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Food & Herbal Park, Village-Padartha, Laksar Road,
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Monographs on Medicinal Plants along Ganga River
Croton bonplandianus Bail.



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along Ganga River

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

Volume 3



Monographs on Medicinal Plants along Ganga River

Croton bonplandianus Baill.

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

Volume 03

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

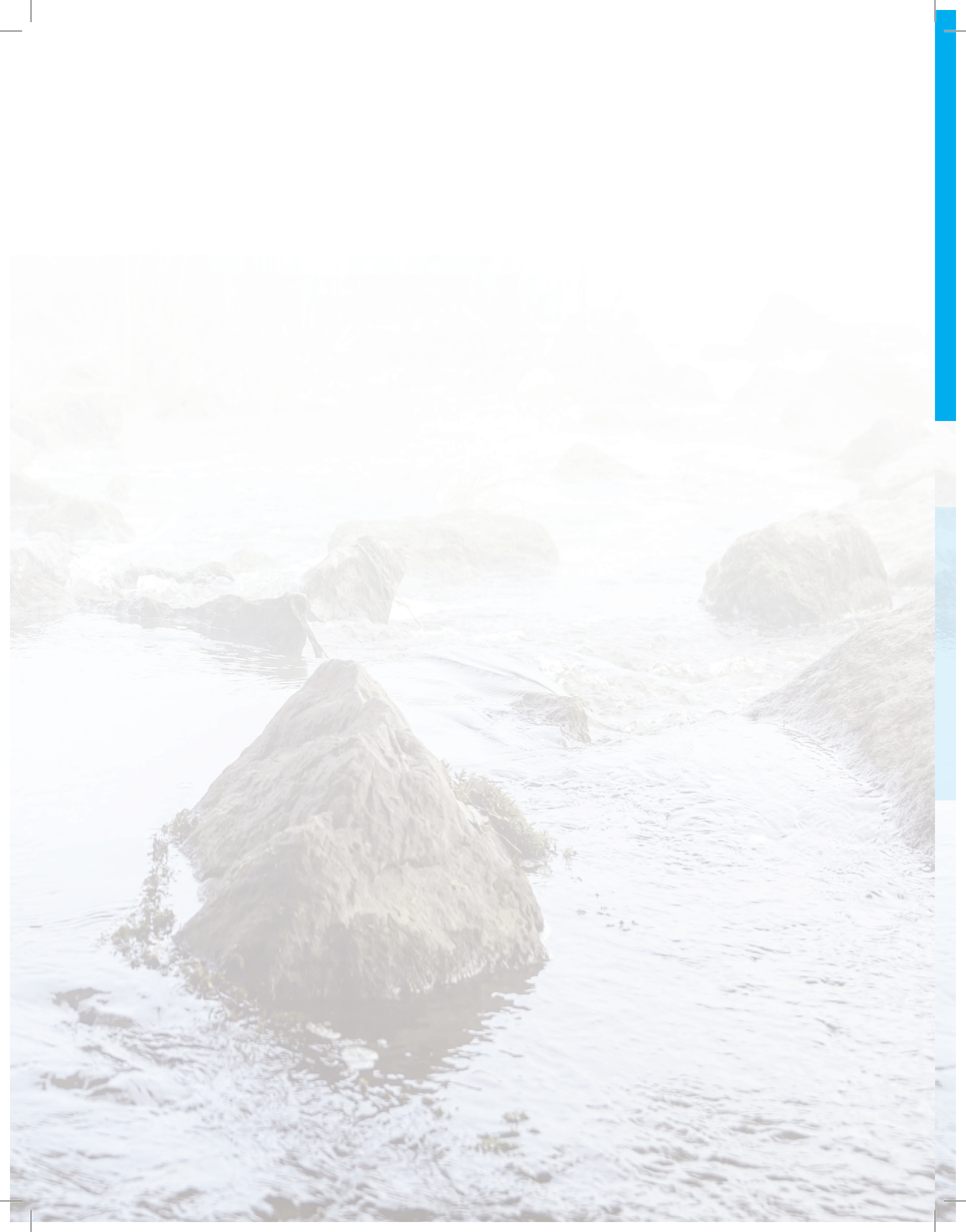
Shri Narendra Modi on Medicinal Plants



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

“

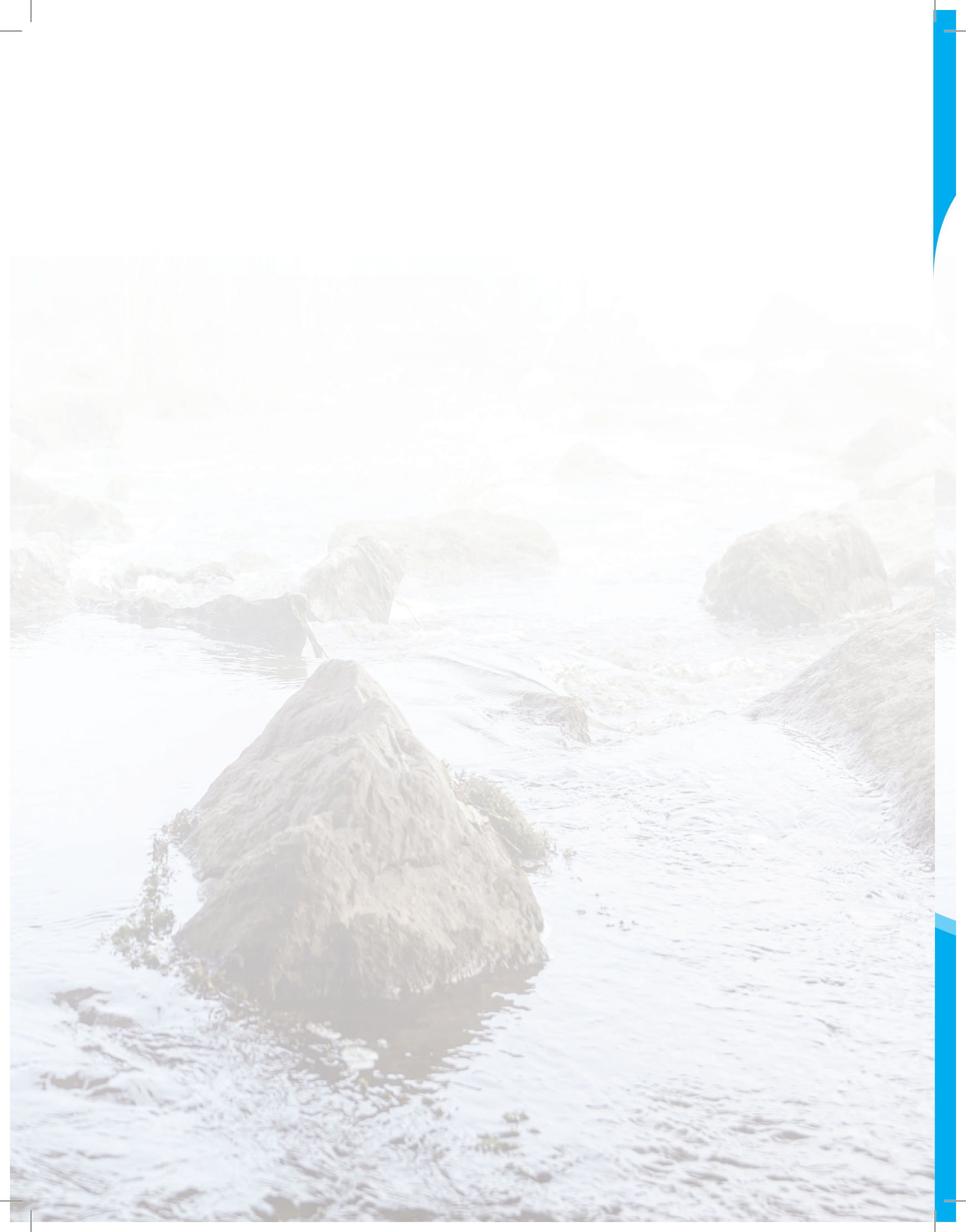
1. We will understand the importance of water, conserve it and ensure rich water resources for future generations.
2. Conservation and enhancement of water resources of our country is a sacred goal. I will work with my dedication and devotion to achieve this.
3. Proper access to water is a strong step 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)

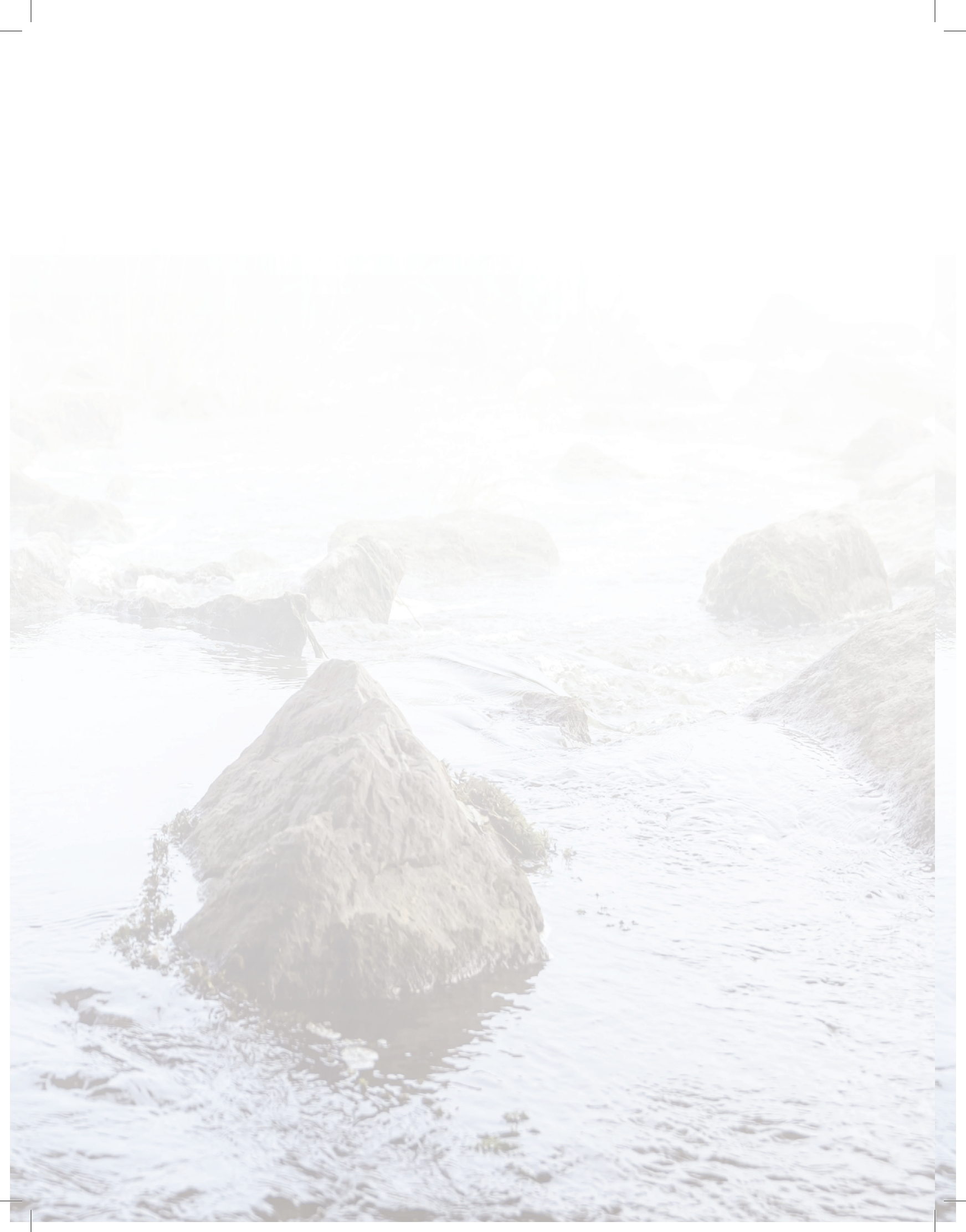


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सत्यमेव जयते
75
आज़ादी का
अमृत महोत्सव

भारत सरकार
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



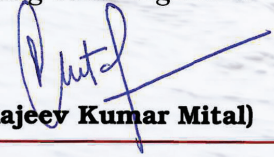
MESSAGE

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.

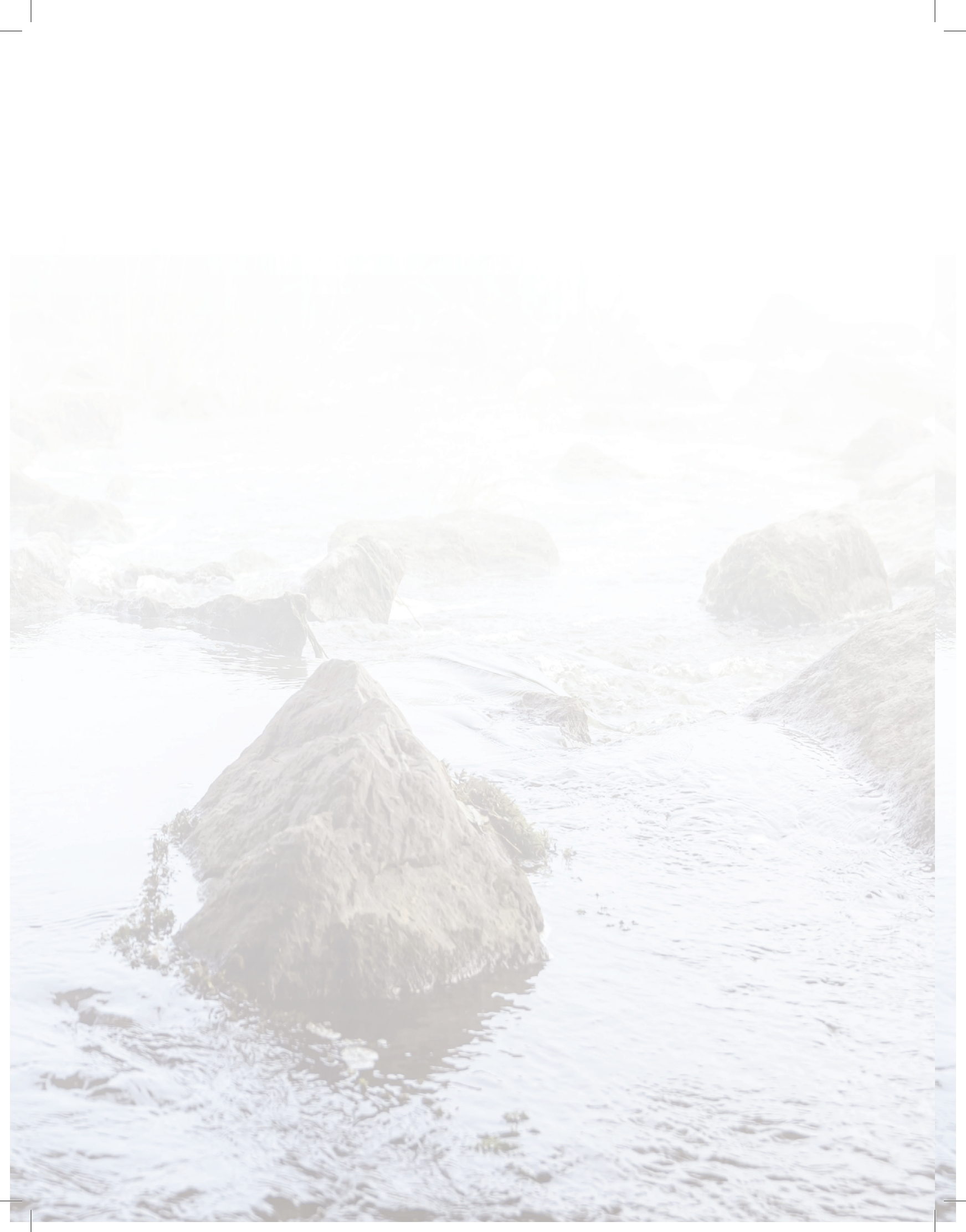
This excellent work documenting the floral diversity along the banks may pave the way for the rejuvenation of the Ganga and set a precedent for biodiversity conservation and socio-economic improvement, potentially benefiting other regions of India as well.


(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.

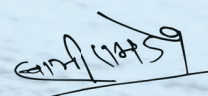
The authors have meticulously documented the plant exploration and botanical study, soil properties analysis, phytochemical analysis, and insilico analysis of the plants found in this region. Their dedication and deep respect for nature's wisdom are evident throughout the pages, making this monograph 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.

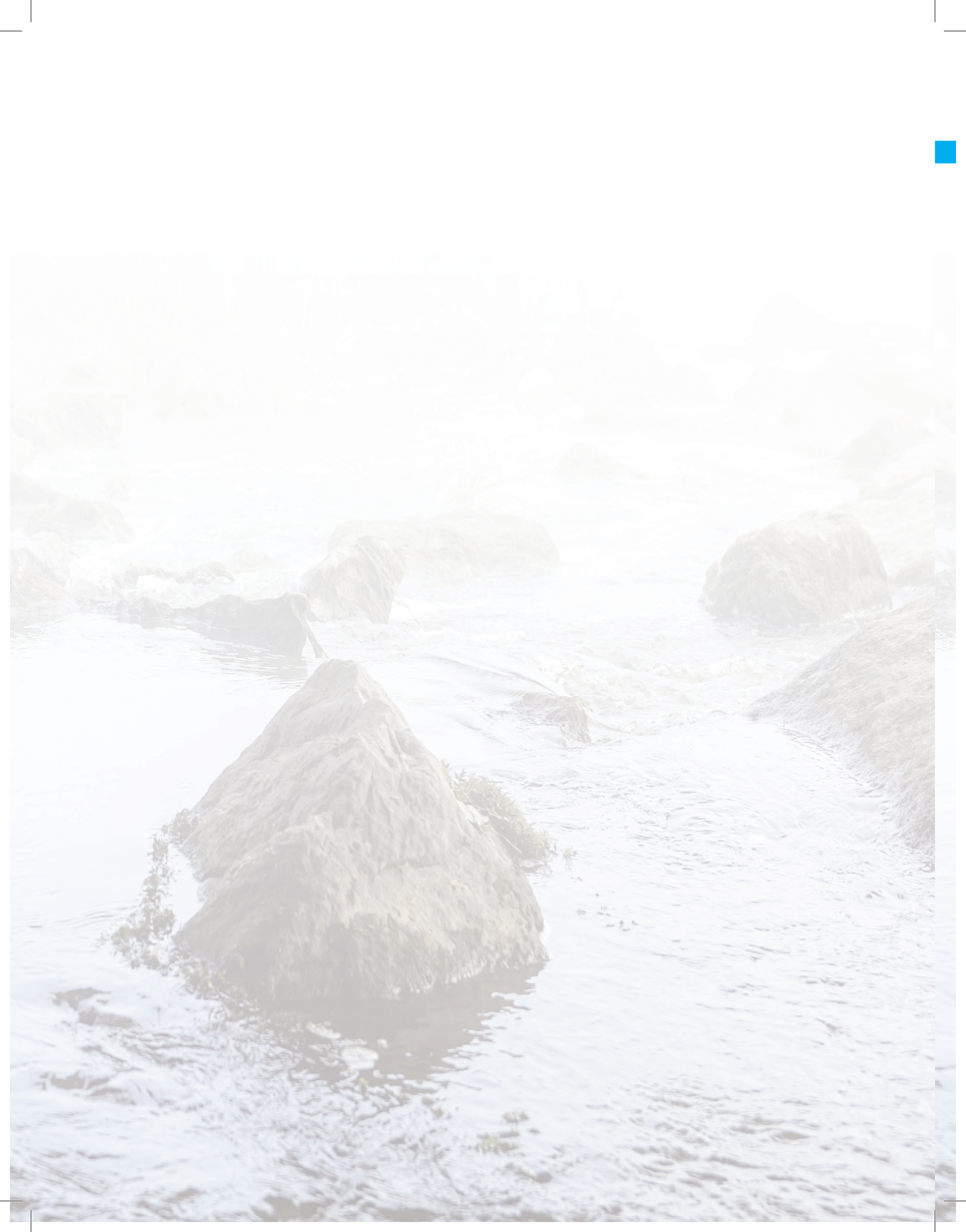
May this monograph serve as a guiding light for researchers, practitioners, and enthusiasts alike, illuminating the path towards a deeper appreciation of nature's pharmacy along the revered Ganga River.

With heartfelt gratitude and blessings,



- Swami Ramdev





PREFACE

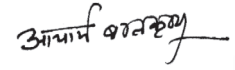
The Ganga River, revered as the lifeblood of India, flows through the heart of the country, nourishing not only the land but also the spirit of the people. It is not just a geographical entity; it is a symbol of life, purity, and sustenance. From its origin in the pristine glaciers of Uttarakhand to 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

Croton bonplandianus Baill.

Vedic name : (Muktatantukaḥ aromavṛntaḥ) (मुक्ततन्तुकः अरोमवृन्तः)

Botanical name : *Croton bonplandianus* Baill.

Vānaspatikavivaraṇaḥ

Muktatantukavamśe'tha romavṛntaśca varṇyate.
 Kāṣṭhīyapādapaścāyaṃ liṅgadvayāśrayī tathā.
 R̥jurupakṣupīyo'yamathavā śākapādapaḥ.
 Dairghyaṃ trimuṣṭito pañcamuṣṭisamaṃ tathaiva ca.
 Kadācit pādapasyāsyā sārddhaikavyāmadīrghatā.
 Pādape'smiṃśca śākhāśca cakrikākṛtikāstathā.
 Parṇavṛntaprarohau ca prārambhe ghanaromaśau.
 Paścādaromaśau lomāvaraṇaṅcīpiṭaṃ tathā.
 Uḍuvatsitaromañca yavato vrīhivigrahi.
 Śākīyapādape cānupatraṃ tathoparomaśam.
 Tantumayānupatrañca prāyo'rddhasarṣapāyatam.
 Ekāntaraṃ dalaṃ prāya ābhāsikūṭacakraki.
 Viparītaṃ dalaṃ prāyaścakrikānvitameva ca.
 Tṛtīyabhāgataḥ parṇavṛntaṃ sārddhaikasarṣapi.
 Muṣṭimitocchritaṃ patrapaṭalaṃ vrīhipañcataḥ.
 Arddhasarṣapataḥ pañcavrīhivistīrṇatā tathā.
 Bhallakamaṇḍavaddīrghavṛttajatastathaiva ca.
 Paṭalamavakīlañca niśītāgraṃ dalasya ca.
 Avāṅge'lpavṛntahīnacakrikābhābhyaṃ dvābhyaṃ tathaiva ca.
 Ādhārīyagranthibhiranvitaṃ patrapaṭalaṃ tathā.
 Picchākāraśīrāyuktaṃ krakaci kuṇṭhadantitaḥ.
 Jhillīmayam tathā'romamūrdhvabhāge tathaiva ca.
 Avāṅge cālparūpeṇa tārāvacchalkaromaśam.
 Gāḍhaharicca kuṇṭhāgramādhāre paṭalaṃ tathā.
 Śīrāṇaṃ sphuṭavinyāso'vāṅgaṃ prati tathaiva ca.
 Pārśvabhāge śīrāḥ ṣaṭtaḥ saptayugmānvitāstathā.
 Tathādhārīyayugmastu trīśīrīyastathaiva ca.
 Asīmākṣaṃ tathā'ntasthaṃ prāyo muṣṭidvayocchritam.

Saptastrīkesarīpuṣpairyuktaṃ dvitastathaiva ca.
 Paṭalaṃ śvetavarṇīyaṃ pītadhavalatastathā.
 Vihāya spaṣṭarūpeṇa romaśāṇḍāśayāṃstathā.
 Alpāromaśataścedaṃ prāyo'romaśameva ca.
 Adhobhāge tṛtīyāṃśe narapuṣpāṇi tatra vai.
 Strīpuṣpāṇyūrdhvbabhāge ca tṛtīyāṃśe dvayostathā.
 Sahapatraṃ trikoṇīyabhallaṃ sarṣapocchritaṃ.
 Upadhānākṛtigranthiyugmānviṭaṃ tathaiva ca.
 Pumpuṣpe granthilotsṛṣṭamarddhasarṣapavigrahi.
 Bāhyabhāgaṃ prati prāyo'romaśato'pyaromaśam.
 Yavasamāyataṃ puṣpavṛntaṅca sarṣapaikataḥ.
 Pañcapāliyuṭaṃ bāhyadalaṅca haripālayaḥ.
 Pālayo'ṇḍavadākāre dīrghavṛttajatastathā.
 Pāliḥ sarṣapataścātra sapādasarṣapāyatā.
 Tathopaniśitāgrā ca sarṣapasamavistrīṭā.
 Śvetāni pañcasaṅkhyāyāṃ pṛṣṭhāvartadalāni ca.
 Aromaśaṃ dalaṃ prāyaḥ dīrghāyatākṛtau tathā.
 Dvisarṣapāyataṅcārddhasarṣaporu tathaiva ca.
 Bimbasthagranthayo muktāstīkṣṇāgrāścopavṛttajāḥ.
 Puṣpe pañcāṣṭataḥ saptanavapuṅkesarāstathā.
 Aromaśāśca puntanturdvisarṣapāyatastathā.
 Sarṣapasya tṛtīyāṃśā parāgakośadīrghatā.
 Strīpuṣpamalpavṛntena yuktamaromapātradhiḥ.
 Prāyo'nupasthitaṃ puṣpavṛntaṃ puṣpe tathaiva ca.
 Trikoṇato'ṇḍavatpañcabāhyadalasya pālayaḥ.
 Sarṣapasamalamborūpaniśitāgrameva ca.
 Nottaravardhi vai prāyo'romaśaṃ dṛśyate tathā.
 Valayākṛtibimbaścānupasthitaṃ dalaṃ tathā.
 Aṇḍāśayastathā copatripālikaḥ pravṛttataḥ.
 Sarṣapaḥ pariṇāho'rddhayavasamāyatastathā.
 Saghanarūpatastārākṛtikaśalkaromaśaḥ.
 Aromaśāśca vistīrṇāḥ prāyo muktāstrivartikāḥ.
 Dvisarṣapocchritāścārddhayavataśca dviśākhitaḥ.
 Śākhāśca tantulā babhruharitphalaṃ tathaiva ca.

Trikoṇīyaṃ phalaṃ dīrghavṛttābhataśca pādape.
 Catuḥsarṣapavistāro dairghyaṃ pañcaśasarṣapam.
 Paṭavidārakeṇaiva rūpeṇa sphuṭitaṃ tathā.
 Tathālpārūpatastārākṛtikaśalkaromaśam.
 Sarṣapadvayato bījañcatuḥsarṣapamāyatam.
 Dabhato dīrghavṛttābhaṃ rūkṣaṃ tathālpārūpataḥ.
 Bījañca dhūsaraṃ bijacolaṃ dvipālikaṃ tathā. (Saumitreyā Mahodadhīḥ: 1-34)

Botanical Characters

A monoecious woody erect, herb or subshrub, usually 30-40 cm tall sometimes up to 1.5 m, branches in whorled. Young shoots and petioles initially densely pubescent, later on glabrous. Indumentum flat, 0.3–0.5 mm in diameter, consisting of stellate, whitish hairs. Stipules subglabrous, filiform, ca 0.5 mm long. Leaves usually pseudo-verticillate, alternate, subopposite or whorled. Petioles 0.3-1.5 cm long. Leaf-blades ovate-elliptic to lanceolate, 2-5.5 x 0.5-2.5 cm, acute, cuneate, with 2 small sessile discoid basal glands beneath, crenate-serrate, membranous, penninerved, glabrous above, sparingly stellate-lepidote beneath, dark green, base obtuse, venation only visible below, side veins 6–7 pairs, basal pair triplinerved. Racemes terminal, up to 15 cm long with 2-7 pistillate flowers, creamy-white, sparsely pubescent to subglabrous except for the distinctly pubescent ovaries, male flowers in lower one-third, female in upper two-third. Bracts triangular lanceolate, 1 mm long, having pair of pillow-shaped glands, 0.5 mm in diameter, eglandular in male flowers. Male flowers subglabrous to glabrous outside, pedicels 1-1.5 mm long, calyx 5-lobed, lobes elliptic-ovate, 1–1.25 x 1 mm, subacute, greenish-yellow; petals 5, oblong, recurved, 2 x 0.5 mm, obtuse, subglabrous, whitish; disc-glands free, subglobose, apiculate; stamens 13-16, filaments 2 mm long, glabrous, anthers 0.3 mm long; receptacle glabrous. Female flowers subsessile; pedicel nearly absent, calyx-lobes 5, triangular-ovate, 1 x 1 mm, subacute, not accrescent, subglabrous; petals 0; disc annular; ovary ellipsoid-subtrilobate, 1.5 x 1 mm, densely stellate-lepidote; styles 3, ± free, 1.5-2 mm long, spreading, glabrous, bifid, the arms filiform. Fruit ellipsoid-trigonous, brownish-green, 5-6 x 4 mm, septicidally dehiscent, sparingly stellate-lepidote. Seeds compressed-ellipsoid, 4 x 2 mm, grey, slightly roughened; caruncle bilobate. ^[1]

Plant Anatomy

Structure of Leaf

Pārśvaśīrāyutaṃ patraṃ pṛthumadhyāśīrānvitam.
 Patrasya paṭalaṃ sūkṣmaṃ sukomalaṃ tathaiva ca.
 Madhyāśīrā tathā'pākṣabhāge kiñcicca vistr̥tā.
 Sthūlā madhyāśīrā'bhyakṣe golachadivadākṛtau.
 Samatalottalā sūkṣmādhicarmanānvitā tathā.

Tanubhittiyutāḥ sūkṣmatarkuvatkośikāstathā.
 Tathā madhyaśirādhāre mṛdūtakīyakośikāḥ.
 Saghanāḥ kośikāḥ sūkṣmabhittiyutāstathaiva ca.
 Sthitāstathā'dhicarmānto bahukoṇīyakośikāḥ. [
 Pṛṣṭhādhāri tathā patrapaṭalaṃ pādapasya ca.
 Tathāpākṣe ca chidriṣṭhaparṇamadyottakasthalaḥ.
 Abhyakṣe paṭalaṃ stambhatalayuktaṃ tathaiva ca.
 Āyatāḥ paṭalasyādhicarmasthakośikāstathā.
 Probhūjinānvitāścāpi chidriṣṭhakośikāstathā.
 Adhicarmatale randhrasthitiśca varṇyate'dhunā.
 Kośikīyaprakāreṇa samāntare ca vistr̥tāḥ.
 Sahāyakośike vā dve pratirandhre'same same.
 Pṛthupravṛttajākāre dvārasthakośikāstathā.
 Patrasya paṭalastambhe'lpasaghanāśca kośikāḥ.
 Saṅkīrṇavellanākāra ekalapaṅktikāstathā.
 Tathā śīthilarūpeṇa vibhājītāśca kośikāḥ.
 Kośikāḥ pāliyuktā vā laghuvṛttākṛtau tathā.
 Mṛdūttakāśca chidriṣṭhāstritaścātuḥstare tathā.
 Mṛduttake ca chidriṣṭhe bṛhadvāyusthalāstathā.
 Patramadyottake cāsthipoṣakā'mśāstathaiva ca.
 Kendre ca cipiṭo dārukendrīsaṃvahanajalāśayaḥ sthitaḥ.
 Ādhārasya tathā'dho'nte sthito dārūttakastathā.
 Saṃvahanījalāśayo dāruyojakānāṃ tathaiva ca.
 Sūkṣmordhvādharekhābhīranekābhīśca nirmitaḥ.
 Koṇīyasthūlasaṅkīrṇā dāruyojakabhittikāḥ.
 Madhyādidāruko'srīyaḥ sampārśviko jalāśayaḥ.
 Pīvarabhittisaṃśliṣṭairanekairdāruyojakaiḥ.
 Samānāntararekhābhīrarīyairnirmitastathā.
 Poṣavāhā viśiṣṭāśca vṛttagrantheradhastale.
 Yeṣāṃ madhye'lparikṭāñca sthānaṃ tatra ca vidyate.
 Asthipoṣakatattvāṃśāstattvāṃśaguhikā hi vā.
 Tathā ca poṣavāhāśca prāpyante hi mṛdūtake.
 Kośikayā talasyātra kośikābhyāṃ vāpi nirmitistathā.
 Saṃvahanījalāśayāvaraṇaṃ dṛḍhakośikātalena. (Saumitreya Mahodadhiḥ: 35-54)

The leaf has lateral veins and a broad midrib. The lamina is delicate and thin. The midrib is somewhat wider on the abaxial side and thicker, dome-shaped, on the adaxial side. There is a planoconvex midrib. The midrib has a thin epidermis, tiny, spindle-shaped cells with thin walls. The ground parenchymatous cells in the midrib are polygonal, compact, thin-walled cells located inside the epidermis. With an abaxial spongy mesophyll zone and an adaxial palisade layer, the lamina is dorsiventral. Lamina's epidermal cells are cutinized and rectangular in shape. In the epidermal layer, the stomata are paracytic type and widely dispersed. There are two equal or unequal subsidiary cells in each stoma. Guard cells have a wide elliptical shape. Palisade cells are less compact and have a single row that is narrowly cylindrical. Three to four layers of loosely distributed, tiny, spherical, or lobed spongy parenchyma cells. The large air spaces are seen in the spongy parenchyma. There are crystals of calcium oxalate in the mesophyll tissue. A flat and amphicribal vascular bundle is located in the centre. At the bottom end of the xylem stand, the vascular bundle is made up of many short, thin vertical lines of xylem components. The xylem components have thick walls, are angular, and are narrow. The bundle is collateral, endarch, and made up of many radial parallel lines of angular, thick-walled xylem components. The phloem is found on the bottom surface of the distinct, small, round lumps that have little spaces between them. The calcium oxalate crystals, or druses, are found in the phloem parenchyma. One or two cells make up the crystal layer. The sclerid layer covers the vascular bundles.^[1]

Structure of Petiole

Kāṣṭhīyapādape parṇavṛntaṃ tathārdhāvṛttajam.

Arddhāvṛttotalākāraṃ bhāge'pākṣe tathaiva ca.

Parṇavṛntasya vai sūkṣmabhittikābhīryutaistathā.

Nirmitiḥ kośikābhiśca tarkuvadadhicarmaṇaḥ.

Granthilā vṛntayuktāścādhicarmakośikāstathā.

Tathaikakośikīyāśca śākhitaromabhīryutāḥ.

Uttakaśca samānāṅgī mṛdūtakīyapoṣaṇaḥ.

Tasya copasthitiścātra nimnabhāge'dhicarmaṇaḥ.

Sūkṣmabhittiyutāścātra mṛdūtakīyakośikāḥ.

Koṇīyāḥ saghanāścāpi vṛttākāre tathaiva ca.

Bahutantuyutaścāsyā saṃvahanījalāśayaḥ.

Prāyaśca parṇavṛnte ṣaṭsaṃvahanījalāśayaḥ.

Sarve viśṛtacāpe te vyūhabaddhāstathaiva ca.

Abhyakṣamādhyakaścāpābhyakṣataśca bṛhattamaḥ.

Cāpe vṛttākṛtau prāyo laghupārśvajalāśayau.

Cipīṭāśca trikoṇīyā bṛhajjalāśayāstathā.

Sarve sampārśvikā dārusaṃvahanījalāśaye.

Ṣaṭtriyaḥkoṣakāḥ prāyaḥ kośikāsu tathaiva ca.

Laghujalāśaye dārughaṭakānām tripaṅktayaḥ.

Puñjāntaḥ spaṣṭaguccheṣu poṣavāhāstathaiva ca

Kṛṣṇakṣīradharaprāptiḥ saṁvardhanottakeṣu ca. (Saumitreya Mahodadhiḥ: 55-65)

The petiole is semicircular, having semicircular convexity on the abaxial side. It is made up of distinct spindle-shaped epidermal cells with thin walls. The epidermal cells having stalked, glandular, unicellular, and branched trichomes are present. There is homogenous, parenchymatous ground tissue present underneath the epidermis. The parenchymatous cells have thin walls, are compact, angular, and round in shape. The vascular system of the petiole is multistrand, consisting of around six vascular bundles grouped in a wide arc. The adaxial median bundle is the biggest of the tiny bundles that are positioned in the adaxial portion of the arc. There are two rather smaller lateral bundles. The smaller bundles have a circular form, whereas the bigger bundles have a flat, triangular contour. The bundles are collateral. There are around six radial multiples of cells that make up the xylem strands. The three rows of xylem components are seen in the smaller bundles. Phloem is found in distinct clusters within the bulk. The lactifers with a black stain are found in the ground tissues. ^[1]

Structure of Stem

Vṛttajākṛtikāṇḍaścādhicarmīyatalānvitaḥ.

Valkuṭīyasthalaḥ poṣavāho dārurdvitīyakaḥ.

Pr̥thumajjā tathā bāhyatamatalo'dhicarma yat.

Sthūlabhittiyutaṁ tarkukośikābhiśca nirmītam.

Kośikāścāyatākāre'dhicarmaṇastathaiva ca.

Tathaikakośikīyaiśca romabhiranvitaṁ tathā.

Ṣaṭto mṛdūtakīasya valkuṭāṣṭastarāstathā.

Khaṇḍairdṛḍhottakīyānām kośikānām sahaiva ca.

Prāpyante vitatā madhye proktāstalāstathaiva ca.

Tritaścatuḥstarāstatra sphaṇṭamṛdūtakasya ca.

Yuktā bhaṇḍāraṇadravyairvarṇahīnāśca te starāḥ.

Tatastritaścatuḥ sphaṇṭastarāśca haritottakāḥ.

Madhyādidārukaścātha saṁpārśvikahṛdasya ca.

Valayo valkuṭāntaśca tatra sthitastathaiva ca.

Ubhayatastathā dāruḥ kāṇḍasaṁracanākrame.

Saghanapoṣavāhānām nityastaraistathāvṛtaḥ.

Vāhikāśca tathā'rīyarūpeṇa suvyavasthitāḥ.

Sthūlabhittiyutā vṛttākārato dīrghavṛttajāḥ.

Ekalā vāhikā'rīyaguṇakeṣu punarhi vā.

Catuṣkoṇīyadāruśca kramaśasantulastathā.

Tathā ca saghanārīyapañkatiṣu suvyavasthitaḥ.

Kāṣṭhīyāḥ kośikāḥ sthūlabhittiyuktāstathaiva ca.

Kāṇḍasya poṣavāhasya dārudvitīyakastathā.

Satatavellanākāraranayāvṛtastathā.

Ghaṭakāḥ poṣavāhasya saṃkṣiptatrijyapañktiṣu.

Mṛdūtakiyamajjorvī khādyasaṃbhārakośajā. (Saumitreya Mahodadhiḥ: 55-79)

The stem is circular consists of an epidermal layer, cortical zone, secondary xylem and phloem and wide pith. The epidermis, the outermost layer, is composed of spindle-shaped cells with relatively thick walls that are rectangular in form. The epidermis having unicellular trichomes. Six to eight layers of parenchymatous cortex, interspersed with tiny patches of sclerenchymatous cells, are found inside the epidermis. Three to four layers of spongy chlorenchyma are layered after the outer three to four layers of colourless spongy parenchyma containing storage materials. A ring of endarch collateral vascular bundles is located inside the cortex. The xylem is surrounded by continuous layers of compacted phloem on both sides. The vessels are arranged radially. The thick-walled, round to elliptical vessels can be found alone or in radial multiples. The squarish xylem fibres are arranged in orderly, compact radial lines. The cells are thick walled and lignified. The xylem is surrounded by a continuous cylinder of secondary phloem. The phloem components are arranged in brief radial rows. The parenchymatous broad pith exhibits food material storage. ^[1]

Structure of Root

Tale viṣamamūlasyāsamabhidāstathaiva ca.

Tathā'samamitañcāsya mūlaṃ kiñcicca vikasitam.

Ekapakṣe'dhikā vṛddhirdvitīyakā'nyatastathā.

Vyutkramarūpato mūlaparicarma ca chidritam.

Nirmāti ca pṛthūttānavivarāṇi tathaiva ca.

Dabhormimatstareṣvevaṃ kośikāḥ paricarmanāḥ.

Laghvākṛti tathā mūle valkuṭīyasthalaṃ hi vai.

Kośikā valkuṭīyasthāḥ saṃśliṣṭāḥ katicitsthale.

Dṛśyate'nyatra vai chāyāyuktākuñcanaṃ tathā.

Tathottamasthitāvatra poṣavāho dvitīyakaḥ.

Vidyante'tra tathā mūle sahagamanakośikāḥ.

Cālanīghaṭakāścātra śliṣṭā mṛdūtakasthayā.

Poṣavāhāṃśabhittiśca dṛḍhā pṛthutarā tathā.

Vistṛtavāhikā dārutantośca bharaṇottake.

Nirmāṇaṃ kriyate tābhirdārudvitīyakasya ca.

Sanīkṛṇavāhikā mūlamadhyabhāge tathaiva ca.

Kāṣṭhīyavāhikāścātra vistīrṇā paridhiṃ prati.

Ekalā vṛttajāḥ sthūlabhittiyutāśca vāhikāḥ.

Riktasaghanasaṅkīrṇakośikātantunirmitiḥ. (Saumitreya Mahodadhiḥ: 79-89)

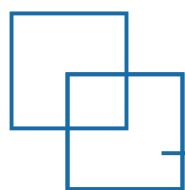
The surface of the rough root has uneven cracks. The root shows somewhat asymmetrical development, with greater secondary growth on one side than the other. The periderm is erratically punctured, creating broad, shallow cracks. Periderm cells are likewise compressed into wavy cell layers. The cortical zone is small, with cortical cells remaining intact in some places and collapsing into shadowy areas in others. The secondary phloem is in excellent form. It contains sieve components connected to the partner cells as well as parenchyma cells. The phloem components have strong walls and a large width. Diffusely scattered vessels in the ground tissue of xylem fibres make up secondary xylem. The vessels in the central part of the root are narrow and those towards the periphery are wider. The vessels are solitary, circular, and thick walled which are lignified. The fibre lumen is narrow and composed of compact and dense cells. ^[1]

References

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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 depends 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 Uttarkashi 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.

Among all the medicinal plants the *Croton bonplandianus* was considered in this study. It is commonly known as 'Ban tulsi' or 'Kala Bhangra' and profoundly grows around the canal, river bank, wastelands, and roadside area. It is an undershrub, stem with 10-12 lined, white-dotted glands, branching pattern is tri or tetracotomous. Medicinally, this plant is used to cure liver disorders, skin diseases, body swelling, bronchitis, asthma, etc. Based on this, the current chapter is focused on the morphology, taxonomy, anatomy, and distribution assessment of *C. bonplandianus* around the Ganga River.

Genus *Croton* L.

Croton L. is a genus of 1150 accepted species that are widely distributed in tropical and subtropical areas to North America (POWO, 2023). The genus belongs to the family Euphorbiaceae. This genus prefers the fertile, well-drained, and moist soil. It comprises herbs, shrubs, trees, and occasionally lianas which are the ecologically prominent part of the secondary vegetation (Webster, 1993; Beyer et al., 2022). Although it is a pantropical genus, its maximum diversity of up to two-thirds of the New World species exists in the Neotropics and in Madagascar (Radcliffe-Smith, 2001; Van Ee et al., 2011). It prefers dry to moist land in tropical and subtropical areas throughout the world. In the field, it can be recognized by its pungent odour, stellate or lepidote pubescence, clear to coloured latex, and leaves that turn orange before dehiscing. Many of the plants of this genus are grown indoors and need bright or indirect light. The outdoor plants grow in partial shade and cool climates and could grow in full sunlight but need moisture. This genus was first time established by Linnaeus with a total of 17 species based on the brief description of male and female flowers, fruits, and seeds (Chakrabarty and Balakrishnan, 1992). Linnaeus's work on this genus was a heterogeneous assemblage because many of the species that were described by him under this genus now shifted to the other genus. After Linnaeus, numerous species were discovered and documented under this genus hence, this genus is one of the largest genera in the family Euphorbiaceae.

In India, firstly J. D. Hooker (1887) mentioned 30 species of this genus in the flora of British India. Chakrabarty and Balakrishnan (1992) revised these plants and suggested the two species i.e., *C. tiglium* and *C. caudatus* are the most widespread species. *C. bonplandianus* is native to South America and was introduced in the Indian continent at Chittagong in Bangladesh around 1897-98 through port introduction (Bruhl, 1908; Joshi, 1934; Croizat, 1940; Kaul, 1967; Chakrabarty and Balakrishnan, 1992). Similarly, *C. glandulosus* var. *hirtus* which is a native of the West Indies and Central & South America, was introduced in India and was first time recorded by Ramachandran et al. (1992) from South India. In recent literature by Dhabak et al. (2021), this genus has a total of 16 taxa in India, among them four species namely *C. gibsonianus* Nimmo, *C. lawianus* Nimmo, *C. malabaricus* Bedd., and *C. scabiosus* Bedd. are endemic. The species like *C. bonplandianus* Baill. and *C. glandulosus* L. are introduced in India which are now naturalized and commonly occur as weeds (Balakrishnan and Chakrabarty, 2007; Nayar et al., 2014; Singh et al., 2015).

Etymology

The generic name, arbitrarily used by Linnaeus to replace the term 'Ricnoides' given to this collection by Tournefort (1700), is derived from the Greek word kroton (a sheep tick), because the seed closely resembled to the tick (Chakrabarty and Balakrishnan, 1992; Moremi et al., 2021).

Habitat and Distribution

Croton is a pantropical genus and widely distributed in tropical and subtropical areas in the world in fertile, well-drained, and moist soil. According to the Plants of the World Online database (POWO, 2023), this genus is native to the Alabama, Andaman Is., Angola, Argentina Northeast, Argentina Northwest, Arizona, Arkansas, Aruba, Assam, Bahamas, Bangladesh, Belize, Benin, Bermuda, Bismarck Archipelago, Bolivia, Borneo, Botswana, Brazil North, Brazil Northeast, Brazil South, Brazil Southeast, Brazil West-Central, Burkina, Burundi, Cabinda, California, Cambodia, Cameroon, Cape Provinces, Caprivi Strip, Caroline Is., Cayman Is., Central African Repu, Chad, Chile Central, Chile North, China South-Central, China Southeast, Christmas Is., Colombia, Colorado, Comoros, Congo, Connecticut, Costa Rica, Cuba, Delaware, District of Columbia, Djibouti, Dominican Republic, East Himalaya, Ecuador, El Salvador, Eritrea, Ethiopia, Fiji, Florida, French Guiana, Gabon, Galápagos, Gambia, Georgia, Ghana, Guatemala, Guinea, Gulf of Guinea Is., Guyana, Hainan, Haiti, Honduras, Idaho, Illinois, India, Indiana, Iowa, Ivory Coast, Jamaica, Jawa, Kansas, Kentucky, Kenya, KwaZulu-Natal, Laos, Leeward Is., Lesser Sunda Is., Liberia,

Louisiana, Madagascar, Malawi, Malaya, Maldives, Mali, Maluku, Marianas, Maryland, Massachusetts, Mauritius, Mexican Pacific Is., Mexico Central, Mexico Gulf, Mexico Northeast, Mexico Northwest, Mexico Southeast, Mexico Southwest, Michigan, Minnesota, Mississippi, Missouri, Mozambique, Myanmar, Namibia, Nansei-shoto, Nebraska, Nepal, Netherlands Antilles, Nevada, New Caledonia, New Guinea, New Jersey, New Mexico, New South Wales, New York, Nicaragua, Nicobar Is., Nigeria, North Carolina, Northern Provinces, Northern Territory, Ohio, Oklahoma, Oman, Oregon, Pakistan, Panamá, Paraguay, Pennsylvania, Peru, Philippines, Puerto Rico, Queensland, Rwanda, Réunion, Senegal, Sierra Leone, Socotra, Solomon Is., Somalia, South Carolina, South Dakota, Southwest Caribbean, Sri Lanka, Sudan, Sulawesi, Sumatera, Suriname, Taiwan, Tanzania, Tennessee, Texas, Thailand, Tonga, Trinidad-Tobago, Turks-Caicos Is., Uganda, Uruguay, Utah, Vanuatu, Venezuela, Venezuelan Antilles, Vietnam, Virginia, Washington, West Virginia, Western Australia, Windward Is., Wisconsin, Wyoming, Yemen, Zambia, Zaïre, Zimbabwe; while it is introduced in the Chagos Archipelago, Guinea-Bissau, Gulf States, Japan, Ontario, Rodrigues, Victoria, West Himalaya (Fig. 1).

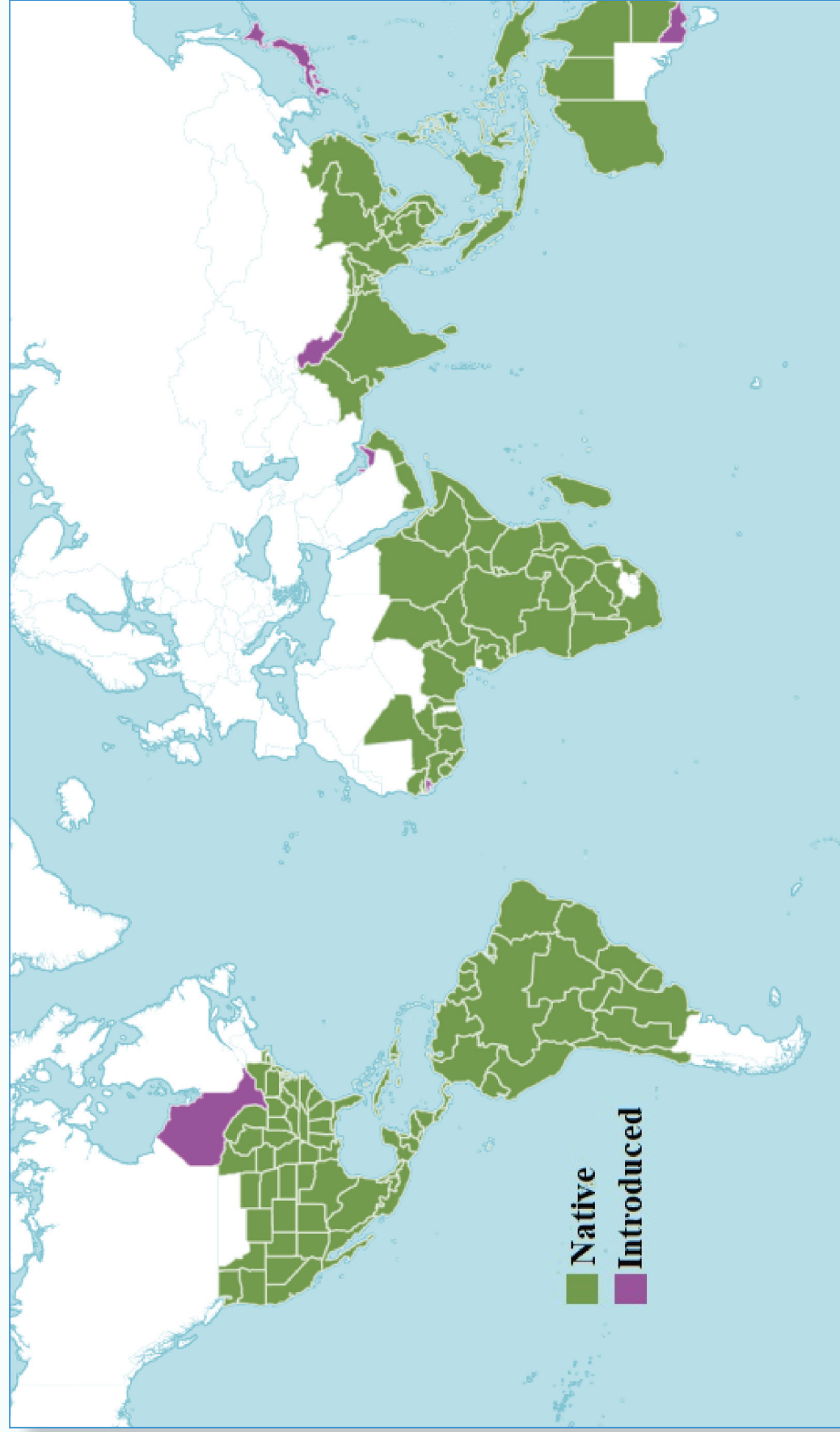


Fig. 1 Global distribution of *Croton*

Botanical Characteristics

Herbs, shrubs, or subshrubs; monoecious or rarely dioecious; annual or perennial. Hairs stellate or scalelike; latex scanty, colorless to reddish, sometimes absent. Leaves persistent, stipulate, or exstipulate, alternate, rarely opposite, semideciduous, or drought deciduous, simple, often with lemony, pungent, older leaves often turning orange before falling; petiole with or without apical paired glands; blade simple sometimes palmately lobed, margin entire crenate, denticulate, serrulate, or serrate-dentate, often with glands; venation pinnate or palmate. Inflorescences unisexual or bisexual, terminal or axillary, raceme like thyrses, often with proximal solitary female flowers and distal male flowers, often in clusters. Staminate flowers: sepals 5, imbricate to valvate, free; petals 5, free, smaller or nearly equal to sepals, white; nectary extrastaminal, disk glandular, usually 5, and opposite sepals; stamens 3-50; distinct, filaments free, inflexed in bud; anthers muticous; pistillode absent. Pistillate flowers: sepals 5, imbricate or valvate, distinct, often narrower than in males, persistent, sometimes accrescent; petals 5 sometimes rudimentary or 0, smaller than in males, distinct or connate basally, mostly reduced or obsolete; white or pale green; nectary annular, glands 5 or absent, disk annular or dissected, receptacle often villous; ovary 1-3 locular; distinct or connate basally, style 1-3, longer and slender, free or nearly so, once to several times bifid, ovules 1 per locule. Fruit a capsule, drupelike or berrylike.

Seeds ellipsoid, oblong, ovoid, globose, or lenticular, usually smooth, carunculate; testa crustaceous; endosperm copious, fleshy; cotyledon broad and flattened.

Ethnomedicinal Importance

The *Croton* has important species that have a broad spectrum of ethnomedicinal uses worldwide. It is extensively used in the treatment of sexually transmitted infections (Iwu, 1993; Ndubani and Hojer, 1999; Langat et al., 2008; Usman et al., 2009; Moremi et al., 2021). Apart from this, it is widely used in the treatment of diabetes, malaria, fever, inflammation, and cancer (Moremi et al., 2021). Extracts of many species as *C. gratissimus* showed antimicrobial activity against pathogens associated with prevalent urogenital/sexually transmitted infections (VAN Vuuren and Naidoo, 2010). *C. gratissimus* is used in controlling blood pressure, gonorrhoea, arthritis, diarrhea, impotency, and urinary tract infections (Iwu, 1993; Usman et al., 2009). The seeds of *C. megalobotrys* are used as a laxative, while the roots are used to turn a thin baby into a fatter one (Watt and Breyer-Brandwijk, 1962). The hepatitis can be mitigated by using the decoction of fresh or dried leaves and the intestinal obstruction can be cleared by the administration of bark decoction (Schmelzer and Gurib-Fakim, 2008).

The leaf infusion of *C. sylvaticus* is taken as a purgative, while its bark or root decoction is used to treat tuberculosis, fever, abnormal swelling, internal inflammation, digestive and abdominal disorders, rheumatism, lethargy, and uterine

disorder [Watt and Breyer-Brandwijk, 1962; Palgrave, 1983; Hutchings et al., 1996]. External application of the leaf sap treats ear infections, and a body wash made from the leaf decoction reduces edema [Watt and Breyer-Brandwijk, 1962; Palgrave, 1983; Mwangi et al., 1998]. *C. macrostachyus* is an important species of the genus that is widely used in African countries and is useful in the control of bleeding, blood clotting, cancer, constipation, diarrhea, epilepsy, malaria, pneumonia, as a purgative, ringworm, skin diseases or infections, stomachache, typhoid, worm expulsion, and help in healing wounds [Maroyi, 2017].

Chemical Constituents

Croton species are reported to possess several medicinal properties owing to their

rich phytochemicals. To date, about 419 chemical compounds have been isolated and identified. Among them, 339 are diterpenoids including clerodane, tiglane, kaurane, crotofolane, labdane, cembrane, abietane, casbane, halimane, pimarane, cleistanthane, grayanane, atisane, phytane, laevinane, and meroditerpenoids. The remaining 17 are terpenoids including sesquiterpenoids, sesterterpenoids, and triterpenoids, 10 flavonoid, 21 glycosides, 10 alkaloids, 3 benzoate derivatives, 3 pyran-2-one derivatives, 2 each of cyclopeptide, tropone derivatives, and limonoids, and 10 miscellaneous compounds [Xu et al., 2018; Moremi et al., 2021]. The chemical's name and sources are elaborated in Table 1.

Table 1 Various chemicals from the *Croton* species

S. No.	Chemical Compounds	Chemical formula	<i>Croton</i> Species
Clerodanes			
1	ent-3,13E-Clerodadiene-15-formate	$C_{21}H_{34}O_2$	<i>C. sylvaticus</i>
2	9-[2-(2(5H)-Furanone-4-yl)eth-yl]-4,8,9-trimethyl-1,2,3,4,5,6,7,8-octahydronaphthalene-4-carboxylic acid	$C_{20}H_{28}O_4$	<i>C. crassifolius</i>
3	9-[2-(2(5H)-Furanone-4-yl)eth-yl]-4,8,9-trimethyl-1,2,3,4,5,6,7,8-octahydronaphthalene-4-carboxylic ester	$C_{21}H_{30}O_4$	<i>C. crassifolius</i>
4	Centrafricine I	$C_{21}H_{24}O_6$	<i>C. mayumbensis</i>
5	Marrubiagenin	$C_{20}H_{28}O_4$	<i>C. glabellus</i>
6	Methyl 15,16-epoxy-3,13(16),14-ent-clerodatrien-18,19-olide-17-carboxylate	$C_{21}H_{26}O_5$	<i>C. oblongifolius</i>
7	Dimethyl 15,16-epoxy-12-oxo-3,13(16),14-ent-clerodatriene-17,18-dicarboxylate	$C_{22}H_{28}O_6$	<i>C. oblongifolius</i>
8	Isoteucvin	$C_{19}H_{20}O_5$	<i>C. jatrophioides</i>

S. No.	Chemical Compounds	Chemical formula	<i>Croton</i> Species
9	Jatrophoidin	$C_{21}H_{22}O_7$	<i>C. jatrophoides</i>
10	8-Epicordatin	$C_{21}H_{26}O_6$	<i>C. palanostigma</i>
11	Laevigatbenzoate	$C_{27}H_{31}O_5$	<i>C. laevigatus</i>
12	3,4,15,16-Diepoxy-cleroda-13(16),14-diene-12,17-olide	$C_{20}H_{26}O_4$	<i>C. oblongifolius</i>
13	Crassifolin A	$C_{21}H_{30}O_4$	<i>C. crassifolius</i>
14	Crassifolin B	$C_{20}H_{29}O_4$	<i>C. crassifolius</i>
15	Crassifolin C	$C_{21}H_{24}O_5$	<i>C. crassifolius</i>
16	Crassifolin D	$C_{21}H_{24}O_6$	<i>C. crassifolius</i>
17	Crassifolin E	$C_{20}H_{23}O_6$	<i>C. crassifolius</i>
18	Crassifolin F	$C_{23}H_{29}O_7$	<i>C. crassifolius</i>
19	Crassifolin G	$C_{19}H_{20}O_6$	<i>C. crassifolius</i>
20	Methyl 3-oxo-12-epibarbascoate	$C_{21}H_{26}O_6$	<i>C. urucurana</i>
21	Laevinoids A	$C_{20}H_{22}O_5$	<i>C. laevigatus</i>
22	Laevinoids B	$C_{20}H_{23}O_5Cl$	<i>C. laevigatus</i>
23	Crotonolide A	$C_{20}H_{18}O_6$	<i>C. laui</i>
24	Crotonolide B	$C_{21}H_{24}O_6$	<i>C. laui</i>
25	Isocrotonolide B	$C_{21}H_{24}O_6$	<i>C. laui</i>
26	Crotonolide C	$C_{23}H_{26}O_8$	<i>C. laui</i>
27	Isocrotonolide C	$C_{23}H_{26}O_8$	<i>C. laui</i>
28	Crotonolide D	$C_{21}H_{26}O_6$	<i>C. laui</i>
29	Isocrotonolide D	$C_{21}H_{26}O_6$	<i>C. laui</i>
30	Crotonolide E	$C_{20}H_{26}O_4$	<i>C. laui</i>
31	Crotonolide F	$C_{20}H_{26}O_4$	<i>C. laui</i>
32	Crotonolide G	$C_{20}H_{32}O$	<i>C. laui</i>
33	Crotonolide H	$C_{20}H_{32}O_4$	<i>C. laui</i>
34	12-Deoxycrotonolide H	$C_{20}H_{32}O_3$	<i>C. laui</i>
35	Crotonoligaketone	$C_{23}H_{26}O_8$	<i>C. oligandrum</i>
36	Crotonpene A	$C_{20}H_{26}O_3$	<i>C. yanhuui</i>
37	Crotonpene B	$C_{21}H_{28}O_5$	<i>C. yanhuui</i>
38	Crassifolin I	$C_{20}H_{22}O_6$	<i>C. crassifolius</i>
39	Crassifolin H	$C_{19}H_{20}O_5$	<i>C. crassifolius</i>

S. No.	Chemical Compounds	Chemical formula	<i>Croton</i> Species
40	Crotoeurin A	$C_{38}H_{36}O_1$	<i>C. euryphyllus</i>
41	Crotoeurin B	$C_{20}H_{24}O_6$	<i>C. euryphyllus</i>
42	Crotoeurin C	$C_{20}H_{22}O_6$	<i>C. euryphyllus</i>
43	3-Oxo-15,16-epoxy-4a,12-dihydroxy-ent-neo-clerodan-13(16),14-diene	$C_{20}H_{30}O_4$	<i>C. limae</i>
44	15,16-Epoxy-3a,4a,12-trihydroxy-ent-neo-clerodan-13(16),14-diene	$C_{20}H_{32}O_4$	<i>C. limae</i>
45	3a,4a,15,16-Tetrahydroxy-ent-neo-cleroda-13E-ene	$C_{20}H_{36}O_4$	<i>C. limae</i>
46	Cracroson A	$C_{19}H_{21}O_6$	<i>C. crassifolius</i>
47	Cracroson B	$C_{20}H_{22}O_6$	<i>C. crassifolius</i>
48	Cracroson C	$C_{19}H_{19}O_4N$	<i>C. crassifolius</i>
49	Crassifolin J	$C_{20}H_{20}O_5$	<i>C. crassifolius</i>
50	Crotocorylifuran-2-one	$C_{22}H_{24}O_8$	<i>C. megalocarpoides</i>
51	Megalocarpoidolide D	$C_{22}H_{22}O_8$	<i>C. megalocarpoides</i>
52	7,8-Dehydrocrotocorylifuran	$C_{22}H_{24}O_7$	<i>C. megalocarpoides</i>
53	Megalocarpoidolide E	$C_{22}H_{24}O_8$	<i>C. megalocarpoides</i>
54	Megalocarpoidolide F	$C_{22}H_{24}O_8$	<i>C. megalocarpoides</i>
55	Megalocarpoidolide G	$C_{22}H_{24}O_9$	<i>C. megalocarpoides</i>
56	Megalocarpoidolide H	$C_{24}H_{28}O_{10}$	<i>C. megalocarpoides</i>
57	Launine K	$C_{27}H_{36}O_3$	<i>C. laui</i>
58	Crassin A	$C_{17}H_{20}O_4$	<i>C. crassifolius</i>
59	Crassin B	$C_{17}H_{20}O_4$	<i>C. crassifolius</i>
60	Crassin C	$C_{21}H_{24}O_6$	<i>C. crassifolius</i>
61	Crassin D	$C_{20}H_{20}O_5$	<i>C. crassifolius</i>
62	Crassin E	$C_{19}H_{20}O_3$	<i>C. crassifolius</i>
63	Crassin F	$C_{19}H_{18}O_7$	<i>C. crassifolius</i>
64	Crassin G	$C_{20}H_{26}O_5$	<i>C. crassifolius</i>

S. No.	Chemical Compounds	Chemical formula	<i>Croton</i> Species
65	Crassin H	$C_{21}H_{30}O_5$	<i>C. crassifolius</i>
66	Crassifolius A	$C_{20}H_{22}O_5$	<i>C. crassifolius</i>
67	Crassifolius B	$C_{21}H_{24}O_6$	<i>C. crassifolius</i>
68	Crassifolius C	$C_{21}H_{26}O_5$	<i>C. crassifolius</i>
69	Crolaevinoid C	$C_{27}H_{28}O_6$	<i>C. laevigatus</i>
70	Crolaevinoid D	$C_{27}H_{32}O_8$	<i>C. laevigatus</i>
71	Crolaevinoid E	$C_{20}H_{28}O_6$	<i>C. laevigatus</i>
72	Crolaevinoid F	$C_{21}H_{30}O_5$	<i>C. laevigatus</i>
73	Norcrassifolin	$C_{19}H_{18}O_4$	<i>C. crassifolius</i>
74	Hypolein A	$C_{20}H_{26}O_4$	<i>C. hypoleucus</i>
75	Hypolein B	$C_{20}H_{28}O_3$	<i>C. hypoleucus</i>
76	Hypolein C	$C_{20}H_{28}O_3$	<i>C. hypoleucus</i>
77	Cracroson E	$C_{19}H_{20}O_6$	<i>C. crassifolius</i>
78	Cracroson F	$C_{19}H_{20}O_6$	<i>C. crassifolius</i>
79	Cracroson G	$C_{21}H_{26}O_7$	<i>C. crassifolius</i>
80	12-Epi-megalocarpoidolide D	$C_{22}H_{22}O_8$	<i>C. oligandrus</i>
81	Crotonolins A	$C_{22}H_{22}O_{10}$	<i>C. oligandrus</i>
82	Crotonolins B	$C_{22}H_{22}O_{10}$	<i>C. oligandrus</i>
Tiglianines			
93	12-O-Isobutyrylphorbol-13-decanoate	$C_{34}H_{52}O_8$	<i>C. tiglium</i>
94	12-O-(2-Methyl)butyrylphorbol-13-octanoate	$C_{33}H_{50}O_8$	<i>C. tiglium</i>
95	12-O-[(2R)-N,N-Dimethyl-3-methylbutanoyl]-4-deoxyphorbol 13-acetate	$C_{29}H_{43}NO_7$	<i>C. ciliatoglandulifer</i>
96	12-O-[(2S)-N,N-Dimethyl-3-methylbutanoyl]-4-deoxyphorbol 13-acetate	$C_{29}H_{43}NO_7$	<i>C. ciliatoglandulifer</i>
97	12-O-[(2R)-N,N-Dimethyl-3-methylbutanoyl]phorbol 13-acetate	$C_{29}H_{43}NO_8$	<i>C. ciliatoglandulifer</i>
98	12-O-[3-Methyl-2-butenoyl]-4-deoxyphorbol 13-acetate	$C_{27}H_{36}NO_7$	<i>C. ciliatoglandulifer</i>
99	12-O-(2-Methyl)butyrylphorbol-13-tiglate	$C_{30}H_{42}O_8$	<i>C. tiglium</i>
100	12-O-Tiglylphorbol-13-propionate	$C_{28}H_{38}O_8$	<i>C. tiglium</i>
101	13-O-Acetylphorbol-20-oleate	$C_{40}H_{62}O_8$	<i>C. tiglium</i>
106	12-O-Tiglyl-4-deoxy-4a-phorbol-13-(2-methyl)butyrate	$C_{30}H_{42}O_7$	<i>C. tiglium</i>

S. No.	Chemical Compounds	Chemical formula	<i>Croton</i> Species
107	Alienusolin	$C_{42}H_{66}O_8$	<i>C. alienus</i>
108	12-O-Acetyl-5,6-didehydro-7-oxophorbol-13-yl 2-methylbutanoate	$C_{27}H_{36}O_9$	<i>C. tiglium</i>
109	12-O-Acetyl-5,6-didehydro-7-oxophorbol-13-yl 2-methylpropanoate	$C_{26}H_{34}O_9$	<i>C. tiglium</i>
110	12-O-Acetyl-5,6-didehydro-6,7-dihydro-7-hydroxyphorbol-13-yl 2-methylbutanoate	$C_{27}H_{38}O_9$	<i>C. tiglium</i>
111	12-O-Decanoyl-7-hydroperoxy-phorbol-5-ene-13-acetate	$C_{32}H_{42}O_{10}$	<i>C. mauritanus</i>
112	20-Deoxy-20-oxophorbol 12-tiglate 13-(2-methyl)butyrate	$C_{30}H_{40}O_8$	<i>C. tiglium</i>
113	12-O-Acetylphorbol-13-isobutyrate	$C_{26}H_{36}O_8$	<i>C. tiglium</i>
114	12-O-Benzoylphorbol-13-(2-methyl)butyrate	$C_{32}H_{40}O_8$	<i>C. tiglium</i>
115	12-O-Tiglyl-7-oxo-5-ene-phorbol-13-(2-methyl)butyrate	$C_{30}H_{40}O_9$	<i>C. tiglium</i>
116	13-O-(2-Methyl)butyryl-4-deoxy-4a-phorbol	$C_{25}H_{36}O_6$	<i>C. tiglium</i>
117	Crotignoid A	$C_{30}H_{42}O_{10}$	<i>C. tiglium</i>
118	Crotignoid B	$C_{29}H_{40}O_{10}$	<i>C. tiglium</i>
119	Crotignoid C	$C_{30}H_{42}O_9$	<i>C. tiglium</i>
120	Crotignoid D	$C_{29}H_{40}O_9$	<i>C. tiglium</i>
121	Crotignoid E	$C_{29}H_{38}O_9$	<i>C. tiglium</i>
122	Crotignoid F	$C_{28}H_{36}O_9$	<i>C. tiglium</i>
123	Crotignoid G	$C_{30}H_{44}O_8$	<i>C. tiglium</i>
124	Crotignoid H	$C_{29}H_{38}O_8$	<i>C. tiglium</i>
125	Crotignoid I	$C_{30}H_{44}O_8$	<i>C. tiglium</i>
126	Crotignoid J	$C_{31}H_{38}O_8$	<i>C. tiglium</i>
127	Crotignoid K	$C_{29}H_{34}O_7$	<i>C. tiglium</i>
128	Crotusin A	$C_{36}H_{54}O_{10}$	<i>C. caudatus</i>
129	Crotusin B	$C_{46}H_{72}O_{11}$	<i>C. caudatus</i>
130	Crotusin C	$C_{36}H_{52}O_{11}$	<i>C. caudatus</i>
131	12-O-Tiglylphorbol-4-deoxy-4β-phorbol-13-acetate	$C_{27}H_{36}O_7$	<i>C. tiglium</i>
132	12-O-Tiglylphorbol-4-deoxy-4β-phorbol-13-hexadecanoate	$C_{41}H_{64}O_7$	<i>C. tiglium</i>

S. No.	Chemical Compounds	Chemical formula	<i>Croton</i> Species
133	13-O-Acetylphorbol-4-deoxy-4 β -phorbol-20-oleate	C ₄₀ H ₆₂ O ₇	<i>C. tiglim</i>
134	13-O-Acetylphorbol-4-deoxy-4 β -phorbol-20-linoleate	C ₄₀ H ₆₀ O ₇	<i>C. tiglim</i>
135	4-Deoxy-20-oxophorbol 12-tiglyl 13-acetate	C ₂₇ H ₃₄ O ₇	<i>C. tiglim</i>
136	7-Oxo-5-ene-phorbol-13-(2-methylbutyrate)	C ₂₅ H ₃₄ O ₈	<i>C. tiglim</i>
137	7-Hydroxyl-phorbol-5-ene-13-(2-methylbutyrate)	C ₂₅ H ₃₆ O ₈	<i>C. tiglim</i>
Kauranes			
138	Caracasine	C ₂₁ H ₃₀ O ₃	<i>C. caracasana</i>
139	Caracasine acid	C ₂₀ H ₂₈ O ₃	<i>C. caracasana</i>
140	Kongensin A	C ₂₂ H ₃₀ O ₅	<i>C. kongensis</i>
141	Kongensin B	C ₂₂ H ₃₀ O ₆	<i>C. kongensis</i>
142	Kongensin C	C ₂₀ H ₂₈ O ₅	<i>C. kongensis</i>
143	Kongensin D	C ₂₀ H ₂₈ O ₄	<i>C. kongensis</i>
144	Kongensin E	C ₂₆ H ₃₆ O ₇	<i>C. kongensis</i>
145	Kongensin F	C ₂₄ H ₃₄ O ₅	<i>C. kongensis</i>
146	Crotonkinin A	C ₂₀ H ₃₀ O ₂	<i>C. tonkinensis</i>
147	Crotonkinin B	C ₂₂ H ₃₂ O ₄	<i>C. tonkinensis</i>
148	14-Epi-hyalic acid	C ₂₀ H ₂₈ O ₄	<i>C. argyrophyllodes</i>
149	14-[[2-Methylbutanoyl]oxy]-3,4-seco-ent-kaura-4(19),16-dien-3-oic acid	C ₂₅ H ₃₉ O ₄	<i>C. megistocarpus</i>
150	14-[[[2Z]-2-Methylbut-2-enoyl]oxy]-3,4-seco-ent-kaura-4(19),16-dien-3-oic acid	C ₂₅ H ₃₇ O ₄	<i>C. megistocarpus</i>
151	ent-11 β -Acetoxykaur-16-en-18-ol	C ₂₂ H ₃₄ O ₃	<i>C. tonkinensis</i>
152	ent-11a-Hydroxy-18-acetoxykaur-16-ene	C ₂₂ H ₃₄ O ₃	<i>C. tonkinensis</i>
153	ent-14 β -Hydroxy-18-acetoxykaur-16-ene	C ₂₂ H ₃₄ O ₃	<i>C. tonkinensis</i>
154	ent-7a-Hydroxy-18-acetoxykaur-16-ene	C ₂₂ H ₃₄ O ₃	<i>C. tonkinensis</i>
155	ent-14S*-Hydroxykaur-16-en-19-oic acid	C ₂₀ H ₃₀ O ₃	<i>C. pseudopulchellus</i>
156	ent-14S*,17-Dihydroxykaur-15-en-19-oic acid	C ₂₀ H ₃₀ O ₄	<i>C. pseudopulchellus</i>
157	ent-3,4-Seco-17-oxo-kaur-4(19),15(16)-dien-3-oic acid	C ₂₀ H ₂₈ O ₃	<i>C. oblongifolius</i>

S. No.	Chemical Compounds	Chemical formula	<i>Croton</i> Species
158	Crotonkinin C	$C_{22}H_{30}O_5$	<i>C. tonkinensis</i>
159	Crotonkinin D	$C_{24}H_{34}O_6$	<i>C. tonkinensis</i>
160	Crotonkinin E	$C_{24}H_{34}O_5$	<i>C. tonkinensis</i>
161	Crotonkinin F	$C_{24}H_{34}O_5$	<i>C. tonkinensis</i>
162	Crotonkinin G	$C_{23}H_{36}O_5$	<i>C. tonkinensis</i>
163	Crotonkinin H	$C_{22}H_{36}O_4$	<i>C. tonkinensis</i>
164	Crotonkinin I	$C_{24}H_{36}O_5$	<i>C. tonkinensis</i>
165	Crotonkinin J	$C_{23}H_{34}O_5$	<i>C. tonkinensis</i>
166	14 β -Hydroxy-3-oxo-ent-kaur-16-ene	$C_{20}H_{30}O_2$	<i>C. kongensis</i>
167	Kongeniod A	$C_{21}H_{30}O_3$	<i>C. kongensis</i>
168	Kongeniod B	$C_{21}H_{30}O_4$	<i>C. kongensis</i>
169	Kongeniod C	$C_{23}H_{32}O_5$	<i>C. kongensis</i>
170	15-Oxo-17(10'- α -pinenyl)-kauran-18-oic acid	$C_{30}H_{44}O_3$	<i>C. limae</i>
171	Micansinoic acid	$C_{40}H_{58}O_7$	<i>C. micans</i>
172	Isomicansinoic acid	$C_{40}H_{58}O_7$	<i>C. micans</i>
173	Dimethylester of micansinoic	$C_{42}H_{62}O_7$	<i>C. micans</i>
174	Methyl-micansinoic acid	$C_{41}H_{60}O_7$	<i>C. micans</i>
175	Ethyl-micansinoic acid	$C_{42}H_{62}O_7$	<i>C. micans</i>
176	Crotonkinensin C	$C_{40}H_{62}O_8$	<i>C. tonkinensis</i>
177	Crotonkinensin D	$C_{44}H_{66}O_{10}$	<i>C. tonkinensis</i>
Crotofolanes			
178	Crotocascarin A	$C_{25}H_{32}O_7$	<i>C. cascarilloides</i>
179	Crotocascarin B	$C_{25}H_{32}O_7$	<i>C. cascarilloides</i>
180	Crotocascarin C	$C_{25}H_{32}O_8$	<i>C. cascarilloides</i>
181	Crotocascarin D	$C_{25}H_{32}O_6$	<i>C. cascarilloides</i>
182	Crotocascarin E	$C_{26}H_{34}O_8$	<i>C. cascarilloides</i>
183	Crotocascarin F	$C_{24}H_{30}O_7$	<i>C. cascarilloides</i>
184	Crotocascarin G	$C_{24}H_{30}O_7$	<i>C. cascarilloides</i>
185	Crotocascarin H	$C_{24}H_{30}O_8$	<i>C. cascarilloides</i>
186	Crotocascarin α	$C_{24}H_{32}O_8$	<i>C. cascarilloides</i>
187	Crotocascarin β	$C_{24}H_{32}O_7$	<i>C. cascarilloides</i>
188	(5 β ,6 β)-5,6:13,16-Diepoxycrotofolan-4(9),10(18),13,15-tetraen-1-one	$C_{20}H_{22}O_3$	<i>C. argyrophyllus</i>

S. No.	Chemical Compounds	Chemical formula	<i>Croton</i> Species
189	(5 β ,6 β)-5,6:13,16-Diepoxy-2-epicrotola-4(9),10(18),13,15-tetraen-1-one	C ₂₀ H ₂₂ O ₃	<i>C. argyrophyllus</i>
190	(5 β ,6 β)-5,6:13,16-Diepoxy-16-hydroxycrotofo-4(9),10(18),13,15-tetraen-1-one	C ₂₀ H ₂₂ O ₄	<i>C. argyrophyllus</i>
191	(5 β ,6 β)-5,6:13,16-Diepoxy-16-hydroxy-2-epicrotola-4(9),10(18),13,15-tetraen-1-one	C ₂₀ H ₂₂ O ₄	<i>C. argyrophyllus</i>
192	Crotocarasin A	C ₂₀ H ₂₂ O ₄	<i>C. caracasanus</i>
193	Crotocarasin B	C ₂₀ H ₂₂ O ₄	<i>C. caracasanus</i>
194	Crotocarasin C	C ₂₂ H ₂₆ O ₅	<i>C. caracasanus</i>
195	Crotocarasin D	C ₂₂ H ₂₆ O ₅	<i>C. caracasanus</i>
196	EBC-162	C ₂₀ H ₂₄ O ₂	<i>C. insularis</i>
197	EBC-233	C ₂₀ H ₂₄ O ₄	<i>C. insularis</i>
198	EBC-300	C ₂₀ H ₂₄ O ₄	<i>C. insularis</i>
199	EBC-240	C ₂₀ H ₂₆ O ₅	<i>C. insularis</i>
200	EBC-241	C ₂₀ H ₂₆ O ₅	<i>C. insularis</i>
201	Crotocascarin I	C ₂₀ H ₂₄ O ₅	<i>C. cascarilloides</i>
202	Crotocascarin J	C ₂₀ H ₂₄ O ₆	<i>C. cascarilloides</i>
203	Crotocascarin K	C ₂₀ H ₂₄ O ₅	<i>C. cascarilloides</i>
204	Crotocascarin γ	C ₁₉ H ₂₄ O ₆	<i>C. cascarilloides</i>
205	Crotocascarin L	C ₂₂ H ₂₆ O ₇	<i>C. cascarilloides</i>
206	Crotocascarin M	C ₂₁ H ₂₆ O ₆	<i>C. cascarilloides</i>
207	Crotocascarin N	C ₂₀ H ₂₂ O ₆	<i>C. cascarilloides</i>
208	Crotocascarin O	C ₂₅ H ₃₄ O ₉	<i>C. cascarilloides</i>
209	Crotocascarin P	C ₂₅ H ₃₄ O ₈	<i>C. cascarilloides</i>
210	Crotocascarin Q	C ₂₅ H ₃₂ O ₇	<i>C. cascarilloides</i>
211	Neocrotocascarin	C ₂₅ H ₃₂ O ₈	<i>C. cascarilloides</i>
212	Crotodichogamoin A	C ₂₀ H ₂₂ O ₄	<i>C. dichogamus</i>
213	Crotodichogamoin B	C ₂₀ H ₂₂ O ₂	<i>C. dichogamus</i>
214	Cascarinoid A	C ₂₈ H ₃₁ NO ₅	<i>C. cascarilloides</i>
215	Cascarinoid B	C ₂₈ H ₃₁ NO ₅	<i>C. cascarilloides</i>
216	Cascarinoid C	C ₂₈ H ₃₁ NO ₆	<i>C. cascarilloides</i>
Labdanes			
217	Labdinine N	C ₂₀ H ₃₄ O ₃	<i>C. laui</i>

S. No.	Chemical Compounds	Chemical formula	Croton Species
218	ent-12,15-Dioxo-3,4-seco-4,8,13-labdatrien-3-oic acid	$C_{20}H_{28}O_4$	<i>C. stipuliformis</i>
219	ent-12,15-Epoxy-3,4-seco-4,8,12,14-labdatetraen-3-oic acid	$C_{20}H_{28}O_3$	<i>C. stipuliformis</i>
220	ent-15-Nor-14-oxo-3,4-seco-4,8,12(E)-labdatrien-3-oic acid	$C_{19}H_{28}O_3$	<i>C. stipuliformis</i>
221	ent-12,15-Dioxo-8,13-labdadien-3a-ol	$C_{20}H_{28}O_3$	<i>C. stipuliformis</i>
222	Crotonlaevin A	$C_{18}H_{30}O_4$	<i>C. laevigatus</i>
223	Crotonlaevin B	$C_{20}H_{32}O_5$	<i>C. laevigatus</i>
224	Crotonlaevin C	$C_{21}H_{34}O_5$	<i>C. laevigatus</i>
225	Crotonlaevin D	$C_{18}H_{30}O_3$	<i>C. laevigatus</i>
226	Crotonlaevin E	$C_{20}H_{32}O_5$	<i>C. laevigatus</i>
227	Crotonlaevin F	$C_{22}H_{34}O_6$	<i>C. laevigatus</i>
228	Crotonlaevin G	$C_{22}H_{36}O_5$	<i>C. laevigatus</i>
229	Crotonlaevin H	$C_{22}H_{36}O_5$	<i>C. laevigatus</i>
230	Crotonlaevin I	$C_{20}H_{34}O_4$	<i>C. laevigatus</i>
231	Crotonlaevin J	$C_{20}H_{30}O_3$	<i>C. laevigatus</i>
232	Crotonlaevin K	$C_{20}H_{28}O_3$	<i>C. laevigatus</i>
233	Crotonlaevin L	$C_{20}H_{30}O_4$	<i>C. laevigatus</i>
234	Crotonlaevin M	$C_{20}H_{30}O_4$	<i>C. laevigatus</i>
235	Crotonlaevin N	$C_{20}H_{30}O_3$	<i>C. laevigatus</i>
236	Crotonlaevin O	$C_{20}H_{30}O_3$	<i>C. laevigatus</i>
237	Crotonlaevin P	$C_{20}H_{30}O_3$	<i>C. laevigatus</i>
238	Crotonolide I	$C_{20}H_{34}O_3$	<i>C. laui</i>
239	Crotonolide J	$C_{19}H_{30}O_3$	<i>C. laui</i>
240	Launine A	$C_{19}H_{32}O_3$	<i>C. laui</i>
241	Launine B	$C_{19}H_{32}O_4$	<i>C. laui</i>
242	Launine C	$C_{20}H_{34}O_3$	<i>C. laui</i>
243	Launine D	$C_{20}H_{34}O_3$	<i>C. laui</i>
244	Launine E	$C_{20}H_{32}O_5$	<i>C. laui</i>
245	Launine F	$C_{20}H_{32}O_5$	<i>C. laui</i>
246	Launine G	$C_{20}H_{30}O_4$	<i>C. laui</i>
247	Launine H	$C_{20}H_{30}O_4$	<i>C. laui</i>
248	Launine I	$C_{20}H_{34}O_3$	<i>C. laui</i>

S. No.	Chemical Compounds	Chemical formula	<i>Croton</i> Species
249	15,16-Epoxy-4-hydroxy-labda-13(16),14-dien-3,12-dione	$C_{20}H_{28}O_4$	<i>C. jacobinensis</i>
250	Crotondecalvatin A	$C_{29}H_{42}O_4$	<i>C. decalvatus</i>
251	Crotondecalvatin B	$C_{30}H_{42}O_6$	<i>C. decalvatus</i>
252	Bicrotonol A	$C_{40}H_{68}O_4$	<i>C. crassifolius</i>
Cembranes			
253	Launine O	$C_{20}H_{34}O_2$	<i>C. laui</i>
254	Launine P	$C_{21}H_{36}O_2$	<i>C. laui</i>
255	Furanocembranoid 1	$C_{20}H_{30}O_2$	<i>C. oblongifolius</i>
256	Furanocembranoid 2	$C_{20}H_{30}O_3$	<i>C. oblongifolius</i>
257	Furanocembranoid 3	$C_{20}H_{32}O_4$	<i>C. oblongifolius</i>
258	Furanocembranoid 4	$C_{20}H_{32}O_5$	<i>C. oblongifolius</i>
259	Laevigatlactone A	$C_{20}H_{30}O_3$	<i>C. laevigatus</i>
260	Laevigatlactone B	$C_{20}H_{30}O_3$	<i>C. laevigatus</i>
261	Laevigatlactone C	$C_{20}H_{30}O_3$	<i>C. laevigatus</i>
262	Laevigatlactone D	$C_{20}H_{30}O_3$	<i>C. laevigatus</i>
263	Laevigatlactone E	$C_{20}H_{30}O_4$	<i>C. laevigatus</i>
264	Laevigatlactone F	$C_{20}H_{30}O_5$	<i>C. laevigatus</i>
265	(+)-[1R*,2S*,7S*,8S*,12R*]-7,8-Epoxy-2,12-cyclocembra-3E,10Zdien-20,10-olide	$C_{20}H_{28}O_3$	<i>C. gratissimus</i>
266	(+)-[1R*,10R*]-Cembra-2E,4E,7E,11Z-tetraen-20,10-olide	$C_{20}H_{28}O_2$	<i>C. gratissimus</i>
267	(+)-[1R*,4S*,10R*]-4-Hydroxycembra-2E,7E,11Z-trien-20,10-olide	$C_{20}H_{30}O_3$	<i>C. gratissimus</i>
268	(-)-[1R*,4R*,10R*]-4-Hydroxycembra-2E,7E,11Z-trien-20,10-olide	$C_{20}H_{30}O_3$	<i>C. gratissimus</i>
269	(-)-[1R*,4R*,10R*]-4-Methoxycembra-2E,7E,11Z-trien-20,10-olide	$C_{21}H_{32}O_3$	<i>C. gratissimus</i>
270	(-)-[1S*,4R*,10R*]-1-Hydroxy-4-methoxycembra-2E,7E,11Z-trien-20,10-olide	$C_{21}H_{32}O_4$	<i>C. gratissimus</i>
271	(-)-[1S*,4S*,10R*]-1,4-Dihydroxycembra-2E,7E,11Z-trien-20,10-olide	$C_{20}H_{30}O_4$	<i>C. gratissimus</i>
272	(-)-[1S*,4S*,10R*]-1,4-Dihydroxycembra-2E,7E,11Z-trien-20,10-olide	$C_{20}H_{30}O_4$	<i>C. gratissimus</i>

S. No.	Chemical Compounds	Chemical formula	<i>Croton</i> Species
273	(+)-(10R*)-Cembra-1E,3E,7E,11Z,16-pentaen-20,10-olide	C ₂₀ H ₂₆ O	<i>C. gratissimus</i>
274	(+)-(10R*)-Cembra-1Z,3Z,7E,11Z,15-pentaen-20,10-olide	C ₂₀ H ₂₆ O	<i>C. gratissimus</i>
275	(+)-(5R*,10R*)-5-Methoxycembra-1E,3E,7E,11Z,15-pentaen-20,10-olide	C ₂₁ H ₃₀ O ₃	<i>C. gratissimus</i>
276	(+)-(1S*,4S*,7R*,10R*)-1,4,7-Trihydroxycembra-2E,8(19),11Z-trien-20,10-olide	C ₂₀ H ₃₀ O ₅	<i>C. gratissimus</i>
277	(-)-(1S*,4S*,7S*,10R*)-1,4,7-Trihydroxycembra-2E,8(19),11Z-trien-20,10-olide	C ₂₀ H ₃₀ O ₃	<i>C. gratissimus</i>
278	(+)-(1S*,4R*,8S*,10R*)-1,4,8-Trihydroxycembra-2E,6E,11Z-trien-20,10-olide	C ₂₀ H ₃₀ O ₅	<i>C. gratissimus</i>
279	Cembranoid 1	C ₂₀ H ₃₀ O ₄	<i>C. longissimus</i>
280	Cembranoid 2	C ₂₀ H ₃₀ O ₃	<i>C. longissimus</i>
281	(-)-(1S*,4R*,10R*)-1-Hydroxy-4-methoxycembra-2E,7E,11Z-trien-20,10-olide	C ₂₁ H ₃₂ O ₄	<i>C. gratissimus</i>
282	(-)-(1S*,4S*,10R*)-1,4-Dihydroxycembra-2E,7E,11Z-trien-20,10-olide	C ₂₀ H ₃₀ O ₄	<i>C. gratissimus</i>
283	(-)-(1S*,4S*,10R*)-1,4-Dihydroxycembra-2E,7E,11Z-trien-20,10-olide	C ₂₀ H ₃₀ O ₄	<i>C. gratissimus</i>
284	(+)-(10R*)-Cembra-1E,3E,7E,11Z,16-pentaen-20,10-olide	C ₂₀ H ₂₆ O	<i>C. gratissimus</i>
285	(+)-(10R*)-Cembra-1Z,3Z,7E,11Z,15-pentaen-20,10-olide	C ₂₀ H ₂₆ O	<i>C. gratissimus</i>
286	(+)-(5R*,10R*)-5-Methoxycembra-1E,3E,7E,11Z,15-pentaen-20,10-olide	C ₂₁ H ₃₀ O ₃	<i>C. gratissimus</i>
287	(+)-(1S*,4S*,7R*,10R*)-1,4,7-Trihydroxycembra-2E,8(19),11Z-trien-20,10-olide	C ₂₀ H ₃₀ O ₅	<i>C. gratissimus</i>
288	(-)-(1S*,4S*,7S*,10R*)-1,4,7-Trihydroxycembra-2E,8(19),11Z-trien-20,10-olide	C ₂₀ H ₃₀ O ₃	<i>C. gratissimus</i>
289	(+)-(1S*,4R*,8S*,10R*)-1,4,8-Trihydroxycembra-2E,6E,11Z-trien-20,10-olide	C ₂₀ H ₃₀ O ₅	<i>C. gratissimus</i>
290	Cembranoid 1	C ₂₀ H ₃₀ O ₄	<i>C. longissimus</i>
291	Cembranoid 2	C ₂₀ H ₃₀ O ₃	<i>C. longissimus</i>
Abietanes			

S. No.	Chemical Compounds	Chemical formula	Croton Species
292	Isolophanthin E	$C_{20}H_{30}O_3$	<i>C. megalocarpooides</i>
293	rel-(1R,4aR,5R,8R)-Methyl-7-(1-(methoxycarbonyl)vinyl)-5,8-diacetoxy-1,2,3,4a,5,6,7,8,9,10,10a-dodecahydro-1,4a-dimethyl-2-oxophenanthrene-1-carboxylate	$C_{26}H_{34}O_9$	<i>C. argyrophylloides</i>
294	Crotontomentosin A	$C_{20}H_{26}O_2$	<i>C. caudatus</i>
295	Crotontomentosin B	$C_{20}H_{30}O_3$	<i>C. caudatus</i>
296	Crotontomentosin C	$C_{20}H_{24}O_2$	<i>C. caudatus</i>
297	Crotontomentosin D	$C_{20}H_{28}O_2$	<i>C. caudatus</i>
298	Crotontomentosin E	$C_{22}H_{32}O_3$	<i>C. caudatus</i>
299	Crotolaevigatone A	$C_{20}H_{24}O_3$	<i>C. laevigatus</i>
300	Crotolaevigatone B	$C_{20}H_{26}O_2$	<i>C. laevigatus</i>
301	Crotolaevigatone C	$C_{20}H_{26}O_3$	<i>C. laevigatus</i>
302	Crotolaevigatone D	$C_{20}H_{28}O_4$	<i>C. laevigatus</i>
303	Crotolaevigatone E	$C_{19}H_{24}O_2$	<i>C. laevigatus</i>
304	Crotolaevigatone F	$C_{20}H_{30}O_4$	<i>C. laevigatus</i>
305	Crotolaevigatone G	$C_{20}H_{30}O_4$	<i>C. laevigatus</i>
Casbanes			
306	1,4-Dihydroxy-2E,6E,12E-trien-5-one-casbane	$C_{20}H_{30}O_3$	<i>C. nepetaefolius</i>
307	4-Hydroxy-2E,6E,12E-5-one-casbane	$C_{20}H_{29}O_3$	<i>C. nepetaefolius</i>
308	1-hydroxy-(2E,6Z,12E)-casba-2,6,12-triene-4,5-dione	$C_{20}H_{28}O_3$	<i>C. argyrophyllus</i>
309	6E,12E-Casba-1,3,6,12-tetraen-1,4-epoxy-5-one	$C_{20}H_{26}O_2$	<i>C. argyrophyllus</i>
310	(2E,5β,6E,12E)-5-hydroxycasba-2,6,12-trien-4-one	$C_{20}H_{30}O_2$	<i>C. argyrophyllus</i>
311	EBC-324	$C_{20}H_{28}O_5$	<i>C. insularis</i>
312	EBC-329	$C_{20}H_{26}O_4$	<i>C. insularis</i>
Halimanes			
313	Crassifoliusin A	$C_{21}H_{24}O_5$	<i>C. crassifolius</i>
314	Crotontomentosin F	$C_{21}H_{30}O_3$	<i>C. caudatus</i>
315	Crolaevinoid A	$C_{27}H_{30}O_7$	<i>C. laevigatus</i>

S. No.	Chemical Compounds	Chemical formula	<i>Croton</i> Species
316	Crolaevinoid B	$C_{20}H_{26}O_4$	<i>C. laevigatus</i>
317	Crothalimene A	$C_{20}H_{26}O_4$	<i>C. dichogamus</i>
318	Crothalimene B	$C_{20}H_{30}O_2$	<i>C. dichogamus</i>
Pimaranes			
319	ent-3 β -Hydroxypimara-8(14),9,15-trien-12-one	$C_{20}H_{28}O_2$	<i>C. insularis</i>
320	EBC-316	$C_{20}H_{26}O_2$	<i>C. insularis</i>
321	EBC-325	$C_{20}H_{26}O_4$	<i>C. insularis</i>
322	EBC-326	$C_{20}H_{26}O_4$	<i>C. insularis</i>
323	EBC-327	$C_{20}H_{24}O_3$	<i>C. insularis</i>
324	EBC-345	$C_{20}H_{30}O_4$	<i>C. insularis</i>
Cleistanthanes			
325	3-Hydroxycleistantha-13(17),15-diene	$C_{20}H_{32}O$	<i>C. oblongifolius</i>
326	3,4-Seco-cleistantha-4(18),13(17),15-trien-3-oic acid	$C_{20}H_{30}O_2$	<i>C. oblongifolius</i>
327	rel-(5 β ,8a,10a)-8-Hydroxy-13-methylpodocarpa-9(11),13-diene-3,12-dione	$C_{18}H_{25}O_3$	<i>C. regelianus</i>
Grayananes			
328	Crotonkinensin A	$C_{20}H_{24}O_4$	<i>C. tonkinensis</i>
329	Crotonkinensin B	$C_{20}H_{26}O_3$	<i>C. tonkinensis</i>
Atisanes			
330	Crotobarin	$C_{22}H_{28}O_5$	<i>C. barorum</i>
331	Crotogoudin	$C_{20}H_{26}O_3$	<i>C. goudotii</i>
Phytanes			
332	Launine L	$C_{20}H_{32}O_3$	<i>C. laui</i>
333	Launine M	$C_{20}H_{32}O_2$	<i>C. laui</i>
Laevinanes			
334	Crolaevinoid G	$C_{20}H_{24}O_6$	<i>C. laevigatus</i>
335	Crolaevinoid H	$C_{21}H_{26}O_6$	<i>C. laevigatus</i>
Meroditerpenoids			
336	Steenkrotin A	$C_{20}H_{24}O_6$	<i>C. steenkampi- anus</i>
337	Steenkrotin B	$C_{20}H_{28}O_7$	<i>C. steenkampi- anus</i>

S. No.	Chemical Compounds	Chemical formula	<i>Croton</i> Species
338	Norcrassin A	$C_{17}H_{22}O_7$	<i>C. crassifolius</i>
339	Cracroson D	$C_{21}H_{26}O_6$	<i>C. crassifolius</i>
Sesquiterpenoids			
340	6 α -Methoxy-cyperene	$C_{16}H_{26}O$	<i>C. muscicarpa</i>
341	rel-(1R,4S,6R,7S,8aR)-Decahydro-1-(hydroxymethyl)-4,9,9-trimethyl-4,7-(epoxymethano)azulen-6-ol	$C_{15}H_{26}O_3$	<i>C. regelianus</i>
342	Blumenol A	$C_{13}H_{20}O_3$	<i>C. pedicellatus</i>
343	Crocrassins A	$C_{15}H_{24}O_3$	<i>C. crassifolius</i>
344	Crocrassins B	$C_{16}H_{26}O_3$	<i>C. crassifolius</i>
345	1,3,5-Cadinatriene-(7R,10S)-diol	$C_{15}H_{25}O_2$	<i>C. dichogamus</i>
346	Cracroson H	$C_{15}H_{22}O_3$	<i>C. crassifolius</i>
Sesterterpenoid			
347	Pseudopulchellol	$C_{25}H_{40}O$	<i>C. pseudopulchellus</i>
Triterpenoid			
348	3 α -Hydroxy-urs-12,15-dien	$C_{30}H_{48}O$	<i>C. bonplandianum</i>
349	Lupeol	$C_{30}H_{50}O$	<i>C. sylvaticus</i> , <i>C. zambesicus</i>
350	β -Sitosterol	$C_{29}H_{50}O$	<i>C. zambesicus</i> , <i>C. steenkampianus</i>
351	Stigmasterol	$C_{29}H_{48}O$	<i>C. zambesicus</i> , <i>C. pseudopulchellus</i>
352	Betulinic acid	$C_{30}H_{48}O_3$	<i>C. zambesicus</i>
353	Betulin	$C_{30}H_{50}O_2$	<i>C. zambesicus</i>
354	Lupenone	$C_{30}H_{48}O$	<i>C. zambesicus</i>
355	α -Amyrin	$C_{30}H_{50}O$	<i>C. zambesicus</i>
356	β -Amyrin	$C_{30}H_{50}O$	<i>C. pseudopulchellus</i>
Flavonoids			
357	Quercetin	$C_{15}H_{10}O_7$	<i>C. steenkampianus</i> , <i>C. sylvaticus</i> , <i>C. menyharthii</i>

S. No.	Chemical Compounds	Chemical formula	<i>Croton</i> Species
358	Kaempferol	$C_{15}H_{10}O_6$	<i>C. gratissimus</i> , <i>C. sylvaticus</i>
359	Tamarixetin	$C_{16}H_{12}O_7$	<i>C. steenkampianus</i>
360	Eriodictyol	$C_{15}H_{12}O_6$	<i>C. steenkampianus</i>
361	Vitexin	$C_{21}H_{20}O_{10}$	<i>C. zambesicus</i>
362	Isovitexin	$C_{21}H_{20}O_{10}$	<i>C. zambesicus</i> , <i>C. gratissimus</i>
363	Quercetin-3-O- β -(p-coumaroyl) glucopyranoside-3'-methylether	$C_{39}H_{32}O_{16}$	<i>C. zambesicus</i>
364	Tiliroside	$C_{30}H_{26}O_{13}$	<i>C. zambesicus</i> , <i>C. gratissimus</i>
365	Myricetrin	$C_{21}H_{20}O_{12}$	<i>C. menyharthii</i>
366	Quercetin-3-O-rhamnoside	$C_{21}H_{20}O_{11}$	<i>C. menyharthii</i>
Glycosides			
367	Cyperenoic acid-9-O- β -d-glucopyranoside	$C_{21}H_{32}O_8$	<i>C. crassifolius</i>
368	3-O- β -d-Xylopyranosylspathodic acid	$C_{35}H_{56}O_9$	<i>C. lachnocarpus</i>
369	Helichrysoside-3'-methylether	$C_{31}H_{28}O_{14}$	<i>C. zambesicus</i>
370	Crotonionoside A	$C_{29}H_{42}O_{11}$	<i>C. cascarilloides</i>
371	Crotonionoside B	$C_{30}H_{44}O_{12}$	<i>C. cascarilloides</i>
372	Crotonionoside C	$C_{24}H_{42}O_{12}$	<i>C. cascarilloides</i>
373	Crotonionoside D	$C_{31}H_{46}O_{14}$	<i>C. cascarilloides</i>
374	Crotonionoside E	$C_{35}H_{52}O_{16}$	<i>C. cascarilloides</i>
375	Crotonionoside F	$C_{24}H_{42}O_{11}$	<i>C. cascarilloides</i>
376	Crotonionoside G	$C_{29}H_{40}O_{11}$	<i>C. cascarilloides</i>
377	Oblongionoside A	$C_{24}H_{42}O_{12}$	<i>C. oblongifolius</i>
378	Oblongionoside B	$C_{24}H_{42}O_{12}$	<i>C. oblongifolius</i>
379	Oblongionoside C	$C_{24}H_{44}O_{11}$	<i>C. oblongifolius</i>
380	Oblongionoside D	$C_{24}H_{44}O_{11}$	<i>C. oblongifolius</i>
381	Oblongionoside E	$C_{19}H_{36}O_8$	<i>C. oblongifolius</i>
382	Oblongionoside F	$C_{19}H_{36}O_8$	<i>C. oblongifolius</i>
383	Blumenol A glucoside	$C_{19}H_{30}O_8$	<i>C. pedicellatus</i>
384	Sparsioside	$C_{53}H_{102}O_{10}$	<i>C. sparsiorus</i>

S. No.	Chemical Compounds	Chemical formula	<i>Croton</i> Species
385	3,12-Dioxo-15,16-epoxy-4a-hydroxy-6-(β-glucopyranosyl)-ent-neo-clerodan-13(16),14-diene	$C_{26}H_{38}O_{10}$	<i>C. limae</i>
386	Isocrotofolane glucoside	$C_{26}H_{38}O_9$	<i>C. cascarilloides</i>
387	2-Methoxyphenol-β-d-(6-O-β-d-apiofuranosyl) glucopyranoside	$C_{18}H_{26}O_{11}$	<i>C. cascarilloides</i>
Alkaloids			
388	Crotamide A	$C_{36}H_{65}NO$	<i>C. sparsiflorus</i>
389	Crotamide B	$C_{38}H_{69}NO$	<i>C. sparsiflorus</i>
390	Crotonimide A	$C_{16}H_{20}N_2O_3$	<i>C. pullei</i>
391	Crotonimide C	$C_{20}H_{20}N_2O_3$	<i>C. alienus</i>
392	Crotonine	$C_{12}H_{14}N_2O_4$	<i>C. tiglium</i>
393	Crotsparsidine	$C_{17}H_{17}O_3N$	<i>C. sparsiflorus</i>
394	6-Hydroxy-1-methyl-2-dimethyl-3,4-tetrahydro-b-carbo-line	$C_{14}H_{19}N_2O$	<i>C. heliotropiifolius</i>
395	N-Trans-feruloyl-3,5-dihydroxyindolin-2-one	$C_{20}H_{20}N_2O_6$	<i>C. echiioides</i>
396	(E)-N-(4-Hydroxycinnamoyl)-5-hydroxytryptamine [trans-N-(p-coumaroyl) serotonin]	$C_{10}H_{12}N_2O$	<i>C. menyharthi</i>
397	Julocrotine	$C_{18}H_{24}N_2O_3$	<i>C. sylvaticus</i>
Benzoate derivatives			
398	2'-(3'',4''-Dihydroxyphenyl)-ethyl-4-hydroxybenzoate	$C_{15}H_{14}O_5$	<i>C. sylvaticus</i>
399	3-(4-Hydroxy-3,5-dimethoxyphenyl)-propyl benzoate	$C_{18}H_{20}O_5$	<i>C. hutchinsonianus</i>
400	3-(4-Hydroxyphenyl)-propyl benzoate	$C_{16}H_{16}O_3$	<i>C. hutchinsonianus</i>
Pyran-2-one derivatives			
401	Crotonpyrone A	$C_{17}H_{28}O_3$	<i>C. crassifolius</i>
402	Crotonpyrone B	$C_{17}H_{26}O_3$	<i>C. crassifolius</i>
403	Crotonpyrone C	$C_{19}H_{28}O_3$	<i>C. crassifolius</i>
Cyclicpeptides			
404	Crotogossamide	$C_{37}H_{56}N_{10}O_{11}$	<i>C. gossypifolius</i>
405	[1-9-NaC]-Crouororb A1	$C_{37}H_{56}N_{10}O_{11}$	<i>C. urucurana</i>
Tropone derivatives			

S. No.	Chemical Compounds	Chemical formula	<i>Croton</i> Species
406	Crototropone	C ₁₀ H ₁₂ O ₄	<i>C. zehntneri</i>
407	Pernambucone	C ₁₅ H ₁₈ O ₂	<i>C. argyroglossum</i>
Limonoids			
408	Musidunin	C ₃₁ H ₃₈ O ₁₁	<i>C. jatrophioides</i>
409	Musiduol	C ₃₀ H ₃₈ O ₁₀	<i>C. jatrophioides</i>
Miscellaneous compounds			
410	Crotoncaudatin	C ₂₂ H ₂₂ O ₉	<i>C. caudatus</i>
411	8S-(-)-8-(4-Hydroxy-3-methoxybenzoyl)-dihydrofuran-8(80H)-one	C ₂₀ H ₃₀ O ₂	<i>C. kongensis</i>
412	Lobaceride	C ₃₅ H ₅₈ O ₆	<i>C. lobatus</i>
413	Laevifolin A	C ₂₉ H ₃₈ O ₄	<i>C. laevifolius</i>
414	Laevifolin B	C ₂₉ H ₃₈ O ₄	<i>C. laevifolius</i>
415	2,6-Dimethyl-1-oxo-4-indanecarboxylic acid	C ₁₂ H ₁₂ O ₃	<i>C. steenkampianus</i>
416	3{3'-Methoxy-5'-phenylfuran-2'-yl}propan-1-ol	C ₁₄ H ₁₆ O ₃	<i>C. oblongifolius</i>
417	Sparsifol	C ₇ H ₁₅ O ₆	<i>C. sparsiflorus</i>
418	Sparsioamide	C ₄₃ H ₈₁ NO ₅	<i>C. sparsiflorus</i>
419	Hexyl Z-ferulate	C ₁₆ H ₂₂ O ₄	<i>C. laevigatus</i>

Species

Genus *Croton* is known to contain a total of 1150 taxonomically accepted species (POWO, 2023) that are as-

1. *C. abaitensis* Baill. in Adansonia 4: 337 (1864)
2. *C. aberrans* Müll.Arg. in C.F.P.von Martius & auct. suc. (eds.), Fl. Bras. 11(2): 232 (1873)
3. *C. abonari* Riina & P.E.Berry in Anales Jard. Bot. Madrid 67: 26 (2010)
4. *C. abutilifolius* Croizat in Darwiniana 6: 448 (1944)
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6. *C. abutilopsis* G.L.Webster in Novon 2: 271 (1992)
7. *C. acapulcensis* M.J.Martinez Gordillo & J.Jiménez Ram. in Anales Inst. Biol. Univ. Nac. Autón. México, Bot. 60: 40 (1990)
8. *C. aceroides* Radcl.-Sm. in Kew Bull. 45: 555 (1990)
9. *C. ackermannianus* (Müll.Arg.) G.L.Webster in Novon 2: 271 (1992)
10. *C. acradenius* Pax & K.Hoffm. in Repert.

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11. *C. acronchioides* F.Muell. in Fragm. 4: 142 (1864)
 12. *C. acuminatissimus* (Pittier) G.L.Webster in Novon 2: 271 (1992)
 13. *C. acunae* Borhidi in Ann. Hist.-Nat. Mus. Natl. Hung. 69: 48 (1977)
 14. *C. acutifolius* Esser in Thai Forest Bull., Bot. 29: 51 (2001)
 15. *C. adabolavensis* Leandri in Ann. Mus. Colon. Marseille, sér. 5, 7(1): 34 (1939)
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 17. *C. adenocalyx* Baill. in Adansonia 4: 344 (1864)
 18. *C. adenodontus* (Müll.Arg.) Müll.Arg. in C.F.P.von Martius & auct. suc. (eds.), Fl. Bras. 11(2): 267 (1873)
 19. *C. adenophorus* Baill. in Recueil Observ. Bot. 1: 153 (1861)
 20. *C. adenophyllum* Spreng. in Syst. Veg., ed. 16. 3: 869 (1826)
 21. *C. adipatus* Kunth in F.W.H.von Humboldt, A.J.A.Bonpland & C.S.Kunth, Nov. Gen. Sp. 2: 68 (1817)
 22. *C. adspersus* Benth. in Pl. Hartw.: 51 (1840)
 23. *C. aemulus* Barbosa & Carn.-Torres in Phytotaxa 365: 262 (2018)
 24. *C. aequatoris* Croizat in J. Arnold Arbor. 21: 88 (1940)
 25. *C. agoensis* Baill. in Adansonia 4: 348 (1864)
 26. *C. agrestis* (Pax & K.Hoffm.) Radcl.-Sm. & Govaerts in Kew Bull. 52: 184 (1997)
 27. *C. agrophilus* Müll.Arg. in C.F.P.von Martius & auct. suc. (eds.), Fl. Bras. 11(2): 175 (1873)
 28. *C. alabamensis* E.A.Sm. ex Chapm. in Fl. South. U.S., Suppl.: 648 (1883)
 29. *C. alainii* B.W.van Ee & P.E.Berry in Bot. Rev. (Lancaster) 74: 158 (2008)
 30. *C. alamosanus* Rose in Contr. U.S. Natl. Herb. 1: 111 (1891)
 31. *C. albellus* Müll.Arg. in C.F.P.von Martius & auct. suc. (eds.), Fl. Bras. 11(2): 180 (1873)
 32. *C. alchorneicarpus* Croizat in Darwiniana 6: 451 (1944)
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 34. *C. aleuritoides* P.E.Berry in Candollea 71: 182 (2016)
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 38. *C. almadinensis* Caruzo & Riina in Ann. Missouri Bot. Gard. 106: 119 (2021)
 39. *C. alnifolius* Lam. in Encycl. 2: 205 (1786)
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 41. *C. alvaradonis* M.E.Jones in Contr. W. Bot. 18: 48 (1933)

42. *C. amaraliae* Riina & P.E.Berry in Ann. Missouri Bot. Gard. 106: 123 (2021)
43. *C. amazonicus* Müll.Arg. in Linnaea 34: 102 (1865)
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49. *C. amplifolius* Airy Shaw in Kew Bull. 27: 79 (1972)
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51. *C. androiensis* (Leandri) Leandri in Adansonia, n.s., 9: 507 (1969 publ. 1970)
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57. *C. ankeranae* Kainul. in Candollea 71: 329 (2016)
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89. *C. astianus* Croizat in J. Arnold Arbor. 21: 95 (1940)
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1013. *C. stenosepalus* Müll.Arg in Linnaea 34: 114 (1865)
1014. *C. stenotrichus* Müll.Arg in Linnaea 34: 105 (1865)
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1016. *C. stipulaceus* Kunth in Nov. Gen. Sp. 2: 85 (1817)
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1020. *C. stoechadis* Baill in Adansonia 4: 314 (1864)
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1067. *C. tetradenius* Baill in Adansonia 4: 343 (1864)
1068. *C. texensis* (Klotzsch) Müll.Arg in Prodr. 15(2): 692 (1866)
1069. *C. thellungianus* (Herter ex Arechav.) Radcl.-Sm. & Govaerts in Kew Bull. 52: 185 (1997)
1070. *C. thoi* Thin in Euphorb. Vietnam : 47 (1995)
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1090. *C. triangularis* Müll.Arg in Linnaea 34: 128 (1865)
1091. *C. trichophilus* (Pax & K.Hoffm.) Radcl.-Sm. & Govaerts in Kew Bull. 52: 186 (1997)
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1094. *C. tridentatus* Mart. ex Müll.Arg in Fl. Bras. 6(2): 219 (1874)
1095. *C. triglandulatus* Vell in Fl. Flumin. 10: 79 (1831)
1096. *C. trigonocarpus* Griseb in Nachr. Königl. Ges. Wiss. Georg-Augusts-Univ. 1: 173 (1865)
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1110. *C. urucurana* Baill in Adansonia 4: 335 (1864)
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1118. *C. vauthierianus* Baill in Adansonia 4: 317 (1864)
1119. *C. velame* Müll.Arg in Fl. Bras. 11(2): 181 (1873)
1120. *C. vellozoanus* Müll.Arg in Prodr. 15(2): 553 (1866)
1121. *C. velutinus* Baill in Adansonia 4: 325 (1864)
1122. *C. venturii* Croizat in J. Arnold Arbor. 21:

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1123. *C. vepretorum* Müll.Arg in Fl. Bras. 11(2): 172 (1873)
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1125. *C. verbascooides* G.L.Webster in Novon 2: 273 (1992)
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1137. *C. viminalis* Griseb in Pl. Wright. 1: 158 (1860)
1138. *C. virgultosus* Müll.Arg in Fl. Bras. 11(2): 104 (1873)
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Croton bonplandianus Baill.

C. bonplandianus belongs to the family Euphorbiaceae which is native to South Bolivia Paraguay, Southwestern Brazil, North Argentina, Bangladesh, South America, India, and Pakistan. It is a perineal monoecious exotic weed, growing up to 1.5 m tall, and commonly occurs in the wild around canals, river banks, big drainages, and wastelands. It is commonly known as 'Van Tulsi' due to its resemblance of the leaves and inflorescence with the 'Tulsi' plant. Three-leaved caper (English), 'Kalabhangre' (Hindi), 'Eliamanakkau' (Tamil), 'Kukka mirapa' (Telugu), 'Alpabedhi soppu' (Kannada), etc. are other common names of this plant (Ghosh et al., 2018). Its stem and branches are used as fuel. Its ash after 4-6 days of storage used as a detergent for cleaning cotton garments. It is rich in phytochemicals like alkaloids, flavonoids, steroids, tannins, and cardiac glycosides that make it strangely medicinal. It possesses immense medicinal value and its stem latex is used in cuts and wounds to stop bleeding (Ghosh et al., 2018). The leaf paste is a purgative, also used to treat lesions, skin disease, bronchitis, and asthma. This plant is very important ecologically, because during the hot climatic conditions it serve as the source of the nectar and food for the pollinators. Its stalked inflorescence is very attractive and attracts the insects.

Classification

Kingdom	-	Plantae
Subkingdom	-	Tracheobionta

Super Division	-	Spermatophyta
Division	-	Magnoliophyta
Class	-	Magnoliopsida
Subclass	-	Rosidae
Order	-	Malpighiales
Family	-	Euphorbiaceae
Subfamily	-	Crotonoideae
Genus	-	<i>Croton</i>
Species	-	<i>bonplandianus</i>

Common Names

Ban tulsi, Kala Bhangra, Reilpoondu, Alpa bedhi soppu, Mirchaiya Jhaar.

Global Distribution

The *C. bonplandianus* is naturalized in paleotropics and has been reported to be native to Southern Bolivia, Paraguay, South Western Brazil, North Argentina, Bangladesh, South America, India, and Pakistan. According to the Plants of the World Online database (POWO, 2023), it is originated from the South American continent and is native to Argentina Northeast, Argentina Northwest, Bolivia, Brazil South, Brazil West-Central, Paraguay, Uruguay; and introduced into Andaman Is., Assam, Bangladesh, Borneo, Cambodia, Comoros, East Himalaya, Gulf States, India, Kenya, Laos, Malaya, Maryland, Mauritius, Myanmar, Nepal, Nicobar Is., Pakistan, Rodrigues, Réunion, Sri Lanka, Sulawesi, Taiwan, Thailand, West Himalaya (Fig. 2).

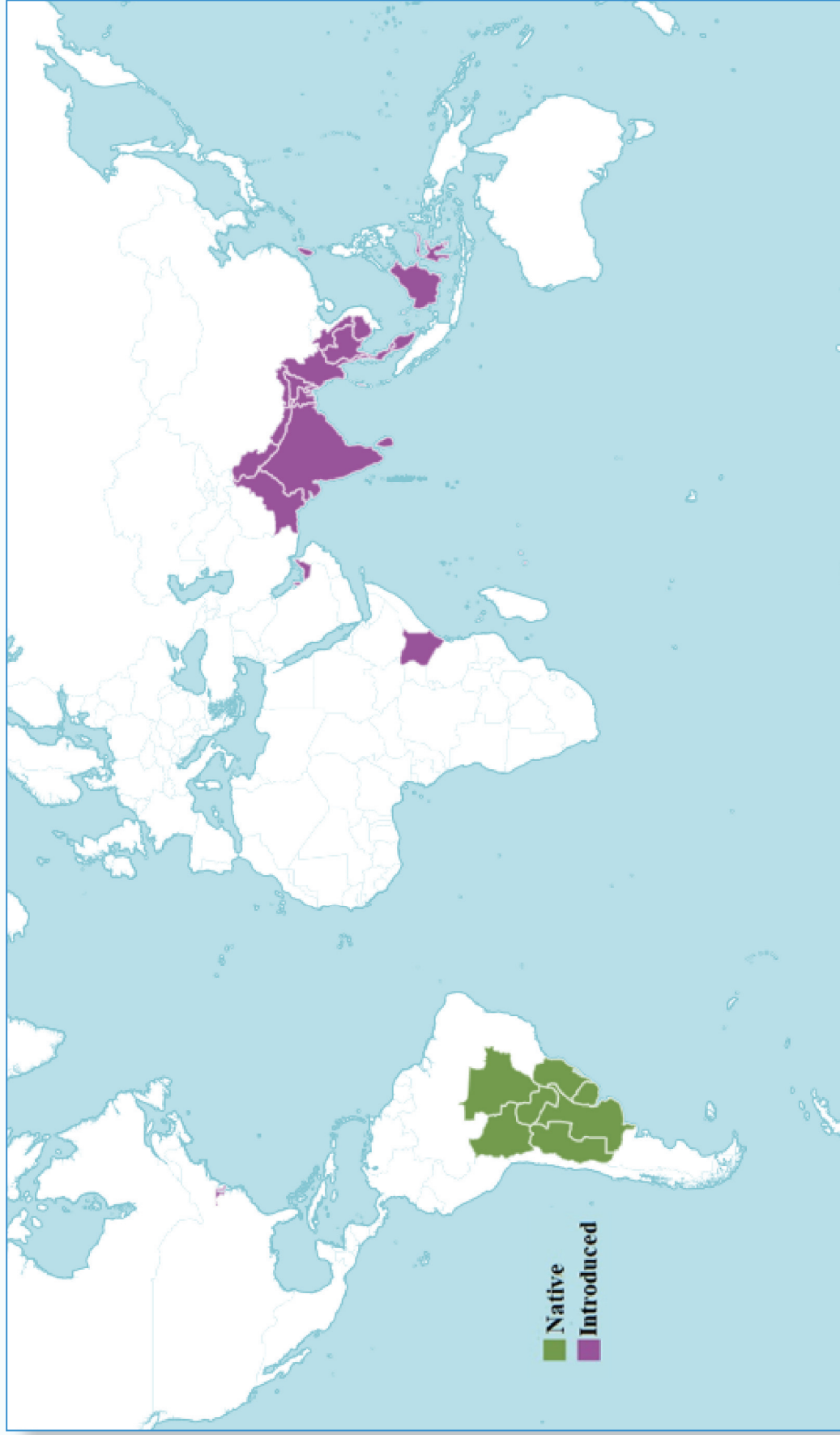


Fig. 2 Global distribution of genus *Croton bonplandianus*

Distribution in India

It is widely distributed in the Sub-Himalayan region of the country in the wastelands of tropical and subtropical areas in plains up to 900 m. It is found in all the plains districts as Andhra Pradesh, Assam, Bihar, Haryana, Karnataka, Kerala, Odisha, Maharashtra, Rajasthan, Tamil Nadu, Uttar Pradesh, Uttarakhand, West Bengal (Rao et al., 2019; Rao and Kumar, 2023).

Ethnopharmacology and Traditional Uses

Medically, The plant *C. bonplandianus* is very important. It has antibacterial (Vadlapudi, 2010), anti-fungal (Asthana et al., 1989), antioxidant (Divya et al., 2011), analgesic (Saggoo et al., 2010), nematicide (Maria et al., 2008), anti-coronary (Nishanta et al., 2002; Chaudhuri, 2007; Bhakat and Sen, 2008), hepatoprotective (Das et al., 2008), and wound healing activities (Reddy, 1995; Chandel et al., 1996). It also has insect-repellent properties (Jeeshna et al., 2010). It has been used to treat skin conditions, ringworm, and liver ailments. This plant's leaves have a great therapeutic value and are used to treat wounds, cuts, and blood pressure. The seeds are used to treat internal abscesses, abdominal dropsy, severe constipation, and jaundice. Tribal communities utilize the fresh juice of this plant to treat acne on the head (Divya et al., 2011). Plant juice is used to treat toothache and helminthiasis (Singh et al., 2011; Dutta and Chaudhuri, 2018). Traditionally, it has been used in the

treatment of liver disorders, and skin diseases including skin infection, swelling, bronchitis, and asthma (Bapuji and Ratnam, 2009; Kumar and Chaturvedi, 2010). The leaves are useful for treating cuts and wounds, venereal sores, and cholera (Asolkar and Chopra, 1992; Rajakaruna et al., 2002). The seeds are important for the treatment of jaundice, acute constipation, abdominal dropsy, and internal abscesses. Its root is utilized by the locals in the isolated parts of West Bengal as a remedy against snake venom, and the leaf extract is used to treat high fevers (Ghosh et al., 2013). The fresh decoction is used in headache (Saggoo et al., 2010; Singh et al., 2011).

Selection of the Sites and Characteristics Studied

The distribution, morphological variations, and association of *C. bonplandianus* 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 26 sites were selected with two sites ranging between around 100 km in distance while the whole distance is about 2600 km (Table 2). The plant diversity was assessed up to 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 2 Different studied sites and their GPS coordinates along with *C. bonplandianus* 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	Absent
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
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	Absent
S25	Hoogly	9	22.91	88.40	Absent
S26	Gangasagar	4	22.19	88.19	Absent

Local Occurrence

The plant was found between 30 and 380 m above sea level, while above 380 m and below 30 m it was absent. The sites 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, and 21 were the sources of the material gathered for the bulk collection while in the remaining sites 1, 2, 3, 4, 22, 23, 24, 25, and 26, it was absent.

Taxonomic Treatment

C. bonplandianus Baill., *Adansonia* 4: 339. 1864; Croizat, J. *Bombay Nat. Hist. Soc.* 41: 573. 1940; Chakrab. & N.P. Balakr. in *Bull. Bot. Surv. India* 34: 31. 1992 (publ. 1997); N.P. Balakr. & Chakrab., *Fam. Euphorb. India*: 212. 2007; Chakrab. & N.P. Balakr. in N.P. Balakr. & al., *FL India* 23: 231. 2012; Khanna, *Geophytology* 47(1): 98. 2017. *C. sparsiflorus* Morong, *Ann. New York Acad. Sci.* 7: 221. 1893; Prain, *Rec. Bot. Surv. India* 3(2): 276. 1905.

Synonyms

The Plants of the World Online database mentioned it with its 1 homotypic and 5 heterotypic synonyms (POWO) as:

Homotypic Synonyms

1. *Oxydectes bonplandiana* (Baill.) Kuntze in *Revis. Gen. Pl.* 2: 610 (1891)

Heterotypic Synonyms

1. *C. pauperulus* Müll.Arg. in *Flora* 47: 485 (1864)
2. *C. rivinoides* Chodat in *Bull. Herb. Boissier*, sér. 2, 1: 395 (1901)

3. *C. sparsiflorus* Morong in *Ann. New York Acad. Sci.* 7: 221 (1893)
4. *Oxydectes pauperula* (Müll.Arg.) Kuntze in *Revis. Gen. Pl.* 2: 612 (1891)
5. *O. sparsiflora* (Morong) Kuntze in *Revis. Gen. Pl.* 3(2): 289 (1898)

Another database i.e., World Flora Online (WFO, 2023) mentioned it with a total of the following six synonyms-

1. *C. pauperulus* Müll.Arg. in *Flora* 47: 485 (1864)
2. *C. rivinoides* Chodat in *Bull. Herb. Boissier*, sér. 2, 1: 395 (1901)
3. *C. sparsiflorus* Morong in *Ann. New York Acad. Sci.* 7: 221 (1893)
4. *Oxydectes bonplandiana* Kuntze in *Revis. Gen. Pl.* 2: 610 (1891)
5. *O. pauperula* Kuntze in *Revis. Gen. Pl.* 2: 612 (1891)
6. *O. sparsiflora* (Morong) Kuntze in *Revis. Gen. Pl.* 3(2): 289 (1898)

Botanical Description

A monoecious woody erect, herb or subshrub, usually 30-40 cm tall sometimes up to 1.5 m, branches in whorled. Young shoots and petioles initially densely pubescent, later on glabrous. Indumentum flat, 0.3-0.5 mm in diameter, consisting of stellate, whitish hairs. Stipules subglabrous, filiform, ca 0.5 mm long. Leaves usually pseudo-vercillate, alternate, subopposite or whorled. Petioles 0.3-1.5 cm long. Leaf-blades ovate-elliptic to lanceolate, 2-5.5 × 0.5-2.5 cm, acute,



Fig. 3A Plant Habit

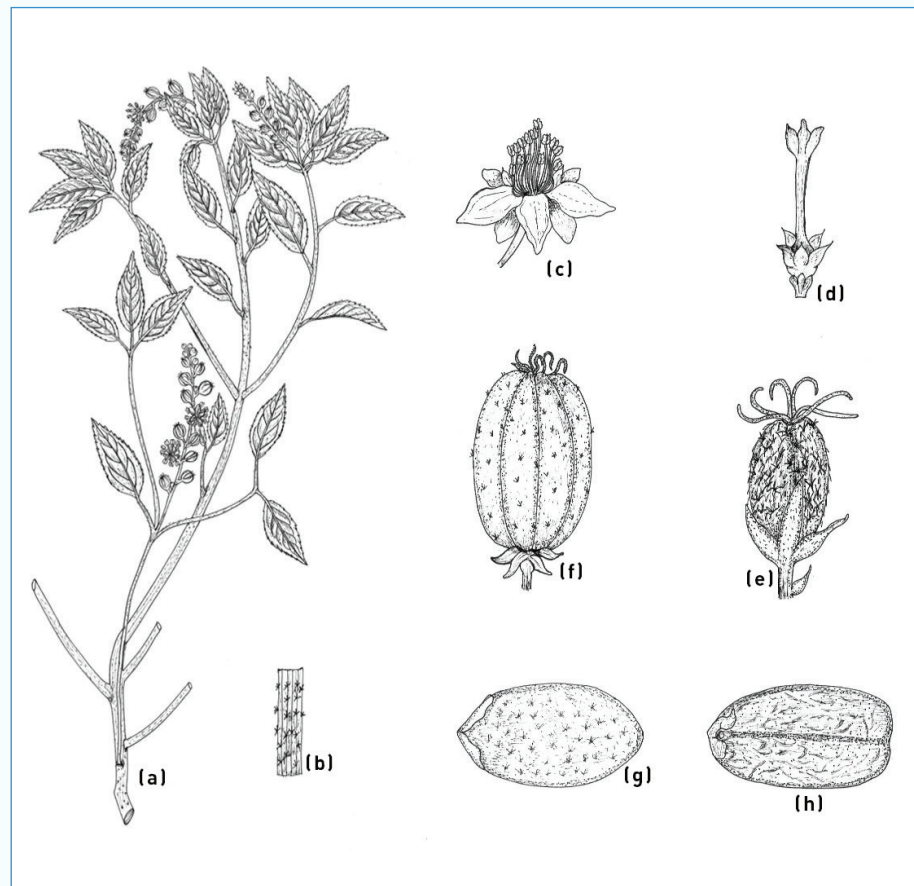


Fig. 3B (a) Plant twig (b) Young twig with stellate hairs (c) Staminate flower (d) Pistillate flower (e) Young fruit (f) Mature fruit (g) Adaxial surface of seed (h) Abaxial surface of seed

cuneate, with 2 small sessile discoid basal glands beneath, crenate-serrate, membranous, penninerved, glabrous above, sparingly stellate-lepidote beneath, dark green, base obtuse, venation only visible below, side veins 6–7 pairs, basal pair triplinerved. Racemes terminal, up to 15 cm long with 2–7 pistillate flowers, creamy-white, sparsely pubescent to subglabrous except for the distinctly pubescent ovaries, male flowers in lower one-third, female in upper two-third. Bracts triangular lanceolate, 1 mm long, having pair of pillow-shaped glands, 0.5 mm in diameter, eglandular in male flowers. Male flowers subglabrous to glabrous outside, pedicels 1–1.5 mm long, calyx 5-lobed, lobes elliptic-ovate, 1–1.25 x 1 mm, subacute, greenish-yellow; petals 5, oblong, recurved, 2 x 0.5 mm, obtuse, subglabrous, whitish; disc glands free, subglobose, apiculate; stamens 13–16, filaments 2 mm long, glabrous, anthers 0.3 mm long; receptacle glabrous. Female flowers subsessile; pedicel nearly absent, calyx-lobes 5, triangular-ovate, 1 x 1 mm, subacute, not accrescent, subglabrous; petals 0; disc annular; ovary ellipsoid-subtrilobate, 1.5 x 1 mm, densely stellate-lepidote; styles 3, $\pm$ free, 1.5–2 mm long, spreading, glabrous, bifid, the arms filiform. Fruit ellipsoid-trigonous, brownish-green, 5–6 x 4 mm, septicidally dehiscent, sparingly stellate-lepidote. Seeds compressed-ellipsoid, 4 x 2 mm, grey, slightly roughened; caruncle bilobate (Fig. 3A, B).

Chromosome Number

The basic chromosome numbers are 20 ($x=10$) (Sidhu, 1983; Soontornchainaksaeng and Chaiyasut, 1999; Bala et al., 2011). Bala et al. (2011) studied the population of *C. bonplandianus* and recorded two cytotypes at different levels of polyploidy; one has $n=10$ and the other population has $n=25$ from Punjab, India.

Anatomical Features

Structure of Leaf

The leaf has lateral veins and a broad midrib. The lamina is delicate and thin. The midrib is somewhat wider on the abaxial side and thicker, dome-shaped, on the adaxial side. There is a planoconvex midrib. The midrib has a thin epidermis, tiny, spindle-shaped cells with thin walls. The ground parenchymatous cells in the midrib are polygonal, compact, thin-walled cells located inside the epidermis. With an abaxial spongy mesophyll zone and an adaxial palisade layer, the lamina is dorsiventral. Lamina's epidermal cells are cutinized and rectangular in shape. In the epidermal layer, the stomata are paracytic type and widely dispersed. There are two equal or unequal subsidiary cells in each stoma. Guard cells have a wide elliptical shape. Palisade cells are less compact and have a single row that is narrowly cylindrical. Three to four layers of loosely distributed, tiny, spherical, or lobed spongy parenchyma cells. The large air spaces are seen in the spongy parenchyma. There are crystals of calcium oxalate in the

mesophyll tissue. A flat and amphicribal vascular bundle is located in the centre. At the bottom end of the xylem stand, the vascular bundle is made up of many short, thin vertical lines of xylem components. The xylem components have thick walls, are angular, and are narrow. The bundle is collateral, endarch, and made up of many radial parallel lines of

angular, thick-walled xylem components. The phloem is found on the bottom surface of the distinct, small, round lumps that have little spaces between them. The calcium oxalate crystals, or druses, are found in the phloem parenchyma. One or two cells make up the crystal layer. The sclerid layer covers the vascular bundles (Fig. 4).

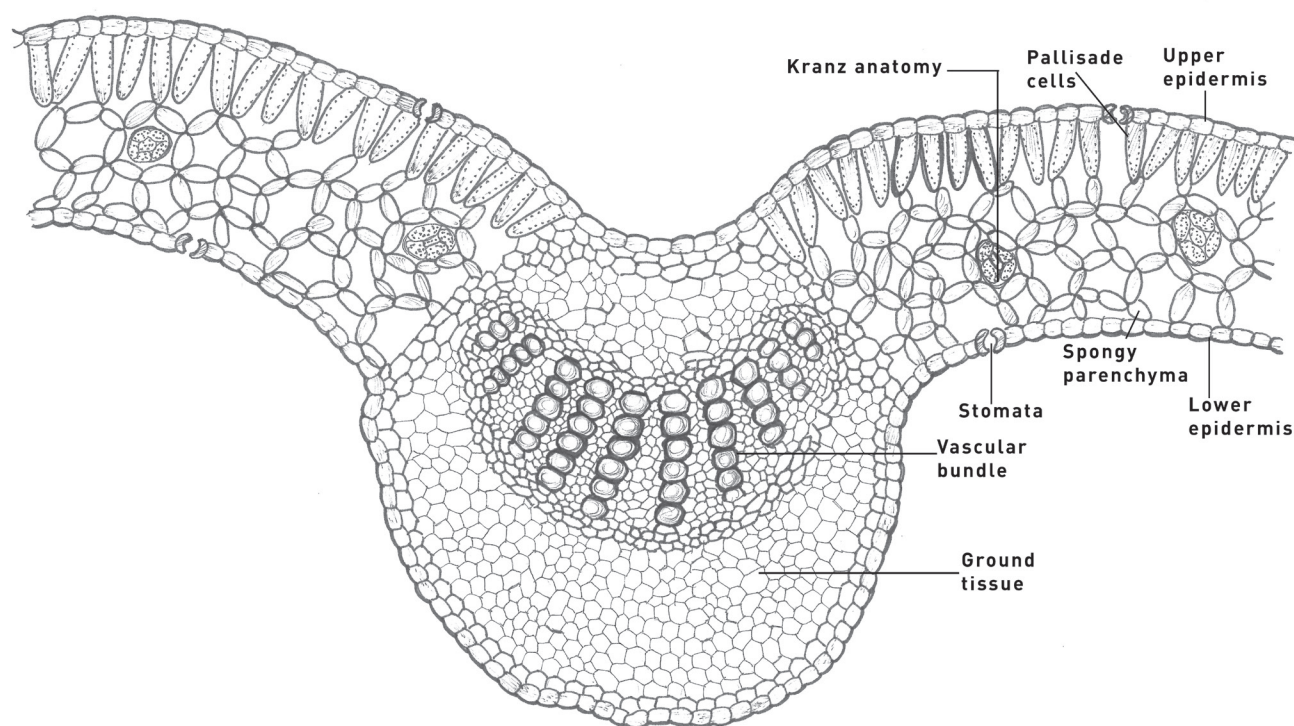


Fig. 4 TS of the leaf

Structure of Petiole

The petiole is semicircular, having semicircular convexity on the abaxial side. It is made up of distinct spindle-shaped epidermal cells with thin walls. The epidermal cells having stalked, glandular, unicellular, and branched trichomes are present. There

is homogenous, parenchymatous ground tissue present underneath the epidermis. The parenchymatous cells have thin walls, are compact, angular, and round in shape. The vascular system of the petiole is multistrand, consisting of around six vascular bundles grouped in a wide arc. The adaxial median

bundle is the biggest of the tiny bundles that are positioned in the adaxial portion of the arc. There are two rather smaller lateral bundles. The smaller bundles have a circular form, whereas the bigger bundles have a flat, triangular contour. The bundles are collateral. There are around six radial multiples of cells

that make up the xylem strands. The three rows of xylem components are seen in the smaller bundles. Phloem is found in distinct clusters within the bulk. The lactifers with a black stain are found in the ground tissues (Fig. 5).

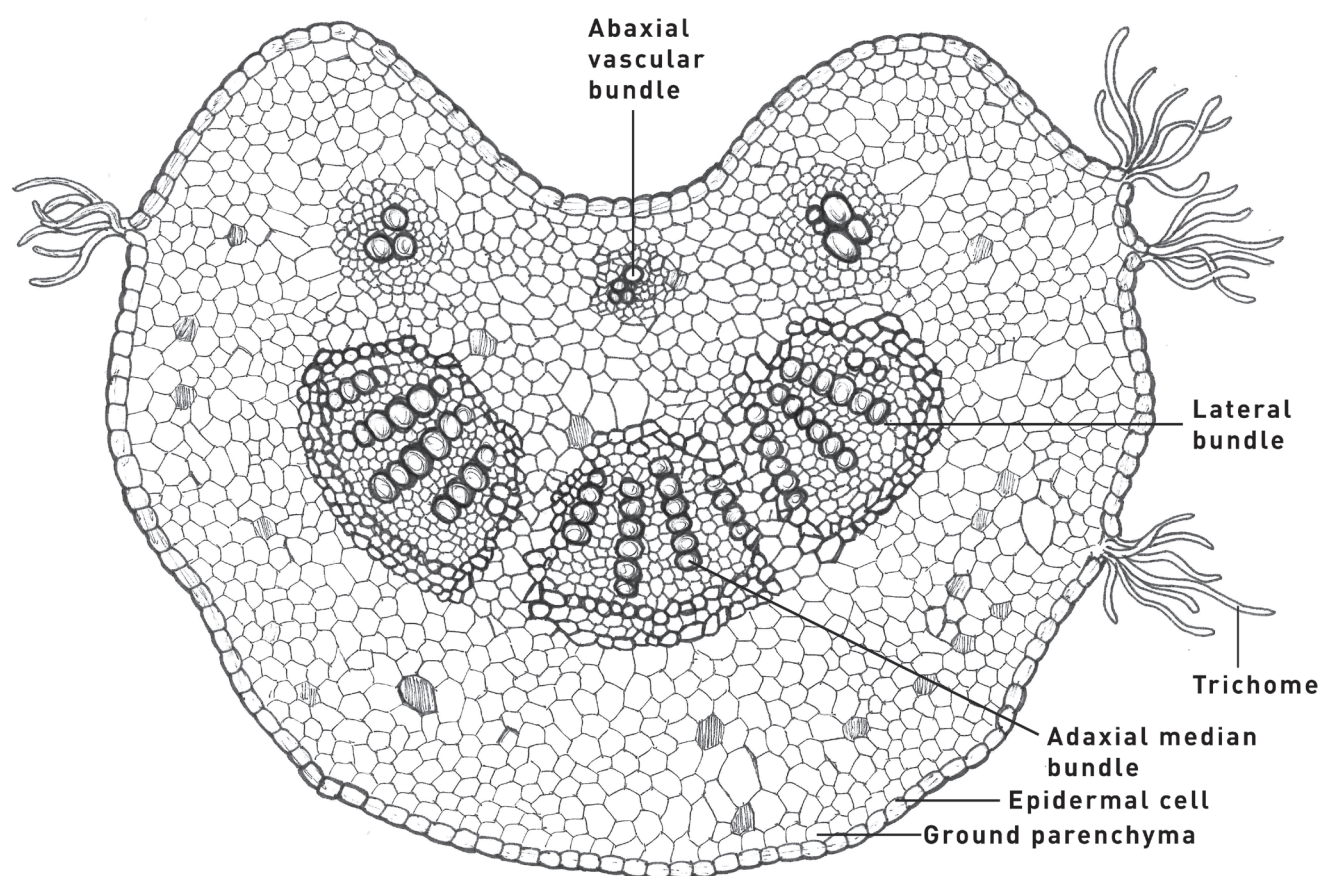


Fig. 5 TS of the petiole

Structure of Stem

The stem is circular consists of an epidermal layer, cortical zone, secondary xylem and phloem and wide pith. The epidermis, the outermost layer, is composed of spindle-shaped cells with relatively thick walls that

are rectangular in form. The epidermis having unicellular trichomes. Six to eight layers of parenchymatous cortex, interspersed with tiny patches of schlerenchymatous cells, are found inside the epidermis. Three to four layers of spongy chlorenchyma are layered after the

outer three to four layers of colourless spongy parenchyma containing storage materials. A ring of endarch collateral vascular bundles is located inside the cortex. The xylem is surrounded by continuous layers of compacted phloem on both sides. The vessels are arranged radially. The thick-walled, round to elliptical vessels can be found alone or in

radial multiples. The squarish xylem fibres are arranged in orderly, compact radial lines. The cells are thick walled and lignified. The xylem is surrounded by a continuous cylinder of secondary phloem. The phloem components are arranged in brief radial rows. The parenchymatous broad pith exhibits food material storage (Fig. 6).

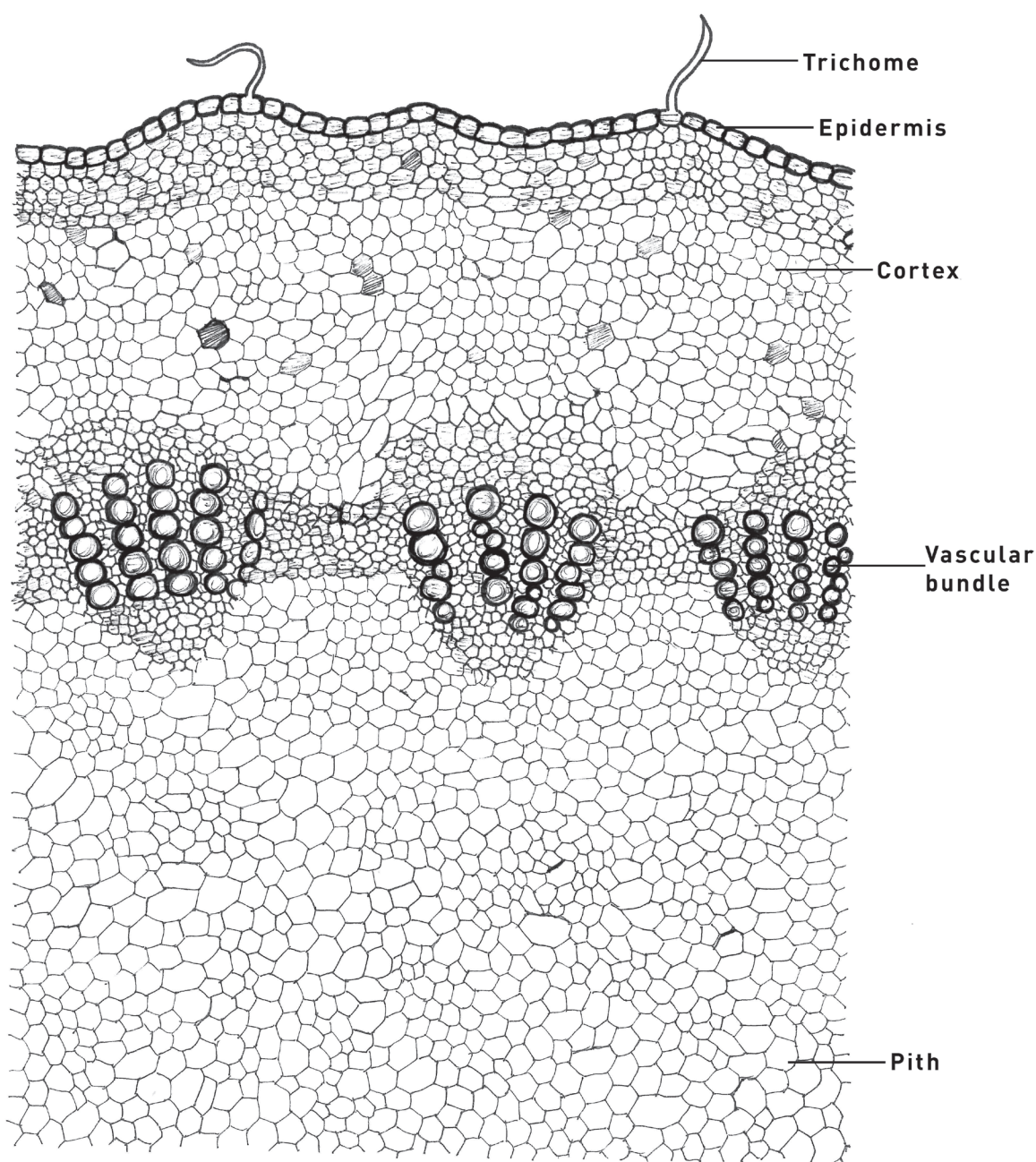


Fig. 6 TS of the Stem

Structure of Root

The surface of the rough root has uneven cracks. The root shows somewhat asymmetrical development, with greater secondary growth on one side than the other. The periderm is erratically punctured, creating broad, shallow cracks. Periderm cells are likewise compressed into wavy cell layers. The cortical zone is small, with cortical cells remaining intact in some places and collapsing into shadowy areas in others. The secondary phloem is in excellent form.

It contains sieve components connected to the partner cells as well as parenchyma cells. The phloem components have strong walls and a large width. Diffusely scattered vessels in the ground tissue of xylem fibres make up secondary xylem. The vessels in the central part of the root are narrow and those towards the periphery are wider. The vessels are solitary, circular, and thick walled which are lignified. The fibre lumen is narrow and composed of compact and dense cells (Fig. 7).

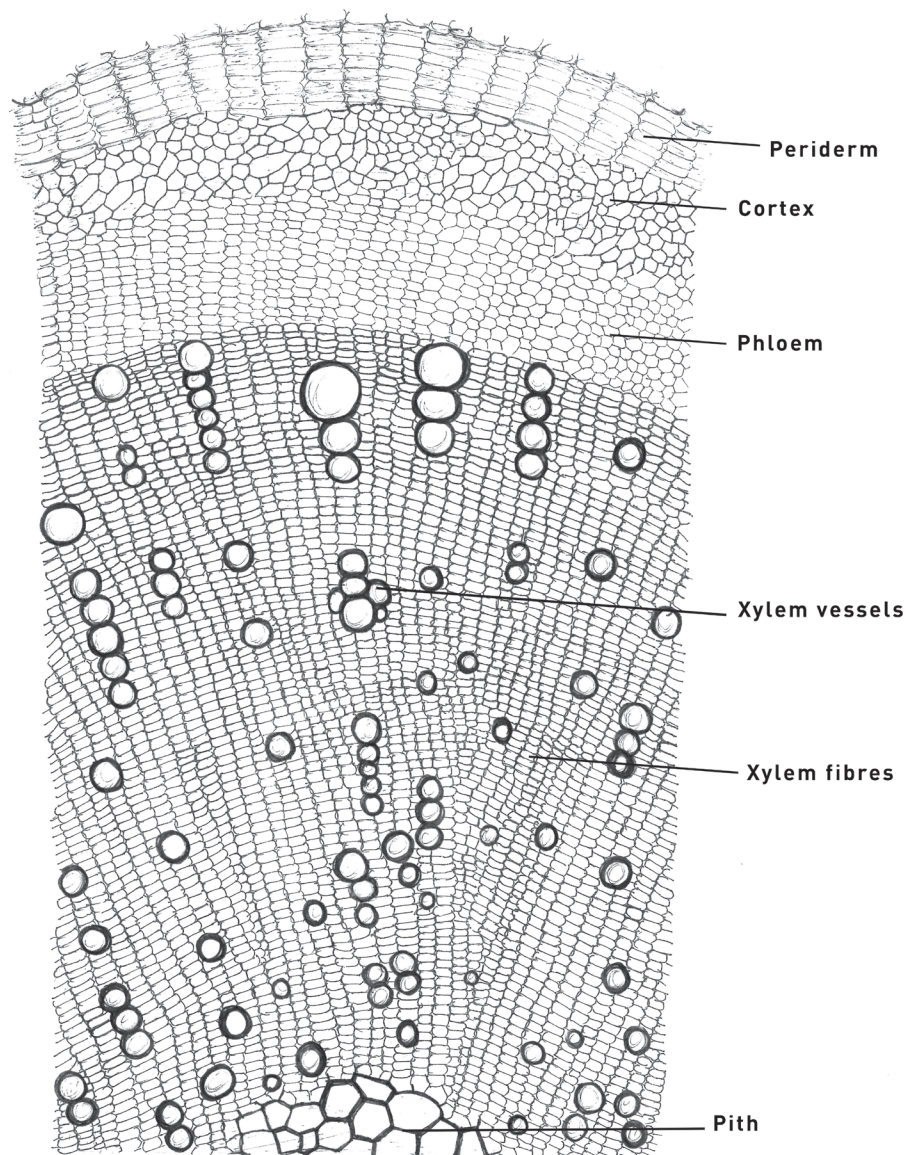


Fig. 7 TS of the Root

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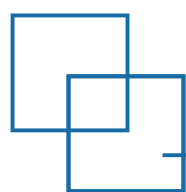
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CHAPTER
02

Bibliometric Analysis



INTRODUCTION

Croton bonplandianus Baill., a plant species belonging to the Euphorbiaceae family, has long been recognized for its traditional medicinal uses in various cultures. The plant, commonly known as "Jamaican Croton" or "Bonpland's Croton," is native to tropical and subtropical regions and has been a subject of growing interest in scientific research due to its rich phytochemical composition and diverse pharmacological properties (Caruzo and Cordeiro, 2013; van Ee and Berry, 2021). As researchers explore the exploration of natural sources for therapeutic compounds, *C. bonplandianus* emerges as a promising candidate with potential applications in medicine and allied fields. *C. bonplandianus* stands out for its unique combination of bioactive compounds including alkaloids, flavonoids, terpenoids, and polyphenols, among others, which have been identified as contributors to the plant's various medicinal attributes (Javed et al., 2023). Traditional uses of *C. bonplandianus* range from treating digestive disorders, skin ailments, and respiratory conditions to addressing more complex health issues (Magwilu et al., 2022). The surge in scientific interest can be attributed to the plant's reported pharmacological activities, such as anti-inflammatory, antioxidant, antimicrobial, analgesic, and antitumor properties (Bezerra et al., 2020). These characteristics make *C. bonplandianus* a compelling subject for researchers seeking novel therapeutic agents and exploring alternative approaches to modern medicine.

Bibliometric analysis stands as a crucial method in contemporary scientific research, offering a holistic evaluation of the scholarly field surrounding a specific subject or topic (Haba et al., 2023). This study explores the research landscape of *C. bonplandianus* through bibliometric analysis, aiming to unravel the expansive body of literature, research trends, and knowledge dissemination associated with this widely studied plant species. By adopting a bibliometric approach, we seek to explore both the quantitative and qualitative dimensions of scholarly publications, enabling a meticulous examination of patterns, trends, and the evolutionary trajectory of scientific literature about *C. bonplandianus* (Barrot et al., 2023). This analytical methodology involves scrutinizing publication metrics, citation patterns,



author collaborations, geographical distributions, and core emphases within the corpus of scientific literature. Moreover, the analysis extends to unveil the impact of research through scholarly production and their impact assessment via citation along with collaborative efforts utilizing various types of networks, shedding light on influential publications and the dissemination of knowledge within the scientific community. Systematically analyzing bibliographic data related to *C. bonplandianus* provides researchers with a comprehensive understanding of the extent and scope of research conducted on this plant species. The insights derived from this bibliometric analysis not only offer a holistic view of existing knowledge but also aid in identifying gaps, guiding future research endeavors, and informing strategic decision-making in various domains, including agricultural practices, environmental conservation, pharmaceutical applications, and turfgrass management (Aparicio et al. 2023).

In this bibliometric exploration of *C. bonplandianus*, our goal is to reveal connections, highlight research themes, and identify gaps to contribute to ongoing scholarly production. The adopted approach provides a quantitative and qualitative analysis of the research landscape, aiding in understanding current states, laying groundwork for future investigations, and emphasizing the importance of bibliometric analysis in scientific inquiry. This analysis focuses on *C. bonplandianus*, offering insights into academic publications, citation patterns, and collaborative networks. The study explores the ecological and medicinal aspects, providing a concise overview of research trends, contributors, and collaboration among them in this specialized field.

Data Sources and Tools for Powerful Insights

The bibliometric exploration of the research landscape on *C. bonplandianus* provides significant insights into the scope, influence, and patterns characterizing scholarly publications on this botanical species. This investigation constituted a bibliometric study employing the specific search terms "*Croton bonplandianus*" OR "*Oxydectes bonplandiana*" or "*Croton pauperulus*" OR "*Croton rivinoides*" OR "*Croton sparsiflorus*" or "*Oxydectes pauperula*" OR "*Oxydectes sparsiflora*" as an exact phrase, conducted through Dimensions.ai, covering the period from 2004 to 2023. Data extraction took place in the last week of October 2023 for research carried out on *C. bonplandianus* over the past two decades. The research methodology encompassed a diverse

array of analytical tools. Microsoft Excel was employed for initial data organization and preliminary assessments, while more advanced functionalities offered by Lens.org and VosViewer (Version 1.6.19) were utilized for in-depth bibliometric analysis (Penfold, 2020; Yu et al., 2020; Mejjad et al., 2022). These tools facilitated the exploration of citation networks, patterns of co-authorship, and the generation of visual representations elucidating bibliographic data. The specific domains of focus within the study were delineated through the utilization of integrated features within Lens.org, which enabled a comprehensive exploration of pivotal research themes and areas.

Holistic Insights from Data Analysis

The comprehensive insights were generated from a collection of 46 scholarly publications, sourced from diverse types of scholarly production such as books and journals. The significance and impact of research on the species in focus are underscored by an impressive average of 9.20 citations per document. The exploration identified a total of 175 authors actively contributing to the realm of *C. bonplandianus* research, emphasizing the collaboration on this specific topic. In this study, three authors stand out for their independent contributions, showcasing both competence and sustained interest in the subject. The scholarly outputs are dispersed across 41

journals affiliated with 58 organizations spanning 13 countries. This extensive network of contributors and institutions attests to the ongoing exploration of the subject matter, reinforcing the continued relevance of *C. bonplandianus* in contemporary scientific discourse.

Temporal Evolution and Growth Analysis

A total of 46 documents were sourced after extensive searches across bibliographic databases via Dimensions.ai. These documents encompass diverse study forms,

comprising 44 articles and 2 chapters. The exponential growth [$R^2=0.1822$] was found in publication production from 2004 to 2023 (Fig. 1). The highest number of scholarly documents were published in 2020 and 2021 witnessing the emergence of 6 publications each year. However, by 2023, the number reduced to 5 publications, indicating that research on species remains a dynamic and evolving field of study. These findings underscore

the comprehensive nature of disseminating research on *C. bonplandianus*, with articles serving as the primary communication medium and being complemented by articles and then chapters. The continuous increase in publications, coupled with the diverse forms of publishing, underscores the significant and sustained interest of the scientific community in this area.

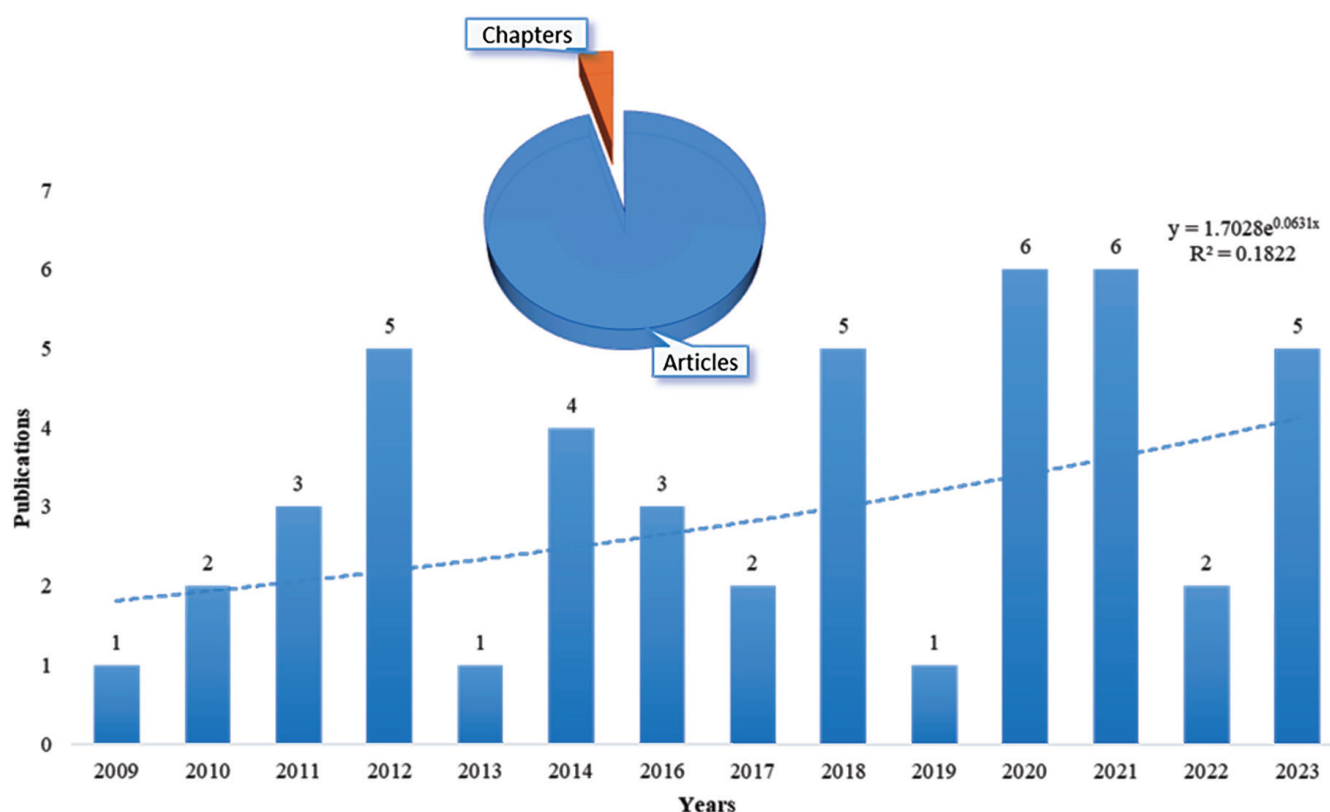


Fig. 1 Publication trends and types in *C. bonplandianus* research

Citation Analysis

The analysis of citations on *C. bonplandianus* research from 2009 to 2023 revealed a dynamic pattern in citation counts over the years. Initially, in 2009, a solitary scholarly article was published, accruing only one citation.

The highest citation scores occurred in 2014, with a remarkable 139 citations, signifying a surge in attention towards the plant. Peaks in citations were observed in 2014 (139 citations) and 2018 (91 citations), possibly attributed to influential research during those periods. The years 2020 to 2023 showed a decline

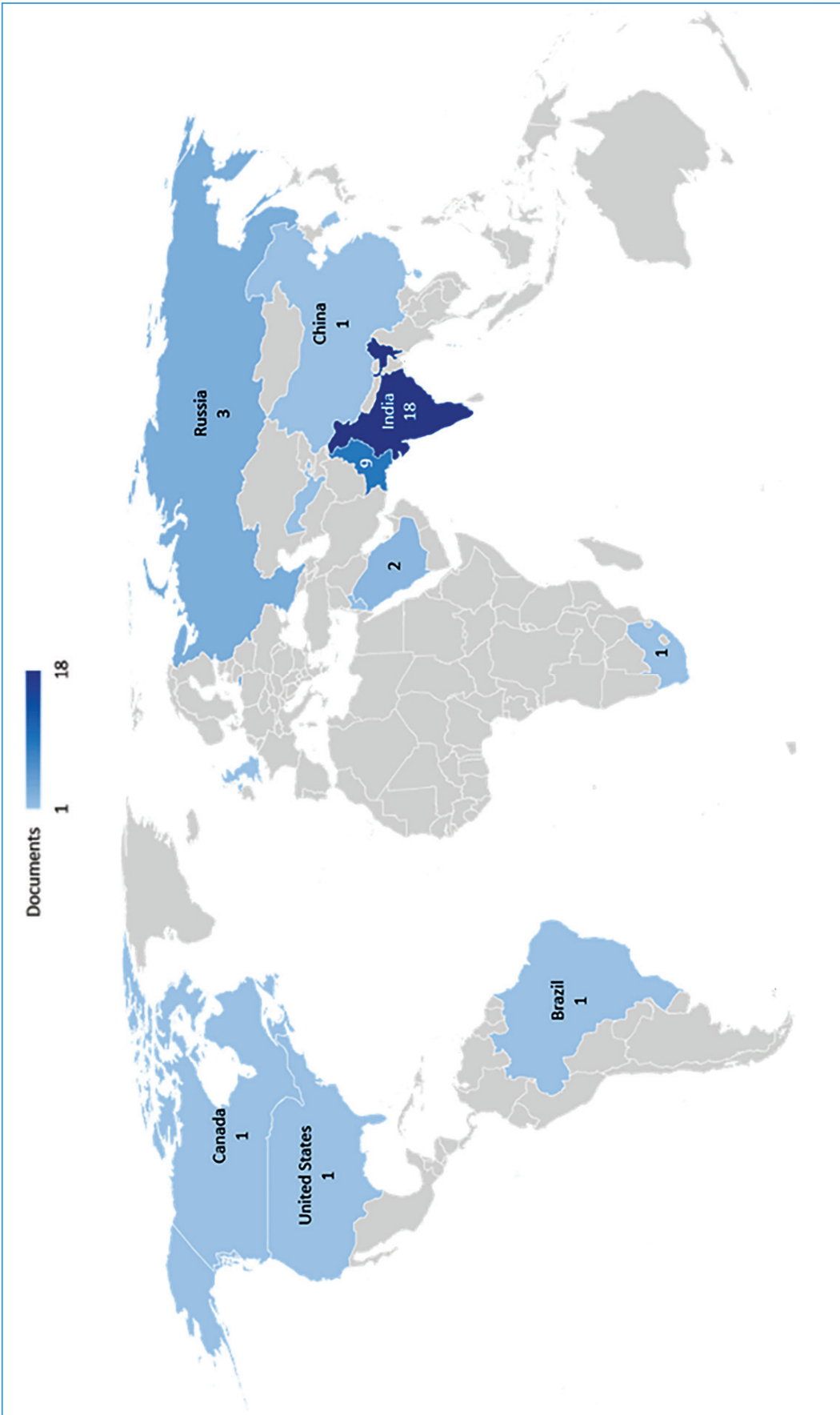


Fig. 3 Global distribution of research on *C. bonplandianus*

in citations, potentially indicating reduced popularity of more recent scholarly articles. The data illustrates fluctuations in citation numbers, highlighting pivotal moments when research on *C. bonplandianus* had a substantial

impact (Fig. 2). Peaks in citations during specific years signify significant contributions and importance, while variations underscore how the field's popularity has evolved over time.

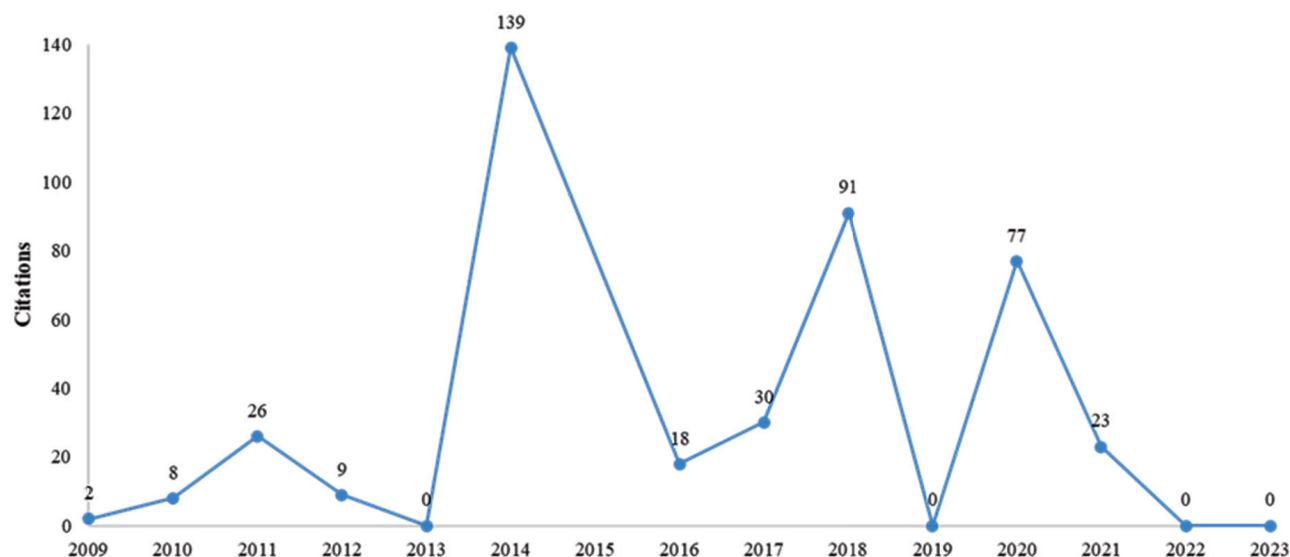


Fig. 2 Citation trends for *C. bonplandianus* research over time

Country-wise Publication Analysis

The analysis of scholarly output concerning research on *C. bonplandianus* across the 13 nations unveiled fascinating patterns and provided valuable insights into the global research contributions and their impact. Among the countries, India emerged as the leader with a total of 18 articles, followed by Pakistan with 9 scholarly articles. Furthermore, significant contributions were made by Russia, contributing 3 publications,

Saudi Arabia with 2 publications, and both China and Jordan each contributed 1 publication. Additionally, countries such as South Africa, South Korea, Brazil, and Canada made substantial contributions (Fig. 3). Despite lower output, these nations might have burgeoning research communities or specialized study areas. This analysis emphasizes the diverse global landscape, showcasing the contributions of various nations influenced by the maturity of their research ecosystem, available resources, and strategic objectives.

Most Prominent Authors

The study showed the rankings of authors based on their research contributions to *C. bonplandianus*. Rashad Mehmood had secured the top position with 4 documents and 13 citations, followed by Abdul Malik with 3 documents and 9 citations. Subsequently, Heminder Singh ranked third with 3 documents and 3 citations, followed by Hina Yasin, who had published 3 documents with one citation. Prominent authors K. Elumalai,

Rob W. Briddon, Shahid Mansoor, N. Chandra Mohana, B.R. Nuthan, and D. Rakshith, who had all made valuable contributions to the area, had published at least two scholarly documents during this period (Fig. 4). The vast range of scholars who were investigating *C. bonplandianus* was reflected in the diversified group of authors. Overall, these results highlighted the significant contribution that these authors had made to the knowledge and comprehension of *C. bonplandianus*.

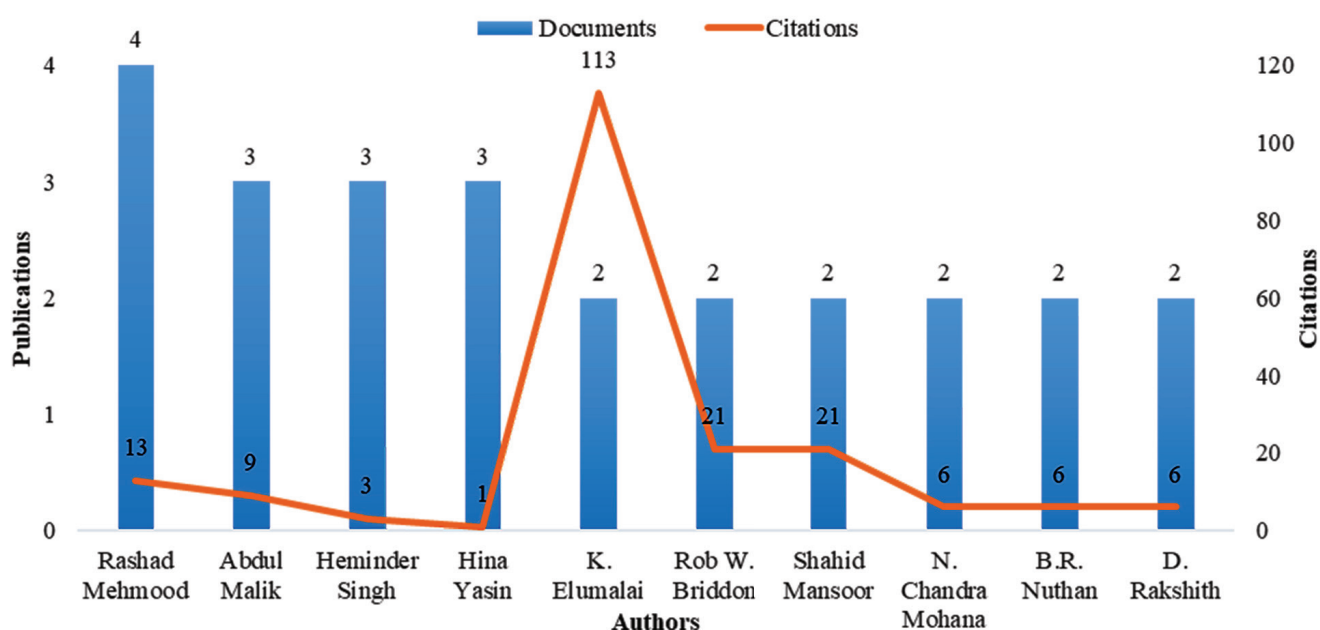


Fig. 4 Most prominent authors based on research contributions to *C. bonplandianus*

Highly Cited Articles

The top 10 highly cited research articles on *Croton bonplandianus* provide a comprehensive overview of its diverse applications and properties. The most highly cited article investigated the influence of *C. bonplandianus* on the "Green Synthesis of Silver Nanoparticles using *Croton sparsiflorus* Morong Leaf Extract

and Their Antibacterial and Antifungal Activities". This study was published in the journal *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* in 2014, acquiring a total of 107 citations (Kathiravan et al., 2014). Following this, the second-most cited article, "Amelioration of CCl_4 induced liver injury in Swiss albino mice by antioxidant-rich leaf extract of *Croton bonplandianus* Baill.,"

published in PLOS ONE in 2018, received 78 citations (Dutta et al., 2018). The third-most cited article, "Green biosynthesis of gold nanoparticles using *Croton sparsiflorus* leaves extract and evaluation of UV protection, antibacterial and anticancer applications" received 36 citations, and was published in Applied Organometallic Chemistry in 2020 (Boomi et al., 2020). Additionally, research on the plant's potential for phytoremediation, its role in metal accumulation strategies, and its chemical composition reveal its ecological and pharmacological significance, gained adequate citations from 18 to 28 (Joshi,

2014; Mukhopadhyay et al., 2017; Mohanty et al., 2020). Moreover, studies elucidating its cytotoxic and pro-apoptotic activities against cancer cells, as well as its bioactive constituents, underscore its potential in pharmaceutical applications and gained comparatively fewer citations of 10 to 13 (Shahwar et al., 2014; Bhavana et al., 2016) (Table 1). These pivotal works, accumulating a substantial total citation count, collectively highlight the multifaceted nature of *C. bonplandianus* and its relevance across various scientific domains.

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

Rank	Title	Journals	Year	Citation	References
1	Green synthesis of silver nanoparticles using <i>Croton sparsiflorus</i> morong leaf extract and their antibacterial and antifungal activities	Spectrochimica Acta Part A Molecular and Biomolecular Spectroscopy	2014	107	Kathiravan et al., 2014
2	Amelioration of CCl ₄ induced liver injury in Swiss albino mice by antioxidant-rich leaf extract of <i>Croton bonplandianus</i> Baill.	PLOS ONE	2018	78	Dutta et al., 2018
3	Green biosynthesis of gold nanoparticles using <i>Croton sparsiflorus</i> leaves extract and evaluation of UV protection, antibacterial and anti-cancer applications	Applied Organometallic Chemistry	2020	36	Boomi et al., 2020
4	Phytoassessment of <i>in situ</i> weed diversity for their chromium distribution pattern and accumulation indices of abundant weeds at South Kaliapani chromite mining area with their phytoremediation prospective	Ecotoxicology and Environmental Safety	2020	28	Mohanty et al., 2020

Rank	Title	Journals	Year	Citation	References
5	Biodiversity variability and metal accumulation strategies in plants spontaneously inhibiting fly ash lagoon, India	Environmental Science and Pollution Research	2017	26	Mukhopadhyay et al., 2017
6	Complete nucleotide sequence of a begomovirus and associated beta-satellite infecting croton (<i>Croton bonplandianus</i>) in Pakistan	Archives of Virology	2011	21	Hussain et al., 2011
7	Chemical Composition of the Essential Oil of <i>Croton bonplandianus</i> from India	Natural Product Communications	2014	18	Joshi, 2014
8	Cytotoxic and pro-apoptotic activities of leaf extract of <i>Croton bonplandianus</i> Baill. against lung cancer cell line A549.	Indian Journal of Experimental Biology	2016	13	Bhavana et al., 2016
9	Bioactive constituents from <i>Croton sparsiflorus</i> Morong	Natural Product Research	2014	10	Shahwar et al., 2014
10	Investigations of Bioactivity of <i>Acalypha indica</i> (L.), <i>Centella asiatica</i> (L.) and <i>Croton bonplandianus</i> (Baill) against Multidrug Resistant Bacteria and Cancer Cells	Journal of Herbal Medicine	2021	9	Suresh et al., 2021

Most Active Journals

The present study showed the top 10 journals for *C. bonplandianus* research based on their publications, citations, average citations per article (ACD), publisher, and impact factor (IF). The highest-ranked journal was "Archives of Phytopathology and Plant Protection," published by Taylor & Francis, with four documents and five citations, maintaining an average of 1.25 citations per document and with an impact factor of 1.09. Following closely was "Environmental Science and Pollution Research," affiliated with Springer

Nature, securing the second position with two documents but an impressive citation count of 32, resulting in an average of 16 citations per document with an elevated impact factor of 3.96. "Natural Product Communications," associated with Bentham Science Publishers, held the third rank with two documents, 24 citations, and an average of 12 citations per document, reflecting a robust impact, complemented by an impact factor of 2.81. "Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy" published by Elsevier stood out in the fourth position, with a single document amassing 107 citations,

resulting in an outstanding average citation per document of 107, and an impact factor of 3.06. "PLOS ONE," under the Public Library of Science, secured the fifth rank with one document and an impressive 78 citations, resulting in a high average citation per document of 78, alongside an impact factor of 3.24. Journals ranking 6-10, each associated with distinct publishers, contributed

significantly to *C. bonplandianus* research, enriching the scientific landscape with diverse impacts, as indicated by their respective average citations per document and impact factors (Table 2). Overall, the study's results assisted readers and researchers in selecting relevant and valuable materials for their *C. bonplandianus* research.

Table 2 Top journals in *C. bonplandianus* research exhibited the highest published documents and citations

Rank	Journals	Publisher	Documents	Citations	ACD	IF
1	Archives of Phytopathology and Plant Protection	Taylor & Francis	4	5	1.25	1.09
2	Environmental Science and Pollution Research	Springer Nature	2	32	16	3.96
3	Natural Product Communications	Bentham Science Publishers	2	24	12	2.81
4	Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy	Elsevier	1	107	107	3.06
5	PLOS ONE	Public Library of Science	1	78	78	3.24
6	Applied Organometallic Chemistry	Wiley	1	36	36	2.15
7	Ecotoxicology and Environmental Safety	Elsevier	1	28	28	4.74

Rank	Journals	Publisher	Documents	Citations	ACD	IF
8	Archives of Virology	Springer Nature	1	21	21	2.80
9	Indian Journal of Experimental Biology	Council of Scientific & Industrial Research	1	13	13	0.90
10	Natural Product Research	Taylor & Francis	1	10	10	2.47

Top Productive Organizations

In this study, the data insights showcased significant contributions from top organizations in *C. bonplandianus* research. The highest-ranked organization was the International Center for Chemical and Biological Sciences in Pakistan, which boasted 6 documents with 23 citations and an average of 3.83 citations per article. Following closely was the University of Karachi (Pakistan) with 5 documents, 22 citations, and a slightly higher average of 4.40 citations per article. The University of Mysore (India) also made a substantial contribution, producing 5 documents, though with a lower citation count of 9 and an average of 1.80 citations per article, suggesting a potentially lower impact compared to the top two organizations. The National Institute for Biotechnology and Genetic Engineering in Pakistan stood out with only 2 documents but an impressive average

of 10.50 citations per article, indicating high impact and recognition in the scientific community. King Saud University (Saudi Arabia) followed with 2 documents, 9 citations, and an average of 4.50 citations per article, showcasing a commendable contribution to *C. bonplandianus* research. Meanwhile, the Dow University of Health Sciences (Pakistan) lagged with 2 documents, a minimal citation count of 1, and an average of 0.50 citations per article, suggesting a lower impact or visibility of their research. Other organizations with 1 document each included Annamalai University (107 citations), Bodoland University (78 citations), North Bengal University (78 citations), Alagappa University (36 citations). This analysis highlighted the diverse and impactful contributions from organizations in both Pakistan and India, with variations in the levels of research impact and visibility within the scientific community (Table 3).

Table 3 Top organizations' contributions to *C. bonplandianus* research

Rank	Organization	Country	Documents	Citations	Average Citations Per Article
1	International Center for Chemical and Biological Sciences	Pakistan	6	23	3.83
2	University of Karachi	Pakistan	5	22	4.40
3	University of Mysore	India	5	9	1.80
4	National Institute for Biotechnology and Genetic Engineering	Pakistan	2	21	10.50
5	King Saud University	Saudi Arabia	2	9	4.50
6	Dow University of Health Sciences	Pakistan	2	1	0.50
7	Annamalai University	India	1	107	107.00
8	Bodoland University	India	1	78	78.00
9	North Bengal University	India	1	78	78.00
10	Alagappa University	India	1	36	36.00

Most Prominent Fields of Study

The current investigation analyzed the co-occurrence of terms in prominent fields of study, examining a dataset comprising 45 scholarly documents sourced from Lens.org. Within these articles, the term "Croton" was identified with a frequency of 26, underscoring

its noteworthy prevalence and significance in academic literature. The field of biology contributed the highest document count with 24 papers, followed by chemistry with 14 articles. This observation suggested a considerable emphasis on biological aspects and chemical applications related to *C. bonplandianus*. Furthermore, the inclusion of

traditional medicine and botany in the study of *C. bonplandianus* indicated a diverse array of disciplines investigating various facets of this grass species. Medicine (7 counts) involved medical sciences and weed (7 counts) pertained to agriculture and ecology. Horticulture (6 counts) focused on plant cultivation. Fields with 5 counts included

antioxidant and biochemistry, indicating research on oxidative stress and chemical processes in living organisms, respectively (Fig. 5). This interdisciplinary interest highlighted the importance of understanding *C. bonplandianus* across various scientific domains, emphasizing its relevance and impact in diverse fields of study.

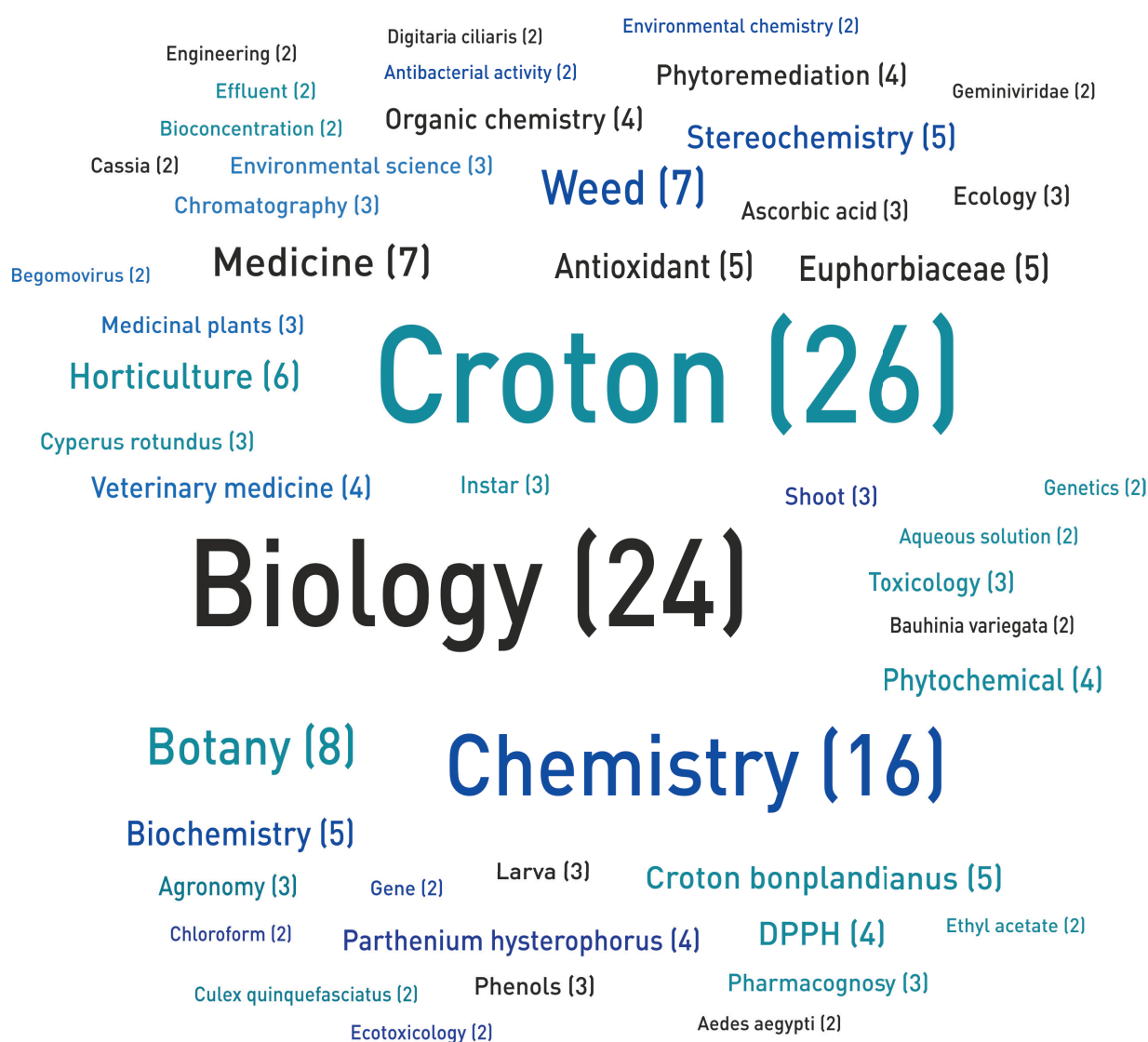


Fig. 5 Terms co-occurrence analysis within prominent areas of study on *C. bonplandianus* research

Research Collaboration Networks

Authors' Collaboration Network

The investigation encompassed an in-depth analysis of 175 authors, focused on a substantial and noteworthy presence of researchers within this subject domain. Co-authorship was used to measure the collaboration extent between authors based on their common citations. The network included authors who had published a minimum of one document on *C. bonplandianus* and had a

minimum citation threshold of one citation per document. The analysis identified 2 clusters within the network, representing groups of interconnected and collaborative authors (Fig. 6). Cluster 1 had eleven members each, while Cluster 2 with 6 members. K. Elumalai emerged as the most prominent author, boasting the highest total link strength of 16. These results reveal a varied research community with differing degrees of specialization and collaboration.

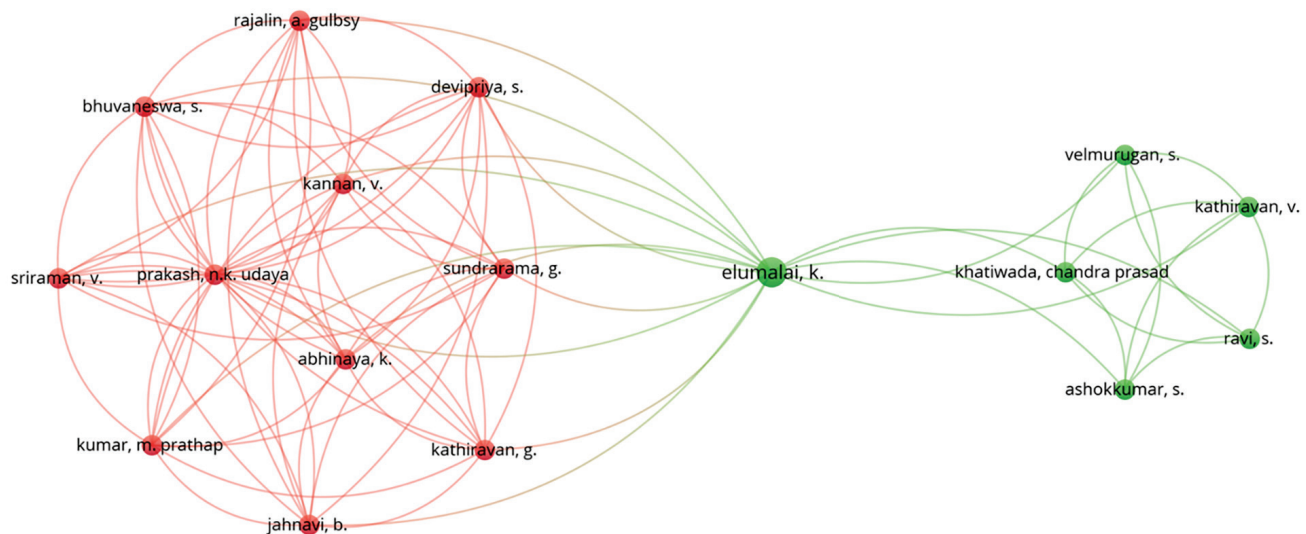


Fig. 6 Collaborative network among the authors on *C. bonplandianus*

Journal-wise Collaboration Network

The journal-wise collaboration network, which analyzed 41 journals studying *C. bonplandianus* research, revealed 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 one document on *C. bonplandianus*, resulting in the identification of 3 distinct clusters in the journal-wise collaboration network. Cluster 1 was found to be the largest cluster, comprised of 5 journals, followed by cluster 2, and cluster 3, encompassing 4 and 3 journals, respectively. This clustering highlighted specialized thematic concentrations within

C. bonplandianus research. "Process Biochemistry" and "South African Journal of Botany" exhibited the strongest connection strength (12), indicating their central roles in fostering cooperative projects. Additionally, the Journal of Asian Natural Products Research, Natural Product Communications, Microscopy Research and Technique,

Microscopy Research and Technique, and Natural Product Research showed considerable influence, boasting total link strengths of 8 for each journal (Fig. 7). The network analysis explored thematic strengths and collaborative potential among journals studying *C. bonplandianus*.

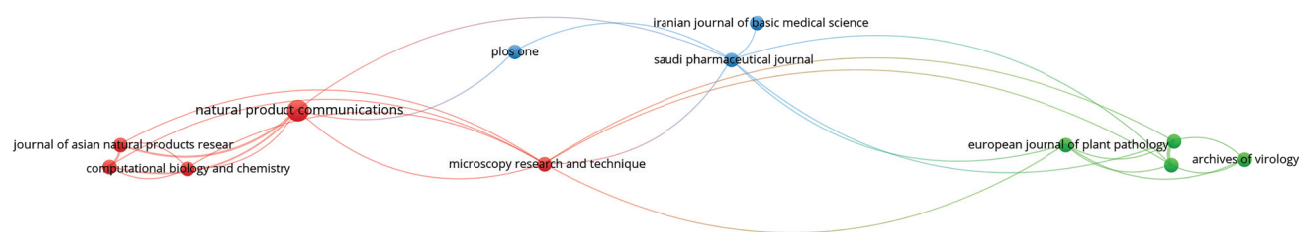


Fig. 7 Journal-wise collaboration network analysis on *C. bonplandianus* research

Organizational Collaborative Network

The study utilized co-authorship analysis to explore the collaborative network among organizations involved in the research on *C. bonplandianus*. The criteria for inclusion in the network required organizations to have published at least one document on *C. bonplandianus*, with each document having a minimum of 5 citations. The analysis encompassed a total of 58 organizations, revealing relationships among 8 of them. The results indicated the presence of a single cluster in the network, comprising

8 organizations. All these organizations exhibited the same link strength, which was measured at 7 (Fig. 8). This finding suggests a cohesive and interconnected collaborative group within the study. For stakeholders such as researchers, institutions, and legislators aiming to enhance research on *C. bonplandianus* through effective partnerships, understanding these collaborative networks can be valuable. Insights derived from the co-authorship analysis may inform strategies for fostering successful collaborations in the pursuit of advancing knowledge on *C. bonplandianus*.

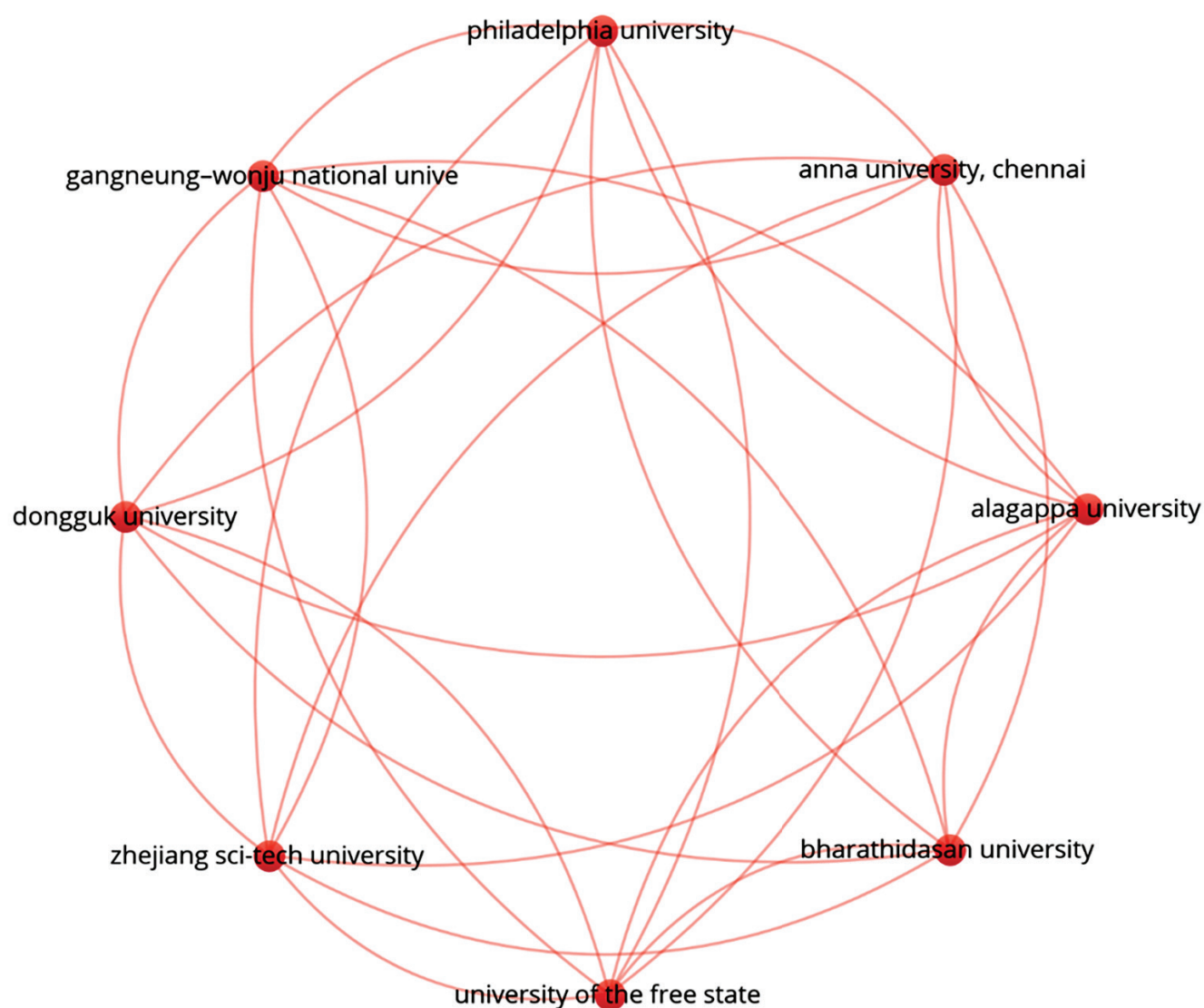


Fig. 8 Clusters of collaborative institutions for *C. bonplandianus* research

Country-wise Collaboration Network

In the analysis of country-wise collaboration networks in *C. bonplandianus* research, a comprehensive analysis involved 13 different countries, uncovering 7 linkages among them. The criteria for inclusion mandated at least one document on *C. bonplandianus* with a minimum of one citation. The study identified 5 distinct clusters, each representing varying

levels of collaboration and research focus based on citation patterns. Clusters 1 and 2 comprised 2 nations each, indicating a specific level of collaboration between them. In contrast, clusters 3, 4, and 5 consisted of individual entities, showcasing more concentrated collaboration networks (Fig 9). Moreover, India and Pakistan showed the strongest connection strength [9], indicating their central roles in this analysis. This analysis provides valuable insights into the

global research landscape surrounding *C. bonplandianus*. By revealing the diversity and interconnectedness of international research collaborations, the study highlights

the complex dynamics within the scientific community and emphasizes the significance of collaborative efforts in advancing the understanding of this species.

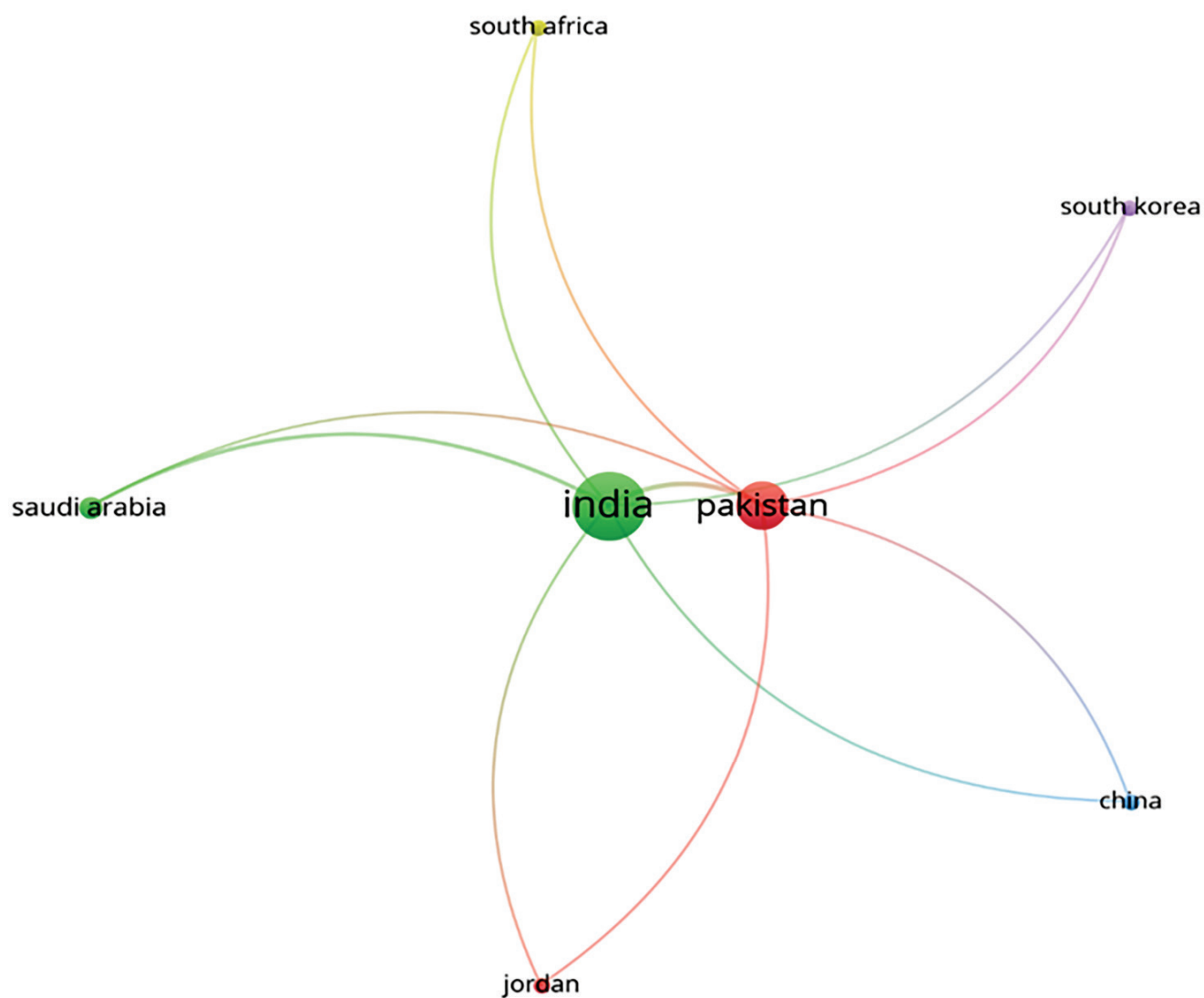


Fig. 9 Country-wise collaboration network in *C. bonplandianus* research

Conclusion

In the present study, the bibliometric analysis conducted on the research landscape of *Croton bonplandianus* provides valuable insights into the scholarly contributions, collaborations, and impact within this field. Analysis of publishing growth, analysis of citations, and analysis of publication by nation were the main topics. *C. bonplandianus* research is becoming more popular, as seen by the statistics showing a consistent rise in publications over time, with large spikes in the years 2020 and 2021. The year 2014 emerges as a pivotal point, marked by the highest citation scores, suggesting a significant peak in the attention and relevance of studies related to *C. bonplandianus* during that period. India takes the lead among the countries with the highest number of scholarly articles, underlining its active and substantial involvement in research on *C. bonplandianus*. Rashad Mehmood stands out as the most prolific author, contributing four documents that garnered 13 citations, showcasing individual scholarly impact. The research article published by Kathiravan et al. in 2014 gained the highest citations, reaching an impressive count of 107, further emphasizing its significance in the academic community. The prominence of "Archives of Phytopathology and Plant Protection" as the highest-ranked journal and the International

Center for Chemical and Biological Sciences in Pakistan as the highest-ranked organization highlights the key players and outlets in the dissemination of research on *C. bonplandianus*. The frequency of the term "Croton" in the articles underscores its noteworthy prevalence and significance in academic literature. The collaborative networks reveal crucial connections within the academic community. K. Elumalai stands out in the authors' collaborative network, while "Process Biochemistry" and "South African Journal of Botany" exhibit robust connections in the journals' collaborative network. All 7 organizations in the organizational collaborative network exhibit equal link strength, emphasizing a balanced and collaborative research environment. In the country-wise collaboration network, the strong connection strength between India and Pakistan indicates their central roles in this analysis, suggesting active collaboration between researchers from these countries. Overall, this bibliometric analysis focused on the key contributors, influential articles, and collaborative dynamics within the research landscape of *C. bonplandianus*, providing a comprehensive understanding of the field's evolution and current state.

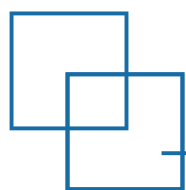
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CHAPTER 03

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 of clay loam soil showed the highest radical scavenging activity. Similar results were obtained from extracts of plant shoots grown on clay loam soil for ABTS radical scavenging. 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. Makgato et al. (2020) showed that inoculation of beneficial soil microbes like *Rhizobium* into soil

can also stimulate plant phytochemical content. 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 understanding plant physiology and the best possible conditions for the cultivation of medicinal plants. In the current study, emphasis 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 rhizosphere 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

S.No.	States	Site	Site Code	Latitude	Longitude
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

Site	Temperature (°C)	Altitude (m)
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
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 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, weighing 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, 100ml 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/cm^3) = 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 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].

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.

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 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 liter.
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 made up to 2 liters with distilled water.

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 g 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.
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.

Calculations

1. Weight of soil taken (W) = 2 g
2. Vol. of N/2 ferrous ammonium sulphate

- used for blank titration = X ml
- Vol. of N/2 ferrous ammonium sulphate used to titrate excess nascent oxygen = Y ml
 - Vol. of 1N $K_2Cr_2O_7$ used for oxidation of organic carbon in soil = $(X-Y)/2$ ml
 - 1 ml of 1 N $K_2Cr_2O_7$ = 0.003 g C
 - % Organic carbon in Soil = $X - Y/2 \times 0.003 \times 100/W$

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

- 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.
- Sodium hydroxide solution (NaOH): 25 g/l of sodium hydroxide pellets were dissolved in distilled water and the volume was made up to 1 liter.
- 0.02 N sulphuric acid (H_2SO_4).
- Mixed indicator: 0.066 g of methyl red and 0.099 g of bromocresol green were mixed in 100 ml of ethanol.

- Boric acid: 25 g/l of boric acid was dissolved in 1 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

- 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.
- 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.
- 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

1. Standard phosphorous solution
 - i. 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 liter.
 - ii. Standard solution of P (5 ppm): 5 ml of 100 ppm P solution was diluted to 100 ml with distilled water.

2. 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.
3. Sulphuric acid (5N): 139 ml of concentrated H_2SO_4 was diluted to 1 liter.
4. Reagent A: Dissolve 12 g of ammonium paramolybdate in 250 ml distilled water. Separately, 0.2908g of potassium antimony tartrate ($\text{KSbO}_3\cdot\text{C}_4\text{H}_4\text{O}_6$) 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 liters.
5. 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. Ten milliliters 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 NaHCO₃ 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 × 5 = 100 times
8. Concentration of P from spectrophotometer = X AU (absorbance units)
9. Available P in soil (ppm) = X (AU) × 100
10. Available P (Kg/ ha) = ppm × 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 (Merwin and Peech, 1951; Black, 1965; Jackson, 1973).

Chemicals and reagents required

1. Neutral normal ammonium acetate solution (CH₃COONH₄): 77.09 g/l of ammonium acetate was dissolved in distilled water and the volume was made up to 1 liter. 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 liter.
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 $\text{CH}_3\text{COONH}_4$ 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 liter.
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 liter.

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.

Calculations

1. Weight of soil taken= 10g
2. Volume of extractant added= 50 ml
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 $\times 2.24$

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).

- Kept at room temperature 20-30 minutes for open digestion.
- Afterwards samples were put in microwave for close digestion.
- After digestion, samples were transferred into the 50ml volumetric flasks and volume make up to the mark.
- 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 × 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

- 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 1 liter 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 liter volumetric flask, 100ml narrow mouth polyethylene bottles, pipettes, electric shaker, Whatman No. 1 filter paper and atomic absorption spectrophotometer.

Procedure

- 12.5 g of air-dried soil sample was taken and transferred to a 100 ml narrow mouth polyethylene bottle.
- 50 ml DTPA solution was added, and the bottle was closed with the stopper.
- The bottle was shaken on an electric shaker for 2 hours at 25°C .
- The contents were then filtered through Whatman No. 42 filter paper to obtain a clear solution.
- 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 × 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 of the 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

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

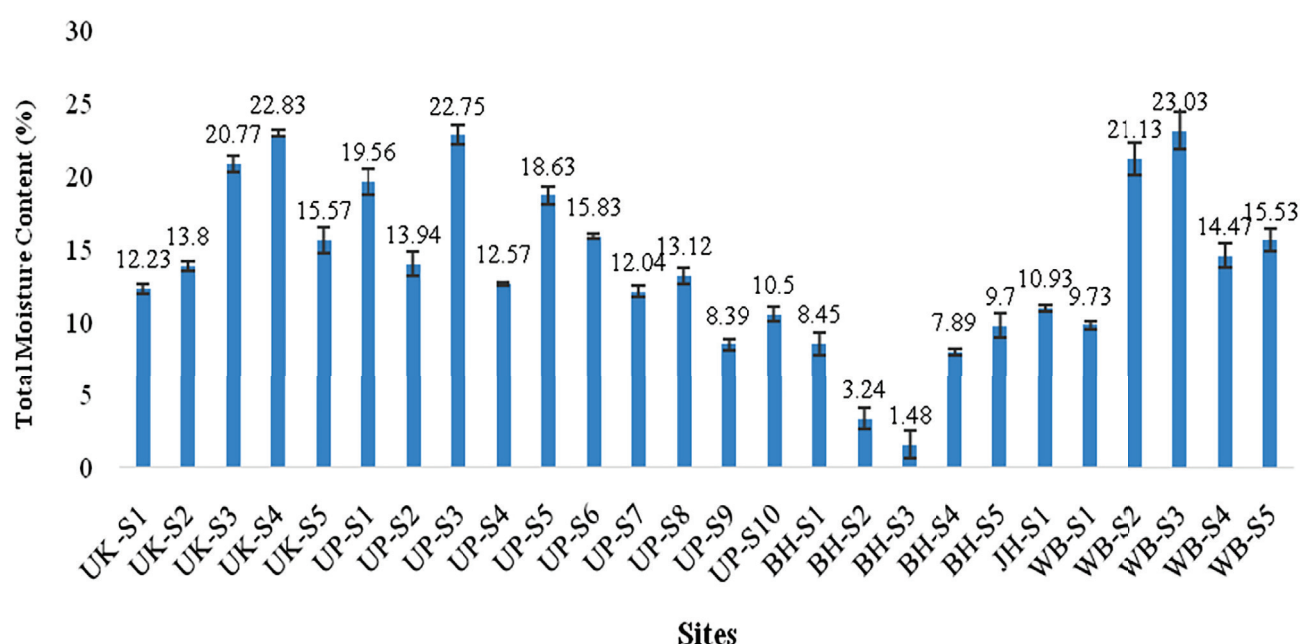


Fig. 1 Total moisture content of different sampling sites

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

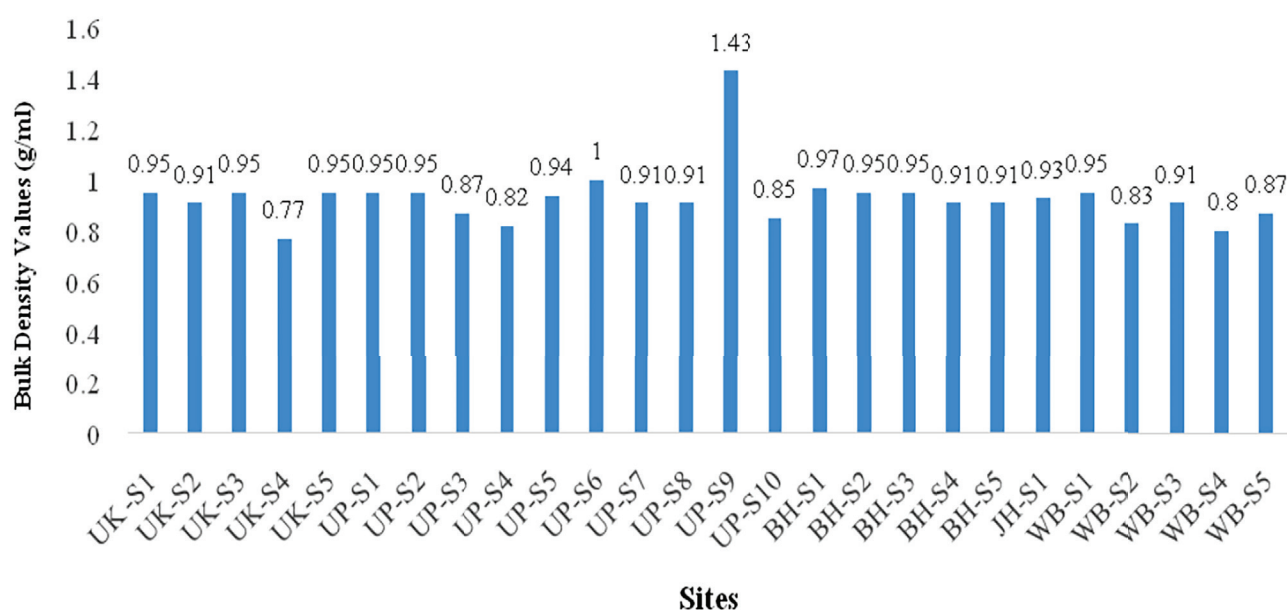


Fig. 2 Soil bulk density of different sampling sites

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

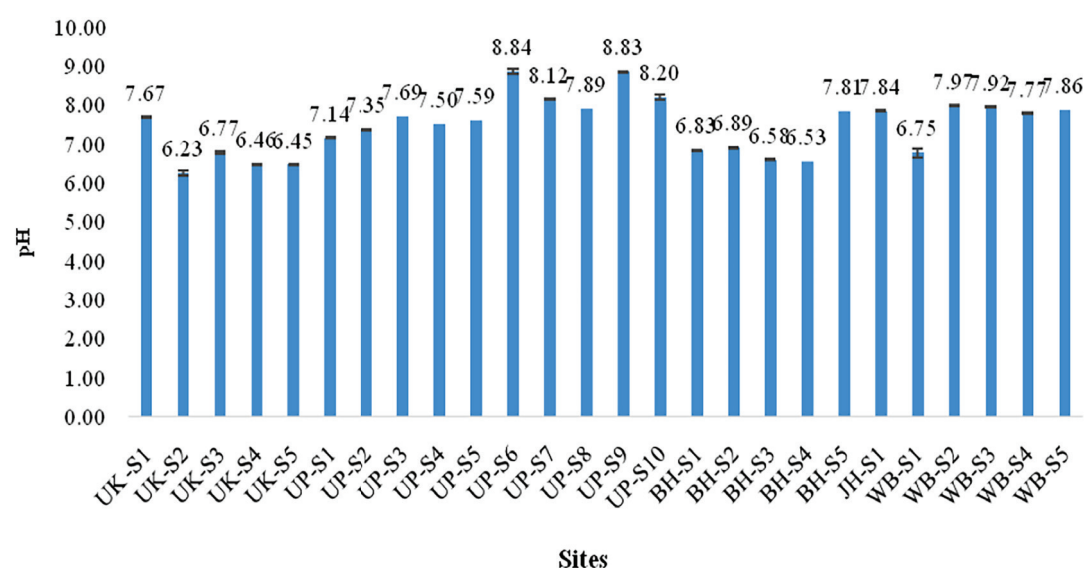


Fig. 3 Soil pH of different sampling sites

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

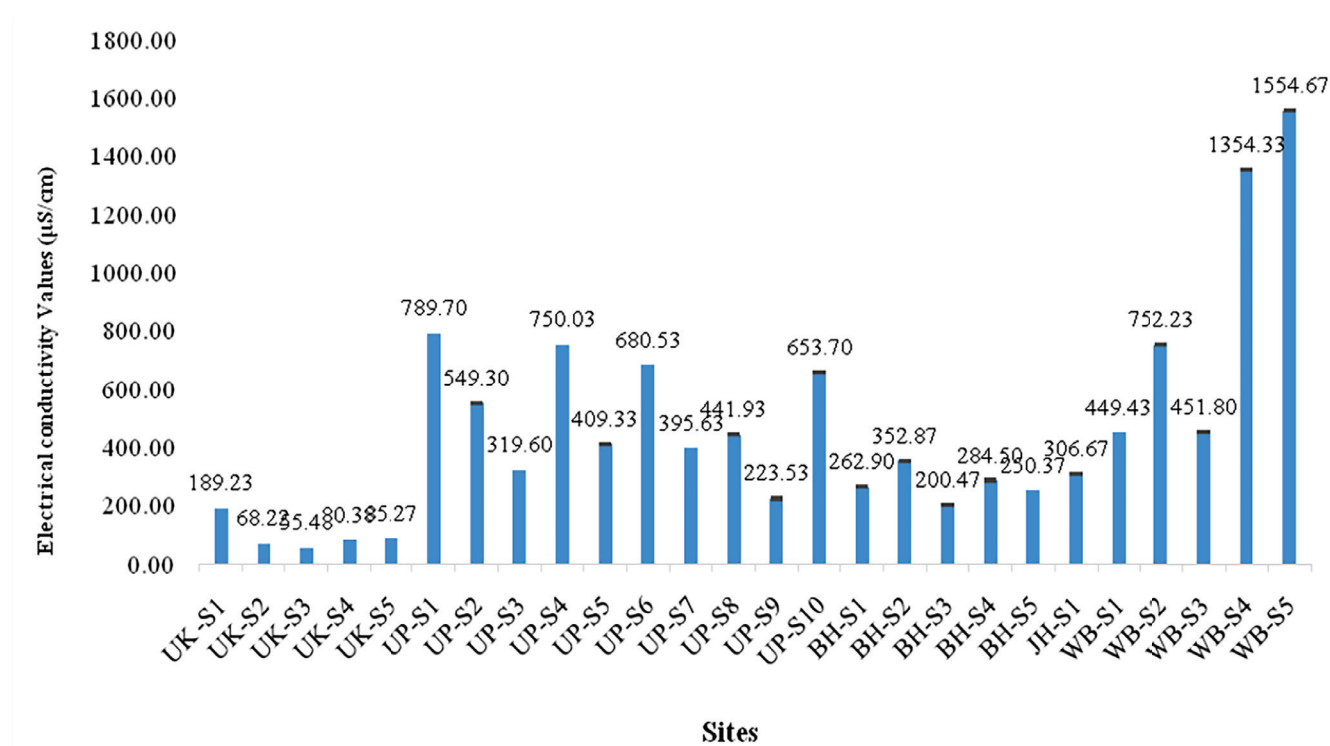


Fig. 4 Soil electrical conductivity at different sampling sites

The electrical conductivity of almost all the soil samples were within the prescribed limits of having EC below $800 \mu\text{S/cm}$. Soil samples from WB-S4 and WB-S5 showed the highest electrical conductivity value of $1354.33 \pm 2.52 \mu\text{S/cm}$ and $1554.67 \pm 2.52 \mu\text{S/cm}$ 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.

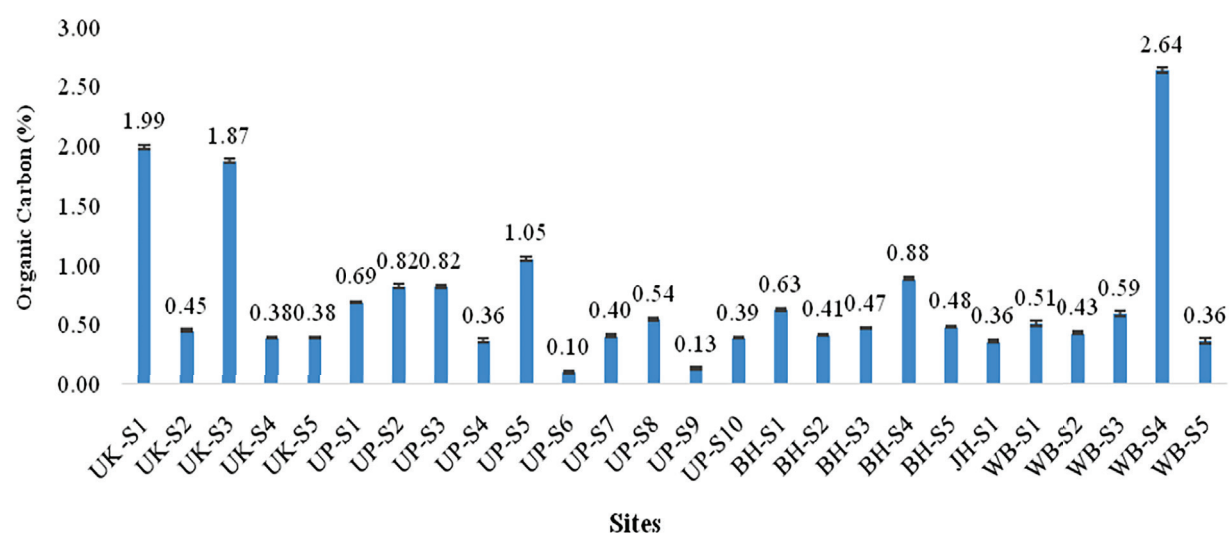


Fig. 5 Soil organic carbon content at different sites

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 ± 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 ± 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 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

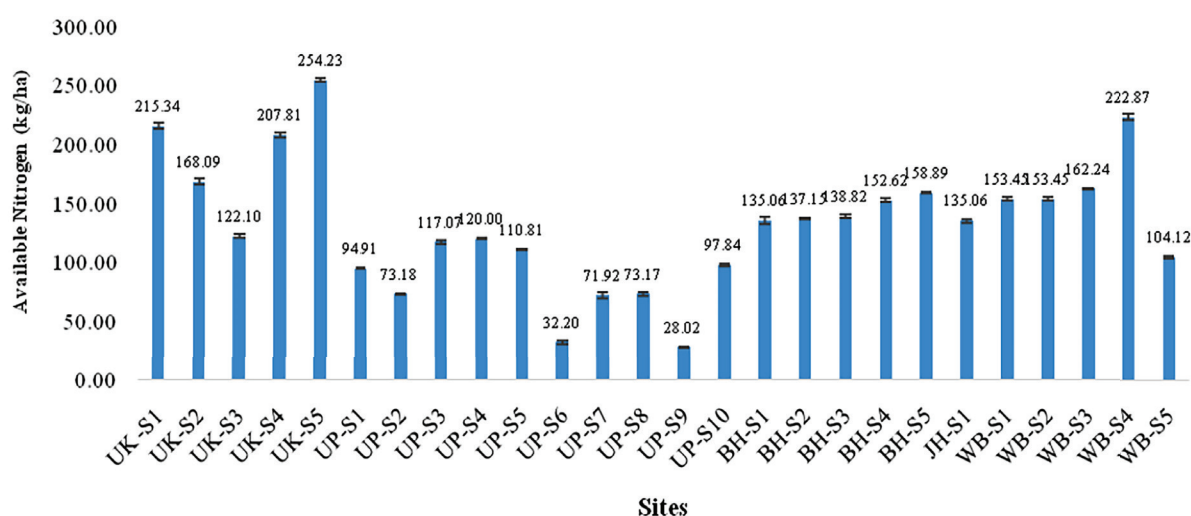


Fig. 6 Soil available nitrogen content at different sites

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

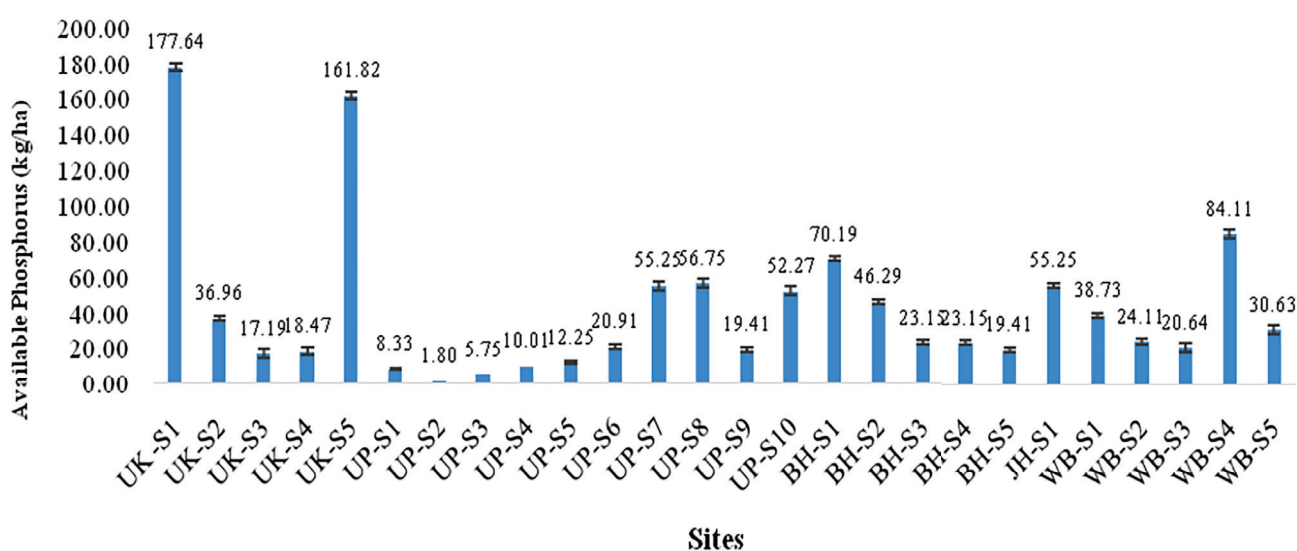


Fig. 7 Soil available phosphorus content at different sites

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

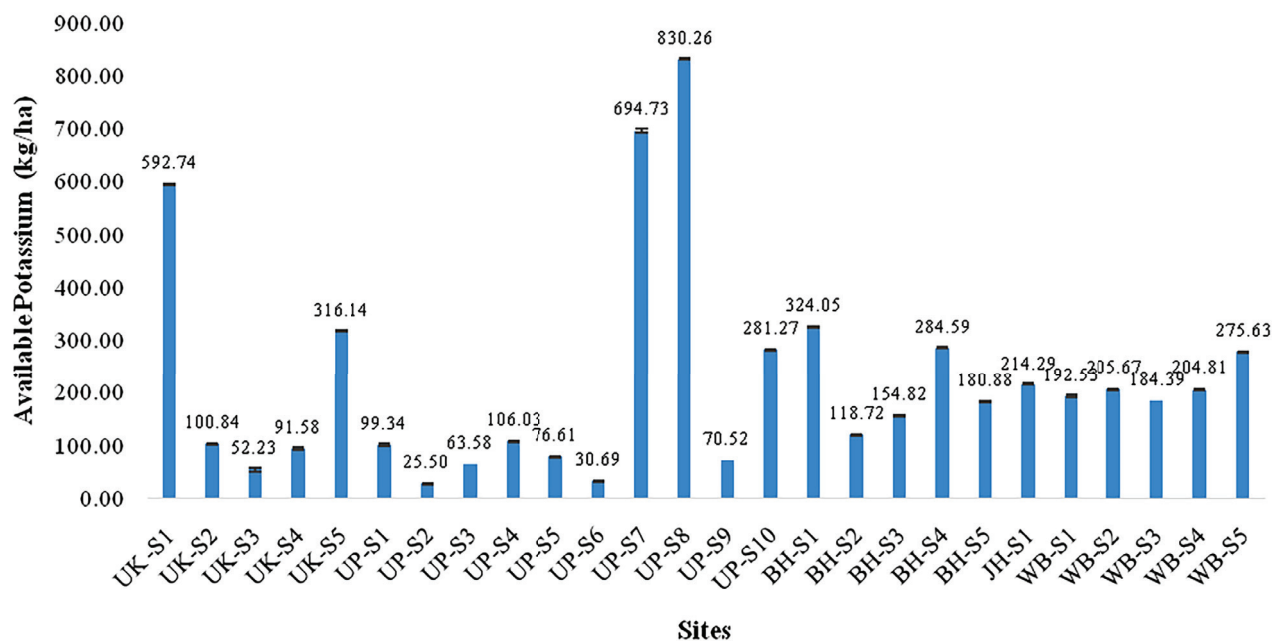


Fig. 8 Soil available potassium content at different sites

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

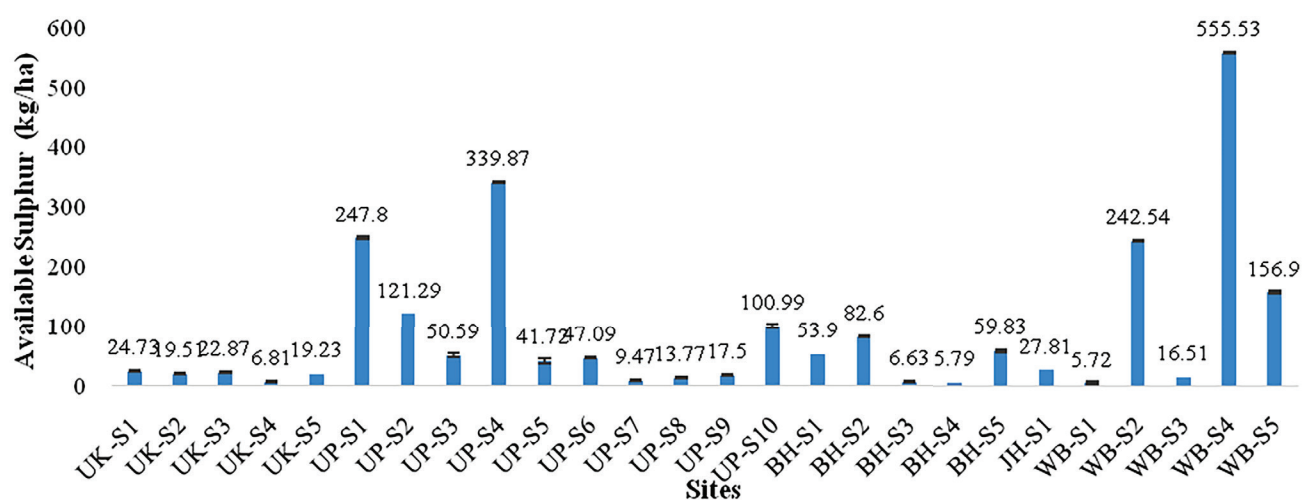


Fig. 9 Soil available sulphur content at different sites

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.

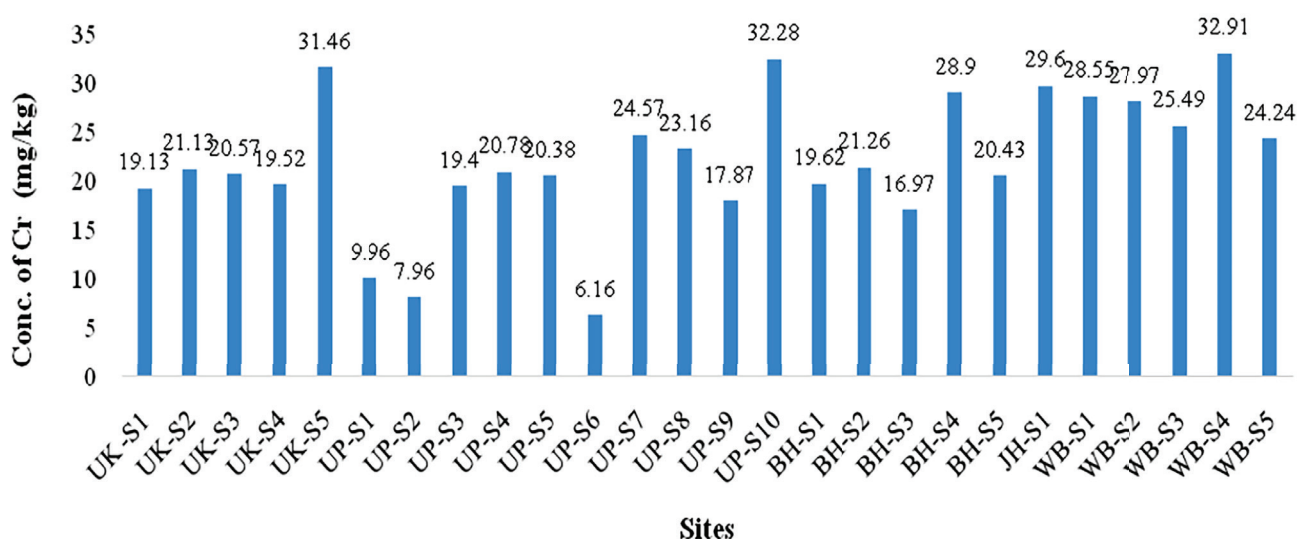


Fig. 10a Concentration of chromium at different sites

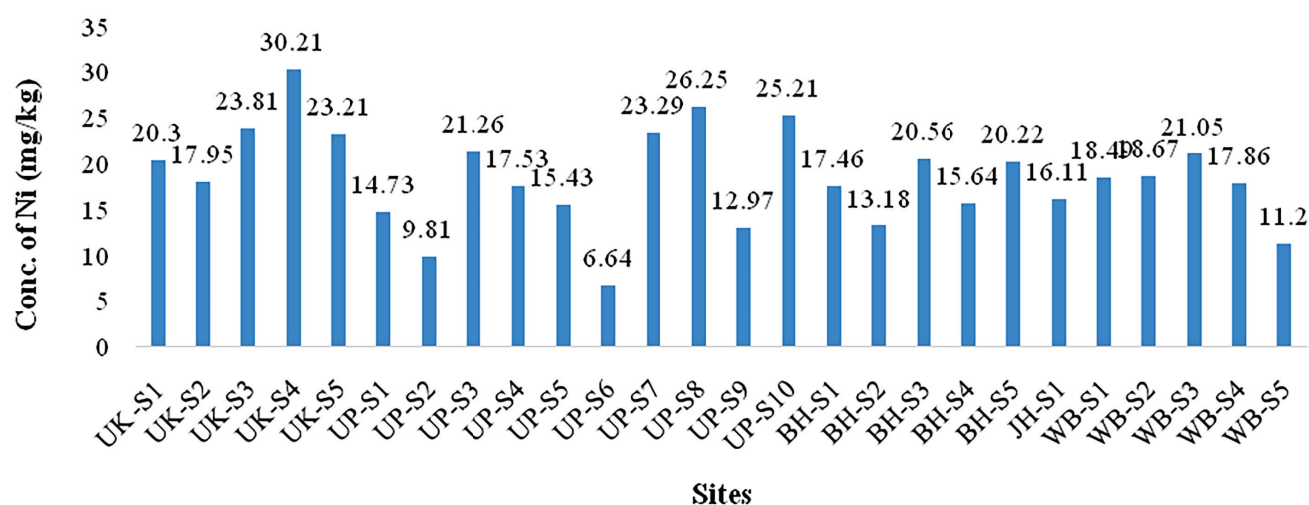


Fig. 10b Concentration of nickel at different sites

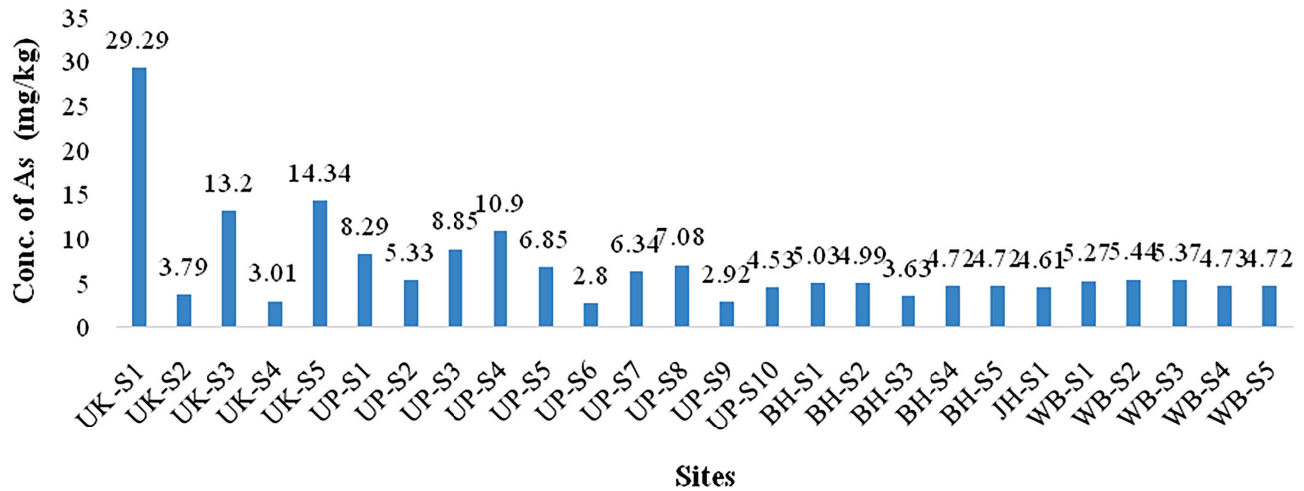


Fig. 10c Concentration of arsenic at different sites

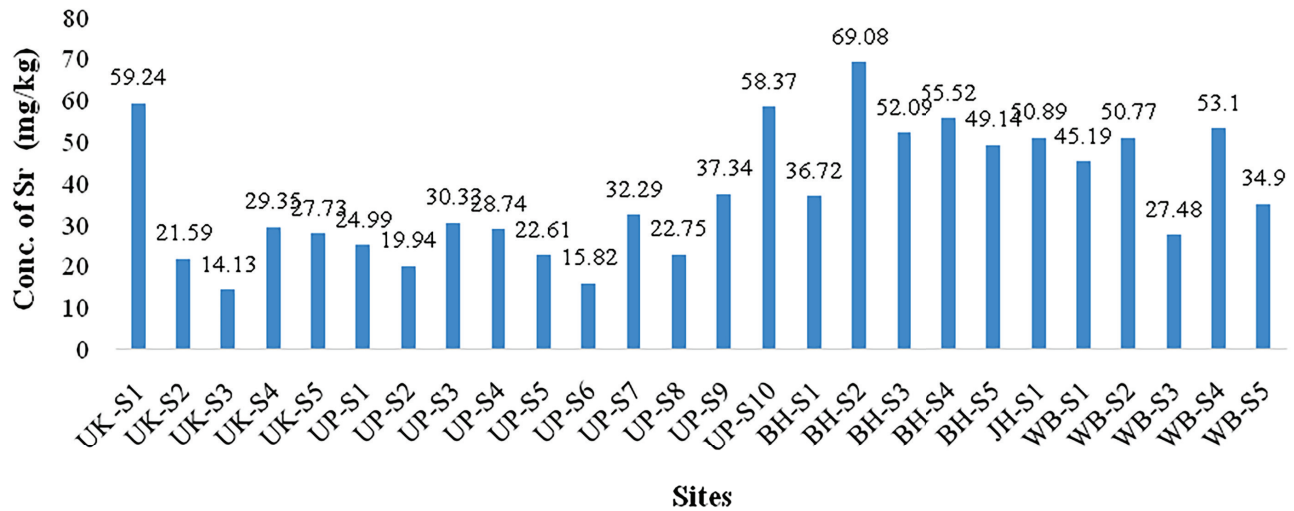


Fig. 10d Concentration of strontium at different sites

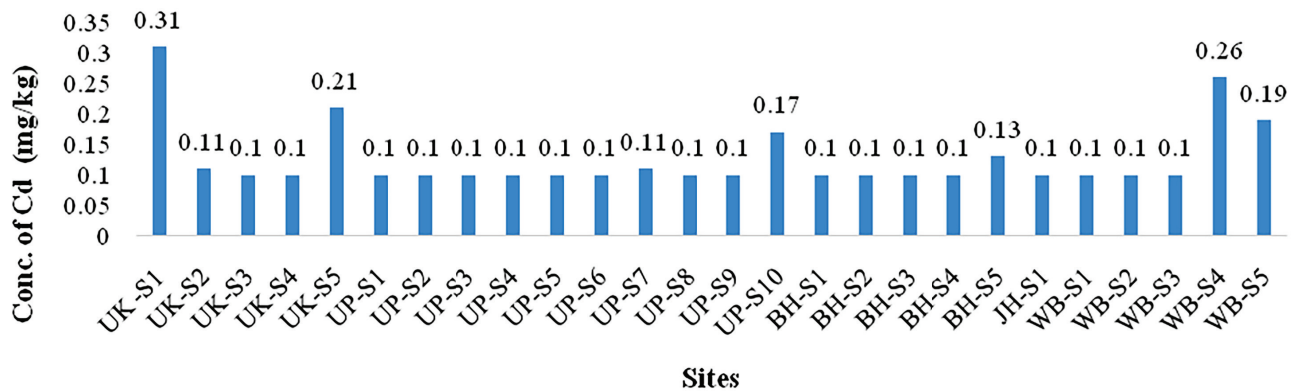


Fig. 10e Concentration of cadmium at different sites

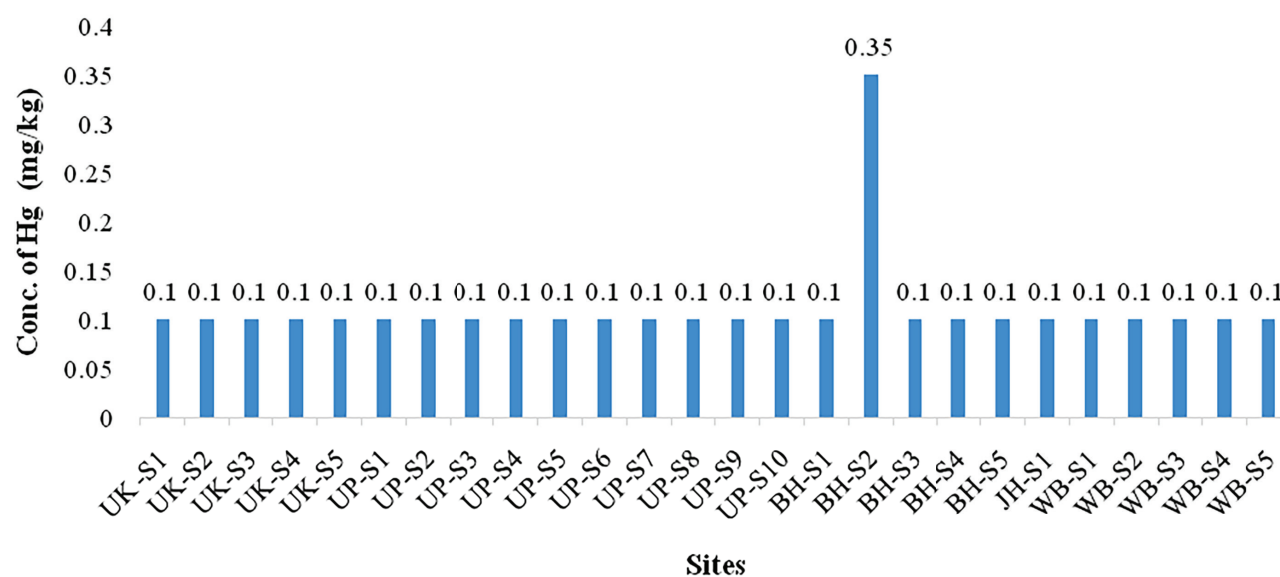


Fig. 10f Concentration of mercury at different sites

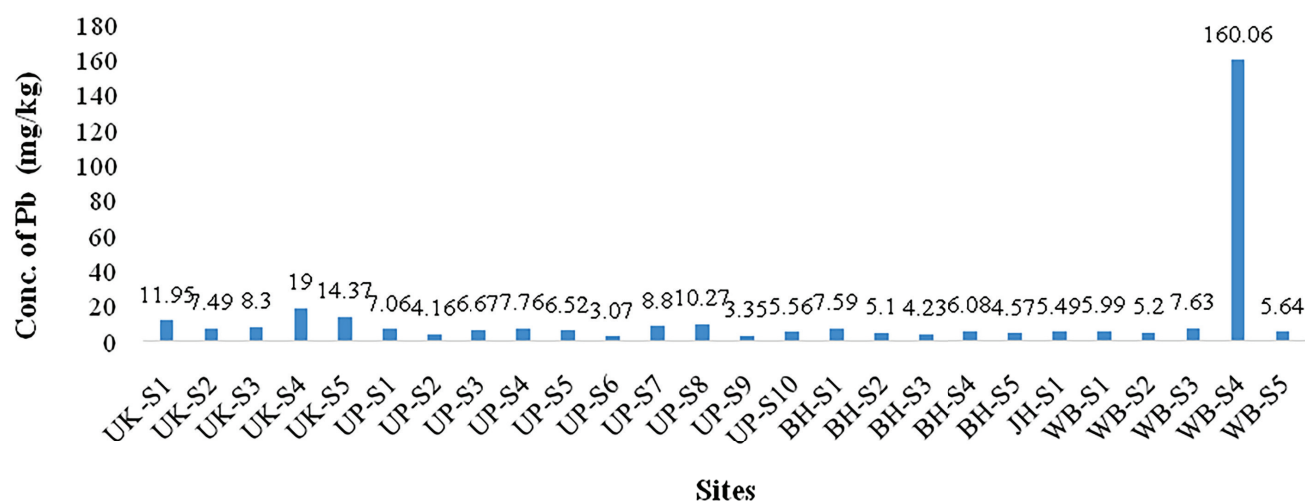


Fig. 10g Concentration of lead at different sites

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.

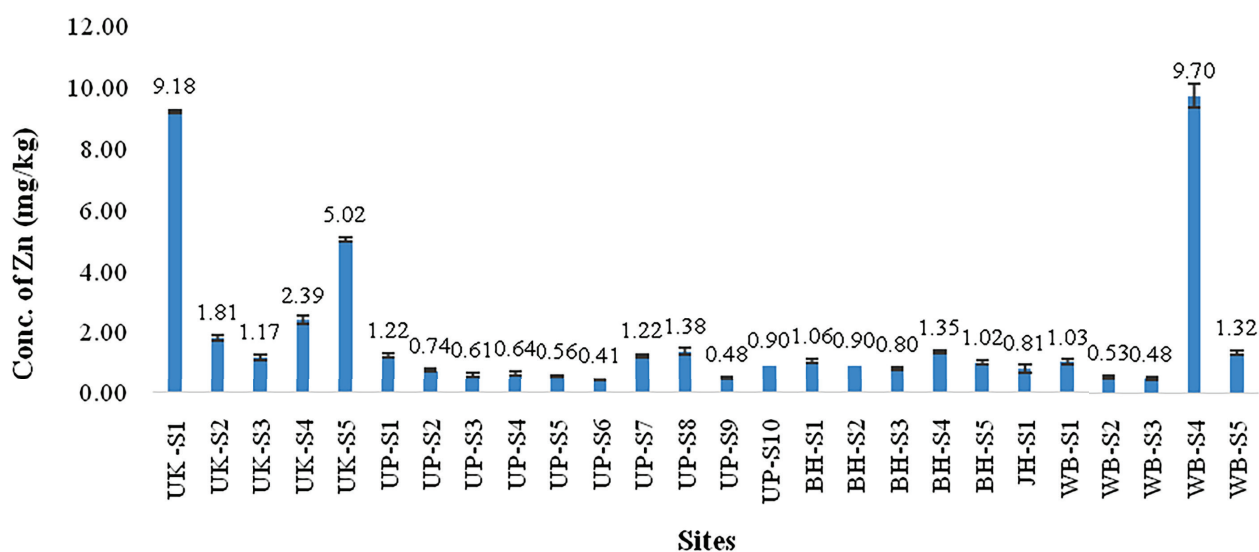


Fig. 11a Concentration of zinc at different sites

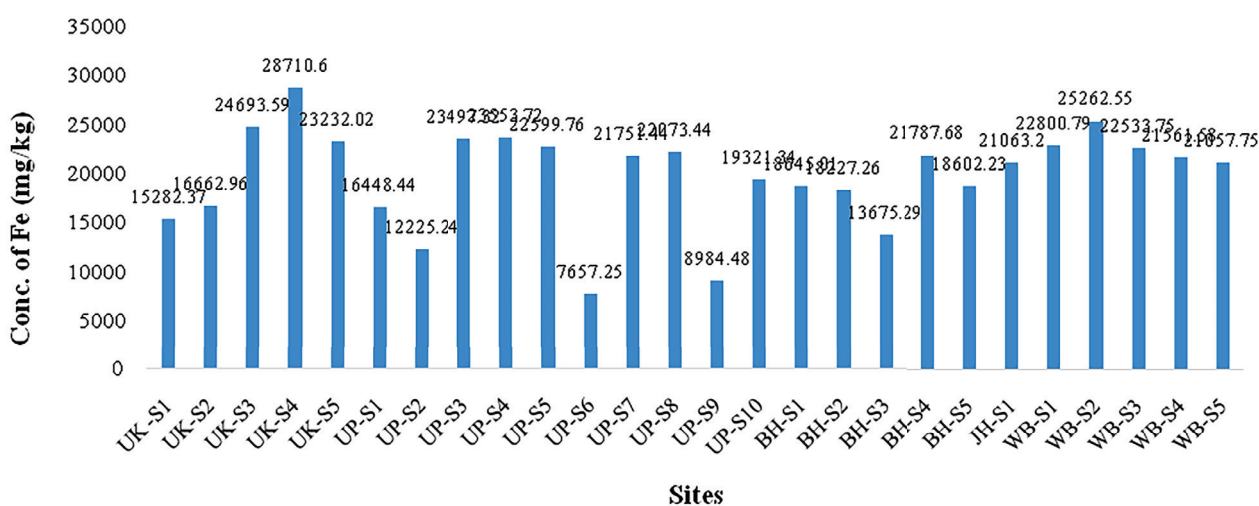


Fig. 11b Concentration of iron at different sites

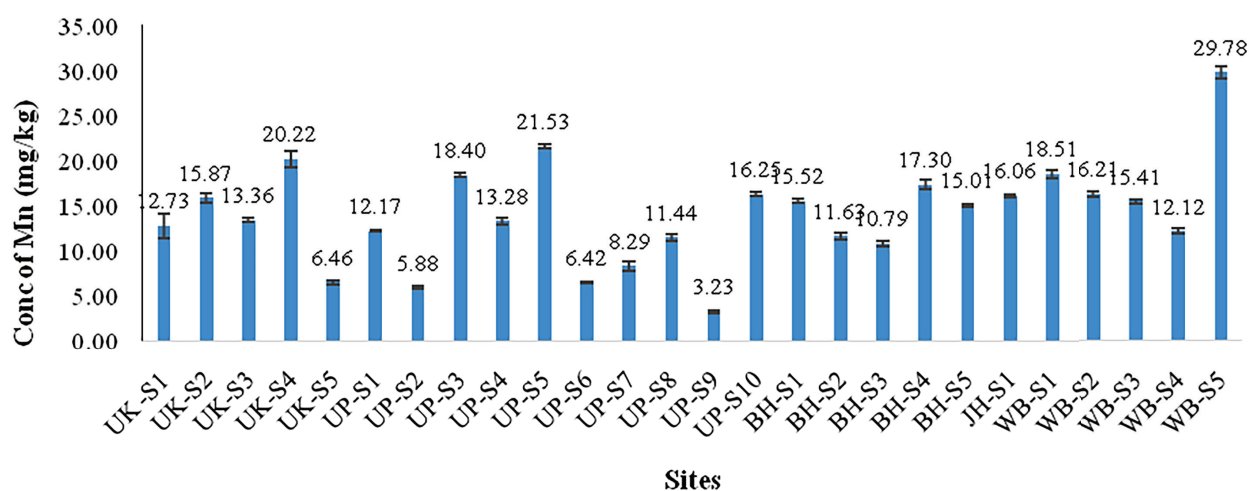


Fig. 11c Concentration of manganese at different sites

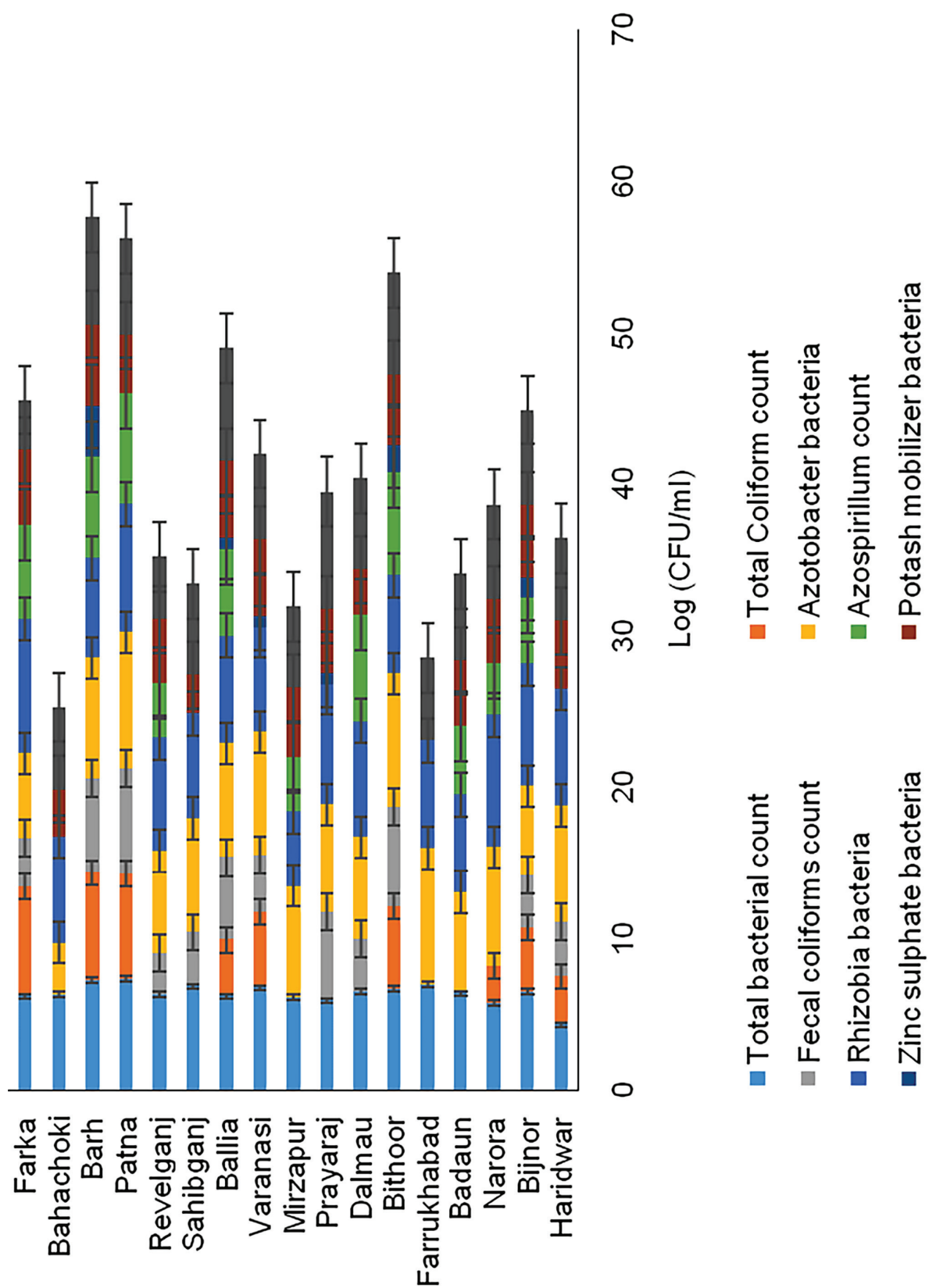


Fig. 12 Total and differential bacterial count

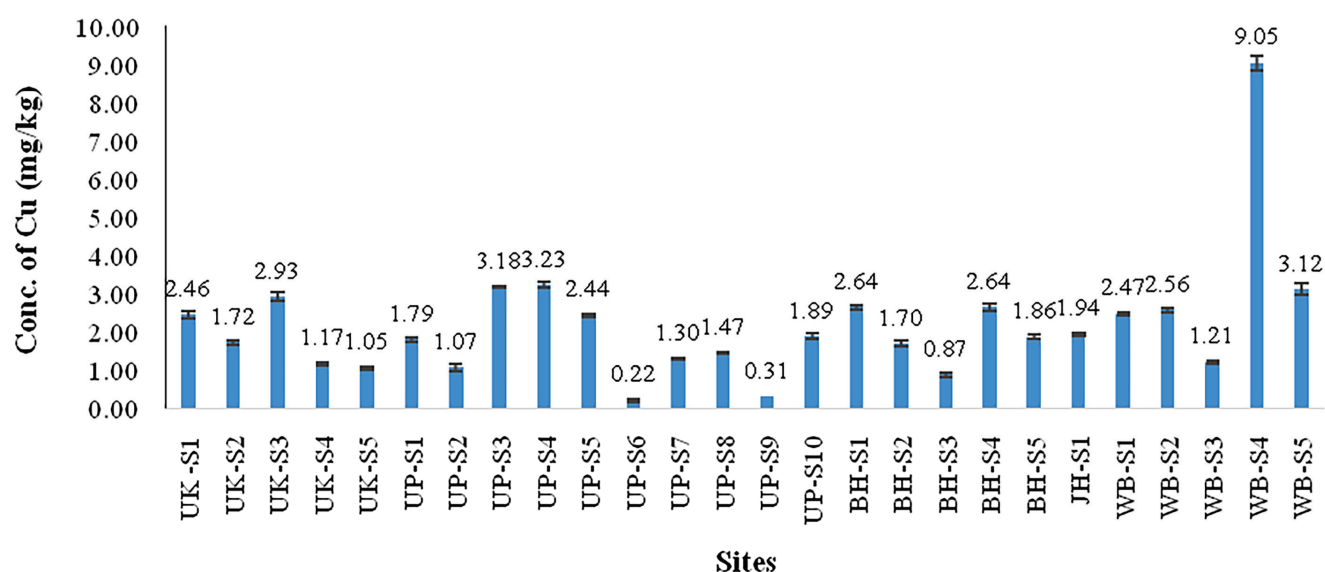


Fig. 11d Concentration of copper at different sites

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

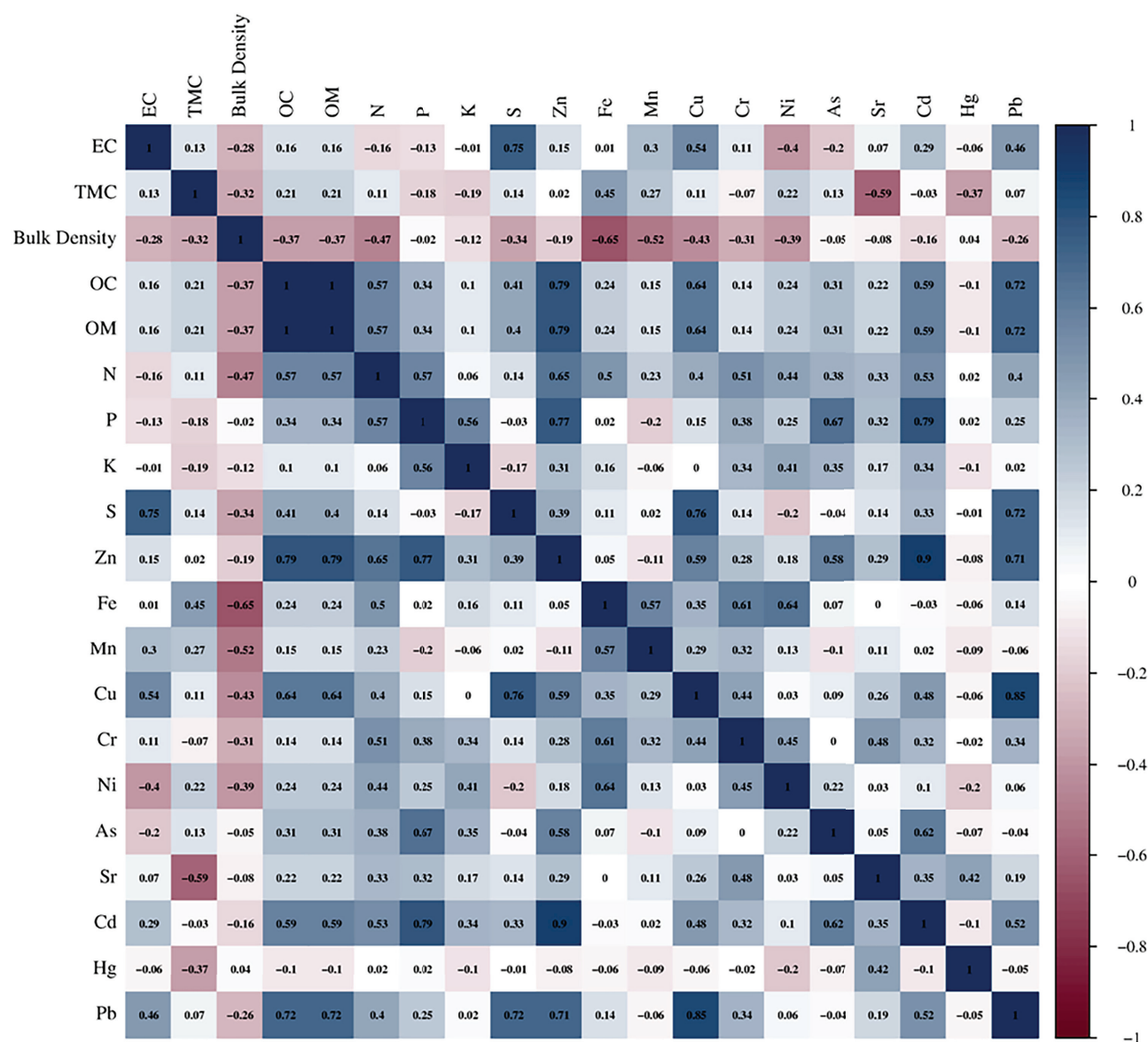
Rhizosphere microbial testing involves analysing the microbial communities in the soil, rhizosphere, and roots of plants. The rhizosphere microbial test is performed in different media to calculate the bacterial count for the *Croton bonplandianus* Baill. in different locations shown in Fig. 12

The total rhizosphere microbial test is performed for the identification of total bacterial count which is found highest in Patna (7.3329 cfu/ml) and lowest in Haridwar

(4.2965 cfu/ml). Whereas the total faecal and coliform count is highest in Patna (6.9177 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 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
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

Sites	pH	EC	OC	N	P	K	S	Zn	Fe	Mn	Cu	Cr	Ni	As	Sr	Cd	Hg	Pb
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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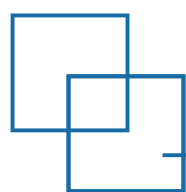
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CHAPTER
04

Traditional and Ethnomedicinal Applications



INTRODUCTION

Plants have long been the main source of food and medicine for humans (Shakya, 2016). Approximately 80% of the global population, or four billion people, use herbal medicine as a part of their healthcare regimen today. Herbs native to various cultures and regions are used to treat common ailments. They have had some degree of success, and over many centuries, some herbal remedies have shown to be far more beneficial than allopathic medications. In this natural healing method, also known as alternative therapy, medicinal plants are used as extracts, decoctions, or powders to treat human and, in certain situations, animal ailments and diseases (Sharma et al., 2020). Some patients have turned to traditional healing practices that use herbal medicines to provide relief after some allopathic drugs failed to provide the desired results or had side effects (Sharma et al., 2018). Herbal and natural products are used in traditional folk medicine to treat a variety of ailments. According to WHO reports, between 70 and 80 percent of the world's population uses herbs as complementary medicine (Divya et al., 2011). In India, over 7500 plant species are being used in traditional medicines (Singh et al., 2011). Apart from food, plant-based products are also being used as complementary medicine. Evaluation of a variety of plant species is still necessary to determine the therapeutic potential of the active ingredients (Danish et al., 2020). Herbal remedies are becoming more and more popular as complementary therapies for a range of illnesses (Grundmann et al., 2014).

It is known that the genus *Croton*, which belongs to the family Euphorbiaceae, comprises about 1300 plant species that are found worldwide in tropical and subtropical regions. Many species of *Croton* have medicinally useful metabolites (Yasin et al., 2022). Heart problems, liver issues, cancer, diabetes, arthritis, skin infections, and antipyretic, anti-inflammatory chest ailments are all treated with this plant (Islam et al., 2019). *Croton bonplandianus* Baill., one of the major species of the genus *Croton*, is native to South Asia, Brazil, Bolivia, Argentina, and Paraguay. It is a member of the Euphorbiaceae family. Growing to a height of 1-2 ft, *C. bonplandianus* is a small annual herb. Growing in waste areas, it is an exotic wild weed (Grant et al., 2000; Govaerts, 2000). *C. bonplandianus* has a high phytochemical



content of secondary metabolites, primarily consisting of terpenoids and alkaloids (Dutta and Choudhuri, 2018). Many plant parts are used to treat different conditions; among their characteristics are antioxidants (Vijayamuthuramalingam et al., 2017), anti-cancer, anti-hypertensive, anti-microbial, antiseptic, and nematocide (Xu et al., 2018). Further, terpenes and phorbol esters, such as 12-orthotrideconeol-13-acetate (TPA) and myristoyl phorbol acetate (MPA), are present in *C. bonplandianus* seed (Mathur and Kapil, 2002). It also demonstrated broad-spectrum anti-bacterial activity against Gram-negative and Gram-positive bacterial isolates (Ghosh et al., 2018).

Traditional and Ayurvedic Benefits

Today, extensive research on the various plant species that are native to local or tribal populations around the world is carried out to evaluate traditional medicine worldwide. They are consumed and used for their therapeutic properties. Indigenous theory holds that herbal remedies are passed down through generations as experiences and beliefs (World Health Organization, 2000). Indian traditional medical systems have always played an important role in meeting global healthcare needs. India has the distinct advantage of having six acknowledged medical systems in this area. They are Yoga, Naturopathy, Homoeopathy, Siddha, Unani, and Ayurveda (Ravishankar and Shukla, 2007). A significant portion of the healers in the folklore stream are not classified under any category, apart from these systems. Folk medicine is the source of the majority of India's traditional medical practices, including Ayurveda. But Ayurveda stands out from other systems because it has a well-defined conceptual framework that has endured over time. It

was probably very advanced conceptually and much ahead of its time. It was one of the first medical systems to support a comprehensive strategy for dealing with health and illness issues (Ramachandran, 2019). The therapeutic applications of various medicinal plants were mentioned in the foundational texts of Ayurveda medicine, 'Susruta Samhita' and 'Charaka Samhita'. *C. bonplandianus* Baill is one such plant that is mentioned in Ayurveda (Dutta and Choudhuri, 2018). *C. bonplandianus* is used to treat skin conditions such as ringworm infection, inflammation of the body, and respiratory issues because of its antiseptic qualities. Cholagogue and purgative are the best properties of its bark and roots (Das et al., 2008; Maurya, 2015). Further, its leaves are used to treat cuts and wounds to stop the bleeding, cholera, and venereal sores. The seeds are used in the treatment of internal abscesses, liver disorders, acute constipation, and abdominal dropsy, while the fresh juice is recommended for headaches (Reddy, 1988; Singh, et al., 2015).

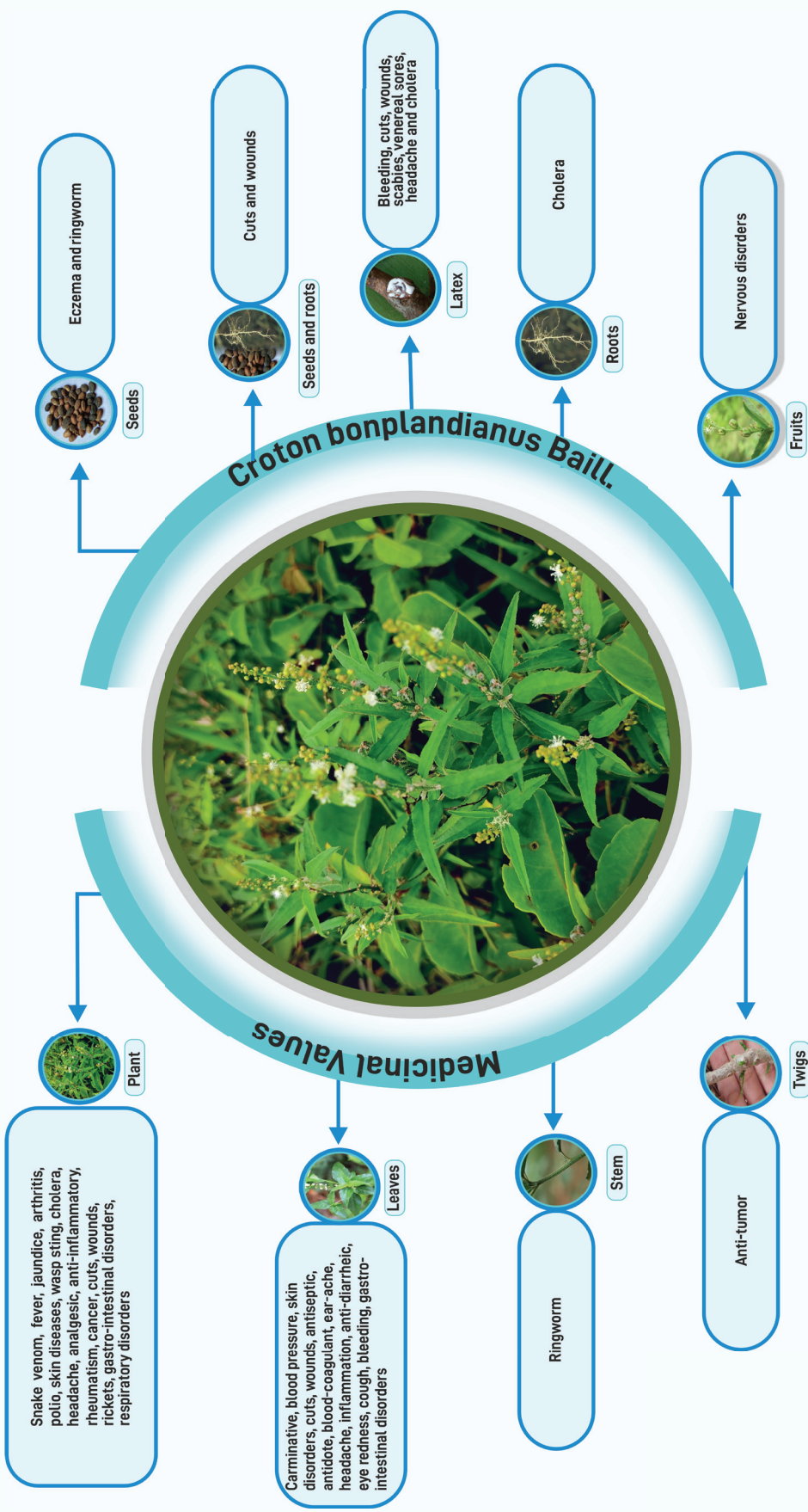


Fig. 1 Medicinal values of *C. bonplandianus* Baill.

[Source: Patil, 2003; Tripathi and srivastava, 2010; Mahmood et al., 2011; Jeeshna et al., 2011; Ghosh et al., 2013; Mathur and Joshi, 2013; Salai et al., 2014; Reddy et al., 2018; Sheikh and Dixit, 2018; Jaya et al., 2019; Subramanian et al., 2019; Sathishkumar and Anbarasu, 2019; Singh et al., 2020; Acharya et al., 2020; Kumar et al., 2021; Ajaib et al., 2021; Alam et al., 2022; Kaur and Ahluwalia, 2022; Das et al., 2022; Ganesan, 2023]

Therapeutic Potential

Plants provide food and fruits that meet human needs. Additionally, plants supply essential ingredients for beverages, cosmetics, and medications. According to the World Health Organization's (WHO) report of 2019, approximately half of the population still believes in plant medicine for basic medical needs. Since the dawn of civilization, medicinal plants have been the foundation of traditional medicine among people who live in rural areas worldwide. Herbs were first used therapeutically in the 3rd millennium BC by the Sumerian and Acadian civilizations. They are still used in traditional Chinese, Egyptian, Ayurvedic, Unani, Siddha, and other medicinal systems to treat a wide range of illnesses. Herbal medicine is now known as complementary and alternative medicine (CAM) in Western nations. Approximately 3.4 billion people, or 88% of the global population, rely primarily on traditional medicines made from plants. The presence of different phytochemicals, including vitamins, terpenoids, phenolic acids, lignin, stilbene, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other secondary metabolites, have been primarily linked to the therapeutic efficacy of

these herbs. Numerous studies have shown these phytochemicals possess antioxidant, anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti-carcinogenic, anti-bacterial, and anti-viral properties (Sala et al., 2002).

Known as 'ban tulsī' in Bengali and 'kukka tulsī' in Telugu, *C. bonplandianus* (Euphorbiaceae) is an exotic weed that is frequently found in wastelands. Its leaves possess immense medicinal value and have demonstrated anti-microbial activity (Vadlapudi, 2010). Other parts of the plant are widely used in traditional medicine for a variety of conditions, including hepatoprotective, swelling of the body, treatment for skin diseases and ringworms, anti-hypertensive, anti-oxidant, wound healing, anti-microbial, anti-diabetic, anti-tumor, anti-cancer, acute constipation, abdominal dropsy, internal abscesses, anti-fertility, anti-spasmodic, antiseptic, antidote, analgesic, repellent property against insects, nematocide, anti-coronary, anti-inflammatory, larvicidal activity, anti-helminth, and so forth. Headache can be treated with the plant's fresh juice depicted in Fig. 1. Furthermore, this plant is in great demand because of its slow rate of conventional multiplication (Ghosh et al., 2018).

Ethnomedicinal and Folk treatments along the Ganga Basin of India

In contemporary times, traditional medicines and natural products derived from ethnomedicinal plants have gained immense importance. Due to their success in treating a wide range of human illnesses, traditional Chinese medicine, Ayurveda, and Unani medicine have gained popularity recently and developed into regulated systems of traditional medicine (Navneet et al., 2020).

In Uttarakhand, *C. bonplandianus* is used by the local people as a treatment for lung cancer (Kumari and Joshi, 2020). The generation-to-generation practice, accumulation, and transmission of traditional medicinal knowledge and management

skills from the area could be crucial for the sustainable utilization and advancement of plant resources. However, many ethnic groups' traditional and folk medical knowledge is in danger of disappearing along with the decline of biodiversity and detrimental effects of mainstream culture. In Uttar Pradesh, the local people have used a decoction of the leaves of this herb for the management of diarrhea (Shukla et al., 2013). Further, in the region of West Bengal, the latex of the plant is used by the tribal people to stop bleeding in new cuts (Manna and Mishra, 2018) whereas in Bihar, the plant sap is applied to cure black rashes/spots on the face (Singh et al., 2020).

Conclusion and Future Perspectives

In conclusion, the overall studies on medicinal and ethnomedicinal features may serve as an important tool for identifying and purify this plant. It is commonly used as a potential source for drugs with anti-inflammatory, anti-cancer, anti-microbial, insectifuge, nematicide, anti-coronary, and wound-healing aid, and possesses hepatoprotective activities.

As a result, more research is required to isolate, identify, characterize, and elucidate the structure of these bioactive compounds responsible for the medicinal properties of *C. bonplandianus*. Further, by using reverse pharmacological approaches in natural drug discovery, a potent and safe drug can be investigated from this plant.

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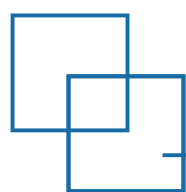
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CHAPTER
05

Phytochemical Analysis



INTRODUCTION

Croton bonplandianus Baill. is a plant native to South America, specifically found in Southern Bolivia, Paraguay, Southwest Brazil, and Northern Argentina. It was accidentally introduced in Chittagong in 1897/8 and has since become naturalized in various other regions, including Kenya, the Mascarene Islands, India, and Southeast Asia (WIKTROP, 2019; POWO, 2024). This plant is a small, scandent herb, and it is an erect, highly branched species, with a woody herb or shrub-like growth habit (India Biodiversity Portal, 2014).

The plant has a rich history of traditional usage in various medicinal systems. Indigenous communities and traditional healers across Asia and South America have employed different parts of this plant for their therapeutic properties. Extracts and formulations derived from this plant have been traditionally used to treat ailments such as inflammation, gastrointestinal disorders, skin conditions, and respiratory issues. The traditional knowledge surrounding the plant's usage reflects its cultural significance and healing potential (Lodhi and Mohanty, 2021).

According to previous studies, the plant is rich in diterpenes, including clerodanes, cembranoid, halimanes, kauranes, labdanes, phorbol esters, trachylobanes, and sarcopetalanes (Salatino et al., 2007). Additionally, it contains bioactive alkaloids, flavonoids, steroid glycosides, and volatile components. These compounds have been found to exhibit a wide range of pharmacological activities, including hepatoprotective, anti-inflammatory, antifungal, wound healing, antimicrobial, antioxidant, and anti-tumor properties (Lodhi and Mohanty, 2021). However, further research is needed to confirm the efficacy and safety of these compounds for medicinal use. The species has also been used for various types of diseases, including acute constipation, abdominal dropsy, dysentery, external wounds, hypercholesterolemia, hypertension, and infectious disorders (Ghosh et al., 2018).



Sampling Sites

Table 1 Sampling sites for the *Croton bonplandianus* plants collected from various state-specific locations spread over the Gangetic course

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

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. 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 1-10g sample was taken and combined with 50 ml of milli-Q water. The mixture was shaken and sonicated for 30 minutes, after which the volume was adjusted to 100 ml and filtered. Subsequently, 10 ml of this filtered solution was taken and mixed with 750 ml of milli-Q water. To this, 25 ml of indigo sulphonc acid was added, and the resulting mixture was shaken. Titration was performed using 0.1 N potassium permanganate solution until the endpoint, characterized by a golden yellow color, was reached. A blank run without the sample was also conducted.

Determination of Saponin Content

A 5 g sample was taken and combined with 50 ml of a 1:1 (methanol:water) solvent. The mixture was refluxed for 1 hour, cooled, and then filtered. This process was repeated three times. The filtrates were combined, concentrated, and evaporated to dryness.

Subsequently, 25 ml of petroleum ether was added, and the mixture was refluxed for 10 minutes. After cooling, the ether layer was decanted. To the remaining residue, 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 taken in a test tube, and 1 ml of Folin–Ciocâlteu reagent was added. The mixture was incubated for 5 minutes. Following this, 1 ml of 10% sodium carbonate solution was added, and the solution was kept in the dark for 1 hour. Absorbance was measured at 760 nm using a UV-visible spectrophotometer. The same steps were repeated for gallic acid to establish linearity.

Determination of Total Flavonoid Content

A 1 ml sample was taken in a test tube, and 0.4 ml of 10% aluminum chloride, 0.4 ml of sodium acetate, and 3 ml of ethanol were added. The mixture was kept at room temperature for 30 minutes. Absorbance was measured at 450 nm using a UV-visible spectrophotometer. The same steps were repeated for quercetin dihydrate to establish linearity.

HPTLC Fingerprinting

The application of high-performance thin-layer chromatography (HPTLC) in the study reflects a prevalent methodology documented in the literature for profiling secondary metabolites. 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 band or spots obtained during the ring test are characteristic of herb. A typical color image of TLC fingerprint provides a clearer guide to the users.

Sample Preparation

Approximately 1 g of sample from each batch was individually dissolved in 10 ml of

methanol. The samples underwent shaking and sonication for 20 minutes. The solution was then centrifuged for 5 minutes at 5000 rpm, resulting in a clear solution that was utilized for analysis. The samples were carefully spotted according to the corresponding serial numbers specified in Table 1.

Methodology and Analytical Conditions

Analysis was performed on CAMAG HPTLC (Muttens, 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 F ₂₅₄ aluminum sheet (1.0554.0007)
Mobile phase	Toluene: Ethyl acetate: Formic acid (5:5:1 v/v/v)
Saturation time	15 minutes
Migration distance	80 mm
Band length	8 mm
Injection volume	10 µl
Visualization	254 nm and 366 nm; under white light before derivatization

High-Performance Liquid Chromatography (HPLC)

Sample and Standard Preparation

Sample: Approximately 500 mg of sample from each batch was individually dissolved in 5 ml of hydro-methanol (20:80). The solutions were sonicated for 30 minutes, followed by centrifugation for 5 minutes at 9000 rpm. Subsequently, the solutions were filtered using a 0.45 µm nylon filter. The resulting filtered solution was employed for analysis.

Standard: Standard magnoflorine and rutin were dissolved in methanol to prepare a solution having a concentration of 50 ppm.

Analytical and Instrumentation Condition

Separation was achieved using a Shodex C18-4E (5 µm, 4.6 ×250 mm) column, subjected to binary gradient elution. The two solvents used for the analysis consisted of water containing 0.1 % acetic acid in water (solvent A) and acetonitrile (solvent B). The flow rate was set at 1.0 ml/min during the analysis. of 20 µl of standard and test solution were injected. The wavelength was set at 257 nm.

Gradient	program		used
Time (Min)	A%	B%	
0	95	05	
5	90	10	
10	88	12	
20	80	20	
40	70	30	
45	95	05	
50	95	05	

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

Test sample: *Croton bonplandianus* (PRF/CHI/0723/0750)

Sample Preparation

A 500 mg of the raw sample was dissolved in 5 ml of methanol:water (80:20) solution and sonicated for 30 min. The solution was centrifuged at 10000 rpm for 5 minutes and filtered through a 0.22 μm nylon filter.

Analytical and Instrumentation Conditions

Analysis was performed on a Xevo G2-XS QToF (Waters Corporation, USA) with Acquity UPLC-I Class and Unifi software. Separation was

carried out using Acquity UPLC HSS-T3 (100 x 2.1 mm, 1.8 μm) column (Waters Corporation, USA) with the flow rate of 0.3 ml/min using gradient elution of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). The column was maintained at 40°C throughout the analysis and sample temperature was kept at 20°C. Detection was carried out by Xevo G2-XS QToF. One microliter of test solution was injected in UPLC/MS-QToF and chromatograms were recorded in positive and negative ionization modes.

Gradient program

Time (min)	Flow (ml/min)	Mobile phase A%	Mobile phase B%
0	0.3	95	5
5	0.3	95	5
15	0.3	85	15
35	0.3	75	25
50	0.3	50	50
65	0.3	20	80
70	0.3	20	80
71	0.3	95	5
75	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

Parameter	Polarity (+ve)	Polarity (-ve)
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 Enkaphalin)	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 phytochemical analysis of *C. bonplandianus* collected from diverse locations within the Gangetic belt revealed significant variations in the concentrations of tannins, saponins, total polyphenols, and total flavonoids. These variations are comprehensively presented in Table 2, reflecting the diverse phytochemical composition influenced by geographical and environmental factors.

For instance, the highest tannin content (0.081% w/w) was observed in samples from Varanasi, Uttar Pradesh, and Farka, Bihar, whereas the lowest (0.019% w/w) was recorded in samples from Farrukhabad, Uttar Pradesh. This high tannin content could be linked to the plant's traditional uses in treating

diarrhea and dysentery due to tannins' known astringent properties. Similarly, the saponin content showed notable variation, with the highest value (11.544% w/w) from Narora, Uttar Pradesh, and the lowest (2.942% w/w) from Varanasi, Uttar Pradesh. The highest saponin content found in the sample from Narora, aligns with the known hemolytic and antimicrobial activities of saponins, making the samples particularly interesting for further pharmacological studies. Further, total polyphenol content was relatively consistent across the samples, ranging from 0.004% to 0.009% w/w, with the highest concentrations again in Narora, Uttar Pradesh, and in Gaumukh, Uttarakhand (Ghosh et al., 2018).

Polyphenols are potent antioxidants, suggesting that the sample could have

significant free-radical scavenging capabilities, supporting its use in traditional medicine for managing oxidative stress-related conditions. Total flavonoid content varied from 0.001% to 0.003% w/w, with the highest levels observed in samples from Narora and Bahachoki, Bihar. Flavonoids are known for their anti-inflammatory and anti-cancer properties, indicating that these samples might be of high interest for further

investigation into their therapeutic potential.

The identified bioactive compounds corroborate the plant's traditional uses and suggest promising avenues for future research and development of herbal medicines. Further studies are warranted to explore the ecological and genetic factors driving these phytochemical variations and their implications for the bioactivity and therapeutic potential of *C. bonplandianus*.

Table 2 Phytochemical analysis of *Croton bonplandianus* collected from different locations

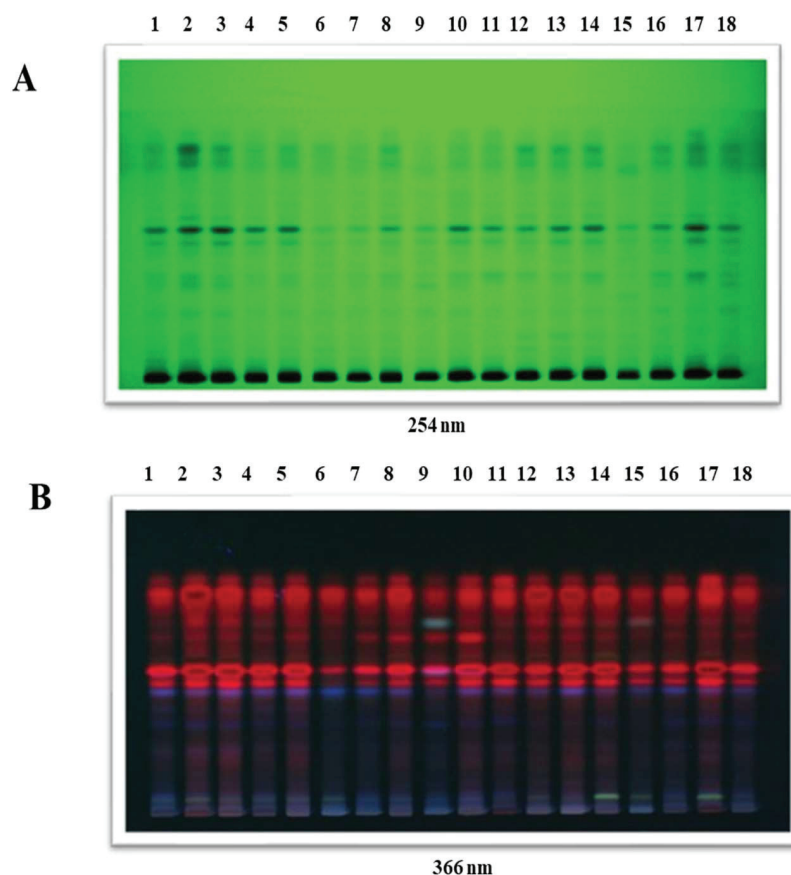
S. No.	Sample code	Tannin content (%w/w)	Saponin content (%w/w)	Total polyphenol content (%w/w)	Total flavonoid content (%w/w)
1.	Bijnor, Uttar Pradesh	0.040	3.360	0.007	0.001
2.	Narora, Uttar Pradesh	0.060	11.544	0.009	0.003
3.	Badaun, Uttar Pradesh	0.020	6.192	0.006	0.003
4.	Farrukhabad, Uttar Pradesh	0.019	8.782	0.008	0.001
5.	Bithoor, Uttar Pradesh	0.038	10.750	0.008	0.001
6.	Dalmau, Uttar Pradesh	0.060	8.518	0.007	0.001
7.	Prayagraj, Uttar Pradesh	0.040	6.624	0.008	0.001
8.	Mirzapur, Uttar Pradesh	0.060	6.524	0.007	0.001
9.	Varanasi, Uttar Pradesh	0.081	2.942	0.008	0.002
10.	Ballia, Uttar Pradesh	0.039	10.831	0.007	0.001
11.	Revelganj, Bihar	0.050	7.886	0.006	0.001
12.	Patna, Bihar	0.080	10.369	0.008	0.001
13.	Barh, Bihar	0.060	9.833	0.006	0.002

S. No.	Sample code	Tannin content (%w/w)	Saponin content (%w/w)	Total polyphenol content (%w/w)	Total flavonoid content (%w/w)
14.	Bahachoki, Bihar	0.040	10.008	0.008	0.003
15.	Farka, Bihar	0.081	7.909	0.007	0.002
16.	Sahibganj, Jharkhand	0.040	9.950	0.004	0.001
17.	Gaumukh, Uttarakhnad	0.050	10.781	0.009	0.002
18.	Farraka, West Bengal	0.070	6.826	0.009	0.002

HPTLC Fingerprint Analysis

The HPTLC analysis at 254 nm, 366 nm, and under white light before derivatization revealed distinct patterns in the chemical composition of the samples. Multiple high-intensity bands were evident across all samples, indicating

the presence of various phytochemicals. Notably, samples from Dalmau, Prayagraj, Varanasi (Uttar Pradesh), and Farka (Bihar) exhibited bands of relatively lower intensity, suggesting lower concentrations of certain phytochemicals in these regions (Fig.1).



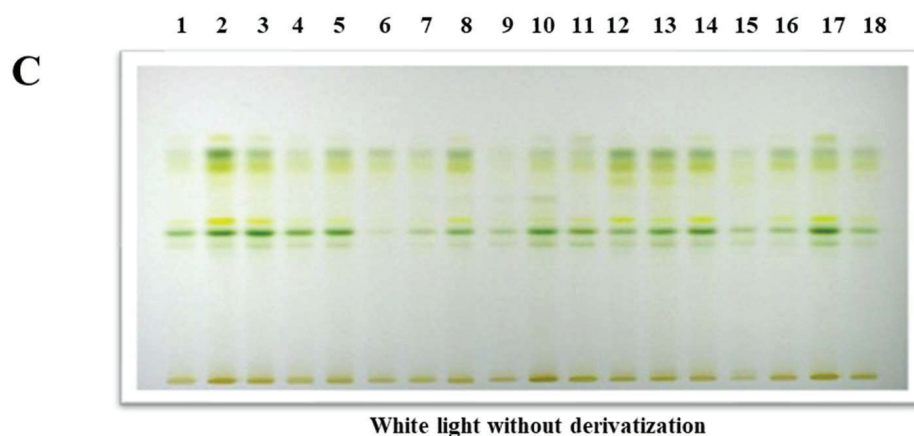


Fig. 1 HPTLC fingerprinting of *Croton bonplandianus* at (a) 254 nm, (b) 366 nm, and (c) White light without derivatization

The HPTLC analysis provided a visual and qualitative assessment of the phytochemical diversity within the species across different geographic locations. The distinct bands observed at various wavelengths highlight the presence of multiple secondary metabolites, which can be linked to the plant's medicinal properties.

- ◆ **High-Intensity Bands:** The presence of multiple high-intensity bands across all samples indicates a rich phytochemical profile. These bands correspond to various bioactive compounds, which may contribute to the therapeutic potential of *C. bonplandianus*.
- ◆ **Regional Variation:** The variation in band intensity among samples from different locations suggests that environmental factors such as soil type, climate, and cultivation practices may influence the phytochemical composition. Specifically, samples from Uttar Pradesh and Bihar exhibited lower intensity bands, indicating regional differences in phytochemical concentrations.

- ◆ **Utility in Standardization:** The HPTLC fingerprints can serve as a reference for the standardization and quality control of *C. bonplandianus*-based herbal products. The characteristic bands can help in identifying and ensuring the consistency of bioactive compounds in commercial formulations.

HPLC Analysis

HPLC analysis using magnoflorine and rutin as reference compounds revealed significant differences in the phytochemical content across samples from different locations. The chromatograms obtained at 257 nm indicated the presence of these reference compounds in varying percentages, as detailed in Table 3 and Fig. 2 and Fig. 3. For instance, samples from Patna, Bihar, exhibited a higher content of magnoflorine, whereas rutin was more prominent in samples from Prayagraj, Uttar Pradesh.

These findings emphasize the role of HPLC in providing precise quantitative data

on specific phytochemicals, contributing to a deeper understanding of the phytochemical landscape of *C. bonplandianus*. The variation in compound concentration across

different samples can be attributed to the diverse environmental conditions and soil compositions of the collection sites.

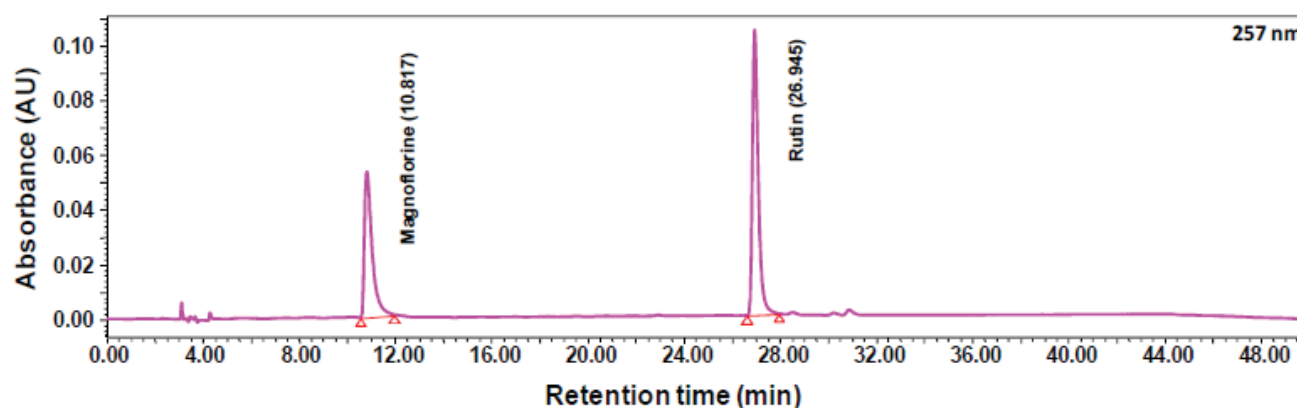


Fig. 2 HPLC chromatogram of standard Magnoflorine and Rutin at 257 nm

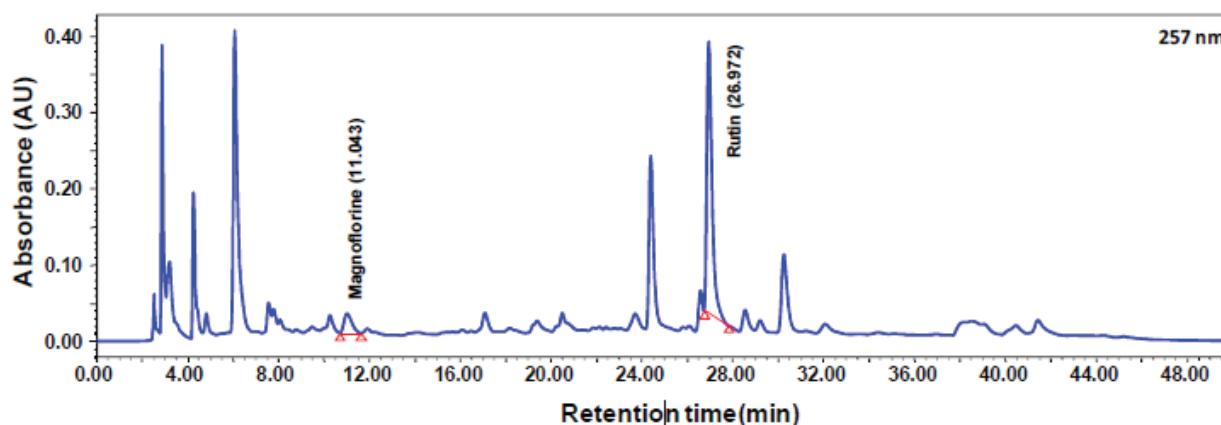


Fig. 3 HPLC chromatogram of *Croton bonplandianus* (PRF/CHI/1123/1101) at 257 nm

Table 3 Phytochemicals content present in *Croton bonplandianus* collected from different locations

S. No	Internal code	External code	Magnoflorine (% w/w)	Rutin (% w/w)
1	PRF/CHI/1123/1099	Bijnor, Uttar Pradesh	0.019	0.285
2	PRF/CHI/1123/1100	Narora, Uttar Pradesh	0.008	0.496
3	PRF/CHI/1123/1101	Badaun, Uttar Pradesh	0.025	0.162
4	PRF/CHI/1123/1102	Farrukhabad, Uttar Pradesh	0.005	0.264
5	PRF/CHI/1123/1103	Bithoor, Uttar Pradesh	0.013	0.061
6	PRF/CHI/1123/1104	Dalmou, Uttar Pradesh	0.011	0.024
7	PRF/CHI/1123/1105	Prayagraj, Uttar Pradesh	0.046	0.137
8	PRF/CHI/1123/1106	Mirzapur, Uttar Pradesh	0.010	0.079
9	PRF/CHI/1123/1107	Varanasi, Uttar Pradesh	0.004	0.022
10	PRF/CHI/1123/1108	Ballia, Uttar Pradesh	0.048	0.063
11	PRF/CHI/1123/1109	Revelganj, Bihar	0.015	0.479
12	PRF/CHI/1123/1110	Patna, Bihar	0.016	0.042
13	PRF/CHI/1123/1111	Barh, Bihar	0.015	0.007
14	PRF/CHI/1123/1112	Bahachoki, Bihar	0.011	0.130
15	PRF/CHI/1123/1113	Farka, Bihar	0.014	0.035
16	PRF/CHI/1123/1114	Sahibganj, Jharkhand	0.038	0.175
17	PRF/CHI/1123/1115	Gaumukh, Uttarakhand	0.013	0.337
18	PRF/CHI/1123/1116	Farraka, West Bengal	0.042	0.152

UPLC/MS-QToF Analysis

The UPLC/MS-QToF analysis provided a detailed qualitative and quantitative profiling of the compounds present in *C. bonplandianus*. This technique enabled high-resolution separation and sensitive detection of various phytochemicals, identifying a total of 25 compounds. The TIC chromatograms in both positive and negative ionization modes facilitated the precise identification of these compounds, as shown in Table 4 and Fig. 4.

Key identified compounds include crotsparsidine, coclaurine, jacularine, and

magnoflorine, among others. For example, crotsparsidine($C_{17}H_{17}NO_3$) was observed with an m/z of 284.1264 and a retention time (RT) of 5.59 minutes, indicating its prominent presence in the sample. Similarly, coclaurine($C_{17}H_{19}NO_3$) had an m/z of 286.1422 and an RT of 8.85 minutes. The high sensitivity and resolution of the UPLC/MS-QToF analysis are evident from the accurate mass errors, which were within acceptable limits, further validating the robustness of the method employed.

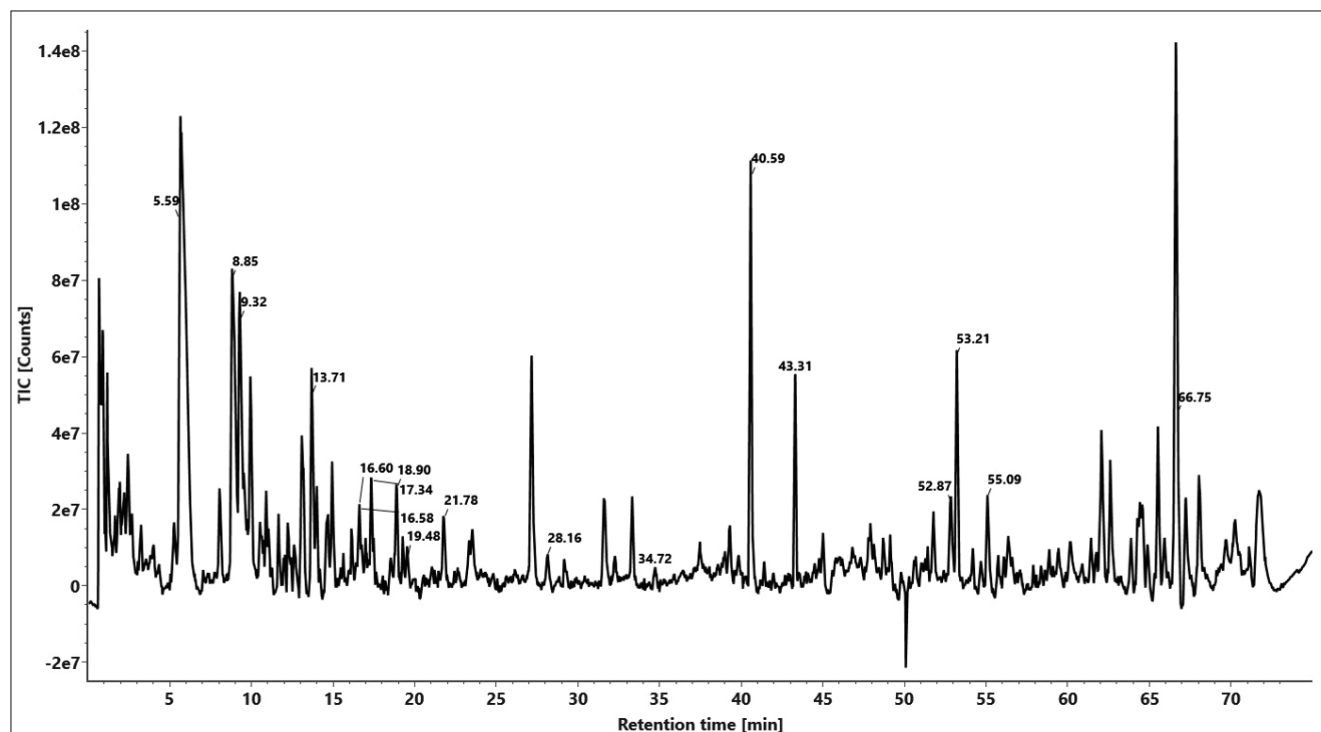


Fig. 4 TIC chromatogram of *Croton bonplandianus* (PRF/CHI/0723/0750) in positive ionization mode

Table 4 Compound-dependent parameters of analytes/identified compounds in *Croton bonplandianus* (PRF/CHI/0723/0750) in positive mode

S. No.	Component Name	Formula	Neutral mass(Da)	Observed m/z	Masserror (mDa)	RT (min)	Response	Adducts
1	Crotsparsidine	C ₁₇ H ₁₇ NO ₃	283.1208	284.1264	-1.7	5.59	2517987	+H
2	Coclaurine	C ₁₇ H ₁₉ NO ₃	285.1365	286.1422	-1.6	8.85	8085984	+H
3	Jacularine	C ₁₇ H ₁₉ NO ₃	285.1365	286.1426	-1.2	9.32	7015752	+H
4	Magnoflorine	C ₂₀ H ₂₃ NO ₄	341.1627	342.1686	-1.4	13.71	4172347	+H
5	p-Coumaric acid	C ₉ H ₈ O ₃	164.0473	165.0545	-0.1	16.58	51625	+H
6	N-Methylcoclaurine	C ₁₈ H ₂₁ NO ₃	299.1521	300.1582	-1.3	16.60	2314084	+H
7	Quercetin3-Glucosyl-(1-4)-Xylosyl-(1-4)-Rhamnoside	C ₃₂ H ₃₈ O ₂₀	742.1956	743.2059	3.0	17.34	1758218	+H
8	Rutin	C ₂₇ H ₃₀ O ₁₆	610.1534	611.1618	1.2	18.90	2075939	+H
9	Isovitexin4'-O-glucoside2''-O-arabinoside	C ₃₂ H ₃₈ O ₁₉	726.2007	727.2104	2.4	19.29	850885	+H
10	Isoquercetin	C ₂₁ H ₂₀ O ₁₂	464.0955	465.1016	-1.1	19.48	96986	+H
11	Kaempferol-3-rutinoside-7-glucoside	C ₃₃ H ₄₀ O ₂₀	756.2113	757.2206	2.1	20.52	118713	+H
12	Nicotiflorin	C ₂₇ H ₃₀ O ₁₅	594.1585	595.1668	1.0	21.78	1451257	+H
13	N-feruloyltyramine	C ₁₈ H ₁₉ NO ₄	313.1314	314.1376	-1.1	28.16	2389855	+H
14	Tribulose	C ₃₀ H ₂₆ O ₁₃	594.1373	595.1453	0.6	34.72	330970	+H
15	Cerberoside	C ₄₂ H ₆₆ O ₁₈	858.4249	859.4364	4.2	40.59	5856622	+H
16	6-O-β-D-Glucopyranosyl-1-O-[[2α,3β,5ξ,6β]-2,3,6,23-tetrahydroxy-28-oxours-12-en-28-yl]-β-D-glucopyranose	C ₄₂ H ₆₈ O ₁₆	828.4507	829.4621	4.1	43.31	1851239	+H
17	Colneleic acid	C ₁₈ H ₃₀ O ₃	294.2195	317.2073	-1.5	52.87	20277	+Na
18	17-Hydroxylinolenic acid	C ₁₈ H ₃₀ O ₃	294.2195	317.2069	-1.8	53.21	72990	+Na
19	18-Hydroxylinoleic acid	C ₁₈ H ₃₂ O ₃	296.2351	319.2231	-1.2	55.09	18996	+Na
20	Pheophorbide A	C ₃₅ H ₃₆ N ₄ O ₅	592.2686	593.2789	3.1	66.75	1831780	+H

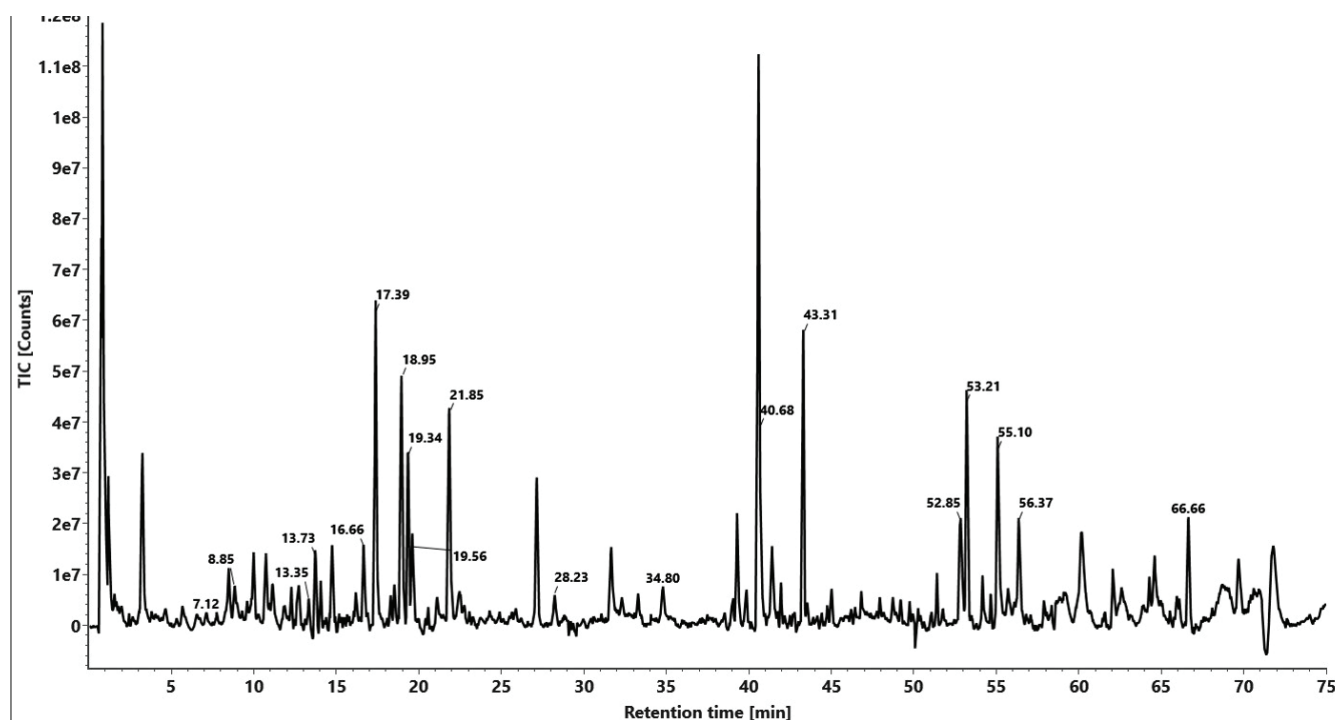


Fig. 5 TIC chromatogram of *Croton bonplandianus* (PRF/CHI/0723/0750) in negative ionization mode

Table 5 Compound-dependent parameters of analytes/identified compounds in *Croton bonplandianus* (PRF/CHI/0723/0750) in negative mode

S. No.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
1	Protocatechui-cacid	C ₇ H ₆ O ₄	154.0266	153.0177	-1.6	7.12	294559	-H
2	3-O-trans-Coumaroylqui-nicacid	C ₁₆ H ₁₈ O ₈	338.1002	337.0914	-1.5	8.85	794651	-H
3	4-p-Couma-roylquinicacid	C ₁₆ H ₁₈ O ₈	338.1002	337.0914	-1.5	11.82	246693	-H
4	5-p-couma-roylquinicacid	C ₁₆ H ₁₈ O ₈	338.1002	337.0913	-1.6	13.35	815350	-H
5	Magnoflorine	C ₂₀ H ₂₃ NO ₄	341.1627	340.1543	-1.1	13.73	1089551	-H
6	p-Coumaricacid	C ₉ H ₈ O ₃	164.0473	163.0385	-1.5	16.66	1500944	-H
7	Querce-tin-3-Gluco-syl-(1-4)-Xylo-syl-(1-4)-Rham-noside	C ₃₂ H ₃₈ O ₂₀	742.1956	741.1922	3.8	17.39	4967170	-H

S. No.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
8	Rutin	C ₂₇ H ₃₀ O ₁₆	610.1534	609.1474	1.3	18.95	6657021	-H
9	Isovitexin4'-O-glucoside2''-O-arabinoside	C ₃₂ H ₃₈ O ₁₉	726.2007	725.1971	3.6	19.34	2974453	-H
10	Isoquercetin	C ₂₁ H ₂₀ O ₁₂	464.0955	463.0876	-0.6	19.56	623559	-H
11	Kaempferol-3-rutinoside-7-glucoside	C ₃₃ H ₄₀ O ₂₀	756.2113	755.2071	3.1	20.57	501654	-H
12	Nicotiflorin	C ₂₇ H ₃₀ O ₁₅	594.1585	593.1523	1.1	21.85	5860830	-H
13	N-Feruloyltyramine	C ₁₈ H ₁₉ NO ₄	313.1314	312.1226	-1.5	28.23	855820	-H
14	Tribuloside	C ₃₀ H ₂₆ O ₁₃	594.1373	593.1315	1.4	34.80	1031907	-H
15	Cerberoside	C ₄₂ H ₆₆ O ₁₈	858.4249	857.4228	5.2	40.68	3279190	-H
16	6-O-β-D-Glucopyranosyl-1-O-[(2α,3β,5ξ,6β)-2,3,6,23-tetrahydroxy-28-oxours-12-en-28-yl]-β-D-glucopyranose	C ₄₂ H ₆₈ O ₁₆	828.4507	827.448	4.5	43.31	3204640	-H
17	Colneleic acid	C ₁₈ H ₃₀ O ₃	294.2195	293.2109	-1.4	52.85	2297811	-H
18	17-Hydroxylinolenic acid	C ₁₈ H ₃₀ O ₃	294.2195	293.2109	-1.4	53.21	3948338	-H
19	18-Hydroxylinoleic acid	C ₁₈ H ₃₂ O ₃	296.2351	295.2265	-1.4	55.1	2918650	-H
20	3-Oxo-octadecanoic acid	C ₁₈ H ₃₄ O ₃	298.2508	297.2418	-1.7	56.37	1576444	-H
21	Pheophorbide A	C ₃₅ H ₃₆ N ₄ O ₅	592.2686	591.2629	1.6	66.66	1575237	-H

Conclusion

Systematic experiments were conducted to elucidate the phytochemical composition of *Croton bonplandianus* across 18 diverse locales within the Gangetic belt. Tannin, total polyphenols, and flavonoid content analyses yielded consistent results across all samples, indicating a uniform botanical profile. However, the saponin content exhibited a substantial range, spanning from 3% to 11% (w/w). Notably, Bijnor and Varanasi (U.P) samples presented the lowest saponin content, underscoring potential intraspecific variability. The HPTLC study provided further insights, particularly showcasing a lower band intensity in the Varanasi samples. This variance in chromatographic patterns may signify inherent differences in the phytochemical composition of *Croton bonplandianus* at this geographical location.

To determine the compound-specific quantification, HPLC analysis focused on magnoflorine and rutin. Ballia (U.P) and Farraka (W.B) samples exhibited the highest magnoflorine content, demonstrating regional disparities in compound abundance.

Conversely, Narora (U.P) and Revelganj (Bihar) emerged as repositories of rutin, contributing to the nuanced understanding of the chemical makeup of this species. Lastly, the study also employed advanced hyphenated techniques, UPLC/MS-QToF, for the identification and quantification of a total of 25 compounds. This advanced methodology allowed for a highly detailed and accurate characterization of the chemical constituents present in the species.

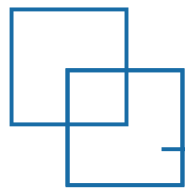
In conclusion, the phytochemical diversity observed in *C. bonplandianus* across different geographical locations within the Gangetic belt highlights the species' potential for various pharmacological applications. The identified bioactive compounds corroborate the plant's traditional uses and suggest promising avenues for future research and development of herbal medicines. Further studies are warranted to explore the ecological and genetic factors driving these phytochemical variations and their implications for the bioactivity and therapeutic potential of *C. bonplandianus*.

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CHAPTER
06

Insilico Analysis Against Japanese Encephalitis



INTRODUCTION

Japanese encephalitis (JE) is one of the most prevalent vector borne zoonotic encephalitic diseases in the world (Boyer et al., 2021; Turtle and Solomon, 2018). The data shared by WHO, 2023 reflects that approximately 3 billion people are at the verge of JE infection, with annual incidences of almost 20,000 clinical cases and 6,000 deaths. Apart from that, only one-third of the affected individuals manages a complete recovery, whereas the mortality rate spans from 5 to 30% and development of neuropsychiatric sequelae is witnessed in 30 to 50% of survivors. The causative agent behind this human viral encephalitis is the Japanese encephalitis virus (JEV), an enveloped, plus-sense, single stranded RNA virus (Folly et al., 2021). JEV belongs to Flaviviridae family of viruses, which also hosts other members like, Dengue virus, ZIKA Virus, Tick Borne Encephalitis Virus, Yellow Fever Virus, and West Nile Virus (Rastogi and Singh, 2019). The virus has established its territory in an area that encompass almost half the world's population, covering from Eastern and Southern Asia, India, Sri Lanka, Maritime Siberia in the north, to South-East Asia and northern Australia, and Pakistan (Mackenzie et al., 2022). Being a vector-borne disease, it is transmitted by culicine, anopheles, aedes, and armigeres mosquito species, however *Culex* is mostly engaged in the process (Hameed et al., 2021). Generally, the water birds serve as a maintenance host, pigs are the amplifying host; humans and horses are the dead-end host. Also, recent studies suspect domestic birds as secondary reservoir host (Mackenzie et al., 2022; Boyer et al., 2021). Even with the current expansion in the domain of science of technology, there is no current sure-shot method for the treatment of JE (Navyashree et al., 2021). Presently, vaccination is the most favorable method for the prevention of JE, however the ever-increasing incidences of JE demands the rigorous research into establishment of innovative options to combat JEV infection (Ashraf et al., 2021).

At a molecular level, the 11 kb viral genome encodes three structural proteins (Capsid, and NS5) (Guo et al., 2020). The Envelope protein (env) mediates binding with the host cell receptor and promotes the transduction. Consequently, it is a major target for the action of neutralizing antibodies (NAbs) in the host (Kumar et al., 2022). Meanwhile, the capsid



protein (cap) is like an armor for RNA genome, aiding the viral assembly. Interestingly, the structure of capsid is conserved for the entire flaviviridae family, and hence is a preferred target for the development of antivirals (Ishida et al., 2019). Furthermore, the non-structural proteins (NSPs) possess a role in formation of assembly and replication complex (Kumar et al., 2022).

Croton bonplandianus Baill., also famous as three-leaved caper, Ban Tulasi and Kala Bhangra is a small annual herb belonging to family Euphorbiaceae (Joshi, 2014; Singh et al., 2011). The plant is reportedly native to southern Bolivia, Paraguay, southwestern Brazil and northern Argentina and is an exotic weed found in wastelands of India, Bangladesh and other South Asian countries (Bhavana et al., 2020; Ghosh et al., 2013). As per various studies, the plant possesses a wealth of medicinal traits. Traditionally, the plant is used to relieve various health issues, such as gastrointestinal disorders, hepatic problems, respiratory diseases, malaria, rheumatism and chicken pox (Singha et al., 2022). In addition, the seeds of this plant are purgative, different ethnic groups employs the plant to treat headache and the latex has the potential to heal cuts and wounds (Saggoo et al., 2010). The plant is also mentioned to be hepatoprotective, anti-helminthic activity and anti-hypertensive properties (Dutta et al., 2018). The leaves of the plant are reported to contain alkaloids, flavonoids, glycosides, steroids, phenols, tannins, and saponins (Jeeshna et al., 2011). Another study showed presence of carbohydrates, cardiac glycosides, and coumarins in the stem (Mohan & Divya, 2017). On the basis of the strong literature support, *C. bonplandianus* and its potential active compounds can provide valuable insights into the development of new drug for the treatment of JEV.

Considering the above mentioned problem of JEV, we have conducted a comprehensive *in silico* study, in which we used molecular docking to target the JEV structural and non-structural proteins with phytoconstituents from *C. bonplandianus*. In addition, to elucidate their dynamic and thermodynamic properties and to support the molecular docking results, dynamics simulation was performed on ursolic acid. Furthermore, to ensure ursolic acid's safety and efficacy in the treatment, its drug-likeness, physicochemical properties, and ADMET were investigated.

Etiology of Disease

A bite from carrier mosquito, results into release of virus particles into the host. These particles interact with the receptors present on the host receptor cells, including dendritic cells, fibroblasts, pericytes, and myeloid cells, the primary points for viral propagation. Post replication, newly assembled virions drift towards the brain cells. After establishment of infection, the virus undergoes an incubation period of 5 to 15 days. Generally, the infections are asymptomatic or with light symptoms (Kumar et al., 2022). On the other hand, approximately 1 in 250 cases are fatal marked by high fever, disorientation, convulsion, seizures, and coma, which may eventually result in death (Kumar et al., 2020). The infection poses as a barrier for normal functioning of various cellular pathways to support the viral replication machinery, which consequently leads to activation of cellular stress responses. The stress pathways have a direct impact on immune responses, cell adhesion, inflammation, cell survival, metabolic pathways, and cytoskeletal organization, which influence the viral pathogenesis (Sharma et al., 2021). Breaching of blood brain barrier (BBB) is

one of the most salient features of JEV. On the environmental front, the enhanced viral replication and dispersal have been found linked to an elevated temperature. In addition, a hike in temperature is responsible for altering human behavior and increasing the frequency of human-mosquito encounters (Tian et al., 2015).

Reported Therapeutic Uses

The efficacy of *C. bonplandianus* has been proven against lung cancer, prostate cancer, liver cancer and breast cancer by *in vitro* studies (Lanchhana et al., 2023). Several studies have reported the larvicidal effect of the plant against vectors, such as, *Aedes aegyptii* and *Anopheles vagus* (Lakshmi et al., 2021; Bagavan and Rahuman, 2011). Furthermore, the plant is a potent anthelmintic agent (Singha et al., 2022; Hapse et al., 2012). The anti-diabetic caliber of the plant has been studied as its alpha-amylase and alpha-glucosidase inhibition potential (Keerthana et al., 2013; Qaisar et al., 2016). In addition, the plant has a role in healing wounds (Divya et al., 2011) and mitigation of urinary tract infection (Ghosh et al., 2018). Table 1 mentions various therapeutic roles of the plant.

Table 1 List of therapeutic uses of *C. bonplandianus*

Plant part	Medicinal Use	Therapeutic use identifiers	Reference
Leaves	Wound Healing	NA	Divya et al., 2011
Leaves	Diabetes Mellitus	NA	Keerthana et al., 2013
Whole plant	Hyperglycemia	NA	Qaisar et al., 2016
Whole plant	Diabetes	NA	Qaisar et al., 2014
Whole plant	Larvicidal (Aedes aegyptii)	NA	Jeeshna et al., 2010
Leaves	Anthelminthic (Pheretima posthuma)	NA	Hapse et al., 2012
Leaves	Larvicidal (An. Vagus, Ar. Subalbatus, Cx. Vishnui)	NA	Jeeshna et al., 2010
Leaves	Urinary Tract Infection	NA	Ghosh et al., 2018
Leaves and Stem	Cancer	NA	Divya & Mohan, 2017
Whole plant	CNS Depressant Activity	NA	Kota et al., 2015
Leaves	Pathogenic Fungus	NA	Mala et al., 2009
Flower	Apnea	MESH:D001049, UMLS:C0003578, DOI:0050847, ICD-11:MD11.0	ISBN:9770972795006
Flower	Chest Pain	MESH:D002637, UMLS:C0008031, ICD-11:MD30.Z	ISBN:9770972795006
Flower	Cough	MESH:D003371, UMLS:C0010200, ICD-11:MD12	ISBN:9770972795006
Flower	Headache	MESH:D006261, UMLS:C0018681, ICD-11:8A81.Z	ISBN:9770972795006
Leaves	Anticoagulants	MESH:D000925, UMLS:C0003280	ISBN:9770972795006

Plant part	Medicinal Use	Therapeutic use identifiers	Reference
Leaves	Antifungal Agents	MESH:D000935, UMLS:C0003308, ICD-11:XM83G4	ISBN:9770972795006
Leaves	Anti-Infective Agents	MESH:D000890, UMLS:C0003204, ICD-11:XM4TV0	ISBN:9770972795006
Leaves	Anti-Infective Agents, Local	MESH:D000891, UMLS:C0003205, ICD-11:XM4VG4	ISBN:9770972795006
Leaves	Antirheumatic Agents	MESH:D018501, UMLS:C0003191, ICD-11:XM95N2	ISBN:9770972795006
Leaves	Antiviral Agents		ISBN:9770972795006
Leaves	Apnea	MESH:D001049, UMLS:C0003578, DOID:0050847, ICD-11:MD11.0	ISBN:9770972795006
Leaves	Arthritis	MESH:D001168, UMLS:C0003864, DOID:848, ICD-11:FA2Z	ISBN:9770972795006
Leaves	Bites and Stings	MESH:D001733, UMLS:C0005659	ISBN:9788190648974
Leaves	Chest Pain	MESH:D002637, UMLS:C0008031, ICD-11:MD30.Z	ISBN:9770972795006
Leaves	Cough	MESH:D003371, UMLS:C0010200, ICD-11:MD12	ISBN:9770972795006
Leaves	Eczema	MESH:D004485, UMLS:C0013595	Contribution To The Medi- co-Botany Of East Godavari And West Godavari Dis- tricts Of Andhra Pradesh, ISBN:9770972795006
Leaves	Fever	MESH:D005334, UMLS:C0015967, ICD-11:MG26	ISBN:9770972795006

Plant part	Medicinal Use	Therapeutic use identifiers	Reference
Leaves	Headache	MESH:D006261, UMLS:C0018681, ICD-11:8A81.Z	ISBN:9770972795006
Leaves	Nasal Disease	MESH:D009668, UMLS:C0028432, DOID:2825, ICD-11:CA0Z	ISBN:9770972795006
Leaves	Scabies	MESH:D012532, UMLS:C0036262, DOID:8295, ICD-11:1G04	ISBN:9770972795006
Leaves	Scorpion Stings	MESH:D065008, UMLS:C0238417, ICD-11:NE61	ISBN:9770972795006
Leaves	Scurvy	MESH:D012614, UMLS:C0036474, DOID:13724, ICD-11:5B56.0	ISBN:9770972795006
Leaves	Skin Diseases	MESH:D012871, UMLS:C0037274, DOID:37, ICD-11:EM0Z	Contribution To The Medi-co-Botany Of East Godavari And West Godavari Districts Of Andhra Pradesh
Leaves	Snake Bites	MESH:D012909, UMLS:C0037379	ISBN:9770972795006
Leaves	Sprains and Strains	MESH:D013180, UMLS:C0038048	ISBN:9770972795006
Leaves	Wounds and Injuries	MESH:D014947, UMLS:C0043251	ISBN:9770972795006
Plant exudate	Furunculosis	MESH:D005667, UMLS:C0016867, ICD-11:1B75.0	ISBN:9770972795006
Plant exudate	Nail Diseases	MESH:D009260, UMLS:C0027339, DOID:4123	ISBN:9770972795006
Plant exudate	Sprains and Strains	MESH:D013180, UMLS:C0038048	ISBN:9770972795006
Plant exudate	Wounds and Injuries	MESH:D014947, UMLS:C0043251	ISBN:9770972795006, ISBN:9788172360481

Plant part	Medicinal Use	Therapeutic use identifiers	Reference
Root	Postpartum Hemorrhage	MESH:D006473, UMLS:C0032797, ICD-11:JA43	ISBN:9770972795006
Seed	Antifungal Agents	MESH:D000935, UMLS:C0003308, ICD-11:XM83G4	ISBN:9770972795006
Seed	Antinematodal Agents	MESH:D000969, UMLS:C0003391, ICD-11:XM16G5	ISBN:9770972795006
Stem	Anti-Inflammatory Agents	MESH:D000893, UMLS:C0003209, ICD-11:XM7XD1	ISBN:9770972795006
Stem	Antirheumatic Agents	MESH:D018501, UMLS:C0003191, ICD-11:XM95N2	ISBN:9770972795006
Stem	Apnea	MESH:D001049, UMLS:C0003578, DOID:0050847, ICD-11:MD11.0	ISBN:9770972795006
Stem	Chest Pain	MESH:D002637, UMLS:C0008031, ICD-11:MD30.Z	ISBN:9770972795006
Stem	Cough	MESH:D003371, UMLS:C0010200, ICD-11:MD12	ISBN:9770972795006
Stem	Headache	MESH:D006261, UMLS:C0018681, ICD-11:8A81.Z	ISBN:9770972795006
Stem	Wounds and Injuries	MESH:D014947, UMLS:C0043251	ISBN:9770972795006
Whole plant	Anti-Bacterial Agents	MESH:D000900, UMLS:C0279516, ICD-11:XM8XH0	ISBN:9770972795006
Whole plant	Wound Healing	MESH:D014945, UMLS:C0043240	ISBN:9770972795006
Whole plant	Wounds and Injuries	MESH:D014947, UMLS:C0043251	ISBN:9770972795006

Plant part	Medicinal Use	Therapeutic use identifiers	Reference
NA	Cardiovascular System	MESH:D002319, UMLS:C0007226, ICD-11:XA4PM9	ISBN:9770972795006
NA	Cathartics	MESH:D002400, UMLS:C0007422, ICD-11:XM6GE9	ISBN:9770972795006
NA	Eczema	MESH:D004485, UMLS:C0013595	Contribution To The Medi-co-Botany Of East Godavari And West Godavari Districts Of Andhra Pradesh
NA	Hypotension	MESH:D007022, UMLS:C0020649, ICD-11:BAZ2	ISBN:9770972795006, ISBN:9788172360481
NA	Insecticides	MESH:D007306, UMLS:C0021576, ICD-11:XM3K66	ISBN:9770972795006
NA	Parasympatholytics	MESH:D010276, UMLS:C0030511, ICD-11:XM3A77	ISBN:9788172360481
NA	Skin Diseases	MESH:D012871, UMLS:C0037274, D0ID:37, ICD-11:EM0Z	Contribution To The Medi-co-Botany Of East Godavari And West Godavari Districts Of Andhra Pradesh
NA	Wounds and Injuries	MESH:D014947, UMLS:C0043251	ISBN:9770972795006

MeSH ID: Medical Subject Headings database ID of National library of medicine in National Institutes of Health; UMLS ID: Unified Medical Language System database ID of National library of medicine in National Institutes of Health; ICD-11: International Classification of Diseases 11th Revision database ID of World Health Organization.

After conducting an extensive study of the reported traditional medicinal uses of *C. bonplandianus*, it was found that this plant exhibits efficacy against a variety of ailments and holds potential for future exploration.

Consequently, we have opted to utilize *C. bonplandianus* in addressing JEV, aiming to contribute to the alleviation of the impact of viral disease.

Material and Methods

Protein Structure Retrieval and Preparation

The crystal structures of JEV env (PDB ID: 3P54; resolution of 2.10 Å), cap (PDB ID: 5OW2; resolution of 1.98 Å) and NS5 (PDB ID: 4K6M; resolution of 2.6 Å) proteins obtained via X-RAY diffraction method were retrieved from RCSB Protein Data Bank (<https://www.rcsb.org/>) (Fig. 1). In order to

generate accurate predictions, any of the associated water molecules or heteroatoms were removed from the structures with the assistance of PyMol (<https://pymol.org/2/>). Further, the protein structures were repaired using Protein Repair and Analysis Server (<https://www.protein-science.com/>) by adding missing hydrogens, side chains and secondary structure elements. The final structure was saved in PDB format for further studies.

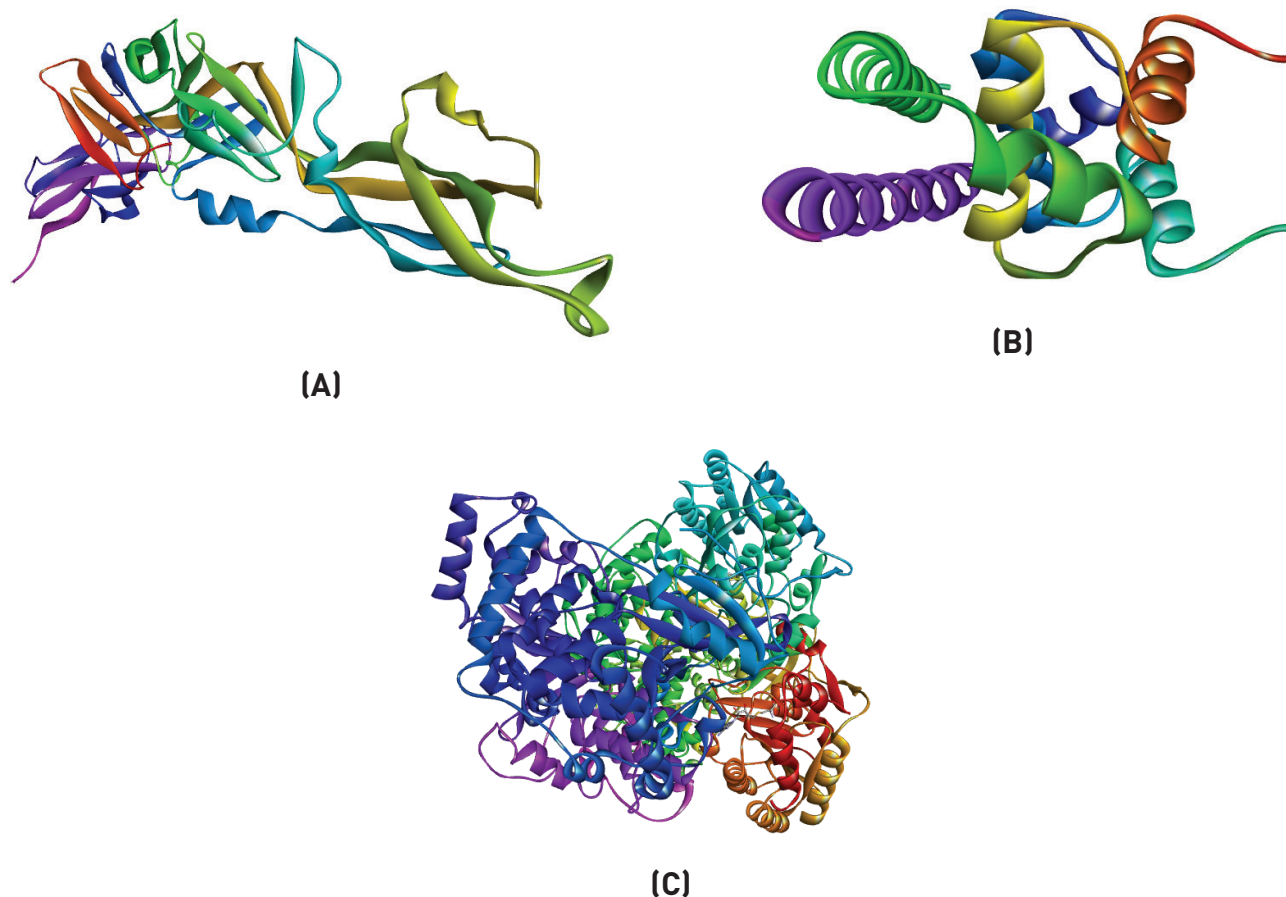


Fig. 1 Protein structures of (A) 3P54; (B) 5OW2; (C) 4K6M

Phytochemical Library Preparation

Extensive literature survey was performed to obtain a list of 96 phytoconstituents present in *C. bonplandianus*. The three dimensional structures of the compounds in SDF format were retrieved from PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). In addition, the SDF files were converted into PDB format by utilizing Open Babel GUI (https://openbabel.org/wiki/Main_Page), in order to facilitate the process of molecular docking.

Rule of Five (R05)

For initial prescreening of phytoconstituents, an online web-based platform, ADMET lab 2.0 (<https://admetmesh.scbdd.com/>) was utilized to predict the drug-likeness of the compounds. For small molecules, it is an empirical rule of thumb to assess their physicochemical aspects, in order to refine their drug ability. In the present study, we analyzed several properties including molecular weight (MW), partition

coefficient (LogP), number of hydrogen bond acceptors (nHA) and number of hydrogen bond donors (nHD). As these attributes form the foundation for the Lipinski rule of five, any molecule deviating from the threshold values was eliminated from further analysis (Lipinski, 2004).

Molecular Docking

In the current study, PyRx software (<https://pyrx.sourceforge.io/>) was utilized for the high-throughput molecular docking study. The prepared PDB structures of target proteins and plant phytoconstituents were imported into the software and were processed to generate the respective PDBQT files. Blind molecular docking was performed with grid box sizes mentioned in table 2. Moreover, the protein-ligand complexes were prepared using PyMol. These complexes were imported to Discovery Studio Visualizer v21.1.0.20298 (<https://discover.3ds.com/discovery-studio-visualizer-download>) for visualization of various interactions formed between the target and phytoconstituents.

Table 2 Grid box dimensions for the target proteins

Target protein	Grid box dimensions
3P54	x = 44.429900732; y = 50.6697712135; z = 9.935888748
50W2	x = 44.1632598305; y = 26.5005692211; z = 42.8220409393
4K6M	x = 132.691782188; y = 90.3213097954; z = 94.1864923573

Bioavailability Radar and Bioactivity Score

Drug-likeness of screened candidate with best binding energy was analyzed in

a comprehensive way using SwissADME web-based tool (<http://www.swissadme.ch/index.php>). In order to do so, physiochemical properties such as size (MW), lipophilicity

(XLOGP3), polarity (TPSA), solubility (LogS), flexibility (fraction Csp3), and saturation (rotatable bonds) were investigated and a bioavailability radar diagram representing these parameters was obtained (Lipinski et al., 2012). The region highlighted in pink delineates the optimal parameter values, and substantial deviations from these values on a significant scale imply that the ligand may not possess favorable oral bioavailability (Daina et al., 2017). The bioactivity scores were determined by using Molinspiration software (<http://www.molinspiration.com/>). Parameters analyzed include Ion channel modulator (ICM), Nuclear receptor ligand (NRL), Protease inhibitor (PI), Enzyme inhibitor (EI), G protein-coupled receptor ligand (GPCR ligand).

In Silico ADMET Analysis

After molecular docking studies of all the compounds with targeted protein of JEV, pharmacokinetics parameters such as ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) of the best candidate were investigated using ADMETlab 2.0 (<https://admetmesh.scbdd.com/>). ADMET constitutes a crucial aspect of drug design and development, encompassing the assessment of significant pharmacokinetic parameters (Olasupo et al., 2020). ADMET properties include Absorption (P-glycoprotein substrate, P-glycoprotein inhibitor, human intestinal absorption, Caco-2 permeability, MDCK Permeability, human oral bioavailability 20%, and human oral bioavailability 30%); Distribution:

(Plasma protein binding, blood–brain barrier penetration, Volume Distribution, and fraction unbound in plasmas); Metabolism (CYP 1A2/2C19/2C9/2D6/3A4 inhibitor and CYP 1A2/2C19/2C9/2D6/3A4 substrate); Excretion (clearance of a drug and half-life of a drug); Toxicity (Skin sensitization, carcinogenicity, respiratory toxicity, rat oral acute toxicity, eye irritation/corrosion, Ames test for mutagenicity and maximum recommended daily dose) (Xiong et al., 2021).

Molecular Dynamics Simulation

On the basis of molecular docking analysis, top phytochemical of *C. bonplandianus* was further selected for molecular dynamics (MD) simulation to examine its stability and intermolecular interactions using Desmond molecular dynamics study software (Bowers et al., 2006). Using the protein preparation wizard of the Desmond-maestro interface, all protein-ligand contacts were preprocessed and improved. The system was configured for each complex using the SPC model of solvent, an orthorhombic box simulation box was calculated using the buffer method with a distance of $10 \times 10 \times 10$ Å. Later, counter ions (Na^+ or Cl^-) were added for neutralization, system's concentration was set to 0.15 M and OPLS3e force field was applied to the system. This generated system was simulated for 100 ns in a minimized system in the NPT ensemble. After simulation, the trajectory

was analyzed using parameters such as Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), protein-ligand interactions, radius of gyration of the

lead compound, and ligand torsion analysis using the simulation interaction diagram utility of the Desmond.

Result and Discussion

Despite of cutting edge research and development taking place to produce innovative solutions to combat viral diseases, JEV remains a significant threat to almost half of the world population. This disease is positioned as one of the most common viral encephalitis in Asia (Joe et al., 2022). Three key viral proteins env, cap and nsp5 proteins, play critical roles in different stages of JEV infection, leading to potential neurological damage. The E protein resides on the viral surface and acts as the key for cellular entry. It binds to specific receptors on host cells, primarily dendritic cells and neuronal cells. Following attachment, the E protein undergoes a conformational change, triggering the fusion between the viral envelope and the host cell membrane. This allows the viral genome to enter the host cell cytoplasm, initiating viral replication. Additionally, the E protein plays a role in viral assembly by interacting with the cap protein during virion formation (Kumar et al., 2022; Leng et al., 2020). The cap protein forms the viral capsid, a protective shell that encloses the JEV genomic RNA. This capsid shields the RNA from degradation and facilitates its delivery to the replication site within the host cell. During viral replication, newly synthesized cap protein molecules assemble around the newly replicated RNA,

forming new viral particles (Ishida et al., 2019; Sarkar et al., 2021). NS5 is a multi-functional enzyme crucial for JEV replication. It acts as the viral RNA-dependent RNA polymerase (RdRp), responsible for synthesizing new copies of the viral RNA genome. Additionally, NS5 possesses methyltransferase activity, modifying the viral RNA cap to evade the host cell's antiviral defenses. NS5 might also play a role in suppressing the host immune response, allowing the virus to replicate more efficiently (Kumar et al., 2022). The sole objective of this study is to identify potential inhibitors of JEV, using diverse computational tools. Thus, an attempt was undertaken to explore diverse phytochemicals from *C. bonplandianus*, targeting the env, cap and nsp5 proteins, playing an indispensable role in the viral life-cycle.

Preliminary Screening of Druglikeness

In the pursuit of creating a highly effective drug with minimal adverse effects, preliminary screening on the basis of drug-likeness is a crucial step. To conduct this preliminary screening, Lipinski's Rule of Five was utilized for evaluating all the phytoconstituents derived from *C. bonplandianus*. Lipinski's rule of five outlines five key parameters that influence a

drug's oral bioavailability. A drug candidate is considered more likely to have good oral absorption if it adheres to most, ideally all of the criteria. According to the rule, a compound demonstrates favorable oral bioavailability when its molecular weight (MW) $\leq$ 500 Da, hydrogen bond donors (HbD) $\leq$ 5, hydrogen bond acceptors (HbA) $\leq$ 10, and an octanol-water partition coefficient log P $\leq$ 5 (Lipinski,

2004). Compounds that violated more than one parameter were excluded, while the remaining compounds were identified as ligands for the subsequent docking study. As illustrated in Table 3, out of the total 96 phytoconstituents, 94 candidates successfully passed Lipinski's rule and were carried forward for further analysis. 45 compounds depicted 0 violations.

Table 3 Screening of phytochemicals based on Lipinski's rule

Phytocompound	LogP	MW	nHA	nHD	LV
2, 4-bis (1, 1-dimethylethyl)phenol	4.832	206.17	1	1	0
9-Eicosene	9.512	280.31	0	0	1
7-hexadecene	7.898	224.25	0	0	1
1-Nonadecene	9.274	266.3	0	0	1
Kaempferol	2.656	286.05	6	4	0
Quercetin	2.155	302.04	7	5	0
Cyclopentylcyclohexane	5.286	152.16	0	0	1
3,7,11-Trimethyl-2,4-dodecadiene	5.788	208.22	0	0	1
Asparagine, N-dl-alanyl	-2.736	203.09	7	6	0
1-Dodecanamine	4.412	185.21	1	2	0
Tetraacetyl-d-xylonic nitrile	-0.226	343.09	10	0	0
5-Eicosene	9.51	280.31	0	0	1
E-15-Heptadecenal	6.575	252.25	1	0	1
Phytol	7.764	296.31	1	1	1
3-Fluorobenzoic acid	2.119	140.03	2	1	0
2-Propenamide	-0.515	71.04	2	2	0
5-Nonadecene	9.114	266.3	0	0	1
Behenic alcohol	9.466	326.35	1	1	1
Hexacosyl acetate	11.702	424.43	2	0	1
Phthalic acid	1.187	166.03	4	2	0
1,1,2,2-Tetra chloro ethanes	2.508	165.89	0	0	0
Pentachloroethane	3.224	199.85	0	0	0
Avlothane	3.822	233.81	0	0	0

Phytocompound	LogP	MW	nHA	nHD	LV
L-Camphor	2.324	152.12	1	0	0
4-Propyl Benzaldehyde	2.96	148.09	1	0	0
Beta Farnesene (E)	6.29	204.19	0	0	1
Curcumene	5.9	202.17	0	0	1
l-zingiberene	5.679	204.19	0	0	1
Cedr-8-ene	5.161	204.19	0	0	1
3,5-Dimethyl benzyl bromide	3.918	198	0	0	0
Beta Curcumene	6.279	204.19	0	0	1
Cadina-1 (10) 4-diene	5.51	204.19	0	0	1
1.1,13 a-Trimethyl-7-methylene deca hydro-1HCyclopropa (a) naphthalene	5.106	204.19	0	0	1
Eudesma-3,7 (11)-diene	5.547	204.19	0	0	1
Cyclo isolongifolene, 8,9-dehydro	4.566	202.17	0	0	1
(E)-Nuciferol	4.535	218.17	1	1	0
Cycloisolongifolene, 8,9-dehydro-9-formyl	3.605	230.17	1	0	0
Gama-Eudesmol	4.055	222.2	1	1	0
Tridecane,2-methyl-2-phenyl	8.409	274.27	0	0	1
Gamma-Gurjunene	4.84	204.19	0	0	1
Vellerdiol	2.589	236.18	2	2	0
(Z)-trans-alpha – Bergamotol	4.276	220.18	1	1	0
Germacrone	4.001	218.17	1	0	0
Bicyclo[7.2.0]undecane, 10,10-dimethyl-2,6-bis(methylene)-, [1S-(1R*,9S*)]	4.926	204.19	0	0	1
Decane, 1-bromo-2-methyl	5.918	234.1	0	0	1
2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8adimethyl-6-(1-methylethenyl)	3.544	218.17	1	0	0
6-[p-Tolyl]-2-methyl-2-heptenol, trans	4.518	218.17	1	1	0
7-Oxabicyclo[4.1.0]heptane, 2,2,6-trimethyl-1-[3-methyl-1,3-butadienyl]-5-methylene	4.512	218.17	1	0	0
1,4-Hexadien-3-one, 5-methyl-1-[2,6,6-trimethyl-2,4- cyclohexadien-1-yl]	4.98	234.2	1	0	0
Isopropyl myristate	6.768	270.26	2	0	1

Phytocompound	LogP	MW	nHA	nHD	LV
Phytol, acetate	8.311	338.32	2	0	1
1-[3,3-Dimethyl-2-(3-methyl-buta-1,3-dienyl)- cyclopentyl]-2-hydroxy-ethanone	3.05	222.16	2	1	0
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	7.764	296.31	1	1	1
Naphthalene, 6-methoxy-2-(1-buten-3-yl)	4.72	212.12	1	0	0
2,6-Xylenol, 4-(2-norbornyl)	4.365	216.15	1	1	0
Phthalic acid, 5-methylhex-2-yl butyl ester	5.208	320.2	4	0	0
E,E,Z-1,3,12-Nonadecatriene-5,14-diol	5.019	294.26	2	2	0
2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hex- a1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	6.912	324.25	1	0	1
Hexanoic acid, 2-ethyl-, anhydride	5.297	270.22	3	0	0
7-Heptadecene, 1-chloro	7.967	272.23	0	0	1
Acetic acid n-octadecyl ester	8.508	312.3	2	0	1
Eicosane	10.033	282.33	0	0	1
Phenol, 2-methyl-5-(1,2,2-trimethylcyclopentyl)-, (S)	4.927	218.17	1	1	0
4,11-Dihexyl-16,16-dimethyl-1,14-dioxa-4,11- di- azacycloheptadecane-3,12-dione	5.483	468.39	6	0	0
Z-5-Nonadecene	9.114	266.3	0	0	1
Cyclotetracosane	11.599	336.38	0	0	1
Cycloeicosane	10.028	280.31	0	0	1
3-Eicosene	9.493	280.31	0	0	1
Z-8—Hexadecene	7.898	224.25	0	0	1
alpha-Thujone	2.261	152.12	1	0	0
alpha-Pinene	4.125	136.13	0	0	1
Myrcene	4.321	136.13	0	0	0
Sabinene hydrate	2.44	154.14	1	1	0
cis-Decahydronaphthalene	4.8	138.14	0	0	1
trans-Sabinol	1.693	152.12	1	1	0
Borneol	2.719	154.14	1	1	0
Isobornyl acetate	3.263	196.15	2	0	0
3-Methoxyacetophenone	1.79	150.07	2	0	0

Phytocompound	LogP	MW	nHA	nHD	LV
Z-beta-Damascenone	3.846	190.14	1	0	0
beta-Bourbonene	4.596	204.19	0	0	1
Arachidic acid	8.389	312.3	2	1	1
alpha1-Sitosterol	7.517	426.39	1	1	1
Oleic acid	7.131	282.26	2	1	1
Linolenic acid	6.156	278.22	2	1	1
12-Octadecadienoic acid	6.687	280.24	2	1	1
Linoleic acid	6.652	280.24	2	1	1
Vomifoliol	1.225	224.14	3	2	0
5(S),9(S),10(S)-15,16-Epoxyclero- da-3,8,13(16),14-tetraene-19,18:20,12(S)-diolac- tone (swassin)	3.157	340.13	5	0	0
Taraxerol	7.479	426.39	1	1	1
Ursolic acid	6.083	456.36	3	2	1
beta-Sitosterol	7.663	414.39	1	1	1
Sparsiflorine	2.046	283.12	4	3	0
(-)-Pronuciferine	1.888	311.15	4	0	0
oleanolic acid	6.486	456.36	3	2	1

MW: Molecular weight; nH-A: H-bone acceptor; nH-D: H-bone donor; LV: Lipinski violations

Molecular Docking Analysis

Molecular docking plays a vital role as a computational tool in the field of drug discovery. It was performed on ligands that had undergone the prescreening. In the current study, the selected targeted protein is docked against screened phytochemicals to examine their binding affinity. The docking results of top 5 lead compounds against each studied protein are detailed in Table 4. On the basis of Lipinski violation and docking results evaluation, it was found that oleanolic acid exhibited the maximum binding affinity of

-10.6 kcal/mol towards nsp5 and formed two hydrogen bonds with residues GLY B:158 and GLU B: 149, followed by ursolic acid (-10.4 kcal/mol), which displayed formation of four hydrogen bonds with residues VAL A:124; GLU A:466; ARG A:355 and MET A: 116 (Fig. 2). Furthermore, Ursolic acid also managed to bind env protein with highest affinity of -8.3 kcal/mol along with 1 hydrogen bond. In case of cap protein, even though oleanolic acid showed the maximum binding energy of -7.7 kcal/mol however, it failed to form any hydrogen bond with the target protein whereas, ursolic acid bound the cap protein with an affinity of

-7.4 kcal/mol and three hydrogen bonds with residues ARG A:98 and TRP B:69. Ursolic acid is a pentacyclic triterpenoid, with promising medicinal properties. The compound is present in a wide variety of medicinal plants (Kashyap et al., 2016) and has been studied for its anti-cancer effect, anti-inflammatory, anti-viral, anti-bacterial, etc. (Panda et al., 2022; Luan et al., 2022; Sycz et al., 2022; Al-Kuraishy et al., 2022). In a computational study against

SARS-CoV-2 main protease, Ursolic acid from *Ocimum basilicum* showed a binding affinity of -8.21 kcal/mol (Matondo et al., 2021), marking its potential against the viral diseases. The binding energy of -10.4 kcal/mol, along with the formation of four hydrogen bonds with nsp5, Ursolic acid emerged as a key compound against JEV and hence, was carried forward for further study.

Table 4 Molecular docking results of top 5 phytoconstituents

Target protein	Compound	Binding affinity (kcal/mol)	H-bond	Interacting residues
Envelope (3P54)	Ursolic acid	-8.3	1	ASN A: 36
	5(S),9(S),10(S)-15,16-Epoxy-cleroda-3,8,13(16),14-tetraene-19,18:20,12(S)-diolactone (swassin)	-8.1	1	GLY A:153
	Oleanolic acid	-7.9	1	LEU A:354
	Sparsiflorine	-6.9	2	GLY A: 352; LEU A: 354
	Beta sitosterol	-6.9	0	-
Capsid (5OW2)	Oleanolic acid	-7.7	0	-
	Ursolic acid	-7.4	3	ARG A:98; TRP B:69
	5(S) 9(S) 10(S)-15 16-Epoxy-cleroda-3 8 13(16) 14-tetraene-19 18:20 12(S)-diolactone (swassin)	-7	1	TRP B:69
	Cyclotetracosane	-6.4	0	-
	Gamma-Eudesmol	-6.2	2	LYS B:55; PHE B:54

Target protein	Compound	Binding affinity (kcal/mol)	H-bond	Interacting residues
nsp5 (4K6M)	Oleanolic acid	-10.6	2	GLY B:158; GLU B: 149
	Ursolic acid	-10.4	4	VAL A:124; GLU A:466; ARG A:355; MET A: 116
	5(S) 9(S) 10(S)-15 16-Epoxy-cleroda-3 8 13(16) 14-tetraene-19 18,20 12(S)-diolactone (swassin)	-9.4	3	LYS A: 105; ARG A: 160
	Kaempferol	-8.9	4	VAL A: 124; MET A: 116; ARG A: 355; GLU A: 466
	Beta sitosterol	-8.8	0	-

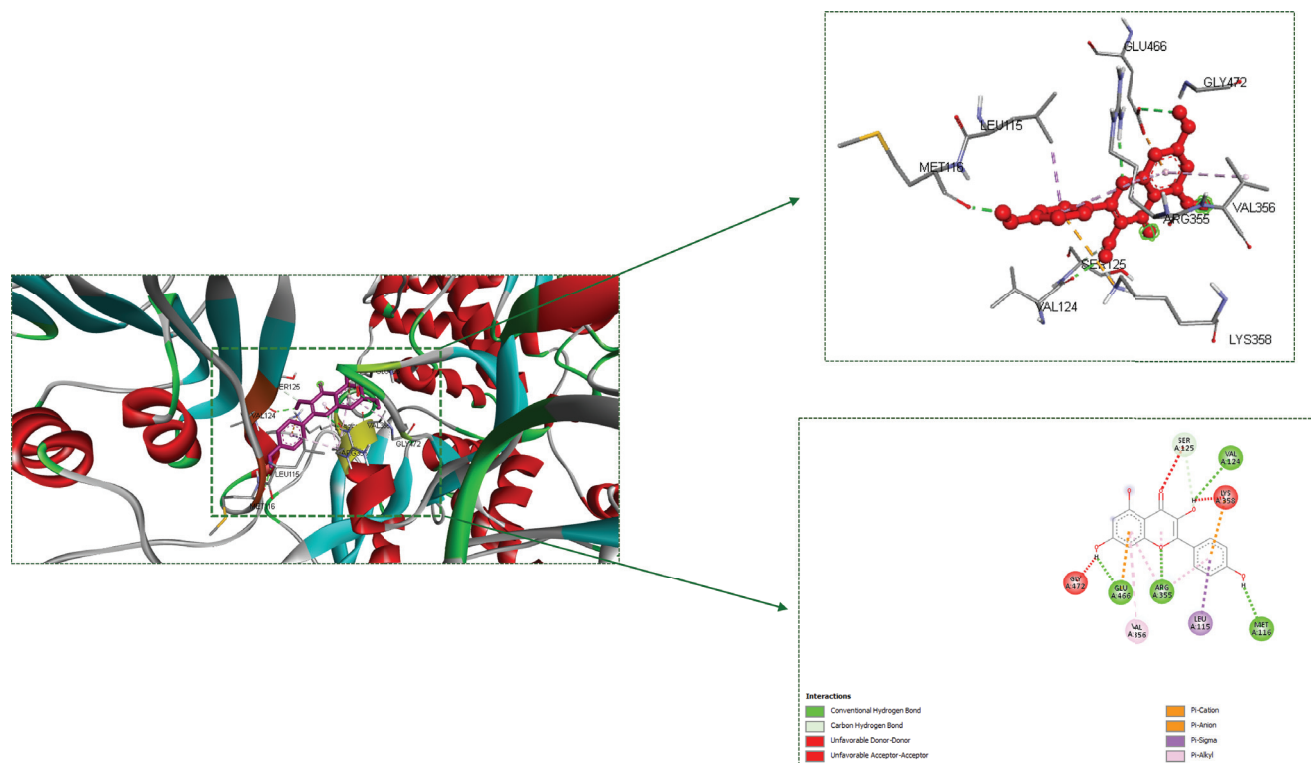


Fig. 2 Illustration of nsp5-ursolic acid docked complex

Bioavailability Radar and Bioactivity

Bioavailability is an important parameter of the drug development process (Wei et al., 2022). In this study, bioavailability was evaluated for the candidate with the best binding affinity i.e., ursolic acid. As shown in Fig. 3, ursolic acid exhibited most of the

bioavailability parameters, including MW, TPSA, fraction Csp3, and rotatable bonds within the optimal prescribed range (Daina et al., 2017). However, the compound depicted a lipophilicity of 7.34 and insolubility of -7.23, slightly deviating from the set range. Overall, the compound was found to possess an acceptable bioavailability with a score of 0.85.

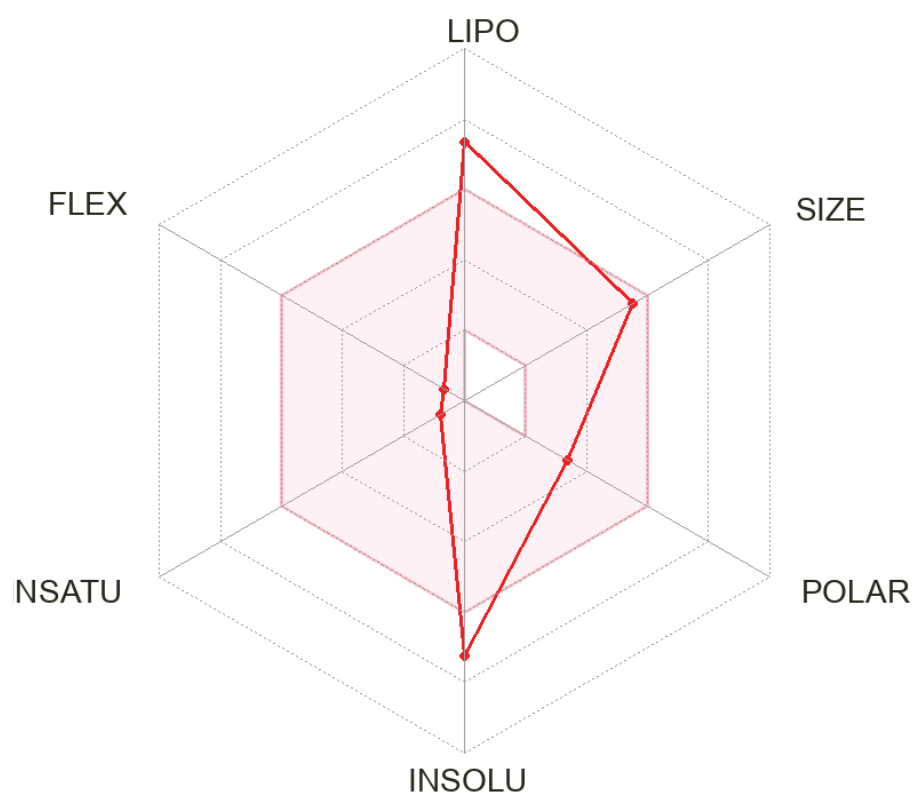


Fig. 3 Bioavailability radar of ursolic acid

In addition, presence of various bioactivities, including G protein coupled receptor (GPCR) ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand and protease inhibition were assessed for ursolic acid using Molinspiration Online tool. In general, organic molecules are considered active when bioactivity score is >0, moderately active if

score is between -5.0 to 0.0 and inactive when the score is <-5.0 (Kulkarni et al., 2021). As elaborated in the Fig. 4, the lead compound possessed potent nuclear receptor ligand, enzyme inhibition, GPCR ligand and protease inhibition activities. Moreover, moderate ion channel modulation and kinase inhibition activities were also found.

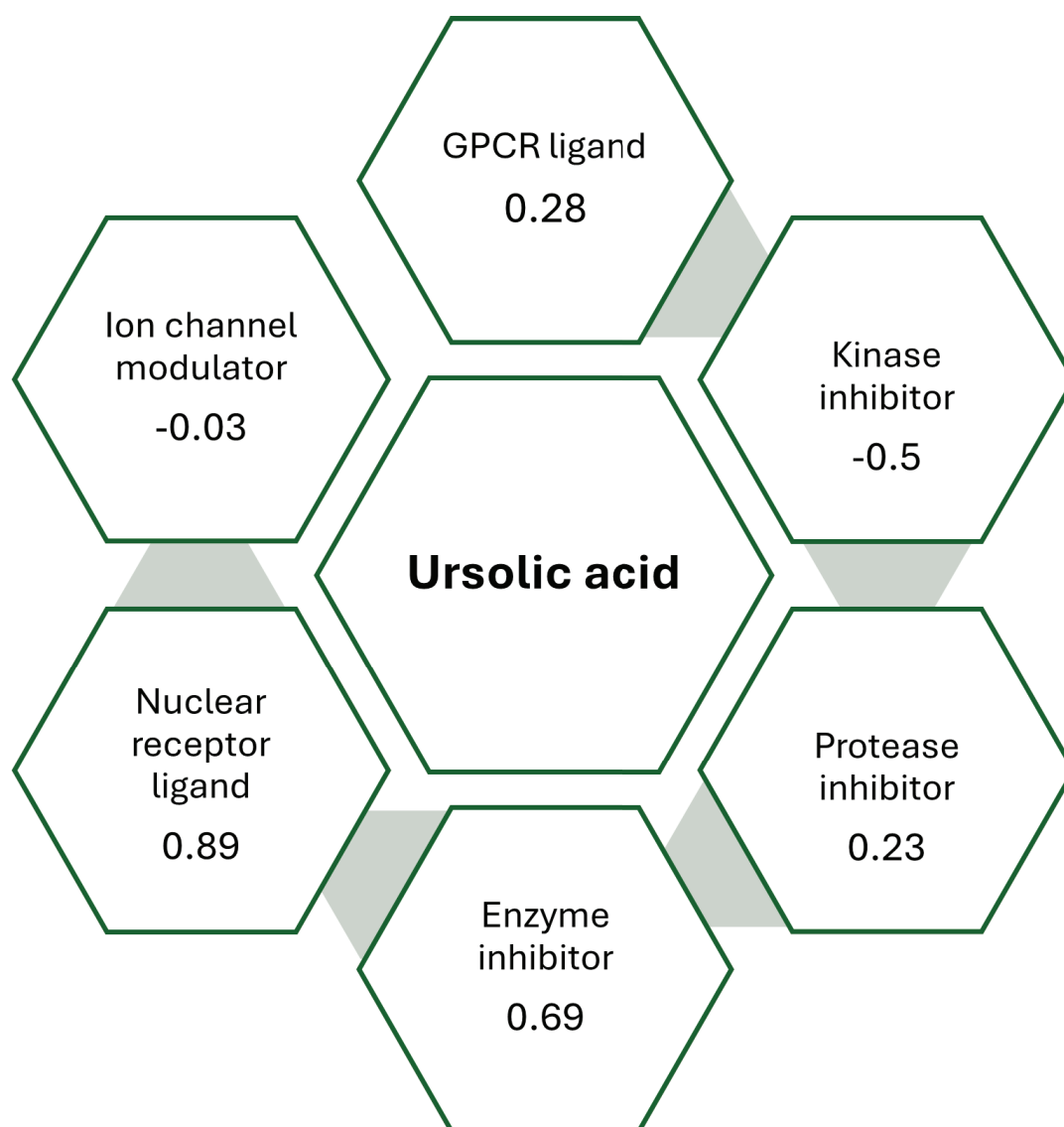


Fig. 4 Bioactivities of ursolic acid

In Silico ADMET Analysis

Administering medication orally is the most convenient method for patients. When a drug is taken orally, it must be absorbed through the epithelial lining of the small intestine (Azman et al., 2022). In the drug discovery pipeline, a critical bottleneck lies in identifying promising lead compounds with favorable pharmacokinetic (ADME) and toxicological (T) properties. Traditionally, these properties

are assessed through extensive *in vitro* and *in vivo* experiments, which are time-consuming, resource-intensive, and often require large quantities of the candidate drug. In silico ADMET analysis offers a powerful alternative approach to evaluate these properties computationally, playing a crucial role in modern drug discovery (Pradeepkiran et al., 2021). Ursolic acid depicted excellent probability of being a Pgp-inhibitor, as well as substrate. P-glycoprotein (P-gp) is a

transmembrane efflux pump expressed in various tissues throughout the body, including the intestinal epithelium and the blood-brain barrier. It plays a critical role in limiting drug absorption and distribution by actively transporting xenobiotics, including many therapeutic drugs, out of cells. Whereas, A P-gp substrate is a molecule recognized and actively transported by P-gp. This leads to reduced intracellular drug accumulation at its site of action, potentially compromising its therapeutic efficacy (Seelig, 2020). Furthermore, the compound displayed potent HIA, F (20%) and MDCK permeability whereas, the F (30%) was found to be medium and Caco-2 permeability was on the lower side. Under the parameters of distribution, the compound showed excellent VDss and medium BBB however, the PPB and Fu were found to be poor. VDss represents the theoretical volume in which a drug would need to be uniformly distributed to achieve its observed plasma concentration. A low VDss indicates high concentration in blood plasma, likely due to high plasma protein binding or water solubility. A high VDss suggests extensive tissue distribution, possibly due to high lipid solubility or tissue binding (Smith et al., 2015). In silico models can predict the likelihood of a drug crossing the BBB based

on its properties. A drug that readily crosses the BBB is more likely to reach its target in the central nervous system, but may also lead to unwanted central nervous system side effects (Pandit et al., 2020). PPB refers to the fraction of a drug bound to plasma proteins like albumin, with only the unbound drug being pharmacologically active, meaning a high PPB results in less free drug available to exert its effect and may lead to a longer duration of action. In addition, FU is the unbound fraction of the drug in plasma, representing the pharmacologically active portion (Di, 2021). To explain the metabolism of ursolic acid, the table depicts probabilities of being CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4 inhibitors and substrates, with values ranging from 0 to 1. A CYP substrate is a compound that is metabolized by a specific CYP enzyme whereas, A CYP inhibitor is a drug or compound that can decrease the activity of a specific CYP enzyme (Deodhar et al., 2020). In addition, the lead compound displayed good T1/2 but a poor CL, among the excretion properties. Lastly, the compound did not show AMES toxicity, rat oral acute toxicity, skin sensitization, carcinogenicity, eye corrosion, eye irritation, exhibited medium recommended daily dose but held the potential of respiratory toxicity (Table 5).

Table 5 *In silico* ADMET screening of ursolic acid

Properties		Ursolic acid
Absorbtion	Pgp-inh	0.001
	Pgp-sub	0
	HIA	0.011
	F(20%)	0.007
	F(30%)	0.624
	Caco-2	-5.221
	MDCK	1.39E-05
Distribution	BBB	0.719
	PPB	98.84%
	VDss	0.815
	Fu	2.24%
Metabolism	CYP1A2-inh	0.008
	CYP1A2-sub	0.434
	CYP2C19-inh	0.018
	CYP2C19-sub	0.95
	CYP2C9-inh	0.113
	CYP2C9-sub	0.701
	CYP2D6-inh	0.002
	CYP2D6-sub	0.276
	CYP3A4-inh	0.137
	CYP3A4-sub	0.404
Excretion	CL	3.671
	T12	0.017
Toxicity	Ames toxicity	0.012
	Rat oral acute toxicity	0.183
	Maximum recommended daily dose	0.61
	Skin sensitivity	0.013
	Carcinogenicity	0.031
	Eye corrosion	0.003
	Eye irritation	0.137
	Respiratory toxicity	0.968

Molecular Dynamics Simulations

On the basis of molecular docking, bioavailability, bioactivity, and ADMET analysis, Ursolic acid fulfilled all the parameters and was therefore, subjected to molecular dynamics simulations for 100 ns. The dynamic behaviors or molecular stability of ursolic acid-nsp5 complex was observed through root-mean square deviation (RMSD), Root mean square fluctuation (RMSF), Protein-Ligand interaction analysis, and Ligand torsion analysis.

RMSD analysis of ursolic acid-nsp5 complex

RMSD analysis gives an idea whether the simulation has equilibrated or not, with acceptable fluctuations ranging between 1 to 3 Å. Fig. 5 depicts RMSD plots for simulated protein-Ursolic acid complex. As clear from

the figure, the trajectory of c-alpha showed increase in the beginning of the reaction, post which it gained stabilization up to 10.60 ns. After that the trajectory depicted minor fluctuations within the permissible range. Finally, the c-alpha trajectory attained stabilization from 85.60 ns until the end of the reaction. Overall, the average RMSD of the entire trajectory was found to be 2.55 Å. In addition, the figure gives insights on the ligand-bound protein RMSD plot, where the complex displayed stability up to first 20.40 ns, post which there was slight increase in the RMSD, peaking at 5.37 Å, making the trajectory overlap with c-alpha trajectory, indicating towards stable complex formation. The overlap was witnessed again from 54.80 to 64.00 ns and 68.50 to 70.70 ns. Finally, the ligand-bound protein RMSD attained equilibrium from 89.10 to the end of the simulation period, with an average RMSD of 3.36 Å.

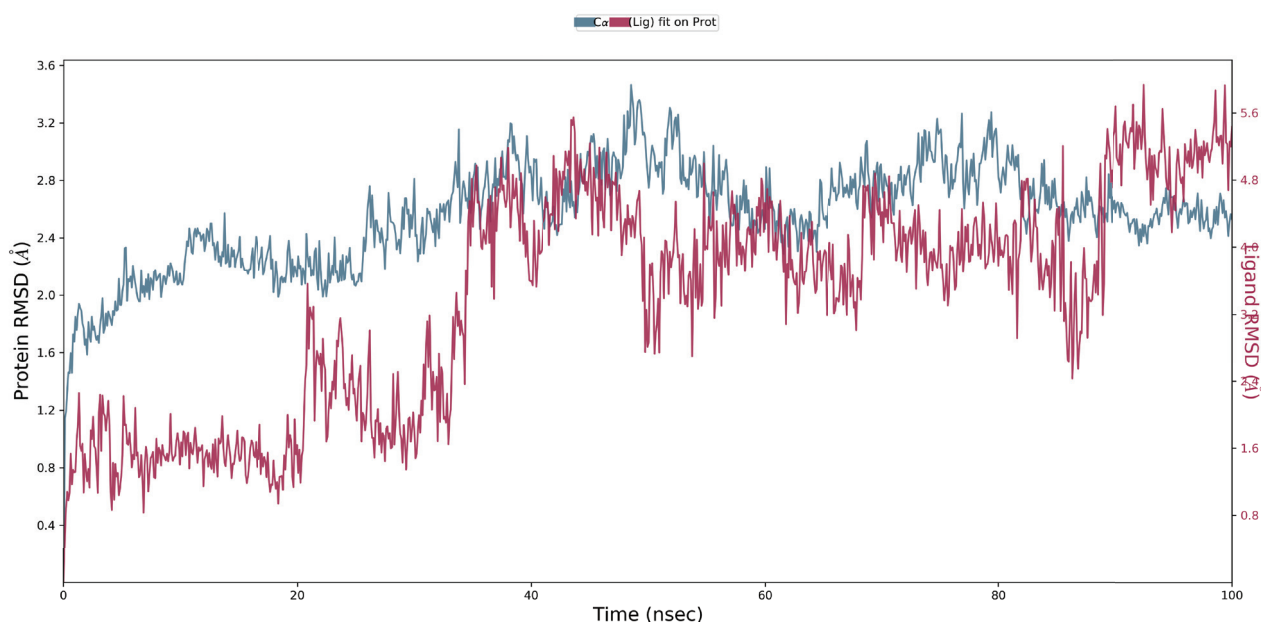


Fig. 5 RMSD Plot of ursolic acid-nsp5 complex

RMSF analysis of ursolic acid-nsp5 complex

RMSF is a measure of the displacement of the reference structure over the number of atoms. In Fig. 6, peaks indicate areas of the protein that fluctuate the most during the simulation. Typically, the tails (*N*- and *C*-terminal) fluctuate more than any other part of the protein. Secondary structure elements like alpha helices and beta strands are usually more rigid than the unstructured part of the protein, and thus fluctuate less than the loop regions. The protein residues

that interact with the ligand are marked with green-colored vertical bars. Generally, RMSF value $>2.5 \text{ \AA}$ points towards less stable bonds (Fatriansyah et al., 2022). As shown in Fig., the RMSF plot of c-alpha depicts six peaks, exceeding the threshold value of $2.5 \text{ \AA}$. These peaks are occupied by ARG 37, ALA 108, LYS 269, GLU 895, GLY 270, and TYR 894. In addition, the residues responsible for making protein-ligand contacts showed RMSF values within the permissible range, depicting stable bonds.

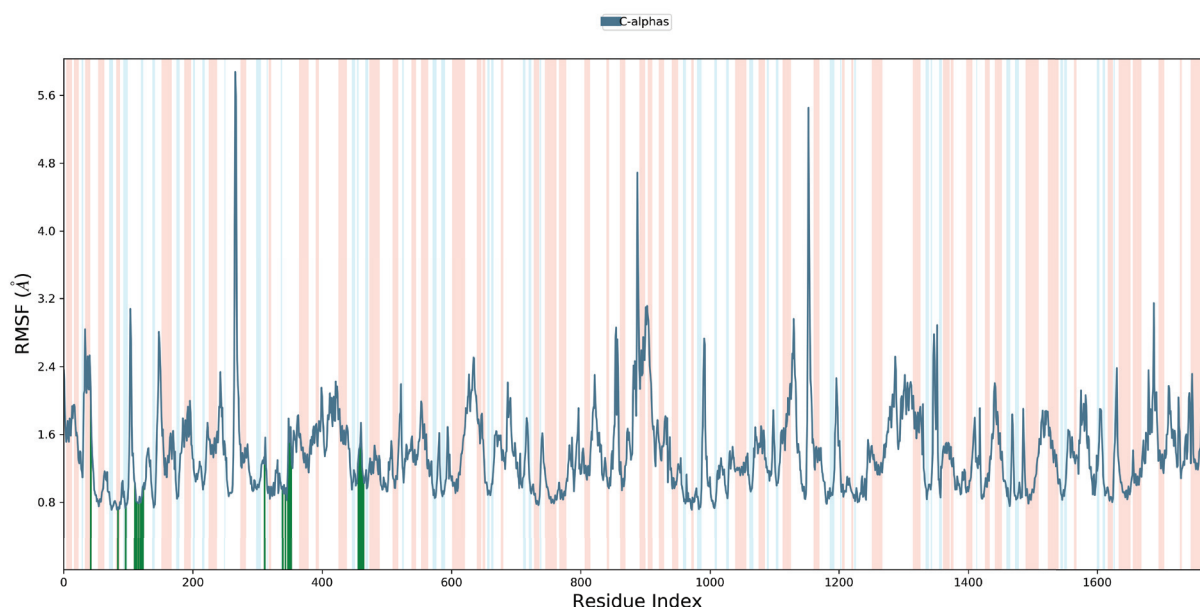


Fig. 6 RMSF Plot ursolic acid-nsp5 complex

Protein-ligand contacts analysis

Protein interactions with the ligand can be monitored throughout the simulation. These interactions can be categorized into four types: Hydrogen Bonds, Hydrophobic, Ionic and Water Bridges. Consideration of hydrogen bonds is importance due to their

strong influence on drug specificity. As shown in Fig. 7, the majority of the significant ligand-protein interactions determined with MD are water bridges, hydrogen bonds, hydrophobic interactions. Formation of ionic interactions was also seen to some extent. In terms of hydrogen bonds interaction, TRP

121 formed the strongest interaction, with in interaction fraction (IF) of 0.838, followed by ALA 470 (0.500), LEU 126 (0.465), GLU 466 (0.383), VAL 124 (0.137), and GLU 359 (0.112). In addition, residues TYR 89, SER 125, LYS

127, LYS 358, LYS 469, LYS 571, GLY 472 also participated in hydrogen bond formation. The protein-ligand interactions maintained for more than 30% of the simulation time are detailed in the figure below.

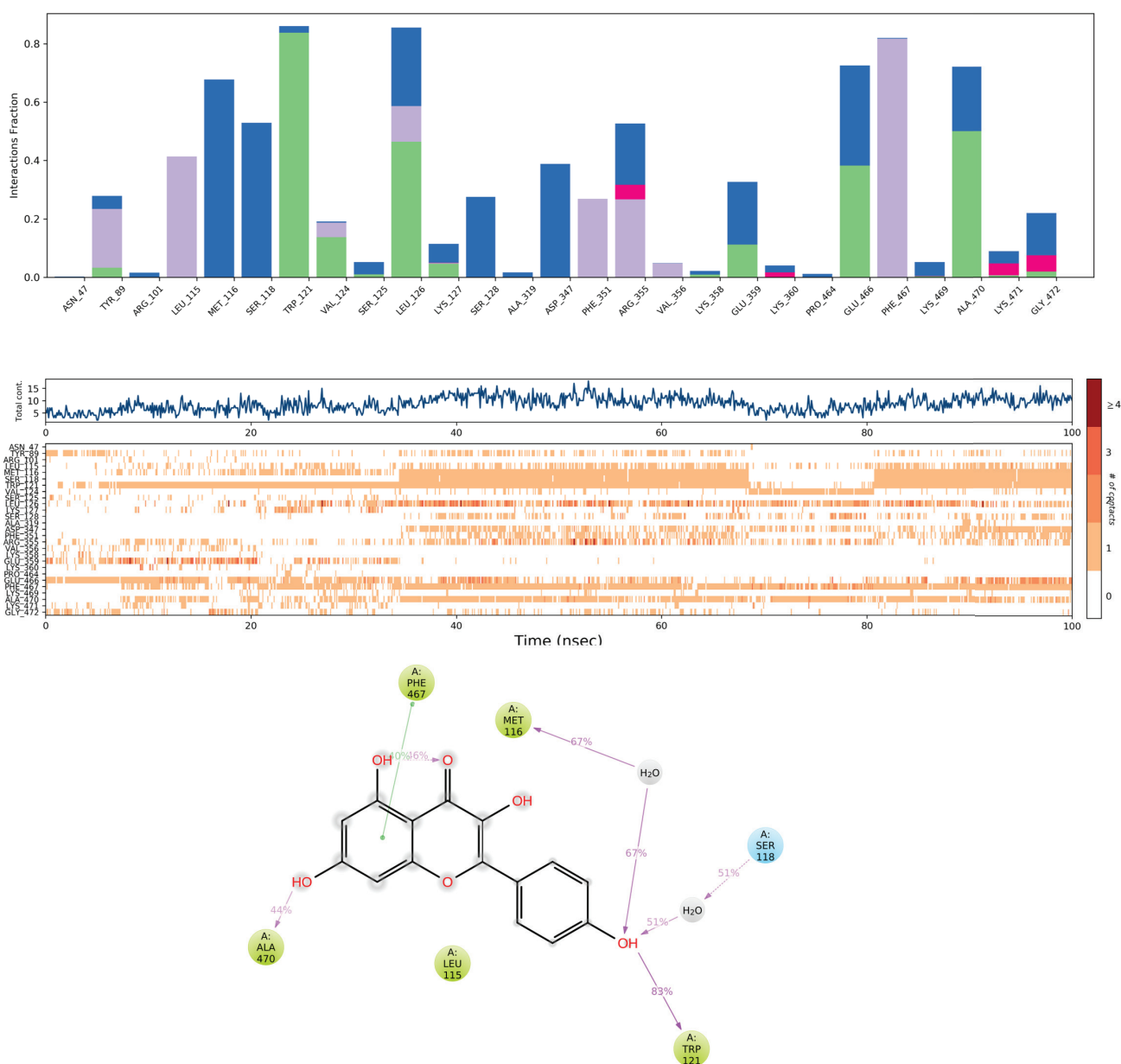


Fig. 7 Protein-Ligand interaction of ursolic acid-nsp5 complex

Ligand torsion analysis

Finally, we analyzed the torsional degree of freedom for the rotatable bonds of ursolic acid. The compound exhibited five rotatable bonds as shown in a color-coded manner in the ligand torsion analysis. Fig. 8 represents dial and bar plots of same color, where the dial plot exhibits the conformation of torsion throughout the simulation period, starting at

the center and evolving radially outwards. In addition, the bar plot briefs the results of dial plots and indicates the probability density of the torsion. In case the torsional potential information is available, it is shown at the y-axis of the bar plot, which may give information about the conformational strain the ligand undergoes in order to maintain a protein-bound conformation.

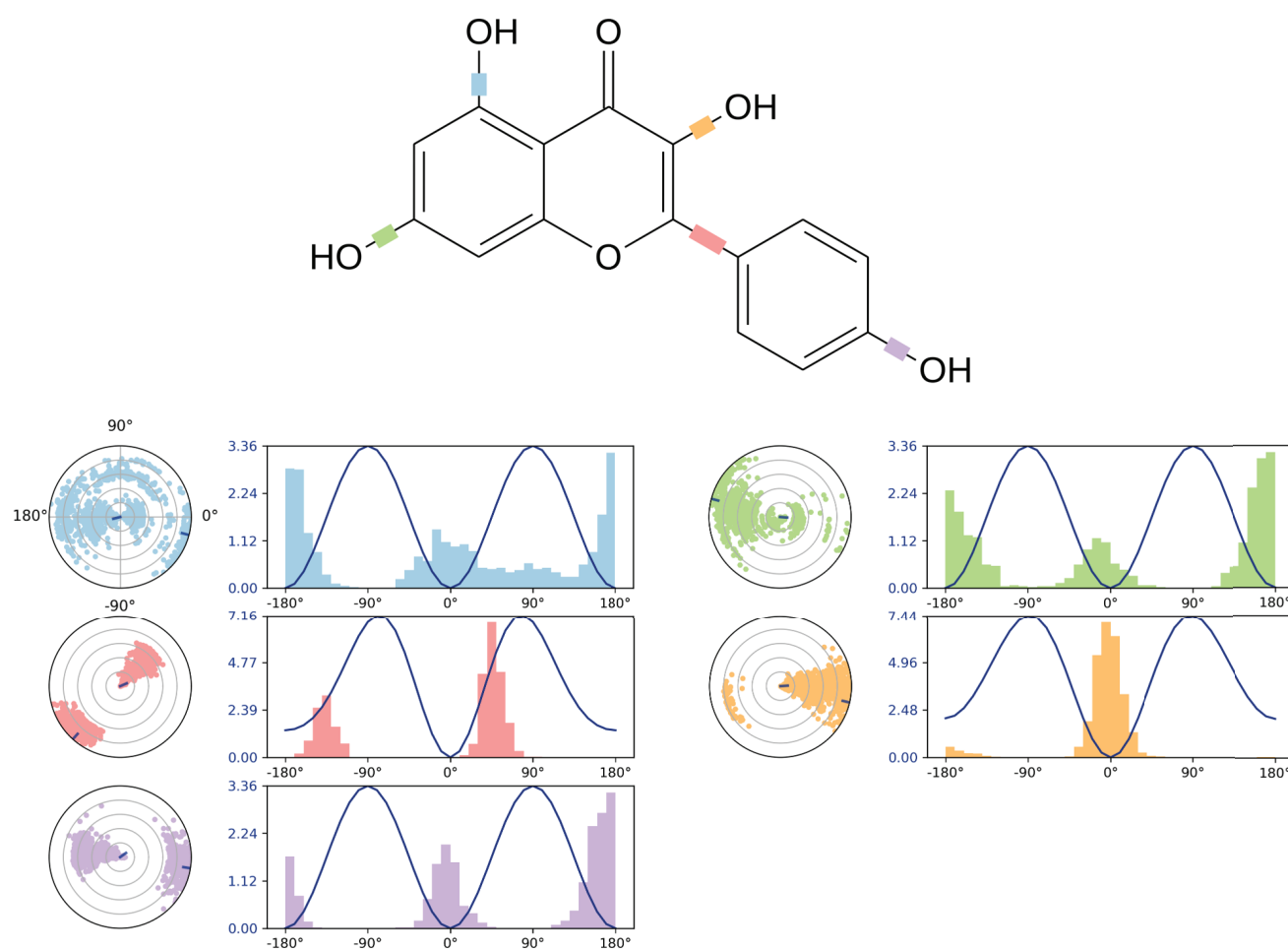


Fig. 8 Ligand torsion analysis of ursolic acid-nsp5 complex

Conclusion

Through a comprehensive computational approach, the present study investigates the promising potential of ursolic acid, a bioactive phytochemical derived from the medicinal plant *C. bonplandianus*, as a viable inhibitor against key proteins associated with Japanese encephalitis virus (JEV). Through molecular docking analysis and molecular dynamics simulation, this study has demonstrated the strong binding affinity of ursolic acid with JEV env, cap, and nsp5 proteins. The utilization of virtual screening methods in drug discovery not only enhances efficiency but also mitigates side effects, reduces costs, and accelerates the overall process. Ursolic acid not only exhibited robust binding to target proteins but also demonstrated favorable pharmacokinetic

properties, bioavailability, and bioactivities, suggesting its safety and potential as a drug candidate. Further, Molecular dynamics simulations confirmed the stability of the protein-ligand complex of ursolic acid with nsp5. This research provides a solid foundation for advancing the exploration of ursolic acid as a therapeutic agent for combating JEV. The identified inhibitory effects on nsp5, a crucial player in JEV progression, offer a promising avenue for the development of targeted therapies for Japanese encephalitis. These findings pave the way for further research and development aimed at translating these insights into practical applications for the treatment of JEV infections.

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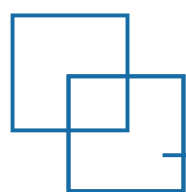
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CHAPTER 07

Reported Pharmacological Profile



INTRODUCTION

The earliest known medical practise is the use of phytomedicine, often known as traditional medicine. Many plant resources are employed for a long and healthy life as well as to treat various disorders. From generation to generation, the traditional understanding of plant-based healing has been passed down. This traditional wisdom has been acquired by many ethnic groups in India and throughout the world as ethnopharmacological knowledge. Even now, many people rely mostly on phytomedicines to treat a variety of illnesses in several other South Asian, South American, and Arabian nations. Ayurveda, the science of long life, has its roots in Indian tradition dating back to the Vedic era. The two founders of the Ayurvedic medical system, 'Susrata' and 'Charaka', discussed the therapeutic use of several medicinal plants. One such plant, *Croton bonplandianus* Baill. (Euphorbeaceae), popularly known as 'Ban-tulsi', is well-known for its effectiveness as a remedy for a variety of ailments in complementary and alternative medicine systems around the globe (Dutta and Chaudhuri, 2018). Known by several names as 'Kala bhanghra' or 'Paitiya' (Marma), this perennial plant primarily grows as a bush and is found around canals, riverbanks, wastelands, and roadside areas. Native to southern Bolivia, Paraguay, South-Western Brazil, and North America, the plant is also found in West Bengal, India's Sub-Himalayan area. The plant's parts are widely used in traditional medicine for a variety of conditions, including swelling of the body, treatment for skin diseases and ringworms, and is antihypertensive, antioxidant, wound healing, antifungal, antimicrobial, antidiabetic, antitumor, anticancer, treat acute constipation, abdominal dropsy, internal abscesses, antispasmodic, antiseptic, antidote, analgesic, insect repellent, nematocide, anti-coronary, anti-inflammatory, hepatoprotective, larvicidal activity, anthelmintic, etc. The plant is in great demand because of its sluggish pace of traditional multiplication (Ghosh et al., 2018).



Pre-clinical Study

Numerous preclinical research has been conducted on *C. bonplandianus* to look at possible medical benefits. Before proceeding with human clinical trials, preclinical investigations are usually carried out on animals or *in vitro* (outside the human body) to evaluate the safety and effectiveness of a chemical. This species may have anti-inflammatory, anti-cancer, and antimicrobial properties, according to recent studies. It usually takes more investigation, including human clinical studies, to validate these effects and establish safe and efficient doses for therapeutic use. Here are some potential areas of study related to *C. bonplandianus*.

Anthelmintic Activity

Hapse et al. (2012) performed the anthelmintic activity of aqueous, ethanolic and petroleum ether extracts (20, 40 and 60 mg/ml) of *C. bonplandianum* leaves against adult Indian earth worm *Pheretima posthuma*. All extracts showed anthelmintic effect with values of 0.65 to 14.33 min (time for paralysis) and, 2 to 18 min (time for death) as compared to albendazole (38 and 43.33 min) used as standard drug.

Anti-cancer Activity

Bhavana et al. (2015) evaluated the anti-cancer activity of acetone leaves extract from *C. bonplandianus* on lung cancer cell line A549 using MTT assay. The extract showed inhibitory effect with IC_{50} of 15.68 $\mu\text{g/ml}$ as compared to cisplatin (2.20 $\mu\text{g/ml}$) as standard drug for

A549 cells. The number of cells in G2/M phase increased with concurrent accumulation of cells in sub G0/G1 phase indicates the induction of apoptosis at G2M phase.

Suresh et al. (2021) studied the anti-cancer effect of ethanol fraction from *C. bonplandianus* leaves against haematological cancer cells; K562 cells (chronic myelogenous leukemia) and Raji cells (Burkitt lymphoma). The fraction exhibited anti-cancer effect with CC_{50} of 86.33 and 470 $\mu\text{g/ml}$ for K562 and Raji cells using MTT assay.

Anti-inflammatory Activity

Thenmozhi et al. (2013) assessed the anti-inflammatory effect of ethanolic extract from *C. bonplandianus* leaves against hypotonicity-induced human red blood cell membrane (HRBC) lysis. The extract prevents hypotonicity induced HRBC membrane lysis to an extent of 83.2% at 200 mg/ml when compared with hydrocortisone (89.7%) used as standard.

Anti-microbial Activity

Suresh et al. (2021) studied the anti-bacterial activity of ethanolic fraction from *C. bonplandianus* leaves against *Escherichia coli*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *K. pneumoniae*, and *Pseudomonas aeruginosa* by broth microdilution assay. The fraction exhibited anti-bacterial effect with MIC ranging from 256 to 1024 $\mu\text{g/ml}$ for tested microorganisms.

Basak et al. (2021) demonstrated the anti-microbial activity of aqueous and methanol extracts of *C. bonplandianus* leaves against *Bacillus cereus*, *Lactobacillus plantarum*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Enterobacter aerogens*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli* using broth dilution method. Both extracts exhibited anti-microbial potential with MICs ranging from 64 to 556 µg/ml for tested microorganisms.

Saggoo et al. (2010) evaluated the anti-microbial potential of aqueous, acetone and methanol leaves and seeds extract of *C. bonplandianum* against *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* by disc-diffusion method. All leaves and seeds extracts exhibited anti-microbial effect with zone inhibitions ranging from 11 to 37 and, 13 to 38 mm for tested microorganisms except *P. aeruginosa* as compared to chloramphenicol (23 to 45 mm) used as standard.

Ghosh et al. (2018) studied the anti-bacterial activity of ethyl acetate, benzene, n-hexane, chloroform, and methanol extracts (25-300 mg/ml) of *C. bonplandianum* leaves against *Staphylococcus haemolyticus* and *Salmonella enterica* ser. *typhi* using agar well diffusion method. All extracts exhibited anti-bacterial potential with zone inhibitions ranged from 15.3 to 18 mm and, 14.6 to 20.6 mm, respectively, for both tested strains.

Anti-nociceptive Activity

Yasin et al. (2022) evaluated the anti-nociceptive activity of n-hexane, chloroform, ethyl acetate and water extracts (81, 54 and 27 mg/kg) of *C. bonplandianus* leaves against acetic acid-induced writhing, formalin-induced hind paw licking and hot plate test in Wistar albino mice. All extracts at dose of 81 mg/kg showed significant reduction ($p < 0.05$ - 0.001) in acetic acid-induced writhes and, abdominal contraction as compared to acetyl salicylic acid (ASA) used as standard. Also, it exhibited marked reduction ($p < 0.05$ - 0.001) in paw licking after formalin induction in treated mice as comparable to ASA. In hot plate method, methanol and aqueous extracts at doses ranging from 27 to 81 mg/kg) showed an increase ($p < 0.01$ and $p < 0.001$) in latency response in mice.

Antioxidant Activity

Vijayamuthuramalingam et al. (2017) evaluated the antioxidant activity of methanol extract (CE) and its n-hexane (HeF), ethyl acetate (EaF) and chloroform fractions (ChF) of *C. bonplandianus* leaves using DPPH, hydroxyl radical, reducing power capacity and nitric oxide scavenging assays. In DPPH and hydroxyl radical assays, ChF showed excellent scavenging effect with IC_{50} of 72.13 and 85.92 µg/ml, followed by other fractions (133.79, 450.21 and 602.34 µg/ml) and (152.91, 295.25, 774.81 µg/ml) as compared to ascorbic acid (3.5 and 6.8 µg/ml) used as standard. All extracts showed reducing power effect with values of 0.79, 0.68, 0.60 and 0.45, whereas

ChF, EaF and CE exert higher nitric oxide scavenging effect (33.80, 57.25 and 75.31 $\mu\text{g/ml}$) but, HeF (344.81 $\mu\text{g/ml}$) was less effective as compared to standard (12.6 $\mu\text{g/ml}$).

Bhavana et al. (2015) evaluated the antioxidant effect of acetone leaves extract of *C. bonplandianus* showed highest DPPH scavenging effect with IC_{50} of 26.97 $\mu\text{g/ml}$ at 1 mg/ml dose as compared to ascorbic acid (1.28 $\mu\text{g/ml}$) used as positive control.

Qaisar et al. (2013) performed the antioxidant activity of methanol and dichloromethane extracts of *C. bonplandianum* using DPPH assay. The methanolic extract showed DPPH scavenging effect with IC_{50} value 396.205 $\mu\text{g/ml}$ as compared to gallic acid used as standard (4.3 $\mu\text{g/ml}$), whereas dichloromethane extract was found to be inactive.

Enzyme Inhibitory Activity

Vijayamuthuramalingam et al. (2017) evaluated the enzyme inhibitory activity of methanol extract, its n-hexane, ethyl acetate and chloroform fractions of *C. bonplandianus* leaves showed highest to least inhibitory effect with IC_{50} values ranging from 95.78 to 242.48 $\mu\text{g/ml}$ and, 126.81 to 679.04 against α -amylase and α -glucosidase inhibitory assays as compared to acarbose (84.50 and 107.24 $\mu\text{g/ml}$) used as standard.

Qaisar et al. (2016) reported the enzyme inhibitory activity of 2H-1-benzopyran-2-one (F), betulin (G) and 3, 5-dimethoxy 4-hydroxy cinnamic acid (I) isolated from

C. bonplandianum. It was reported that they possess more potent α -glucosidase inhibitory effect with values ranging from 23 to 26.7 μM than acarbose (38.2 μM) used as positive control.

Qaisar et al. (2014) screened the enzyme inhibitory effect of dichloromethane and methanol extracts of *C. bonplandianus* against α -glucosidase inhibition assay. Only dichloromethane extract exhibited α -glucosidase inhibition with IC_{50} of 14.93 mg/ml as compared to acarbose (38.25 mg/ml) used as reference.

Haemolytic Activity

Ghosh et al. (2018) evaluated the haemolytic activity of ethyl acetate, chloroform, and benzene extracts (300, 150, 75, and 25 $\mu\text{g/ml}$) of leaves of *C. bonplandianum* against human erythrocytes. All extracts exhibited very low haemolytic effect with percentage haemolysis to be 162.5, 131.5 and 156.25% at 300 $\mu\text{g/ml}$ dose.

Hepatoprotective Activity

Dutta et al. (2018) investigated the hepatoprotective activity of hydro-methanolic extract (50-200 $\mu\text{g/ml}$) of *C. bonplandianus* against carbon tetrachloride (CCl_4)-induced liver injury in albino Swiss mice. The extract showed reduction in liver marker enzymes and biochemical parameters except protein and albumin. Treatment with extract increased the enzymatic activity of catalase but, reduced glutathione. Further, the extract

also decreased TNF- α , nitric oxide (NO) and lipid peroxidation (MDA levels) but, peroxidase activity was elevated.

Larvicidal Activity

Deepa et al. (2015) evaluated the larvicidal activity of methanol leaves extract of *C. bonplandianus* against third instars larvae of *Aedes aegypti* mosquito. The extract exhibited larvicidal effect with LC₅₀ value of 131.72 ppm and, 40.5% mortality at 300 ppm.

Patel et al. (2000) performed the larvicidal effect of methanol leaves extract of *C. bonplandianus* against fourth instars larvae of *Aedes aegypti* mosquito. The extract exhibited larvicidal effect with LC₅₀ value of 123.8 ppm and 96.6% mortality at 200 ppm.

Jeeshna et al. (2010) studied the larvicidal activity of methanol leaves extract of *C.*

bonplandianus against fourth instar larvae of the mosquito *Aedes aegypti*. The extract exhibited larvicidal effect with LC₅₀ value of 123.8 ppm.

Nephroprotective Activity

Yasin et al. (2023) investigated the nephroprotective activity of aqueous extract of *C. bonplandianus* leaves against gentamicin-induced liver and kidney injuries in albino Wistar rats. The extract significantly reduced ($p < 0.01$) the serum levels of SGPT, ALT, GGT, bilirubin, creatinine, and urea, while no significant changes found in levels of ions and uric acids. However, the extract exhibited alterations in liver and kidneys tissues of treated rats. In addition, histological examination of liver and kidney confirmed the biochemical changes in treated rats.

Toxicological Profile

C. bonplandianus is known to contain several toxic compounds, including diterpenes, tiglane, and phorbol esters. These compounds can cause a variety of toxic effects in humans and animals, including skin irritation, dermatitis, and gastrointestinal upset. Yasin et al. (2023) evaluated the brine shrimp lethality of n-hexane, chloroform, ethyl acetate and water extracts (12.5-800 mg/kg) of *C. bonplandianus* leaves against *Artemia salina* (brine shrimp) eggs. All extracts showed LC₅₀

values of 303.78, 337.23, 419.03, 1392.22, and 334.91 $\mu\text{g/ml}$ as compared to vincristine (1.974 $\mu\text{g/ml}$) used as standard. Qaisar et al. (2013) evaluated the toxicity of methanolic extract of *C. bonplandianum* and reported its toxicity with LD₅₀ value of 115.76 $\mu\text{g/ml}$ against *A. salina*. Ajoy and Padma (2013) reported that the brine shrimp lethality assay of 50% ethanolic extract of *C. bonplandianus* was very effective with LC₅₀ at the concentration of 46.7 mg/l.

Summary and Future Outlook

C. bonplandianus is a small shrub or herb native to South America, from southern Bolivia to Uruguay. It is found in a variety of habitats, including grasslands, forests, and wetlands. The plant is known for its small, white flowers and its leaves, which are lanceolate or sub-lanceolate and have a serrate margin. This species has a long history of use in traditional medicine. The leaves are used to treat a variety of ailments, including fever, diarrhea, and stomachache. The plant is also used as a pesticide and a hair tonic. More recently, this species has been studied for its potential use as a source of bioactive compounds. The plant has been found to contain several compounds with potential anticancer, anti-inflammatory, and antimicrobial properties. This species is a promising plant with a wide range of potential

uses. Further research is needed to fully understand the plant's bioactive compounds and their potential therapeutic applications. Additionally, more work is needed to develop sustainable cultivation practices for this plant. This species has been shown to inhibit the growth of cancer cells *in vitro*. Further research is needed to determine if the plant is effective in treating cancer in humans. Also, this species has been shown to reduce inflammation in animal models. Further research is needed to determine if the plant is effective in treating inflammatory diseases in humans. Also, this species has been shown to be effective against several bacteria and fungi. Further research is needed to determine if the plant can be used to treat infectious diseases in humans.

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