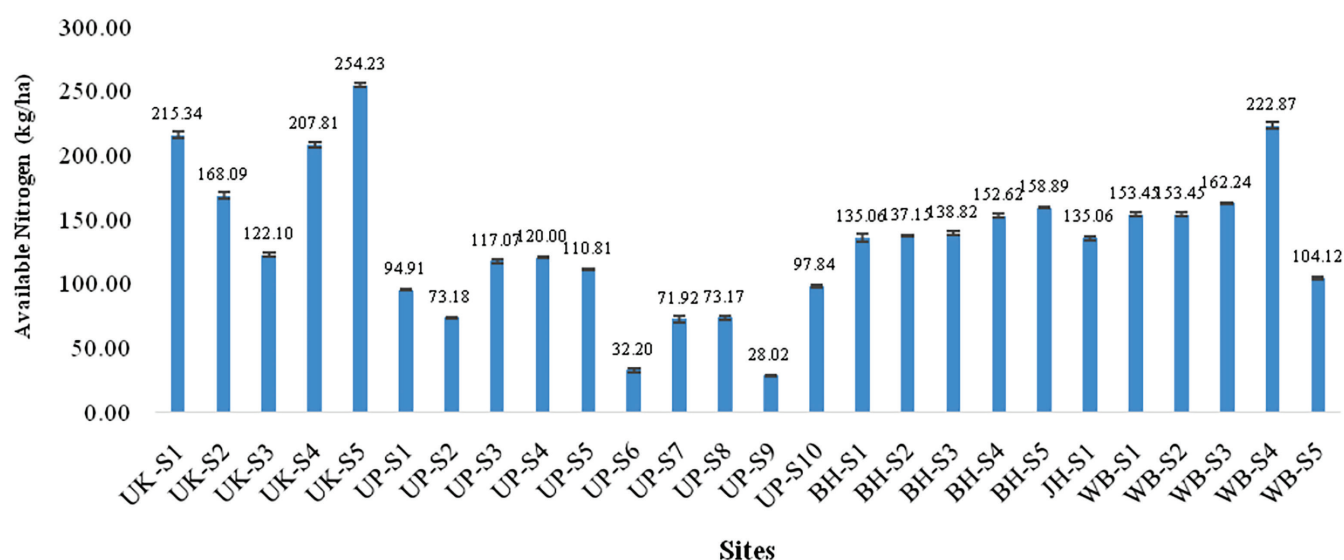


Fig. 6 Soil available nitrogen content at different sites

From the available data, it was observed that site UK-S5 had the maximum available nitrogen content (254.23 ± 1.45 kg/ha) and as per soil categories, the content was low. For all the other types of soil, the available nitrogen content was low. This could be because all these samples were randomly picked from different sites, which necessarily did not receive any fertilization.

The next parameter tested was that of phosphorous which is another important

plant macronutrient. Phosphorous is a macronutrient that controls the water use efficiency, winter hardiness and promotes root formation (Chakraborty and Prasad, 2021). In case of medicinal plants, enough phosphorous in soils promotes development of leaf biomass which is the main part of the medicinal plant in most cases (Stewart, 2003). The concentration of available phosphorous in soils from the different sites is shown in Fig. 7.

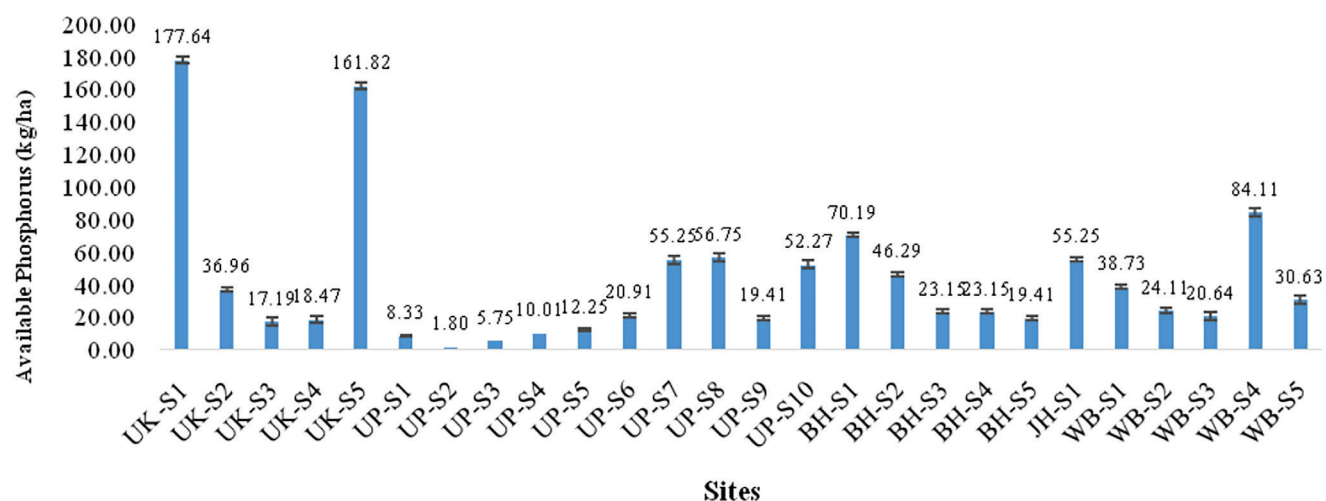


Fig. 7 Soil available phosphorus content at different sites

As per the data obtained and divisions demarcating phosphorous content in soil, the maximum P was recorded in site UK-S1 (177.64 ± 1.83 kg/ha) while a very less amount was recorded in site UP-S2 (1.80 ± 0.06 kg/ha). The remaining sites have enough amount of the P in soil. Four of the sites had available phosphorus content in low range, while 6 sites were having available phosphorus in medium range. Similarly, 4 sites had their range in high category and the remaining sites had available phosphorus in very high category.

The next parameter is the third major macronutrient for plants, potassium. In general, potassium plays a critical role in the closing and opening of stomata and thus regulates the uptake of water by plants. It also regulates plant growth and yield (Perrenoud, 1977). Literature also suggests that proper potassium content in soil results in higher concentration of essential oils in medicinal plants. Thus, such plants when grown in properly fertilized soil will result in good quality of medicinal plants for maximum benefit of consumers. The potassium content of the different soil samples is shown in Fig. 8.

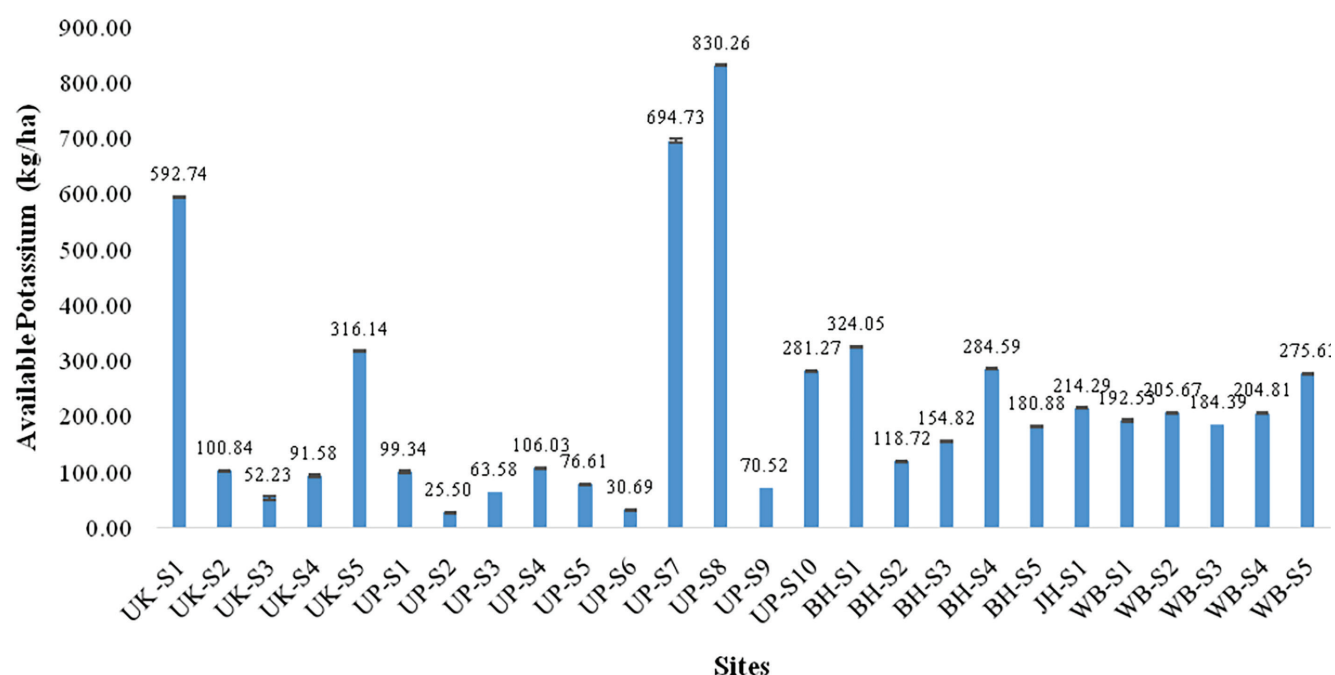


Fig. 8 Soil available potassium content at different sites

From the obtained results, it was seen that site UK-S1, UP-S7 and UP-S8 had high potassium content. As per the divisions of potassium content in soil, the potassium content was medium at twelve sites. The remaining sites had low potassium content.

Sulphur content in soil plays a role as it is up taken by plants for the formation of sulphur containing amino acids and hence the buildup of proteins, chlorophyll, and oils (Tabatabai, 1984). The sulphur content in the soil is shown in Fig. 9.

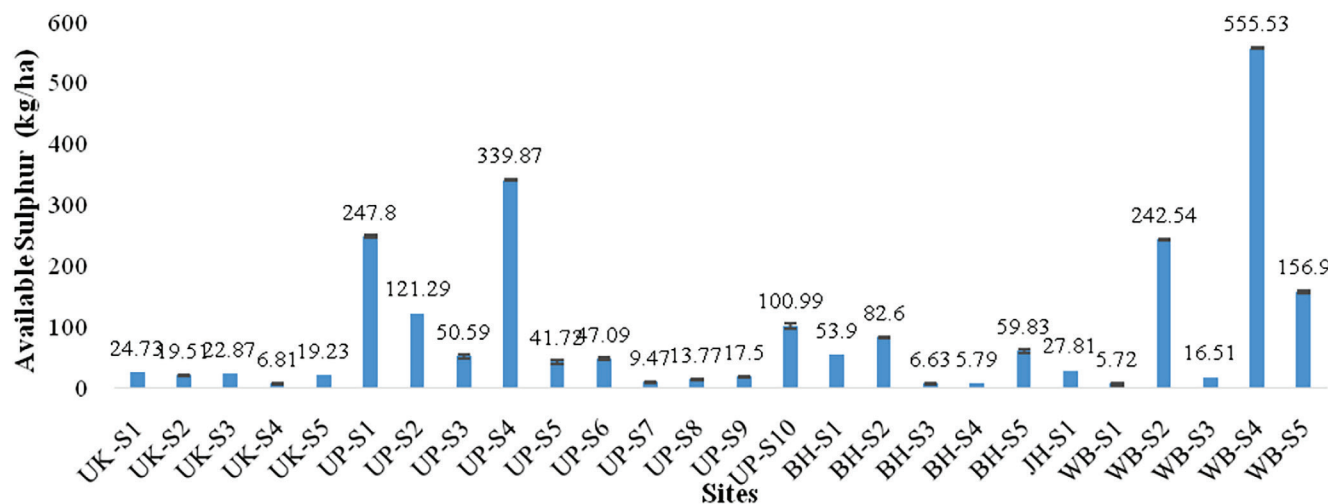


Fig. 9 Soil available sulphur content at different sites

For the results obtained for the sulphur content, it was found that 10 sites were sulphur deficient. The remaining sites had enough sulphur in their soil. Further, correlation studies will help us to understand the effect of pH, EC, or even microbial parameters on sulphur content and vice versa. Additionally, the impact of both high and low sulphur on the composition of phytochemicals can be

examined.

Fig. 10 a-g shows the different heavy metals content in all the sampling sites. The concentration of 7 heavy metals Cr, Ni, As, Sr, Cd, Hg and Pb were determined using ICP-MS. Their concentration further talks about the level of pollution and contamination in the soil sampling sites.

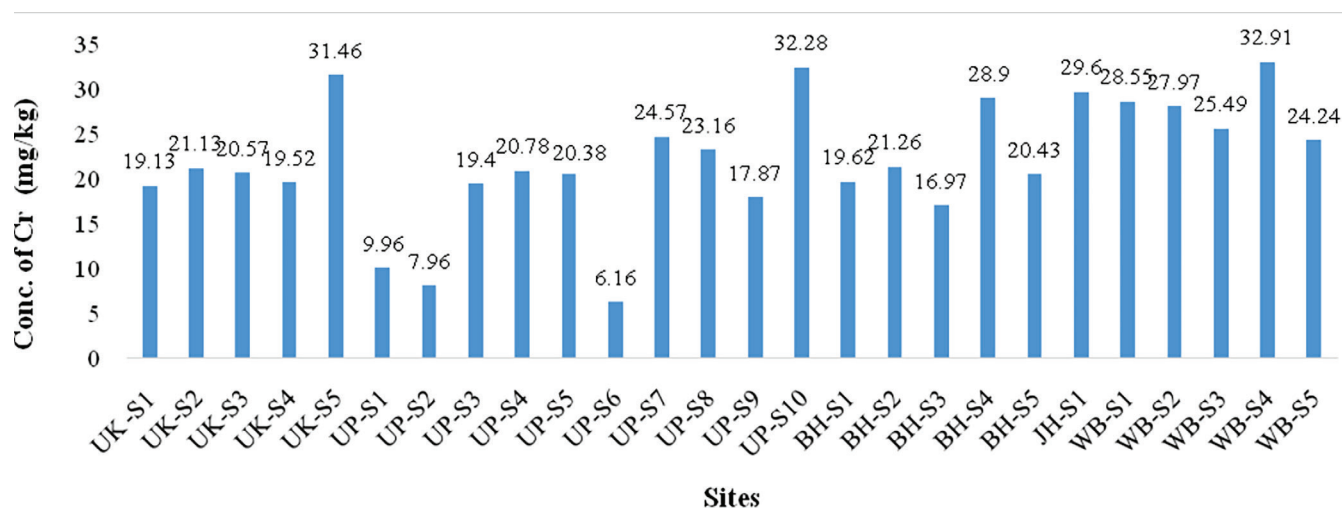


Fig. 10a Concentration of chromium at different sites

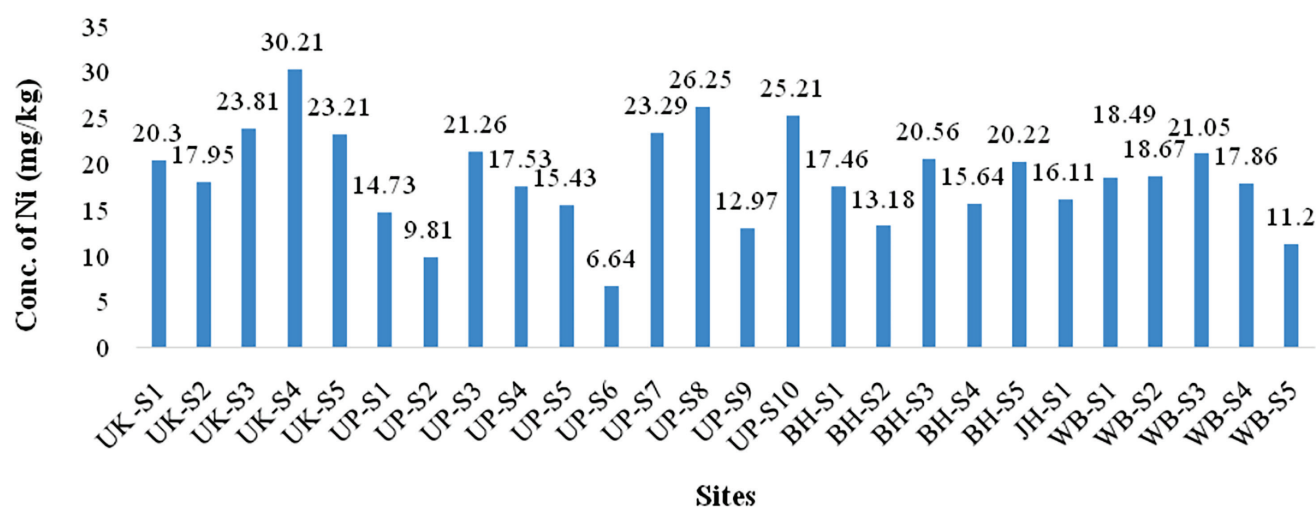


Fig. 10b Concentration of nickel at different sites



Fig. 10c Concentration of arsenic at different sites

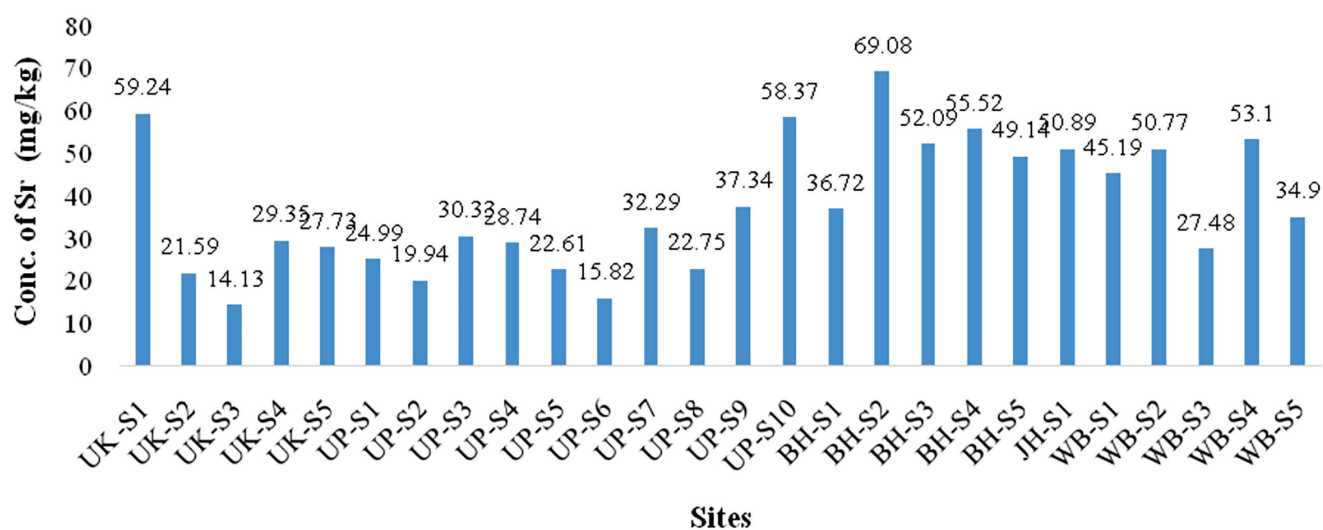


Fig. 10d Concentration of strontium at different sites

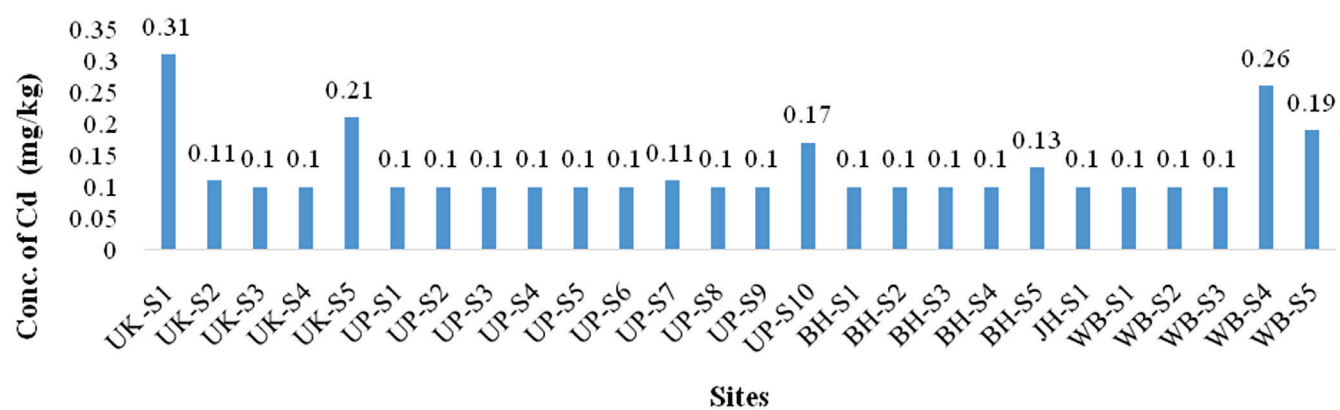


Fig. 10e Concentration of cadmium at different sites

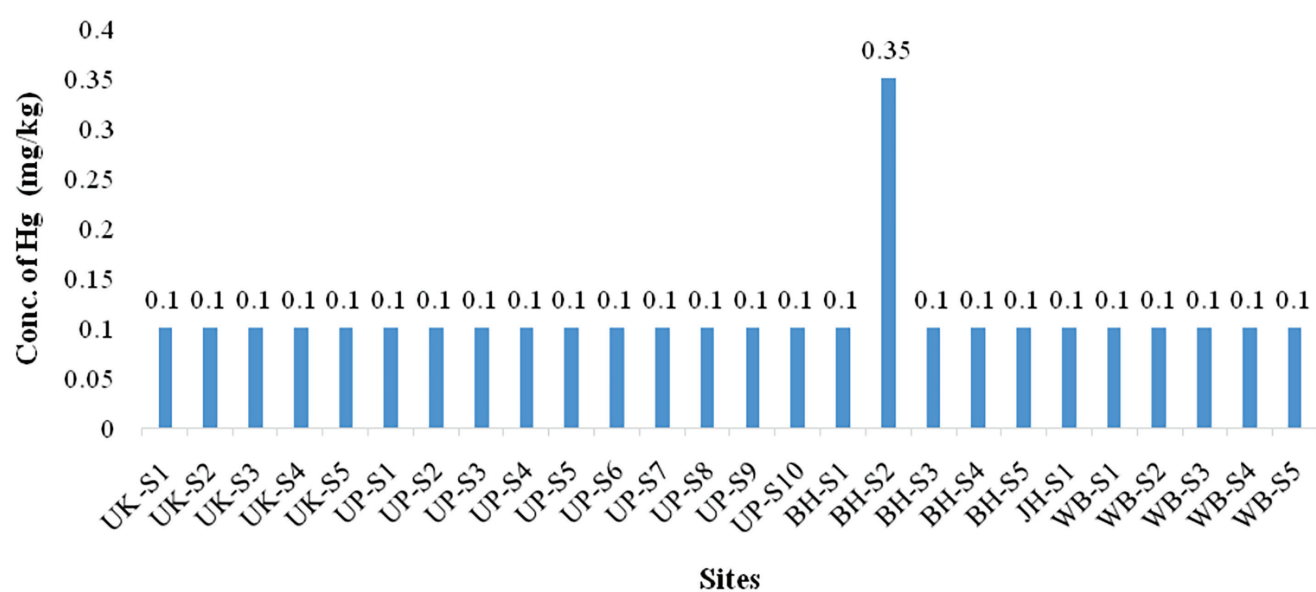


Fig. 10f Concentration of mercury at different sites

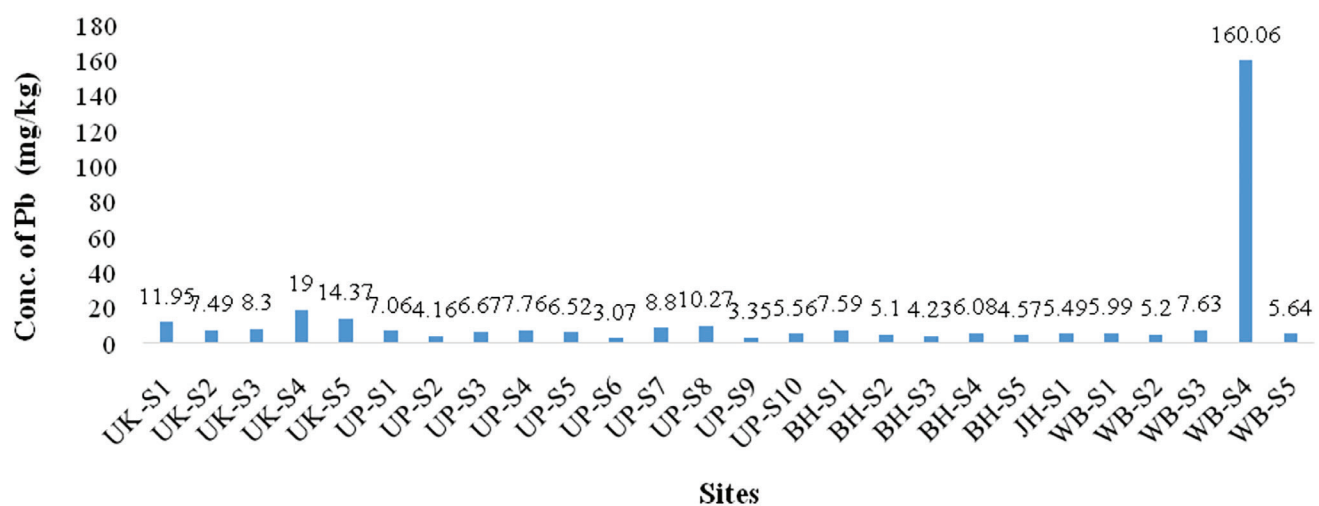


Fig. 10g Concentration of lead at different sites

Micronutrients are elements which are required by plants in very small quantities. They mainly act as co-factor of enzymes. When available to plants in the right quantity, they help in proper metabolism and bring about optimal plant growth and yield. The

concentration of the four micronutrients Zn, Fe, Mn and Cu were determined using atomic absorption spectrophotometer. The values obtained for all the sites are represented in Fig. 11 a, b, c and d.

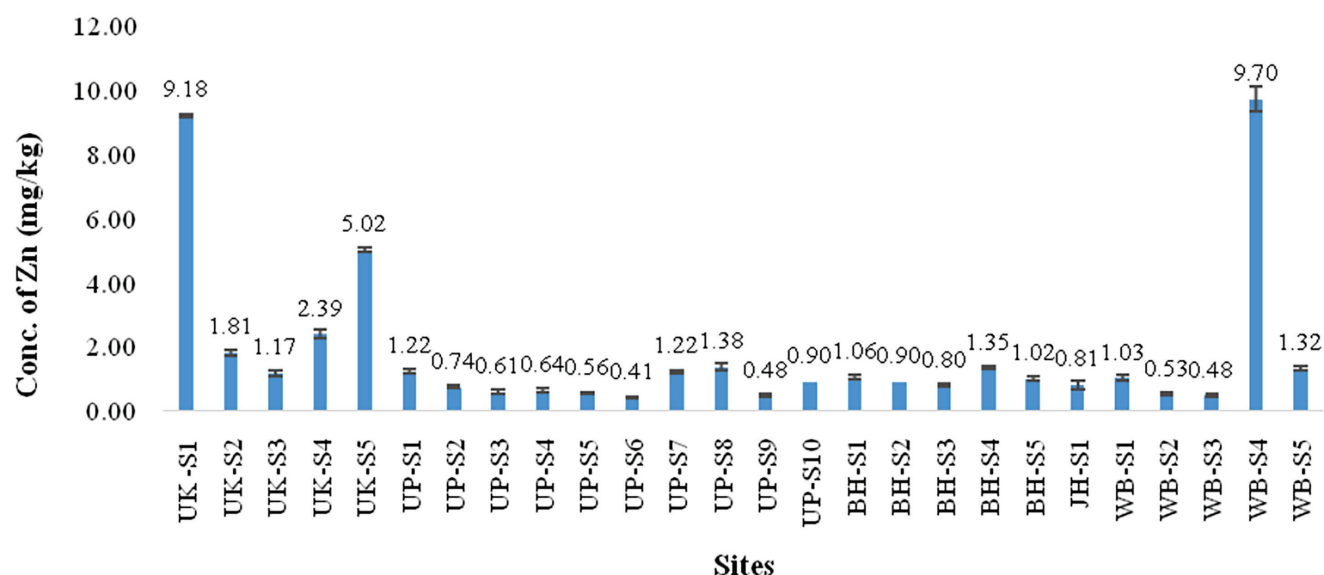


Fig. 11a Concentration of zinc at different sites

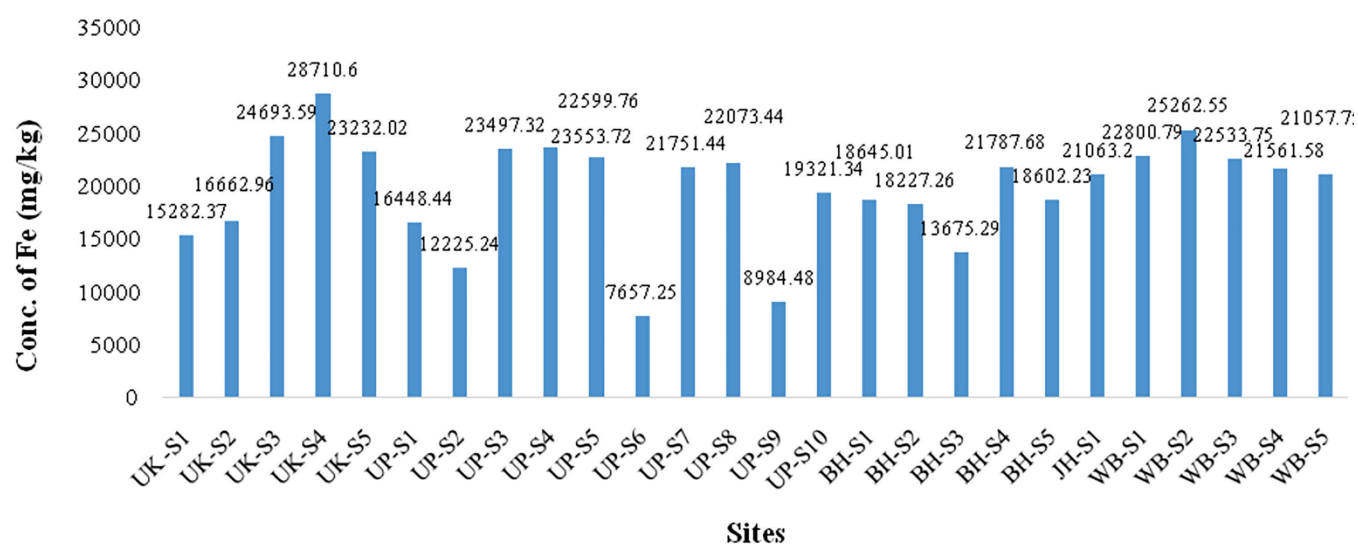


Fig. 11b Concentration of iron at different sites

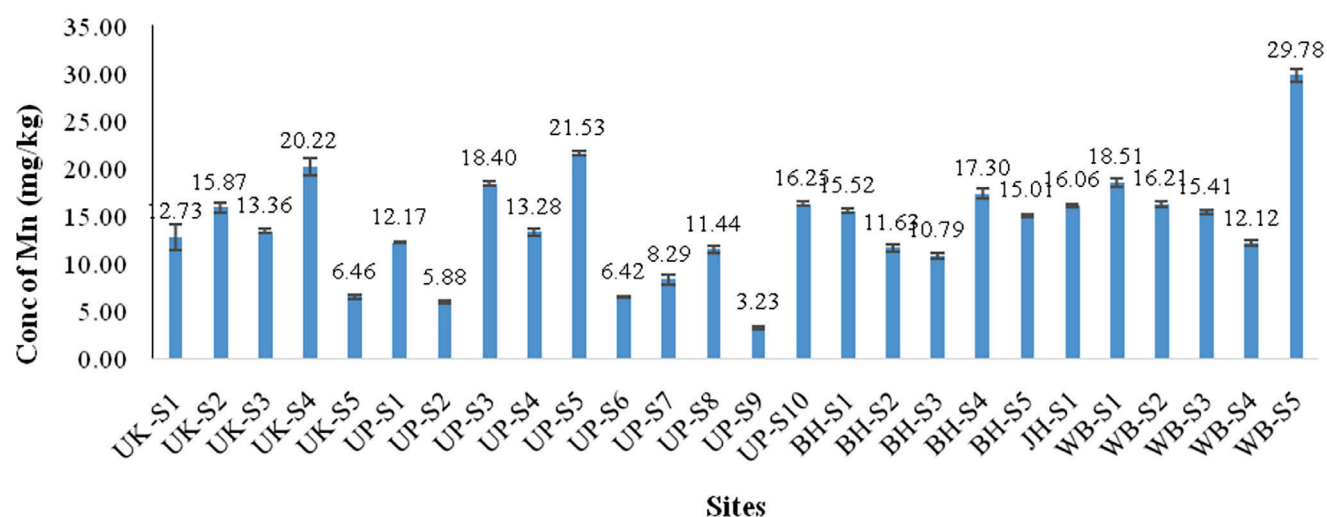


Fig. 11c Concentration of manganese at different sites

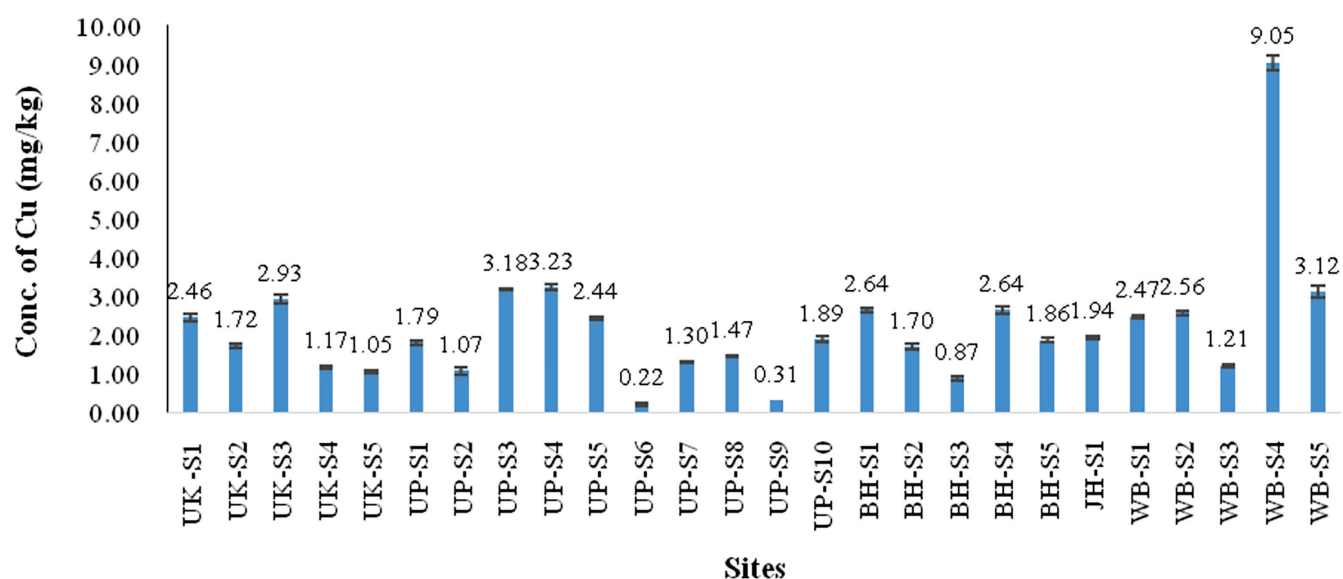


Fig. 11d Concentration of copper at different sites

As per the requirement of micronutrients by plants, there are different prescribed limits. The minimum amount of a particular micronutrient to be present in the soil is considered as the threshold. Deficient soils are those with concentrations below them, whereas sufficient soils have concentrations above them.

Though micronutrients are required by plants in very small quantities, they are

important for proper metabolic functioning. Soils where micronutrients are present in insufficient quantities will result in plant having inadequate metabolism and hence, less of phytochemical content too. Thus, cultivation of medicinal plants must be done in soils with proper nutritional content (Katyal et al., 1991).

Total and Differential Bacterial Count from Rhizosphere

Rhizosphere microbial testing involves analysing the microbial communities in the

soil, rhizosphere, and roots of plants. The rhizosphere microbial test is performed in different media to calculate the bacterial count for the *Cynodon dactylon* (L.) Pers. in different locations shown in Fig. 12

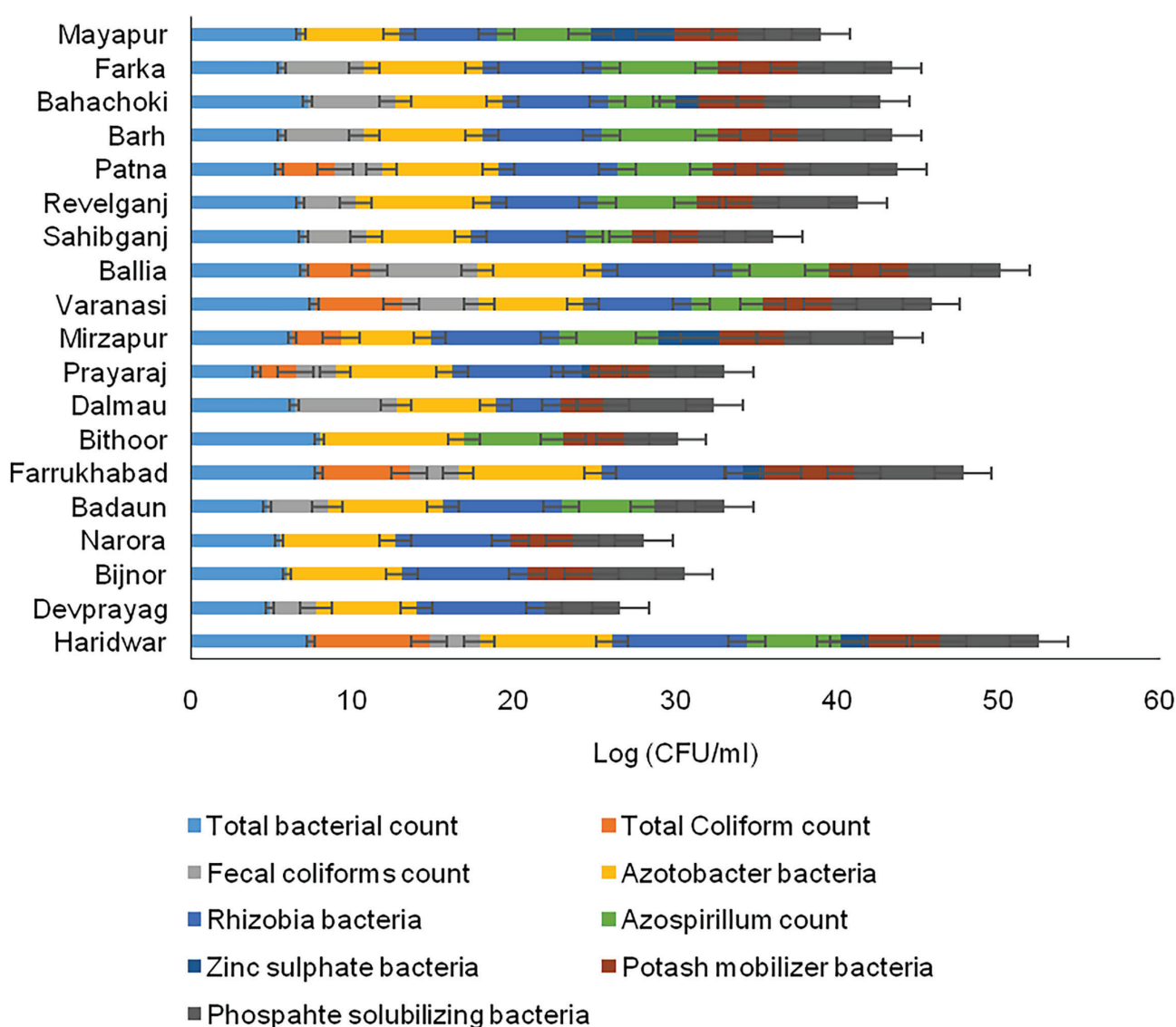


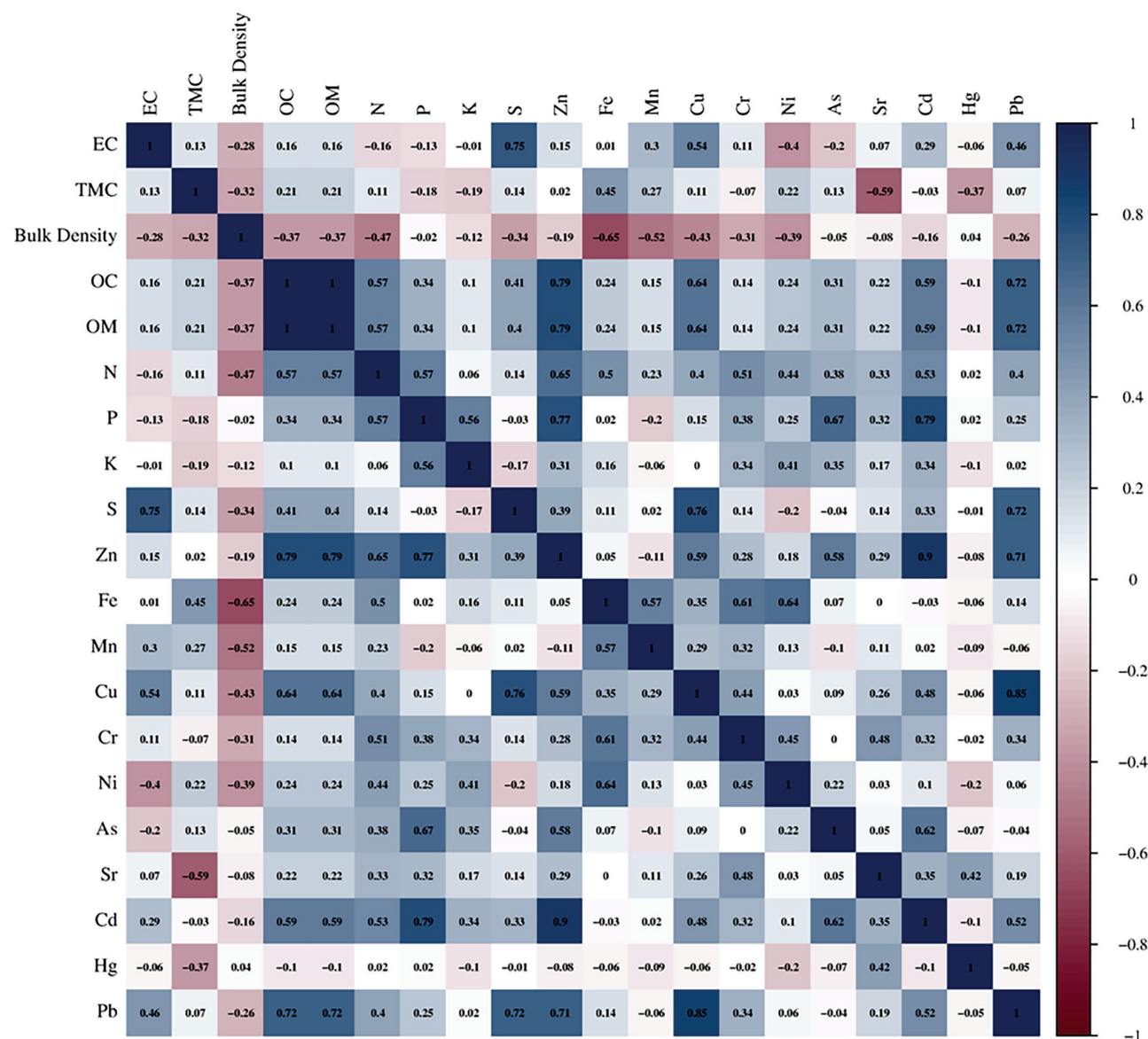
Fig. 12 Total and differential bacterial count

The total rhizosphere microbial test is performed for the identification of total bacterial count which is found highest in Bithoor 7.9192 cfu/ml and lowest in Prayaraj 4.0107 cfu/ml. Whereas the total faecal and

coliform count is highest in Haridwar and Ballia recorded as 7.3329 and 6.6789 cfu/ml. The results of correlation studies have been represented as a matrix in Table 4.

Correlation Coefficient Matrix

Table 4 Correlation coefficient matrix



The correlation matrix in Table 6 clearly shows the micronutrient status at different sites and the correlation matrix in Table 6 clearly shows the micronutrient status at different sites and according to the correlation coefficient values of one parameter against the other, the values have been colour coded. The colour coding key also shown above shows the colour codes

as per the range of the correlation coefficient value. According to correlation coefficient values of each parameter against the other, few observations can be made. These are relevant for every parameter from every site because the values were determined using information gathered for every site for every parameter.

- pH showed weak negative correlation with total moisture content, potassium, sulphur, and lead while it showed moderate positive correlation with electrical conductivity.
- A high degree of strong positive correlation between electrical conductivity and sulphur was observed, while moderate positive correlation was seen between the former and copper and lead.
- Total moisture content showed moderate positive correlation with iron; furthermore, bulk density showed weak negative correlation with mercury.
- Organic carbon showed negative correlation with mercury, moderate positive correlation with nitrogen, phosphorus, sulphur and cadmium and strong positive correlation with zinc, copper, and lead.
- Nitrogen showed a very strong positive correlation with zinc. Phosphorus showed the same with zinc, arsenic, and cadmium. Potassium had moderate positive correlation with zinc, chromium, nickel, arsenic, and cadmium.
- A strong positive correlation was seen between sulphur, copper, and cadmium. The same was also observed between zinc, cadmium, and lead. Iron, chromium, and nickel. Manganese and chromium shared moderate positive correlation between each other.
- Strong positive correlation was seen between copper and lead. Chromium, arsenic, and mercury had negative correlation amongst themselves. The same was also observed between nickel and mercury. A strong positive correlation was seen between arsenic and cadmium.
- Strontium showed moderate positive correlation between cadmium and mercury. The same was also seen between cadmium and lead. Mercury on the other hand showed negative correlation with lead.

Conclusion

From the physicochemical and microbial analysis of soil, it was found that the parameters were different at each site. If the soil of each site is characterized as per the availability of nutrients and bacteria

according to the recommendations, then the best soil as per the area can be determined. The Table 5 shows the conclusive results of all the results obtained for different parameters from all the sites.

Table 5 Conclusive results for different parameters from all sites

Sites	pH	EC	OC	N	P	K	S	Zn	Fe	Mn	Cu	Cr	Ni	As	Sr	Cd	Hg	Pb
UK -S1	AB	WR	AR	BR	Very AR	AR	WR	WR	WR	WR	WR	WR	WR	AB	WR	WR	WR	WR
UK-S2	BR	WR	WR	BR	AR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UK-S3	WR	WR	BR	BR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UK-S4	BR	WR	AR	BR	WR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UK-S5	BR	WR	BR	BR	Very AR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S1	WR	WR	WR	BR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S2	WR	WR	AR	BR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S3	AB	WR	AR	BR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S4	WR	WR	BR	BR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S5	AB	WR	AR	BR	BR	BR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S6	AB	WR	BR	BR	WR	BR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S7	AB	WR	WR	BR	Very AR	AR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S8	AB	WR	WR	BR	Very AR	AR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S9	AB	WR	BR	BR	WR	BR	BR	BR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S10	AB	WR	BR	BR	Very AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR

Sites	pH	EC	OC	N	P	K	S	Zn	Fe	Mn	Cu	Cr	Ni	As	Sr	Cd	Hg	Pb
BH-S1	WR	WR	WR	BR	Very AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
BH-S2	WR	WR	WR	BR	AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	AB	WR
BH-S3	WR	WR	WR	BR	AR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
BH-S4	WR	WR	AR	BR	AR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
BH-S5	AB	WR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
JH-S1	AB	WR	BR	BR	Very AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
WB-S1	WR	WR	WR	BR	AR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
WB-S2	AB	WR	WR	BR	AR	WR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
WB-S3	AB	WR	WR	BR	WR	WR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
WB-S4	AB	CSSC	AR	BR	Very AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
WB-S5	AB	CSSC	BR	BR	AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR

The given parameter range is expressed in CSSC (Critical for Salt-Sensitive Crops): This range is vital for salt-sensitive crops. WR (Within Range): The parameter falls within the acceptable range. AR (Above Range): The parameter exceeds the recommended range. BR (Below Range): The parameter is below the recommended range. These parameter ranges are associated with the studies conducted by the Katyal et al., 1991, Hossain et al., 2022, Bowen, 2016, Varley, 1972; Jackson, 1973, Chopra and Kanwar, 1976; Richards, 1954, Jackson, 1973; Walkley and Black, 1934, Subbaiah, 1956, Bray and Kurtz, 1945; Black, 1965, Black, 1965; Merwin and Peech, 1951; Jackson, 1973, Lisle et al., 1994 and Retka et al., 2010.

The comprehensive investigation can also provide a better understanding of how soil quality factors, environmental fluctuations, and altitudinal variations affect the phytochemical composition of medicinal plants.

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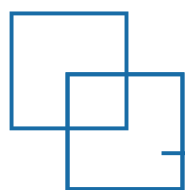
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CHAPTER

04

Traditional and Ethnomedicinal Applications



INTRODUCTION

Medicinal plants contain a wealth of potential pharmaceuticals and offer safer, more wholesome substitutes for synthetic drugs (Rai et al., 2007). Plants and natural products are the basis of both traditional and modern medicine. These materials are also frequently used in the production of pharmaceuticals intended for commercial use. Reputable, scientific research indicates that about 25% of medications prescribed worldwide are made from herbs (Galor et al., 2011, Rengasamy et al., 2019). As documented in the literature, the World Health Organization estimates that approximately 80% of the world's population, particularly in Latin America, Africa, Asia, and the Middle East, depends on herbs for their primary healthcare needs (Rahman et al., 2014). India has an abundance of biodiversity, including the traditional healers' indigenous knowledge. It has a long history and a wealth of empirical knowledge regarding the application of medicinal plants to treat a wide range of illnesses. Research on the chemical properties of Indian medicinal plants provides valuable information for the identification and creation of novel natural medicine products (Krishnaraju et al., 2005). Plants generate a wide variety of bioactive compounds, which makes them an abundant source of various medications. The resurgence of interest in medicinal plants across the globe is indicative of the acceptance of numerous conventional assertions about the benefits of natural products in healthcare (Azmathullah et al., 2011). Nearly 95% of the traditional Unani, Ayurvedic, homeopathic, and Siddha prescriptions in India were plant-based (Savithramma et al., 2011).

A genus of perennial plants known by the name *Cynodon* Rich. (Poaceae) is widely adapted to a variety of environments and is typical of tropical and subtropical regions. Due to its high morphological variability, it can be used in a wide range of global regions for a variety of purposes, including gardens, golf courses, pasture, and stored fodder (Wu et al., 2011; Jewell et al., 2013). *Cynodon dactylon* (L.) Pres., a member of the Gramineae family, is one of the significant species of this genus. Bermuda grass is the common name for this plant, and 'Doorva' is its Hindi name (Kaur et al., 2012). This resilient perennial grass is among the most frequently found weeds in India. It is frequently referred to as



'harialil', 'doob', or 'dhub' (Mane and Dangare, 2020). Although it grows everywhere, its native climates are mild temperate and tropical, with the lower Himalayas being its highest point at up to 1500 meters (Auddy et al., 2003; Bethel et al., 2006). It is native to Australia, Asia, South and East Africa, and Asia (Jarald et al., 2008). Most soils used to grow *C. dactylon* are lateritic, nutrient-poor, and have a favourable iron oxide content. Sedimentary soils are found adjacent to streams and rivers. Flavonoids, alkaloids, glycosides, terpenoids, triterpenoids, steroids, saponins, tannins, resins, phytosterols, reducing sugars, carbohydrates, proteins, volatile oils, and fixed oils are all found in *C. dactylon*, according to the phytochemical analysis (Al-Snafi et al., 2016). There are also numerous pharmacological properties of this herb including anti-inflammatory, antipyretic, anti-ulcer, antioxidant, anti-tumor, anti-diabetic, antihyperlipidemic, anti-cancer, anti-microbial, anti-hypertensive, anti-bacterial, and anticonvulsant (Parihar et al., 2021). In traditional ayurvedic aspects, this herb is used as a folk remedy for the management of diarrhea, bronchitis, hemorrhages, urogenital disorders, cancer, convulsions, hemorrhoids, hypertension, asthma, tumors, inflammations, etc. (Saikia et al., 2006; Rajakumar et al., 2009).

Traditional and Ayurvedic Benefits

Plants have long been used as a food source and source of medicine by humans. All plant species produce a range of secondary metabolites or bioactive compounds. Humans use this property of plants to make herbal remedies, flavourings, and recreational drugs. Herbal remedies are more cost-effective, less hazardous, and more efficient (Sridhar et al., 2016).

'Durva', the Sanskrit term for 'Doob', signifies "cut or eaten by the animal." The plants that are sacred to Visnu, Ganesha, and Lord Sankara are 'tulsi', 'vilva', and 'durva'; these plants relieve 'vata', 'pitta', and 'kapha doshas', respectively. Hindus have its religious significance of its leaves as a devotion to the God Ganesha. Also, as cited in Dhanvantari, Kaiyadeva, and Raja Nighantus,

this plant is known for its cooling, hemostatic, diuretic, and tonic qualities. There are two varieties of 'durva' mentioned in Ayurvedic texts: white and green. The herb *Cynodon* is described as warming, aromatic, bitter, pungent, appetizer, vulnerary, anthelmintic, antipyretic, and alexiteric in Ayurveda. It eliminates bad breath and is beneficial for leucoderma, bronchitis, piles, asthma, tumors, and splenic enlargement. It is used in homeopathic medical systems to treat all kinds of bleeding and skin conditions (Ghosh et al., 1998). Among the folk remedies for diarrhea, bronchitis, anasarca, calculus, dropsy, hemorrhage, carbuncles, cramps, cystitis, dysentery, epilepsy, hemorrhoids, hypertension, hysteria, measles, rubella, snakebite, warts,

wounds, eye disorders, weak vision, and dandruff are among the conditions for which *C. dactylon* is used. Additionally, it helps to relieve children's gripe, toothaches, and inflammations (Saikia et al., 2006; Rajakumar et al., 2009). The plant was traditionally used to treat wounds, dysentery, and hyperplasia. Its fresh juice was used as an astringent, and demulcent as well

as used to treat chronic catarrhal ophthalmia. It can also be applied topically to cuts and wounds. Likewise, root decoctions were used to treat vesical calculus, secondary syphilis, pile bleeding, and urinary tract irritation (Warrier et al., 1994; Auddy et al., 2003), some of which are mentioned in Table 1.

Table 1 Some important preparations used against various diseases in ancient Ayurveda

S. No.	Diseases/ Conditions	Mode of Administration/Uses	References
1.	Nasal disorders	Harītakīdāḍīmapuḍpadūrvālākḍārasonasyavidhānayo-gāt.Nivārayatyevacirapraḍṭtamathāḍunāsān-taraśoḍitaugham- instillation of 2-3 nasal drops prepared from <i>Terminalia chebula</i> , <i>Punica granatum</i> flower, <i>C. dactylon</i> juice and <i>Lākṣā</i> juice, used against epistaxis.	Mishra, 2009
2.	Abdominal disorders	Pittacchadirvrajeddūrvātaḍḍulodakapānataḍ.Pibecharditrayeśītaḍ, dūrvāvātaḍḍulāmbunā- juice of <i>C. dactylon</i> along with rice water, against vomiting.	Tripathi, 2012a; Tripathi, 2012b
3.	Anorectal disorders	Dūrvāghḍtapradehaḍ.....Vyajanapavanaḍsuśītor-aktasrāvaḍjayatyāḍu- <i>Ghrita</i> (Clarified butter) processed with juice of <i>C. dactylon</i> or topical application of <i>C. dactylon</i> juice, mixed with <i>Ghrita</i> (Clarified butter), useful in hemorrhages caused due to hemorrhoids.	Śāstrī et al., 2011
4.	Reproductive system disorders	Saraktapradarāvā'pisāsaḍksrāvā ca garbhiḍī. Dūrvāyāḍpiḍḍakaḍprāḍyanāsaḍksrāveḍapīḍyate- rice pounded with <i>C. dactylon</i> juice, useful against hemorrhages and menorrhagia.	Tripathi, 2010
5.	Dermatological disorders	Dūrvāsvarasasiddhaḍ ca ghḍtaḍsyādvraḍa-ropaḍam- <i>Ghrita</i> (Clarified butter) processed with <i>C. dactylon</i> juice, useful in erysipelas; Svarasena hi dūrvāyāḍpacettaḍcaturguḍam. Kacchūvicar-cikāpāmāabhyaḍgādevanāśayet- massage with oil processed from <i>C. dactylon</i> juice, useful against pruritus, eczema, and cholera; Dūrvāniḍāyutolepaḍkacchūpāmāvināśanaḍ. Kḍmidadrūharaḍcaivaśītapit-taharaḍsmḍtaḍ- topical application of <i>C. dactylon</i> and <i>Curcuma longa</i> (Turmeric) paste, useful in pruritus, eczema, worm infestation, ringworm and	

S. No.	Diseases/ Conditions	Mode of Administration/Uses	References
		urticarial; Dūrvāsvarasasiddhaḍvātailaḍ pradhānaḍvraḍaropaḍam - topical application of <i>Sesamum indicum</i> oil processed with <i>C. dactylon</i> paste and juice, enhances wound healing; Visarpakagranthiviḍādirogānlepādinātailaghḍtāpahārāt. Vināśayeddugdhayutā''ḍuśubhrādūrvāmasūrānapipittajātā - topical application of paste prepared from <i>C. dactylon</i> , <i>Terminalia chebula</i> , rock salt, <i>Cassia tora</i> seed, <i>Ocimum basilicum</i> leaf pounded with buttermilk, useful to cure pruritus and ringworm; Dūrvā'bhayāsaindhavaḍ ca cakramardaḍkaḍherakaḍ. Ebhistakrayutolepaḍkaḍḍūdadrūvināśanaḍ - topical application of paste prepared from an equal quantity of <i>C. dactylon</i> and <i>Berberis aristata</i>	Śāstrī et al., 2011; Tiwari et al., 2007a; Tiwari et al., 2007b; Tiwari et al., 2007c; Sharma, 2012; Tripathi et al., 2013a; Tripathi et al., 2013b; Mishra, 2009
		pounded with buttermilk, useful in pruritus, eczema, worm infestation, ringworm and urticaria; Dūrvāniḍāyutolepaḍkaḍḍūpāmāvināśanaḍ. Kḍmi-dadruharāḍcaivaśītapittāpahāḍsmḍtaḍ - topical application of a paste prepared from arsenic trisulphide, <i>C. dactylon</i> , rock salt pounded with cow urine, useful against chronic ringworm and pruritus; Gomūtrapiḍḍairharitāladūrvāsindhūdbhavairlepitamādareḍa. Prayātināśaḍniyataḍnarāḍḍadruḍcapāmāsuciraprardhā - topical application of a paste prepared from <i>C. dactylon</i> , mixed with oil and <i>Ghrita</i> , used in erysipelas, smallpox and cyst or intake of <i>C. dactylon</i> (Devil grass) with milk, useful in <i>Pitta</i> associated smallpox.	
6.	Generalized body disorders	 nasyaḍ dūrvāsvarasasyacaiva - arrhine of <i>C. dactylon</i> juice, useful in <i>Raktapitta</i> ; Lihyāccadūrvāvaḍa-jāḍḍcapallavānmadhudvitīyān <i>C. dactylon</i> and <i>Ficus benghalensis</i> leaf juice or paste, mixed with honey, useful in <i>Raktapitta</i> .	Śāstrī et al., 2011; Sharma et al., 2012

Therapeutic Potential

In the field of human health care, medicinal plants are essential. Approximately, 8% of people on the planet are dependent on traditional medicine, which is mostly made of plant materials (Surendra et al., 2008). Numerous medicinal plants have been demonstrated to have positive therapeutic potential, as have their purified constituents. Interest in the medicinal properties of medicinal plants as an antioxidant to lessen tissue damage caused by free radicals has increased. In addition to the widely recognized and conventionally utilized

natural antioxidants found in tea, wine, fruits, vegetables, and spices, these can be purchased commercially as dietary supplements or as antioxidant additives (Proestos et al., 2006). There are several medicinal benefits associated with the perennial grass *C. dactylon* (L.) Pers. It is grown in every tropical and subtropical region. For medicinal purposes, the entire plant as well as its root stalk is used (Singh et al., 2009). There are several therapeutic values of *C. dactylon* along with their preparations, depicted in Table 2.

Table 2. Medicinal importance along with their preparation and mode of administration of *C. dactylon* (L.) Pers.

S. No.	Parts Used	Preparations	Diseases Treated and Therapeutic Uses	References
1.	Plant	-	Anasarca, calculus, cancer, carbuncles, convulsions, cough, cramps, cystitis, diarrhea, dysentery, epilepsy, hemorrhoids, leucoderma, headache, hemorrhage, hypertension, bronchitis, asthma, tumors, measles, snakebites, sores, stones, urogenital disorders, warts, wounds, eye disorders, pain, inflammation, toothache, gripe in children, appetizer, antipyretic, alexiteric, laxative, expectorant, carminative, brain and heart tonic, bleeding, skin trouble, antiseptic, cough, cramps, astringent, aperient (constipation), demulcent, emollient, vulnerary, hysteria, diuretic, anthelminthic, psoriasis, herpes, allergies, rashes, itching, menorrhagia, irregular menstrual cycle, abortion, laxative, emetic, carminative, coolant, expectorant, emmenagogue, inflammation, hyperdipsia, dandruff, fever, measles, leukoderma, to stop bleeding from wounds	Nagori et al., 2011; Hossain et al., 2018; Sharif et al., 2022; Qureshi et al., 2010; Reshu et al., 2018; Malik et al., 2019; Rajakumar et al., 2009; Saikia et al., 2006; Shendye et al., 2014; Phondani et al., 2010; Das et al., 2009; Kumar et al., 2021; Semwal et al., 2020; Tewari et al., 2014; Dangwal et al., 2022; Uniyal et al., 2011

S. No.	Parts Used	Preparations	Diseases Treated and Therapeutic Uses	References
		Mixed with powder of <i>Syzygium aromaticum</i>	Anthelmintic	
		3-4 spoons juice mixed with a bit of 'sonth'	Stomach pain and infection	
		Grind with water	Wound healing, skin problems	
		Mixed with starch of rice	Vomiting	
		Juice	Astringent, bleeding cuts, wounds, menstrual over-bleeding, nose bleeding, fever, burning sensation, catarrhal ophthalmic problems,	
		Juice with milk	Epilepsy and as a memory enhancer	
		Decoction	Diuretic, syphilis, dropsy, vesical calculus, stop bleeding of piles, treat urinary irritations	
		3-4 spoons juice taken in an empty stomach	Acidity, ulcer, colitis, stomach infections	
		Mixed with black pepper	Ascites	
		Juice mixed with potash alum	Mouth ulcer	
		Extract	Dysentery, nose bleeding, and anemia	
		Juice mixed with honey	Menorrhagia	
		Paste mixed with honey	Epistaxis	

S. No.	Parts Used	Preparations	Diseases Treated and Therapeutic Uses	References
		Paste	Bleeding in vagina, headache, cuts, wounds, stress	
		Mixed with sugar	Urine retention	
2.	Leaves	-	Dropsy, piles, throat problem, wounds, to boost immunity, diabetes, burning sensation, dysentery, vomiting, poisonous bites	Devi et al., 2015; Upadhyay et al., 2023; Shendye et al., 2014; Tewari et al., 2014; Chauhan et al., 2022; Shukla et al., 2012; Biswakarma et al., 2015; Pala et al., 2019; Singh, et al., 2022; Nagori et al., 2011
		Juice	Blood purification, diabetes	
		Crushed	Wounds, to stop bleeding, anti-inflammatory	
		Paste along with oil of <i>Jatropha curcas</i>	Cracked heel pain	
		Paste	Cuts and wounds, bleeding, vomiting, indigestion, piles, asthma	
3.	Roots	-	Fever, injuries	Sharif et al., 2022; Shendye et al., 2014; Dobhal et al., 2007; Chauhan et al., 2009; Johnsy et al., 2013
		Decoction	Diuretic, dropsy, secondary syphilis	

S. No.	Parts Used	Preparations	Diseases Treated and Therapeutic Uses	References
		Infusion	To stop bleeding from piles, sarsaparilla, diarrhea, dysentery	
		Juice	Fresh cuts and wounds, demulcent, astringent, dropsy, catarrhal ophthalmia, secondary syphilis, chronic dysentery	
		Paste	Headache, toothache, to reduce bleeding in vagina	
		Paste mixed with honey	Epitaxis	
		Juice mixed with honey	Menorrhagia	
		Decoction mixed with honey	Urolithiasis	
		Decoction mixed with sugar	Urine retention	
4.	Leaves, root, flowers and seeds	-	Cancer, convulsions, cough, cramps, stones, dysentery, epilepsy, headache, hypertension, urogenital disorders, warts, wounds	Nagori et al., 2011; DAS et al., 2013
5.	Rhizomes	-	Diuretic, diaphoretic, anti-rheumatism, anti-pustules, hypoglycemic	Singh et al., 2008
6.	Root, Leaves	-	Piles, fistula, fissure, chronic gleet	Upadhyay et al., 2023
7.	Aerial parts	Paste	Headache, cuts, wounds, skin diseases	Singh et al., 2017

(-) Not mentioned

Ethnomedicinal and Folk treatments along the Ganga Basin of India

Over 18% of the Indian subcontinent is covered by the Himalayas, a dynamic region that is home to about 8000 species of angiosperms, 1748 of which are used medicinally (Malik et al., 2015). Since ancient times, the area has been well-known for its abundant ethnomedicinal flora (Basant et al., 2009). Because of necessity, observation, the experience of elder ethnic people, and trial and error, ethnic people derive economic values and primary healthcare benefits from the plants in their environment (Kunwar et al., 2016). Around 65% of Indians receive their medical care through traditional means (Timmermans et al., 2003).

In the hilly state of Uttarakhand, there are low-lying plains, high-altitude glaciers, and lush forests with rivers that flow year-round. Amazingly diverse, both the flora and wildlife make up one of the country's largest reservoirs of biodiversity. Garhwal Himalaya is referred to as the "land of gods" because it is home to numerous important religious sites next to the point where five tributaries of the sacred Ganges River converge. Numerous items found in nature are revered in Hinduism. The holy sites of Badrinath, Kedarnath, Yamunotri, and Gangotri are among them, as are the Ganges River and its tributaries. Rich biodiversity and dense vegetation can be found throughout the Himalayas and its foothills (Bartwal et al., 2011). According to Mehra et al. (2014), *C.*

dactylon was traditionally used by the people of Uttarakhand as a folk remedy for several ailments, such as nasal bleeding. Decoction of the plant was given to remove calculi, as well as the roots were boiled in honey to treat urolithiasis (Chauhan et al., 2009; Khajuria et al., 2016).

Similarly, the state Uttar Pradesh, situated in the north-central part of India, has long been a thriving centre of vibrant Indian culture. This region, known as the "rainbow land," is endowed with diverse geographical features and a multitude of cultural diversities. One particular area of interest is Eastern Uttar Pradesh, which exhibits unique characteristics that span from 23°510 N to 28°300 N and 81°310 E to 84°390 E, falling within the boundaries of Uttar Pradesh (Forster et al., 2014; Dubey et al., 2020). In this state, people use a wide range of plants as medicines for the treatment of skin and eye problems, piles, and other bleeding diseases (Singh et al., 2023). The inhabitants of Sonbhadra district employ this herb, together with *Zingiber officinalae* and *Foeniculum vulgare*, to eradicate diarrhea (Kant et al., 2021).

The other state with a land area of 7.97 million hectares and a population of over 2,69,09,428 people, Jharkhand is a state located in eastern India. Approximately 28% of all people are scheduled tribes (Bhatt et al., 2002). To maintain a reasonable level of

health, over 70% of the state's population exclusively relies on herbs and traditional healers (Tomar et al., 2002). People in Jharkhand employ *C. dactylon* for a variety of conditions, including epilepsy, paralysis, and jaundice (Tomar et al., 2012). It is also used to treat eye conditions like conjunctivitis, redness, edema, pain, and inflammation (Singh et al., 2008).

Moreover, Bihar, India's third-largest state by population, is located between latitudes 27°-31'-15" N and longitudes 88°-17'-40" E. Of the 9359.57 thousand hectares that make up Bihar's total area, 14.2% are islands, 6.6% are forests, and 1.37% are used for agriculture. The indigenous people still use their traditional knowledge of medicinal herbs to treat wounds and illnesses. Different types of medicinal plants are grown in different parts of the Saran district in Bihar to treat different kinds of illnesses. One of the reasons behind the decline in medicinal plants in the Saran district is climate change. People of Bihar use a wide variety of plants as traditional medicines. The leaves of *C. dactylon* were used medicinally to treat a variety of conditions, including skin diseases and bleeding (Singh et al., 2018).

Due to its significantly varied climate, which stretches from the coastal regions to the Himalayas, West Bengal is home to a wide variety of aromatic and medicinal plants that are used throughout the Indian

medical system (Mukherjee et al., 2018). Changing socioeconomic conditions and developmental activities have an impact on traditional knowledge. Thus, it is critical to maintain these knowledge traditions, particularly for indigenous populations that still rely on their traditional medical system for conditions related to the liver, gynecology, and dermatology (Biswakarma et al., 2017; Vineeta et al., 2018). According to reports, the most common medical conditions among the indigenous communities in West Bengal are cuts and wounds, bites and stings, stomach-related issues, dermatological issues, liver-related issues, and gynecological issues. These conditions are typically treated with ethnomedicinal plant-based therapies (Shukla et al., 2022). Their tribes use a vast range of plants for medicinal purposes such as using a paste of the leaves to stop bleeding (Mandal et al., 2020). In cases of hysteria, epilepsy, insanity, and genito-urinary disorders, its juice is used as an astringent, against dropsy, and anasarca. Its roots are used as a diuretic to stop bleeding. Young stems are used as a headache treatment, and the leaves and stems are used to treat epilepsy and ear pain (Annon et al., 1950). Moreover, *C. dactylon* leaf extract and *Jatropha curcas* leaf sap are combined to treat wounds, hematuria, cuts, wounds, and bleeding, respectively (Pattanayak et al., 2012; Mandal et al., 2020).

Conclusion and Future Perspectives

Ever since the dawn of human civilization, medicinal plants have been a major source of information to combat illness. *C. dactylon* is a plant that has been shown to have a wide range of potential medicinal properties. It has been widely used in Indian ayurvedic medicine since ancient times to treat a variety of illnesses in people. It has a wide range of therapeutic and medical uses, but these can only be realized after thorough safety testing, extensive pharmacological activity, mechanism, and bioactivity research. However, focused

research is ongoing, and after more extensive studies on pharmacological and biological activities, it would be easier to develop new medications. Numerous investigations proved that *C. dactylon* is a naturally occurring crude drug with various pharmacological and biological uses. As a result, it is projected that it will be utilized as a novel medication shortly to treat various illnesses, including wound healing, ulcerative colitis, diabetes, cancer, and infections.

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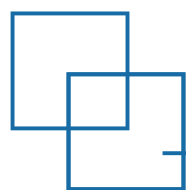
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CHAPTER

05

Phytochemical Analysis



INTRODUCTION

Cynodon dactylon, commonly known as “bermuda grass” or *durva*, is a versatile plant with significant medicinal and traditional uses. Geographically, it thrives in various regions worldwide, including tropical and subtropical areas. This perennial grass, belonging to the Poaceae family, is characterized by its creeping nature and resilience in diverse ecological conditions, making it a common sight in fields, open areas, parks, and along road shoulders [Zhang et al., 2019].

Phytochemical analysis of *Cynodon dactylon* has revealed a rich composition of bioactive compounds such as flavonoids, alkaloids, glycosides, terpenoids, saponins, tannins, and more. These compounds contribute to the plant's pharmacological properties, including diuretic, antidiabetic, antibacterial, antimicrobial, antioxidant, and wound-healing activities. Traditional medicine extensively uses the species for various purposes, such as treating biliousness, itching, diarrhea, gonorrhea, conjunctivitis, and stomachaches. Additionally, it is valued for its cardioprotective properties and blood-purifying effects [Mozafari et al., 2018].

The plant's traditional usage spans different medicinal systems like Ayurveda, Unani, Nepalese, and Chinese medicine, highlighting its broad spectrum of therapeutic applications. Its bioactive compounds and diverse chemical composition make it a subject of interest for further research, particularly in areas like diabetes, atherosclerosis, wound healing, and potential anticancer properties. Its adaptability, coupled with its medicinal benefits and historical significance in traditional healing practices, underscores the importance of exploring its phytochemical profile and therapeutic potential for both human health and environmental benefits [Savadi et al., 2020].



Sampling Sites

Table 1 Samples of the *Cynodon dactylon* were collected from various state-specific locations

S. No.	Sampling Sites		
1.	Bijnor, Uttar Pradesh	11.	Revelganj, Bihar
2.	Narora, Uttar Pradesh	12.	Patna, Bihar
3.	Badaun, Uttar Pradesh	13.	Barh, Bihar
4.	Farrukhabad, Uttar Pradesh	14.	Bahachoki, Bihar
5.	Bithoor, Uttar Pradesh	15.	Farka, Bihar
6.	Dalmau, Uttar Pradesh	16.	Sahibganj, Jharkhand
7.	Prayagraj, Uttar Pradesh	17.	Gangasagar, West Bengal
8.	Mirzapur, Uttar Pradesh	18.	Gaumukh, Uttarakhand
9.	Varanasi, Uttar Pradesh	19.	Gangotri, Uttarakhand
10.	Ballia, Uttar Pradesh		

Phytochemical Analysis

Phytochemical components like tannin (by titration), total saponins (by gravimetry), total polyphenols, and total flavonoids (by UV-visible spectrophotometer), were determined for their respective contents via in-house protocols using API standards and literature, developed in Chemical Science Division, Drug Discovery and Development Department, Patanjali Research Foundation, Haridwar. Advanced methods and techniques were employed for the identification and quantification of secondary metabolites and active components within the collected samples. High-performance thin layer chromatography (HPTLC) served to detect marker compounds and provide a chromatographic fingerprint of the plant sample. The hyphenated techniques have been proven as a powerful tool for the identification and structural characterization of known compounds. Hence, for further identification and quantification of compounds

high-performance liquid chromatography (HPLC) and ultra performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC/MS-QToF) were employed.

Determination of Tannin Content

A 1-10 g sample was taken and mixed with 50 ml of Milli-Q water. The mixture was then shaken and sonicated for 30 minutes before being brought up to a volume of 100 ml and filtered. From this solution, 10 ml was extracted and combined with 750 ml of Milli-Q water. Next, 25 ml of indigo sulfonic acid was added, and the solution was shaken. The mixture was titrated with a 0.1N potassium permanganate solution until a golden yellow colour endpoint was reached. A blank run was conducted without a sample.

Determination of Saponin Content

A 5 g sample was taken and combined with 50 ml of a 1:1 mixture of methanol and water solvent. The mixture was then refluxed for 1 hour, cooled, and filtered. This process was repeated three times. The filtrates were combined, concentrated, and evaporated to dryness. Next, 25 ml of petroleum ether was added, and the mixture was refluxed for 10 minutes. After cooling, the ether layer was decanted. Then, 10 ml of methanol and 100 ml of acetone were added, and the mixture was filtered. The filter paper with residue was dried at 80°C for 1 hour and weighed.

Determination of Total Polyphenol Content

A 1 ml sample was placed in a test tube and mixed with 1 ml of Folin–Ciocâlteu reagent,

then incubated for 5 minutes. Following this, 1 ml of a 10% sodium carbonate solution was added. The mixture was kept in the dark for 1 hour. Absorbance was measured at 760 nm using a UV-visible spectrophotometer. The same procedure was repeated for gallic acid to establish the linearity of various concentrations of the standard.

Determination of Total Flavonoid Content

A 1 ml sample was placed in a test tube, followed by the addition of 0.4 ml of 10% aluminium chloride, 0.4 ml of sodium acetate, and 3 ml of ethanol. The mixture was then kept at room temperature for 30 minutes. Subsequently, the absorbance was measured at 450 nm using a UV-visible spectrophotometer. These steps were repeated for quercetin dihydrate to establish the linearity of different concentrations of the standard.

HPTLC Fingerprinting

The application of HPTLC in the study reflects a prevalent methodology documented in the literature for profiling secondary metabolites. The use of HPTLC not only enables the rapid separation of compounds but also aligns with the commonly reported diverse chemical fingerprints found in *Cynodon* species. This technique has been recognized for its effectiveness in exploring the qualitative aspects of secondary metabolites in various plant species. As per WHO Technical Report Series, No. 1010, [2018], HPTLC chromatographic

patterns, generally refer as “fingerprints”, are used for the identification of phytochemicals. The bands or spots obtained during the test are characteristic of a particular herb. A colour image of a typical TLC fingerprint provides a clearer guide to the users.

Sample Preparation

About 1 g of sample from each batch was dissolved individually in 10 ml of methanol. The samples were shaken and sonicated for 20 min. The solution was then centrifuged

for 5 min at 5000 rpm, and a clear solution obtained was used for analysis.

Methodology and Analytical Conditions

Analysis was performed on CAMAG HPTLC (Muttenez, Switzerland), equipped with

an Automatic TLC Sampler (ATS 4), TLC scanner 4 and TLC visualize. Data processing acquisition and visualization were achieved using win-CATS software (version 1.4.10). The chromatographic conditions for the HPTLC analysis were as follows:

Stationary phase	TLC Silica gel 60 F254 aluminium sheet (1.0554.0007)
Mobile phase	Toluene: Formic acid: Ethyl acetate (5:1:5)
Saturation time	15 minutes
Migration distance	70 mm
Band length	8 mm
Injection volume	10 µl
Visualization	254 nm and 366 nm and under white light without derivatization

High-Performance Liquid Chromatography (HPLC)

Sample and Standard Preparation

Sample preparation: Approximately 2 g of the sample were dissolved in 25 ml of methanol, sonicated for 2 hours at 60°C, and filtered through a 41-number Whatman filter paper. The extraction process was repeated once more, and all the extracts were combined to make up a total volume of 50 ml. The solution was then filtered through a 0.45 µ filter paper and used for analysis.

Standard preparation: The stock solution was prepared by dissolving 10 mg of schaftoside in 10 ml of methanol. Further 0.05 ml of stock solution was diluted to 1 ml of methanol to prepare the working standard.

Analytical and Instrumentation Condition

Analysis was conducted using the Prominence-i HPLC system manufactured by Shimadzu in Japan. The separation process was carried out utilizing a Shodex C18-4E column (5 µm, 4.6 × 250 mm) with binary gradient elution. The two solvents employed for the analysis were water containing 0.1% acetic acid (solvent A) and acetonitrile (solvent B). The column temperature was maintained at 35°C, and the flow rate was set at 1.0 ml/min throughout the analysis. A volume of 10 µl of both the standard and test solutions was injected, with the wavelength set at 270 nm.

Gradient program

Time (Min)	A%	B%
0.01	95	5
10	88	12
15	88	12
30	85	15
50	80	20
60	70	30
65	30	70
66	95	5
70	95	5

Identification of Compounds by Ultra Performance Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (UPLC/MS-QToF)

This analysis aimed to identify the major compounds in the *Cynodon dactylon* test sample (PRF/CHI/1123/1163).

Sample Preparation

500 mg powdered sample was dissolved in 10 ml methanol and sonicated for 30 min. The solution was centrifuged at 10000 rpm for 5 minutes and filtered through 0.22 µm nylon filter.

Analytical and Instrumentation Conditions

The analysis was performed using a Xevo G2-XS QToF system (Waters Corporation, USA)

equipped with an Acquity UPLC-I Class and Unifi software. A separation was carried out using an Acquity UPLC HSS-T3 column (100 x 2.1 mm, 1.8 µm) from China, with a flow rate of 0.3 mL/min. The separation used a gradient elution of 0.1% v/v formic acid in water (mobile phase A) and 0.1% v/v formic acid in acetonitrile (mobile phase B). The column temperature was maintained at 40°C throughout the analysis, and the sample temperature was kept at 20°C. Detection was performed using the Xevo G2-XS QToF system. A 1 µl test solution was injected into the UPLC/MS-QToF, and chromatograms were recorded in both positive and negative ionization modes.

Gradient Program

Time (min)	Flow (ml/min)	Mobile phase A%	Mobile phase B%
0	0.3	95	5
10	0.3	90	10
20	0.3	70	30
35	0.3	60	40
40	0.3	40	60
45	0.3	30	70
46	0.3	95	5
50	0.3	95	5

Xevo G2-XS QToF Parameters

Parameter	Polarity (+ve)	Polarity (-ve)
Ionisation type	ESI	ESI
Mode	MS ^E	MS ^E
Mass range (m/z)	50-1200 m/z	50-1200 m/z
Scan time	0.5 s	0.5 s
Cone Voltage	40 V	40 V
Capillary	1.0 kV	2.0 kV
Low CE	6.0 eV	6.0 eV
High CE	15-60 eV(ramp)	15-60 eV(ramp)
Source temperature	120°C	120°C
Desolvation Temperature	500°C	500°C
Cone gas flow	50 L/h	50 L/h
Desolvation gas flow	900 L/h	900 L/h
Lock Spray (Leucine Enkephalin)	556.2766 m/z	554.2620 m/z
Lock mass scan time	0.5 s	0.5 s
Lock mass interval	30 s	30 s

Results and Discussion

Phytochemical Analysis

The quantitative phytochemical analysis of the samples exhibited the presence of tannins,

saponins, total phenolics, and total flavonoids in diverse percentages as reflected accurately in the table given below (Table 2).

Table 2 Phytochemical analysis of *Cynodon dactylon* collected from different locations

S. No.	Samples	Tannin content (%w/w)	Saponin content (%w/w)	Total polyphenol content (%w/w)	Total flavonoid content (%w/w)
1.	Bijnor, Uttar Pradesh	0.020	1.698	0.195	0.055
2.	Narora, Uttar Pradesh	0.019	6.093	0.212	0.075
3.	Badaun, Uttar Pradesh	0.039	5.519	0.235	0.038
4.	Farrukhabad, Uttar Pradesh	0.040	5169	0.235	0.027
5.	Bithoor, Uttar Pradesh	0.040	3.342	0.222	0.058
6.	Dalmau, Uttar Pradesh	0.015	1.353	0.049	0.050
7.	Prayagraj, Uttar Pradesh	0.038	3.310	0.094	0.004
8.	Mirzapur, Uttar Pradesh	0.038	2.128	0.081	0.044
9.	Varanasi, Uttar Pradesh	0.020	1.872	0.162	0.043
10.	Ballia, Uttar Pradesh	0.040	2.267	0.190	0.045
11.	Revelganj, Bihar	0.020	4.028	0.207	0.040
12.	Patna, Bihar	0.038	4.293	0.167	0.048
13.	Barh, Bihar	0.021	7.505	0.177	0.063

S. No.	Samples	Tannin content (%w/w)	Saponin content (%w/w)	Total polyphenol content (%w/w)	Total flavonoid content (%w/w)
14.	Bahachoki, Bihar	0.061	4.160	0.603	0.063
15.	Farka, Bihar	0.057	2.332	0.168	0.021
16.	Sahibganj, Jharkhand	0.057	5.532	0.165	0.044
17.	Gangasagar, West Bengal	0.080	3.402	0.189	0.054
18.	Gaumukh, Uttarakhand	0.060	6.989	0.220	0.084
19.	Gangotri, Uttarakhand	0.056	5.532	0.204	0.054

The quantitative analysis of *C. dactylon* revealed varying levels of tannins, saponins, total phenolics, and total flavonoids across different sampling locations. For instance, the tannin content ranged from 0.019% to 0.080%, while saponin content varied significantly, with the highest recorded at 6.989% in Gaumukh, Uttarakhand. This variation suggests that environmental factors such as soil composition and climate may influence the phytochemical profiles of plants, as supported by studies on other species like *Euphorbia hirta*, which also showed geographical variations in phytochemical content.

HPTLC Fingerprint Analysis

During the HPTLC analysis, bands of phytochemical compounds were detected at 254 nm, 366 nm, and under white light without derivatization. It was noted that the intensity of these bands was lower in samples 4 to 12. Specifically, at 366 nm and R_f 0.70, a blue band was observed with varying intensity across different samples. Interestingly, this band was most pronounced in samples 9 and 10 (Fig. 1).

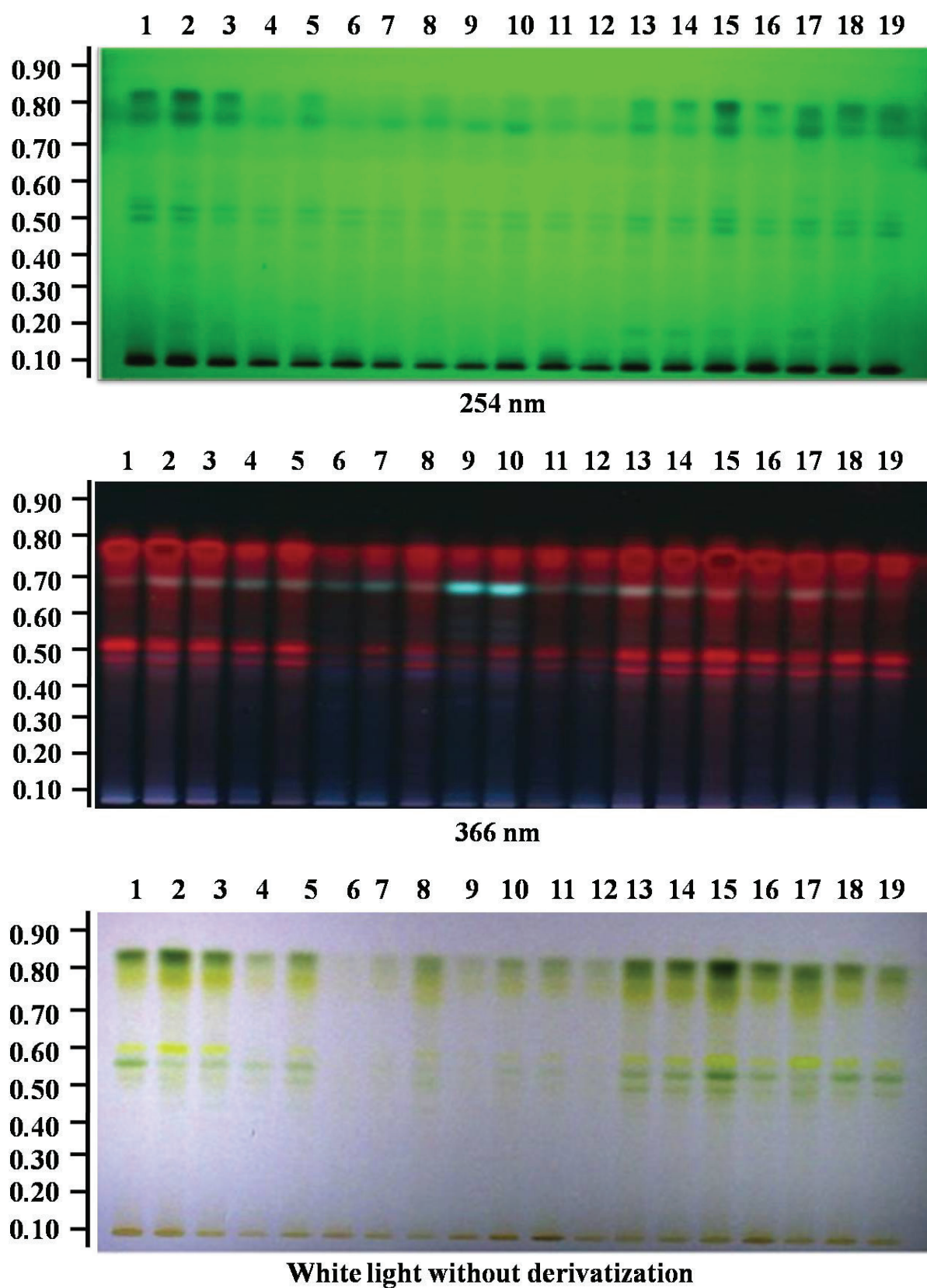


Fig. 1 HPTLC fingerprinting of methanolic extract of *Cynodon dactylon*. A: 254 nm, B: 366 nm C: Under white light without derivatization

The HPTLC fingerprinting provided a visual representation of the phytochemical diversity within the plant samples. Distinctive bands observed under different wavelengths indicated the presence of various compounds, corroborating findings from similar studies on species like *Andrographis paniculata*, where HPTLC have been used to identify multiple active constituents. This technique is crucial for quality control and standardization of herbal medicines, ensuring consistency in therapeutic efficacy.

HPLC Analysis

The HPLC study employed the reference standard schaftoside to analyse a variety of plant samples. Schaftoside was successfully identified and quantified in all samples of *Cynodon dactylon*. Notably, the sample from Gaumukh, Uttarakhand exhibited the highest concentration of schaftoside. Conversely, the samples from Varanasi, Uttar Pradesh, and Ballia, Uttar Pradesh contained the lowest levels of schaftoside (Fig 2 and 3).

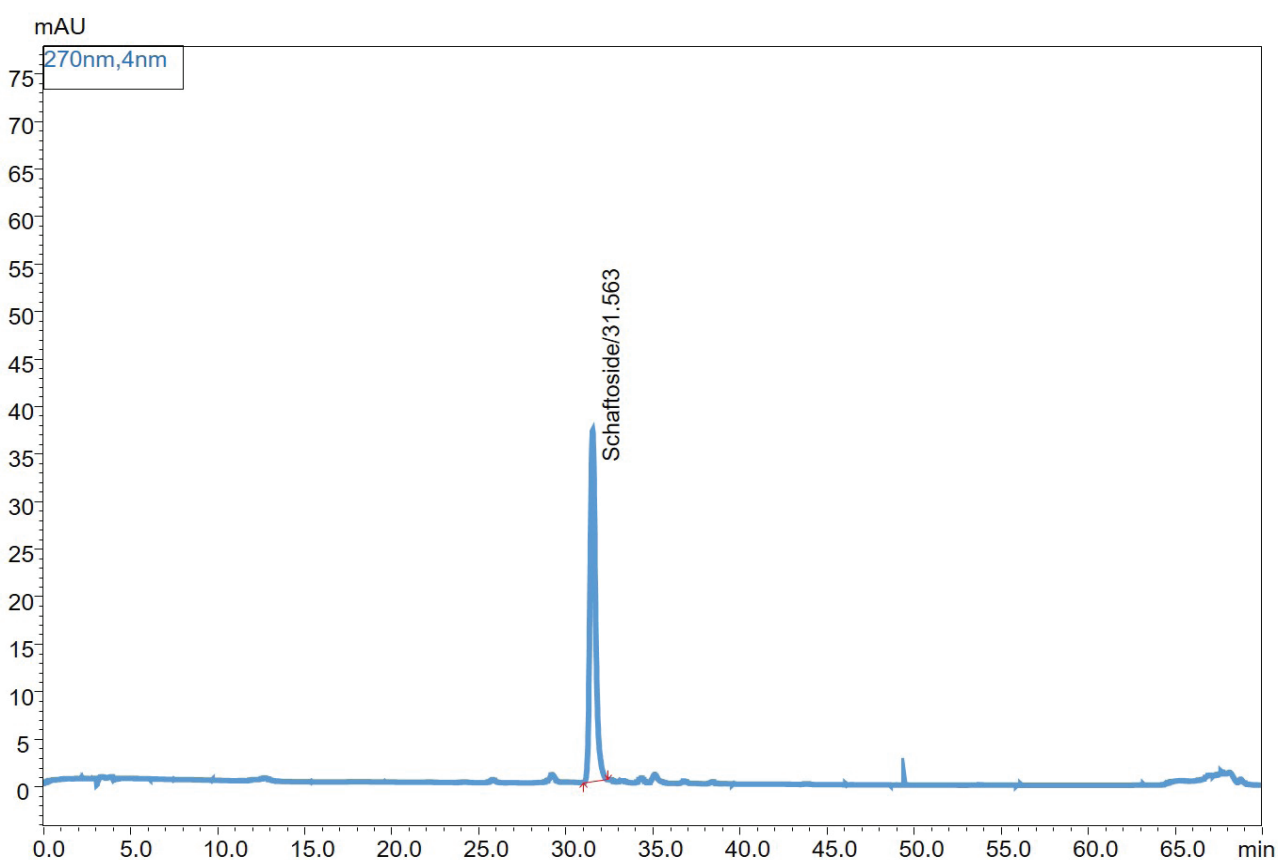


Fig. 2 HPLC profile of the reference standard

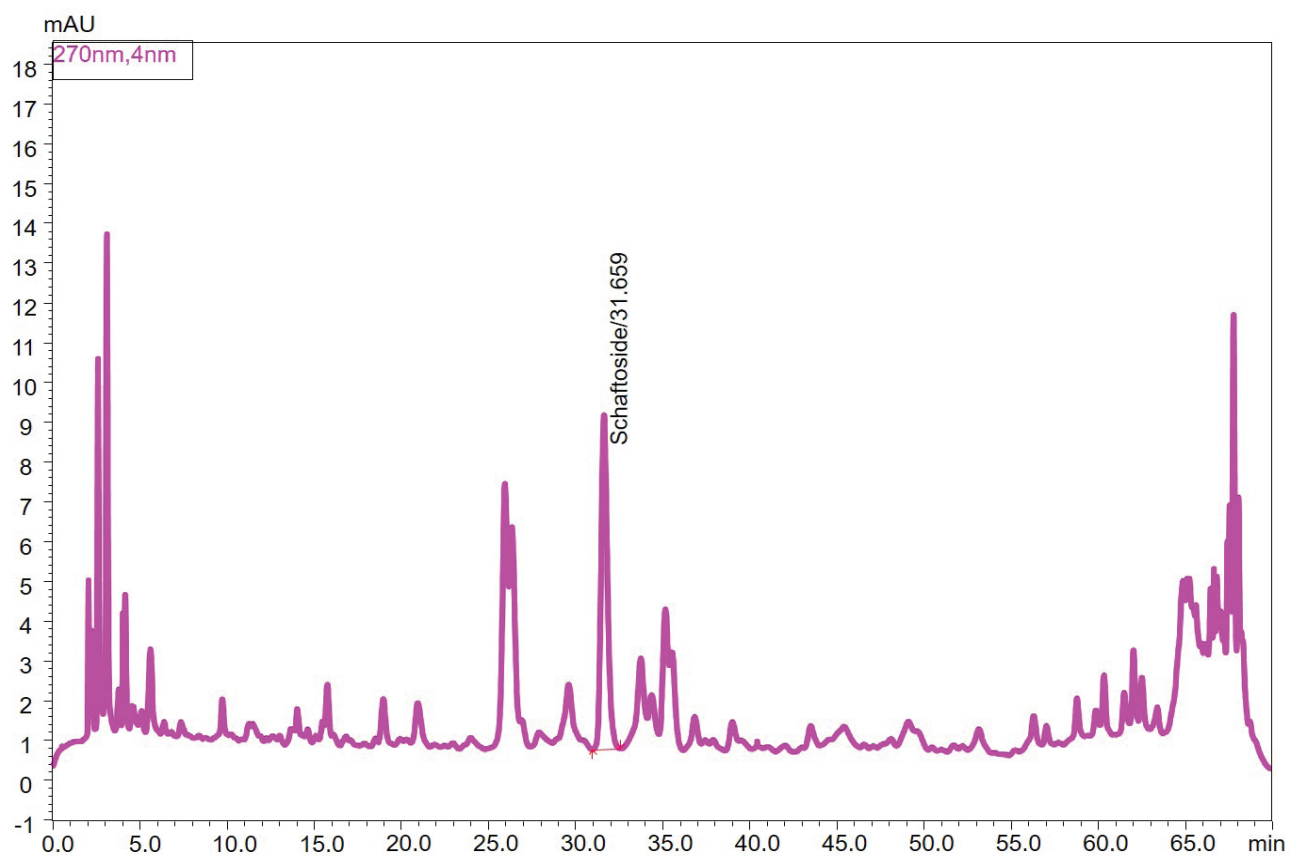


Fig. 3 HPLC profile of *Cynodon dactylon* raw material

Table. 3 Content of phytochemicals (in µg/mg) present on *Cynodon dactylon* collected from the different locations

S. No.	Internal code	Samples	Schaftoside content (µg/mg)
1.	PRF/CHI/1123/1163	Bijnor, Uttar Pradesh	0.098
2.	PRF/CHI/1123/1164	Narora, Uttar Pradesh	0.100
3.	PRF/CHI/1123/1165	Badaun, Uttar Pradesh	0.106
4.	PRF/CHI/1123/1166	Farrukhabad, Uttar Pradesh	0.157
5.	PRF/CHI/1123/1167	Bithoor, Uttar Pradesh	0.175
6.	PRF/CHI/1123/1168	Dalmau, Uttar Pradesh	0.027

S. No.	Internal code	Samples	Schaftoside content (µg/mg)
7.	PRF/CHI/1123/1169	Prayagraj, Uttar Pradesh	0.062
8.	PRF/CHI/1123/1170	Mirzapur, Uttar Pradesh	0.027
9.	PRF/CHI/1123/1171	Varanasi, Uttar Pradesh	0.014
10.	PRF/CHI/1123/1172	Ballia, Uttar Pradesh	0.014
11.	PRF/CHI/1123/1173	Revelganj, Bihar	0.274
12.	PRF/CHI/1123/1174	Patna, Bihar	0.103
13.	PRF/CHI/1123/1175	Barh, Bihar	0.067
14.	PRF/CHI/1123/1176	Bahachoki, Bihar	0.083
15.	PRF/CHI/1123/1177	Farka, Bihar	0.116
16.	PRF/CHI/1123/1178	Sahibganj, Jharkhand	0.207
17.	PRF/CHI/1123/1179	Gangasagar, West Bengal	0.051
18.	PRF/CHI/1123/1180	Gaumukh, Uttara-khand	0.369
19.	PRF/CHI/1123/1181	Gangotri, Uttara-khand	0.240

UPLC/MS-QToF Analysis

The UPLC/MS-QToF analysis was conducted to identify and quantify major bioactive compounds. This high-resolution technique allows for the precise separation and identification of compounds based on their mass-to-charge ratio (m/z) and retention time (RT). Each compound was characterized by specific retention times and m/z values, allowing for accurate identification. For instance, compounds with m/z values corresponding to known flavonoids and phenolic acids were detected, which aligns

with previous research indicating that *C. dactylon* is rich in these classes of compounds known for their antioxidant and anti-inflammatory properties. Overall, the analysis revealed a complex profile of phytochemicals and enabled high-resolution separation and identification of 23 major compounds in *Cynodon dactylon*. The TIC chromatogram (Fig. 4) and compound-dependent parameters (Table 4) provide detailed insights into the phytochemical composition.

The compounds like quercetin, kaempferol, and various phenolic acids have been

previously documented for their health benefits, including anti-cancer, anti-diabetic, and anti-microbial activities, reinforcing the therapeutic potential of the plant. The identification and quantification of these

compounds not only support the traditional uses of the plant but also pave the way for further pharmacological studies to explore their potential health benefits.

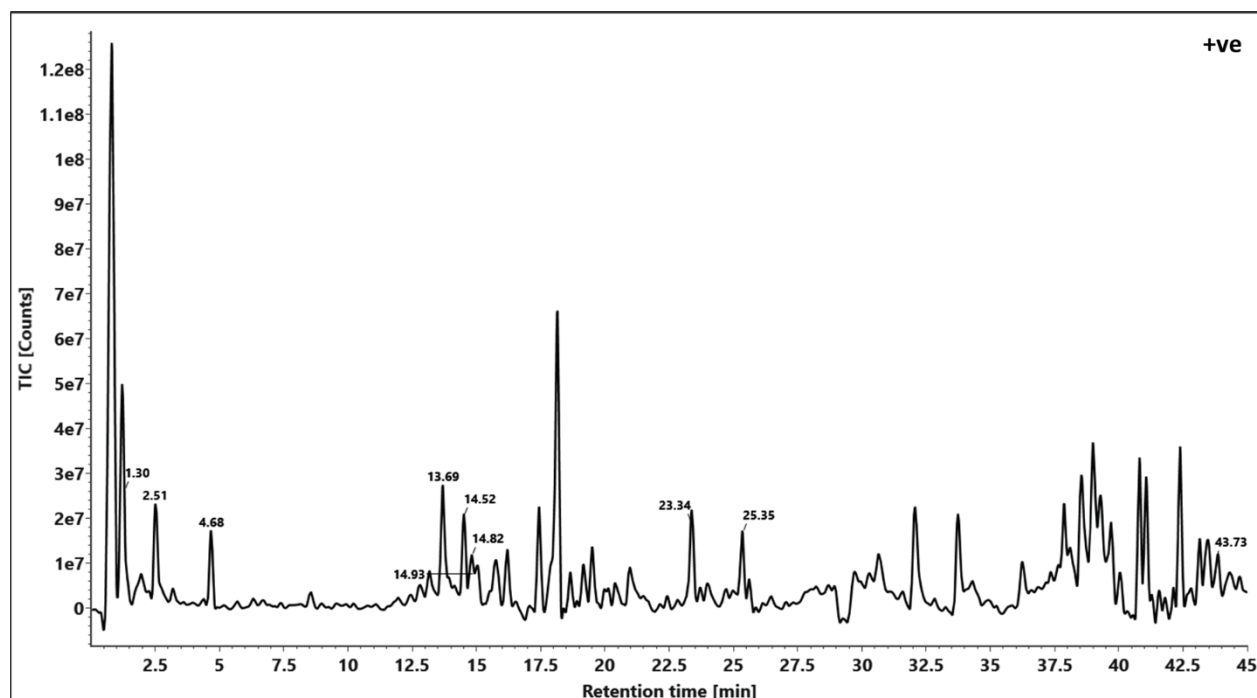


Fig. 4 TIC chromatogram of *Cynodon dactylon* (PRF/CHI/1123/1163) in positive ionization mode

Table 4 Compound-dependent parameters of analytes/identified compounds in *Cynodon dactylon* (PRF/CHI/1123/1163) in positive ionization mode

S. N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
1	N-(1-De-oxy-1-fructosyl) isoleucine	$C_{12}H_{23}NO_7$	293.1475	294.1558	1.1	1.30	703923	+H
2	Phenylalanine	$C_9H_{11}NO_2$	165.0790	166.0862	-0.1	2.51	682789	+H
3	Tryptophan	$C_{11}H_{12}NO_2$	204.0899	205.0970	-0.1	4.68	239227	+H
4	Kaempferol 3-xylosylglucoside	$C_{26}H_{28}O_{15}$	580.1428	581.1506	0.5	13.69	3777494	+H
5	Schaftoside	$C_{26}H_{28}O_{14}$	564.1479	565.1557	0.6	14.52	2275715	+H

6	Apigenin-7-O-gentiobioside	$C_{27}H_{30}O_{15}$	594.1585	595.1663	0.6	14.82	505036	+H
7	Apigenin 6-C-beta-arabinopyranosyl-8-C-beta-galactopyranoside	$C_{26}H_{28}O_{14}$	564.1479	565.1560	0.8	14.93	573076	+H
8	Syringetin 3-(6''-acetylglucoside)	$C_{25}H_{26}O_{14}$	550.1323	551.1398	0.3	15.05	614466	+H
9	Apigenin 6,8-di-C-alpha-L-arabinopyranoside	$C_{25}H_{26}O_{13}$	534.1373	535.1450	0.4	15.84	831119	+H
10	Isoschaftoside	$C_{26}H_{28}O_{14}$	564.1479	565.1552	0.1	16.14	651527	+H
11	Quercitrin	$C_{21}H_{20}O_{11}$	448.1006	449.1082	0.3	16.20	628217	+H
12	Kaempferol 3-glucuronide	$C_{21}H_{18}O_{12}$	462.0798	463.0874	0.3	16.24	737202	+H
13	3,5,6-Trihydroxy-4',7-dimethoxyflavone	$C_{17}H_{14}O_7$	330.0740	331.0815	0.3	23.34	1060037	+H
14	6,7,10-Trihydroxy-8-octadecenoic acid	$C_{18}H_{34}O_5$	330.2406	353.2301	0.2	25.35	139237	+Na
15	Hellebrigenin glucoside	$C_{30}H_{42}O_{11}$	578.2727	579.2827	2.7	43.73	231917	+H

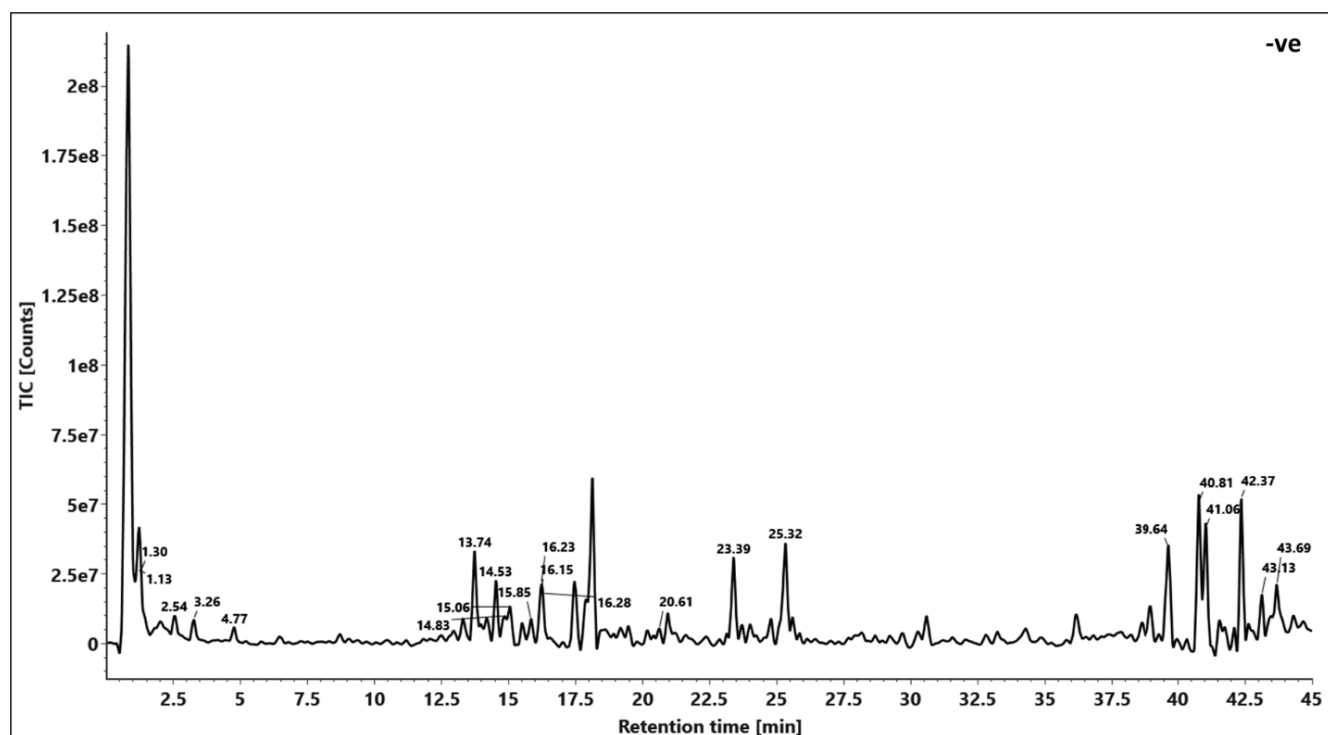


Fig. 5 TIC chromatogram of *Cynodon dactylon* (PRF/CHI/1123/1163) in negative ionization mode

Table 5 Identified compounds in *Cynodon dactylon* (PRF/CHI/1123/1163) in negative ionization mode

S. N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass Error (mDa)	RT (min)	Response	Adducts
1	Malic acid	C ₄ H ₆ O ₅	134.0215	133.0137	-0.5	1.13	467255	-H
2	N-(1-Deoxy-1-fructosyl) isoleucine	C ₁₂ H ₂₃ NO ₇	293.1475	292.1389	-1.3	1.30	67499	-H
3	Phenylalanine	C ₉ H ₁₁ NO ₂	165.0790	164.0709	-0.8	2.54	338766	-H
4	Protocatechuic acid 3-glucoside	C ₁₃ H ₁₆ O ₉	316.0794	315.0713	-0.8	3.26	792953	-H
5	Tryptophan	C ₁₁ H ₁₂ N ₂ O ₂	204.0899	203.0818	-0.8	4.77	446052	-H
6	Kaempferol 3-xylosylglucoside	C ₂₆ H ₂₈ O ₁₅	580.1428	579.1358	0.2	13.74	5151645	-H
7	Schaftoside	C ₂₆ H ₂₈ O ₁₄	564.1479	563.1409	0.3	14.53	2990625	-H

S. N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass Error (mDa)	RT (min)	Response	Adducts
8	Apigenin-7-O-gentiobioside	C ₂₇ H ₃₀ O ₁₅	594.1585	593.1508	-0.4	14.83	702405	-H
9	Apigenin 6-C-beta-arabinopyranosyl-8-C-beta-galactopyranoside	C ₂₆ H ₂₈ O ₁₄	564.1479	563.1403	-0.3	14.95	984213	-H
10	Syringetin 3-(6''-acetylglucoside)	C ₂₅ H ₂₆ O ₁₄	550.1323	549.1251	0.1	15.06	1047755	-H
11	Apigenin 6,8-di-C-alpha-L-arabinopyranoside	C ₂₅ H ₂₆ O ₁₃	534.1373	533.1297	-0.4	15.85	1253961	-H
12	Isoschaftoside	C ₂₆ H ₂₈ O ₁₄	564.1479	563.1401	-0.5	16.15	808875	-H
13	Quercitrin	C ₂₁ H ₂₀ O ₁₁	448.1006	447.0927	-0.6	16.23	1064066	-H
14	Kaempferol 3-glucuronide	C ₂₁ H ₁₈ O ₁₂	462.0798	461.0720	-0.5	16.28	1023154	-H
15	Cynodontin	C ₁₅ H ₁₀ O ₆	286.0477	285.0397	-0.7	20.61	639683	-H
16	3,5,6-Trihydroxy-4',7-dimethoxyflavone	C ₁₇ H ₁₄ O ₇	330.0740	329.0661	-0.6	23.39	1214616	-H
17	6,7,10-Trihydroxy-8-octadecenoic acid	C ₁₈ H ₃₄ O ₅	330.2406	329.2324	-0.9	25.32	4924433	-H
18	Formosanin C	C ₅₁ H ₈₂ O ₂₀	1014.5400	1059.5427	4.5	39.64	4043199	+HCOO
19	2-Hydroxylinolenic acid	C ₁₈ H ₃₀ O ₃	294.2195	293.2110	-1.3	40.81	4663381	-H

S. N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass Error (mDa)	RT (min)	Response	Adducts
20	17-Hydrox-ylinolenic acid	C ₁₈ H ₃₀ O ₃	294.2195	293.2111	-1.2	41.06	2874776	-H
21	18-Hydrox-ylinoic acid	C ₁₈ H ₃₂ O ₃	296.2351	295.2267	-1.2	42.37	5001556	-H
22	Hexopyrano-syl 6-O-pal-mitoylhexo-pyranoside	C ₂₈ H ₅₂ O ₁₂	580.3459	625.3448	0.8	43.13	718839	+HCOO
23	Hellebrigenin glucoside	C ₃₀ H ₄₂ O ₁₁	578.2727	577.2691	3.7	43.69	4264866	-H

Conclusion

The comprehensive analysis of *Cynodon dactylon* has elucidated its rich phytochemical profile, characterized by significant levels of tannins, saponins, total phenolics, and flavonoids, which vary across different geographical locations. The application of advanced techniques such as HPTLC and UPLC/QToF has not only confirmed the presence of key bioactive compounds, including schaftoside, but also highlighted the influence of environmental factors on the plant's chemical composition.

These findings underscore the potential therapeutic applications of *Cynodon dactylon*,

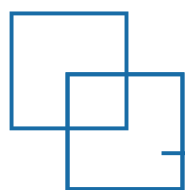
particularly in the realms of anti-inflammatory and antioxidant therapies, as supported by the identified compounds. The geographical variations in phytochemical content suggest that specific regions may yield samples with enhanced medicinal properties, warranting further investigation into the pharmacological activities of these compounds. Overall, this study reinforces the importance of *Cynodon dactylon* as a valuable resource in herbal medicine and encourages future research to explore its full therapeutic potential.

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CHAPTER
06

Insilico Analysis Against Ovarian Cancer



INTRODUCTION

Ovarian cancer (OC) poses a significant threat to women's health, ranking the list of cancers that affect the female reproductive system and representing the highest rates of recurrence, morbidity, and mortality. Approximately, 85%-90% of OCs are of epithelial origin. Due to the inadequacy of efficient early screening methods and the absence of distinct symptoms during the initial phases of epithelial ovarian cancer (EOC), patients are diagnosed at the advanced stage (stage III-IV) (Huang et al., 2023). Epithelial ovarian cancer (EOC) constitutes 90% of all ovarian cancer cases. EOC can be categorized into four subtypes based on its origin: serous type, mucinous type (MUC), endometrioid type (END), and clear cell type (CLC). Serous OC is further divided into high-grade serous type (HGS) and low-grade serous type (LGS). The subtypes of EOC exhibit heterogeneity not only in their cellular origins but also in their distribution. High-grade serous (HGS) EOC may stem from the epithelium of the fallopian tube. Conversely, the origin of low-grade serous (LGS) EOC is linked to a stepwise progression from benign to borderline tumour, and finally to non-invasive and invasive stages. These subtypes display heterogeneity in their clinical progression, with the high-grade serous (HGS) subtype exhibiting the most adverse clinical outcomes. Additionally, the four subtypes exhibit distinct molecular alterations and gene expression patterns, suggesting diverse carcinogenic mechanisms proposed across the subtypes, informed by their genetic and molecular backgrounds (Sung et al., 2023).

Plants are often considered as nature's factories, producing various chemical components. Anti-tumour compounds extracted and tested from plants show potential for enhancing the effectiveness of ovarian cancer treatment while mitigating the adverse effects associated with the disease (Ding et al., 2023). *Cynodon dactylon* (L.) Pers., belongs to the family Poaceae and has huge religious importance and is utilized in Ayurvedic, Siddha, Unani, Nepalese, and Chinese medicinal traditions. It is reported that this plant has anticancer properties. Medicinal herb such as *C. dactylon* encompasses a variety of bioactive compounds, including flavonoids, alkaloids, terpenoids, and phenolic acids (Kalimuthu et al., 2022). These constituents showcased diverse therapeutic activities,



such as antioxidant, anti-inflammatory, anti-cancer, and immunomodulatory effects (Gao and Homayoonfal, 2023). It is used as medicine for dropsy, headache, urogenital disorders, cough and bronchitis, cancer, cramps, urogenital disorders, epilepsy, miscarriage, hypertension, leukoderma, convulsions, and calculus (Sen et al., 2011; Malpani et al., 2020). While preliminary research suggested potential health benefits of *C. dactylon*, particularly its anti-cancer properties, it was crucial to acknowledge the limited extent of scientific evidence.

Etiology of Disease

Epithelial ovarian cancer is an important concern of women's health and results from a complex interplay of genetic, environmental, and lifestyle factors (Duarte Mendes et al., 2023). A familial history of ovarian, breast, or colorectal cancers can also elevate susceptibility to ovarian cancer, indicating potential hereditary predispositions. Age serves as another leading factor, with ovarian cancer predominantly affecting older women, especially those over 50 years old, with a higher incidence among women over 60 (Ponti et al., 2023). Reproductive factors such as early onset of menstruation, late onset of menopause, and infertility have been associated with altered ovarian cancer risk profiles. Hormonal influences, including hormone replacement therapy and estrogen-only therapy post-menopause, may marginally

heighten the risk (Mills et al., 2023). Moreover, conditions like endometriosis have been linked to increased susceptibility to specific types of ovarian cancer. Lifestyle factors, such as smoking, unhealthy diet, and obesity, also play significant roles, with smoking being correlated to mucinous ovarian cancer and unhealthy diet and obesity possibly exacerbating hormonal imbalances and chronic inflammation (Almanza-Aguilera et al., 2023). Despite the recognition of these risk factors, it's essential to emphasize that many women diagnosed with ovarian cancer lack identifiable risk factors, while not all women with risk factors develop the disease. Thus, early detection through regular screenings and heightened awareness of symptoms remain pivotal in ovarian cancer management and treatment strategies.

Reported Therapeutic Uses

Cynodon dactylon, known for its diverse therapeutic properties, has been traditionally employed across various medicinal applications (Vinayagam et al., 2021). The whole plant, particularly its leaves,

is utilized for alleviating abdominal pain, treating abscesses, combating acne vulgaris, managing amenorrhea, and addressing anorexia. Its rhizomes serve as anthelmintics and aid in managing asthma (Das et al.,

2021). The roots of *C. dactylon* are used as antipyretics and contraceptives, while the leaves act as antirheumatic agents and help in managing diabetes mellitus and diarrhea. The plant's stems and leaves contribute to digestive system health, while its roots, leaves, and whole plant act as diuretics, beneficial for conditions like cystitis and dysuria (Monari et al., 2022). Moreover, this plant is also used in the treatment of dysentery, epilepsy,

fever, and hemorrhoids (Table 1). It also aids in wound healing and is applied to manage wounds, injuries, and ulcers. Furthermore, the plant demonstrates efficacy against various ailments including eye infections, fatigue, muscle cramps, and skin diseases. With its broad spectrum of therapeutic uses, *C. dactylon* holds significant potential in traditional and alternative medicine practices (Singh et al., 2021).

Table 1 List of previously reported therapeutic uses of *C. dactylon*

Plant Parts	Therapeutic use	Therapeutic use identifiers	References
Whole Plant, Leaf	Abdominal pain	MESH:D015746, UMLS:C0000737, SYMP:0000457, ICD-11:MD81.4	ISBN:9770972795006
Whole Plant	Abortifacient agents	MESH:D000019, UMLS:C0000782, ICD-11:JA00.1	ISBN:9770972795006, ISBN:9788173717031
Whole Plant	Abscess	MESH:D000038, UMLS:C0000833, SYMP:0000672, ICD-11:1B75.3	ISBN:9770972795006
Whole Plant	Acne vulgaris	MESH:D000152, UMLS:C0702166, D0ID:6543, ICD-11:ED80.Z	ISBN:9770972795006
Whole Plant	Amenorrhea	MESH:D000568, UMLS:C0002453, D0ID:13938, ICD-11:GA20.0	ISBN:9770972795006, ISBN:9788171360536
Whole Plant	Anorexia	MESH:D000855, UMLS:C1971624, D0ID:8689, ICD-11:MG43.7	ISBN:9770972795006
Rhizome	Anthelmintics	MESH:D000871, UMLS:C0003158, ICD-11:XM4EC0	ISBN:9770972795006
Whole Plant	Anticonvulsants	MESH:D000927, UMLS:C0003286, ICD-11:XM07T3	ISBN:9788172362133
Whole Plant, Leaf	Antifungal agents	MESH:D000935, UMLS:C0003308, ICD-11:XM83G4	ISBN:9770972795006
Whole Plant	Anti-infective agents	MESH:D000890, UMLS:C0003204, ICD-11:XM4TV0	ISBN:9788172362133
Whole Plant	Anti-infective agents, local	MESH:D000891, UMLS:C0003205, ICD-11:XM4VG4	ISBN:9789327275590

Whole Plant	Antimutagenic agents	MESH:D016587, UMLS:C0085168	ISBN:9770972795006
Whole Plant	Antioxidants	MESH:D000975, UMLS:C0003402	ISBN:9770972795006
Root	Antipyretics	MESH:D058633, UMLS:C0003419, ICD-11:XM1RS7	ISBN:9770972795006
Leaf	Antirheumatic agents	MESH:D018501, UMLS:C0003191, ICD-11:XM95N2	ISBN:9770972795006
Whole Plant	Antiviral agents	MESH:D000998, UMLS:C0003451	ISBN:9770972795006
Whole Plant	Aphrodisiacs	MESH:D001046, UMLS:C0003567	ISBN:9770972795006
Leaf	Appendicitis	MESH:D001064, UMLS:C0003615, DOID:8337, ICD-11:DB10	ISBN:9770972795006
Whole Plant	Arm injuries	MESH:D001134, UMLS:C0003794	ISBN:9770972795006
Whole Plant, Leaf	Asthenia	MESH:D001247, UMLS:C0004093, ICD-11:MG22	ISBN:9770972795006
Rhizome	Asthma	MESH:D001249, UMLS:C0004096, DOID:2841, ICD-11:CA23	ISBN:9788172360481
Whole Plant, Leaf	Astringents	MESH:D001252, UMLS:C0004110, ICD-11:XM0VK6	ISBN:9770972795006
Whole Plant	Brain diseases	MESH:D001927, UMLS:C0006111, DOID:936	ISBN:9770972795006
Root, Shoot	Burns	MESH:D002056, UMLS:C0006434, ICD-11:NE2Z	ISBN:9770972795006
Whole Plant	Carbuncle	MESH:D002270, UMLS:C0007078, DOID:2176, ICD-11:1B75.1	ISBN:9770972795006, ISBN:9788172360481
Whole Plant	Chemoprevention	MESH:D018890, UMLS:C0282515	ISBN:9770972795006
Whole Plant	Coma	MESH:D003128, UMLS:C0009421, ICD-11:MB20.1	ISBN:9770972795006
Whole Plant, Root	Common cold	MESH:D003139, UMLS:C0009443, DOID:10459, ICD-11:CA00	ISBN:9770972795006
Whole Plant	Conjunctivitis	MESH:D003231, UMLS:C0009763, DOID:6195, ICD-11:9A60	ISBN:9770972795006, ISBN:9788171360536, ISBN:9788172362133, ISBN:9788173717031

Root	Contraceptive agents	MESH:D003270, UMLS:C0009871	ISBN:9770972795006
Whole Plant	Cooling effect on body	UMLS:C0678568	ISBN:81-204-0828-4
Root	Cough	MESH:D003371, UMLS:C0010200, ICD-11:MD12	ISBN:9770972795006
Whole Plant	Cystitis	MESH:D003556, UMLS:C0010692, D0ID:1679, ICD-11:GC00	ISBN:9780387706375, ISBN:9789327275590
Whole Plant	Delayed puberty	MESH:D011628, UMLS:C0034012, ICD-11:5A91	ISBN:9770972795006
Whole Plant	Demulcents	MESH:D065106, UMLS:C0302232	ISBN:81-204-0828-4
Whole Plant	Dermatitis	MESH:D003872, UMLS:C3875321, D0ID:2723, ICD-11:EA8Z	ISBN:9788172360481
Leaf	Diabetes mellitus	MESH:D003920, UMLS:C0011849, D0ID:9351, ICD-11:5A14	ISBN:9770972795006
Whole Plant, Leaf	Diarrhea	MESH:D003967, UMLS:C0011991, D0ID:13250, ICD-11:ME05.1	ISBN:9770972795006
Stem, Leaf	Digestive system diseases	MESH:D004066, UMLS:C0012242, ICD-11:DE2Z	ISBN:9770972795006
Whole Plant, Root	Diuretics	MESH:D004232, UMLS:C0012798, ICD-11:XM4D06	ISBN:9770972795006, ISBN:9788172361266
Whole Plant, Leaf	Dysentery	MESH:D004403, UMLS:C0277526, D0ID:12384, ICD-11:1A40.Z	ISBN:9770972795006
Whole Plant	Dysentery, amoebic	MESH:D004404, UMLS:C0013370, ICD-11:1A36.00	ISBN:9770972795006
Whole Plant, Root, Leaf	Dysuria	MESH:D053159, UMLS:C0013428, ICD-11:MF50.7	ISBN:9770972795006
Whole Plant	Eczema	MESH:D004485, UMLS:C0013595	ISBN:9770972795006
Whole Plant, Root, Leaf	Edema	MESH:D004487, UMLS:C0013604, ICD-11:MG29.1	ISBN:9770972795006
Whole Plant	Endophthalmitis	MESH:D009877, UMLS:C0014236, D0ID:4692, ICD-11:9C21	ISBN:9770972795006, ISBN:9788171360536, ISBN:9788172361266, ISBN:9788172362140

Whole Plant, Leaf	Epilepsy	MESH:D004827, UMLS:C0014544, D0ID:1826, ICD-11:8A6Z	ISBN:9770972795006
Whole Plant, Leaf	Epistaxis	MESH:D004844, UMLS:C0014591, ICD-11:MD20	ISBN:9770972795006
Stem	Erectile dysfunction	MESH:D007172, UMLS:C0242350, ICD-11:HA01.1	ISBN:9770972795006
Whole Plant	Erysipelas	MESH:D004886, UMLS:C0014733, D0ID:11330, ICD-11:1B70.0	ISBN:9770972795006
Whole Plant	Exanthema	MESH:D005076, UMLS:C0015230	ISBN:9770972795006
Whole Plant	Eye burn	MESH:D005126, UMLS:C0015395, ICD-11:NE00	ISBN:9770972795006
Whole Plant	Eye diseases	MESH:D005128, UMLS:C0015397, D0ID:5614, ICD-11:9E1Z	ISBN:81-204-0828-4
Whole Plant	Eye infections	MESH:D015817, UMLS:C0015403, ICD-11:9C20.2	ISBN:81-204-0828-4
Whole Plant	Fatigue	MESH:D005221, UMLS:C0015672, ICD-11:MG22	ISBN:9770972795006
Whole Plant	Fertility agents	MESH:D005299, UMLS:C0015896	ISBN:9770972795006
Whole Plant, Root, Leaf	Fever	MESH:D005334, UMLS:C0015967, ICD-11:MG26	ISBN:9770972795006
Whole Plant	Furunculosis	MESH:D005667, UMLS:C0016867, ICD-11:1B75.0	ISBN:9770972795006
Whole Plant, Leaf	General tonic for rejuvenation	MESH:D012060, UMLS:C0035016	ISBN:9770972795006
Whole Plant, Root	Gonorrhoea	MESH:D006069, UMLS:C0018081, D0ID:7551, ICD-11:1A7Z	ISBN:9770972795006, ISBN:9788172361266
Whole Plant	Hallucinations	MESH:D006212, UMLS:C0018524, ICD-11:MB27.2Z	ISBN:9770972795006, ISBN:9788172362133
Whole Plant	Headache	MESH:D006261, UMLS:C0018681, ICD-11:8A81.Z	ISBN:9770972795006, ISBN:9788172360481, ISBN:9788173717031
Whole Plant	Hematologic diseases	MESH:D006402, UMLS:C0018939, D0ID:74, ICD-11:3C0Z	ISBN:9770972795006

Whole Plant, Root	Hematuria	MESH:D006417, UMLS:C0018965, ICD-11:MF50.4Z	ISBN:9770972795006
Whole Plant	Hemoptysis	MESH:D006469, UMLS:C0019079, ICD-11:MD22	ISBN:9788171360536, ISBN:9788173717031
Whole Plant	Hemorrhage	MESH:D006470, UMLS:C0019080, ICD-11:MG27	ISBN:9770972795006
Whole Plant, Root, Leaf	Hemorrhoids	MESH:D006484, UMLS:C0019112, D0ID:9746, ICD-11:DB60.Z	ISBN:9770972795006
Whole Plant, Stem, Leaf	Hemostasis	MESH:D006487, UMLS:C0019116	ISBN:9770972795006
Whole Plant, Root, Leaf	Hemostatics	MESH:D006490, UMLS:C0019120	ISBN:9770972795006
Whole Plant	Hysteria	MESH:D007046, UMLS:C0020701	ISBN:81-204-0828-4
Whole Plant	Insecticides	MESH:D007306, UMLS:C0021576, ICD-11:XM3K66	ISBN:9770972795006
Whole Plant, Leaf	Jaundice	MESH:D007565, UMLS:C0022346, ICD-11:ME10.1	ISBN:9770972795006
Whole Plant	Laxatives	MESH:D054368, UMLS:C0282090, ICD-11:XM62T3	ISBN:9770972795006, ISBN:9788172362140
Whole Plant	Leprosy	MESH:D007918, UMLS:C0023343, D0ID:1024, ICD-11:1B20	ISBN:9770972795006, ISBN:9788171360536, ISBN:9788172362133, ISBN:9788173717031
Whole Plant	Leukorrhea	MESH:D007973, UMLS:C0023533, D0ID:3766, ICD-11:MF3A	ISBN:9770972795006, ISBN:9788172362133
Whole Plant	Lithiasis	MESH:D020347, UMLS:C0023869	ISBN:9788172360481
Root	Malaria	MESH:D008288, UMLS:C0024530, D0ID:12365, ICD-11:1F4Z	ISBN:9770972795006
Whole Plant, Root	Menorrhagia	MESH:D008595, UMLS:C0025323, ICD-11:GA20.50	ISBN:9780387706375
Whole Plant	Menstruation disturbances	MESH:D008599, UMLS:C0025345, ICD-11:GA20.30	ISBN:9770972795006
Whole Plant	Mental disorders	MESH:D001523, UMLS:C0004936, ICD-11:6E8Z	ISBN:81-204-0828-4

Whole Plant	Metrorrhagia	MESH:D008796, UMLS:C0025874, ICD-11:GA22	ISBN:9780387706375
Leaf	Miliaria	MESH:D008883, UMLS:C0026113, D0ID:1382, ICD-11:EE02	ISBN:9770972795006
Whole Plant	Muscle cramp	MESH:D009120, UMLS:C0026821, ICD-11:MB47.3	ISBN:9770972795006, ISBN:9788172360481
Stem	Muscle strength	MESH:D053580, UMLS:C0517349	ISBN:9770972795006
Whole Plant	Musculoskeletal diseases	MESH:D009140, UMLS:C0026857, D0ID:17, ICD-11:FC0Z	ISBN:9770972795006
Whole Plant	Nail diseases	MESH:D009260, UMLS:C0027339, D0ID:4123	ISBN:9788172360481
Whole Plant	Nausea	MESH:D009325, UMLS:C0027497, ICD-11:MD90.0	ISBN:9770972795006
Whole Plant	Neoplasms	MESH:D009369, UMLS:C0027651, D0ID:162, ICD-11:2E2Z	ISBN:9789327275590
Whole Plant	Nephritis	MESH:D009393, UMLS:C0027697, D0ID:10952, ICD-11:GB40	ISBN:9780387706375
Whole Plant	Nervous system diseases	MESH:D009422, UMLS:C0027765, D0ID:863, ICD-11:8E7Z	A Report On Medicinal Plants Of Kachchh (Gujarat)
Whole Plant	Pain	MESH:D010146, UMLS:C0030193, ICD-11:MG3Z	ISBN:81-204-0828-4, ISBN:9770972795006
Whole Plant	Pruritus	MESH:D011537, UMLS:C0033774, ICD-11:EC90	ISBN:9770972795006
Leaf	Rectal diseases	MESH:D012002, UMLS:C0034882, D0ID:1285, ICD-11:DD92.Z	ISBN:9770972795006
Rhizome	Rhinitis	MESH:D012220, UMLS:C0035455, D0ID:4483, ICD-11:CA09.0	ISBN:9788172360481
Whole Plant	Scabies	MESH:D012532, UMLS:C0036262, D0ID:8295, ICD-11:1G04	ISBN:81-204-0828-4, ISBN:9780387706375, ISBN:9789327275590
Whole Plant	Scorpion stings	MESH:D065008, UMLS:C0238417, ICD-11:NE61	ISBN:9770972795006

Whole Plant, Root	Sexually transmitted diseases	MESH:D012749, UMLS:C0036916, ICD-11:1A9Z	ISBN:9788172361266, ISBN:9788172362140
Whole Plant	Skin diseases	MESH:D012871, UMLS:C0037274, D01D:37, ICD-11:EM0Z	ISBN:9770972795006, ISBN:9780387706375, ISBN:9789327275590
Whole Plant	Snake bites	MESH:D012909, UMLS:C0037379	ISBN:9770972795006, ISBN:9788172360481
Whole Plant, Leaf	Stomach diseases	MESH:D013272, UMLS:C0038354, D01D:76, ICD-11:DA4Y	ISBN:9770972795006
Whole Plant, Leaf	Stomach ulcer	MESH:D013276, UMLS:C0038358, ICD-11:DA60	ISBN:9770972795006
Whole Plant	Suppuration	MESH:D013492, UMLS:C3203360	ISBN:81-204-0828-4
Whole Plant	Syncope	MESH:D013575, UMLS:C0039070, ICD-11:MG45.Z	ISBN:9770972795006
Whole Plant, Root	Syphilis	MESH:D013587, UMLS:C0039128, D01D:4166, ICD-11:1A6Z	ISBN:9770972795006
Whole Plant	Thirst	MESH:D013894	ISBN:9770972795006
Leaf	Thrombosis	MESH:D013927, UMLS:C0040053, D01D:0060903	ISBN:9770972795006
Leaf	Tuberculosis	MESH:D014376, UMLS:C0041296, D01D:399, ICD-11:1B1Z	ISBN:9770972795006
Whole Plant, Rhizome	Ulcer	MESH:D014456, UMLS:C0041582	ISBN:9770972795006
Root	Urinary bladder calculi	MESH:D001744, UMLS:C0005683, ICD-11:GB71.0	ISBN:9788172362140
Whole Plant	Urinary retention	MESH:D016055, UMLS:C0080274, ICD-11:MF50.3	ISBN:9788172362133
Leaf	Urinary tract infections	MESH:D014552, UMLS:C0042029, D01D:0080784, ICD-11:GC08	ISBN:9770972795006
Whole Plant	Urination disorders	MESH:D014555, UMLS:C0042035, ICD-11:GB0Y	ISBN:9770972795006
Rhizome	Urogenital system	MESH:D014566, UMLS:C0042066	ISBN:9770972795006, ISBN:9788172360481

Whole Plant	Urticaria	MESH:D014581, UMLS:C0042109, DOID:1555, ICD-11:EB05	ISBN:9770972795006
Whole Plant	Uterine diseases	MESH:D014591, UMLS:C0042131, DOID:345, ICD-11:GA1Y	ISBN:9770972795006
Whole Plant	Vaginal diseases	MESH:D014623, UMLS:C0042251, DOID:121, ICD-11:GA1Y	ISBN:9788173717031
Whole Plant, Root, Leaf	Vomiting	MESH:D014839, UMLS:C0042963, ICD-11:MD90.1	ISBN:9770972795006
Leaf	Warts	MESH:D014860, UMLS:C3665596, DOID:11165, ICD-11:1E80	ISBN:9788172360481
Whole Plant	Whitlows	UMLS:C0152448, ICD-11:EE12.0	ISBN:9788172360481
Whole Plant, Leaf	Wound healing	MESH:D014945, UMLS:C0043240	ISBN:9770972795006
Whole Plant, Rhizome, Leaf	Wounds and injuries	MESH:D014947, UMLS:C0043251	ISBN:9770972795006

Numerous research has investigated the therapeutic efficacy of phytochemicals extracted from diverse medicinal plants to combat EOC. Despite these efforts, as of now, the therapeutic efficacy of *C. dactylon* against EOC has not been explored. At this outset, the present study is dedicated to employing big data techniques to systematically examine extant targets and pathways associated with the phytoconstituents of *C. dactylon* to

combat EOC. The investigation focused on utilizing ADMET, molecular docking, and molecular dynamics simulations to provide a comprehensive mechanistic overview of how these phytochemicals help to prevent the pathogenesis of EOC at the molecular level. The overall process of employing multifaceted computational approaches has been depicted in Fig. 1.

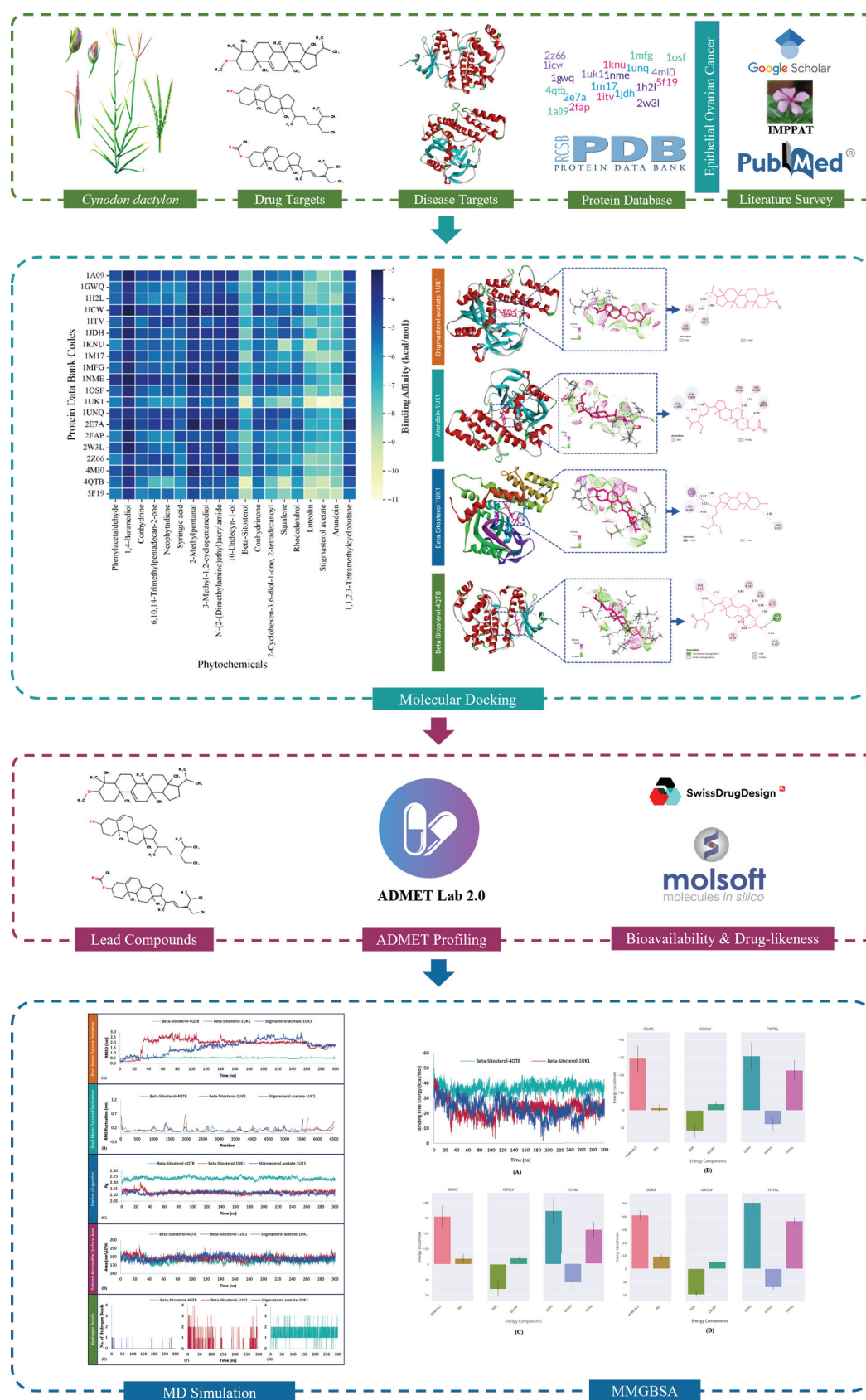


Fig. 1 General procedure of computational approaches

Materials and Methods

Assembling of Phytochemicals Library

All potential phytochemicals of *Cynodon dactylon* were sourced from the IMPPAT, Dr Duke, and KNApSACk databases (Hartono Wijaya et al., 2016; Mohanraj et al., 2018; Lans and van Asseldonk, 2020). Furthermore, an extensive review of the available literature was undertaken to compile a library of phytochemicals (Malpani et al., 2020; Savadi et al., 2020; Singh et al., 2021). To conduct a comprehensive analysis of each phytocompound, we utilized the PubChem database to gather crucial information such as 3D structures, Canonical SMILE, molecular weight, and PubChem CID (Kim & Bolton, 2024). The canonical SMILES were specifically acquired for assessing the ADMET profiling of all phytochemicals. Furthermore, canonical SMILES were also utilized to conduct drug-likeness and bioavailability screening for lead compounds. Additionally, the 3D structures were essential for conducting molecular docking investigations. This approach enabled a detailed analysis of compound characteristics, aiding the assessment of their potential as lead compounds in drug-related evaluations.

Lipinski's Rule of Five

The canonical SMILES of phytochemicals were analysed according to Lipinski's rule of five

criteria, which include molecular weight (MW), hydrogen bond donors (nHD), hydrogen bond acceptors (nHA), and octanol-water partition coefficient (LogP). If phytochemicals did not meet more than one criterion of Lipinski's rule of five, they were considered to have Lipinski violations (LV). Furthermore, phytochemicals that satisfied Lipinski's rule of five criteria underwent for molecular docking analysis.

Disease Targets Exploration

Upon extensive literature survey, 20 disease targets have been identified as significant contributors to the development of epithelial ovarian cancer. Among the disease targets identified, mitogen-activated protein kinase 3 (MAPK3) and poly (ADP-ribose) polymerase 1 (PARP1) have been highlighted as playing crucial roles in ovarian cancer, with MAPK3, encoding ERK1, being essential for cell growth, survival, immune response, and neuronal function (Hu et al., 2023; Li et al., 2023). Dysregulation related to cancer, and inflammation, making it a therapeutic target for diseases (Redenšek Trampuž et al., 2023). The PARP1, a critical enzyme in DNA repair, cell death, and genomic stability, pivotal in cancer therapy, aging, and inflammatory responses. Its inhibitors show promise in treating BRCA-mutated cancers and other DNA repair-deficient tumours (Grypari et al., 2023).

Protein Data Extraction and Preparation

The 3-D protein structures of genes were obtained in 'pdb' format from the Protein Data Bank (Safran et al., 2021). In cases where any residues were found to be missing in the protein structure, MODELLER (version 10.5) was employed for protein structure modelling purposes (Webb and Sali, 2016). Subsequently, the proteins were prepared using UCSF Chimera X software (version 1.7) by removal of water molecules, co-ligands, and heteroatoms, and the addition of charge and polar hydrogen, ensuring the optimal preparation of all target proteins for molecular docking (Meng et al., 2023).

Molecular Docking

Molecular docking was carried out to assess the interactions between phytochemicals and disease targets. The 3D conformations of FDA-approved drugs and screening phytochemicals were obtained from PubChem, serving as ligands in the molecular docking process. Energy reduction was executed using UCSF ChimeraX software, ensuring a stable protein structure by eliminating undesirable torsion angles and nonstandard residues. This stabilization facilitated the binding of ligands to the receptor molecule, eliminating steric clashes (Meng et al., 2023). The molecular docking procedure employed Autodock Vina, while Open Babel was utilized to minimize the energy of ligands, as well as converting receptor and ligands from pdb to pdbqt format (Tanchuk et al., 2016; Yoshikawa and Hutchison, 2019).

Blind docking was performed by enlarging the grid box size to encompass the entire protein structure. The comprehensive evaluation of ligand-protein binding affinities encompassed to assess docking score (Kcal/mol) and relative mean square deviation (RMSD). The resulting protein-ligand complexes were analysed and visualized using Discovery Studio 2021 Client (Baroroh et al., 2023).

ADMET Profiling of Lead Compounds

In the absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiling, parameters such as P-glycoprotein inhibitor (Pgp-inh), P-glycoprotein substrate (Pgp-sub), human intestinal absorption (HIA), Fraction absorbed in the upper 20% of the small intestine (F20%), Caco-2 permeability (Caco-2), plasma protein binding (PPB), volume of distribution (VD), fraction unbound (Fu), cytochrome P450 1A2 inhibitor (CYP1A2-inh), cytochrome P450 1A2 substrate (CYP1A2-sub), cytochrome P450 2C19 inhibitor (CYP2C19-inh), cytochrome P450 2C19 substrate (CYP2C19-sub), cytochrome P450 2C9 inhibitor (CYP2C9-inh), cytochrome P450 2C9 substrate (CYP2C9-sub), cytochrome P450 2D6 inhibitor (CYP2D6-inh), cytochrome P450 2D6 substrate (CYP2D6-sub), cytochrome P450 3A4 inhibitor (CYP3A4-inh), cytochrome P450 3A4 substrate (CYP3A4-sub), clearance (CL), half-life (T12), Ames test for mutagenicity (Ames), route of administration (ROA), skin sensitization (SkinSen), carcinogenicity assessment (Carcinogenicity), p53 suppressor

ratio (SR-p53), and genotoxic carcinogenicity mutagenicity were considered (Xiong et al., 2021). Compounds meeting all criteria in the medium range of empirical decisions as per ADMETlab 2.0 were chosen for further analysis.

Lead Compounds Drug Profiling

The lead compounds were screened based on drug-likeness (DL) and oral bioavailability (OB). Drug-likeness is the degree to which a drug's chemical structure resembles that of other, well-known drugs. Oral bioavailability contrasts with the dosage of medications taken orally, which reach the bloodstream and have an impact on local tissues and organs before having a comparable pharmacological effect (Ahmed et al., 2022). To evaluate a compound's potential therapeutic value, both properties are crucial. Screening thresholds for DL and OB are ≥ 0.18 and ≥ 0.30 respectively. Molsoft L.L.C. was utilized for DL, and for OB analysis SwissADME tool was used (Gfeller et al., 2014).

Molecular Dynamics Simulation

The molecular dynamics (MD) simulation was conducted using GROMACS software, version 2023.3, on a Linux operating system. The results obtained were visualized through Microsoft Excel. The force fields employed for the simulation included the CHARMM36 force field for the protein structure and the CHARMM all atoms force field for ligand parameterization. To prepare the system for

simulation, the TIP 3-point solvent model was used for solvation. Post-solvation, neutralization of the system was achieved by adding appropriate quantities (0.1M) of Na^+ and Cl^- ions. Energy minimization of the system was performed using the steepest descent minimization algorithm, involving 50,000 steps. The convergence criterion was set at a maximum force threshold of 10.0 kJ/mol. Equilibration of the system was carried out in two phases. First, a 1000 ps NVT equilibration was conducted under constant volume and temperature (310K) conditions. Subsequently, a 1000 picoseconds (ps) NPT equilibration was performed under constant pressure (1 bar) and temperature (310K). During the simulation run, the integrator employed was the leap-frog integrator for MD simulations. The total number of steps undertaken was 150,000,000, corresponding to a simulation time of 300 ns with a time step of 2 femtoseconds (fs). Output control parameters included saving energy data every 100 ps, updating log files every 100 ps, and saving coordinate data every 100 ps. To maintain bond constraints, the LINCS algorithm was used, and hydrogen bonds were constrained. Neighbor searching and van der Waals interactions were managed using a Verlet cutoff scheme and a grid-based approach, respectively. Temperature coupling was achieved using a modified Berendsen thermostat (V-rescale) with a time constant of 0.1 ps. Pressure coupling utilized the Parrinello-Rahman method with a time constant of 2.0 ps, maintaining pressure at 1.0 bar. Periodic boundary conditions (PBC) were applied in three dimensions.

Long-range electrostatic interactions were accounted for using the Particle mesh Ewald (PME) algorithm. Post-simulation analysis encompassed various parameters including root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), hydrogen bonds (HB), and solvent accessible surface area (SASA). For Principal Component analysis (PCA), the subsequent examination concentrated on crucial factors, including eigenvalues and eigenvectors, aiming to reveal the principal components that dictate the dynamics of the system. All MD simulation analysis were performed using the built-in tools of GROMACS version 2023.3 (Páll et al., 2020).

Binding Free Energy Analysis

We utilized the gmx_MMPBSA package (version 1.6.2) to compute the binding free energy (ΔG) analysis on ligand-protein complexes. Binding free energy was analysed

utilizing MM/GBSA (molecular mechanics/generalized born surface area) method (Valdés-Tresanco et al., 2021). The *GROMACS trajectory*, topology, and index files of the target complexes were employed in this methodology. The determination of the total binding free energy (ΔG total) involved subtracting ΔG receptor from ΔG complex, followed by subtracting ΔG ligand from the obtained result. Subsequently, the data underwent analysis using the gmxMMPBSA ana module. The energy components such as changes in van der Waals energy ($\Delta VDWAAALS$), electrostatic (Coulombic) energy (ΔEEL), Generalized Born (GB) solvation energy (ΔEGB), solvent-accessible surface area (SASA) term ($\Delta ESURF$), gas-phase free energy ($\Delta GGAS$), solvation free energy ($\Delta GSOLV$), and Overall change in total free energy ($\Delta TOTAL$) were analysed in the term of average value with standard deviation to assess the binding free energy.

Results and Discussion

In the past few years, there has been a continuous rise in both morbidity and mortality rates associated with EOC, significantly affecting women's reproductive health (Keyvani et al., 2023). Effective treatment options for advanced and recurrent EOC are currently limited. Consequently, there remains a need for the development of natural drugs to address the challenges in treating EOC. Plants contain various phytochemicals which

show therapeutic effects. The application of medicinal plants involves a broad category of practices, spanning the use of various plant parts such as roots, bark, leaves, flowers, and fruits, along with their extracts (Moiketsi et al., 2023). These plants have various phytochemicals with therapeutic properties. *C. dactylon* has various phytochemicals having medicinal properties that can be used as drug (Alex et al., 2023).

Potential Phytochemical Profiling

In this study, a dataset comprising 89 phytochemicals of *Cynodon dactylon* was extracted from various databases, including IMPPAT, Dr. Duke, and KNApSACk, along with extensive literature survey. Out of the initial 89

phytochemicals, 84 were deemed acceptable based on parameters such as MW, nHD, nHA, and LogP, in accordance with Lipinski's rule of five. The rejection of Lipinski's rule led to the elimination of five phytochemicals (Table 2). These identified phytochemicals were then subjected to further analysis in molecular docking.

Table 2 Screening of phytochemicals based on Lipinski's rule

Compounds	LogP	MW	nHA	nHD	LV
Acetoacetic acid	-0.68	102.03	3	1	0
4-Hydroxybenzoic acid	1.51	138.03	3	2	0
2-Methoxy-4-vinylphenol	2.25	150.07	2	1	0
Dihydroxyacetone	-1.27	90.03	3	2	0
Palmitic Acid	6.73	256.24	2	1	1
Phenylacetaldehyde	1.16	120.06	1	0	0
9,12-Octadecadienoic acid	6.65	280.24	2	1	1
D-Glucose	-2.50	180.06	6	5	0
N,N-Dimethylformamide	-1.01	73.05	2	0	0
Mestranol	4.18	310.19	2	1	0
Thymol	3.15	150.10	1	1	0
2,4-Di-tert-butylphenol	4.83	206.17	1	1	0
Furfuryl alcohol	0.35	98.04	2	1	0
Furfural	0.82	96.02	2	0	0
1,4-Butanediol	-0.68	90.07	2	2	0
Ethylene glycol diacetate	-0.13	146.06	4	0	0
Maltol	0.11	126.03	3	1	0
Vanillic Acid	1.40	168.04	4	2	0
Conhydrine	0.73	143.13	2	2	0
2,3-Dihydrobenzofuran	2.32	120.06	1	0	0

Compounds	LogP	MW	nHA	nHD	LV
6,10,14-Trimethylpentadecan-2-one	6.48	268.28	1	0	1
Neophytadiene	8.01	278.30	0	0	1
Ascaridole	2.55	168.12	2	0	0
Syringic acid	1.21	198.05	5	2	0
Methyl 2-chloropropionate	0.99	122.01	2	0	0
2-Methylpentanal	1.68	100.09	1	0	0
2,4,7,9-Tetramethyl-5-decyne-4,7-diol	3.21	226.19	2	2	0
3-Methyl-1,2-cyclopentanediol	-0.05	116.08	2	2	0
2-Pentadecanone	5.68	226.23	1	0	1
2-Oxo-octanoic acid	2.05	158.09	3	1	0
N-(2-(Dimethylamino)ethyl)acrylamide	-0.14	142.11	3	1	0
10-Undecyn-1-ol	3.01	168.15	1	1	0
3,6,9-Trioxaundecanedioic acid	-1.20	222.07	7	2	0
Friedelin	7.55	426.39	1	0	1
2-(4-Hydroxyphenyl)propanoic acid	1.58	166.06	3	2	0
21-Diazopregn-4-ene-3,20-dione	3.47	340.22	4	0	0
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	-0.68	144.04	4	1	0
Ethane, 1-chloro-2-nitro-	0.71	108.99	3	0	0
Isovitexin	0.41	432.11	10	7	1
Beta-Sitosterol	7.66	414.39	1	1	1
5-Hydroxymethylfurfural	0.15	126.03	3	1	0
Ethyl 4-hydroxycinnamate	2.70	192.08	3	1	0
Conhydrinone	0.79	141.12	2	1	0
gamma-Coniceine	2.73	125.12	1	0	0
Ergometrine	1.04	325.18	5	3	0
Retinol	5.79	286.23	1	1	1
Oleic Acid	7.13	282.26	2	1	1
Ferulic acid	1.80	194.06	4	2	0

Compounds	LogP	MW	nHA	nHD	LV
1-(Cyclopropyl-nitro-methyl)-cyclopentanol	1.62	185.11	4	1	0
Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butyryl]-	-0.41	210.14	4	1	0
Tetradec-13-en-11-yn-1-ol	4.48	208.18	1	1	0
1-[3-(Cyclohexylamino)propyl]guanidine	-0.56	198.18	4	5	0
7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	3.92	276.17	3	0	0
9-Oximino-2,7-diethoxyfluorene	3.63	283.12	4	1	0
Dasycarpidan-1-methanol, acetate (ester)	3.75	326.20	4	1	0
12-Dimethylamino-10-oxododecanoic acid	0.46	257.20	4	1	0
6-Octadecynoic acid, methyl ester	6.60	294.26	2	0	1
8-Methylenedispiro[2.1.2.4]undecane	3.89	162.14	0	0	0
2-Cyclohexen-3,6-diol-1-one, 2-tetradecanoyl-	5.70	338.25	4	4	1
Elaidic Acid	7.13	282.26	2	1	1
p-Coumaric acid	1.92	164.05	3	2	0
beta-Ionone	3.54	192.15	1	0	0
Squalene	12.25	410.39	0	0	1
Rhododendrol	1.50	166.10	2	2	0
1-Chloro-2-methylpropyl chloroformate	2.38	169.99	2	0	0
Phytol	7.76	296.31	1	1	1
Vitexin	0.33	432.11	10	7	1
Apigenin	3.31	270.05	5	3	0
Luteolin	2.90	286.05	6	4	0
Linoleic Acid	6.65	280.24	2	1	1
Tricin	3.06	330.07	7	3	0
Alfacalcidol	5.67	400.33	2	2	1
Pyrrolidine, 1-(1-oxo-2,5-octadecadienyl)-	6.96	333.30	2	0	1
3,7,11,15-Tetramethyl-2-hexadecen-1-OL	7.76	296.31	1	1	1
1,3-Diazocane-2-thione	0.61	144.07	2	2	0

Compounds	LogP	MW	nHA	nHD	LV
Ergometrine	1.04	325.18	5	3	0
Stigmasterol acetate	7.75	454.38	2	0	1
Desulphosinigrin	-0.76	279.08	7	5	0
2-O-(alpha-D-glucopyranosyl)glycerol	-2.70	254.10	8	6	1
Arundoin	7.71	440.40	1	0	1
1,1,2,3-Tetramethylcyclobutane	3.90	112.13	0	0	0
2-Allyl-5-t-butylhydroquinone	3.70	206.13	2	2	0
Ascorbic Acid	-1.42	176.03	6	5	0
r-Limonene	2.34	184.11	3	0	0

LogP: Partition coefficient; MW: Molecular weight; nH-A: H-bond acceptor; nH-D: H-bond donor; LV: Lipinski violations

Molecular Docking Analysis

The molecular docking process utilized drug targets with disease-related targets corresponding to the 20 genes. Nineteen phytochemicals were docked with protein structures of key hub genes, including AKT1, EGFR, TNF, CTNNB1, CASP3, BCL2, ESR1, HIF1A, HSP90AA1, ERBB2, SRC, MAPK3, MMP9, PPARG, MTOR, PTGS2, PARP1, TLR4, EZH2, and CXCL8, identified by PDB IDs including 1UNQ, 1M17, 2E7A, 1JDH, 1NME, 2W3L, 1GWQ, 1H2L, 1OSF, 1MFG, 1A09, 4QTB, 1ITV, 1KNU, 2FAP, 5F19, 1UK1, 2Z66, 4MI0, and 1ICW, respectively. The resulting interactions were visualized in a heat map, depicting affinity between phytochemicals and targeted proteins associated with the 20 genes, with darker colours indicating lower receptor-ligand affinity in kcal/mol (Fig. 2). Molecular docking interactions between selected bioactive compounds and their

respective target proteins, Stigmasterol acetate (PubChem ID 6437330) exhibited the highest binding affinity with poly [ADP-ribose] polymerase-1, a corresponding protein (PDB ID: 1UK1) of PARP1 gene, at -10.9 kcal/mol, while arundoin (PubChem ID 12308619) and beta-sitosterol (PubChem ID 222284) both demonstrated strong affinities with the same targeted protein at -10.4 kcal/mol and -10.1 kcal/mol, respectively. Despite similar total bond numbers, indicating comparable overall binding interactions with 1UK1 protein, these ligands exhibited distinct characteristics. Moreover, beta-sitosterol, when interacting with mitogen-activated protein kinase 3 (MAPK3; PDB ID: 4QTB) protein, exhibited a binding affinity of -10.1 kcal/mol, forming 2 hydrogen bonds and a total of 10 bonds formed through interaction between ligand and 10 residues. Rucaparib, an FDA-approved drug represented by PubChem ID 9931954,

interacted with PARP1 with a binding affinity of -9.7 kcal/mol, forming 2 hydrogen bonds and a total of 8 bonds (Table 3). The 3D and 2D structures of the targeted protein, along with

the corresponding bonds formed during the interaction with neighbouring amino acids, were depicted in Fig. 3A-E.

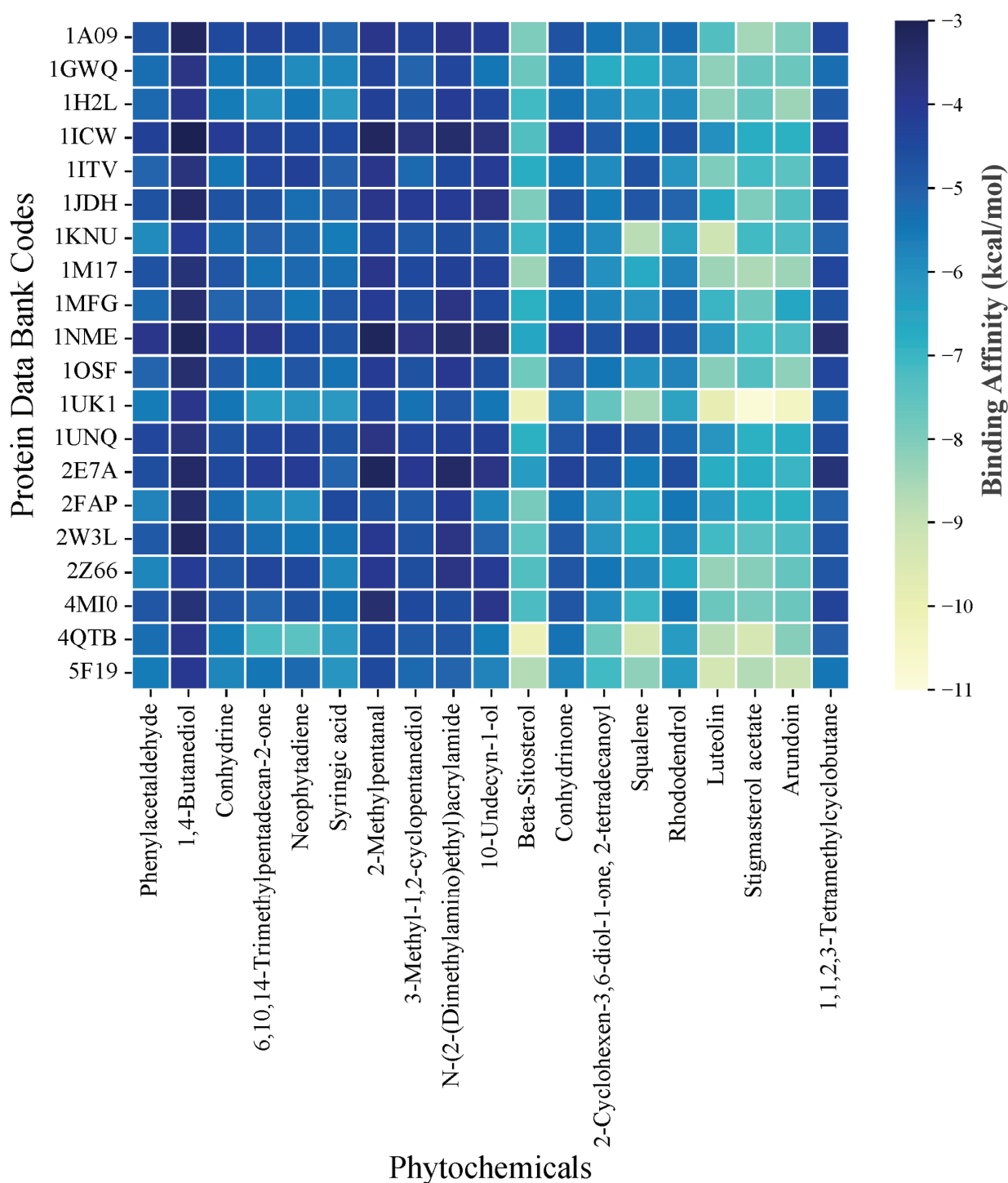


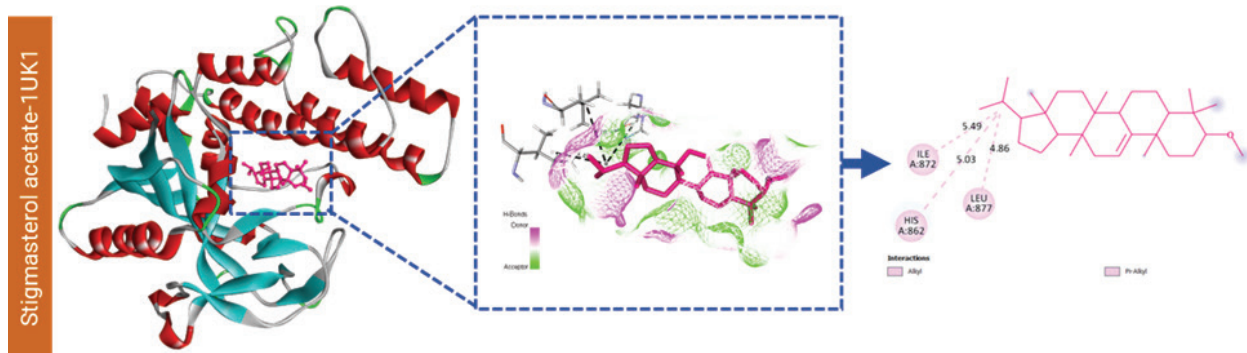
Fig. 2 Heat map of molecular docking illustrates interactions between phytochemicals and proteins linked to the top 20 hub genes

Table 3 Interactions between selected bioactive compounds and target proteins

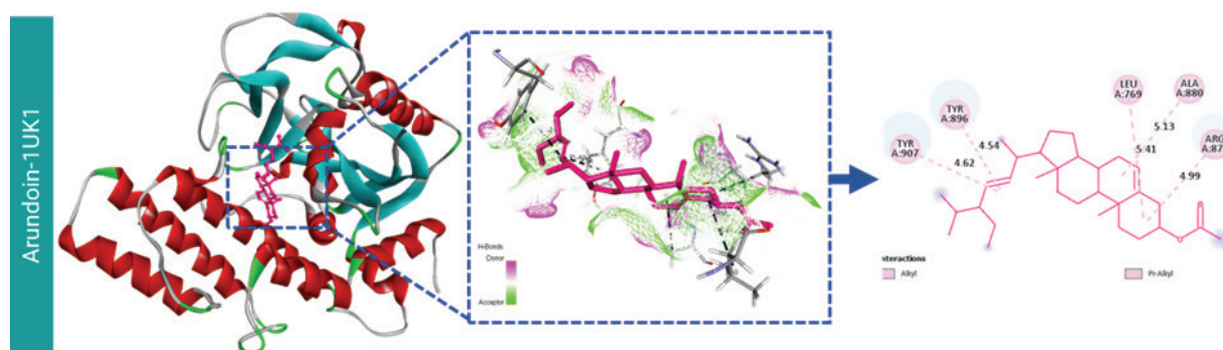
S. N.	Ligand	PubChem ID	Target (PDB ID)	Binding affinity (kcal/mol)	Hydrogen bonds	Total no. of bonds	Type of Bond	Interacting Residues	Bond Length
1.	Stigmasterol acetate	6437330	PARP1 (1UK1)	-10.9	0	3	Alkyl	N:UNK1 - A:ILE872	5.49
							Alkyl	N:UNK1 - A:LEU877	4.86
							Pi-Alkyl	A:HIS862 - N:UNK1	5.03
2.	Arundoin	12308619	PARP1 (1UK1)	-10.4	0	5	Alkyl	A:LEU769 - N:UNK1	5.41
							Alkyl	A:ARG878 - N:UNK1	4.99
							Alkyl	A:ALA880 - N:UNK1	5.13
							Pi-Alkyl	A:TYR896 - N:UNK1	4.54
							Pi-Alkyl	A:TYR907 - N:UNK1	4.62
3.	Beta-Sitos-terol	222284	PARP1 (1UK1)	-10.1	0	5	Pi-Sigma	N:UNK1:C - A:TYR907	3.92
							Alkyl	A:ARG878 - N:UNK1	4.98
							Pi-Alkyl	A:TYR896 - N:UNK1	4.60
							Pi-Alkyl	A:TYR907 - N:UNK1	5.46
							Pi-Alkyl	A:TYR907 - N:UNK1	5.11

S. N.	Ligand	PubChem ID	Target (PDB ID)	Binding affinity (kcal/mol)	Hydrogen bonds	Total no. of bonds	Type of Bond	Interacting Residues	Bond Length
4.	Beta-Sitosterol	222284	MAPK3 (4QTB)	-10.1	2	10	Conventional Hydrogen Bond	N:UNK1:H - A:MET125:O	2.01
							Carbon Hydrogen Bond	A:THR127:HA - N:UNK1:O	3.03
							Alkyl	A:ILE48 - N:UNK1	5.42
							Alkyl	A:VAL56 - N:UNK1	4.79
							Alkyl	A:LEU173 - N:UNK1	4.86
							Alkyl	A:CYS183 - N:UNK1	4.88
							Alkyl	N:UNK1 - A:ILE48	4.93
							Alkyl	N:UNK1 - A:LEU173	4.58
							Pi-Alkyl	A:TYR53 - N:UNK1	4.74
							Pi-Alkyl	A:TYR53 - N:UNK1	4.55
5.	Rucaparib (FDA Approved Drug)	9931954	PARP1 (1UK1)	-9.7	2	8	Carbon Hydrogen Bond	N:UNK1:C - A:ASP766:O	3.57
							Carbon Hydrogen Bond	N:UNK1:C - A:ASP770:OD2	3.39
							Halogen (Fluorine)	A:PHE897:C - N:UNK1:F	3.33
							Pi-Pi Stacked	A:TYR896 - N:UNK1	5.26
							Pi-Pi Stacked	A:TYR907 - N:UNK1	4.49
							Pi-Pi Stacked	N:UNK1 - A:TYR896	5.35
							Pi-Pi Stacked	N:UNK1 - A:TYR907	3.90
							Pi-Pi T-shaped	A:HIS862 - N:UNK1	4.93

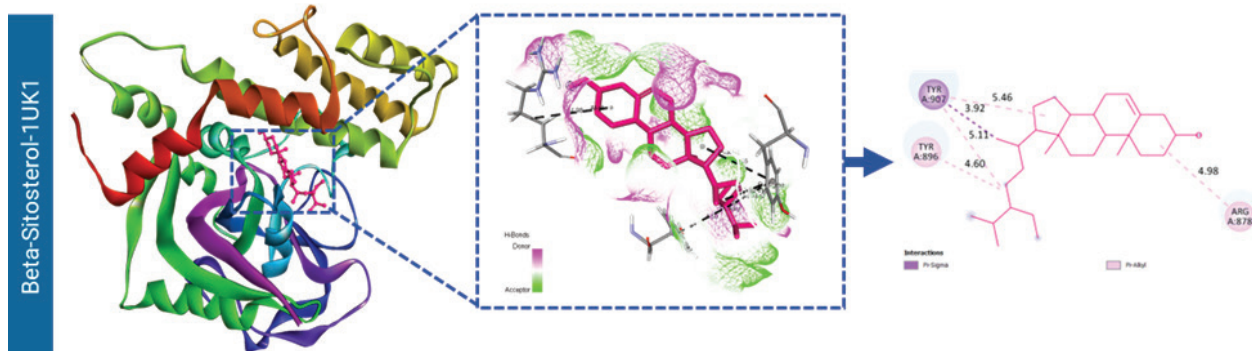




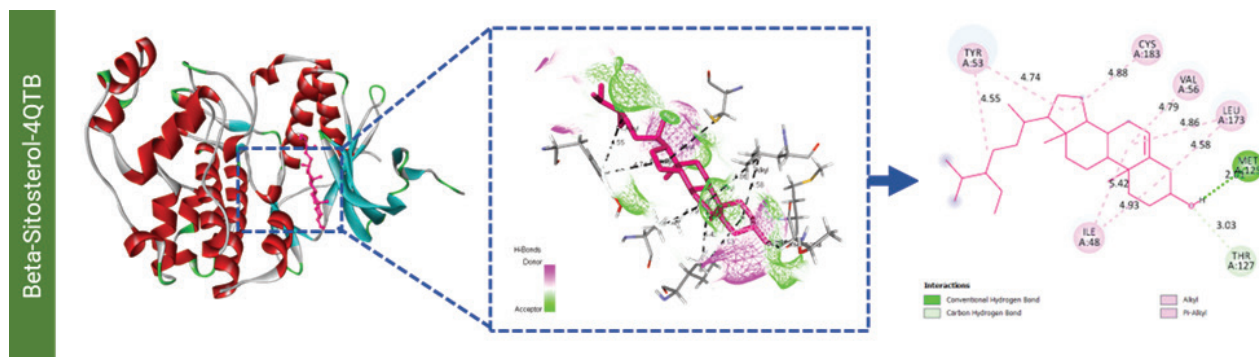
(A)



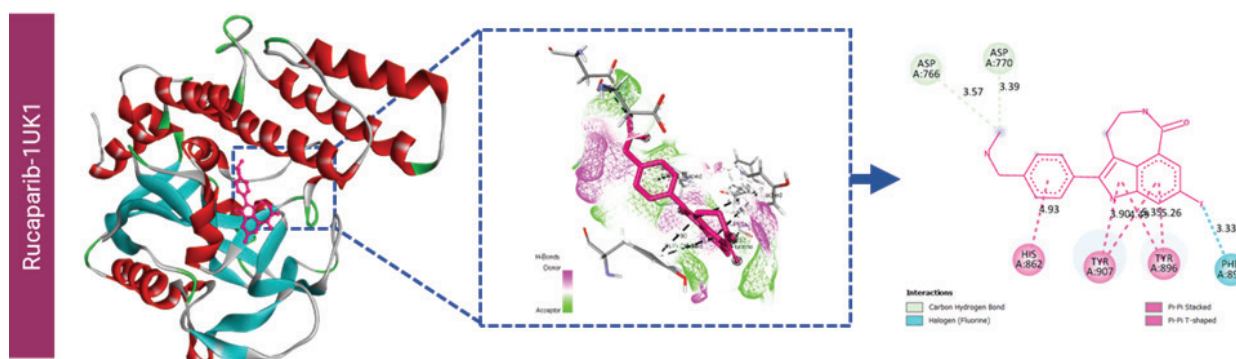
(B)



(C)



(D)



(E)

Fig. 3 (A-E) 3D and 2D structures of targeted protein docked with different phytochemicals. (A) Stigmasterol acetate with the target protein 1UK1 (B) Arundoin with the target protein 1UK1 (C) Beta-Sitosterol with the target protein 1UK1 (D) Beta-Sitosterol with the target protein 4QTB (E) Rucaparib (FDA-approved drug) with the protein 1UK1

In several molecular docking studies, phytosterols like stigmasterol acetate, and beta-sitosterol showed significant binding affinities with SIRT1, AMPK, and other disease targets which showed an agreement with our study in which stigmasterol acetate, arundoin, and beta-sitosterol demonstrated remarkable binding affinities with specific target proteins, such as poly [ADP-ribose] polymerase-1 (PDB ID: 1UK1) and mitogen-activated protein kinase 3 (MAPK3; PDB ID: 4QTB), highlighting their potential as candidates for therapeutic applications (Wang et al., 2022; Anggreini et al., 2023). In another study, AKT1-stigmasterol complex demonstrates stable binding in pneumonia treatment (Peng et al., 2022). Additionally, phytosterols such as β -sitosterol, α -amyrin, and stigmasterol have been identified as potential inhibitors of cancer targets (Oladipupo et al., 2023). Moreover, beta-sitosterol from *Polygonum hydropiper* L. exhibited prominent interaction with BACE1 compared to stigmasterol, supporting its

potential against BACE1 and MAO drug targets (Ayaz et al., 2022).

Drug Profiling of Lead Compounds

The comprehensive assessment of lead compounds for drug development, including arundoin, beta-sitosterol, and stigmasterol acetate, entailed examining their PubChem ID, Rader Chart, 2D Structure, Bioavailability, and Drug-likeness. Arundoin had a moderate bioavailability (0.55), indicating potential for absorption and distribution, but its drug-likeness score of -0.13, falling below the threshold of 0.18, rendered it unsuitable for drug development. Beta-sitosterol showed bioavailability score of 0.55, suggesting comparable potential for absorption and having a drug-likeness score of 0.78, indicating a higher resemblance to known drugs compared to arundoin and positioning it as a more favourable drug development

candidate. Moreover, stigmasterol acetate, with a bioavailability score of 0.55 and a high drug-likeness score of 0.95, exhibited significant similarities to established drugs, indicating its potential as a promising candidate for further exploration in drug development (Table 4). These leading compounds also exhibited values below the moderate threshold for various ADMET parameters, including Pgp-inh, Pgp-sub, HIA, F(20%), Caco-2, PPB, VD, Fu, CYP1A2-inh, CYP1A2-sub, CYP2C19-inh, CYP2C19-

sub, CYP2C9-inh, CYP2C9-sub, CYP2D6-inh, CYP2D6-sub, CYP3A4-inh, CYP3A4-sub, CL, T1/2, Ames, ROA, SkinSen, carcinogenicity, SR-p53, and genotoxic, mutagenicity (Table 5). Similar results were obtained in previous studies in which beta-sitosterol and stigmasterol from *Tripterygium wilfordii* and 'Wenxin Keli' were considered as drugs based on bioavailability and drug-likeness against diabetic kidney disease and atrial fibrillation, respectively (Zhang et al., 2020).

Table 4 Profiling of lead compounds for drug development

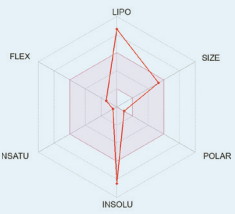
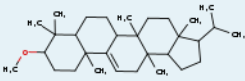
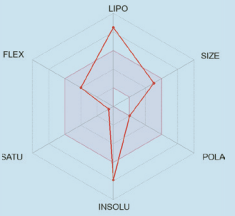
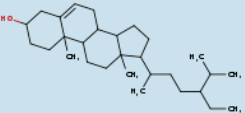
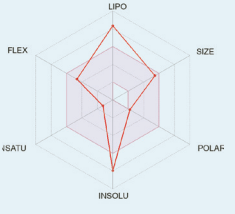
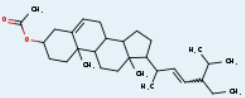
S. No.	Ligand	PubChem ID	Rader Chart	2D Structure	Bioavailability	Drug-likeness
1	Arundoin	12308619			0.55	-0.13
2	Beta-Sitosterol	222284			0.55	0.78
3	Stigmasterol acetate	6437330			0.55	0.95

Table 5 Characterization of lead compounds through ADMET profiling

Category	Parameters	Arundoin	Beta-Sitosterol	Stigmasterol acetate
Absorption	Pgp-inh	0.53	0.34	0.45
	Pgp-sub	0.00	0.00	0.00
	HIA	0.01	0.00	0.00
	F (20%)	0.39	0.01	0.01
	Caco-2	-4.93	-4.76	-4.66
Distribution	PPB	1.00	0.98	0.98
	VD	2.10	1.96	2.64
	Fu	0.01	0.01	0.02
Metabolism	CYP1A2-inh	0.02	0.044	0.026
	CYP1A2-sub	0.482	0.491	0.377
	CYP2C19-inh	0.055	0.074	0.073
	CYP2C19-sub	0.977	0.958	0.934
	CYP2C9-inh	0.063	0.096	0.092
	CYP2C9-sub	0.064	0.314	0.142
	CYP2D6-inh	0.046	0.005	0.058
	CYP2D6-sub	0.616	0.409	0.535
	CYP3A4-inh	0.139	0.202	0.355
	CYP3A4-sub	0.776	0.784	0.753
Excretion	CL	15.85	16.69	7.85
	T12	0.01	0.01	0.01
Toxicity	Ames	0.02	0.03	0.03
	ROA	0.28	0.02	0.02
	Skin Sensitivity	0.06	0.13	0.03
	Carcinogenicity	0.02	0.05	0.07
	SR-p53	0.006	0.013	0.069
	Genotoxic Carcinogenicity Mutagenicity	0.00	0.00	0.00

Structural Stability Analysis

Molecular dynamics simulations were employed to scrutinize interactions between compounds and proteins at a molecular scale. This investigation specifically concentrated

on the most significant interactions, namely the binding of stigmasterol acetate and beta-sitosterol with poly[ADP-ribose] polymerase-1 (PDB ID: 1UK1), as well as the binding of beta-sitosterol with mitogen-activated

protein kinase 3 (PDB ID: 4QTB). Throughout a 300-nanoseconds (ns) simulation, a total of 3000 frames were recorded to assess the structural stability of complexes such as stigmasterol acetate-1UK1, beta-sitosterol-1UK1, and beta-sitosterol-4QTB using various parameters including RMSD, RMSF, Rg, SASA, HB, and PCA.

Root Mean Square Deviation (RMSD)

The RMSD is a crucial metric in molecular dynamics (MD) simulations, assessing the structural stability and fluctuations of biomolecular systems over time. In analysing the backbone RMSD for three complexes, specifically stigmasterol acetate-1UK1, the average RMSD was 1.6 nm with a range of 0.1 to 3.0 nm and standard deviation (SD) of 0.66 nm, indicating the highest level of structural variations among them. The complex remained stable for the first 30 nanoseconds, but afterwards, an increase in RMSD was observed, indicating a decline in structural stability. Similarly, beta-sitosterol-1UK1 showed an average RMSD of 1.9 nm, with a range of 0.1 nm to 3.0 nm, and a lower SD of 0.60, representing more consistent behaviour with a high degree of variability during the simulation. Contrastingly, beta-sitosterol-4QTB exhibited a lower average RMSD of 0.5 nm, with a range of 0.3 nm to 0.7 nm, as well as a low SD of 0.04 nm, indicating a more stable conformation and a highly consistent backbone behaviour throughout the MD simulation (Fig. 4A).

Root Mean Square Fluctuation (Rmsf)

The RMSF is an essential metric employed in MD simulations to assess the dynamic behaviour and flexibility of specific atoms or residues within biomolecular systems. During the 300 nanoseconds MD simulation reaction, the average RMSF values for all three compounds stigmasterol acetate-1UK1, beta-sitosterol-1UK1, and beta-sitosterol-4QTB were found uniform at 0.1 nm, indicating a moderate overall flexibility in their molecular structures. Minimum RMSF values of 0.0 nm suggested that certain atoms or residues remained stable or exhibited minimal deviation from their initial positions during the simulation. On the other hand, maximum RMSF values, such as 0.7 nm for beta-sitosterol-1UK1, highlighted regions with higher degrees of motion or flexibility. The SD values of 0.05, 0.08, and 0.06 nm respectively indicated relatively consistent fluctuations across atoms or residues, providing insights into the uniformity of the dynamic behaviour. The RMSF analysis suggested that these complexes exhibited a moderate level of flexibility during the MD simulation, with specific regions showing varying degrees of motion, while other regions remain stable (Fig. 4B).

Radius of Gyration (Rg)

The radius of gyration is a structural parameter that characterizes the overall dimensions and distribution of mass in a molecular system. Stigmasterol acetate-1UK1 and beta-sitosterol-1UK1 exhibited an average Rg of 2.1

nm, with minimal variation, as indicated by the identical minimum and maximum Rg values of 2.1 and 2.2 nm, respectively. The small SD value of 0.01 nm further emphasized the stability and compactness of these molecules throughout the simulation. In contrast, beta-sitosterol-4QTB showed a slightly higher average Rg of 2.2 nm, with a range from 2.2 to 2.3 nm implying a relatively larger structural variability during the MD simulation. However, the small SD of 0.01 nm implied that, despite its variability, beta-sitosterol-4QTB maintains a stable structural behaviour (Fig. 4C).

Solvent Accessible Surface Area (SASA)

Solvent Accessible Surface Area is a critical metric in MD simulations, providing insights into the accessibility of a molecule's surface to solvent molecules. The average SASA values revealed subtle distinctions, with beta-sitosterol-1UK1 displaying a slightly higher average (178.4 nm²) compared to stigmasterol acetate-1UK1 (178.0 nm²) and beta-sitosterol-4QTB (175.4 nm²). These complexes exhibited minimum SASA values of 169.1, 169.4, and 165.2 nm², respectively, indicating potential structural changes or compact conformations during the simulation. Additionally, stigmasterol acetate-1UK1, beta-sitosterol-1UK1, and beta-sitosterol-4QTB exhibited maximum SASA values of 190.2, 193.5, and 187.4 nm², respectively. Higher maximum SASA values may indicate greater flexibility or conformational changes, suggesting possible interactions with the surrounding solvent

molecules. Elevated standard deviation values across all complexes suggested increased structural flexibility or dynamic behaviour during the MD simulation, particularly remarkable in the case of beta-sitosterol-1UK1 (Fig. 4D).

Hydrogen Bonds (HB)

Hydrogen bonds provide imperative insights into binding affinity, helping to optimize the interactions between drug candidates and their target receptors. For the stigmasterol acetate-1UK1 complex, a single hydrogen bond was consistently formed, occurring 33 times throughout the simulation. No instances of two or more hydrogen bonds were recorded throughout MD simulation (Fig. 4E). In the case of beta-sitosterol-1UK1, one-hydrogen bond interactions were observed 215 times, while two, three, and four hydrogen bond interactions occurred 179, 17, and 5 instances respectively, resulting in a total of 416 hydrogen bonds (Fig. 4F). The beta-sitosterol-4QTB system exhibited a substantial number of hydrogen bond formations. Specifically, one-hydrogen bond interactions were observed 2187 times, while two-hydrogen bond interactions occurred 728 times. Further, three-hydrogen bond interactions were noted 44 times, and no instances of four or more hydrogen bonds were recorded, resulting in a total of 2959 hydrogen bonds (Fig. 4G). Among the three complexes, the beta-sitosterol-4QTB complex seems more stable due to the significant number of hydrogen bonds formed with a focus on one and two-hydrogen bond interactions.

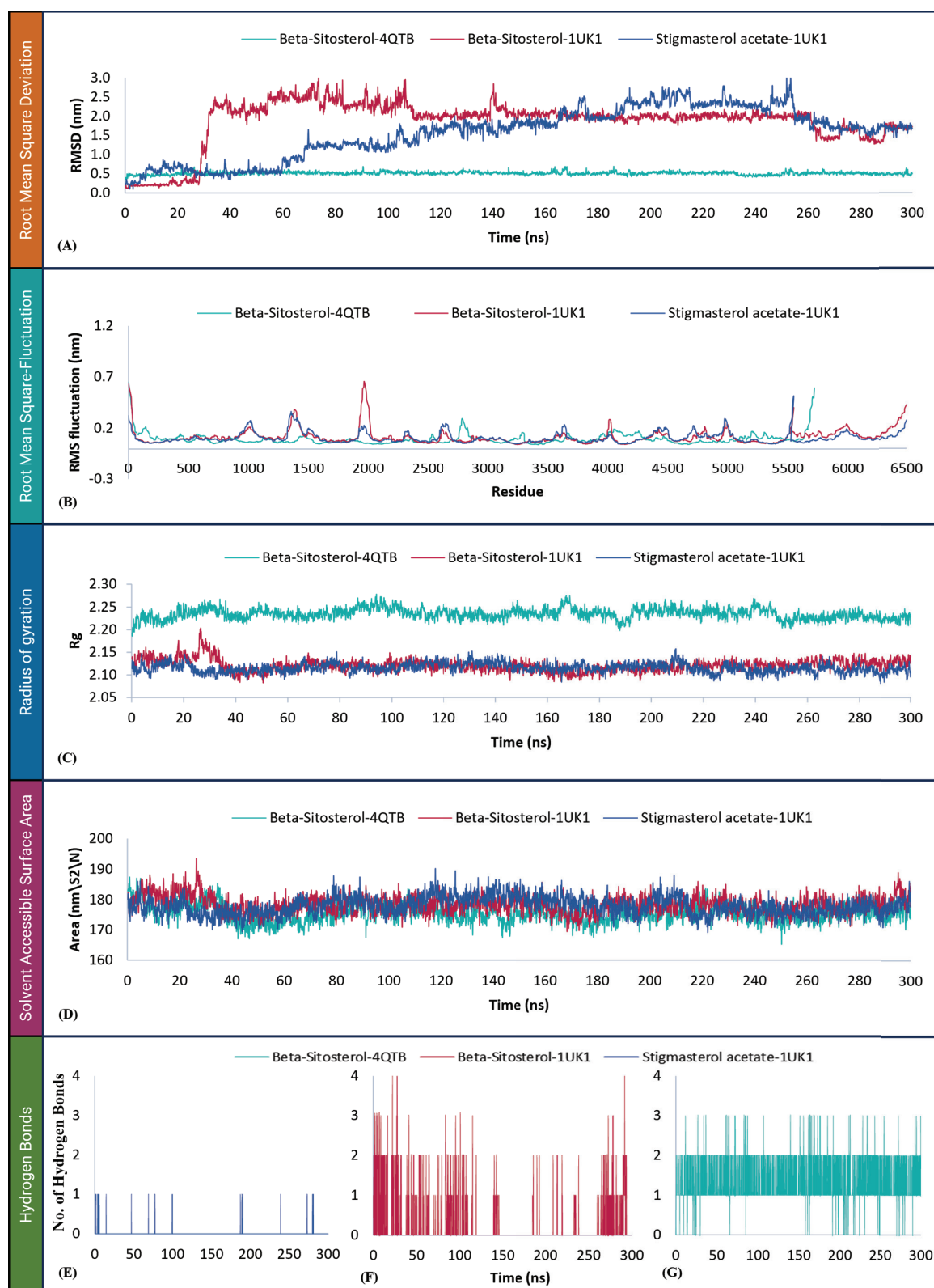


Fig. 4 (A-G) Molecular dynamics simulations endpoints as (A) Root Mean Square Deviation, (B) Root Mean Square Fluctuation, (C) Radius of Gyration (total and around axes), (D) Solvent Accessible Surface, (E) Hydrogen bond formed in Stigmasterol acetate-1UK1 (F) Hydrogen bond formed in beta-sitosterol-1UK1, and (G) hydrogen bond formed in beta-sitosterol-4QTB complex.

The findings of this study are in excellent agreement with those of Shi et al. (2022), who observed stable RMSD values in MD simulations of various compounds binding to PTK2 (Shi et al., 2022). In another study, based on RMSD and RMSF results, beta-sitosterol is more stable compared to other phytochemicals suggesting its inhibitory nature towards diabetes (Ravi et al., 2023). Likewise, The RMSF analysis of natural phytocompounds to treat COVID-19, revealed stable complexes of beta-sitosterol, with fluctuating residues having no visible impact on the protein-ligand complex (Sankar et al., 2021). In several studies based on SASA analysis, evaluating surface accessibility to solvent molecules, aligns with studies examining the compactness of protein-ligand interfaces (Khan et al., 2020; Al-Wahaibi et al., 2022). Similarly, Somadder et al. (2023) explored various ligands' binding to protein targets, noting that ligands with lower SASA values formed more tightly bound interfaces (Somadder et al., 2023). Chen et al. 2022 reported a similar decline in SASA values of beta-sitosterol against gastric cancer. The findings of this study, corroborated by another research that demonstrated beta-sitosterol from *Nyctanthes arbor-tristis* leaves exhibited strong binding affinity and potential to inhibit human DNA topoisomerase I activity, indicate its promise as a cancer inhibitor (Pareek et al., 2023).

Interaction Energy Analysis

The binding free energy (BFE) trajectory analysis utilizing MMGBSA was conducted for 3 ligands such as stigmaterol acetate-1UK1, beta-sitosterol-1UK1, and beta-sitosterol-4QTB

interacting with their respective receptors. The average binding free energy values revealed that stigmaterol acetate-1UK1 and beta-sitosterol-1UK1 exhibited similar average affinities of -22.7 kcal/mol with a range from -40.9 to -2.2, and -43.7 to -3.2 kcal/mol respectively, while beta-sitosterol-4QTB showed a stronger average binding affinity at -36.6 kcal/mol with a range from -49.9 to -23.6 kcal/mol (Fig. 5A). Moreover, stigmaterol acetate-1UK1, beta-sitosterol-1UK1, and beta-sitosterol-4QTB demonstrated SD values of 5.87, 5.03, and 3.16 kcal/mol, respectively. A lower SD value for beta-sitosterol-4QTB indicated a more stable binding interaction, suggesting less fluctuation in binding energy compared to the other ligands.

In the comprehensive analysis of the binding free energy components for each complex, stigmaterol acetate-1UK1 exhibited a robust interaction profile including van der Waals Energy (VDWAALS) value of -29.39 ± 7.47 kcal/mol signifies a strong non-bonded atom interaction, contributing favourably to the binding free energy. Electrostatic energy (EEL) further supported this binding with a negative value of -1.32 ± 1.93 kcal/mol, indicating strong electrostatic interactions. However, the generalized born solvation energy (EGB) introduced a moderately unfavourable effect, measuring at 11.64 ± 3.95 kcal/mol, while the solvent-accessible surface area (ESURF) demonstrated a favourable decrease at -3.65 ± 0.87 kcal/mol. The gas-phase free energy (GGAS) change was found relatively low at -30.72 ± 7.77 kcal/mol. Solvation free energy (GSOLV) contributed unfavourably at 7.99 ± 3.39 kcal/mol. The overall binding free

energy (TOTAL) was observed favourable at -22.72 ± 5.87 kcal/mol (Fig. 5B). Moreover, beta-sitosterol-1UK1 exhibited a similarly robust interaction profile with VDWAALS value of -31.06 ± 7.04 kcal/mol that indicated strong non-bonded atom interactions, while the EEL value of -3.79 ± 3.11 kcal/mol showed significant electrostatic contributions. The EGB established a moderately unfavourable effect at 16.15 ± 4.93 kcal/mol, but this was compensated by a favourable decrease in surface area (ESURF) at -3.97 ± 0.86 kcal/mol. The GGAS value of -34.84 ± 8.43 kcal/mol indicated a relatively low gas-phase free energy change, and solvent effects (GSOLV) contributed unfavourably at 12.18 ± 4.25 kcal/mol. The overall binding free energy (TOTAL) was found favourable at -22.66 ± 5.03 kcal/mol ((Fig. 5C).). In contrast, beta-sitosterol-4QTB

exhibited the strongest interaction profile among the 3 complexes. The VDWAALS value of this complex was found the most negative at -41.2 ± 3.4 kcal/mol, signifying the strongest non-bonded atom interactions. The EEL value of -9.4 ± 1.84 kcal/mol indicated dominant electrostatic interactions. However, the EGB showed a relatively higher unfavourable effect at 19.36 ± 1.77 kcal/mol. The ESURF demonstrated the most favourable decrease in surface area at -5.37 ± 0.43 kcal/mol, and the GGAS value of -50.6 ± 3.53 kcal/mol indicated the lowest gas-phase free energy change. Solvent effects (GSOLV) contributed unfavourably at 13.99 ± 1.55 kcal/mol. Overall, beta-sitosterol-4QTB exhibited the most favourable binding free energy at -36.61 ± 3.16 kcal/mol (Fig. 5D).

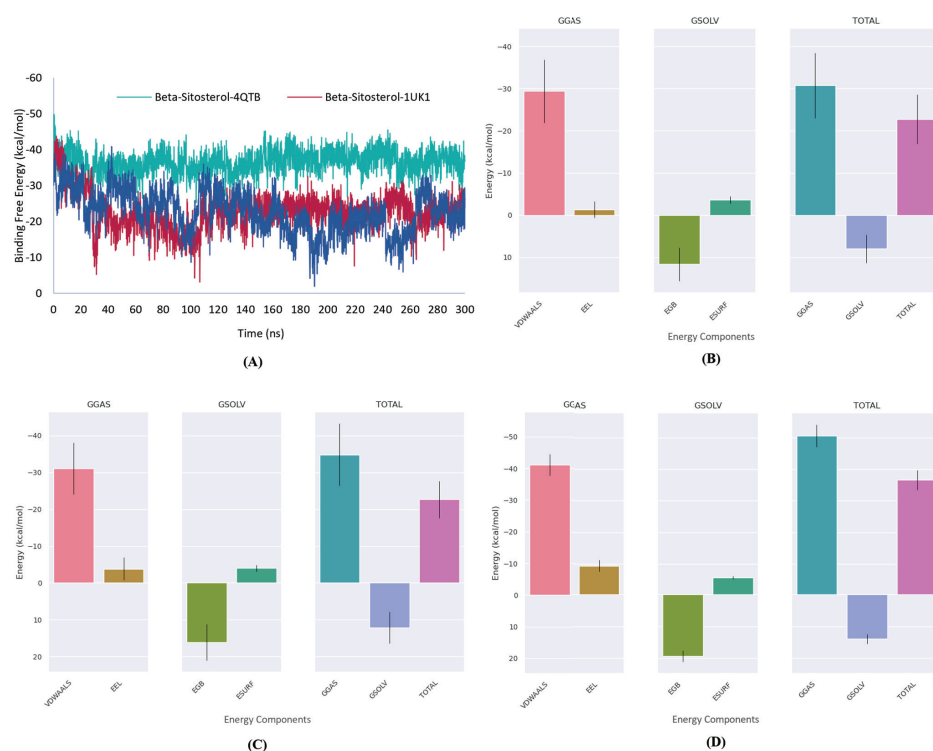


Fig. 5 (A) BFE trajectory of Stigmasterol acetate-1UK1, Beta-Sitosterol-1UK1, and Beta-Sitosterol-4QTB complexes. Binding Free Energy components of (B) Stigmasterol acetate-1UK1 (C) Beta-Sitosterol-1UK1, (D) and Beta-Sitosterol-4QTB

The binding free energy was also separated into three components: ΔG Complex, ΔG Receptor, and ΔG Ligand, with ΔG Total representing the cumulative binding free energy. For stigmasterol acetate-1UK1, the ΔG complex (-6257.56 ± 254.29 kcal/mol) was influenced by the conjugation of the receptor (-6384.24 ± 253.63 kcal/mol) and ligand (149.4 ± 7.96 kcal/mol), resulting in a ΔG Total of -22.72 ± 2.63 kcal/mol that implied a favourable binding interaction. Similarly, beta-sitosterol-1UK1 shows a ΔG complex of -6296.86 ± 267.27 kcal/mol, with the receptor and ligand contributions being -6382.02 ± 266.15 and 107.82 ± 7.36 kcal/mol, respectively. The overall ΔG Total is -22.66 ± 2.49 kcal/mol was also suggested a favourable binding interaction. For beta-sitosterol-4QTB, the ΔG complex is -8448.52 ± 210.12 kcal/mol, with the receptor and ligand contributing -8519.42 ± 210.03 and 107.51 ± 7.16 kcal/mol, respectively. The

resulting ΔG Total was found -36.61 ± 2.41 kcal/mol, also indicating a favourable binding interaction (Table 6). Overall, these findings indicated that beta-sitosterol-4QTB consistently maintained a more favourable interaction with its receptor throughout the simulation. Various studies utilizing molecular docking and MD simulations explored the interactions between phytochemicals from herbs and molecular targets related to cancer, and showed similar results as described by our study (Sankar et al., 2021; Pareek et al., 2023). In conclusion, this investigation integrated molecular docking and MD simulations to dissect the mechanism of action of *C. dactylon* phyto-compounds in treating EOC. These findings described beta-sitosterol's pivotal role as a cancer suppressor, targeting multiple pathways, and emphasizing its multifaceted potential in EOC treatment.

Table 6 Net binding free energies of both the complexes (kcal/mol)

Complex	ΔG Complex	ΔG Receptor	ΔG Ligand	ΔG Total
Stigmasterol acetate-1UK1	-6257.56 ± 254.29	-6384.24 ± 253.63	149.4 ± 7.96	-22.72 ± 2.63
Beta-Sitosterol-1UK1	-6296.86 ± 267.27	-6382.02 ± 266.15	107.82 ± 7.36	-22.66 ± 2.49
Beta-Sitosterol-4QTB	-8448.52 ± 210.12	-8519.42 ± 210.03	107.51 ± 7.16	-36.61 ± 2.41

Conclusion

The current study investigates the unexplored therapeutic potential of *C. dactylon* phytosterols against epithelial ovarian cancer. The systematic curation of a dataset comprising 89 phytochemicals from *Cynodon dactylon*, adhering to strict criteria including

Lipinski's rule of five and ADMET parameters, led to the identification of 19 promising phytochemicals. MAPK3 and PARP1 emerged as pivotal targets, prompting the need for molecular docking investigations to elucidate their interactions and therapeutic potential

against epithelial ovarian cancer. Molecular docking studies revealed that stigmasterol acetate, arundoin, and beta-sitosterol exhibited remarkable binding affinities with specific target proteins, particularly PARP1 and MAPK3, suggesting their potential as therapeutic candidates. Bioavailability and drug-likeness analysis favoured beta-sitosterol and stigmasterol acetate, with the latter showing the highest potential for drug development. In MD simulations, beta-sitosterol-4QTB demonstrated superior stability and lower structural variability

compared to stigmasterol acetate-1UK1 and beta-sitosterol-1UK1. Moreover, hydrogen bond analysis indicated enhanced stability in the beta-sitosterol-4QTB complex. Binding free energy and decomposition analyses also consistently supported its superior affinity and stability. These findings collectively support the therapeutic potential of *Cynodon dactylon* phytosterols, particularly beta-sitosterol, in targeting MAPK3 and PARP1 for combating epithelial ovarian cancer, warranting future experiments.

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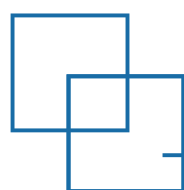
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CHAPTER
07

**Reported
Pharmacological
Profile**



INTRODUCTION



Cynodon dactylon (L.) Pers., commonly known as 'Bermuda grass', couch grass, or dog's tooth grass, is a perennial herb with a wide distribution across tropical and subtropical regions. It has been used in traditional medicine for centuries and possesses a diverse range of pharmacological activities. The pharmacological properties of *C. dactylon* are attributed to its rich phytochemical composition (Khatun et al., 2020). Several studies have identified a plethora of bioactive compounds in the plant, including flavonoids, alkaloids, glycosides, terpenoids and triterpenoids, steroids, and some other compounds. Flavonoids such as apigenin, luteolin, quercetin, and rutin found in this species are potent antioxidants with anti-inflammatory, anti-allergic, and anti-cancer properties. This plant contains alkaloids which exhibit various pharmacological effects, including analgesic, anti-inflammatory, and anti-tumor activities. Glycosides from this species have demonstrated diuretic, anti-diabetic, and anti-inflammatory properties. Terpenoids and triterpenoids namely cyperene, β -sitosterol, and stigmasterol have diverse biological activities, including anti-inflammatory, anti-microbial, and anticancer effects (Al-Snafi, 2016). Steroids present in this plant play important roles in various physiological processes such as anti-inflammatory and anti-allergic properties. Other compounds like saponins, tannins, resins, phytosterols, reducing sugars, carbohydrates, proteins, volatile oils, and fixed oils (Mozafari et al., 2018) contributes to the overall pharmacological profile of the plant. Its diverse phytochemical composition and various pharmacological activities make it a valuable resource for the development of new drugs and therapeutic strategies. However, further research is needed to fully elucidate the mechanisms of action of the various bioactive compounds and to validate the clinical efficacy of the plant for various diseases.



Pre-Clinical Study

Extensive pre-clinical research examining the safety and efficacy of *C. dactylon* have been conducted because of the increased scientific interest in the plant's possible medicinal effects. Animal models are the main tool used in pre-clinical research on *C. dactylon* to evaluate its pharmacological actions and possible toxicity. Pre-clinical research has generally shown encouraging findings about *C. dactylon*'s medicinal potential. To completely comprehend its mechanisms of action, optimize dose and delivery techniques, and validate its efficacy and safety in clinical studies, more study is necessary. Preclinical research has demonstrated its promising potential as a viable natural treatment for a range of illnesses. Its therapeutic usefulness is suggested by its comparatively low toxicity profile and varied range of pharmacological actions. To convert this preclinical finding into clinical applications, more research is necessary.

Analgesic Activity

Garg and Khosa (2008) evaluated the analgesic activity of aqueous extract (200-600 mg/kg) of *C. dactylon* roots against acetic acid induced writhing and hot plate test in Albino Wistar rats. The aqueous extract at 600 mg/kg exhibited 82.10% inhibition of writhing as compared to aspirin (10 mg/kg, 82.96% inhibition). It was quite evident from the result that the extract at 600 mg/kg showed comparable effectiveness to standard drug. The extract exerts a significant increase in the hot plate reaction time.

Angiogenic Activity

Soraya et al. (2015) evaluated the angiogenic effect of aqueous extract of *C. dactylon* rhizomes on vascular endothelial growth factor (VEGF) expressions in human umbilical vein endothelial cells (HUVECs) and, also on angiogenesis in carrageenan induced air-pouch model in rats. The extract at 400 mg/kg oral dose, significantly increased angiogenesis ($p < 0.05$), markedly decreased neutrophil ($p < 0.05$) and total leukocyte infiltration ($p < 0.001$) in air pouch rats. Moreover, the extract increased expression of total VEGF in HUVECs at 100 μ l/ml dose.

Anthelmintic Activity

Abhishek and Thakur (2012) reported the anthelmintic activity of aqueous extract (5-50 mg/ml) of *C. dactylon* against adult Indian earthworm *Pheretima posthuma*. The extract at 50 mg/ml showed paralysis at 25.20 min. and death at 48.50 min. in *P. posthuma* as compared to albendazole (37.52 and 65.83 min.), used as standard drug.

Anti-arthritis Activity

Bhangale and Acharya (2014) investigated the anti-arthritis activity of ethanolic extract (100, 200 and 400 mg/kg, orally) of *C. dactylon* leaves against Freund's complete adjuvant-induced arthritis in rats. The extract showed reduction in paw swelling, joint pain and, body weight in rats. The levels of WBC, ESR,

C-reactive protein (CRP) and TNF α which were significantly suppressed by extract. The histopathological examination showed that extract reduces inflammation and cartilage damage in rats.

Sindhu et al. (2009) studied the anti-arthritic potential of methanolic extract (20 mg/kg, b.w.) of *C. dactylon* against complete Freund's adjuvant-induced arthritis in rats. The extract showed anti-arthritic effect with percentage inhibition of 77% for paw edema at 211 days. Further, the extract significantly decreased ($p < 0.05$) levels of 5-LOX and total COX and TBARS, whereas increased concentration of vitamins C and E and glutathione content as well as SOD, CAT and GPx activities in rats. Also, decreased the CRP, ceruloplasmin and nitrite levels as compared with adjuvant treated rats.

Anti-atherosclerotic Activity

Pashaie et al. (2017) investigated the anti-atherosclerotic potential of ethanolic extract (100-400 mg/kg) of *C. dactylon* against high cholesterol diet (HCD)-induced hypercholesterolemia in Wistar rats. Treatment with extract at tested doses lowered total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol, (LDL-C) and high-density lipoprotein cholesterol (HDL-C) in treated rats.

Anti-cancer Activity

Albert-Baskar and Ignacimuthu (2010) demonstrated the anti-cancer property of methanolic extract from *C. dactylon* against

DMH-induced colon carcinogenesis albino rats. Treatment with extract reduced the levels of lipid peroxide and increased the antioxidant enzymes in circulation. The extract also significantly ($p < 0.05$) decreased the extent of lipid peroxidation and, a significant ($p < 0.05$) increase in the activities of free radical metabolizing enzymes.

Baskar et al. (2012) demonstrated the anti-cancer effect of n-hexane, ethyl acetate and methanol extracts of *C. dactylon* roots against human cancer cell lines: COLO 320 DM (colon), MCF-7 (breast), AGS (stomach), A549 (lung) and VERO (monkey normal kidney epithelial) using MTT assay. All extracts showed inhibitory effect with IC₅₀ values ranging from 4 to 684 μ g/ml for all tested cell lines.

Khelifi et al. (2013) investigated the anti-cancer effect of water, petroleum ether, methanol, dichloromethane, and acetone extracts of *C. dactylon* whole plant against human breast cancer cells (MCF7) using MTT assay. Only acetone and petroleum ether extracts exhibited inhibitory effect with IC₅₀ values of 39 and 44 μ g/ml as compared to doxorubicin (0.22 μ g/ml) used as standard, whereas other extracts were found to be inactive (> 100 μ g/ml) for tested cell line.

Anti-diabetic Activity

Singh et al. (2007) evaluated the anti-diabetic potential of aqueous extract (250, 500 and 1000 mg/kg) of *C. dactylon* against normal and streptozotocin-induced diabetic rats. The extract lowered blood glucose level with 31% after 4 h in normal rats. Further, the extract

at 500 mg/kg dose showed fall of 23% in blood glucose level within 1 h during oral glucose tolerance test in mild diabetic rats, with similar effect of tolbutamide (250 mg/kg) used as standard drug. The extract exhibit reduction in the urine sugar level and, increased the body weight of severe diabetic rats as well as, total cholesterol, low density lipoprotein and triglyceride levels were decreased by 35, 77 and 29%, respectively in severely diabetic rats whereas, cardioprotective, high density lipoprotein was increased by 18% in rats.

Karthik and Ravikumar (2011) performed the anti-diabetic activity of ethanolic extract of *C. dactylon* leaves (450 mg/kg, b.w.) against alloxan-induced diabetic albino rats. The extract exerts a significant diminution of fasting blood sugar level and, also significant increase in HDL and LPO in plasma while, decrease ($p < 0.05$) in cholesterol, triglyceride, LDL, and VLDL levels. The levels of AST, ALT, ALP, AP, LDH, and CPK along with protein content, SOD, CAT, GPx, and GSH were significantly decreased ($p < 0.05$) by extract.

Anti-diarrheal Activity

Babu et al. (2009) evaluated the anti-diarrheal potential of methanol extract (200 and 300 mg/kg, b.w.) of *C. dactylon* whole plant in various diarrhoeal models of Albino Wistar rats. The extract attained anti-diarrheal effect with 73.89 and 82.76% inhibitions in castor oil-induced diarrhoeal model. Further, the extract also showed reduction with 55.67 and 49.73% in gastrointestinal motility by charcoal meal and, decrease in weight of intestinal contents

with 48.25% at 300 mg/kg in enter pooling as comparable to atropine sulphate (33.54%) used as standard.

Fertility Promoting Activity

Chidrawar et al. (2011) studied the fertility enhancing effect of methanolic (CDME), benzene and water (CDWE) extracts (100, 200 and 500 mg/kg) of *C. dactylon* aerial parts against stress-induced infertility in male Swiss albino rats. The CDME at 500 mg/kg dose decreased latency of first intromission in comparison to control male rats. Further, the extract (200 mg/kg) showed significant restoration of sperm count ($p < 0.05$) and fructose content ($p < 0.001$) in rats. In addition, CDME also inhibited the stress-induced reduction in wet weight of isolated accessory sexual organs to body weight ratio of rats.

Anti-fertility Activity

Malpani et al. (2020) evaluated the anti-fertility effect of ether (ErCD), chloroform (CeCD) and ethyl alcohol (EyCD) extracts (200 and 400 mg/kg, orally) of *C. dactylon* whole plant in female albino Wistar rats. Only EyCD exhibited maximum reduction in pregnancy (83.33%), number of implants as well as number of litters born. The extract exhibits a significant ($p < 0.01$) increase in uterine weight and uterine proliferation whereas, reduced deciduoma formation in rats. EyCD attained a significant ($p < 0.01$) increase in the diameter of the uterus, diameter of endometrium, its thickness and height of endometrium in ovariectomized rats. Also, a significant rise in serum estrogen level

of immature ovariectomized rats. EyCD exerts a significant ($p < 0.0001$) decrease in serum LH and FSH level of rats. Further, the extract showed insignificant decrease in the serum cholesterol when compared to control. Also, the extract exhibits a significant ($p < 0.001$) decrease in level of serum alkaline phosphate.

Anti-inflammatory Activity

Garg and Paliwal (2011) investigated the anti-inflammatory effect of aqueous extract (200, 400 and 600 mg/kg, oral) of whole plant of *C. dactylon* against carrageenan, serotonin, histamine and dextran-induced rat paw edema and cotton pellet models. The extract showed a significant ($p < 0.001$) reduction in the formation of edema induced by carrageenan, serotonin, histamine, and dextran after 3 and 5 hrs. Also, the extract produced maximum effect with 46.40% inhibition in dry weight cotton pellet formation at 600 mg/kg dose as compared to indomethacin (10 mg/kg) used as standard drug with 46.27% inhibition.

Antimalarial Activity

Khelifi et al. (2013) examined the anti-malarial potential of water, petroleum ether, methanol, dichloromethane, and acetone extracts of *C. dactylon* whole plant against *Plasmodium falciparum*. Only acetone and petroleum ether extracts exhibited inhibitory effect with IC_{50} values of 38 and 48 $\mu\text{g/ml}$ as compared to chloroquine (0.10 $\mu\text{g/ml}$) used as standard drug, whereas other extracts were found to be inactive ($> 100 \mu\text{g/ml}$) for tested model.

Anti-microbial Activity

Vinayagam et al. (2021) evaluated the anti-bacterial activity of synthetic gold nanoparticles (AuNPs; 0.625-100 $\mu\text{g/ml}$) containing an aqueous extract of *C. dactylon* against *Enterobacter cloacae*, *Staphylococcus haemolyticus*, *Staphylococcus petrasii* subsp. *pragensis* and *Bacillus cereus* using disc-diffusion assay. AuNPs exhibited anti-bacterial effect with zone inhibitions as 13, 12, 13 and 12 mm, respectively for tested microbial strains.

Marasini et al. (2015) studied the anti-bacterial potential of ethanolic extract along with n-hexane, chloroform, n-butanol, and water fractions of *C. dactylon* whole plant against *Citrobacter freundii*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *S. pyogenes*, *Salmonella enteritidis*, *S. typhimurium* and *Vibrio cholera* using agar-well diffusion. The extract and its fractions showed inhibitory effect with MIC values ranging from 391 to 6250 $\mu\text{g/ml}$ and, 32 to 500 $\mu\text{g/ml}$ for tested organisms.

Pranita et al. (2012) performed the anti-bacterial effect of ethanolic extract (5, 7.5 and 10 mg/ml) from *C. dactylon* whole plant against *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes* using agar well diffusion method. The extract showed anti-bacterial effect with zone inhibitions for *E. coli* (17.7 mm), *S. aureus* (17.5 mm), and *S. pyogenes* (16.7 mm) when compared to povidone-iodine (14.8, 15.2 and 14 mm) used as standard drug.

Rao et al. (2011) investigated the anti-microbial activity of aqueous extract of *C. dactylon* whole plant against *E. coli*, *S. aureus*, *K. pneumoniae*, *Proteus mirabilis* and *Candida albicans* using agar well diffusion method. The extract showed inhibitory effect with 40, 12 and 10 mm respectively for tested microorganisms, except *C. albicans*.

Antioxidant Activity

Biswas et al. (2017) evaluated the antioxidant potential of aqueous extract of *C. dactylon* using DPPH, ABTS and reducing power assays. The extract showed scavenging effect with IC_{50} values of 2.29 and 0.40 for DPPH and ABTS assays, where reducing power value was found to be 12.96 mg/gm dry extract (AAE equivalent).

Albert-Baskar and Ignacimuthu (2010) demonstrated the antioxidant activity of n-hexane, ethyl acetate and methanol extracts of *C. dactylon* using DPPH and nitric oxide radical (NO) assays. Only methanol extract exhibited strong antioxidant effect with IC_{50} of 63.03 and 111.22 $\mu\text{g/ml}$ as compared to vitamin C (5 and 25 $\mu\text{g/ml}$) as standard for both assays.

Baskar et al. (2012) studied the antioxidant effect of n-hexane, ethyl acetone and methanol extracts of *C. dactylon* using DPPH and NO scavenging assay. All extracts showed scavenging effect with values ranging from 63.03 to 874.15 $\mu\text{g/ml}$ and, 98.89 to 450.08 $\mu\text{g/ml}$ for both assays as compared to vitamin C (5 and 25 $\mu\text{g/ml}$) as standard drug.

Khelifi et al. (2013) investigated the antioxidant activity of water, petroleum ether, methanol, dichloromethane, and acetone extracts of *C. dactylon* whole plant using DPPH and ABTS assays. The water extract showed good scavenging effect with IC_{50} of 57.21 $\mu\text{g/ml}$, followed by acetone extract (179.77 $\mu\text{g/ml}$) for DPPH assay. Further, the water extract exerts ABTS scavenging effect of 152.96 $\mu\text{g/ml}$, followed by methanol extract (203.29 mg/l) and acetone extract (206.69 $\mu\text{g/ml}$). However, petroleum ether and dichloromethane extracts attained poor scavenging potential with 921.76 and 1053.12 $\mu\text{g/ml}$, respectively.

Anti-Parkinson's Activity

Sharma and Bafna (2012) evaluated the anti-Parkinson's activity of aqueous extract (210 mg/kg; i.p.) of *C. dactylon* against rotenone-induced Parkinson's disease in rats. The extract showed reduction in the TBARS level and, an increase in the GSH, SOD and CAT levels of brain. The extract exhibits a significant ($p < 0.001$) decrease in catalepsy and muscle rigidity along with a significant ($p < 0.001$) increase in locomotion in treated rats.

Anti-pyretic Activity

Garg and Khosa (2008) performed the anti-pyretic potential of aqueous extract of *C. dactylon* roots against yeast-induced hyperthermia in Albino Wistar rats. The extract at 600 mg/kg dose showed decrease in rectal temperature of treated rats which was like paracetamol (100 mg/kg) used as standard drug.

Anti-ulcer Activity

Venkateswarlu and Chandrasekhar [2016] reported the anti-ulcer potential of n-hexane, chloroform, and methanol extracts (200 and 400 mg/kg, orally) of *C. dactylon* aerial parts against paracetamol-induced ulcer in Albino rats. All extracts at highest dose showed potent anti-ulcer effect with reduction of ulcer in rats as compared to pantoprazole (240 mg/kg, b.w.) used as standard drug.

Anti-urolithiatic Activity

Khajavi et al. [2011] reported the anti-urolithiatic potential of ethanolic extract (3.2 and 12.6 mg/kg) of *C. dactylon* roots against ethylene glycol-induced urolithiasis in albino Wistar rats. The extract significantly decreased ($p < 0.05$) the weight of kidney and urine oxalate level in nephrolithiatic rats. Also, the extract lowered the number of deposits and serum magnesium concentration in rats.

Bronchodilatory Activity

Patel et al. [2013] examined the bronchodilatory activity of chloroform extract of *C. dactylon* against acetylcholine (ACh), histamine-induced bronchospasm in guinea pigs as well as carbachol (CCh) and high K^+ -induced contraction of rat tracheal strip. The extract showed bronchodilatory effect with ED_{50} value of 9.884 mg/kg, higher than atropine (4.23 mg/kg) towards ACh-induced bronchospasm in guinea pigs. Further, the extract inhibited high K^+ and

Ca^{+2} induced contractile response in rat. However, the extract was found to be inactive for histamine-induced model.

Cardioprotective Activity

Garjani et al. [2009] studied the cardioprotective activity of hydroalcoholic extract (100 and 200 mg/kg; orally) from *C. dactylon* rhizomes against monocrotaline (50 mg/kg)-induced cardiac contractility in isolated normal hearts and cardiac functions in right ventricular heart failure of Wistar rats. The extract showed an increase in the contraction force by increasing the left ventricular developed pressure (LVDP) and parallel decrease of end diastolic pressure (LVEDP) as well as, heart rate and elevated mean arterial pressure in monocrotaline-injected rats. The $RVdP/dt_{max}$, and $RVdP/dt/P$ as indices of myocardial contractility increased by extract treatment. The extract also reduced heart and lung congestion by decreasing tissue wet/dry and wet/body weight ratios ($p < 0.01$). In isolated rat hearts, the extract produced a remarkable ($p < 0.001$) positive inotropic effect concomitant with a parallel decrease in LVEDP.

Cytotoxicity

Vinayagam et al. [2021] studied the cytotoxicity of *C. dactylon*-loaded AuNPs (0.625 to 100 $\mu\text{g/ml}$) against human adenocarcinoma (MCF-7) cell line using MTT assay. AuNPs showed cytotoxic effect with IC_{50} value of 31.34 $\mu\text{g/ml}$ for tested cell line.

Diuretic Activity

Sadki et al. (2010) performed the diuretic activity of aqueous extract (0.125, 0.250 and 0.500 g/kg b.w.) of *C. dactylon* on urinary output and electrolytes (Na⁺ and K⁺) in Wistar rats. Treatment with extract at 0.500 g/kg dose significantly ($p < 0.05$) increased urinary output and electrolytes excretion in rats when furosemide (0.015 g/kg) used as standard.

Effects on Central Nervous System (CNS)

Pal (2008) examined the anti-depressant effect of ethanol extract (25, 50 and 75 mg/kg, b.w.) of aerial parts of *C. dactylon* against pentobarbitone sodium, diazepam, and meprobamate-induced sleeping time in mice. The extract potentiated the sleeping time in mice induced by pentobarbitone sodium, diazepam, and meprobamate. Further, the extract reduced the number of writhes and stretches induced in mice with 1.2% acetic acid. The extract also increased the latency but, decreased the duration of seizures induced by maximal electroshock and pentylenetetrazole. Biochemical analysis showed that extract increased the levels of serotonin and γ -aminobutyric acid (GABA) in mice brain.

Kothari (20215) evaluated the anti-depressant potential of aqueous extract (200 and 400 mg/kg) of *C. dactylon* using elevated plus maze (EPM) and light dark box, forced swim (FST) and tail suspension (TST) tests in albino mice. The extract showed an increase

in entries and stay in open arms (EPM) as well as in light compartment of light and dark box as compared to gum acacia (GA10, 10 ml/kg) used as standard. Further, the extract also lowered the mean immobility time as compared to GA10 for both FST and TST.

Kothari and Sahu (2022) studied the memory enhancing activity of aqueous extract (200 and 400 mg/kg) of *C. dactylon* on normal and scopolamine-induced impaired memory in albino mice. Treatment with extract exhibited memory enhancement with reduction of escape latency in Morris water maze and transfer latency during elevated plus maze test in normal and scopolamine-induced mice as comparable to piracetam (200 mg/kg) used as standard drug.

Sharma et al. (2011) demonstrated the anti-cataleptic potential of aqueous extract (150 and 300 mg/kg; i.p.) of *C. dactylon* against reserpine-induced catalepsy in Swiss albino mice. The extract significantly ($p < 0.001$) reduced the severity for reserpine induced catalepsy at tested doses with cataleptic scores of 56.33 and 18.50, respectively.

Enzyme Inhibitory Activity

Wang et al. (2021) evaluated the enzyme inhibitory activity of cysestermerol A from *C. dactylon* whole herb against α -glucosidase *in vitro*. The extract exhibited strong inhibitory effect with IC₅₀ of 99.58 mg/l as compared to acarbose (67.23 mg/l) used as standard for tested model.

Hepatoprotective Activity

Kowsalya et al. (2015) performed the hepatoprotective activity of methanolic extract (50 mg/kg; orally) from *C. dactylon* against diethyl nitrosamine (DEN)-induced liver cancer in Swiss albino mice. The extract enhanced levels of antioxidant enzymes such as AST, ALT, GST, GPx and CAT, while reduced the amount of serum amino transaminase, SGOT and SGPT values in DEN-induced cancer mice.

Javalgikar et al. (2021) studied the hepatoprotective effect of ethanolic and aqueous extracts (200 and 400 mg/kg) of leaves of *C. dactylon* against rifampicin-induced liver damage in albino rats. The extract showed hepatoprotective effect with improvement in biochemical parameters like SGPT, SGOT, ALP, and BIT levels of rats.

Devi et al. (2017) reported the hepatoprotective potential of aqueous extract (200 and 400 mg/kg, b.w.) of *C. dactylon* aerial parts against paracetamol-induced hepatotoxicity in albino rats. Treatment with extract attained hepatoprotective effect with reduction in the values of SGOT, SGPT, and ALP as compared to N-acetylcysteine (100 mg/kg) used as standard.

Immunomodulatory Activity

Mangathayaru et al. (2009) performed the immunomodulatory activity of fresh juice (250 and 500 mg/kg, b.w.) from *C. dactylon* against doxorubicin-induced DNA damage in BALB/cmice. Fresh juice at both doses

increased humoral antibody response upon antigen challenge in mice. Further, fresh juice showed an increase in antibody titre for haemagglutination antibody and plaque forming cell assays.

Santhi and Annapoorani (2010) studied the immunomodulatory potential of protein fraction from *C. dactylon* leaves on neutrophil adhesion, HA titre and DTH response to antigenic challenge by sheep RBCs in Swiss albino mice. The fraction showed an increase in neutrophil adhesion, haemagglutinating antibody titre and delayed type hypersensitivity response. In mice immunized with sheep RBC, the fraction enhanced humoral antibody response to the antigen and, potentiated the cellular immunity by facilitating the food pad thickness response to sheep RBC in sensitized mice.

Devi et al. (2011) investigated the immunomodulatory effect of ethyl acetate fraction (70 µl/70 µl) of *C. dactylon* leaves against pyrogallol-induced immunosuppression in BALB/cmice. The fraction at tested dose prevented the immunosuppression caused by pyrogallol in mice.

Larvicidal Activity

Bagavan and Rahuman (2011) evaluated the larvicidal potential of ethyl acetate and methanol extracts (1000 µg/ml) of *C. dactylon* leaves, against fourth instar larvae of *Anopheles vagus* and *Armigeres subalbatus*. Both extracts at tested dose exhibited larvicidal effect with LC₅₀ values of 68.12, 21.67 µg/ml and, 41.13, 32.62 µg/ml for *A. vagus* and *A. subalbatus*, respectively.

Neuroprotective Activity

Sumathi et al. (2011) studied the neuroprotective activity of aqueous extract (300 and 750 mg/kg; orally) of *C. dactylon* against aluminium-induced neurotoxicity in cerebral cortex, hippocampus, and cerebellum of rat brain. Treatment with extract at 750 mg/kg showed an increase in the levels of GSH, SOD, GPx, GST status, activities of membrane-bound enzymes (Na^+/K^+ ATPase and Mg^{2+} ATPase) but decreased the level of LPO when compared with aluminium-induced rats.

Radioprotective Activity

Rao et al. (2008) investigated radioprotective potential of hydroalcoholic extract (40 and 50 $\mu\text{g}/\text{ml}$) of *C. dactylon* against radiation-induced cytogenetic damage in Chinese hamster lung fibroblast (V79) cells and human peripheral blood lymphocytes (HPBLs). The extract significantly ($p < 0.001$) reduced the frequency of micronucleated binucleate cells (MNBNCs) at 40 $\mu\text{g}/\text{ml}$ in HPBLs and, 50 $\mu\text{g}/\text{ml}$ in V79 cells, respectively.

Wound healing Activity

Biswas et al. (2017) evaluated the wound healing potential of aqueous extract of *C. dactylon* on full thickness punch wound in Wistar rats. The extract showed a significant ($p < 0.05$) reduction in wound contraction while, an increase in tensile strength when compared to framycetin sulfate (1%, w/w), used as standard drug. Further, the extract decreased the DNA, RNA, total protein levels but, increased hydroxyproline level in punch wound of rat. Also, the extract exerts remarkable appearance of epithelialisation and collagenesis with genesis of new vascular tissues in rats.

Dande and Khan (2012) studied the wound healing effect of aqueous and alcoholic extracts gel (10 and 20%) from fresh herb of *C. dactylon* on excision and incision wound healing models of albino Wistar rats. Both extracts gel showed a significant increase in the rate of wound healing for excision ($p < 0.05$) and incision ($p < 0.01$) wound healing models.

Clinical Study

In a clinical trial, 20 male patients aging 25-50 years who had had clinical presentation of repeated attacks of renal colic and dysuria with 750 ml aqueous extract of *C. dactylon* showed 90% improvement in prognosis of lower ureteric stones based on clinical and ultrasound finding reported by Majeed et al. (2014). Another randomized, double-blind, placebo-controlled clinical trial reported

in 12 patients aged 18-65 years with acute or chronic wounds. Patients received 15% (w/w) *C. dactylon* topical ointment for treatment of ulcers. At dose of 15%, w/w ointment significantly reduced wound area, as compared to framycetin. Treatment with ointment significantly promoted the formation of granulation tissue, significantly accelerated complete wound closure

(epithelialization). The ointment significantly improved haematological parameters as polymorph count (%), lymphocyte count (%)

and erythrocyte sedimentation rate (ESR) (Biswas et al.,2017).

Summary and Future Outlook

C. dactylon is a widely distributed plant used in traditional medicine for various ailments. Scientific research has confirmed several pharmacological activities of *C. dactylon*, including wound healing, anti-inflammatory, analgesic, bronchodilatory, antioxidant, and antidiabetic potential. Extracts of this species have been shown to accelerate wound healing, promote collagen synthesis, and reduce inflammation. The plant exhibits anti-inflammatory effect through various mechanisms, including inhibition of pro-inflammatory mediators and modulation of immune response as well as, possesses analgesic and antipyretic properties, potentially useful for pain and fever management. The plant extracts demonstrate bronchodilatory effect through mechanisms like muscarinic antagonism and calcium channel blocking. This species contains various antioxidants that protect against free radical damage and oxidative stress. Studies suggested potential

antidiabetic effects of the plant, including lowering blood glucose levels, and improving insulin sensitivity. Despite promising research findings, further studies are needed to validate the efficacy and safety of *C. dactylon* for various medicinal applications. Clinical trials are crucial to evaluate the therapeutic potential of the plant and determine appropriate dosages and formulations. Identification and isolation of active components responsible for specific pharmacological activities will enable the development of targeted therapies. Exploring synergistic combinations of *C. dactylon* with other medicinal plants or conventional drugs could lead to enhanced therapeutic effects. Further research is needed to understand the underlying mechanisms of action of this species, particularly for its anti-inflammatory and bronchodilatory properties. Overall, this species shows significant potential as a valuable resource for developing natural medicines for various health conditions.

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