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Monographs on Medicinal Plants along Ganga River
Ranunculus sceleratus L.

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

Volume 6

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Volume 06

Sponsored by

National Mission for Clean Ganga (NMCG),
Department of Water Resources, River Development & Ganga Rejuvenation,
Ministry of Jal Shakti, New Delhi

Code: Ad-35013/4/2022-KPMG-NMCG

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Publisher	:	**Patanjali Organic Research Institute Private Limited (PORI)** Food & Herbal Park, Village-Padartha, Laksar Road, Haridwar-249407, Uttarakhand (India) Tel: + 91-8755904985
Website	:	www.patanjalibio.com
Email	:	porihr@patanjalibio.com
Research Supported by	:	**Patanjali Research Foundation Trust**
Publication Support	:	The Ministry of Jal Shakti, Government of India Website: https://jalshakti-dowr.gov.in/
Year	:	2024

This monograph is an outcome of the project entitled

"Scientific Exploration of Floral Diversity Near the Ganga Riverbanks for Ethnobotanical Purposes along withtheir Conservation and Economic Development of the Region via SkillDevelopment Programs" of The Ministry of Jal Shakti, Government of India

Implemented by Patanjali Organic Research Institute Pvt. Ltd. (PORI)

Food & Herbal Park, Village-Padartha, Laksar Road, Haridwar-249407, Uttarakhand (India)

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'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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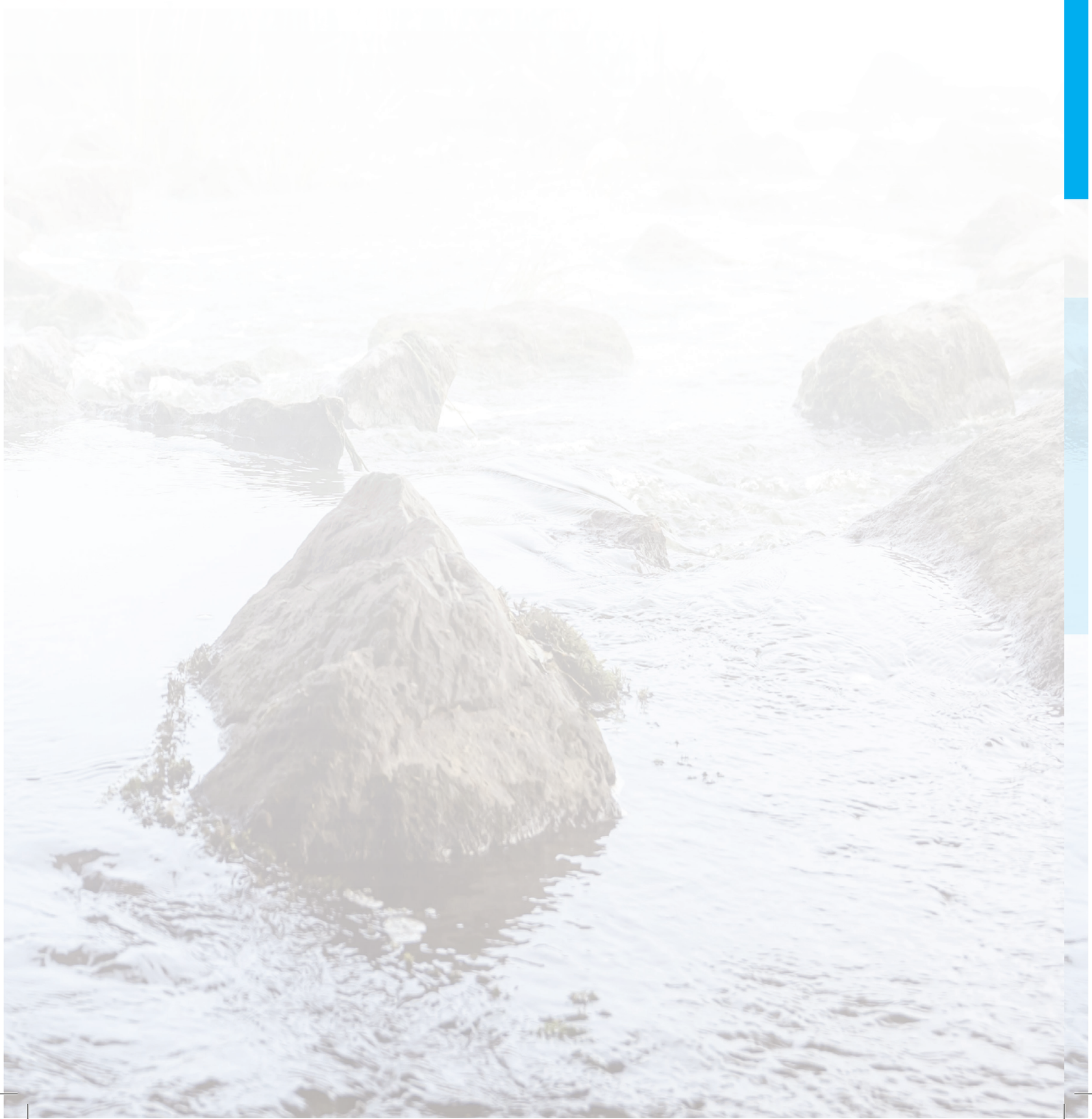

Vision of Union Minister for Jal Shakti

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.

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

Ministry of Jal Shakti (MoJS)

- Shri Chandrakant Raghunath Patil, Hon'ble Minister
- Ms. Debashree Mukherjee, Secretary

National Mission for Clean Ganga (NMCG)

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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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Rajeev Kumar Mital, IAS
DIRECTOR GENERAL
NATIONAL MISSION FOR CLEAN GANGA

सत्यमेव जयते

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)

FOREWORD

In 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 andbotanical 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, insilico 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.

आचार्य बालकृष्ण

Dr. Acharya Balkrishna

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Classical Ayurvedic Insight

Ranunculus sceleratus L.

Vedic name : (Kāṇḍīrakaḥ dīrghavṛntaḥ) (काण्डीरक: दीर्घवृन्त:)

Botanical name : *Ranunculus sceleratus* L.

Utpatti sthānam

Mūlato madhyamodīcyayūrope cīnabhūmiṣu.
Jāpāne brahmadeśe ca pākistāne tathaiva ca.
Pārasīkābhidhe deśe nepāle sīriyābhidhe.
Misradeśe ca labhyeta kāṇḍīravaṃgastaru.
Bhārate saritāṃ prānte tathā coṣṇahimālaye.
Parvatīyāsu ghāṭīṣu jammūkaśmīrayorapi.
Vaṅge cā'samasaṃkhyātaprānteṣvapi ca sarvata. (Saumitreya Mahodadhiḥ: 1-4)

Distribution

It is indigenous to central and northern Europe also occurs in Japan, China, Myanmar, Nepal, Pakistan, Iran, Syria and Egypt. In India it found form on the bank of the dryers and in warm valleys of Himalaya from Jammu Kashmir to Bengal, Assam an altitude of 1500 m and throughout Northern India. [1-4]

Vānaspatika vivaraṇam

1. Kāyika lakṣaṇam

Sahasravyāmakoddeśe labhyate nātra saṃśaya.
Māṃsalaścāpi varṣāyuḥ śākīyaścikkaṇastathā.
Aratnilambitāt sārdhadvihastalambitastaru.
Kāṇḍaṃ ca māṃsalaṃ sthūlaṃ śākhitaṃ cordhvata khalu.
Kadācit sūkṣmaromāḍhyaṃ pratibhāti svarūpata.
Kāṇḍantathaiva śākhāśca suṣirānvitavigrahāḥ.
Sāndrakhātayutāścāpi śūnyaromatayoditāḥ.
Patraṃ stambhānubandhīha mūlājjātantathaiva ca.
Viṣamapatrasaṃyuktaṃ yavonāṅgulavigrahāt.
Yavonadvyaṅgulavyāsaṃ vṛkkākārayutantathā.
Ādhāreṣu tridhā nūnaṃ pravibhājitakāyakam.
Khaṇḍā adhomukhāṇḍābhāḥ phaṇākārayutā api.
Vṛntahīnāstathā naikapāliyuktāstathā'thavā.
Sadantāḥ pratibhāsante parṇavṛntantu tatra vai.

Sapādāṅgulakocchrāyād dvimuṣṭilambitaṃ puna.

Aromaśaṃ ca vidyeta tadūrdhvastambhagaṃ kila.

Patraṃ vai vṛntahīnañca tridhaiva pravibhājitam.

Saṅkucadrūpasaṃbhinnadīrghāyāmīyakhaṇḍayuk.

Aromaśantathā pūrṇamathavā danturaṃ bhavet. (Saumitreya Mahodadhiḥ: 4-13)

Botanical Description

1. Vegetative Characters

An annual, erect, succulent, glabrous herb 30-90 cm high. Stem stout, fleshy and branched, rarely puberulous above. Stem and branches hollow, deeply furrowed, glabrous. Leaves basal and cauline, heterophyllous. Radical leaves 18-37 mm in diameter, reniform, 3-partite almost to the base, segments obovate, cuneate, variously lobed or notched. Petioles glabrous of the radical leaves variable in length, from 2.5-15 cm long. Upper cauline leaves sessile, 3-partite, segments narrow-oblong, entire or toothed, glabrous. [1-4]

2. Puṣpīya lakṣaṇam

Puṣpīyalakṣaṇe puṣpaṃ triyavavyāsata kila.

Sayavārdhāṅgulavyāsaṃ hrasvarūpeṇa tiṣṭhati.

Pāṇḍurapītavarṇāsu samaśikhāsu sarvathā.

Śākhāsu vai samutpannaṃ bhāsate nātra saṃśaya.

Bāhīkadalapuñjantu pañcabāhyadalānvitam.

Bāhyadalaṃ natantāvad dīrghāyatakalevaram.

Dalena tulyamānañca rājatyeva tu romaśam.

Āśupāti ca vidyeta dalānāntu catuṣṭayam.

Teṣāṃ vai pañcakaṃ kvāpi sapādayavalambitāt.

Ardhayavāyatāt kvāpi sārdhayavamitāyatam.

Dīrghavṛttānusambaddhadīrghāyatasuvigraham.

Śvetavarṇatayotpannaṃ pratibhāti svarūpata.

Makarandasya ca granthirdalānāṃ paṭale sthita.

Śalkajātantathā svalpavikasadgolasaṃnibhaiḥ.

Kaṇṭakairmakarandānāṃ granthibhāgaṃ vihāya vai.

Āvṛṇotyanyabhāgāṃśca naike puṃskesarā matāḥ.

Puṃstantustu bṛhadrūpa prasūnadhūlikośaka.

Bṛhadrūpadhara pītavarṇo'tra pratibhāsate.

Yuktāṇḍapisvarūpāsta aṇḍapā bahava kila.

Anupasthitarūpaivaṃ vartikā parikīrtitā.

Phalaṃ dīrghāyatāṇḍābhamekalaśīrṣakaṃ puna.

Vellanākārasaṃpuṣṭaṃ triyavocchrāyata khalu.

Sārdhacaturyavocchrāyamanekaṃ hrasvakantathā.

Kuṇṭhāgramathavā nūnaṃ niśitāgrañca romaśam.

Ekalañcāpi bhāseta tatprasūnaphalodbhava.

Mārgaśīrṣāt samārabhya yāvaccaitraṃ pratīyate. (Saumitreya Mahodadhiḥ: 14-26)

2. Floral Characters

Flower small, 6-12 mm in diameter, pale yellow born in corymb branches. Calyx consists of 5 sepals reflexed, oblong, about equalling the petals, pubescent, caducous. Petals 4-5, 2.5×1-3 mm long and wide, elliptic-oblong, white. Nectar gland present on petal surface, scale poorly developed and forming crescent-shaped or circular ridge surrounding but not covering nectary. Stamens numerous. Filament large. Anthers large, yellow in colour. Carpels many, syncarpous. Style absent. Fruits oblong-ovoid, head of achenes cylindric, 6-9 mm long, achenes small, numerous, obtuse or apiculate, hairy. Flowering / Fruiting - November - March. [1-4]

Plant Anatomy

Prayojyāṅgam

Kāṇḍīrakasya taddīrghavṛntasya kāṇḍapatrakam.

Prayojyāṅgaṃ matañcāntaḥsaṃracanopavarṇyate. (Saumitreya Mahodadhiḥ: 1)

Antaḥ saṃracanekṣaṇe

(a) Kāṇḍabhāgaḥ

Vṛttīyaparidhau kāṇḍaprabhāgaḥ paridṛśyate.

Adhicarmaprabhāgo'tra tanūpacarmaṇāvṛtaḥ.

Tanmajjakośikā naikakośikīyābhidṛśyate.

Vāyavyasthānasaṃyuktā hyadhastvacā parisphuṭā.

Nopasthitaidhikā cātra saṃvahanakapūlakāḥ.

Saṃyuktapūlakaprotā bahiḥpravāhibandhagāḥ.

Te punastalabhāgeṣūpasthitā laghavastathā.

Subṛhatpūlakā atraikāntaritā iva sthitāḥ.

Saṃvahanakapūlāśca dṛḍhotakīyarūpakaiḥ.

Kośikānicayairdṛśyāḥ parivṛtā yathāyatham.

Majjā subṛhatī vīkṣyā kendrabhāge'nupasthitā.

Kāṇḍīrakasyakāṇḍāntaḥsaṃracanā prabhāṣitā. (Saumitreya Mahodadhiḥ: 2-7)

Plant Parts

Stem, Leaf

Microscopic Characters

Stem: Stem shows circular in outline with a thin cuiticle covering epidermis. Cortex is multicellular, having air-cavities. Hypodermis is distinct and cambium absent.Vasculer bundles are conjoint, collateral and closed. Vasculer bundles are present in peripheral region consisting of smaller and larger bundles arranged alternately. Vasculer bundles are surrounded by sclerenchyma. Pith is large with central hollow region. [1,5]

(b) Patram

Sṛṣṭiḥ pṛṣṭhādharīyāsya laghvī tathaikakośagā.

Tanubhittimayī dṛśyā granthilai romasañcayaiḥ.

Parivṛtā tathā randhravyūho'niyatakośikāḥ.

Kāṇḍīrakasyapatrāntaḥsaṃracanā pracoditā. (Saumitreya Mahodadhiḥ: 8-9)

Leaf

Dorsiventral structure is covered with small, unicellular, thin walled, glanduler hairs. Stomata are anomocytic. [1,5]

Medicinal Uses

Thoracic Diseases

Tryūṣaṇaṃ triphalāṃ drākṣāṃ kāśmaryāṇi paruṣkam. .....

.....brāhmī tāmalakīṃ medāṃ kākanāsāṃ śatāvarīm. .....

.....jvaragulmāruciplīhaśirohṛtpārśvaśūlanut.

Kāmalārśo'nilāṣṭhīlākṣataśoṣakṣayāpaham.

Tryūṣaṇaṃ nāma vikhyātametad ghṛtamanuttamam.

Iti tryūṣaṇādyaṃ ghṛtam. (Ca.Ci. 18:39-42)

Cough- *Ghṛta* (Clarified butter) cooked with *Zingiber officinale* (Dry ginger root), *Piper nigrum* (Black pepper), *Piper longum* (Long pepper), *Martynia annua* (Devils claws), *Asparagus racemosus* (Asparagus), *Tribulus terrestris* (Caltrop) and other herbs taken in an appropriate dose is useful in treating cough, fever, *Gulma*, anorexia, splenomegaly, cephalic diseases, etc. [6] (Ca.Ci. 18:39-42)

Musculoskeletal Diseases

Bhūtikairaṇḍavarṣābhūrāsnāvṛṣakarohiṣaiḥ. .....

Sahācaravarīviśvākākanāsāvidāribhiḥ.Yavamāṣātasīkolakulatthaiḥ kvathite śṛtam.

Jīvanīyapratīvāyaṃ tailaṃ kṣīraṃ caturguṇam.

Jaṅghorutrikapārśvāṃśabāhumanyāśiraḥsthitān.

Hanyādvātavikārāṃstu bastiyogairniṣevitam. (Su.Ci. 37:19-22)

Vāta associated disorders- *Cymbopogon schoenanthus* (Camel grass), *Ricinus communis* (Castor), *Boerhavia diffusa* (Hogweed), *Pluchea lanceolata* (Rasna), *Martynia annua* (Devils claws), *Hordeum vulgare* (Barley), *Vigna mungo* (Black gram), *Macrotyloma uniflorum* (Horse gram) and other flavouring agents (Prakṣepaka dravya) cooked in 4 parts of water and used for enema therapy (vasti) to treat the diseases of leg, thigh, sacral region, flanks, dorsal part, shoulder, sternomastoid region, *Vāta* associated disorders of cephalic region. [7] (Su.Ci.37:19-22)

Dematological Diseases

Pūtīkadārujaṭilāḥ pakvasurā kṣaudramudgaparṇyoṃ ca.

Lepaḥ sakākanāso maṇḍalakuṣṭhāpahaḥ siddhaḥ. (Ca.Ci. 7:122)

Maṇḍala Kuṣṭha (Type of leprosy)- Paste prepared from the equal quantity of *Cedrus deodara* (Deodar), *Nardostachys jatamansi* (Spikenard), *Martynia annua* (Devils claws) *Caesalpinia bonducella* (Bonduce nut) and other herbs applied externally is very much beneficial in treating *Maṇḍala Kuṣṭha* (a specific type of Kuṣṭha explained in ayurvedic texts). [6] (Ca.Ci. 7:122)

Part Used

Leaf.

Dose

Powder 1-3gm or as directed by the physician.

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Abbreviation

A.Hṛ.Ci.	:	AṣṭāṇgaHṛdayaCikitsā
A.Hṛ.U.	:	AṣṭāṇgaHṛdaya Uttara Tantra
As.Hr.Ut.	:	AṣṭāṇgaHṛdaya Uttara Tantra
Bhā.Pra.Ci.	:	BhāvaprakśaCikitsā
Śā.Sa.Ut.Kha.	:	ŚāraṇgadharaSamhitā Uttara Khaṇḍa
Ca.Ci.	:	Caraka SamhitāCikitsāSthāna
Ca.Da.Ci.	:	CakradattaCikitsā
Ca.Si.	:	Caraka Samhitā Siddhi Sthāna
Ca.Sū.	:	Caraka SamhitāSūtraSthāna
Ck.Dt.Ci.	:	CakradattaCikitsā
Ga.Ni.Kā.Ci.	:	GadanigrahaKāyacikitsā
Ga.Ni.Kau.Taṃ.	:	GadanigrahaKaumaryatantra
Ga.Ni.Śā.Ta.	:	GadanigrahaŚālākya Tantra
Ga.Ni.Śa.Taṃ.	:	GadanigrahaŚalyatantra
Hā.Sa.	:	HārāitaSamhitā
Hā.Saṃ.	:	HārāitaSamhitā
Kā.Saṃ.Khilasthāna	:	KāśayapaSamhitāKhilasthāna
Rā.Mā.	:	RājaMārtaṇḍa
Ra.Ra.Sa.	:	Rasa Ratna Samuccaya
Śā.Ni.Guḍūcyādivarga	:	ŚāligrāmaNighaṇṭuGuḍūcyādivarga
Śā.Sa.U.Kha.	:	ŚāraṇgadharaSamhitā Uttara Khaṇḍa
Śā.Saṃ.Ma.Kha.	:	ŚāraṇgadharaSamhitā Madhyam Khaṇḍa
Sd.Bh.Mm. Ch. Gr.Ci.	:	Siddha BheṣajaMaṇimālāCaturthaGucchaGrahaṇāiCikitsā
Sd.Bh.Mm. Ch. Nd.Vr.Ci.	:	Siddha BheṣajaMaṇimālāCaturthaGuccha Nāḍāivraṇa Cikitsā
Sd.Bh.Mm. Ch.Nt.Rg.	:	Siddha BheṣajaMaṇimālāCaturthaGuccha Netra RogaCikitsā
Sd.Bh.Mm.Ch. Ars.Ci.	:	Siddha BheṣajaMaṇimālāCaturthaGucchaArśaCikitsā
Sd.Bh.Mm.Ch. Vrn.Ci.	:	Siddha BheṣajaMaṇimālāCaturthaGucchaVraṇaCikitsā
Sh.Gr.Ng. Gd.Vg.	:	ŚāligrāmaNighaṇṭuGuḍūcyādivarga
Su.Ci.	:	SuśrutaSamhitāCikitsāSthāna

Su.Ka.	:	SuśrutaSamhitā Kalpa Sthāna
Su.Śā.	:	SuśrutaSamhitāSārīraSthāna
Su.Ut.	:	SuśrutaSamhitā Uttara Tantra
Vai.Jī. Caturthavilāsa	:	Vaidya JāivanamCaturthavilāsa
Vai.Jī. Dvitīyavilāsaḥ	:	Vaidya JāivanamDvitīyavilāsaḥ
Vai.Ma.	:	Vaidya Manoramā
Vaṃ. Se. Āmavātarogādhikāraḥ	:	VaṃgasenaSamhitāĀmavātarogādhikāraḥ
Vaṃ.Se. Vātavyādhyidhikāraḥ	:	VaṃgasenaSamhitāVātavyādhyidhikāraḥ
Vaṃ.Se.Karṇarogaḥ	:	VaṃgasenaSamhitāKarṇarogādhikāraḥ
Vaṃ.Se.Medarogaḥ	:	VaṃgasenaSamhitāMedorogādhikāraḥ
Vaṃ.Se. Netrarog	:	VaṃgasenaSamhitāNetrarogādhikāra
Vaṃ.Se. Śothādhikāraḥ	:	VaṃgasenaSamhitāŚothādhikāraḥ
Vaṃ.Se. Strīrogaḥ	:	VaṃgasenaSamhitāStrīrogaḥ
Vaṃ.Se.Vātavyādhiḥ	:	VaṃgasenaSamhitāVātavyādhyidhikāraḥ
Vṛ.Mā.	:	Vṛndamādhava

CHAPTER

01

Plant Exploration and Botanical Study

INTRODUCTION

Water is the key resource required to sustain life on this planet. It is found in most of the earth as in the ocean, rivers, ponds, streams, ice, etc. The river Ganga is an important source of water in India that makes an important river system from the Himalaya to the Bay of Bengal. The Indian civilization and economy depended on this river system due to its water availability throughout the year (Paul and Sinha, 2013). The river Ganga alone accounts for 25% of India's total water resources (Paul, 2017). Globally more than 300 million people from India, Nepal, and Bangladesh depend on the river Ganga (Gopal, 2000). This is the thirtieth longest river in the world and covers a basin area of 861,404 km^2 (Rahaman, 2009a). This basin is the most populated area in the world with an average density of 520 persons/km^2 (Das and Tamminga, 2012). The basin is very rich in heritage, cultural, and religious values. India's about one-fourth area drains by the river Ganga.

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

Up to Haridwar it flows in the hills after here it enters the plain area. From here it flows southwards, passing through the plains of Uttar Pradesh starting from Bijnor district. Up to Ballia it continuously flows in Uttar Pradesh through different districts after here it enters Bihar near Chausa of Buxar district. It flows up to Katihar district in Bihar and from here it enters the Sahebganj district of Jharkhand. Then it turns southwards and enters 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 m^3/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 m^3 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 *Ranunculus sceleratus* was considered in this study. It is a member of Ranunculaceae that is native to North America and Eurasia and is commonly known as celery-leaved buttercup, cursed crowfoot, cursed buttercup, and ditch crowfoot. The plant can grow up to 20 inches tall, with smooth, hollow stems that branch above and support many flowers. Although this plant is harmful in general, eating it will not be very dangerous. Burning mouth, stomachache, vomiting, and bloody diarrhea are some of the symptoms that can occur from touching the plant and produce mild skin irritation that lasts for a few minutes. The leaves and roots are used topically as an antirheumatic in medicine. The tonic-like seed is used to cure spermatorrhoea, rheumatism, colds, and general debility. On this basis, the current chapter is focused on the morphology, taxonomy, anatomy, and distribution assessment of *R. sceleratus* around the Ganga River.

Genus *Ranunculus* L.

The genus *Ranunculus* includes almost 1750 species found worldwide, ranging from temperate and subtropical regions to tropical mountains in their natural habitat (POWO, 2024). Among the worldwide data, around 41 species and 4 varieties are found in India (Srivastava, 2010). It is an annual or perennial, terrestrial and/or aquatic herb. Its rootstock is tuberous or horizontal and the roots are often fibrous. The stem is erect, decumbent, prostrate, creeping, or stoloniferous. The leaves are radical and cauline, whorled or alternate on stem, often palmatifid or palmatipartite, ternate on lobed or dissected. The flowers are white or yellow in color.

Twenty-five species of *Ranunculus* from India, Afghanistan, Tibet, and Sri Lanka were included in Hook. f. and Thomson's (1855) *Flora Indica* under the four divisions of Batrichium, Ceratocephalus, Hecatonia, and Echinella. They subsequently listed 21 species and 6 varieties from India and its subcontinents in Flora of British India vol. I (1872). Several of the species listed in *Flora Indica*—namely, *R. radicans*, *R. caespitosus*, *R. subpinnatus*, *R. fibrosus*, and *R. chinensis*—have been classified in Flora British India either as synonyms or as varieties. Hara and Williams (1979) listed 17 species and 7 varieties from Nepal, among these 14 species and 5 varieties are known to exist in India. In the Flora of India, Rau (1993) listed 33 species and 1 variety from over the current Indian border. Several intermediate forms under *R. pulchellus* have been proposed by him. In addition, the genus was the subject of taxonomic inquiry on the Asian continent by several scholars. Reidl (1991) listed 28 species in Flora West Pakistan, of which 22 are found in India. In Flora of China, Mao et al. (2001) listed 125 species, of which 20 species and 4 variants were also known to exist in India (Srivastava, 2010).

Traditional medicinal use and decorative plant cultivation are the two most popular uses of *Ranunculus* species. *Ranunculus* accounted for 0.4% of the overall turnover of cut flowers and leaves in 2014, according to data from FloraHolland in the Netherlands. With around 60 million stems, it was the 6th most popular plant for summer flowers sold at the Dutch Flower Auction. The Netherlands, Israel, and Italy are the top three countries from which Royal FloraHolland imports its product, according to data gathered between January 2015 and May 2017. Only a tiny portion (about 6-8%) come from Turkey, Tunisia, Ethiopia, and Kenya. Ecuador is a growing market for *Ranunculus*, cultivating 2.5–3 million tuberous roots annually, whereas Japan cultivates around 1 million tuberous roots annually on an estimated 10 hectares of cultivation space. During the 2014–2015 production season, which spanned from September 2014 to March 2015, in the Hyères market in France, fewer than a million stems from 35–40 different types were sold (SICA Marché aux Fleurs,

official statistics). With 132 million stems and 300–350 hectares of farmed land, *Ranunculus* is the most popular cut flower in the world, produced mostly in Italy, which is currently the world's leading producer (Source: ISPF 2014). At the Sanremo Flower Market, around 150 hectares of farmed area, fifteen million stems are documented (Beruto et al., 2018).

Etymology

The Latin terms "rana" (meaning frog) and "unculus" (meaning tiny) are combined to form the word "*Ranunculus*", which means little frog. The name is said to have originated from the species' favored growth areas near bodies of water; in the spring, they were frequently spotted along streams, like little frogs (Floral Design Institute, 2024).

Habitat and Distribution

The genus *Ranunculus* is universal, found across the world from the Tropics to the Arctic and subantarctic zones, and it is found on all continents. Temperate to meridional zones are particularly species-rich for this genus (Ovczinnikov, 1937; Iranshahr et al., 1992; Whittemore, 1997). Certain species can only be found in high mountain regions in tropical regions (Tamura, 1993, 1995). Numerous morphological adaptations to diverse environments are displayed by *Ranunculus* species, which are found in a range of wet to dry habitats from lowland to high alpine zones (Paun et al., 2005). Although widespread species are also rather abundant at lower elevations, endemism plays a significant role in the significant species variety found in mountainous regions (Emadzade et al., 2011). According to POWO (2024), it is native to Afghanistan, Alabama, Alaska, Albania, Alberta, Aleutian Is., Algeria, Altay, Amsterdam-St.Paul Is., Amur, Angola, Antipodean Is., Argentina Northeast, Argentina Northwest, Argentina South, Arizona, Arkansas, Assam, Austria, Azores, Baleares, Baltic States, Bangladesh, Belarus, Belgium, Bolivia, Borneo, Botswana, Brazil Northeast, Brazil South, Brazil Southeast, Brazil West-Central, British Columbia, Bulgaria, Burundi, Buryatia, California, Cameroon, Canary Is., Cape Provinces, Central African Republic, Central European Rus, Chatham Is., Chile Central, Chile South, China North-Central, China South-Central, China Southeast, Chita, Colombia, Colorado, Connecticut, Corse, Costa Rica, Crozet Is., Cuba, Cyprus, Czechoslovakia, Delaware, Denmark, District of Columbia, Dominican Republic, East Aegean Is., East European Russia, East Himalaya, Ecuador, Egypt, El Salvador, Eritrea, Ethiopia, Falkland Is., Finland, Florida, France, Free State, Føroyar, Galápagos, Georgia, Germany, Great Britain, Greece, Greenland, Guatemala, Gulf of Guinea Is., Gulf States, Haiti, Hawaii, Heard-McDonald Is., Honduras, Hungary, Iceland, Idaho, Illinois, India, Indiana, Inner Mongolia, Iowa, Iran, Iraq, Ireland, Irkutsk, Italy, Jamaica, Japan, Jawa, Juan Fernández Is., Kamchatka, Kansas, Kazakhstan, Kentucky, Kenya, Kerguelen, Khabarovsk, Kirgizstan,

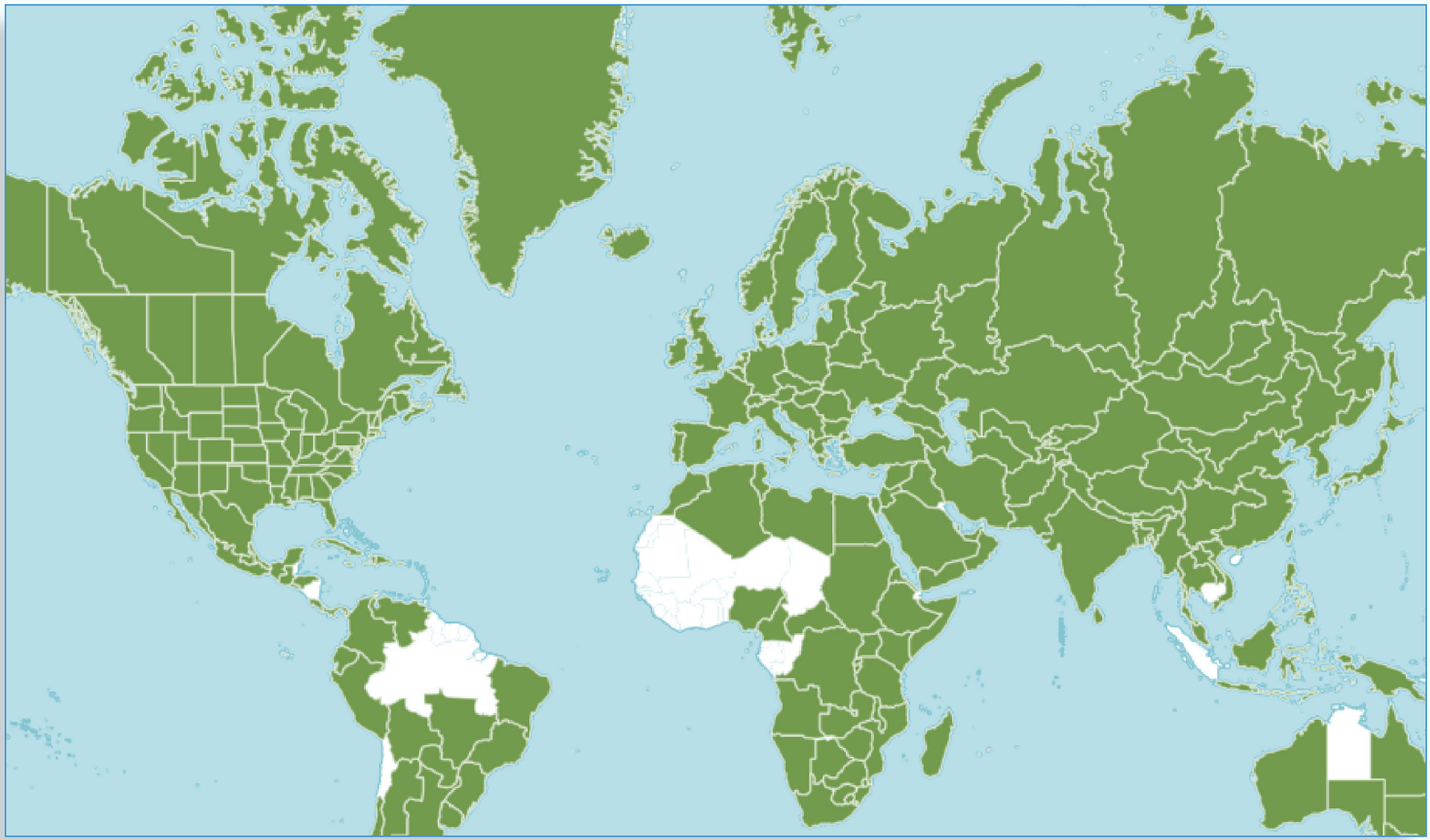

Fig. 1 Global distribution of the genus *Ranunculus*

Korea, Krasnoyarsk, Kriti, Krym, Kuril Is., KwaZulu-Natal, Labrador, Laos, Lebanon-Syria, Lesotho, Lesser Sunda Is., Libya, Louisiana, Macquarie Is., Madagascar, Madeira, Magadan, Maine, Malawi, Malaya, Maluku, Manchuria, Manitoba, Marion-Prince Edward, Maryland, Massachusetts, Mexican Pacific Is., Mexico Central, Mexico Gulf, Mexico Northeast, Mexico Northwest, Mexico Southeast, Mexico Southwest, Michigan, Minnesota, Mississippi, Missouri, Mongolia, Montana, Morocco, Mozambique, Myanmar, Namibia, Nansei-shoto, Nebraska, Nepal, Netherlands, Nevada, New Brunswick, New Guinea, New Hampshire, New Jersey, New Mexico, New South Wales, New York, New Zealand North, New Zealand South, Newfoundland, Nigeria, North Carolina, North Caucasus, North Dakota, North European Russi, Northern Provinces, Northwest European R, Northwest Territories, Norway, Nova Scotia, Nunavut, Ogasawara-shoto, Ohio, Oklahoma, Oman, Ontario, Oregon, Pakistan, Palestine, Panamá, Paraguay, Pennsylvania, Peru, Philippines, Poland, Portugal, Primorye, Prince Edward I., Puerto Rico, Qinghai, Queensland, Québec, Rhode I., Romania, Rwanda, Réunion, Sakhalin, Sardegna, Saskatchewan, Saudi Arabia, Sicilia, Sinai, Somalia, South Australia, South Carolina, South Dakota, South European Russi, South Georgia, Spain, Sri Lanka, Sudan, Sulawesi, Svalbard, Swaziland, Sweden, Switzerland, Tadzhikistan, Taiwan, Tanzania, Tasmania, Tennessee, Texas, Thailand, Tibet, Transcaucasus, Tristan da Cunha, Tunisia, Turkey, Turkey-in-Europe, Turkmenistan, Tuva, Uganda, Ukraine, Uruguay, Utah, Uzbekistan, Venezuela, Vermont, Victoria, Vietnam, Virginia, Washington, West Himalaya, West Siberia, West Virginia, Western Australia, Wisconsin, Wyoming, Xinjiang, Yakutskiya, Yemen, Yugoslavia, Yukon, Zambia, Zaïre, Zimbabwe; and introduced into Bermuda, Mauritius, New Caledonia, Norfolk Is., St. Helena (Fig. 1)

Botanical Characteristics

Perennial or annual, terrestrial, or rarely aquatic herb. Stems leafy. Leaves basal and cauline, spirally arranged; basal leaves petiolate, petiole expanded into a sheath at base; leaf blade simple, palmately divided, 1- or 2-ternate or, rarely, pinnate; cauline leaves with sheathing bases. Inflorescence solitary terminal or a simple or compound monochasium. Flowers bisexual and actinomorphic. Receptacle ± convex, sometimes forming androgynophore (*R. angustisepalus*). Sepals 3-7, usually greenish, occasionally dark reddish or purple, very rarely abaxial sepal appendiculate (*R. angustisepalus*), deciduous or, rarely, persistent. Petals 3-10, yellow, rarely white, exceptionally red, base shortly clawed, with foveolate adaxial nectary pit, which is sometimes covered by a scale. Stamens are numerous or rarely few. Carpels numerous, sessile, or rarely stalked; ovule 1 per carpel, basal; style usually present, with adaxial stigmatic tissue, sometimes absent; distinct stigma usually absent. Fruit aggregate, globose, ovoid, or cylindric,

with numerous achenes. Fruit of numerous achenes with usually glabrous styles; achenes ovoid, obovoid, or slightly to strongly bilaterally compressed, smooth, sometimes tuberculate, or spiny, sometimes marginate or winged along sutures, usually greenish, black. Seeds with copious endosperm and small embryos.

Ethnomedicinal Importance

In traditional medicine, several *Ranunculus* species have been used to cure various illnesses and symptoms, including gout, rheumatism, asthma, jaundice, nebula, edema, malaria, cancer, and hypertension (Goo, 2022). Furthermore, studies have shown that extracts from *Ranunculus* exhibit anti-inflammatory, anti-mutagenic, antibacterial, and antitumoral, cardioprotective, and wound-healing qualities (Newall and Beedles, 1996; Sezik et al., 2001; Prieto et al., 2003; Barbour et al., 2004; Gürhan et al., 2004; Zou et al., 2007). The most prevalent use of *Ranunculus* species is for managing intermittent fever, as a rubefacient, and to treat rheumatism. The herb is often prepared as a decoction for this purpose. Additionally, it is recommended as antihemorrhagic (*R. repens*), diaphoretic, anti-spasmodic, and neuralgia pain (*R. bulbosus*), anthelmintic, vermifacient (*R. hirtellus*). Tympany, ocular conjunctivitis (*R. laetus*). Treat acute icteric hepatitis (*R. sceleratus*), internal abscess, malaria, scrofula, and snake or scorpion poison (Aslam et al., 2012).

Most of the species of this genus are important for their therapeutic uses. Some plants show poisonous activities, as *R. sceleratus* is poisonous if used fresh. The plant should be used as a folk medicine to treat various diseases after heating or drying (Prieto et al., 2003). Certain species work well for a variety of purposes. Hemorrhoids can be treated topically or orally using *R. ficaria*, an herbal astringent (Tita et al., 2009). Native people employ *R. muricatus*, a significant medicinal plant, as a folk remedy for a variety of conditions, including cough, asthma, heart disease, jaundice, diarrhea, dysentery, urinary infection, eczema, lymphatic tuberculosis, dental diseases, ringworm infection, leprosy (Iqbal and Sher, 2011; Rahman et al., 2016). It also exhibits cytotoxic, analgesic, antibacterial, antifungal, antioxidant, and anti-inflammatory properties (Ibrar and Samreen, 2012; Nazir et al., 2014; Khan et al., 2016; Nasreen et al., 2020). Traditional Chinese medicine employs *R. ternatus* because of its positive benefits on a variety of medical diseases, including malignant lymphoma, leukemia, pulmonary tuberculosis, breast tumors, goiters, esophageal tumors, lung disease, gastric problems, and other health conditions (Pan and Sun, 1986; Zhang and Wan, 1993; Chen et al., 2002; Tong et al., 2013).

R. arvensis is commonly used to treat rheumatism, gastrointestinal disorders, psoriasis, arthritis, asthma, and hay fever (Bhatti et al., 2015a). Additionally, the extract of *R. arvensis* show anticarcinogenic and antioxidant properties (Bhatti et al., 2015a, b). Certain adverse effects of this plant have also been identified, including skin irritation, skin burns, and damage to mucous membranes when applied topically (Kocak et al., 2016;

Polat, 2016; An et al., 2018). Significant anti-inflammatory and wound-healing properties possessed by *R. pedatus* (Akkol et al., 2012). Some plants in this genus grow in extreme environments, as *R. hyperboreus* is a subarctic and subalpine plant that thrives in harsh environments. The extract from this plant induces anti-inflammatory activity by controlling the expression of genes linked to inflammation, like iNOS and COX-2, and proinflammatory cytokines, like TNF-α, IL-1ß, and IL-6 (Kong et al., 2018).

Chemical Constituents

Ranunculus are rich sources of alkaloids, phenol, flavonoids, and saponins. β-*sitosterol,* hexadecanoic acid and anemonin are the compounds isolated from *Ranunculus bulbosus*. A huge bunch of flavonoid glycoside isolated from *Ranunculus chinensis* that are 3-O-α-larabinopyranosyl-(1→2)-ß-D-galactopyranosyl-7-O-ß-D glucopyranosyl kaempferol, 3-O-α-L-arabinopyranosyl-(1→2)-{4-O-[(E)-caffeoyl] β-*D*-galactopyranosyl}-7-O-ß-D glucopyranosyl quercetin, 3-O-{2-O-[(E)-caffeoyl]-α-larabinopyranosyl(1→2)-ß-D-galactopyranosyl}-7-O-ß-D glucopyranosyl kaempferol, 3-O-{2-O-[(E)-caffeoyl]-α-larabinopyrannosyl-(1-2)-ß-D-galactopyranosyl} kaempferol (Zou et al., 2007). It is interesting to note that *Ranunculus laetus* consists of jacein, jacedin-5-O-ß-D-glucoside, centaurein, 6,7-dimethoxycoumarin, ß-amyrin, and ß-sitosterol-3-O-ß-Dglucoside (Hussain et al., 2009). *Ranunculus muricatus* contains a phenolic class of compounds such as stigmasterol-4-ene-3, 6-dione, stigmasterol, anemonin, aescin lactone dimethyl ether, , protocatechuic aldehyde, protocatechuic acid, and luteolin factors (Lingjie et al., 2009). R (+)-dalbergi phenol, R (+)-4methoxydalbergione, methyl3,4,5,-trihydrobenzoate,4-hydroxy-2-methoxybenzoic acid, p-hydroxycinnamic acid, ß-sitosterol, and ranupenin are chemical constituents isolated from *Ranunculus repens* (Wagner et al., 1977; Noor et al., 2006). The most interesting species among all genus is *Ranunculus sceleratus* containing 5-hydroxy tryptamine (Bhargava et al., 1965), apigenin, apigenin 4′-O-α-rhamnopyranoside, apigenin 7-O-ß-glucopyranosyl-4′-O-α-rhamnopyranoside, tricin 7-O-ß-glucopyranoside, isoscopoletin, tricin, protocatechuyl aldehyde (Li et al., 2005), protoanemonin (Aslam et al., 2012). It is interesting to note that ß-sitosterol is isolated from 3 of the species of *Ranunculus* namely *Ranunculus bulbosus*, *Ranunculus laetus,* and *Ranunculus repens* (Aslam et al., 2012).

Species

Genus *Ranunculus* is known to contain a total of 1782 taxonomically accepted species including hybrids (POWO, 2024) that are as-

1. *R. abbaianus* Dunkel, Webbia 65: 207 (2010).
2. *R. abchasicus* Freyn, Oesterr. Bot. Z. 43: 373 (1893).
3. *R. abditus* (Markl.) Ericsson, Ann. Bot.

Fenn. 29: 129 (1992).

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Ranunculus sceleratus L.

Ranunculus sceleratus commonly known as the "celery-leaved buttercup", is a flowering plant species distributed over the Northern Hemisphere. It grows in moist areas including ditches, streams, and the areas surrounding ponds and lakes. A fleshy, glossy-leaved buttercup resembles a young celery plant on the outside. It is an annual plant that reaches a height of half a meter. The leaves include short blades that are strongly lobed or split into three leaflets, and they are mostly glabrous (hairless). Their long petioles carry them. The flowers are 5–10 mm in diameter, with reflexed sepals and five or fewer yellow petals that are a few millimeters long. The fruit is an achene that is carried in many clusters. Fresh plant material is toxic in every part; however, poisons can be eliminated by drying or heat treatment (Altmann, 1980; Grieve and Leyel, 1984). Additionally, the plant produces a potently acidic liquid that can burn skin (Frohne and Pfänder, 1984; Facciola, 1990).

It biosynthesizes and releases functional biomolecules such as ranunculin, protoanemonin, and anemonin. It has great therapeutic properties and is frequently used in traditional Chinese medicine (Wu Gang et al., 1999; Mei et al., 2012). *R. sceleratus* mostly contains flavonoids, the glycoside ranunculin, and steroids such as pyrogallol tannins (Mahran et al., 1968). After *R. sceleratus* leaves are dried or crushed, ranunculin is hydrolyzed and produces protoanemonin, which is linked to buttercup's poisonous qualities. Due to its instability, protoanemonin dimerizes into the non-irritating form of anemonin (Minakata et al., 1983; Martín et al., 1990; Goo, 2022). This plant, whether fresh or dried, can be utilized to cure breast and esophageal cancer (Li, 1999). Apart from its therapeutic properties, it has further potential uses.

Classification

Kingdom	-	Plantae
Subkingdom	-	Tracheophyta
Super Division	-	Spermatophyta
Division	-	Mangoliophyta
Class	-	Magnoliopsida
Subclass	-	Magnoliidae
Order	-	Ranunculales
Family	-	Ranunculaceae
Genus	-	*Ranunculus*
Species	-	*sceleratus*

Common Names

Celery-Leaved Buttercup, Cursed Buttercup, Poisonous buttercup, Celery-leaved buttercup, Blister buttercup; Shim, Aglaon, Jaldhaniya (Hindi); Lalukaoba (Manipuri); Khajakollathi, Kulagi (Marathi); Nakkore, Naakure, Shamphu Jhaar, Tharuni (Nepali); Kandakatuka, Kandira, Nasasamvedana (Sanskrit).

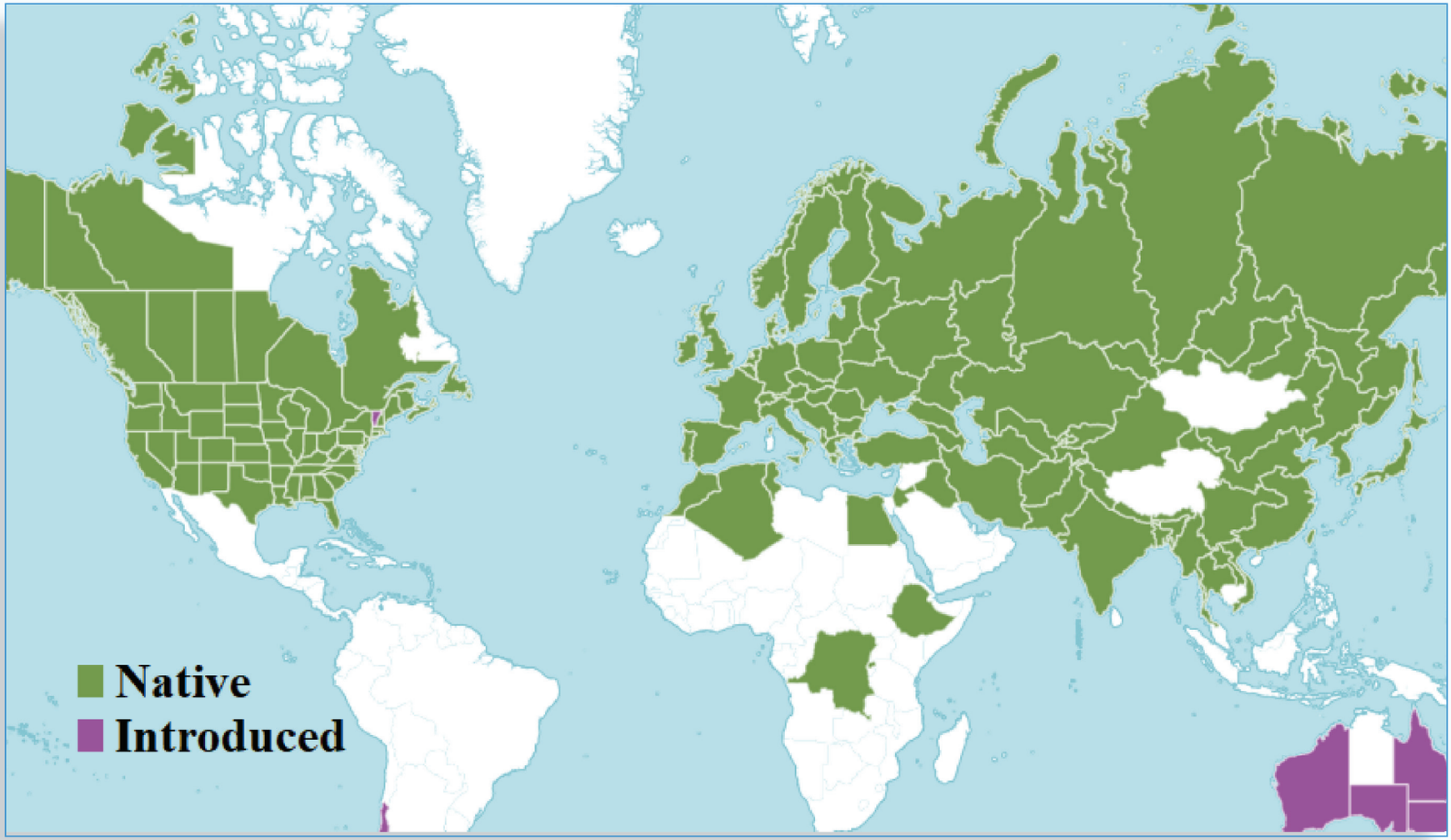

Fig. 2 Global distribution of *R. sceleratus*

Global Distribution

The native range of *R. sceleratus* is Temperate Eurasia, North Africa, Ethiopia to Rwanda, and Central and East Canada to Central and East United State of America. It primarily grows in the temperate biome. According to POWO (2024) it is native to Afghanistan, Alabama, Alaska, Albania, Alberta, Algeria, Altay, Amur, Arizona, Arkansas, Assam, Austria, Baleares, Baltic States, Bangladesh, Belarus, Belgium, British Columbia, Bulgaria, Buryatia, California, Central European Rus, China North-Central, China South-Central, China Southeast, Chita, Colorado, Connecticut, Corse, Czechoslovakia, Delaware, Denmark, District of Columbia, East European Russia, East Himalaya, Egypt, Ethiopia, Finland, Florida, France, Georgia, Germany, Great Britain, Greece, Hungary, Idaho, Illinois, India, Indiana, Inner Mongolia, Iowa, Iran, Iraq, Ireland, Irkutsk, Italy, Japan, Kamchatka, Kansas, Kazakhstan, Kentucky, Khabarovsk, Kirgizstan, Korea, Krasnoyarsk, Krym, Kuril Is., Laos, Louisiana, Magadan, Maine, Manchuria, Manitoba, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Montana, Morocco, Myanmar, Nansei-shoto, Nebraska, Nepal, Netherlands, Nevada, New Brunswick, New Hampshire, New Jersey, New Mexico, New York, Newfoundland, North Carolina, North Caucasus, North Dakota, North European Russi, Northwest European R, Northwest Territorie, Norway, Nova Scotia, Ohio, Oklahoma, Ontario, Oregon, Pakistan, Palestine, Pennsylvania, Poland, Portugal, Primorye, Québec, Rhode Is., Romania, Rwanda, Sakhalin, Saskatchewan, Sicilia, South Carolina, South Dakota, South European Russi, Spain, Sweden, Switzerland, Tadzhikistan, Taiwan, Tennessee, Texas, Thailand, Transcaucasus, Tunisia, Turkey, Turkey-in-Europe, Turkmenistan, Tuva, Ukraine, Utah, Uzbekistan, Vietnam, Virginia, Washington, West Himalaya, West Siberia, West Virginia, Wisconsin, Wyoming, Xinjiang, Yakutskiya, Yugoslavia, Yukon, Zaïre; and introduced into Chile Central, New South Wales, New Zealand North, Queensland, South Australia, St. Helena, Tasmania, Vermont, Victoria, Western Australia (Fig. 2).

Distribution in India

R. sceleratus is grow in across India, especially around rivers, canals, ponds, mainly in wet and moist clayey soil in full sunlight.

Ethnopharmacology and Traditional Uses

This plant has a particular application in ethnobotany that takes advantage of both its pharmacological and toxicological characteristics. The ethnopharmacological uses include the prescription of extracts in Turkey and Iraq as emmenagogues and galactagogue, external application of blistering oil as a counterirritant by natives of British Columbia (Turner, 1984), and its use as an antirheumatic and antineuralgic agent in the traditional medicine of northeastern Italy (Cappelletti et al., 1982). Socially, beggars use it to disfigure themselves with blisters to gain sympathy owing to itstoxic properties, for

centuries (Prieto et al., 2003). *R. sceleratus* is used in Asian traditional medicine to treat blood stasis, internal abscess, scrofula, malaria, snakebites or scorpion stings, and severe icteric hepatitis (Aslam et al., 2012). Ranunculin, protoanemonin, and anemonin are biosynthesized by the plant (Mei et al., 2012). Antibacterial and anti-inflammatory properties are present in the aerial portion of *R. sceleratus* (Aslam et al., 2012). In Iraq, it has traditionally been employed to treat intestinal disorders, rheumatism, asthma, and high fever (Al-Snafi, 2021). Additionally, it is utilized as a natural remedy for myalgia and the common cold, as well as for hemorrhoids, and abscess drainage (Tanker et al., 1998; Metın et al., 2021; Al-Snafi, 2022).

Sharif et al. (2020) conducted an *in vivo* investigation to assess the effects of hypertension therapy using fructose-induced hypertensive and normotensive rats. The most intriguing results were observed in the aqueous fraction. Moreover, muscarinic receptor involvement, inhibition of the angiotensin-converting enzyme, ganglionic block, and nitric oxide production are responsible for the hypotensive response elicited by *R. sceleratus*, according to mechanistic investigations conducted with different pharmacological antagonists. Additionally, substances produced from *R. sceleratus*, such as isoscopoletin, apigenin 7-O-beta-glucopyranosyl-4'-O-α-rhamnopyranoside, apigenin 4'-O-α-rhamnopyranoside, tricin 7-O-beta-glucopyranoside, and tricin, showed inhibitory activity against the hepatitis B virus (Bonora et al., 1988). Furthermore, fresh *R. sceleratus* for TianJiu therapy, which included, applying its herbal paste on specific acupoints, which resulted in therapeutic effect on intrahepatic cholestasis in rats, even though the fresh form of *R. sceleratus* is known as an irritant (Zhang et al., 2020). The precise processes of the extract used to elicit irritating or nonirritant reactions is still unknown. Therefore, a methanolic extract of *R. sceleratus* was utilized to illustrate the mechanism of the extract's irritating and non-irritant effects on cutaneous inflammation to explain this phenomenon. The extract's response to arachidonic acid-induced inflammation was neutral or proinflammatory (Goo, 2022).

Selection of the Sites and Characteristics Studied

The distribution and morphological variations of *R. sceleratus* were investigated across the Ganga River. The Ganga originates from the Gomukh in Gangotri glacier and ends 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 1). The plant diversity was assessed up to 10 km on both sides of each site. The plants were identified and deposited in the Patanjali Research Foundation Herbarium with an acronym of PRFH for future records. A bulk sample of the plant was taken from each site in a large polybag which was deposited in the analytical laboratory for phytochemical profiling.

Table 1 Different studied sites and their GPS coordinates along with *R. sceleratus* 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	Absent
S6	Bijnor	225	29.37	78.13	Present
S7	Narora	174	28.20	78.38	Absent
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	Absent
S14	Varanasi	81	25.32	83.01	Present
S15	Ballia	67	25.76	84.15	Present
S16	Revelganj	52	25.78	84.67	Absent
S17	Patna	53	25.61	85.14	Absent
S18	Barh	47	25.48	85.72	Absent
S19	Bahachouki	55	25.30	86.36	Present
S20	Farka	42	25.23	87.09	Present
S21	Sahebganj	16	25.25	87.65	Absent
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	Hoogli	9	22.91	88.40	Absent
S26	Gangasagar	4	22.19	88.19	Absent

Local Occurrence

The plant was recorded in majority of the sites, but it was collected for the analytical studies only from the sites 6, 8, 9, 10, 11, 12, 14, 15, 19, and 20 due to these sites being the source of its bulk collection. For the remaining sites (1, 2, 3, 4, 5, 7, 13, 16, 17, 18, 21, 22, 23, 24, 25, and 26), it was either absent or only a representative.

Taxonomic Treatment

Ranunculus sceleratus L., Sp. Pl.: 551 (1753). C.B. Clarke in Hook.f., Fl. Brit. India 1: 19. 1871; Duthie, Fl. Gangetic Plain 1: 19. 1903; Haines, Bot. Bihar Orissa (Repr. ed.) 1: 6.1961; Uniyal & al., Fl. Pl. Uttarakhand, Checkl.: 68. 2007; Sinha et. al, Fl. Uttar Pradesh 1: 122.2016.

Synonyms

The Plants of the World Online database mentioned it with its four homotypic synonyms (POWO, 2024) as-

1. *Batrachium sceleratum* (L.) Th.Fr. ex A.Pihl in Bot. Not. 1893: 5 (1893)
2. *Hecatonia scelerata* (L.) Fourr. in Ann. Soc. Linn. Lyon, n.s., 16: 325 (1868)
3. *Ranunculus sceleratus* var. *typicus* L.D.Benson in Amer. Midl. Naturalist 40: 203 (1948), not validly publ.
4. *R. sceleratus* subsp. *typicus* Á.Löve & D.Löve in Rit Landbúnaoard. Atvinnud. Háskólans, B 3: 109 (1948), not validly publ.

Another database World Flora Online (WFO, 2024) mentioned it with a total of sixteen synonyms as-

1. *Adonis palustris* Raeusch in Nomencl. Bot., ed. 3: 162 (1797)
2. *Batrachium sceleratum* Th.Fries ex A.Pohl in Bot. Not.: 5. (1893)
3. *Hecatonia scelerata* Fourr. in Ann. Soc. Linn. Lyon, n.s., 16: 325 (1868)
4. *H. palustris* Lour. in Fl. Cochinch. : 303 (1790)
5. *Ranunculus apiophyllus* St.-Lag. in Étude Fl., éd. 8, 2: 12 (1889)
6. *R. eremogenes* var. *longissimus* Lunell in Amer. Midl. Naturalist 1: 206 (1910)
7. *R. holophyllus* Hance in Ann. Sci. Nat., Bot., sér. 4, 15: 220 (1861)
8. *R. indicus* Roxb. in Fl. Ind. ed. 1832, 2: 671 (1832)
9. *R. oryzetorum* Bunge Enum. Pl. China Bor.: 2 (1833)
10. *R. sceleratus* f. *sceleratus*
11. *R. sceleratus* var. *eremogenes* Garrett in Spring Fl. Wasatch (ed. 1) 25. (1911)
12. *R. sceleratus* var. *longissimus* (Lunell) L.D.Benson in Bull. Torrey Bot. Club 69: 313 (1942)
13. *R. sceleratus* var. *sceleratus*
14. *R. sceleratus* var. *sinensis* H.Lév. & Vaniot in Bull. Herb. Boissier, sér. 2, 6(6): 505 (1906)
15. *R. umbellatus* Roxb. ex Willd. in Enum. Pl.: 588 (1809)
16. *R. carnosus* Wall. in Numer. List [Wallich] n. 4699. (1831)

Varieties of *Ranunculus sceleratus* L.

A total of two varieties are accepted in Plants of the World Online (POWO, 2024) and World Flora Online (WFO, 2024) database as-

1. *R. sceleratus* var. *multifidus* Nutt. in J.Torrey & A.Gray, Fl. N. Amer. 1: 19 (1838)
2. *R. sceleratus* var. *sceleratus*

Botanical Description

Fleshy, erect, annual herb. Roots fibrous. Stem up to 90 cm tall, glabrous, or sparsely pubescent, stout, branched, hollow, deeply furrowed outside. Radical leaves petiolate, reniform, up to 5 cm in diameter, 3-lobed; segments obovate, bluntly 3-5-toothed; lateral lobes sometime deeply bilobed again, lobes irregularly shallow crenate; petioles 2.5-5.0 cm long, progressively shortened and ultimately sessile in cauline leaves, auricles scarious; cauline leaves sessile, 3-lobed or 3-5 partite, lobes linear-oblong, entire or deeply crenate or lobulate. Flowers numerous, ca 1 cm in diameter, bright yellow, diffusely racemose; pedicel up to 1.5 cm long, glabrous; sepals 5, ca 4 mm long, ovate-elliptic, ovate, pubescent outside, reflexed, caduceus; petals 5, imbricate, shorter or as long as sepals, obovate, 4-6 × 3-4 mm, apex rounded or shallowly notched, claw inconspicuous; nectary pit small, pocket-like without nectary scale. Stamens 10-19; anthers ellipsoid. Aggregate fruit ovoid-cylindrical to cylindrical, 3-11 × 1.5-4 mm; carpels numerous. Fruit achenes, small, slightly bilaterally compressed, obliquely obovoid, up to 1.3 mm in diameter, beak inconspicuous, glabrous, compressed, smooth to 2- or 3-rugose, somewhat turgid along sutures; arranged in an oblong to shortly cylindrical, 7-8 mm long head; style short, minutely beaked; stigma persistent (Fig. 3A, B).

Fig. 3A Plant habit

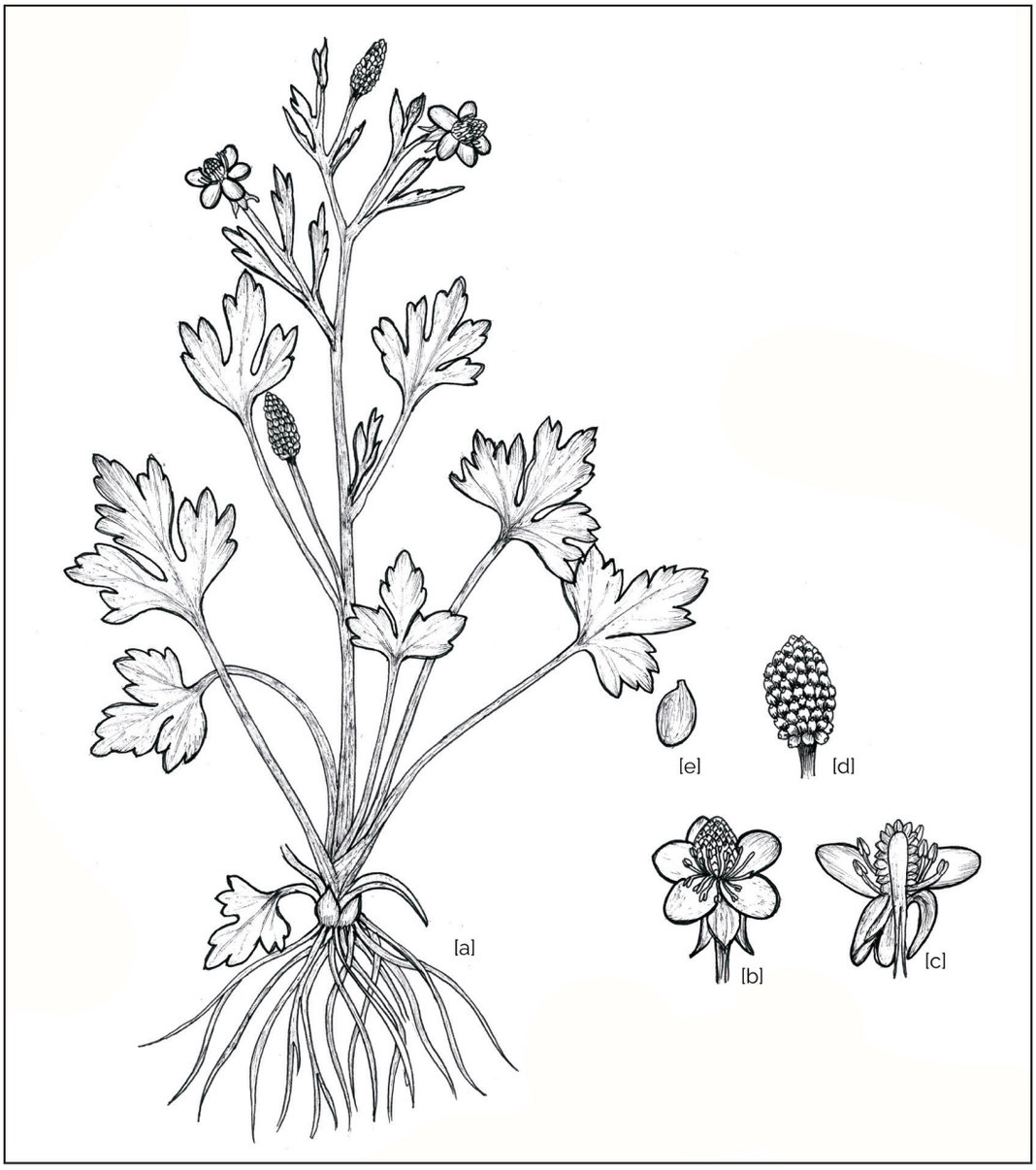

Fig. 3B [a]Plant twig [b] Flower [c] L.S of flower [d] Fruit [e] Achene

Anatomical Features

Structure of the Leaf

The leaf has well-developed tissues. Well-developed, unistratified epidermis with stomata covers both surfaces of the leaf. The mesophyll differentiates into lacunar tissue and palisade tissue made up of one-row cells. The mesophyll tissues have a well-developed vascular bundle at the center (Fig. 4).

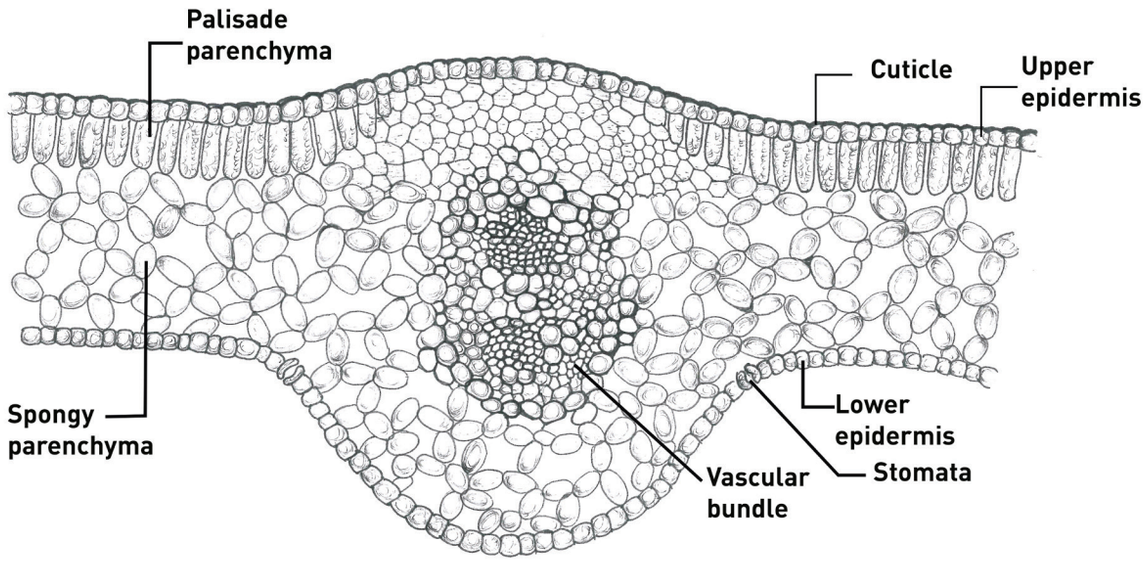

Fig. 4 T.S. of leaf

Structure of the Stem

The outer layer is the epidermis, which is unistratified and composed of square, thin-walled cells. The unlignified parenchymatous cortex, which has larger air spaces oriented towards the exterior and smaller air spaces oriented towards the core cylinder, is located underneath the epidermis. A few open collateral vascular bundles are embedded in parenchymatous tissues with thin walls and tiny intercellular spaces around the core. A thin-walled sclerenchymatous sheath with partly lignified walls encircles the vascular bundles. Large sieve tubes and smaller companion cells comprise the phloem, whereas lignified vessels comprise the xylem (Fig. 5).

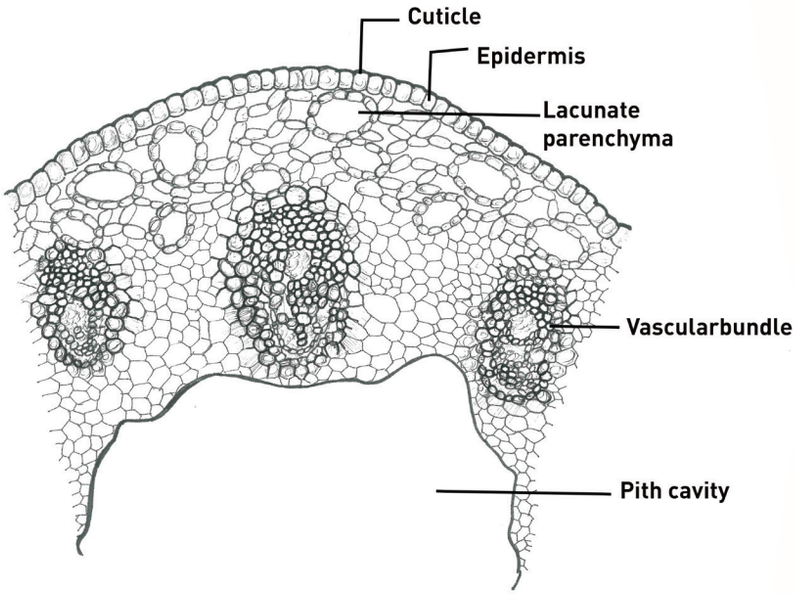

Fig. 5 T.S. of stem

Structure of the Root

The roots are fibrous. The parenchymatous cortex is found inside the epiblema, which is the outer layer. Large auriferous canals, organized in one or two rows, are characteristic of the well-developed aerenchyma in the cortical region. The endodermis, which contains caspary thickness, is the inner layer of the cortex. Four wooden fascicles and four liberian fascicles make up the centered vascular cylinder (Fig. 6).

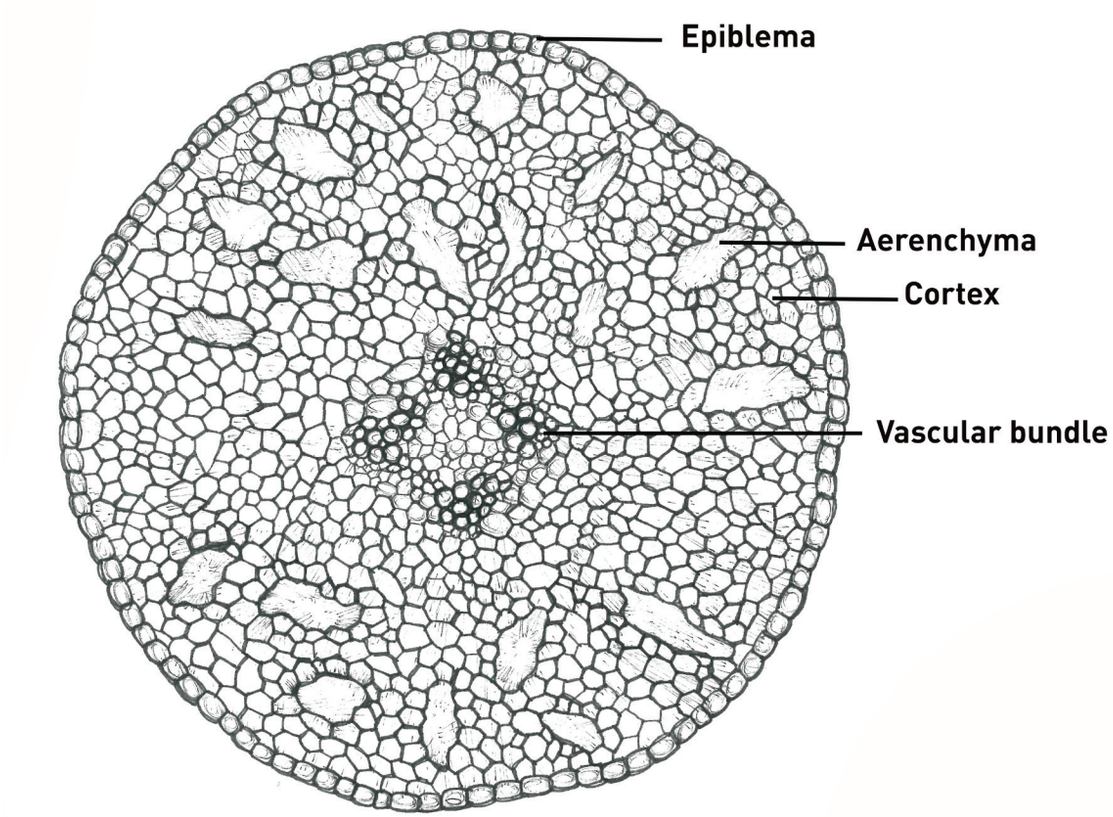

Fig. 6 T.S. of root

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CHAPTER

02

Bibliometric Analysis

INTRODUCTION

The botanical family *Ranunculaceae* is universally distributed across the world, including a total of 59 genera and 2500 species. Genetically, this taxonomic group exhibit significant diversity and a broad geographical range, contributing to the complexity of its classification (Almerekova et al., 2020). *Ranunculus sceleratus* L., is an annual semi-aquatic species characterized by a relatively short-lived life cycle, commonly occurring in salt-affected coastal habitats (Laime, 2013; Rove, 2013). *R. sceleratus* is known for its medicinal applications in treating a variety of conditions, including plague, malaria, scorpion bites, blood stasis, acute icteric hepatitis, and internal abscesses (Madhulika et al., 2020). Additionally, historical medicinal practices have attributed the stimulant and diuretic properties of this plant. The juice of its leaves has been traditionally utilized to alleviate symptoms associated with asthma, dysuria, pneumonia, rheumatism, and sciatica. Moreover, the seeds of this plant are used in the treatment of kidney disorders (Gangwar et al., 2008). Both dried and fresh plants have been reportedly employed in the treatment of esophageal and breast cancer, along with malaria-induced jaundice (Zhang et al., 2020). Furthermore, as a medicinal plant, it has validated the capability to mitigate the onset of degenerative diseases through its antibacterial and antioxidant properties (Shahid et al., 2015). These plants exhibit considerable pharmacological activity and hold promise as a potential candidate for novel drugs (Goo, 2022). *R. sceleratus*, has a broad spectrum of therapeutic effects, including antibacterial, antifungal, anti-inflammatory, antioxidant, antiparasitic, and antiviral properties (Al-Snafi, 2022). It has been utilized as an antiphlogistic, anti-diarrheal agent, mastitis remedy, and for alleviating articular effusion (Mei et al., 2012; Neag et al., 2017). The initial phytochemical examination of *R. sceleratus* revealed the presence of various biochemical compounds including amino acids, proteins, alkaloids, phytosterols, flavonoids, steroids, fatty acids, terpenoids, phenols, saponins, tannins, and resins (Zayat et al., 2015; Madhulika et al., 2020).

Bibliometric analysis of the research landscape on *R. sceleratus*, a widespread and ecologically significant wetland plant, revealed diverse and evolving scholarly interest (Xu et al., 2022). This species, commonly known as celery-leaved buttercup, has been

the subject of studies spanning various disciplines, including ecology, phytochemistry, and environmental science. A review of literature indexed in major databases indicates a steady increase in publications over the past two decades, reflecting growing awareness of its ecological roles and medicinal potential (Sharifi et al., 2021). Key research themes include its phytoremediation capabilities, given its propensity to thrive in contaminated environments, and its biochemical properties, which have implications for natural product chemistry and pharmacology. Collaboration patterns, as indicated by co-authorship networks, show a predominance of research output from institutions, highlighting regional interests in the species' applications and management (Borrett et al., 2014). Overall, the bibliometric profile of *R. sceleratus* research emphasized a dynamic and multidisciplinary field, with ongoing investigations likely to expand its applications in environmental and health sciences.

The objective of this bibliometric analysis was to systematically evaluate and map the research landscape of *R. sceleratus*, a wetland plant known for its medicinal properties and ecological significance. By employing bibliometric techniques, this study aimed to quantify the scientific output, identify key research trends, and elucidate the collaboration networks within the scholarly community. Specifically, the analysis focused on tracking publication growth, discerning the most influential articles, authors, and institutions, and examining the thematic evolution of research topics related to *R. sceleratus* over time. Additionally, the study sought to reveal geographical patterns in research activity and to highlight the potential gaps in the existing literature, thereby providing a comprehensive overview that could guide future research directions and policy decisions in the field of botany and environmental science.

Insights with Data Source and Tools

The study explored the scholarly landscape of *R. sceleratus* using bibliometric analysis. The articles published from 2000 to 2023 were collected from Dimensions.ai with the search term "*Ranunculus sceleratus*" ensuring a comprehensive overview of academic publications on this species. Lens.org and VosViewer (Version 1.6.19) were employed for analytical assessment. Lens.org provided a detailed exploration of research fields identifying, and categorizing key thematic areas, while VosViewer created visual depictions of bibliographic data. This included co-authorship networks, and citation analyses among journals, organizations, and countries, highlighting influential insights within the academic community. Microsoft Excel was used for managing and processing the dataset, ensuring systematic organization for analysis. The integration of these tools revealed significant trends and patterns in *R. sceleratus* research, tracing the evolutionary trajectory of scholarly interest and demonstrating shifts in scientific understanding and priorities over the years (Yu et al., 2020; Hajkowicz et al., 2023). The analysis emphasized the growing importance of *R. sceleratus* in academic literature, highlighting its burgeoning role in various research domains. This comprehensive approach combined systematic data collection with advanced analytical tools, providing invaluable insights into the breadth, influence, and evolving trends of research on *R. sceleratus*, enriching the understanding of its scientific significance.

Comprehensive Data-driven Insights

The comprehensive analysis of scholarly publications from 2000 to 2023 provides a detailed and insightful overview of global research on *R. sceleratus*. During this period, 66 scholarly articles were authored by 245 researchers from 91 organizations across 23 countries. Especially, 5 authors were recognized for their independent and significant contributions, highlighting their expertise and the advancements they have brought to the field. The research findings were published in 61 esteemed journals, indicating the high regard for *R. sceleratus* studies within the scientific community. The average citation rate of 16.53 citations per document further reflects the high impact and quality of the research being conducted. The scope of *R. sceleratus* research has expanded significantly, encompassing a wide range of disciplines such as biology, *Ranunculus sceleratus*, chemistry, ecology, botany, environmental science, aquatic plants, and horticulture. This interdisciplinary approach emphasizes the plant's diverse applications and the broad interest in its properties and benefits. Moreover, the data revealed strong collaborative networks among researchers and organizations, which have facilitated

knowledge exchange, and resource sharing. These collaborations are crucial for advancing knowledge and fostering innovation in *R. sceleratus* research.

Temporal Evolution and Growth Analysis

A thorough search across bibliographic databases using Dimensions.ai revealed a total of 67 documents. These documents comprised various types of studies including 58 articles, 7 chapters, 1 preprint, and 1 proceeding. The bibliometric analysis of the research landscape for *R. sceleratus*, spanning the years 2000 to 2023, revealed a fluctuating yet progressively increasing trend in publication numbers. Initially, research activity was sparse, with only 3 publications in 2000. The subsequent years saw minimal activity, peaking briefly in 2005 with another 3 publications before experiencing a slight decline. This period of relative inactivity persisted through the late 2000s, with publication numbers varying between one and two annually. A resurgence in research interest became apparent starting in 2012, which marked the beginning of a more consistent upward trend. In 2012, the number of publications rose to 4, demonstrating renewed academic interest. This increase was followed by a remarkable peak in 2020 and 2023, each year recording the highest number of publications at nine. These peaks underscored significant bursts of research activity. The years leading up to 2020 also saw moderate increases, with 5 publications each in 2021 and 2022 (Fig. 1). Overall, the data highlighted an initial phase of low research output, a subsequent period of intermittent interest, and a marked increase in the latter years, reflecting growing scientific engagement and interest in *R. sceleratus*.

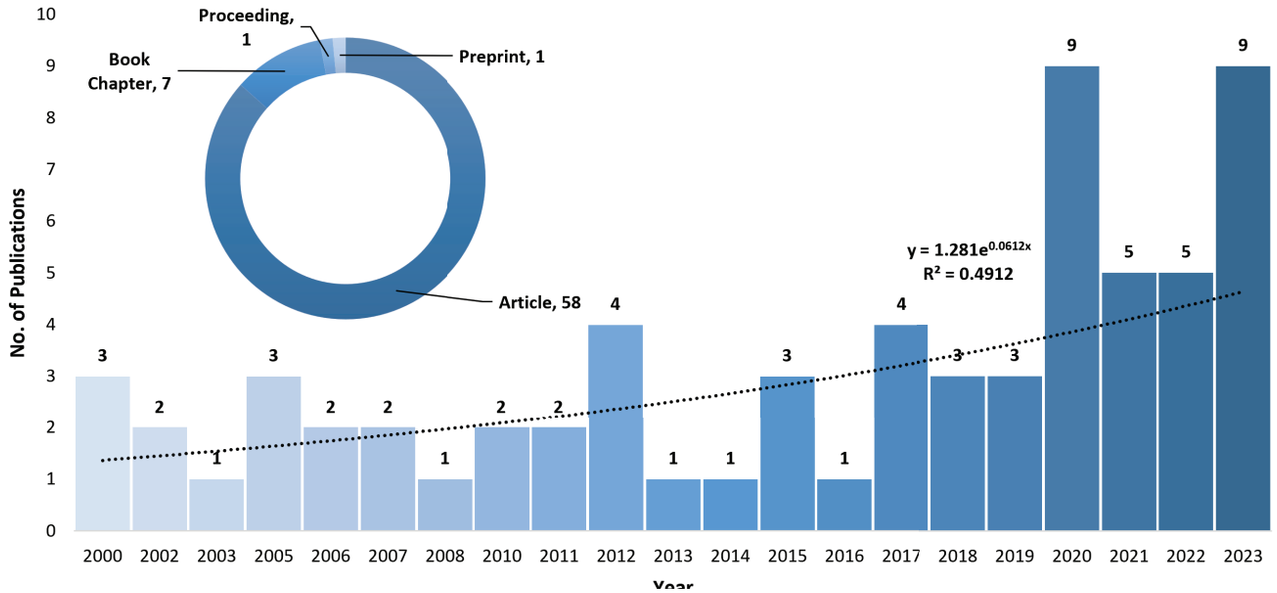

Fig. 1 Publication trends and distribution types in *R. sceleratus* research

Citation Analysis

In examining the research landscape of *R. sceleratus* through a bibliometric analysis, citation patterns over the years revealed significant fluctuations. Starting from the year 2000, there was a remarkable peak with 274 citations, indicating substantial research interest or a pivotal publication during that period. This was followed by a dramatic decline to 43 citations in 2002 and a further drop to 29 citations in 2003. In 2005, citations saw a resurgence to 67, but this interest waned again, reaching only 12 citations in 2006. Interestingly, 2007 marked another significant rise with 136 citations, yet this was short-lived as citations plummeted to single digits in 2008. The subsequent years showed sporadic increases and decreases; for instance, 2010 saw a modest rise to 35 citations, while 2011 and 2013 both dipped to 5 citations each. A slight recovery occurred in 2015 with 31 citations, and a more pronounced peak was observed in 2017 with 182 citations, suggesting renewed interest or significant developments in the research on *R. sceleratus*. However, the citation counts remained inconsistent in the following years, with counts like 98 in 2020 and 41 in 2021, before settling down to more moderate figures such as 28 in 2023 (Fig. 2). This fluctuating citation trend highlighted the episodic nature of research focus and attention on *R. sceleratus* over the years.

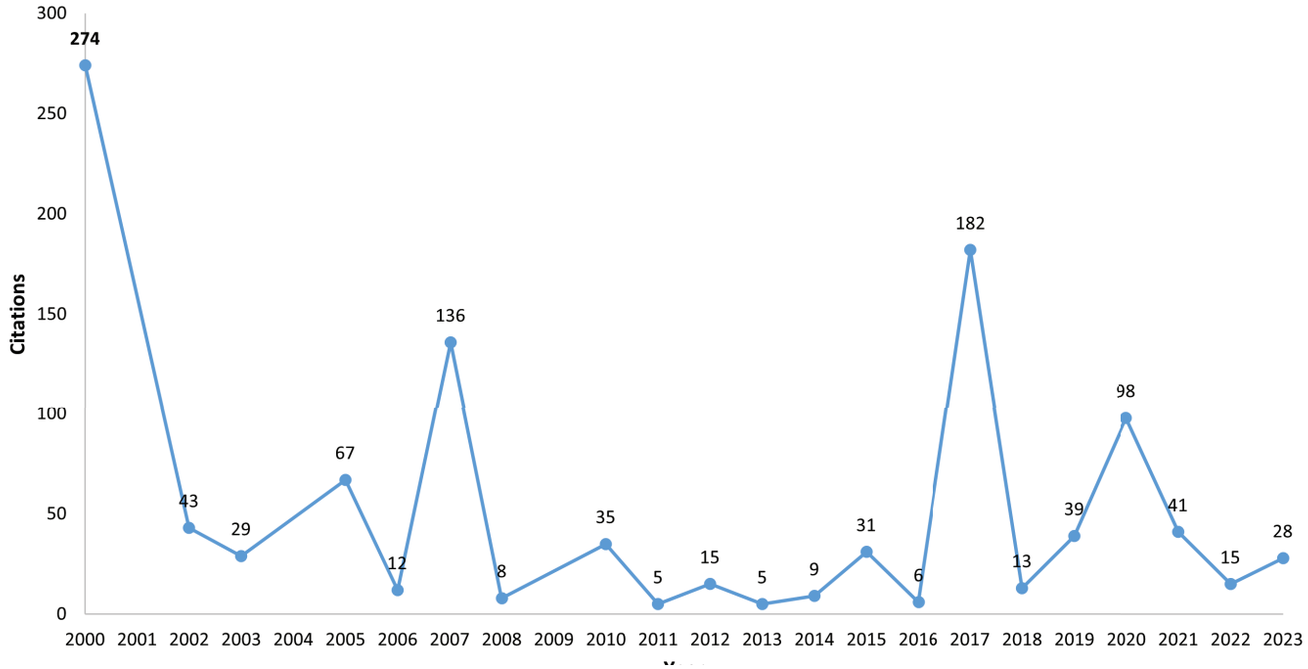

Fig. 2 Citation trends for *R. sceleratus* research over time

Country-wise Publication Analysis

The bibliometric analysis of research publications on *R. sceleratus* revealed remarkable contributions from various countries. China led the field with 13 documents, and collected a significant 264 citations, reflecting its strong research output and influence. Germany, India, and the United States each published 5 documents, although their impact varied widely. Germany has publications with 41 citations, while India's attracted 72 citations, indicating a higher average influence per document. The United States, despite its high publication count, had a relatively low citation total of 16. Pakistan and Spain both contributed 4 documents, yet Pakistan's work was cited 166 times, and Spain's was highly impactful with 206 citations and established substantial academic influence. Australia, with only 3 documents, achieved an impressive 426 citations, showcasing exceptional research quality and impact. Latvia and South Korea also contributed 3 documents each, with 15 and 22 citations respectively, reflecting moderate engagement in the field (Fig. 3). Egypt had 2 documents that were cited 33 times, indicating a respectable influence relative to the number of publications. This analysis highlights the diverse global contributions to the study of *R. sceleratus*, with varying levels of impact and research productivity across different countries.

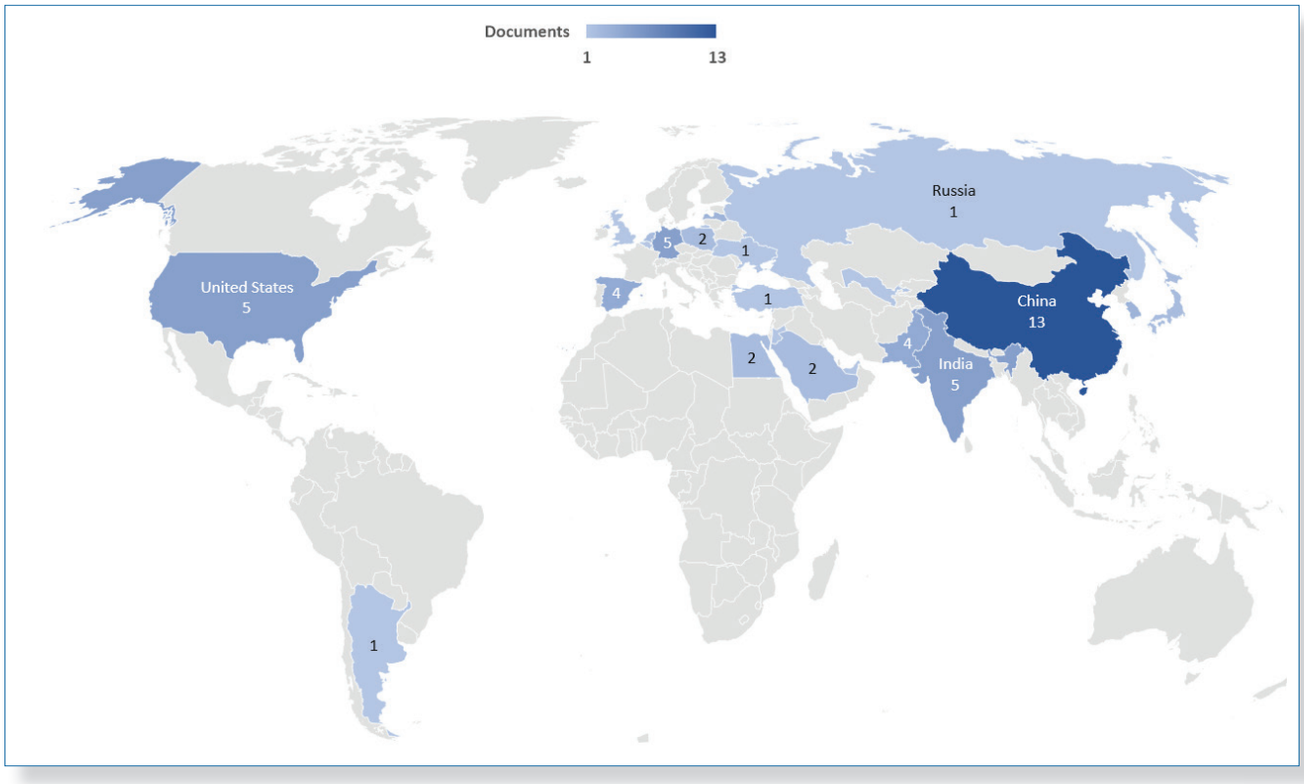

Fig. 3 Global distribution of research on *R. sceleratus*

Most Prominent Authors

In the bibliometric analysis concerning *R. sceleratus*, several prominent authors emerged based on their contributions and citation counts. Topping the list were Wenming Cheng and Qunlin Zhang, both with 3 documents each, and both collected 18 citations each (Fig. 4). Their collective work significantly influenced the scholarly discourse on the subject. Following closely behind were Gederts Ievinsh, Pooja Sharma, and Sonam Tripathi, each with 2 documents. Sharma and Tripathi's contributions were particularly significant, accumulating an impressive 91 citations for their work, indicative of its impact on the scientific community. Yu Zhao, J.M Prieto, and J.L RíOs also made significant contributions, with each author having 2 documents and accruing citations in the range of 62 to 67. Florian Beyer and Florian Jansen also published 2 documents each and 15 citations each. Collectively, these authors played pivotal roles in shaping the understanding and advancement of research on *R. sceleratus*. Their collective efforts have enriched the scholarly landscape and lined the way for further exploration and discovery in this field.

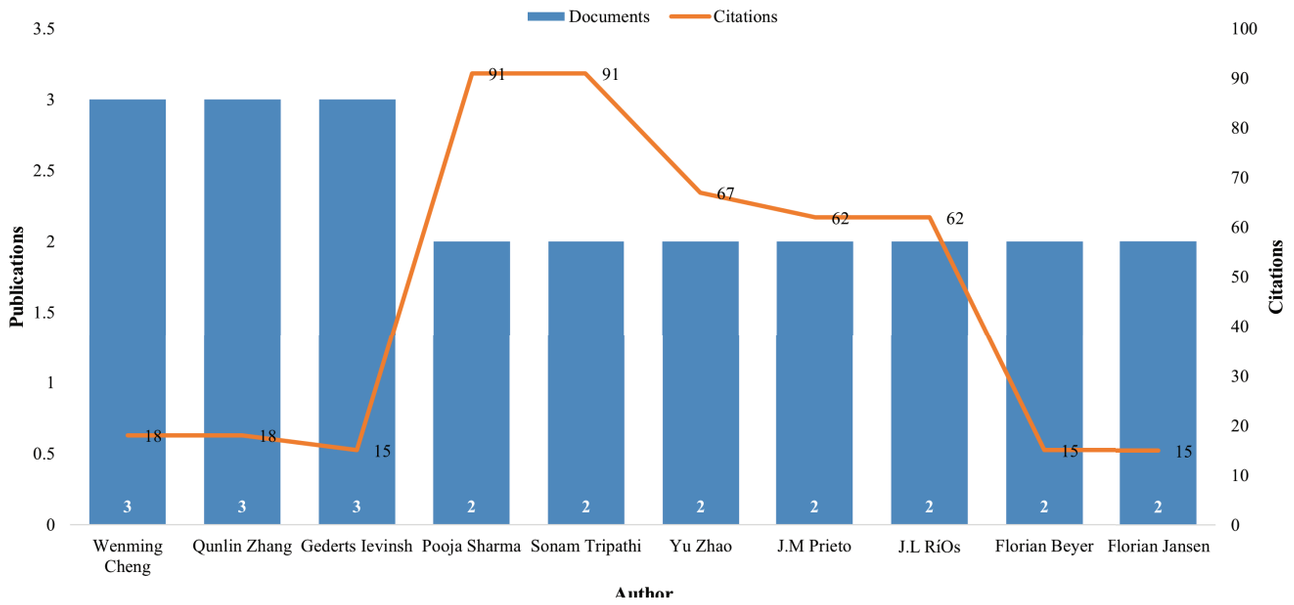

Fig. 4 Most prominent authors based on research contributions to *R. sceleratus*

Highly Cited Articles

In a bibliometric analysis focusing on the research landscape of *R. sceleratus*, a wetland plant species of ecological significance, several highly cited articles emerged, on various facets of its biology, ecology, and medicinal properties (Table 1). Among the highly cited articles, influential work published in Plant Cell & Environment in 2000, which investigated the "Changes in growth, porosity, and radial oxygen loss from adventitious roots of wetland

species", offering insights into their adaptation strategies (Visser et al., 2000). Subsequently, a comprehensive ethnobotanical survey was documented in PLOS ONE in 2017, detailing the "Indigenous medicinal plants in the Hafizabad district of Punjab, Pakistan, including *R. sceleratus*" (Umair et al., 2017). The role of waterbirds in dispersing invertebrates and plants in arid Australia was elucidated in Freshwater Biology in 2007, underlining the ecological interactions involving *R. sceleratus* (Green et al., 2007). A study has explored how *R. sceleratus* can help clean up heavy metals in the pulp and paper industry, as reported in the Heliyon Journal in 2020. Additionally, its antiviral properties were documented in a 2005 article in Planta Medica, highlighting its various uses (Li et al., 2005; Sharma et al., 2020). Furthermore, research into environmental reservoirs for pathogens, published in the Environmental Science and Technology journal in 2010, along with its pharmacological properties discussed in the Journal of Ethnopharmacology in 2003, has enhanced our understanding of this plant species (Prieto et al., 2003; Singh et al., 2010). These influential articles collectively contribute to our understanding of the ecological, medicinal, and biotechnological importance of *R. sceleratus* across different ecosystems.

Table 1 Top 10 highly cited research articles published on *R. sceleratus*

Rank	Title	Source	Year	Citations	Reference
1	Changes in growth, porosity, and radial oxygen loss from adventitious roots of selected mono- and dicotyledonous wetland species with contrasting types of aerenchyma	Plant Cell & Environment	2000	273	Visser et al., 2000
2	An ethnobotanical survey of indigenous medicinal plants in Hafizabad district, Punjab-Pakistan	Plos one	2017	152	Umair et al., 2017
3	The potential role of waterbirds in dispersing invertebrates and plants in arid Australia	Freshwater Biology	2007	136	Green et al., 2007
4	Phytoremediation potential of heavy metal accumulator plants for waste management in the pulp and paper industry	Heliyon	2020	74	Sharma et al., 2020
5	Evaluation of Antiviral Activity of Compounds Isolated from Ranunculus *sieboldii* and *Ranunculus sceleratus*	Planta Medica	2005	56	Li et al., 2005

Rank	Title	Source	Year	Citations	Reference
6	Environmental Reservoirs for Enterotoxigenic *Escherichia coli* in South Asian Gangetic Riverine System	Environmental Science and Technology	2010	35	Singh et al., 2010
7	Inhibition of *Trypanosoma cruzi* growth by medical plant extracts	Fitoterapia	2002	33	Schinella et al., 2002
8	Pharmacological approach to the pro- and anti-inflammatory effects of *Ranunculus sceleratus* L.	Journal of Ethnopharmacology	2003	29	Prieto et al., 2003
9	Trace metal accumulation by *Ranunculus sceleratus*: implications for phytostabilization	Environmental Science and Pollution Research	2017	25	Farahat and Galal, 2017
10	Late Pleniglacial and Late Glacial lake-mire transformations in south-eastern Poland reflected in aquatic and wetland vegetation changes	Quaternary International	2015	19	Kołaczek et al., 2015

Most Active Journals

Bibliometric analysis revealed several prominent journals that have actively contributed to the dissemination of scholarly work in this area. Among these, the "Journal of Ethnopharmacology" published by Elsevier emerged as a standout, boasting an impact factor of 4.36 and featuring 3 documents dedicated to the study of this plant species. Elsevier's "Biochemical Systematics and Ecology" also played a significant role, with an impact factor of 1.37 and 2 documents related to *R. sceleratus*. Additionally, the "Plants" journal by MDPI, with an impact factor of 4.66, and "Planta Medica" by Thieme, with an impact factor of 2.49, contributed substantially to the literature, each published 2 documents. Decent contributions also came from journals such as "Egyptian Journal of Biological Pest Control" (Springer), "Plant Cell & Environment" (Wiley), "PLOS One" (PLOS), "Freshwater Biology" (Wiley), "Heliyon" (Elsevier), and "Environmental Science and Technology" (ACS Publications), each featuring 1 document on *R. sceleratus* research (Table 2). These journals collectively represent a diverse spectrum of publishers and scholarly platforms that have facilitated the dissemination and exchange of knowledge on the subject, highlighting the multidisciplinary nature of research about *R. sceleratus*.

Table 2 Top journals in *R. sceleratus* research having highest published document and citations

Rank	Source	Publisher	Impact factor	Documents	Citations	Citations per Document
1	Journal of Ethnopharmacology	Elsevier	4.36	3	42	14.00
2	Egyptian Journal of Biological Pest Control	Springer	1.14	2	11	5.50
3	Biochemical Systematics and Ecology	Elsevier	1.37	2	9	4.50
4	Plants	MDPI	4.66	2	3	1.50
5	Plant Cell & Environment	Wiley	6.36	1	273	273.00
6	Plos One	PLOS	3.75	1	152	152.00
7	Freshwater Biology	Wiley	3.66	1	136	136.00
8	Heliyon	Elsevier	2.85	1	74	74.00
9	Planta Medica	Thieme	2.49	1	56	56.00
10	Environmental Science and Technology	ACS Publications	9.03	1	35	35.00

Top Productive Organizations

In the bibliometric analysis, several productive organizations emerged as key contributors, each making significant strides in advancing knowledge in this field. Among the top organizations, Anhui Medical University published the highest 4 documents with 18 citations, showcasing their dedication to exploring the work of this botanical subject. Subsequently, the University of Valencia published 3 documents and gathered a remarkable 70 citations, indicating the high impact of their research efforts. The University of Latvia also demonstrated a strong presence, with 3 documents and 15 citations, further enriching the scholarly discourse on *R. sceleratus*. Other institutions include Zhejiang University, the Indian Institute of Toxicology Research, Helwan University, Yonsei University, the University of Rostock, King Saud University, and the

University of Sargodha, each contributing significantly to the collective understanding of this plant species through their respective publications and citations (Table 3). Through their collaborative efforts and rigorous research activities, these organizations have played pivotal roles in shaping the research landscape of *R. sceleratus*, paving the way for further exploration and discovery in this field.

Table 3 Top organizations' contributions to *R. sceleratus* research

Rank	Organization	Country	Documents	Citations	Citations per Document
1.	Anhui Medical University	China	4	18	4.50
2.	University of Valencia	Spain	3	70	23.33
3.	University of Latvia	Latvia	3	15	5.00
4.	Zhejiang University	China	2	67	33.50
5.	Indian Institute of Toxicology Research	India	2	52	26.00
6.	Helwan University	Egypt	2	33	16.50
7.	Yonsei University	South Korea	2	22	11.00
8.	University of Rostock	Germa-ny	2	15	7.50
9.	King Saud University	Saudi Arabia	2	7	3.50
10.	University of Sargodha	Pakistan	2	7	3.50

Top Fields of Study

The study examined a dataset comprising 86 scholarly documents sourced from Lens.org, aiming to investigate the prevalence of terms across significant academic domains related to plant species *R. sceleratus*. Biology emerged as the most prominent field, with 65 documents indicating a strong focus on the biological aspects of the species, including its physiology, genetic makeup, and interactions with other organisms. Subsequently, *Ranunculus sceleratus*, with 54 documents, highlighted a significant portion of research dedicated to understanding this species, its distribution, unique characteristics, and ecological role. Botany, represented by 41 documents, was another major field, reflecting the interest in the plant's classification, morphology, and place within the plant kingdom. Both the Chemistry and Ecology fields had 18 documents each, showing that the chemical properties of *R. sceleratus* and its ecological interactions were equally significant research areas. Environmental Science, with 12 documents,

and Environmental Chemistry, with 11 documents, highlighted the environmental impact and chemical properties related to the species, possibly in terms of pollution tolerance, bioaccumulation, and effects on water quality. The general study of the genus *Ranunculus*, with 9 documents, indicated some research focused on broader genus-level questions that included multiple species, providing comparative insights. Research in the context of aquatic plants and horticulture, each with 8 documents, suggested a focus on the species' role in aquatic ecosystems, its growth conditions, and interactions within these habitats (Fig. 5). Overall, the research landscape for *R. sceleratus* was predominantly biological, with significant contributions from botany and specialized studies directly on the species. The interdisciplinary interest encompassing chemistry, ecology, and environmental sciences indicated a comprehensive approach to understanding its characteristics, environmental interactions, and potential uses or impacts.

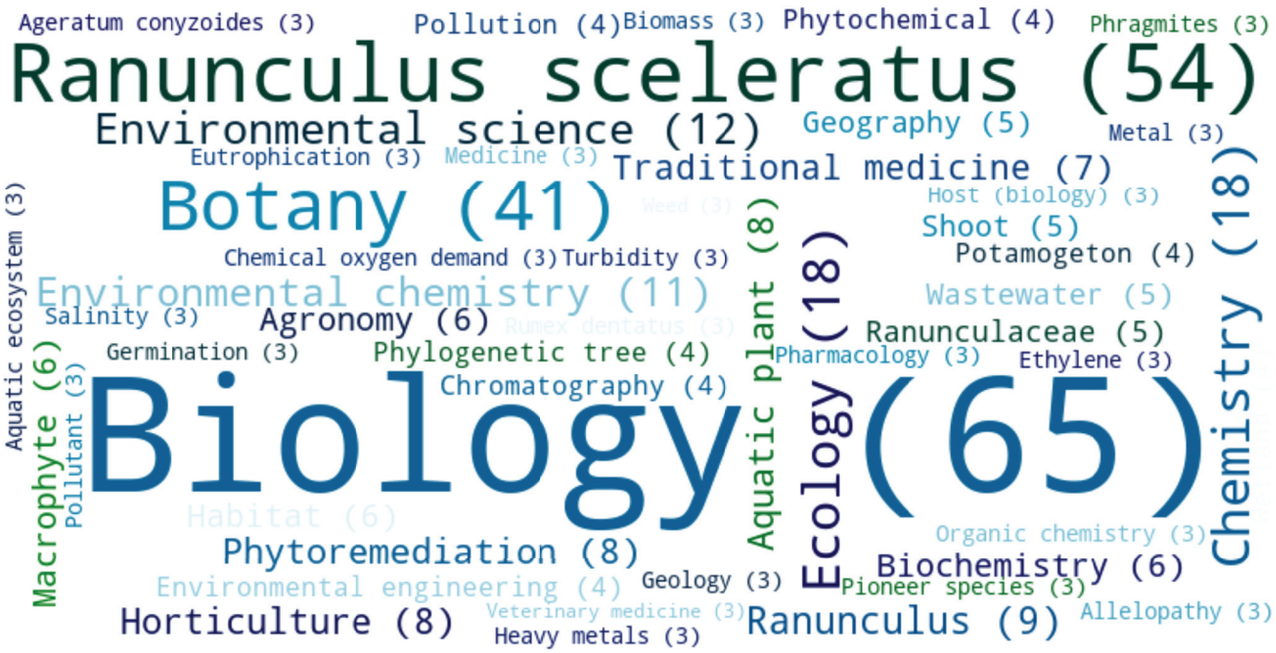

Fig. 5 Terms co-occurrence analysis within prominent terms

Research Collaboration Networks

Authors' Collaboration Network

The study on *R. sceleratus* offers insights into the dynamics of scholarly interaction within its research community. Analyzing 245 authors and their publications, the study examined the interconnectedness and collaborative efforts among researchers. Inclusion criteria required authors to have at least one document on *R. sceleratus* with a minimum of one citation per document. The network revealed 19 authors with 115 links, forming 2 distinct clusters, indicating cohesive author groups. Cluster 1, the largest, consisted of 14 authors, while cluster 2 included 5 authors, showing diversity in collaboration. Wenming Cheng and Qunlin Zhang emerged as the most influential authors, each with a high total link strength of 23. Yu Zhao and Hua Bai also stood out, with link strengths of 18 and 14 respectively, emphasizing the field's diverse expertise and collaborative networks (Fig. 6). The findings highlight a dynamic and varied research community characterized by different levels of specialization and collaboration. This network analysis provides valuable insights into collaborative dynamics and knowledge dissemination, facilitating future collaborations and interdisciplinary exchanges within the *R. sceleratus* research community.

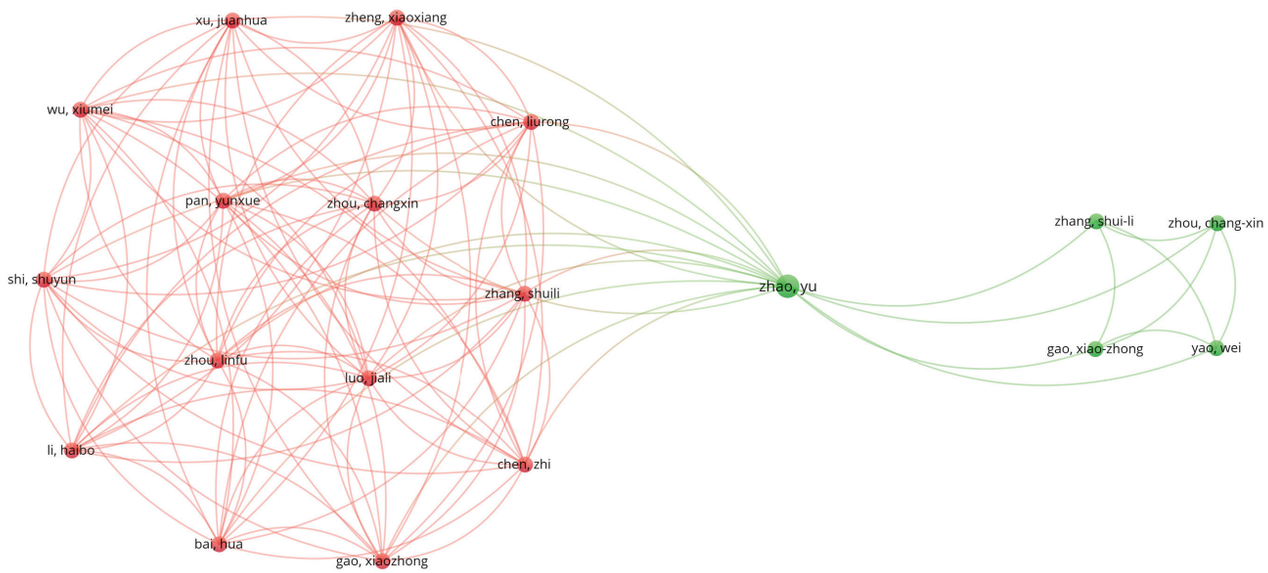

Fig. 6 Collaborative network among the authors of *R. sceleratus* research

Journal-wise Collaboration Network

An extensive examination of journal collaboration in *R. sceleratus* research was carried out using a dataset comprising 61 journals. This thorough investigation showed 118 connections among 21 journals through bibliographic coupling analysis. To be included in the collaborative network, journals had to publish at least one document on *R. sceleratus*, with each receiving a minimum of one citation. The analysis distinguished 6 distinct clusters within the network, showcasing groups of journals with robust interconnections. Clusters 1 and 2 emerged as the largest, comprising 7 and 4 journals respectively, while clusters 3 and 4 consisted of 3 journals each. Moreover, clusters 5 and 6 encompassed 2 journals each (Fig. 7). Among the journals, "Soil Biology" emerged as the most influential journal, boasting the highest connection strength at 35. Subsequently, "Life" exhibited a total link strength of 32, while "Biogeosciences" and "EGUsphere" also showed noteworthy influence, each with total link strength of 27. This network analysis revealed collaborative dynamics and thematic strengths among *R. sceleratus* research journals, highlighting key journals and clusters for potential future collaborations.

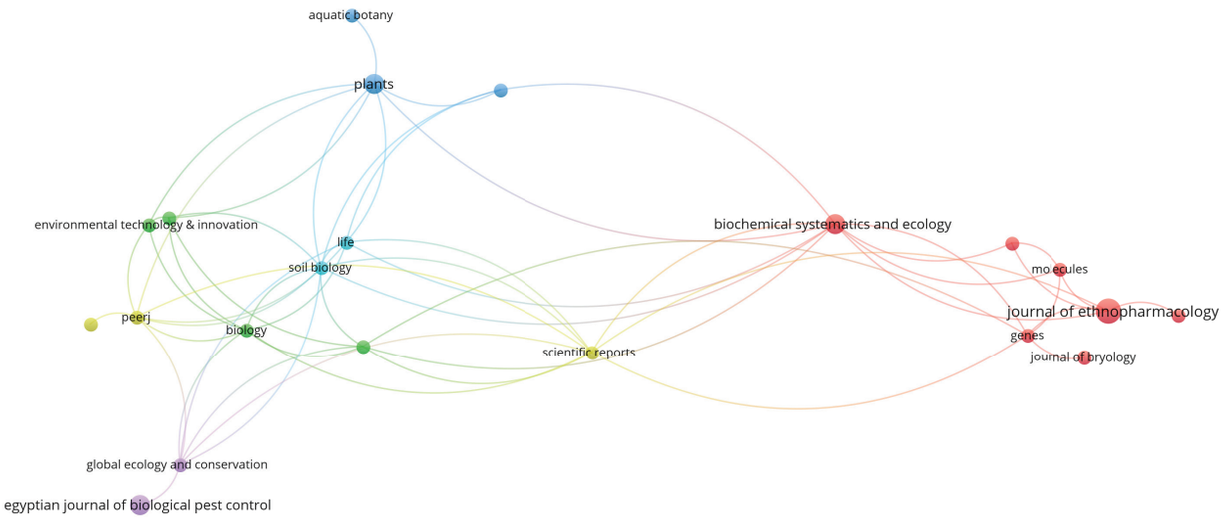

Fig. 7 Journal-wise collaboration network analysis on *R. sceleratus* research

Organizational Collaborative Network

The study used bibliographic coupling analysis to explore the collaborative network among organizations in *R. sceleratus* research. Among 451 organizations, 47 met the criteria of publishing at least one document with at least one citation each. The analysis identified 4110 connections among these 47 organizations, divided into 8 clusters. Cluster 1, the largest with 11 organizations,

showed significant inter-organizational cooperation, indicating a highly collaborative environment. Clusters 2 and 3, with 8 and 7 organizations respectively, showed intense internal connections, suggesting specialized research areas within scholarly publication on *R. sceleratus*. Clusters 4 and 5, each with 5 organizations, likely represent specialized or emerging areas of research, requiring focused collaboration. Clusters 6, 7, and 8 included 4, 3, and 2 organizations respectively, also showing strong internal connections, implying specialized or emerging collaborative networks. Yonsei University emerged as the most influential, with the highest connection strength and a total link strength of 515. Helwan University followed with 473 connections, and Alexandria University with 425, highlighting their significant contributions and influence (Fig. 8). Understanding this network proceeds effective partnerships, directs resources to key organizations, advances *R. sceleratus* research, and emphasizes areas for new collaborations and emerging research clusters.

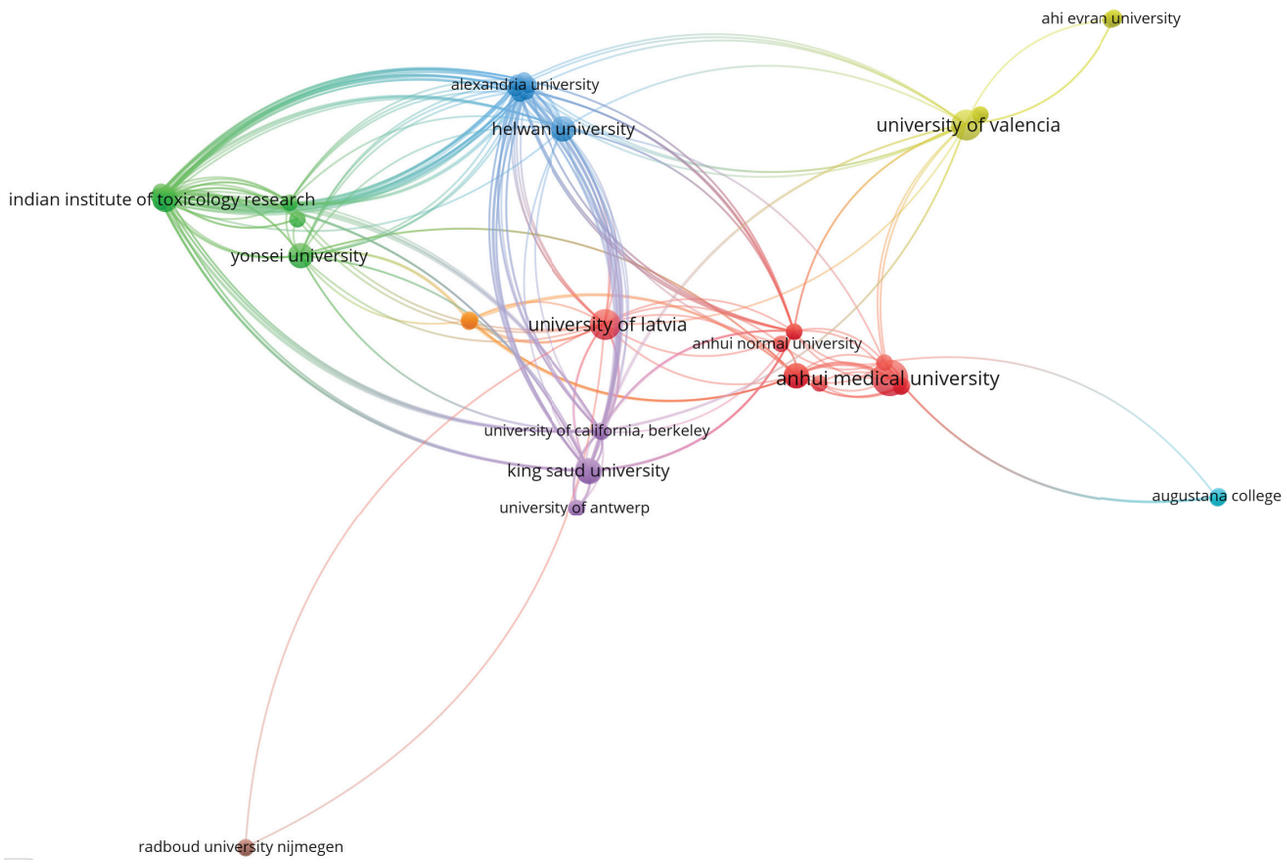

Fig. 8 Clusters of collaborative institutions for *R. sceleratus* research

Country-wise Collaboration Network

The country-wise collaboration network analysis in *R. sceleratus* research highlights global interconnectedness and cooperation among 12 nations. Through bibliographic coupling analysis, the study identified 36 collaborative connections, ensuring that only significant contributions were included by requiring each country to publish at least one document with a minimum of one citation. Six distinct clusters of collaboration emerged, reflecting varying degrees of interconnectedness and research focus. Clusters 1 and 2, with 3 countries each, exhibited the most robust collaborative activities. Clusters 3 and 4, with 2 countries each, showed moderate collaboration, while clusters 5 and 6, each with a single country, indicated more focused but isolated research efforts. China and Spain were outstanding for their strong collaboration strengths, each forming 14 links, highlighting their dominant roles in global *R. sceleratus* research. This emphasizes the significance of their contributions and leadership in this field (Fig. 9). The analysis highlights the value of international collaboration in driving research progress, enhancing knowledge sharing, techniques, and resources, and fostering innovation and deeper scientific understanding of *R. sceleratus*.

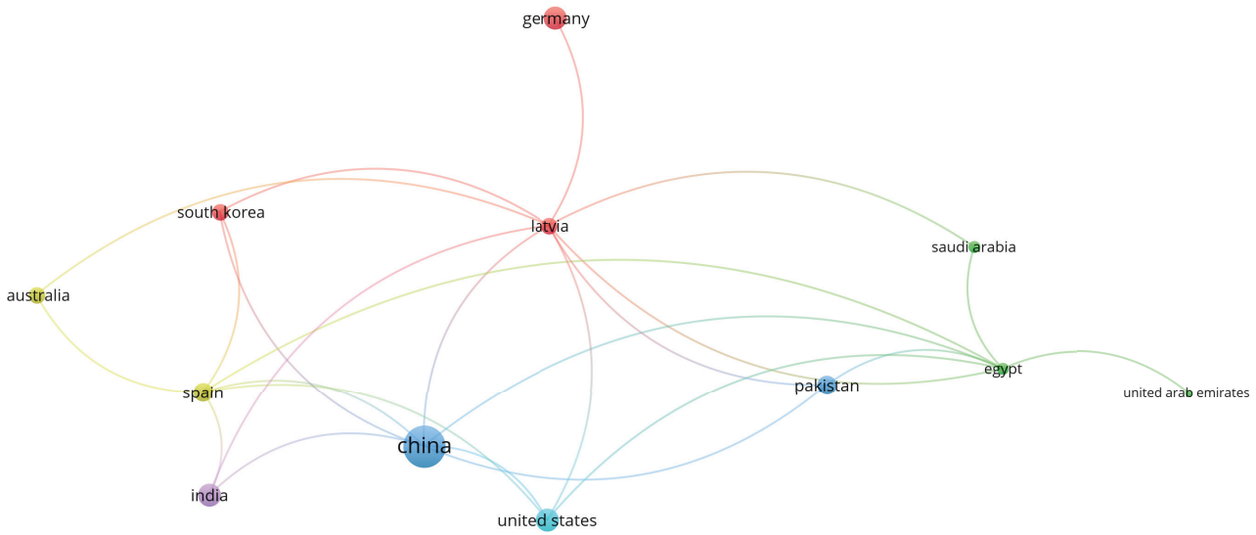

Fig. 9 Country-wise collaboration network in *R. sceleratus* research

Conclusion

The bibliometric analysis of the research landscape concerning *R. sceleratus* provides a comprehensive overview of its scholarly impact and global research trends from 2000 to 2023. This study, based on data collected from Dimensions.ai, encompassed 66 scholarly articles authored by 245 researchers from 91 institutions across 23 countries, reflecting a broad international interest in this plant species. The analysis revealed that the consistent publication output and citation rates emphasized the enduring scientific relevance of *R. sceleratus*. The average citation rate of 16.53 citations per document indicates the substantial impact and recognition within the academic community, driven by pivotal publications and ongoing research contributions. The highest publications around 2020 and 2023 suggest intensified research activity, possibly influenced by significant discoveries or emerging research priorities. Moreover, the geographical distribution highlighted China's prominent role in *R. sceleratus* research, leading both in publication output and citations, indicative of its strong research capabilities in this botanical field. Moreover, specific authors such as Wenming Cheng and Qunlin Zhang, along with institutions like Anhui Medical University, made prominent contributions, emphasizing diverse global participation and expertise in this botanical research. The analysis also identified key publications and journals that have shaped the discourse on *R. sceleratus*, with remarkable works appearing in prestigious journals like Plant Cell & Environment and Journal of Ethnopharmacology. These publications have enriched our understanding of *R. sceleratus* from various disciplinary perspectives, including biology, chemistry, ecology, and botany, highlighting its ecological, medicinal, and horticultural significance. Collaborative networks among authors, journals, organizations, and countries have played a crucial role in advancing *R. sceleratus* research and facilitating knowledge exchange and innovation. Such collaborations are pivotal in driving future advancements and applications of *R. sceleratus* in diverse fields. Overall, this bibliometric analysis highlights the global importance and collaborative nature of *R. sceleratus* research, laying a robust foundation for continued exploration and innovation in harnessing the potential of this botanical species. As research continues to evolve, interdisciplinary approaches will further deepen our understanding and utilization of *R. sceleratus*, contributing to its conservation, medicinal applications, and ecological management worldwide.

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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 pwlant 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 possiwble conditions for the cultivation of medicinal plants. In the current study, emphasize on studying the physicochemical variations in soil quality from different altitudes of sampling sites is given in Table 2. Soil physicochemical parameters like pH, electrical conductivity, organic carbon content, available nitrogen, phosphorous, potassium, sulphur and micronutrients, total moisture content, heavy metal, and bulk density have been studied to understand the quality at different altitudes and how they may influence vegetation in the different sites.

Sampling Sites

The bulk soil and plants (along with the rhizospheric soil) were collected from a total of 26 sites. The numbering of the sites, name of sites along with the 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	78.85346
2.		Gangotri	UK-S2	31.03920	79.62818
3.		Uttarkashi	UK-S3	30.74619	80.27700
4.		Devprayag	UK-S4	30.13581	81.12184
5.		Haridwar	UK-S5	29.91694	81.53151
6.	Uttar Pradesh	Bijnor	UP-S1	29.28574	82.32432
7.		Narora	UP-S2	28.14650	83.13147
8.		Badaun	UP-S3	27.93941	83.56120
9.		Farrukhabad	UP-S4	27.41044	84.49599
10.		Bithoor	UP-S5	26.61589	85.12415
11.		Dalmau	UP-S6	26.34853	85.59791
12.		Prayagraj	UP-S7	25.25373	86.20763
13.		Mirzapur	UP-S8	25.85879	87.09083
14.		Varanasi	UP-S9	25.15181	87.39595
15.		Ballia	UP-S10	25.35455	87.91067
16.	Bihar	Revelganj	BH-S1	25.43876	88.22134
17.		Patna	BH-S2	25.38077	88.38087
18.		Barh	BH-S3	25.23107	88.38949
19.		Bahachoki	BH-S4	25.17823	88.19586
20.		Farka	BH-S5	25.23225	78.85346
21.	Jharkhand	Sahibganj	JH-S1	25.14606	79.62818
22.	West Bengal	Farraka	WB-S1	24.82274	80.27700
23.		Hazarduari	WB-S2	23.99688	81.12184
24.		Mayapur	WB-S3	23.41293	81.53151
25.		Hoogly	WB-S4	22.84913	82.32432
26.		Gangasagar	WB-S5	22.17743	83.13147

The temperature of a particular site is dependent on its altitude as well as climate conditions. At the time of sample collection, the temperature of the sites as well as altitude of the sites as shown in live GPS map via GPS map camera app was noted down. The altitude and temperature of different sites for sample collection are shown in the Table 2.

Table 2 Temperature and altitude of different sampling sites

Site	Temperature (°C)	Altitude (m)
UK -S1	09	3794
UK –S2	17	2506
UK –S3	18	1232
UK –S4	23	1014
UK –S5	34	265
UP-S1	29	217
UP-S2	31	179
UP-S3	25	162
UP-S4	27	136
UP-S5	28	113
UP-S6	31	91
UP-S7	33	91
UP-S8	32	91
UP-S9	38	65
UP-S10	34	67
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±5°C. Almost all soil tests detect the natural moisture content of the soil, which is critical knowledge for all soil mechanics. The natural moisture content indicates the condition of the soil in the field (Hossain et al., 2022).

Apparatus and equipment required: non-corrodible vented container, thermostatically controlled drying oven that maintains temperatures between 105°C to 115°C, balance of sufficient sensitivity (sensitive to 0.01 g) and container handling apparatus.

Procedure

1. Clean, dry and weigh W_1 the container. The balance needs to be tared before it is used to measure the weight.
2. Weigh W_2 a 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 = (Mw g/ Ms g) × 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 cm^3).
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. Bilk 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 to according to the temperature of the solution (Varley, 1972; Jackson, 1973).

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

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

Procedure

1. 25 g of the soil was weighed in a 100 ml beaker and make it to 50 ml final volume by adding of distilled water. The mixture was stirred well for at least four times within a 30-minute period to allow the soil and water to reach equilibrium.
2. In the meantime, the pH meter was switched on.

3. Initially the instrument was calibrated with buffer solution of known pH 4, 7.0, and 9.2.
4. The electrodes were washed with distilled water and wiped dry with a tissue paper.
5. Then, the electrode was dipped in the sample and the readings were taken.
6. Finally, the electrodes were washed with distilled water and placed back into a beaker containing 4.00-7.00 pH buffer or 3-4M KCl.

Interpretation

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

Electrical Conductivity

Principle: A conductivity meter known as "Solu Bridge" is used to measure the electrical conductivity of a soil solution. It is based on the Wheat Stone Bridge principle, in which alternating current is utilized instead of direct current to prevent electrode polarization and electrolysis of the solution. In a branched circuit with the conductance cell having resistance Rx, two fixed resistances R1 and R2 and a variable resistance (Rv) are linked. The variable resistance (Rv) is adjusted until no current flows through it and the reading is taken. The resistance or conductance (Rv) 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; µS/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 $K_2Cr_2O_7$): 49.04 g of analytical grade $K_2Cr_2O_7$ was dissolved in distilled water and the volume was made up to 1 litre.
2. Concentrated sulphuric acid (H_2SO_4).
3. Orthophosphoric Acid (85%) or sodium fluoride (NaF).
4. Diphenylamine indicator: 0.5 g diphenylamine indicator was dissolved in a mixture of 100 ml concentrated sulphuric acid and 20 ml distilled water. This was stored in an amber colour bottle.
5. Ferrous ammonium sulphate solution [0.5 N, $FeSO_4(NH_4)_2SO_4.6H_2O$]: 392 g analytical grade ferrous ammonium sulphate was dissolved in distilled water, to which 15 ml concentrated sulphuric acid was added and volume to made up to 2 litres with distilled water.

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

Procedure

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

1. 2 gm of dried, ground, and sieved soil was taken in a 250 ml conical flask.
2. To it, 10 ml 1 N $K_2Cr_2O_7$ 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

3. Vol. of N/2 ferrous ammonium sulphate used to titrate excess nascent oxygen = Y ml
4. Vol. of 1N $K_2Cr_2O_7$ used for oxidation of organic carbon in soil = (X-Y)/2 ml
5. 1 ml of 1N $K_2Cr_2O_7$ =0.003 g C
6. % Organic Carbon in Soil= X-Y/2 x 0.003 × 100/W

Interpretation

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

Available Nitrogen

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

Chemicals and reagents required

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

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

Procedure

1. The Kjeldahl assembly was prepared by dipping the respective inlet tubes, one in 0.32% $KMnO_4$ and second in 2.5% NaOH reagent tanks.
2. 5 g soil sample was weighed and put into distillation tube carefully so that the soil sample does not stick to the sides of the tubes. Soil particles attached to the sides of the tube were washed down with distilled water.
3. The distillation tube was fixed in distillation unit and the sample was moistened with distilled water by pressing the dilution key.
4. 25 ml of 2.5% boric acid was taken in a conical flask and the receiving end of the distillation tube was dipped in it.

5. Next, 25 ml of $KMnO_4$ and 25 ml NaOH were added in the sample by pressing the respective keys.
6. The heating unit was switched on, and the process ran for 6 minutes.
7. The ammonia gas released from the sample was distilled and collected into the receiver containing acid. After complete digestion, the receiver flasks were removed.
8. The ammonium borate formed in the receiver flask was titrated against 0.02 N H_2SO_4 acid and note the volume of 0.02 N H_2SO_4 utilized. The colour changes from bluish green to wine red.

Calculations

1. Weight of soil taken = 5 g
2. Vol. of 2.5% boric acid taken = 25 ml
3. Vol. of 0.02 N H_2SO_4 used to titrate ammonium borate = X ml
4. 1 ml of 0.02 N H_2SO_4 = 0.00028 g of N
5. Available N (%) = 0.00028 g of N
6. Available N (ppm) = percentage N × 10,000
7. Available N (Kg/ha) = ppm × 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 litre.
   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 litre.
4. Reagent A: Dissolve 12 g of ammonium paramolybdate in 250 ml distilled water. Separately, 0.2908g of potassium antimony tartrate ($KSbO.C_4H_4O_6$) was dissolved in 100 ml of distilled water. Both these dissolved reagents were added to 1 litre of 5 N sulphuric acid. Both were mixed thoroughly and diluted with distilled water to 2 litres.
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. 10 ml of distilled water was added while washing the neck of the flask and 4 ml of reagent B and the volume was made up to the mark with distilled water and mixed. All flasks developed a blue colour of variable intensity. The intensity of blue colour was read on spectrophotometer at 880 nm. A standard curve was plotted using P concentration against absorbance value to establish their relationship.

b) **Analysis of the test sample**

1) 1 g of soil sample was taken in a 100 ml flask.
2) To it, a pinch of Darco-G 60 and 20 ml of 0.5 N $NaHCO_3$ solution were added.
3) The flask was placed on an electric shaker and mixed at a constant speed for half an hour. Then the contents were filtered through Whatman No. 1.
4) A blank was prepared by following all the steps without addition of soil.
5) In case, the filtrate was not clear, a pinch of Darco – G was added.
6) 5 ml of the filtrate was taken in a 25 ml volumetric flask and proceed further for colour development as described under preparation of standard curve.

Calculations

1. Weight of soil sample taken = 1 g
2. Vol. of 0.5N $NaHCO_3$ solution added = 20 ml

3. First dilution = 20 times
4. Vol. of the filter taken for colour development = 5 ml
5. First volume made = 25 ml
6. Second volume = 5 times
7. Total dilution = 20 × 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 (CH_3COONH_4): 77.09 g/l of ammonium acetate was dissolved in distilled water and the volume was made up to 1 litre. The pH of the solution was adjusted to 7 with ammonium solution or acetic acid.
2. Standard solution of K (1000 ppm K): 1.91 g of potassium chloride (KCl) was dissolved in distilled water and the volume was made to 1 litre.
3. Working standard solution of K: The stock solution was diluted 100 times to get 10 ppm K solution.

Apparatus and equipment required: A weighing balance, 150 ml conical flasks, a shaker, funnels, beaker, Whatman filter paper No 1, pipettes and a flame photometer.

Procedure

1. 5 g of soil was weighed in a 150 ml conical flask.
2. To this, 25 ml of neutral normal ammonium acetate solution was added.
3. The mixture was shaken for 5 minutes on an electric shaker and then filtered through Whatman No. 1 filter paper.
4. 5 ml of the filtered extract was taken in a 25 ml volumetric flask and the volume

was made up with distilled water. This solution was fed into the atomizer of the flame photometer and readings were noted down.

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

Calculations

1. Weight of soil sample taken = 5 g
2. Volume of the neutral normal CH_3COONH_4 solution added = 25 ml
3. Dilution = 5 times
4. Reading of K (ppm) in flame photometer = Y
5. In ppm K = Y × total dilution-A
6. In kg/ha = A × 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 ($CaCl_2.2H_2O$) was dissolved in distilled water and volume made up to 1 litre.
2. Barium chloride ($BaCl_2$)
3. Gum acacia solution (0.25%): 0.25 g of gum acacia was dissolved in distilled water and diluted to 100 ml.
4. Standard sulphur solution (100 ppm): 0.5434 g of the reagent grade potassium sulphate (K_2SO_4) was dissolved in distilled water and diluted to 1 litre.

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

Procedure

a) **Preparation of standard curve**

1. 0.25, 0.5, 1.0, 2.5 and 5.0 ml of 100 ppm S solution were taken in different 25 ml volumetric flasks. It was theoretically calculated to give 0, 1, 2, 4, 7, 10, 20 ppm concentration of sulphur in the final volume.

2. To every flask 10 ml 0.15% $CaCl_2$ solution and 1 g $BaCl_2$ were added.
3. Mixed for 1 minute to dissolve all the crystals.
4. Next, 1 ml 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. 10 g 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= 10 g
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 × 1.25= 6.25 times
8. ppm of S from standard curve= Y
9. ppm of S in soil= Y × 6.25
10. S in kg/ha= ppm of S × 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).

3. Kept at room temperature 20-30 minutes for open digestion.
4. Afterwards samples were put in microwave for close digestion.
5. After digestion, samples were transferred into the 50 ml volumetric flasks and volume make up to the mark.
6. Samples were vortexed properly and run of ICP-MS against the linearity.

Calculations

Calculate the concentration of the elements as follows.

Sample Conc = (Sample reading-reagent blank reading × 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 $CaCl_2.2H_2O$ 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 $CaCl_2.2H_2O$ helps avoiding dissolution of $CaCO_3$ and thus excludes from the estimation of the occluded micronutrients, which do not form a part of the pool that is available for absorption by plant roots. After that the contents of the micronutrients cations in the soil extract are estimated on atomic absorption spectrophotometer (Katyal and Sharma, 1991).

Chemicals and reagents required

1. DTPA extraction solution: This solution was prepared to contain 0.005M DTPA, 0.01M $CaCl_2$ and 0.1M TEA (Triethanolamine) and its pH was adjusted to 7.3. For preparing one litre of this solution, 13.3 ml $(HOCH_2CH_2)_3N$ i.e. TEA, 1.967 g DTPA and 1.47 g $CaCl_2.2H_2O$ were dissolved in about 500 ml of deionized water. Sufficient time was given for DTPA to dissolve, and the contents are diluted to approximately 900 ml. The pH of the solution was then adjusted to 7.30 ± 0.05 by adding 6N HCL while stirring and finally the contents were diluted to 1 litre. This solution was stable for several months.

Apparatus and equipment required: 1 litre volumetric flask, 100 ml narrow mouth polyethylene bottles, pipettes, electric shaker, Whatman No. 1 filter paper and atomic absorption spectrophotometer.

Procedure

1. 12.5 g of air-dried soil sample was taken and transferred to a 100 ml narrow mouth polyethylene bottle.
2. 50 ml DTPA solution was added, and the bottle was closed with the stopper.
3. The bottle was shaken on an electric shaker for 2 hours at 25°C.
4. The contents were then filtered through Whatman No. 42 filter paper to obtain a clear solution.

5. Also, a blank was run with only DTPA solution without the soil.

Calculations

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

1. Different growth medium is used to grow different types of microorganisms is given in Table 3.
2. The medium is sterilized in an autoclave.
3. Petri dishes are used to hold the growth media.
4. A small number of bacteria is needed to inoculate the growth media.
5. 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.
6. The inoculated growth media is incubated at the optimal temperature and conditions for the bacteria to grow.
7. 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 various growth media that are used to cultivate the various kinds of microorganisms

S. 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.	*Rhizobium*	Rhizobium	HiMedia (M408-500G)
7.	*Azotobacter*	Azotobacter	HiMedia (M1944-500G)
8.	*Azospirillum*	Azospirillum + KOH	HiMedia (M1720-500G + M1720-500G)
9.	Phosphate Solubilizes	Pikovskaya	HiMedia (GM1719-500G)

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

Calculation

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

Correlation Studies and Statistical Analysis

The tests have been performed in triplicates and the mean of values along with the standard deviation has been represented graphically. A total of 8 parameters have been studied for 26 sites and each parameter has shown considerable variation. The effect of one parameter on the other may be studied through correlation. A correlation coefficient is an indicator of the relationship between two variables. The correlation coefficient is a statistical measure that indicates the strength and direction of the relationship between two variables. Its value ranges from -1 to +1, representing different degrees and types of correlation. When the correlation coefficient is closer to +1, it signifies a strong positive relationship between the variables. This means that as one variable increases, the other tends to increase as well. A correlation coefficient around 0 implies no linear relationship between the variables. Changes in one variable do not predict or affect changes in the other. As the correlation coefficient approaches -1, it denotes a strong negative relationship. This suggests that as one variable increases, the other tends to decrease.

Results and Discussion

Physicochemical Characterization of Soil

Moisture content is one of the most essential index qualities for determining the relationship between soil behaviour and index values. Soil moisture content expresses the phase relationships of water, air, and solids in each volume or weight of material. The consistency of a specific soil, combined with its liquid and plastic limitations, is used to represent its relative consistency in cohesive soil (Hossain et al., 2022). The moisture content of different sampling sites is shown in Fig. 1

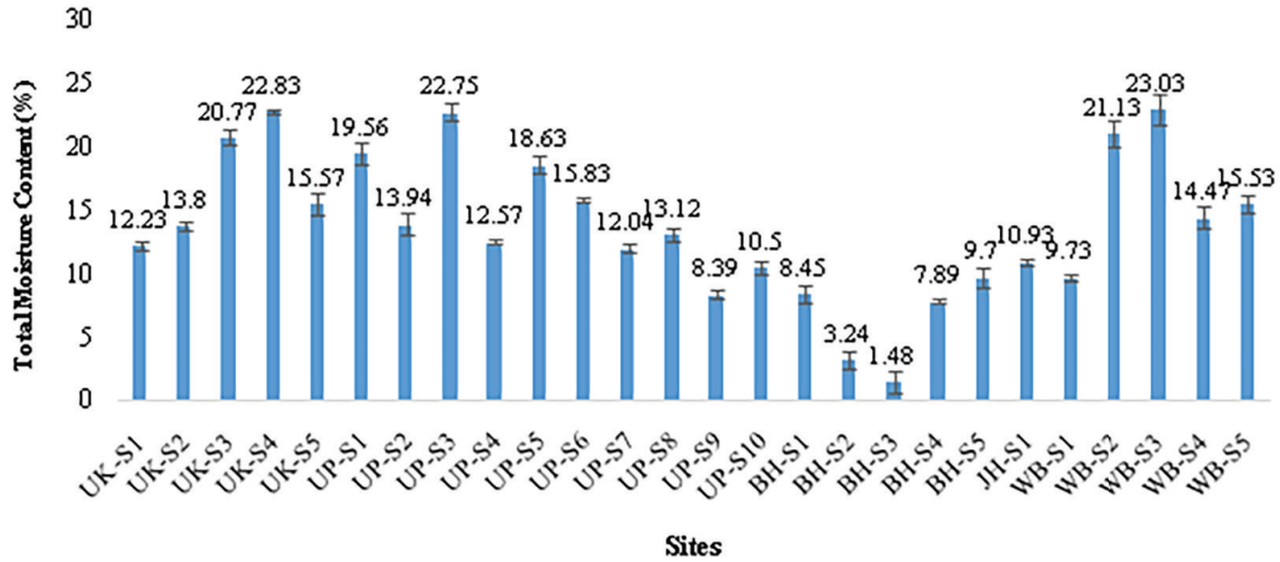


Fig. 1 Total moisture content of different sampling sites

The TMC% was found to be in the range of 1.48±0.94% (lowest) to 23.03±1.25% (highest). Soil sample from BH-S3 had very little moisture content i.e., 1.48±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.

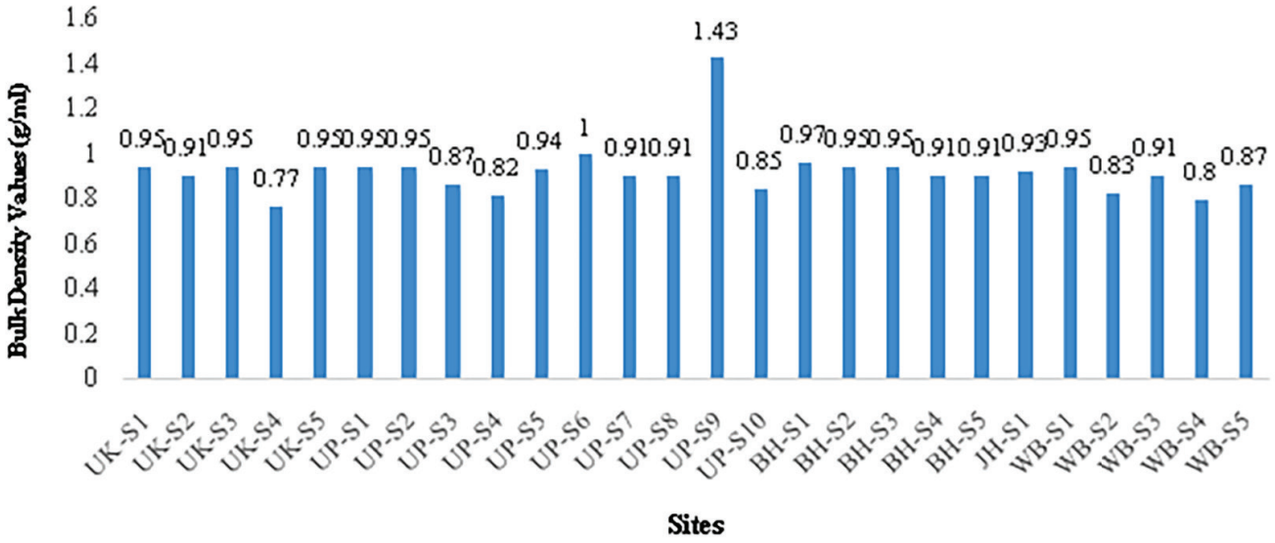


Fig. 2 Soil bulk density of different sampling sites

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.

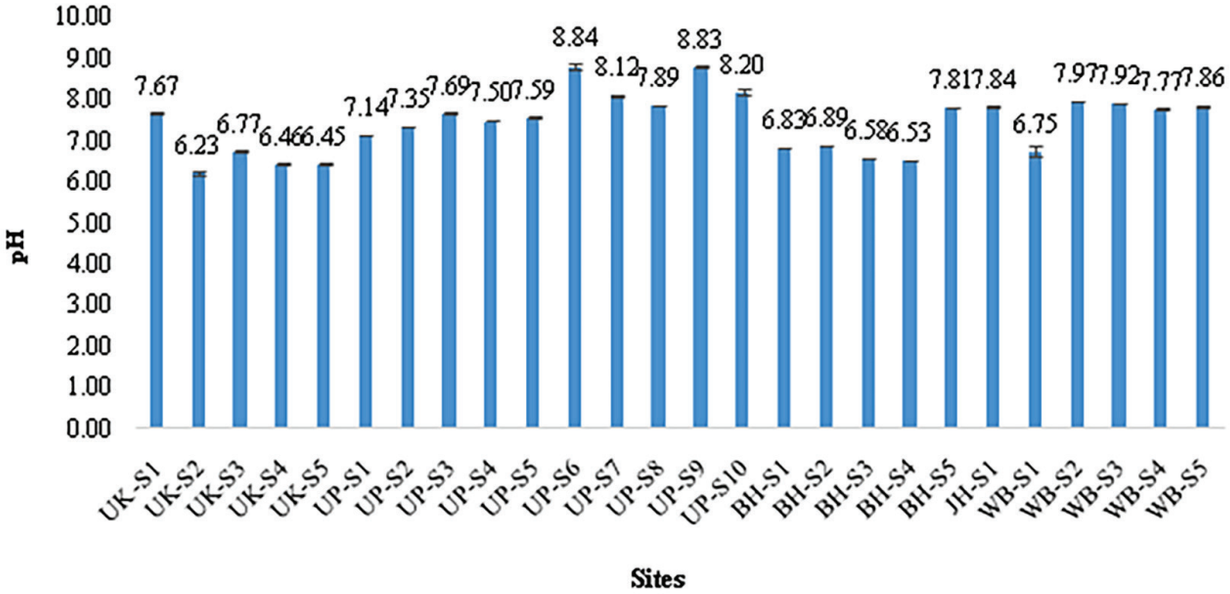


Fig. 3 Soil pH of different sampling sites

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.

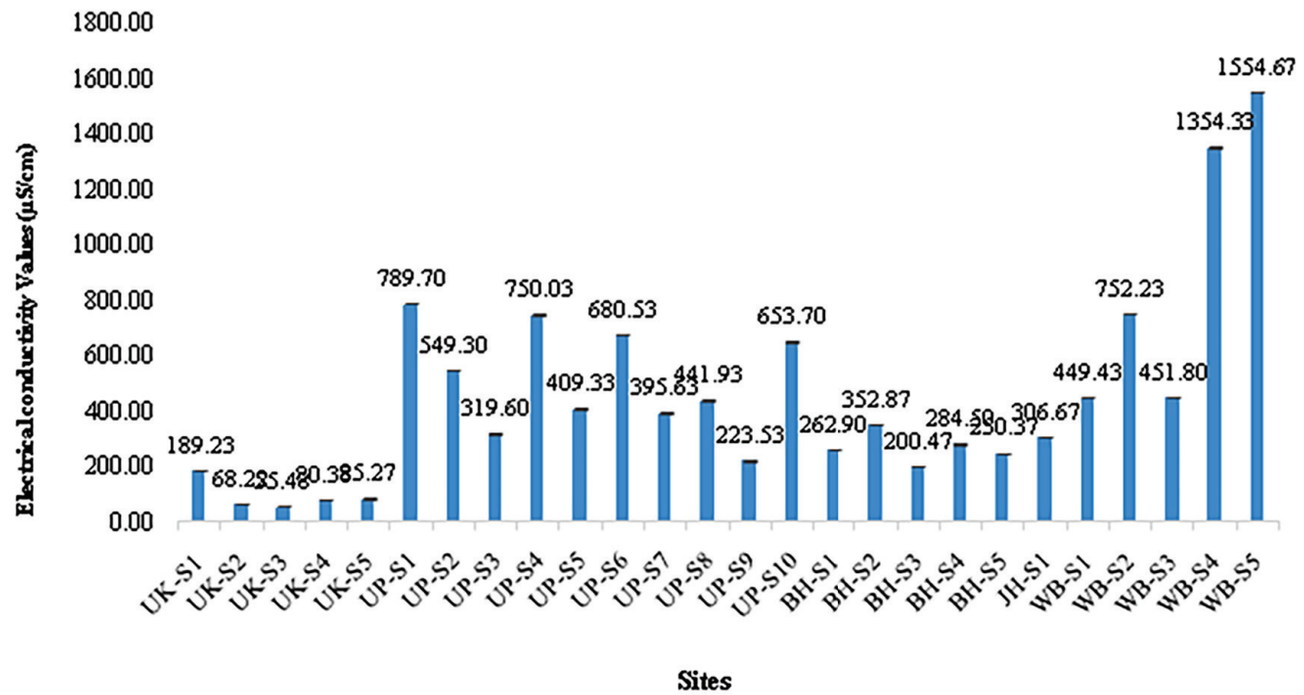


Fig. 4 Soil electrical conductivity at different sampling sites

The electrical conductivity of almost all the soil samples were within the prescribed limits of having EC below 800 µS/cm. Soil samples from WB-S4 and WB-S5 showed the highest electrical conductivity value of 1354.33±2.52 µS/cm and 1554.67±2.52 µS/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.

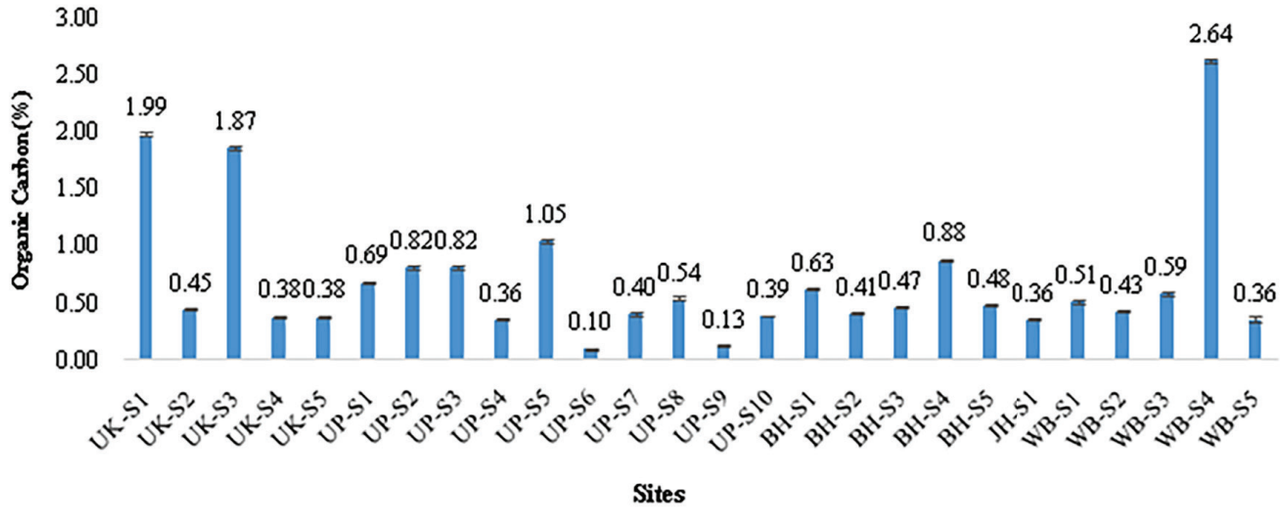


Fig. 5 Soil organic carbon content at different sites

According to the data, the organic carbon content of soil ranged from 0.10±0.01% to 2.64±0.03%. UP-S6, which had the highest pH value of 8.84 ± 0.01, had the lowest organic carbon content of 0.10±0.01%. The highest quantity of organic carbon content was discovered in WB-S4 (2.64±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 (Baričevič 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.

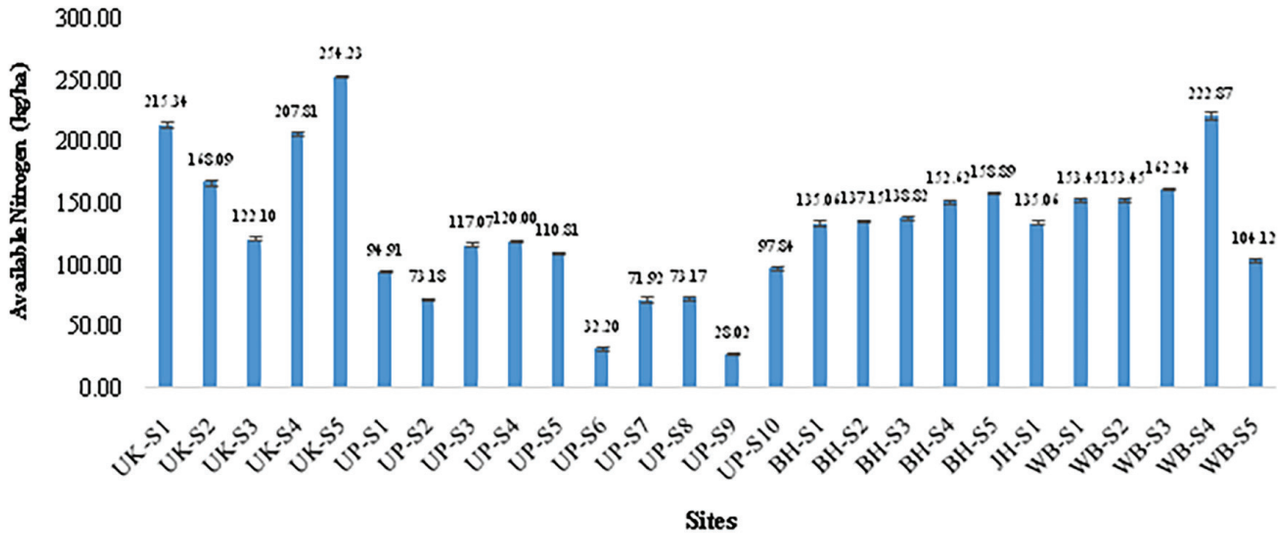


Fig. 6 Soil available nitrogen content at different sites

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.

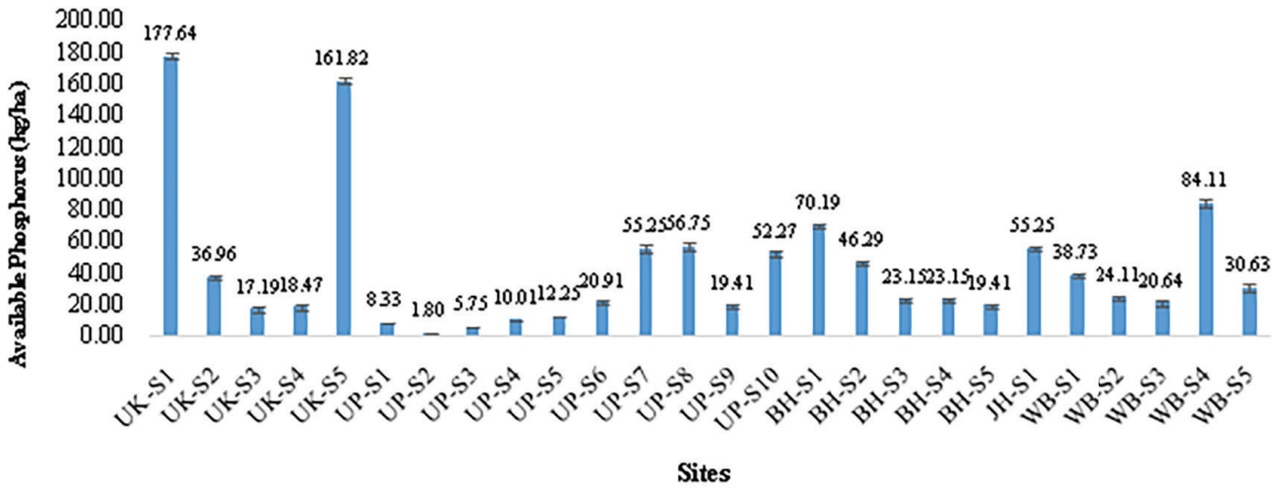


Fig. 7 Soil available phosphorus content at different sites

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.

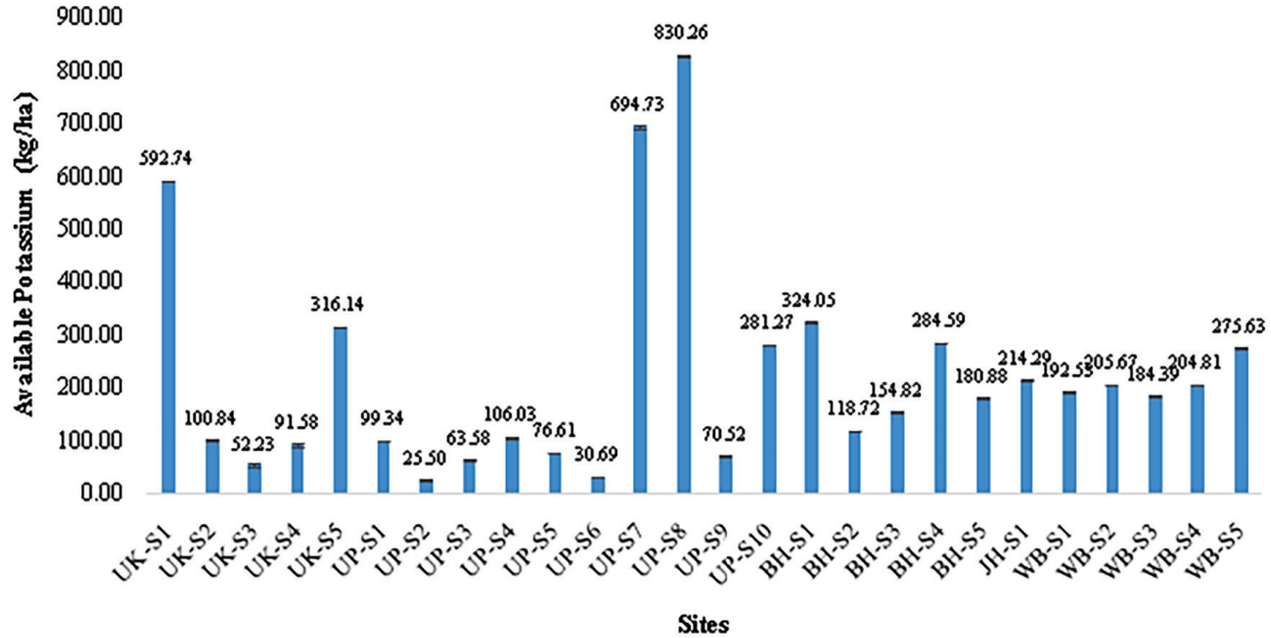


Fig. 8 Soil available potassium content at different sites

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.

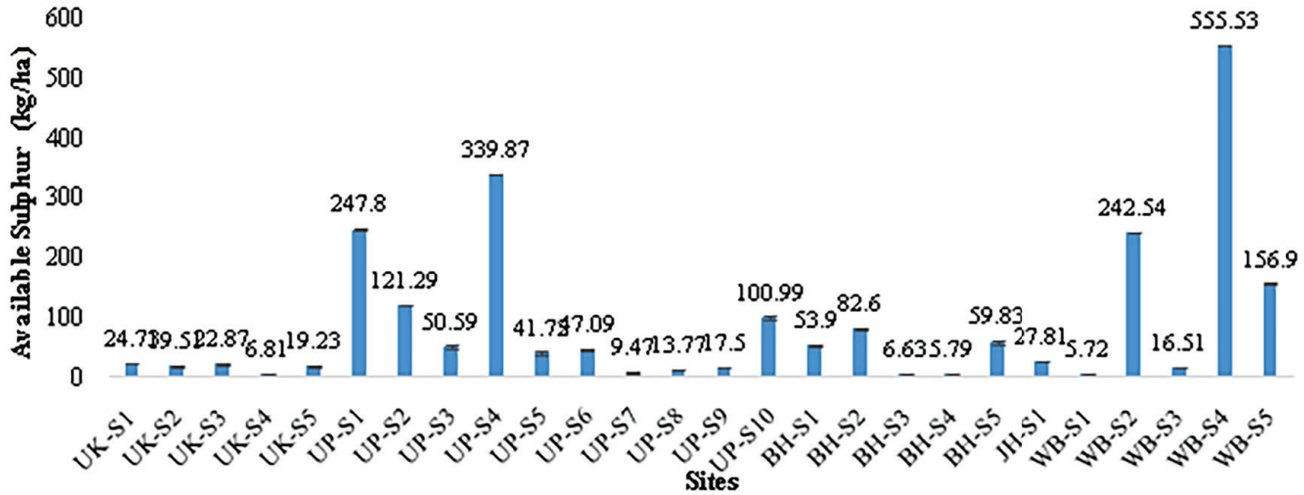


Fig. 9 Soil available sulphur content at different sites

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.

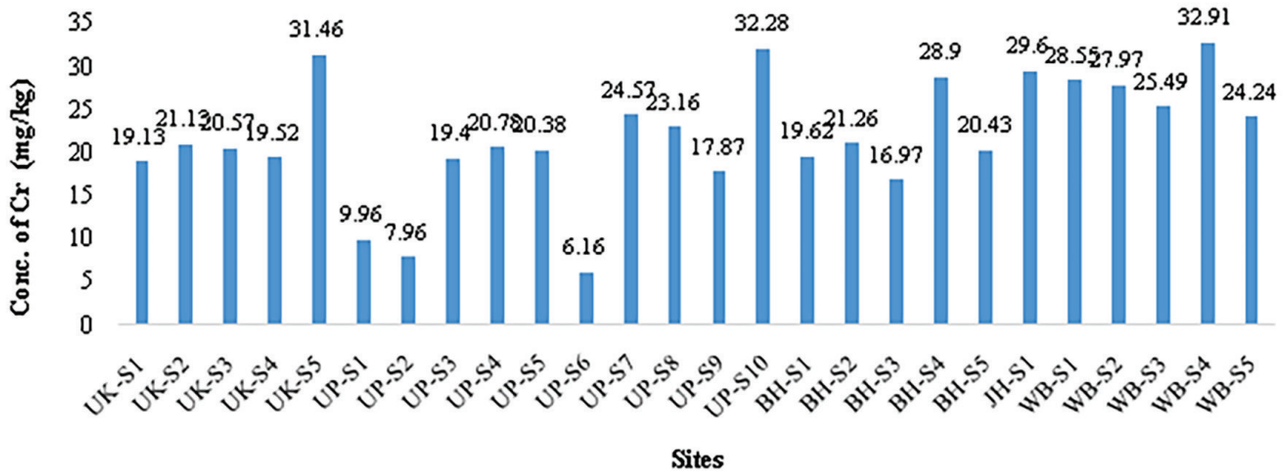


Fig. 10a Concentration of chromium at different sites

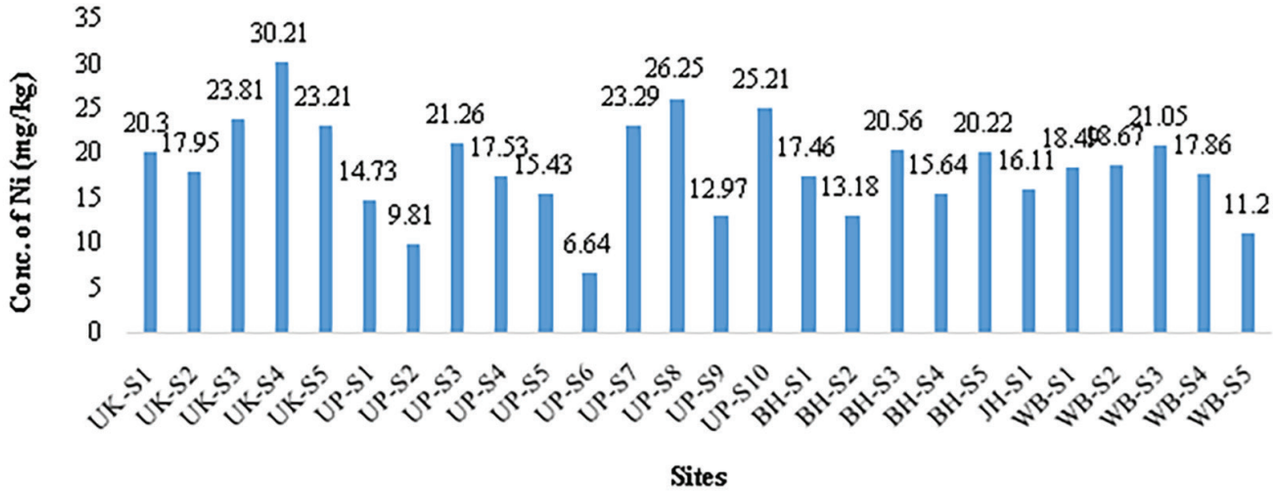


Fig. 10b Concentration of nickel at different sites

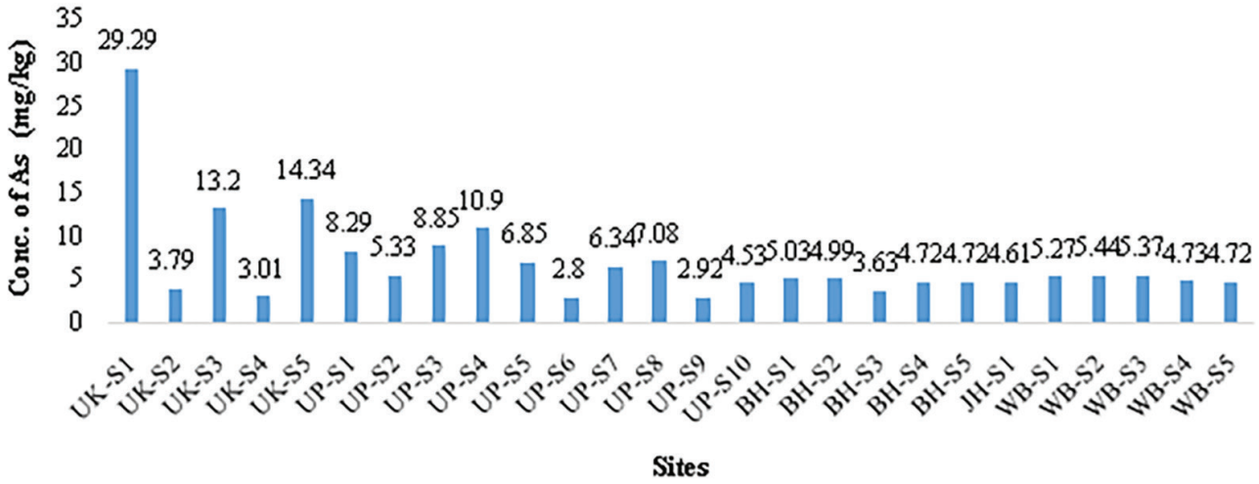


Fig. 10c Concentration of arsenic at different sites

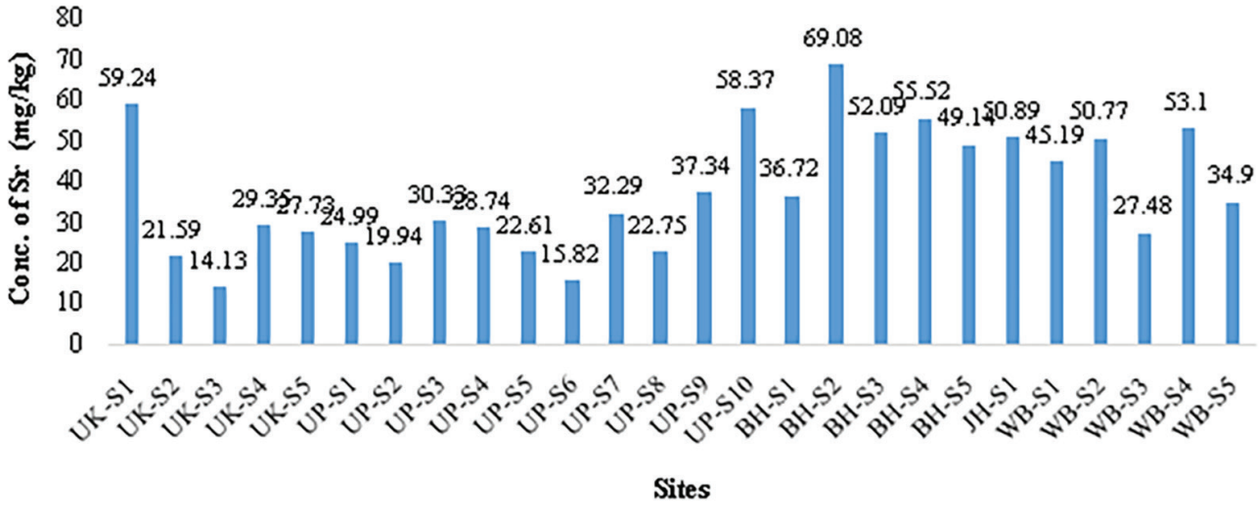


Fig. 10d Concentration of strontium at different sites

Fig. 10e Concentration of cadmium at different sites

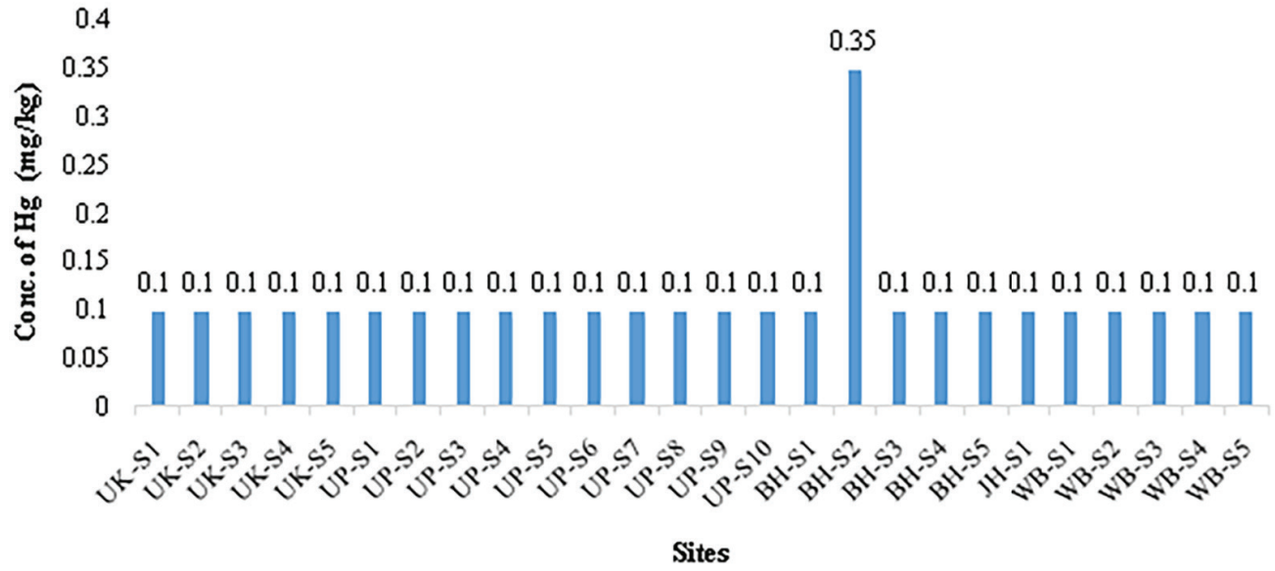


Fig. 10f Concentration of mercury at different sites

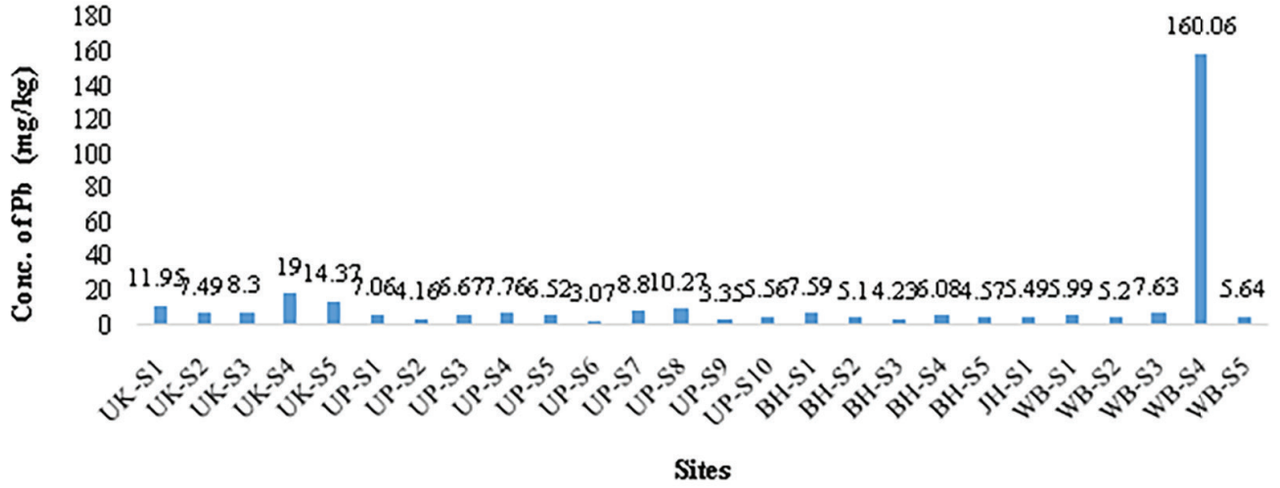


Fig. 10g Concentration of lead at different sites

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.

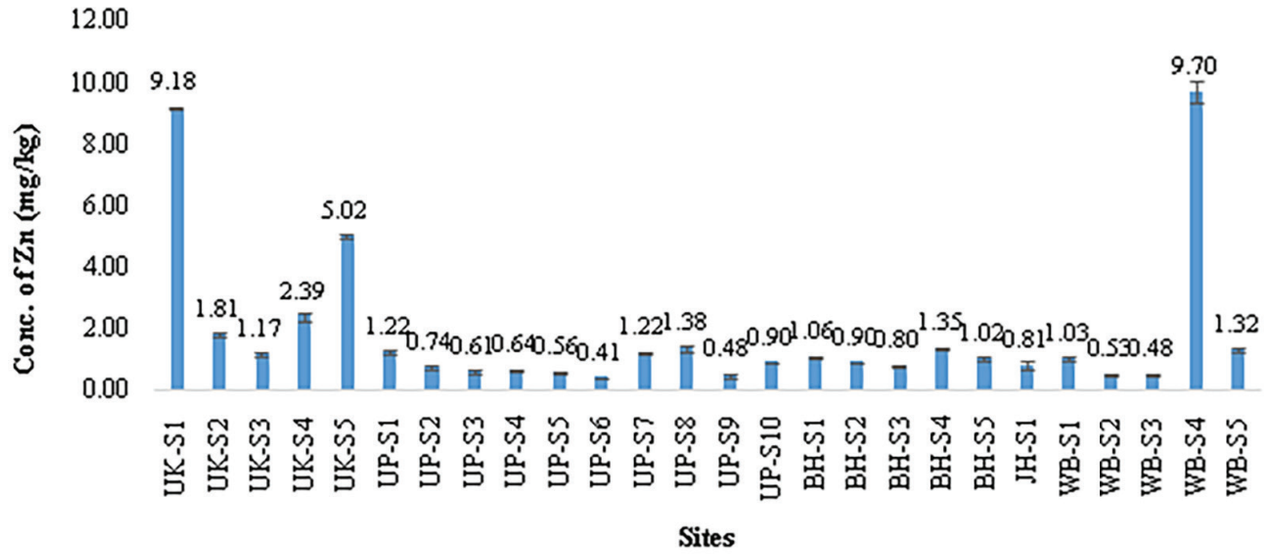


Fig. 11a Concentration of zinc at different sites

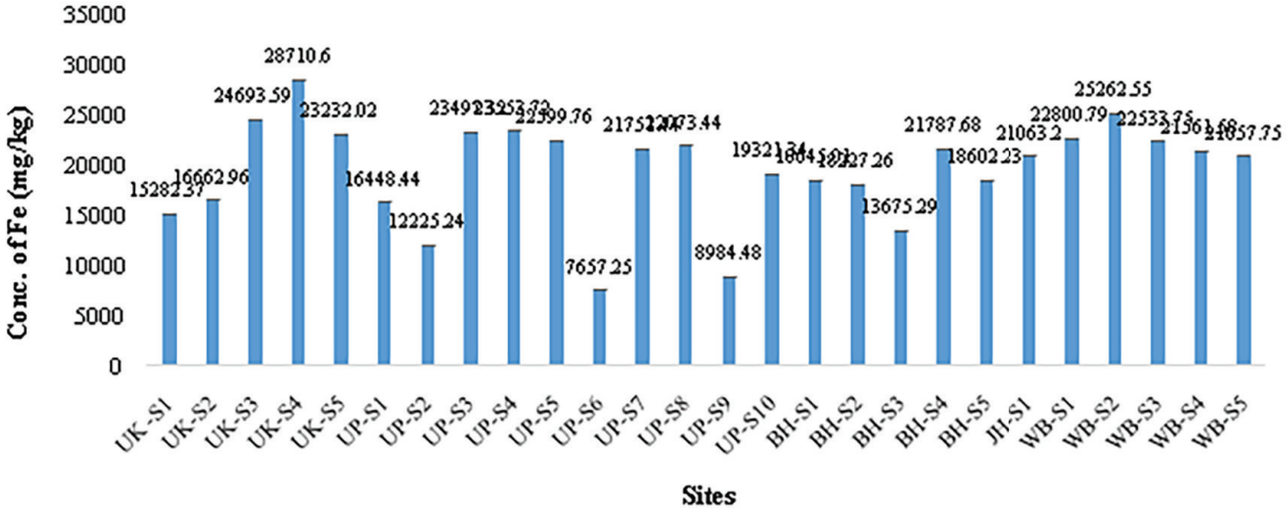


Fig. 11b Concentration of iron at different sites

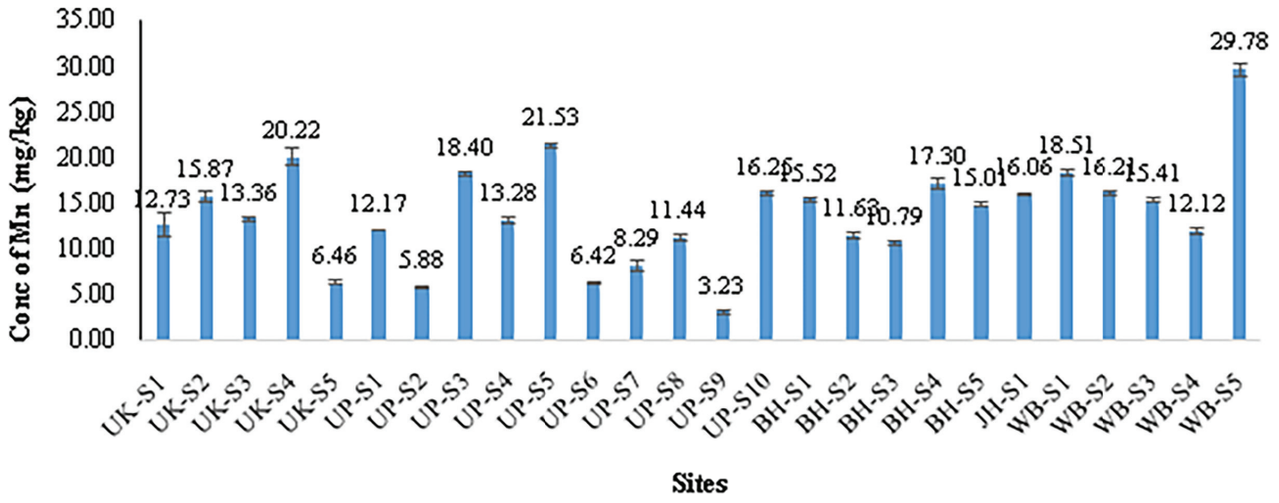


Fig. 11c Concentration of manganese at different sites

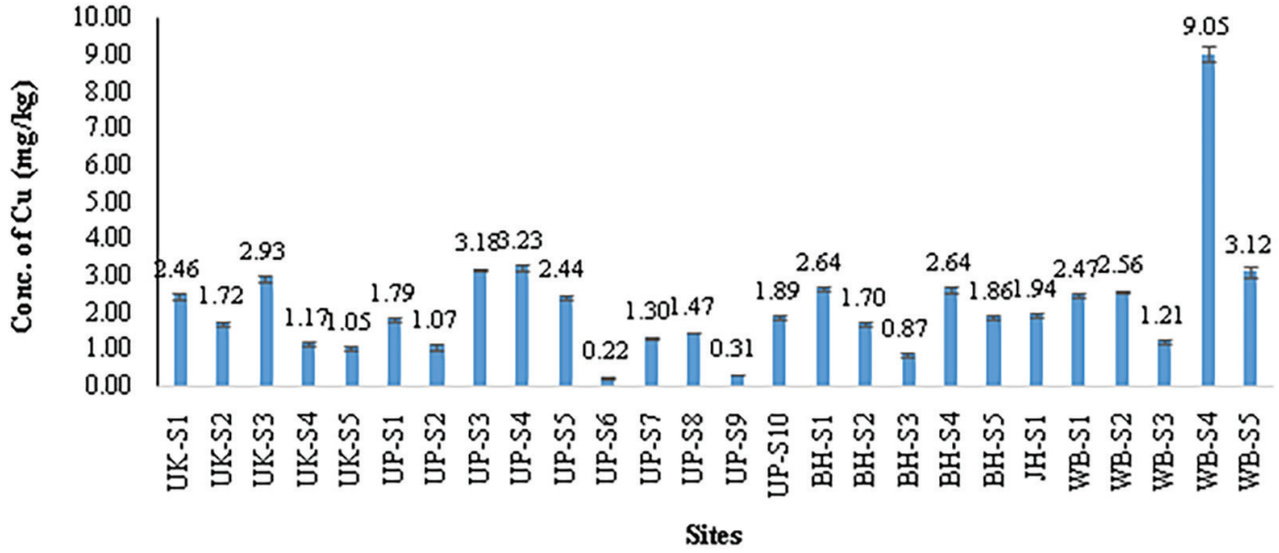


Fig. 11d Concentration of copper at different sites

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 *Ficus racemosa* L. in different locations shown in Fig. 12

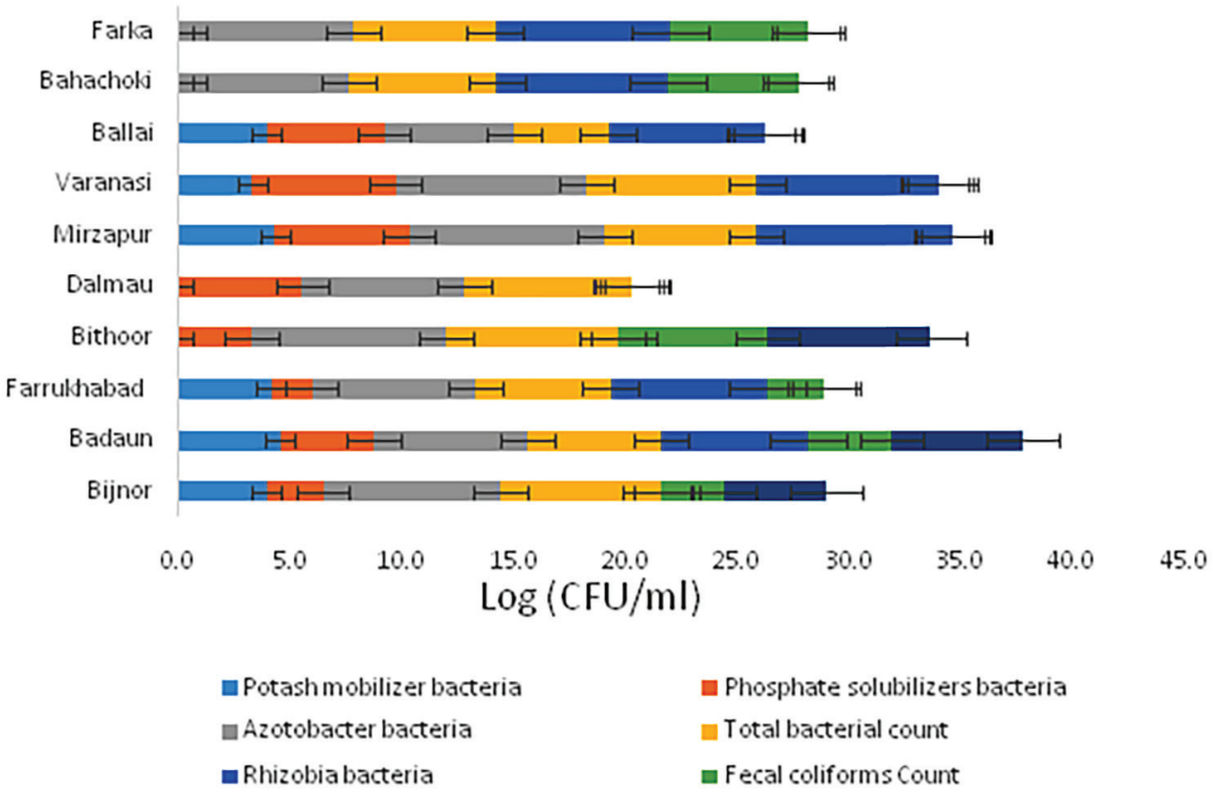
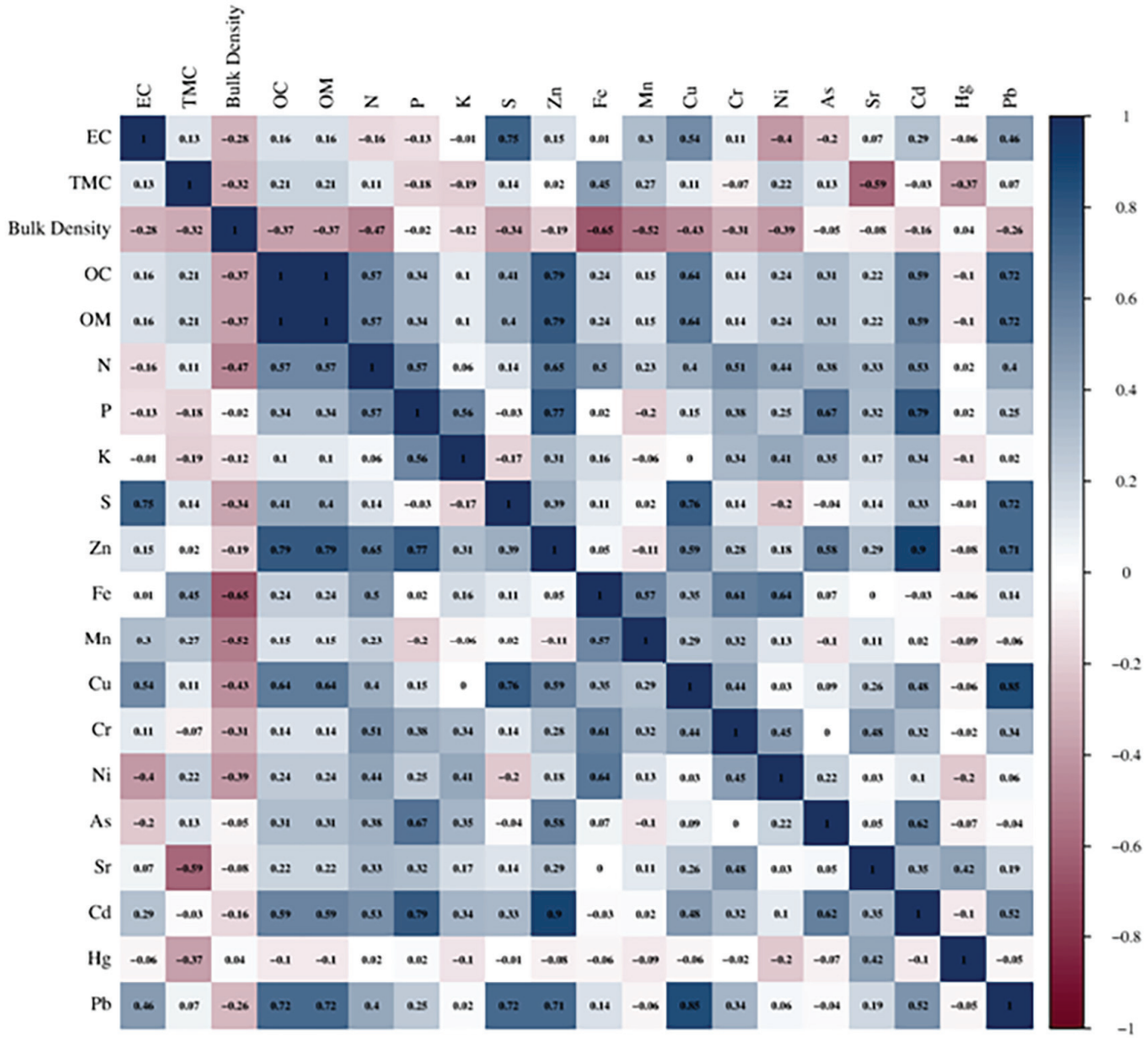


Fig. 12 Total and differential bacterial count

The total rhizosphere microbial test is performed for the identification of total bacterial count which is found highest in Bithoor is 7.7103 cfu/ml and lowest in Badaun is 6.0 cfu/ml. Whereas the total fecal and coliform count is highest in Bithoor is 6.686 and 7.3324 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

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

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

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

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CHAPTER

04

Traditional and Ethnomedicinal Applications

INTRODUCTION

Since ancient times, plants have been used as food and possess great medicinal value. Plants are a rich source of several bioactive chemical compounds and are frequently used as pure active principles or in conventional formulations. Whether cultivated or wild, plants that are readily available in the area can serve as a reasonable alternative to artificial preparations. Numerous professionals have affirmed the therapeutic benefits of conventional herbal remedies. Over the past 20 to 25 years, there has been a significant increase in the use of herbs as complementary and alternative therapy (Jimoh et al., 2011).Furthermore, the indiscriminate use of antibiotics has led to the rise of numerous drug-resistant strains of microbes, which has rekindled interest in herbal remedies(Chopra et al., 1997). Approximately, 50,000 to 80,000 flowering plants are utilized medicinally worldwide. A great deal of traditional knowledge from numerous indigenous and local cultures can be used in the advancement of biotechnology, agriculture, pharmaceutical research, and healthcare (Kumar and Bhagat, 2012). Worldwide, the genus *Ranunculus* has over 600 species. According to recent taxonomic assessments, this genus, which is comprised of two subgenera and 17 sections, is monophyletic in origin (Kubitzki et al., 2013).In folk medicine, several *Ranunculus* species have been used to cure a wide range of illnesses and symptoms, including rheumatoid arthritis, asthma, jaundice, nebula, edema, malaria, cancer, and hypertension. Furthermore, studies have shown that extracts from *Ranunculus* contain anti-inflammatory, anti-mutagenic, anti-bacterial, anti-tumoral, cardioprotective, and wound-healing qualities(Prieto et al., 2003; Gürhanand Ezer, 2004).Popularly known as "celery-leaved buttercup", *R. sceleratus* is a species of flowering plant found throughout the Northern Hemisphere. According to Mahran et al., 1998, the primary components of *R. sceleratus* are flavonoids, steroids such as pyrogallol tannins, and the glycoside ranunculin (Minakata et al., 1983; Martín et al., 1990). Furthermore, myristic acid is abundant in 70% ethanolic extracts from the aerial parts of *R. sceleratus*(Marrelli et al., 2022). However, the presence of the chemical protoanemonin makes *R. sceleratus* toxic; this is especially true for the 'cursed buttercup', which has a 2.5% protoanemonin

content and is the most toxic buttercup. Crumpled, broken, or twisted leaves cause ugly blisters and ulcers on people's skin.Written during the Western Han Dynasty, the Shennong Traditional Herbal Scriptures describe *Ranunculus sceleratus* L., an annual herbaceous plant in the *Ranunculus* family, as one of the best herbs. According to the Ming Dynasty of ancient China, Shizhen Li wrote *Compendium of Materia Medica* that described how fresh *R. sceleratus* was rubbed and then pasted onto the acupuncture point of Cunkou overnight, causing blistering at the skin surrounding Cunkou and a sensation of fire scald. This treatment, known as "vesiculating moxibustion," was used to treat jaundicecaused by malaria (Zhang et al., 2020).

Traditional and Ayurvedic Benefits

Due to their uniqueness and fewer side effects, traditional uses of herbs and related medicinal products are becoming more and more popular in both developed and developing nations. Owing to its intricate structural makeup, non-toxic nature, and wide-spectrum antibacterial action, herbal remedies are considered potential treatments (Mukhtar et al., 2008). Traditionally, it was used as a mastitis treatment, an antiphlogistic, an anti-diarrheal, and a relief for articular effusion(Mei et al., 2012; Neag et al., 2017).In addition, the herb was utilized to treat internal abscesses, blood stasis, acute icteric hepatitis, malaria, plague, and scorpion bites, and used as diuretics,and stimulants. Also, the juice of the leaves was used to treat pneumonia, rheumatism, sciatica, and dysuria (Gangwarand Joshi, 2008).Table 1 illustrates some of the other significant medical applications of ancient Ayurveda, described below.

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

S. No.	Diseases/ Conditions	Mode of Administration/Uses	References
1.	Thoracic diseases	*Ghṛta* (Clarified butter) cooked with *Zingiber officinale* (Dry ginger root), *Piper nigrum* (Black pepper), *P. longum* (Long pepper), *Martynia annua* (Devils claws), *Asparagus racemosus* (Asparagus), *Tribulus terrestris* (Caltrop) and other herbs, in an appropriate dose, useful in cough, fever, *Gulma*, anorexia, splenomegaly, cephalic diseases, etc	Śāstrī and Chaturvedi, 2011a

S. No.	Diseases/ Conditions	Mode of Administration/Uses	References
2.	*Vāta* associated disorders	*Cymbopogon schoenanthus* (Camel grass), *Ricinus communis* (Castor), *Boerhaviadiffusa*(Hogweed), *Pluchea lanceolata* (Rasna), *Martynia annua* (Devils claws), *Hordeum vulgare* (Barley), *Vigna mungo* (Black gram), *Macrotyloma uniflorum* (Horse gram) and other flavouring agents (Prakṣepakadravya) cooked in 4 parts of water and used for enema therapy (vasti) as a treatment forthe diseases of leg, thigh, sacral region, flanks, dorsal part, shoulder, sternomastoid region, and *Vāta* associated disorders of cephalic region	Sharma, 2012
3.	MaṇḍalaKuṣṭha (Type of leprosy)	Paste prepared from the equal quantity of *Cedrus deodara* (Deodar), *Nardostachysjatamansi* (Spikenard), *M. annua*, *Caesalpinia bonducella* (Bonduce nut), and other herbs applied externally for treating *MaṇḍalaKuṣṭha* (a specific type of Kuṣṭha explained in ayurvedic texts)	Śāstrī and Chaturvedi, 2011

Therapeutic Potential

In many countries, traditional medicine evolved from folk medicine (Ahmad et al., 2009). Several reasons, including environmental pollution, famine, overcrowding, and lifestyle changes, have contributed to the recent fall in people's overall health. As a result, there are several alternatives for treating illnesses using medications made from plants based on conventional medical theories. People nowadays understand the importance of medicinal plants in treating and avoiding common diseases, particularly in India (Das, 1972).In indigenous medical systems like Ayurveda, Unani, and others, the use of herbal components for the treatment of various diseases has a long history in India (Chopra, 1982).The earliest Chinese literature in this field mentions this traditional Chinese medicinal plant, which has excellent therapeutic properties. This herb is good-natured, bitter, and possesses a unique toxicity. By eliminating blood stasis, driving out cold, reducing edema, and eliminating excess heat from the liver and gall bladder, it can increase blood circulation, some of the important therapeutic values are mentioned in Table 2. Furthermore, it is used to treat internal abscesses, scrofula, malaria, snake or scorpion stings, and severe icteric hepatitis. They also used fresh *R. sceleratus* with an 80%

cure rate to treat hydrops articulation of the knee (PRI, 2003; Du, 2009).

The substance found in *R. sceleratus* substance significantly increases the affected area's stimulation and modifies the blood flow in the area that is sore, which helps to reduce swelling and inflammation and promotes the healing of aberrant tissue (Misra and Dixit, 1980). Although all portions of *R. sceleratus* are toxic when raw, the plant can be heated or dried and used in traditional medicine to cure a variety of ailments (Prieto et al., 2003). Protonanemonin and anemonin, the two ranunculins, have demonstrated fungicidal, anti-bacterial, anti-mutagenic, and antipyretic qualities (Misra et al., 1980; Minakata et al., 1983; Martín et al., 1990).

Table 2 Medicinal importance of *Ranunculussceleratus*L., along with their preparation and mode of administration

S. No.	Parts Used	Preparations	Diseases Treated and Therapeutic Uses	References
1.	Whole plant	-	Boils, remove skin scaring,-decrease hypersensitivity of penis, urogenital disorders, anti-spasmodic, diaphoretic, anti-rheumatic, cold,urinary disorders, blisters, sores, stimulant, vermifuge, diuretic, cutaneous disorders, phlegm, menstrual disorders, diuresis, kidney, urethra problems, respiratory problems, anti-rheumatic, spermatorrhoea, swelling, removing excessive heat fromliver & gall bladder, anodyne, anti-helminthic, promoting blood circulation by removing blood stasis, internal abscesses, malaria, scrofula, snake or scorpion venom, acute icteric hepatitis, anti-spasmodic, induces sweating, dyspnea,tuberculosis, jaundice, scrofula, intermittent malarial fever, gout	Kala, 2005; Khumbongmayum et al., 2005; Bala and Mukherjee, 2007; Adhikari et al., 2010; Dangwal et al., 2010; Kumar et al., 2011; Yasari and Vahedi, 2011; Unial et al., 2011; Kumar and Bhagat, 2012; Mei et al., 2012; Singh et al., 2015; Chang et al., 2017; Jan et al., 2018; Umair et al., 2019; Ali et al., 2020; Behara and Satapathy, 2021; Kumar et al., 2021; Lin et al., 2021; Rautela and Tiwari, 2021
		Infusion	Febricity, body tonic, asthma, muscle hamstring, urinary incontinence, anthelmintic	

S. No.	Parts Used	Preparations	Diseases Treated and Therapeutic Uses	References
		Decoction	Swelling, fever	
		Juice	Sciatica, rheumatism, dysuria, asthma,pneumonia, skin disorders,bronchitis	
2.	Leaves	-	Pain, cough, urinary troubles, gout, asthma,scabies,andother skin diseases	Singh et al., 2008; Vardhan, 2008; Pandey et al., 2012; Ikram et al., 2014; Das et al., 2016; Verma, 2017; Kumar and Singh, 2022
		Juice	Ringworm, eczema	
		Paste	Remove pus of boils, bubble-sandabscesses	
3.	Leaves and root	Powder	Urinary disorders, skin diseases	Kumar and Sharma, 2015
4.	Seeds	-	Tonic,stomachic, kidney troubles, stomach pain	Bala and Mukherjee, 2007; Unial et al., 2011; Kumar and Bhagat, 2012;Das et al., 2016; Kumar and Singh, 2022
5.	Root, leaves, and seeds	-	Sneezing, catarrh, kidney problems, toothache, leukoderma, wounds, scabies	Kumar and Chander, 2018; Dey et al., 2021
6.	Aerial parts	-	Anti-sciatica	Pieroni et al., 2004
7.	Rhizome	Powder	Diarrhea, dysentery	Das et al., 2016
8.	Stem	Juice	Asthma, pneumonia, rheumatism	Kumar and Singh, 2022
9.	Roots	-	Hypercritic dermatitis	Khanday and Singh, 2017
10.	Roots, leaves, and fruits	-	Diarrhea, cough, eye diseases, sore throat	Khanum et al., 2024
11.	Leaves and fruits	-	Earache, gout, liver pain, nose ulceration	Shah et al., 2016

Ethnomedicinal and Folk treatments along the Ganga Basin of India

Uttar Pradesh is the fourth largest and most populous state in India (Sachan et al., 2015). The people of Uttar Pradesh uses a variety of medicinal plants, such as *R. sceleratus*, to treat a wide range of illnesses. The juice of this plant was utilized by the locals in Ghaziabad to treat asthma and pneumonia, and the seeds were used to treat renal issues (Chaudhary and Kumar, 2015). The leaves of this plant are also utilized to treat skin conditions, pneumonia, and asthma (Rao, 2021). In Saharanpur, ringworm, eczema,rheumatism, and asthma are treated with the stem juice. Seeds are used to alleviate renal issues and stomach pain (Kumar and Singh, 2021),as well as renal, stomach, and rheumatic discomfort,asthma and other conditions (Kumar, 2006). Additionally, the herb known as 'jaldhania' is widely utilized in the Noornagar and Shakarpur region of Muzaffarnagar regions to alleviate swelling and eliminate excess heat from the liver and gall bladder (Kumar et al., 2021). Likewise, in Sambhal area of Uttar Pradesh, it is alsoutilized to cure rhinitis, arthritis, ulcers, skin conditions, and rheumatic disorders (Yadav and Chand, 2020). In Agra, it is used against liver illnesses associated with cholestasis, such as cystic fibrosis, drug-induced liver injury, intrahepatic cholestasis of pregnancy, and primary biliary cirrhosis (Zhang et al., 2020). Its juice is used in Bundelkhand to treat skin problems, asthma, rheumatism, dysuria, and pneumonia (Unial et al., 2011).

Bihar has rich tribal diversity of India. The principal tribes include Santhal, Oraon, Munda Paharia (Sauriya, Mal, and Kumar Bhag), Kol, Ho, Asur, and Baiga. The districts of Santhal Pargana and Chotanagpur have a dense population. The extensive medicinal plant lore of the aboriginal people of Bihar is largely attributed to their reliance on ethnomedicinal plants for the treatment of various diseases. Small, dense woods with natural shelter for medicinal plants are abundant in Bihar.These surrounding woodlands provide the tribal people with the botanical medicines they need. The tribes transmit their knowledge of ethnomedicinal plants orally from one generation to the next. Undoubtedly, one of India's great legacies is the invaluable knowledge of the ethnomedicinal plants found here. Tribal groups have strong faith in ethnomedicine. The residents used a variety of plants to cure a wide range of illnesses. *R. sceleratus* was used to treat boils (Upadhyay et al., 1998). Additionally, its leaves are used to treat scabies and other skin conditions in the Darbhanga area of North Bihar (Verma, 2017).

Conclusion and Future Perspectives

Despite the availability of contemporary healthcare facilities, the indigenous people continue to rely on traditional medicine, highlighting the importance of plant-based conventional recipes. Utilizing plant extracts, researchers have examined the chemical and biological properties of *R. sceleratus*. In addition to antioxidant and anticarcinogenic qualities, modern studies on the biological activity of the species' extracts have revealed a wide range of activities, including antibacterial, antiviral, and antiprotozoal effects.The plant's most fascinating properties were its ability to treat cancer, gastrointestinal problems, and skin illnesses. However, further study is needed into this plant's molecular mechanism, medication administration, drug-drug interactions, and toxicity investigations. Raising more and more knowledge about its medical use can help medical practitioners efficiently address the difficulties associated with treating skin and gastrointestinal disorders.

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CHAPTER

05

Phytochemical Analysis

INTRODUCTION

Ranunculus sceleratus, commonly known as the "celery-leaved buttercup", is a medicinal plant with a broad geographical distribution, found in temperate and subarctic regions of the Northern Hemisphere, including North America, Europe, and Asia. It is a member of the Ranunculaceae family, characterized by its bright yellow flowers and lobed leaves (Goo, 2022).

Phytochemical analysis of the species has revealed the presence of various bioactive compounds, including flavonoids, organic acids, coumarins, lignans, nitrogenous compounds, and anthraquinones. These compounds contribute to the plant's pharmacological properties, such as anti-inflammatory, antioxidant, and antimicrobial activities (Prieto et al., 2003; Cao et al., 2022). Traditional medicine widely uses *R. sceleratus* for various purposes, such as treating skin diseases, wounds, and inflammation. In traditional Chinese medicine, the plant is used to treat jaundice induced by malaria, among other ailments (Goo, 2022). Research on the plant species has highlighted its potential therapeutic effects on intrahepatic cholestasis induced by α-naphthylisothiocyanate in rats. The plant's chemical profile, however, has not been fully clarified, which impedes further research on its therapeutic potential. A recent study has successfully characterized sixty-nine compounds, including flavonoids and lignans, which were identified for the first time in the plant (Cao et al., 2022).

Sampling Sites

Table 1 Samples of the *Ranunculus sceleratus* collected from various state-specific locations

S. No.	Sampling Sites
1.	Bijnor, Uttar Pradesh
2.	Badaun, Uttar Pradesh
3.	Farrukhabad, Uttar Pradesh
4.	Bithoor, Uttar Pradesh
5.	Dalmau, Uttar Pradesh
6.	Prayagraj, Uttar Pradesh
7.	Varanasi, Uttar Pradesh
8.	Ballia, Uttar Pradesh
9.	Bahachoki, Bihar
10.	Farka, Bihar

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 of the samples collected, advanced methods and techniques were used. High performance thin layer chromatography (HPTLC) was used to detect the presence of marker compound and provide a chromatographic fingerprint of the plant sample. For further identification of compounds high-performance liquid chromatography (HPLC) was employed.

Determination of Tannin Content

A 1-10 g sample was taken and mixed with 50 ml of Milli-Q water. The mixture was shaken and sonicated for 30 minutes before being brought up to a volume of 100 ml and filtered. Subsequently, 10 ml of this solution was combined with 750 ml of Milli-Q water. Twenty-five milliliters of indigo sulfonic acid was added, and the solution was shaken. The mixture was titrated with 0.1N potassium permanganate solution until a golden yellow color endpoint was reached. A similar analysis was conducted using water as a blank instead of a sample.

Determination of Saponin Content

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

Determination of Total Polyphenol Content

A 1 ml sample was placed in a test tube and mixed with an equal volume of Folin-Ciocalteu reagent, then incubated for 5 minutes. Following this, 1 ml of a 10% sodium carbonate solution was added. The mixture was then kept in the dark for 1 hour. Absorbance was measured at 760 nm using a UV-visible spectrophotometer. This procedure was repeated with various concentrations of gallic acid as reference standards to create a linearity curve for determining the content in the sample.

Determination of Total Flavonoid Content

A 1 ml sample was placed 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 to it. The mixture was left at room temperature for 30 minutes. The absorbance was measured at 450 nm using a UV-visible spectrophotometer. The procedure was repeated using various concentrations of quercetin dihydrate as reference standards. A linearity curve was plotted to calculate the content in the sample.

HPTLC Fingerprinting

The application of HPTLC in the study reflects a prevalent methodology documented in the literature for profiling secondary metabolites. The use of HPTLC not only enables the rapid separation of compounds but also aligns with the commonly reported diverse chemical fingerprints found in the plant species. This technique has been recognized for its effectiveness in exploring the qualitative aspects of secondary metabolites in various plant species. As per WHO Technical Report Series, No. 1010, (2018), HPTLC chromatographic patterns, generally refer as “fingerprints”, are used for the identification of phytochemicals. The bands or spots obtained during the test are characteristic of a particular herb. A color image of a typical TLC fingerprint provides a clearer guide to the users.

Sample Preparation

About 1 g of sample from each batch was dissolved individually in 10 ml of methanol. The samples were then shaken and sonicated for 20 min. The solution was further centrifuged for 5 min at 5000 rpm. A clear solution thus obtained was used for the analysis.

Methodology and Analytical Conditions

Analysis was performed on CAMAG HPTLC (Muttenz, Switzerland), equipped with an Automatic TLC Sampler (ATS 4), TLC scanner 4, and a TLC visualizer. Data processing acquisition and visualization were achieved using win-CATS software (version 1.4.10). The chromatographic conditions for the HPTLC analysis were as follows:

Stationary phase	TLC Silica gel 60 F254 aluminium sheet (1.0554.0007)
Mobile phase	Toluene: Formic acid: Ethyl acetate (7.5:2.5:5)
Saturation time	15 minutes
Migration distance	70 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 preparation: About 600 mg of sample from each batch was dissolved individually in 6 ml of hydromethanol (20:80). The solution was sonicated for 30 minutes, centrifuged for 5 min at 9000 rpm, and filtered using a 0.45 µm nylon filter. The filtered solution was further used for analysis.

Standard preparation: Protocatechuic acid was dissolved in methanol to prepare a solution having a concentration of 50 ppm.

Analytical and Instrumentation Condition

Separation was achieved using a Shimadzu C8 (3 µm, 3.0 ×100 mm) column, subjected to binary gradient elution. The two solvents used for the analysis consisted of water containing 0.1 % acetic acid in water and acetonitrile (solvent B). The flow rate was set at 1.0 ml/min during the analysis. Ten microlitres of standard and test solution were injected. The wavelength was set at 270 nm.

Gradient Program

Time (Min)	A%	B%
0	95	5
10	90	10
20	82	18
35	82	18
40	65	35
50	50	50
55	35	65
56	95	5
60	95	5

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

The objective was to identify the major compounds in *Ranunculus sceleratus* test sample (PRF/CHI/1223/1277) using UPLC/MS-QToF analysis.

Sample Preparation

500 mg powdered sample was dissolved in 10 ml methanol and sonicated for 30 min. The solution was centrifuged at 10000 rpm for 5 minutes and filtered through 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 (China) with the flow rate of 0.3 ml/min using gradient elution of 0.1% v/v formic acid in water (mobile phase A) and 0.1% v/v formic acid in acetonitrile (mobile phase B). The column 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 mode.

Gradient program

Time (min)	Flow (ml/min)	Mobile phase A %	Mobile phase B%
0	0.3	95	5
10	0.3	90	10
20	0.3	90	10
35	0.3	60	40
40	0.3	40	60
50	0.3	20	80
51	0.3	95	5
55	0.3	95	5

Xevo G2-XS QToF Parameters

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

Results and Discussion

Phytochemical Analysis

The quantitative phytochemical analysis of samples collected from different locations revealed the presence of tannins, saponins, total phenolics, and total flavonoids in varying concentrations (Table 2). The tannin content ranged from 0.039 to 0.060% w/w, saponin content varied from 1.732 to 15.373% w/w, total polyphenol content was between 0.001 to 0.004% w/w, and total flavonoid content was found maximum as 0.004% w/w across the samples. The variations in phytochemical content among the samples indicate potential environmental and genetic influences on the plant's phytochemical profile.

Table 2 Phytochemical analysis of *Ranunculus sceleratus* collected from different locations

S. No.	Samples	Tannin content (%w/w)	Saponin content (%w/w)	Total polyphenol content (%w/w)	Total flavonoid content (%w/w)
1.	Bijnor, Uttar Pradesh	0.040	6.545	0.004	0.002
2.	Badaun, Uttar Pradesh	0.060	15.248	0.004	0.003
3.	Farrukhabad, Uttar Pradesh	0.061	15.373	0.002	0.004
4.	Bithoor, Uttar Pradesh	0.040	6.555	0.003	0.004
5.	Dalmau, Uttar Pradesh	0.060	7.497	0.004	0.001
6.	Prayagraj, Uttar Pradesh	0.040	3.881	0.004	0.001
7.	Varanasi, Uttar Pradesh	0.059	4.472	0.002	0.001
8.	Ballia, Uttar Pradesh	0.060	1.732	0.001	0.001
9.	Bahachoki, Bihar	0.039	8.313	0.002	0.001
10.	Farka, Bihar	0.039	2.226	0.003	0.002

HPTLC Fingerprint Analysis

The HPTLC fingerprints reveal distinct bands of phytochemicals at 254 nm, 366 nm, and under white light. The banding pattern of phytochemicals was found to be similar at 254 nm and under white light, with variations in the intensity of the bands. At 366 nm, blue, red, and yellow, fluorescent bands were observed. Notably, a yellow band at Rf 0.20 was exclusively present in samples 5 and 9, while a yellow band at Rf 0.65 was only observed in sample 5. Additionally, a blue intense band at Rf 0.71 was observed in samples 1, 5, 7, 8, and 9. The banding pattern at 366 nm exhibited variability from sample to sample (Fig. 1).

The HPTLC analysis showed distinct chromatographic patterns under multiwavelength detection (254 nm and 366 nm UV light). The presence of high-intensity bands across all samples suggests a rich diversity of

chemical compounds. These results provide a preliminary indication of the complex phytochemical composition of the plant and underline the importance of further detailed analyses to identify the specific compounds responsible for the observed bands.

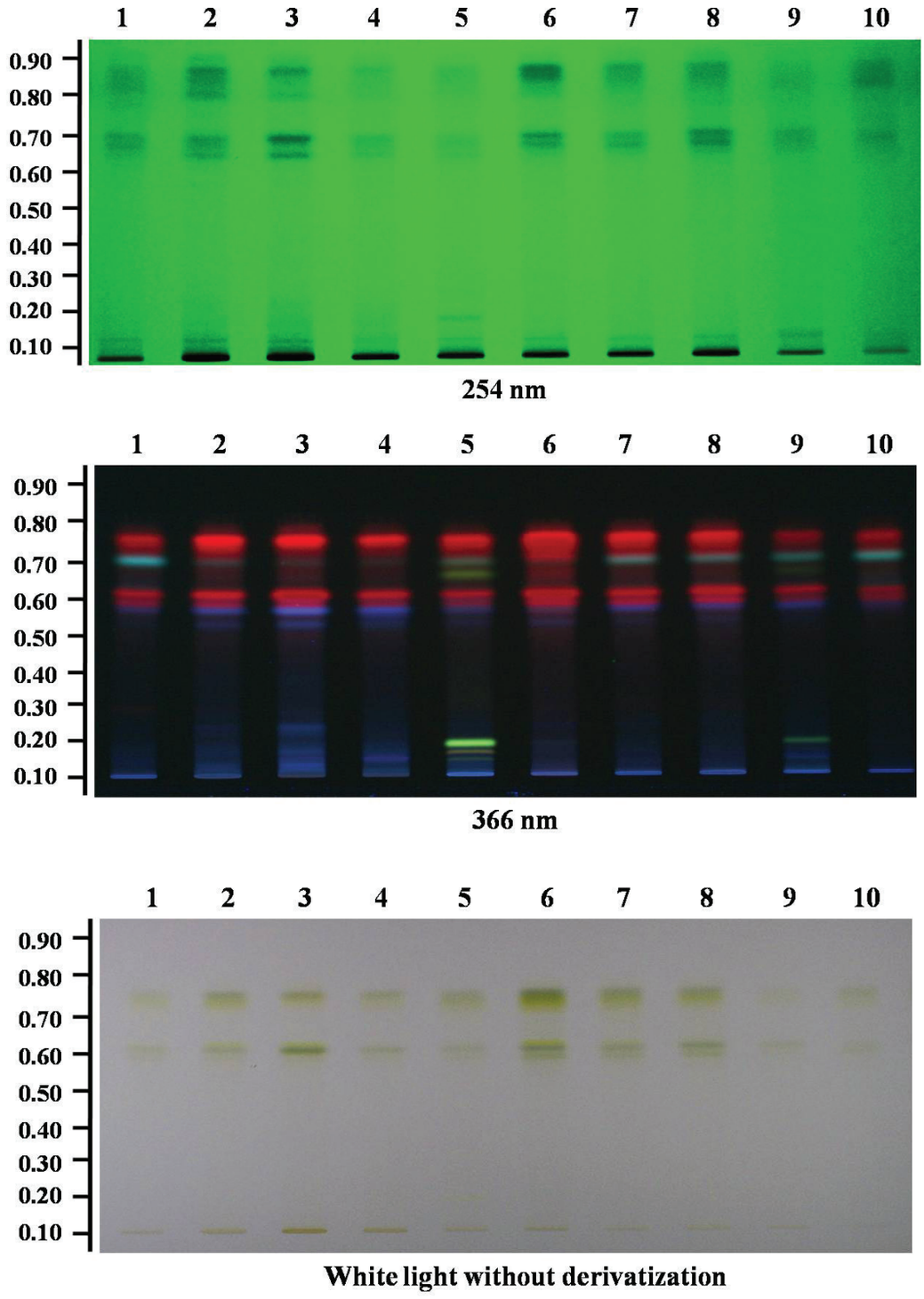

Fig. 1 HPTLC fingerprinting of *Ranunculus saleratus*. **A:** 254 nm, **B:** 366 nm, **C:** Under white light before derivatization

The HPTLC fingerprint patterns observed suggest a high degree of chemical diversity, which is consistent with the plant's reported pharmacological activities. The presence of multiple high-intensity bands indicates the presence of bioactive compounds that may contribute to the therapeutic effects of the plant. The qualitative data from HPTLC, combined with the quantitative insights from HPLC and UPLC/MS-QToF, provide a robust framework for our understanding.

HPLC Analysis

The protocatechuic acid compound was observed to be maximum in Farrukhabad, Uttar Pradesh sample. A detailed result has been presented in Table 3. HPLC analysis using protocatechuic acid as the reference compound provided quantitative data on the presence of this specific compound across samples (Table 3). The chromatograms displayed characteristic peaks corresponding to protocatechuic acid, indicating its presence in varying amounts. This analysis not only confirms the presence of this compound but also highlights the differences in its concentration across different geographical locations, which could be attributed to environmental factors affecting the biosynthesis of secondary metabolites in the plant (Fig 2, 3).

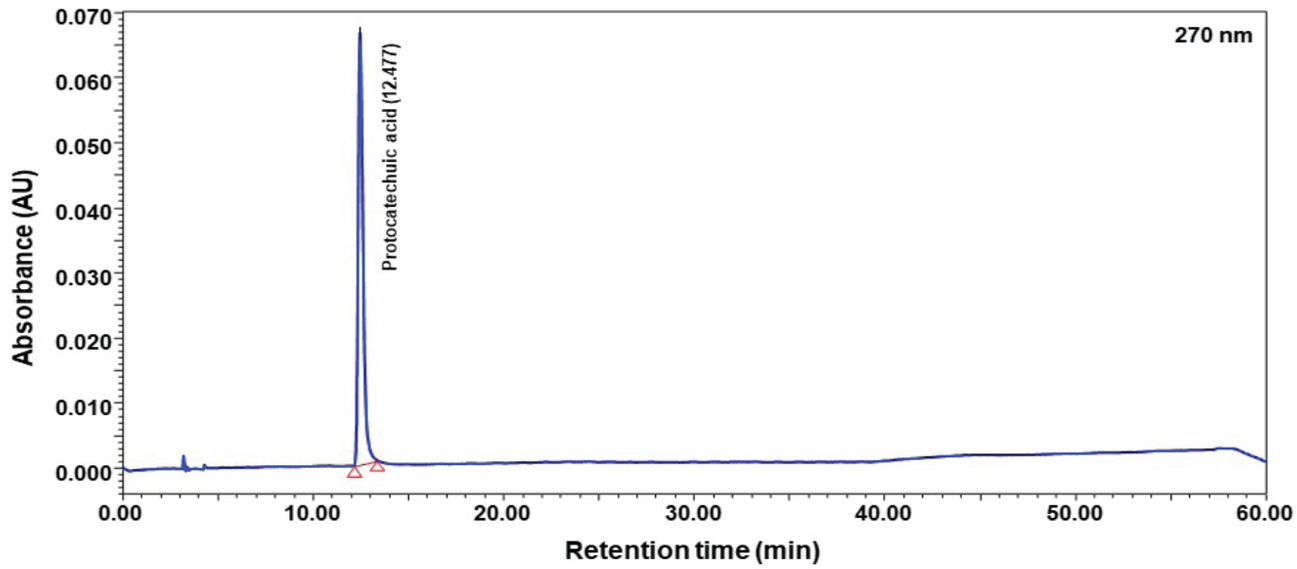

Fig. 2 HPLC chromatogram of the standard Protocatechuic acid at 270 nm

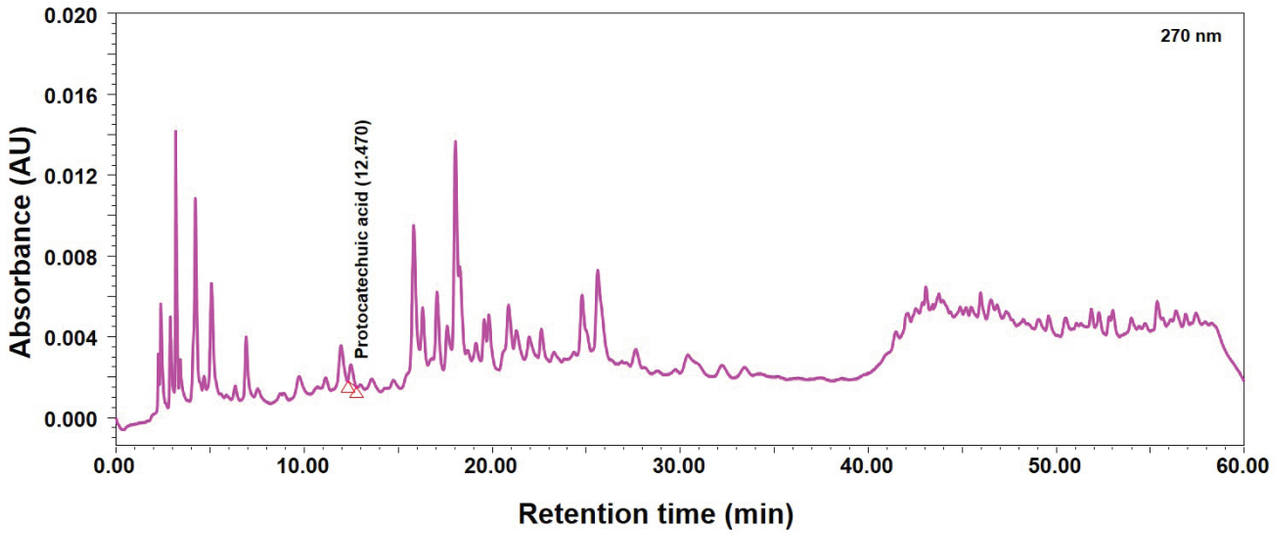

Fig. 3 HPLC chromatogram of *Ranunculus sceleratus* (PRF/CHI/1223/1277) at 270 nm

Table 3 Content of phytochemicals (in µg/mg) present in *Ranunculus sceleratus* collected from the different locations

S. No.	Internal code	Samples	Protocatechuic acid (µg/g)
1.	PRF/CHI/1223/1275	Bijnor, Uttar Pradesh	5.37
2.	PRF/CHI/1223/1276	Badaun, Uttar Pradesh	22.47
3.	PRF/CHI/1223/1277	Farrukhabad, Uttar Pradesh	110.08
4.	PRF/CHI/1223/1278	Bithoor, Uttar Pradesh	7.37
5.	PRF/CHI/1223/1279	Dalmau, Uttar Pradesh	2.53
6.	PRF/CHI/1223/1280	Prayagraj, Uttar Pradesh	< 0.01
7.	PRF/CHI/1223/1281	Varanasi, Uttar Pradesh	4.34
8.	PRF/CHI/1223/1282	Ballia, Uttar Pradesh	8.27
9.	PRF/CHI/1223/1283	Bahachoki, Bihar	3.71
10.	PRF/CHI/1223/1284	Farka, Bihar	3.31

The varying concentrations of protocatechuic acid across different samples of *R. sceleratus* highlight the influence of geographical and environmental factors on phytochemical synthesis. This variation could have implications for the standardization and quality control of *R. sceleratus* -based herbal products.

UPLC/MS-QToF Analysis

UPLC/MS-QToF analysis was employed to identify the major compounds present in *R. sceleratus* samples. The high-resolution separation achieved in this method allowed for the identification of 30 compounds, including phenylalanine, tryptophan, caffeic acid, and several quercetin derivatives. The accurate mass measurements and retention times provided a comprehensive profile of the phytochemicals present, demonstrating the utility of this technique in unravelling the complex chemical composition of plant extracts.

Studies on related species within the Ranunculaceae family have also reported the presence of similar bioactive compounds, such as flavonoids and phenolic acids. For instance, research on *R. japonicus* identified quercetin and its glycosides as major constituents, corroborating the findings in *R. sceleratus* (Goo, 2022; Xu et al., 2023). The detection of caffeic acid and quercetin derivatives is consistent with phytochemical analysis of other medicinal plants used in traditional medicine, such as *Camellia sinensis* (green tea) and *Ginkgo biloba*. These compounds are associated with significant health benefits, including cardiovascular protection and neuroprotection (Dal Belo et al., 2009; Liu et al., 2022).

- Major compounds identified: The UPLC/MS-QToF analysis identified 30 significant compounds in *R. sceleratus*, including phenylalanine, tryptophan, caffeic acid, and several quercetin derivatives. The detailed identification of these compounds underscores the rich phytochemical diversity of the plant.
- Phenylalanine and Tryptophan: Phenylalanine and tryptophan are essential amino acids that play crucial roles in protein synthesis and metabolic pathways. Their presence in *R. sceleratus* suggests potential contributions to the plant's therapeutic properties, particularly in anti-inflammatory and neuroprotective activities.
- Caffeic acid: Caffeic acid is a well-known phenolic compound with strong antioxidant properties. Its detection aligns with previous studies that have highlighted its role in mitigating oxidative stress and providing anti-inflammatory benefits. The presence of caffeic acid in *R. sceleratus* adds to the plant's medicinal value, particularly in conditions involving oxidative damage.
- Quercetin derivatives: Quercetin and its derivatives are prominent flavonoids recognized for their antioxidant, anti-inflammatory, and anticancer properties. The identification of quercetin derivatives in *R. sceleratus* supports its use in traditional medicine for treating various ailments, including inflammation and infections.

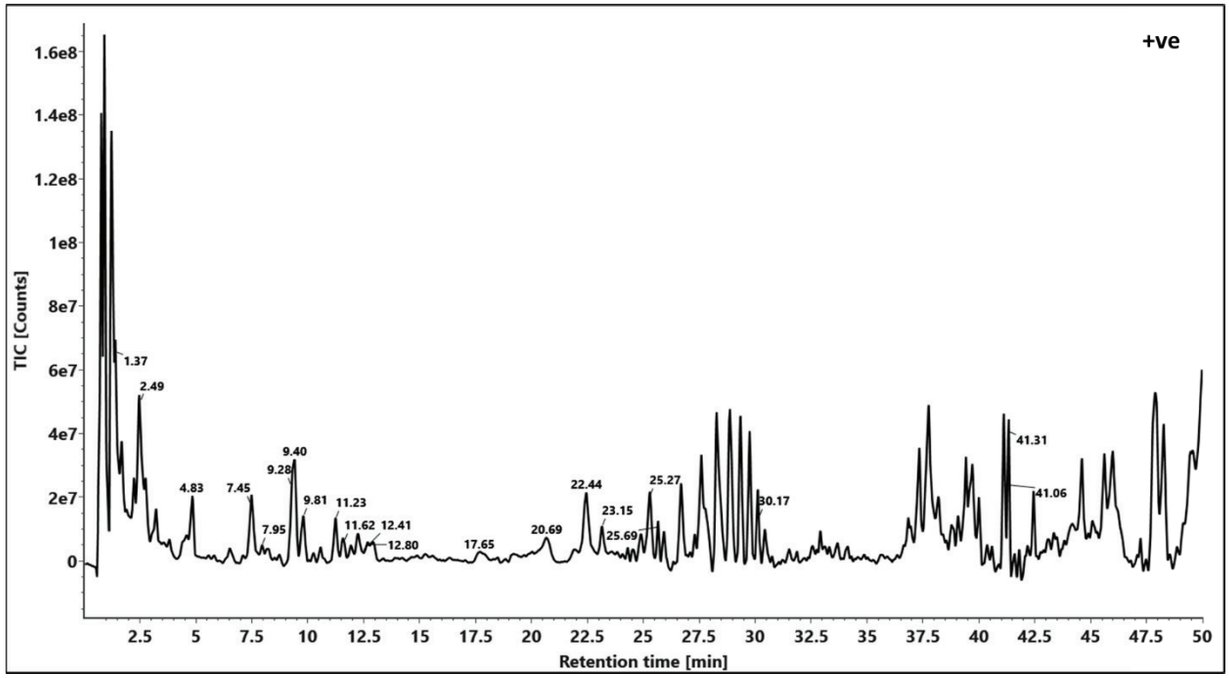

Fig. 4 TIC chromatogram of *Ranunculus sceleratus* (PRF/CHI/1223/1277) in positive ionization mode

Table 4 Compound-dependent parameters of analytes/identified compounds in *Ranunculus sceleratus* (PRF/CHI/1223/1277) in positive ionization mode

S. N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
1	N-(1-Deoxy-1-fructosyl) isoleucine	$C_{12}H_{23}NO_7$	293.1475	294.1545	-0.3	1.37	7247017	+H
2	Phenylalanine	$C_9H_{11}NO_2$	165.0790	166.0856	-0.7	2.49	671202	+H
3	Tryptophane	$C_{11}H_{12}N_2O_2$	204.0899	205.0964	-0.8	4.83	325552	+H
4	Quercetin 3-sophoroside-7-glucoside	$C_{33}H_{40}O_{22}$	788.2011	789.2058	-2.6	7.45	830249	+H
5	Quercetin 3-sophorotrioside	$C_{33}H_{40}O_{22}$	788.2011	789.2052	-3.2	7.95	434990	+H
6	Caffeic acid	$C_9H_8O_4$	180.0423	181.0491	-0.5	9.28	103832	+H
7	Quercetin 3-(2'''-(E)-caffeylsophoroside]-7-glucoside	$C_{42}H_{46}O_{25}$	950.2328	951.2375	-2.6	9.40	2828693	+H
8	Quercetin 3-(6''''-caffeylsophorotrioside)	$C_{42}H_{46}O_{25}$	950.2328	951.2364	-3.7	9.81	1097390	+H

S. N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
9	Quercetin 3-(2'''-ferulylsambubioside)-7-glucoside	$C_{42}H_{46}O_{24}$	934.2379	935.2403	-4.9	11.23	503042	+H
10	Quercetin 3-p-coumarylsophoroside-7-glucoside	$C_{42}H_{46}O_{24}$	934.2379	935.2401	-5.1	11.62	288976	+H
11	Quercetin 3-[2'''-(E)-feruloylsophoroside]-7-glucoside	$C_{43}H_{48}O_{25}$	964.2485	965.2497	-6.1	12.41	123871	+H
12	Quercetin 3-(6''''-ferulylsophorotrioside)	$C_{43}H_{48}O_{25}$	964.2485	965.2488	-7.0	12.80	134045	+H
13	Herbacetin 7-methyl ether 8-sophoroside	$C_{28}H_{32}O_{17}$	640.1640	641.1693	-1.9	17.65	494306	+H
14	Quercetin-3,5-diglucoside	$C_{27}H_{30}O_{17}$	626.1483	627.1546	-1.0	20.69	878819	+H
15	Quercetin-3,7-diglucoside	$C_{27}H_{30}O_{17}$	626.1483	627.1549	-0.7	21.92	322694	+H
16	Quercetin 3-(2'''-caffeylsophoroside)	$C_{36}H_{36}O_{20}$	788.1800	789.1852	-2.0	22.44	1110842	+H
17	Quercetin 3-(6''-caffeoylsophoroside)	$C_{36}H_{36}O_{20}$	788.1800	789.1845	-2.8	23.15	259720	+H
18	Sophoraflavonoloside	$C_{27}H_{30}O_{16}$	610.1534	611.1600	-0.7	24.52	568227	+H
19	Quercetin 3-O-α-(6'''-caffeoylglucosyl-ß-1,2-rhamnoside)	$C_{36}H_{36}O_{19}$	772.1851	773.1906	-1.7	25.27	248980	+H
20	Isoquercitrin	$C_{21}H_{20}O_{12}$	464.0955	465.1033	0.5	25.69	659046	+H
21	Quercitrin	$C_{21}H_{20}O_{11}$	448.1006	449.1080	0.1	25.85	335076	+H

S. N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
22	Isorhamnetin-7-O-ß-D-glucopyranoside	$C_{22}H_{22}O_{12}$	478.1111	479.1186	0.2	27.49	268976	+H
23	Isorhamnetin-3-O-glucoside	$C_{22}H_{22}O_{12}$	478.1111	479.1184	0.0	30.17	194801	+H
24	17-Hydroxylinolenic acid	$C_{18}H_{30}O_3$	294.2195	317.2064	-2.3	41.06	131040	+Na
25	Colneleic acid	$C_{18}H_{30}O_3$	294.2195	317.2075	-1.3	41.31	110195	+Na

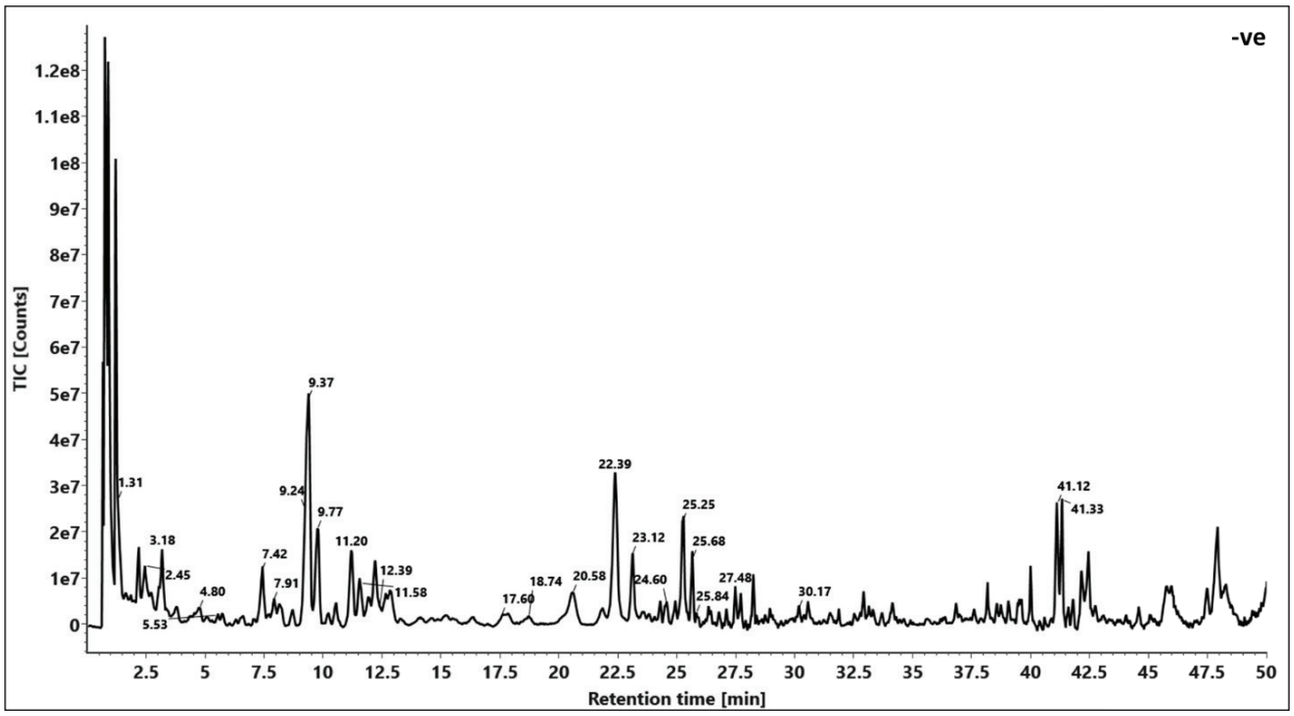

Fig. 5 TIC chromatogram of *Ranunculus sceleratus* (PRF/CHI/1223/1277) in negative ionization mode

Table 5 Compound-dependent parameters of analytes/identified compounds in *Ranunculus sceleratus* (PRF/CHI/1223/1277) in negative ionization mode

S.N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
1	N-(1-Deoxy-1-fructosyl) isoleucine	$C_{12}H_{23}NO_7$	293.1475	292.1391	-1.1	1.31	301300	-H
2	Phenylalanine	$C_9H_{11}NO_2$	165.0790	164.0704	-1.3	2.45	209063	-H
3	Syringic acid	$C_9H_{10}O_5$	198.0528	197.0444	-1.2	3.18	299732	-H
4	Tryptophane	$C_{11}H_{12}N_2O_2$	204.0899	203.0817	-0.9	4.80	138898	-H
5	Caffeic acid 3-glucoside	$C_{15}H_{18}O_9$	342.0951	341.0871	-0.7	5.53	101054	-H
6	Protocatechuic acid	$C_7H_6O_4$	154.0266	153.0183	-1.0	6.29	35243	-H
7	Quercetin 3-sophoroside-7-glucoside	$C_{33}H_{40}O_{22}$	788.2011	787.1972	3.4	7.42	944729	-H
8	Quercetin 3-sophorotrioside	$C_{33}H_{40}O_{22}$	788.2011	787.1971	3.3	7.91	261887	-H
9	Caffeic acid	$C_9H_8O_4$	180.0423	179.0341	-0.9	9.24	815994	-H
10	Quercetin 3-(2'''-(E)-caffeylsophoroside]-7-glucoside	$C_{42}H_{46}O_{25}$	950.2328	949.2323	6.8	9.37	7113815	-H
11	Quercetin 3-(6''''-caffeylsophorotrioside)	$C_{42}H_{46}O_{25}$	950.2328	949.2317	6.1	9.77	2668960	-H
12	Quercetin 3-(2'''-ferulylsambubioside)-7-glucoside	$C_{42}H_{46}O_{24}$	934.2379	933.2360	5.3	11.20	1512087	-H
13	Quercetin 3-p-coumarylsophoroside-7-glucoside	$C_{42}H_{46}O_{24}$	934.2379	933.2362	5.6	11.58	760915	-H
14	Quercetin 3-[2'''-(E)-feruloylsophoroside]-7-glucoside	$C_{43}H_{48}O_{25}$	964.2485	963.2474	6.2	12.39	229413	-H
15	Quercetin 3-(6''''-ferulylsophorotrioside)	$C_{43}H_{48}O_{25}$	964.2485	963.2480	6.8	12.77	274110	-H

S.N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
16	Herbacetin 7-methyl ether 8-sophoroside	$C_{28}H_{32}O_{17}$	640.1640	639.1566	-0.1	17.60	259677	-H
17	Quercetin 3-(2'''-caffeylsambubioside)-7-glucoside	$C_{41}H_{44}O_{24}$	920.2223	919.2195	4.5	18.74	54629	-H
18	Quercetin-3,5-diglucoside	$C_{27}H_{30}O_{17}$	626.1483	625.1410	0.0	20.58	1439555	-H
19	Quercetin-3,7-diglucoside	$C_{27}H_{30}O_{17}$	626.1483	625.1415	0.5	21.84	498367	-H
20	Quercetin 3-(2'''-caffeylsophoroside)	$C_{36}H_{36}O_{20}$	788.1800	787.1761	3.4	22.39	5984305	-H
21	Quercetin 3-(6''-caffeoylsophoroside)	$C_{36}H_{36}O_{20}$	788.1800	787.1755	2.8	23.12	1682881	-H
22	Sophoraflavonoloside	$C_{27}H_{30}O_{16}$	610.1534	609.1462	0.1	24.53	335715	-H
23	Quercetin 3-(2'''-p-coumarylsambubioside)-7-glucoside	$C_{41}H_{44}O_{23}$	904.2273	949.2301	4.6	24.60	147842	+HCOO
24	Quercetin 3-O-α-(6'''-caffeoylglucosyl-ß-1,2-rhamnoside)	$C_{36}H_{36}O_{19}$	772.1851	771.1802	2.4	25.25	2004441	-H
25	Isoquercitrin	$C_{21}H_{20}O_{12}$	464.0955	463.0886	0.4	25.68	816691	-H
26	Quercitrin	$C_{21}H_{20}O_{11}$	448.1006	447.0933	0.0	25.84	140967	-H
27	Isorhamnetin-7-O-ß-D-glucopyranoside	$C_{22}H_{22}O_{12}$	478.1111	477.1039	0.0	27.48	368710	-H
28	Isorhamnetin-3-O-glucoside	$C_{22}H_{22}O_{12}$	478.1111	477.1035	-0.3	30.17	200220	-H
29	17-Hydroxylinolenic acid	$C_{18}H_{30}O_3$	294.2195	293.2124	0.2	41.12	1486246	-H
30	Colneleic acid	$C_{18}H_{30}O_3$	294.2195	293.2122	0.0	41.33	1514072	-H

The UPLC/MS-QToF analysis of *Ranunculus sceleratus* provides a detailed and comprehensive insight into its phytochemical composition. The diverse phytochemical profile of *R. sceleratus* as revealed by UPLC/MS-QToF analysis underscores its potential for developing natural therapeutic agents. The presence of multiple bioactive compounds suggests synergistic effects that could enhance the plant's medicinal efficacy. The identification of compounds like caffeic acid and quercetin derivatives highlights the potential for *R. sceleratus* to be used in formulations aimed at combating oxidative stress-related diseases and inflammatory conditions. Further research into the specific mechanisms of action and clinical efficacy of these compounds could pave the way for new therapeutic applications.

Conclusion

The comprehensive phytochemical analysis of *Ranunculus sceleratus* leaf samples collected from various locations across the Ganga River basin has provided valuable insights into the chemical composition of this medicinal plant species. Through rigorous testing for tannins, saponins, polyphenols, and flavonoids, coupled with HPTLC fingerprinting and HPLC analysis for protocatechuic acid, a detailed characterization of the leaf samples was achieved.

The quantitative analysis revealed varying percentages of tannins, saponins, total phenolics, and total flavonoids across the sampled locations, indicating geographical influences on phytochemical content. Additionally, the HPTLC analysis detected distinct bands of phytochemical compounds under different wavelengths and white light, further highlighting the chemical diversity within the species. Of particular significance is the successful identification and quantification of protocatechuic acid in all samples, with the highest concentration observed in the sample from Farrukhabad, Uttar Pradesh. Conversely, the samples from Prayagraj, Uttar Pradesh exhibited the lowest levels of protocatechuic acid.

These findings underscore the importance of geographical location in shaping the phytochemical profile of *R. sceleratus*, suggesting potential variations in therapeutic properties across different regions. Further research into the pharmacological activities of protocatechuic acid and other phytochemicals present in this plant species could offer valuable insights for its medicinal applications.

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CHAPTER

06

Insilico Analysis Against Melanoma

INTRODUCTION

Melanoma, a malignant tumour of melanocytes, represents a significant public health challenge due to its increasing incidence and mortality rates (Centeno et al., 2023). Melanocytes, the cells responsible for producing melanin, are primarily found in the skin but can also occur in the eyes, ears, gastrointestinal tract, leptomeninges, and oral and genital mucous membranes (Mihulecea and Rotaru, 2023). Although melanoma accounts for a smaller proportion of skin cancer cases compared to basal cell carcinoma and squamous cell carcinoma, it is responsible for majority of skin cancer deaths due to its high metastatic potential (Kurva et al., 2024). In recent years, the global burden of melanoma has shown a concerning upward trend. According to the World Health Organization (WHO), there were approximately 324,635 new cases of melanoma diagnosed worldwide in 2020 (Koczkodaj et al., 2023). The incidence rates vary significantly by geographic region, with the highest rates observed in countries with predominantly fair-skinned populations, such as Australia, New Zealand, and parts of Northern Europe (Huang et al., 2023). The five-year survival rate for early-stage melanoma is relatively high at approximately 99%, but this rate drops significantly for advanced stages. Novel therapeutic approaches, including targeted therapies and immunotherapies, have shown promise in improving outcomes for patients with advanced melanoma. However, the challenge of melanoma highlights the need for continued research focused on understanding its pathogenesis, identifying at-risk populations, and developing more effective prevention and treatment strategies (Kim and Kim, 2024).

In recent years, natural products have been widely used in medicine and pharmacology because of their potential chemotherapeutic activity. As an alternative therapy in modern medicine, natural products have been shown to have better antitumor activity with fewer side effects (Hu et al., 2022a). *Ranunculus sceleratus* L., commonly known as 'celery-leaved buttercup' or 'cursed buttercup', is a member of the Ranunculaceae family, typically found in damp habitats such as marshes, ditches, and the edges of ponds (Kim et al., 2023). *R. sceleratus* has been used in traditional medicine across various cultures, particularly for its vesicant properties, which cause blistering and were applied

to treat warts and other skin conditions. The therapeutic potential of *R. sceleratus* lies in its rich phytochemical composition, including alkaloids, saponins, flavonoids, and glycosides (Hachelaf et al., 2013). *R. sceleratus* exhibits significant biological activity, such as antimicrobial and anti-inflammatory effects. This is particularly relevant in an era where antibiotic resistance is a growing concern. Emerging research also suggests that *R. sceleratus* may have potential applications in cancer therapy, with some studies showing that its extracts can induce apoptosis (programmed cell death) in certain cancer cell lines (Dai et al., 2024). However, *R. sceleratus* usages in medicine is not without challenges, given its high toxicity, which necessitates careful dosage control and a thorough understanding of its pharmacological effects. Despite these challenges, *R. sceleratus* presents a compelling case for the therapeutic potential hidden within traditionally toxic plants. The genus *Ranunculus* includes approximately 600 species globally, and recent taxonomic reports suggest that this genus has a monophyletic origin, divided into two subgenera and 17 sections (Paun et al., 2005). Owing to its wide distribution, the genus has high genetic diversity. Several *Ranunculus* species have been used in folk medicine to treat various diseases or symptoms such as jaundice, nebula, edema, malaria, asthma, pain, gout, rheumatism, inflammatory skin disorders, cancer, and hypertension. Additionally, researchers have reported that *Ranunculus* extracts possess antioxidant, anti-inflammatory, antimutagenic, antimalarial, antibacterial, antitumoral, cardioprotective, and wound-healing properties. Over the last decade, various studies have investigated the chemical components and pharmacological activities of *Ranunculus* species (Goo, 2022).

Etiology of Disease

Melanoma, a malignant tumour originating from melanocytes, the pigment-producing cells of the skin, poses a significant health challenge worldwide (Schadendorf et al., 2015). Its etiology is multifaceted, influenced by various genetic, environmental, and lifestyle factors. A substantial body of evidence supports the genetic basis of melanoma susceptibility, with individuals having a family history of the disease at significantly higher risk (Landi et al., 2020). Genetic mutations in genes like CDKN2A, CDK4, and BRAF are implicated in familial cases, emphasizing the importance of genetic screening (Khaddour et al., 2021). UV radiation exposure from sunlight remains the primary environmental risk factor for melanoma, inducing DNA damage in skin cells that can trigger malignant

transformation. Intermittent, intense sun exposure, especially during childhood, coupled with a history of sunburns, elevates melanoma risk. Artificial UV sources like tanning beds further exacerbate this risk, emphasizing sun protection measures. Phenotypic characteristics like fair skin, light eyes, and a high density of moles or freckles are associated with increased susceptibility to melanoma due to lower melanin levels (Khalesi et al., 2013). Immune dysfunction, including chronic inflammation and impaired immune surveillance, facilitates tumour growth and progression. Lifestyle factors such as smoking, alcohol consumption, and obesity also contribute to melanoma risk, highlighting the need for comprehensive preventive strategies (Sawada and Nakamura, 2021). By understanding the intricate interplay of genetic, environmental, and lifestyle factors, researchers and healthcare professionals can develop targeted approaches to combat melanoma and reduce its impact on public health.

Therapeutic Uses

Ranunculus sceleratus exhibited a range of therapeutic uses attributed to different parts of the plant. The aerial parts are utilized as insecticides. The leaves are particularly valuable in treating skin diseases, promoting the expulsion of urinary bladder calculi, and healing wounds and injuries (Mei et al., 2012). The stems serve as galactogogues, stimulating milk production, and as menstruation-inducing agents (Goo, 2022). The whole plant is used for its counterirritant properties, providing relief from irritation by inducing mild inflammation. Additionally, the entire plant is used to combat poisoning, and promote lactation and menstruation. These traditional uses highlight the broad spectrum of therapeutic potential that *R. sceleratus* offers in addressing various health conditions, making it a valuable resource in herbal medicine practices (Table 1).

Table 1 List of previously reported therapeutic uses of *R. sceleratus*

Plant part	Therapeutic use	Therapeutic use identifiers	References
Leaves	Skin diseases	MESH:D012871, UMLS:C0037274, DOID:37, ICD-11:EM0Z	ISBN:9788172361266
Whole plant, Leaves	Urinary bladder calculi	MESH:D001744, UMLS:C0005683, ICD-11:GB71.0	ISBN:9788172361266, ISBN:9780387706375, ISBN:9788172363130
Leaves	Wounds and injuries	MESH:D014947, UMLS:C0043251	ISBN:9788172363130

Plant part	Therapeutic use	Therapeutic use identifiers	References
Whole plant, Stem	Galactogogues	MESH:D056669, UMLS:C2717873	ISBN:9788172363130, ISBN:9788172361266
Whole plant, Stem	Menstruation-inducing agents	MESH:D008600, UMLS:C0025346	ISBN:9788172363130, ISBN:9788172361266
Whole plant	Counterirritant	UMLS:C0010215	ISBN:9780387706375
Whole plant	Anti-poisoning	MESH:D011041, ICD-11:NE6Z	ISBN:9788172363130, ISBN:9788172361266

Several studies have reported that phytochemicals from *R. sceleratus* show significant cytotoxic effects against various type of cancer (Goo, 2022; Dai et al., 2024). Despite these efforts, as of now, the therapeutic efficacy of *R. sceleratus* against melanoma has not been explored. This study aimed to investigate the potential of *R. sceleratus* in treating melanoma by examining the genes associated with its phytoconstituents against melanoma. The present study utilized ADMET analysis, molecular docking, and molecular dynamics simulations to provide a detailed understanding of how these phytochemicals might alleviate pathogenesis of melanoma. The multidimensional computational methods used in this chapter were represented in Fig. 1.

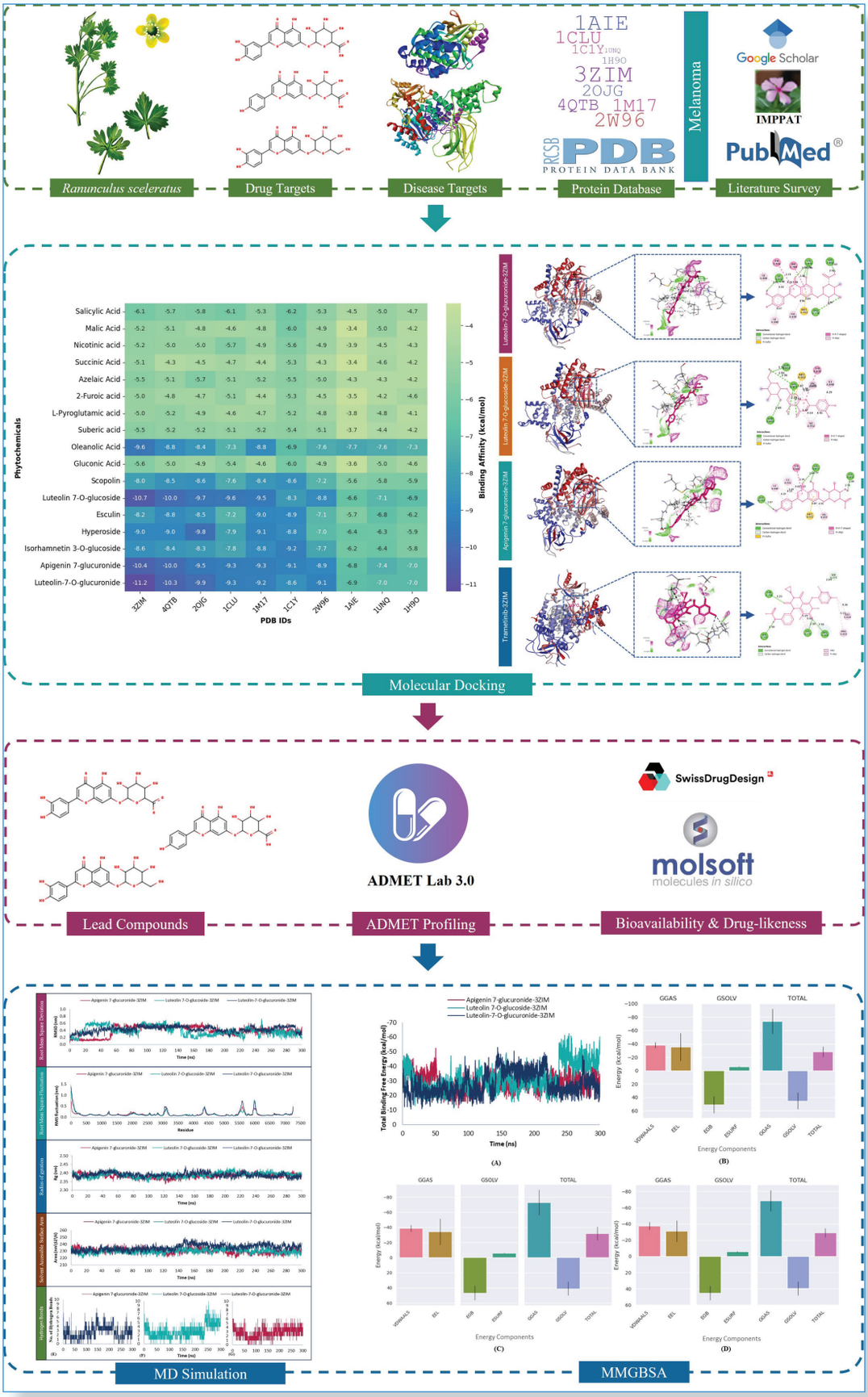


Fig. 1 General procedure of computational approaches

Materials and Methods

Assembling of Phytochemicals Library

All phytochemicals from *R. sceleratus* were collected from IMPPAT, and Dr. Duke databases, and a thorough literature review was conducted to compile a comprehensive library (Prieto et al., 2003; Lans and van Asseldonk, 2020; Cao et al., 2022; Vivek-Ananth et al., 2023). The PubChem database was utilized to retrieve canonical SMILES, 3D structures, molecular weights, and PubChem CIDs for comprehensive analysis (Kim et al., 2016). Canonical SMILES were employed to assess the ADMET profiles (absorption, distribution, metabolism, excretion, and toxicity) of all phytochemicals, as well as for screening potential lead compounds for drug-likeness and bioavailability. Moreover, the use of 3D structures aided molecular docking studies, enabling a thorough assessment of compound properties, thus supporting their potential as lead compounds in drug development.

Lipinski's Rule of Five

Canonical SMILEs of phytochemicals were subjected to analysis based on Lipinski's rule of five parameters, including molecular weight (MW), hydrogen bond donors (nHD), hydrogen bond acceptors (nHA), and the octanol-water partition coefficient (LogP). Phytochemicals not meeting Lipinski's rule of five criteria were classified as Lipinski violations (LV), while those meeting the criteria underwent molecular docking analysis (Lipinski, 2004).

Protein Data Extraction and Preparation

A total of 10 disease targets were selected for melanoma, according to comprehensive review of the literature. The 3-D protein structure of genes was obtained from the Protein Data Bank (Burley et al., 2017). Structure modelling was conducted using MODELLER (version 10.5), followed by preparation with UCSF ChimeraX software (version 1.7). This involved adding any missing residues in the protein structure and eliminating water molecules, co-ligands, and heteroatoms. Moreover, polar hydrogen atoms and charges were added to confirm the ideal preparation of all target proteins for molecular docking (Madhavi Sastry et al., 2013).

Molecular Docking

Molecular docking was performed to evaluate the interactions between phytochemicals and target proteins linked to specific disease. The initial three-dimensional conformations of both FDA-approved drugs and screened phytochemicals were acquired from the PubChem database and used as ligands. The software UCSF ChimeraX was used to optimize the structures of protein through the modification of unfavourable torsional angles

and nonstandard residues, consequently reducing energy and assuring structural stability (Meng et al., 2023). The optimization process enhanced the binding of ligands to receptor molecules, preventing steric clashes efficiently. Molecular docking analyses were conducted using Autodock Vina, a widely utilized software for ligand-protein interaction predictions. Ligand energy minimization and the conversion of receptor-ligand complexes from the pdb to the pdbqt format were facilitated by Open Babel, a versatile toolkit for molecular modelling tasks (Butt et al., 2020; Chaurasia et al., 2023). Blind docking was performed by expanding the grid box to cover the structure of protein. The assessment of ligand-protein binding affinities involved examining docking scores (kcal/mol) and relative mean square deviation (RMSD). Afterwards, protein-ligand complexes were analyzed and visualized using Discovery Studio 2021 Client software.

ADMET Profiling of Lead Compounds

In the absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiling, parameters such as P-glycoprotein inhibition (pgp_inh) and substrate (pgp_sub), fraction unbound in plasma (fu), BBB, BCRP, and drug metabolism-related parameters such as inhibition (inh) and substrate (sub) for CYP1A2, and CYP2D6, as well as plasma clearance (cl-plasma), skin sensitivity Ames test, route of administration (ROA), and assessments of carcinogenicity, and hematotoxicity, were considered (Xiong et al., 2021). Compounds that met all criteria within the medium range of empirical decisions according to ADMETlab 2.0 were selected for further analysis.

Lead Compounds Drug Profiling

All compounds were evaluated for their drug-likeness (DL) and oral bioavailability (OB) to identify potential leads. Drug-likeness refers to the degree of similarity between a compound's chemical structure and that of well-known drugs. Oral bioavailability relates to the amount of medication administered orally that reaches the bloodstream and impacts local tissues and organs before producing a similar pharmacological effect (Ahmed et al., 2022). The therapeutic potential of phytochemicals is evaluated based on their DL and OB, with screening thresholds set at ⩾ 0.18 for DL and ⩾ 0.30 for OB and analysed respectively using Molsoft L.L.C. and the SwissADME tool (Daina et al., 2017).

Molecular Dynamics Simulation

The molecular dynamics (MD) simulation was conducted using GROMACS software, version 2023.3, in a Linux environment. The force fields employed for the simulation included the CHARMM36 force field for the protein structure and the CHARMM all atoms force field for ligand parameterization. The system was prepared for simulation using the TIP 3-point solvent model for solvation, followed by neutralization through the addition of appropriate quantities (0.1M) of Na^+ and Cl^-

ions. Post-simulation analysis encompassed various parameters including root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), hydrogen bonds (HB), and solvent accessible surface area (SASA). The principal component analysis (PCA) focused on determining key factors such as eigenvalues and eigenvectors to explore the principal components governing system dynamics. All MD simulation analyses were performed using the built-in tools of GROMACS version 2023.3 (Balkrishna et al., 2023).

Binding Free Energy Analysis

The current study utilized the gmx_MMPBSA package (version 1.6.2) to compute the binding free energy (ΔG) analysis on ligand-protein complexes. Binding free energy was analyzed utilizing the molecular mechanics generalized born surface area (MM/GBSA) method (Valdés-Tresanco et al., 2021). The GROMACS trajectory, topology, and index files of the target complexes were employed to evaluate overall change in total free energy (ΔTOTAL) with standard deviation. The energy components such as changes in van der Waals energy (ΔVDWAALS), electrostatic (Coulombic) energy (ΔEEL), generalized Born (GB) solvation energy (ΔEGB), solvent-accessible surface area (SASA) term (ΔESURF), gas-phase free energy (ΔGGAS), solvation free energy (ΔGSOLV), and overall change in total free energy (ΔTOTAL) were analyzed in the term of average value with standard deviation to assess the binding free energy.

Results and Discussion

Ranunculus sceleratus, rich in phytochemicals, has been traditionally used for various ailments. Recent studies highlight its bioactive compounds with potential applications in disease treatment, including cancer. Known for its anti-inflammatory properties, *R. sceleratus* shows promise in treating skin conditions and reducing swelling (Anwar et al., 2024; Shi et al., 2024). However, its mechanisms in treating melanoma remain unexplored. Melanoma, a highly metastatic skin cancer, is the leading cause of skin cancer mortality. Early-stage melanoma can often be treated surgically (Koshenkov et al., 2016), but advanced melanoma has poor prognosis due to treatment resistance (Schadendorf et al., 2015). Current therapies have limited efficacy and significant side effects, highlighting the need for novel treatments. Herbal medicines, rich in bioactive compounds, are valuable for oncology research (Rathor, 2021). Many herbal components control tumor progression. Computational methods like molecular docking and dynamics simulations can explore phytochemical-protein interactions, advancing drug development and clinical research for melanoma treatments (Parida et al., 2020).

Potential Phytochemical Profiling

A total of 75 phytochemicals were retrieved from various databases, along with an extensive literature survey. The obtained phytochemicals were screened based on Lipinski's rule of five and ADMET parameters (Table 2). After screening, only 17 phytochemicals were found, and these phytochemicals were used for molecular docking analysis.

Table 2 Screening of phytochemicals based on Lipinski's rule and ADMET

Phytochemical	Compound Id	MW	nHA	nHD	logP	LV	ADMET
2,4-Dinitrophenylhydrazine	3772977	198.04	8	3	1.48	0	NA
24-Ethylcholest-5-en-3beta-ol	22012	414.39	1	1	8.11	0	NA
2-Furoic acid	6919	112.02	3	1	0.67	0	A
3-(4-Hydroxyphenyl)propionic acid	10394	166.06	3	2	1.21	0	NA
3,4-Dihydroxybenzoic acid	72	154.03	4	3	1.00	0	NA
8-Hydroxypinoresinol	3010930	374.14	7	3	0.94	0	NA
Aglycone	139597845	584.33	8	3	4.49	0	NA
Anemonin	10496	192.04	4	0	0.85	0	NA
Apigenin	5280443	270.05	5	3	2.98	0	NA
Apigenin 7-glucuronide	5319484	446.08	11	6	0.47	1	A
Asparagine	6267	132.05	5	5	-3.72	0	NA
Azelaic Acid	2266	188.1	4	2	1.36	0	A
Caffeic Acid	689043	180.04	4	3	1.02	0	NA
Choline	305	104.11	2	1	-1.29	0	NA
Chryseriol	5280666	300.06	6	3	2.67	0	NA
cis-Aconitic acid	643757	174.02	6	3	-0.43	0	NA
cis-Aconitic anhydride	65163	156.01	5	1	0.04	0	NA
Citric Acid	311	192.03	7	4	-2.05	0	NA
Coumarin	323	146.04	2	0	1.47	0	NA
Cytosine	597	111.04	4	3	-1.21	0	NA
Diosmetin	5281612	300.06	6	3	2.63	0	NA
Emodin	3220	270.05	5	3	3.81	0	NA
Esculetin	5281416	178.03	4	2	0.64	0	NA
Esculin	5281417	340.08	9	5	-1.39	0	A
Ferulic acid	445858	194.06	4	2	1.48	0	NA
Gluconic Acid	10690	196.06	7	6	-1.68	0	A

Phytochemical	Compound Id	MW	nHA	nHD	logP	LV	ADMET
Glutamic Acid	33032	147.05	5	4	-3.37	0	NA
Guanine	135398634	151.05	6	4	-0.90	0	NA
Hyperoside	5281643	464.1	12	8	-0.09	1	A
Isoorientin	114776	448.1	11	8	-0.19	1	NA
Isorhamnetin	5281654	316.06	7	4	1.82	0	NA
isorhamnetin 3-O-glucoside	5318645	478.11	12	7	0.11	1	A
Isoscopoletin	69894	192.04	4	1	0.84	0	NA
Kaempferol	5280863	286.05	6	4	1.97	0	NA
Kaempferol-3,7-di-o-glucoside	91872956	610.15	16	10	-1.22	1	NA
Limonene, (+/-)-	22311	136.13	0	0	4.54	0	NA
L-Pyroglutamic acid	7405	129.04	4	2	-1.06	0	A
Luteolin	5280445	286.05	6	4	2.25	0	NA
Luteolin 7-O-glucoside	5280637	448.1	11	7	0.05	1	A
Luteolin-7-O-glucuronide	13607752	462.08	12	7	0.25	1	A
Maleic Anhydride	7923	98	3	0	0.69	0	NA
Malic Acid	525	134.02	5	3	-1.28	0	A
Matairesinol	119205	358.14	6	2	1.60	0	NA
Matairesinoside	486612	520.19	11	5	-0.52	1	NA
N-Acetyl-L-phenylalanine	74839	207.09	4	2	0.23	0	NA
Niacinamide	936	122.05	3	2	-0.38	0	NA
Nicotinic acid	938	123.03	3	1	0.28	0	A
Oleanolic Acid	10494	456.36	3	2	4.22	0	A
Palmitic Acid	985	256.24	2	1	6.65	0	NA
p-Coumaric acid	637542	164.05	3	2	1.32	0	NA
Phenylalanine	6140	165.08	3	3	-1.67	0	NA
Physcione	10639	284.07	5	2	3.99	0	NA
Pinocembrin	68071	256.07	4	2	2.89	0	NA
Proline	145742	115.06	3	2	-2.78	0	NA
Protoanemonin	66948	96.02	2	0	0.98	0	NA
Protocatechualdehyde	8768	138.03	3	2	1.12	0	NA
Pyrogallol	1057	126.03	3	3	0.85	0	NA
Quercetin	5280343	302.04	7	5	1.45	0	NA

Phytochemical	Compound Id	MW	nHA	nHD	logP	LV	ADMET
Ranunculin	441581	276.08	8	4	-2.02	0	NA
Rhamnetin	5281691	316.06	7	4	1.98	0	NA
Salicylic Acid	338	138.03	3	2	2.26	0	A
Scoparone	8417	206.06	4	0	1.43	0	NA
Scopoletin	5280460	192.04	4	1	0.86	0	NA
Scopolin	439514	354.1	9	4	-1.03	0	A
Serotonin	5202	176.09	3	4	0.32	0	NA
Stigmast-4-ene-3,6-dione	5490007	426.35	2	0	6.85	0	NA
Stigmasterol	5280794	412.37	1	1	7.29	0	NA
Suberic acid	10457	174.09	4	2	0.82	0	A
Succinic Acid	1110	118.03	4	2	-0.65	0	A
Threonine	6288	119.06	4	4	-2.86	0	NA
Tricin	5281702	330.07	7	3	2.48	0	NA
Tryptophan	6305	204.09	4	4	-1.20	0	NA
Uracil	1174	112.03	4	2	-1.10	0	NA
Warfarin	54678486	308.1	4	1	2.27	0	NA

LogP: Partition coefficient; MW: Molecular weight; nH-A: H-bond acceptor; nH-D: H-bond donor; LV: Lipinski violations; A: Accepted; NA: Not Accepted

Molecular Docking Analysis

The molecular docking process utilized drug targets with disease-related targets corresponding to the 10 genes and 17 phytochemicals which were docked with protein structures of key hub genes, including TP53, HRAS, PIK3CA, CCND1, AKT1, EGFR, RAF1, PIK3R1, MAPK3, and MAPK1 identified by PDB IDs including 1AIE, 1CLU, 3ZIM, 2W96, 1UNQ, 1M17, 1C1Y, 1H9O, 4QTB, and 2OJG respectively. The resulting interactions were visualized in a heat map, depicting affinity between phytochemicals and targeted proteins associated with the 10 genes, with darker colours indicating lower receptor-ligand affinity in kcal/mol (Fig. 2). The molecular docking analysis of 3 phytochemicals and FDA-approved drug, trametinib, with the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) receptor (PDB ID: 3ZIM) revealed insightful differences in binding affinity and interaction characteristics. Luteolin-7-O-glucuronide (PubChem ID: 13607752) exhibits the highest binding affinity at -11.2 kcal/mol, forming 7 hydrogen bonds and a total of 16 bonds, indicating strong interaction stability, and suggesting a significant inhibitory effect on the PIK3CA target. Luteolin 7-O-glucoside

(PubChem ID: 5280637) showed a slightly lower binding affinity of -10.7 kcal/mol, with 6 hydrogen bonds and 14 total bonds, highlighting its potential efficacy as a PIK3CA inhibitor. Apigenin 7-glucuronide (PubChem ID: 5319484) demonstrated a binding affinity of -10.4 kcal/mol, forming 5 hydrogen bonds and 13 total bonds, making it the third most effective among the tested natural compounds. Despite its slightly lower interaction stability compared to the luteolin derivatives, it remains a promising candidate. In contrast, the FDA-approved drug trametinib (PubChem ID: 11707110) has the lowest binding affinity of -8.6 kcal/mol, with 6 hydrogen bonds and a total of 10 bonds (Table 3). The findings suggested that trametinib's interaction with PIK3CA is less robust compared to the tested phytochemicals. The 3D and 2D structures of the targeted protein, along with the corresponding bonds formed during the interaction with neighbouring amino acids, were depicted in Fig. 3 (A-D).

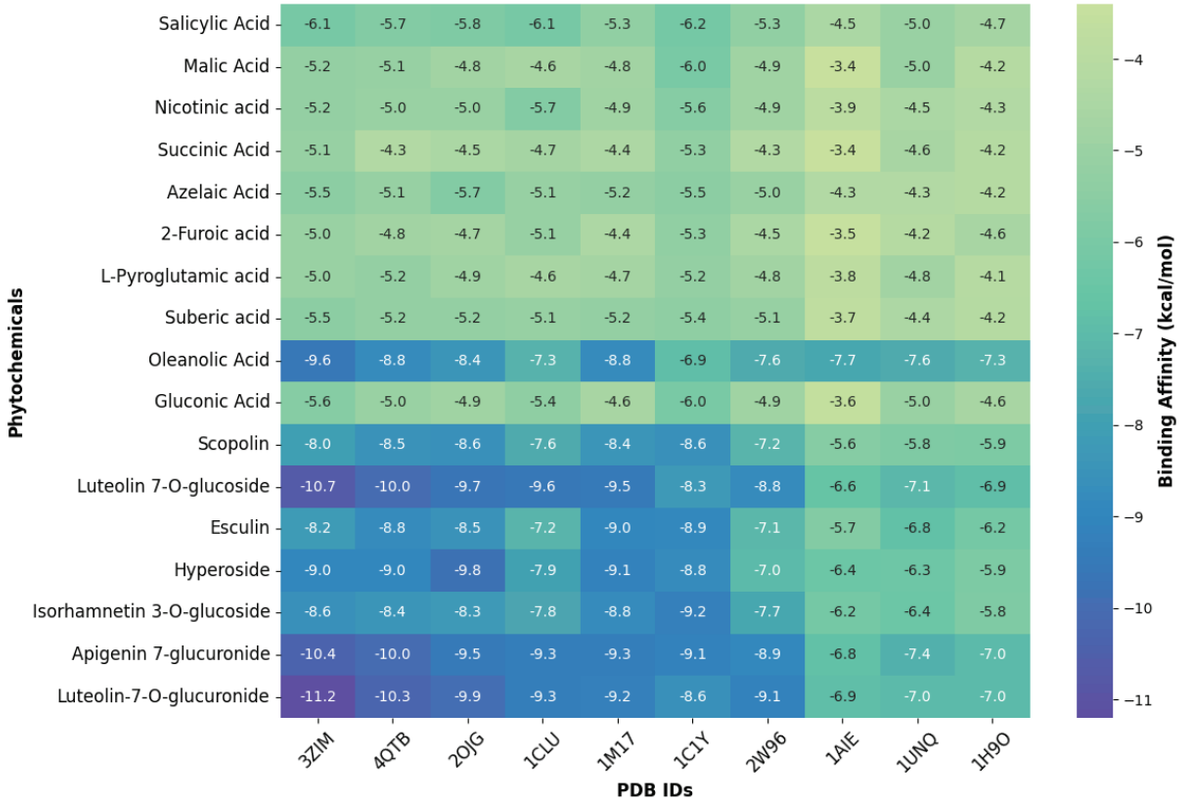


Fig. 2 Heat map of molecular docking illustrates interactions between phytochemicals and proteins linked to the top 10 hub genes

Table 3 Interactions between selected bioactive compounds and target proteins

S.N.	Ligand	PubChem ID	Target (PDB ID)	Binding affinity (kcal/mol)	Hydrogen bonds	Total no. of bonds	Type of Bond	Interacting residues	Bond Length
1.	Luteolin-7-O-glucuronide	13607752	PIK3CA (3ZIM)	11.2	7	16	Conventional Hydrogen Bond	A:ARG770:HH22 - N:UNK1:O	2.87
							Conventional Hydrogen Bond	A:VAL851:HN - N:UNK1:O	2.49
							Conventional Hydrogen Bond	A:VAL851:HN - N:UNK1:O	2.06
							Conventional Hydrogen Bond	N:UNK1:H - A:SER854:O	2.62
							Conventional Hydrogen Bond	N:UNK1:H - A:VAL851:O	2.36
							Conventional Hydrogen Bond	N:UNK1:H - A:ILE932:O	2.49
							Carbon Hydrogen Bond	N:UNK1:C - A:SER854:O	2.97
							Pi-Sulfur	A:MET922:SD - N:UNK1	3.84
							Pi-Pi T-shaped	A:TRP780 - N:UNK1	5.45
							Pi-Pi T-shaped	A:TYR836 - N:UNK1	5.71
							Pi-Alkyl	N:UNK1 - A:ILE800	5.48
							Pi-Alkyl	N:UNK1 - A:ILE932	4.24
							Pi-Alkyl	N:UNK1 - A:VAL850	4.96
							Pi-Alkyl	N:UNK1 - A:ILE932	5.48
							Pi-Alkyl	N:UNK1 - A:ILE848	4.27

S.N.	Ligand	PubChem ID	Target (PDB ID)	Binding affinity (kcal/mol)	Hydrogen bonds	Total no. of bonds	Type of Bond	Interacting residues	Bond Length
2.	Luteolin 7-O-glucoside	5280637	PIK3CA (3ZIM)	10.7	6	14	Conventional Hydrogen Bond	A:VAL851:HN - N:UNK1:O	2.5262
							Conventional Hydrogen Bond	A:SER854:HN - N:UNK1:O	1.90859
							Conventional Hydrogen Bond	A:GLN859:HE22 - N:UNK1:O	2.8931
							Conventional Hydrogen Bond	N:UNK1:H - A:VAL851:O	2.45663
							Conventional Hydrogen Bond	N:UNK1:H - A:VAL851:O	2.19541
							Carbon Hydrogen Bond	N:UNK1:C - A:SER854:O	2.9289
							Pi-Sulfur	A:MET922:SD - N:UNK1	3.86786
							Pi-Pi T-shaped	A:TYR836 - N:UNK1	5.69156
							Pi-Alkyl	N:UNK1 - A:MET922	5.44474
							Pi-Alkyl	N:UNK1 - A:ILE932	4.14307
							Pi-Alkyl	N:UNK1 - A:VAL850	4.96166
							Pi-Alkyl	N:UNK1 - A:ILE932	5.47189
							Pi-Alkyl	N:UNK1 - A:ILE848	4.29467
							Pi-Alkyl	N:UNK1 - A:ILE932	4.44667
							Pi-Alkyl	N:UNK1 - A:ILE932	4.43

S.N.	Ligand	PubChem ID	Target (PDB ID)	Binding affinity (kcal/mol)	Hydrogen bonds	Total no. of bonds	Type of Bond	Interacting residues	Bond Length
3.	Apigenin 7-glucuronide	5319484	PIK3CA (3ZIM)	10.4	5	13	Conventional Hydrogen Bond	A:VAL851:HN - N:UNK1:O	2.80
							Conventional Hydrogen Bond	N:UNK1:H - A:SER854:O	2.89
							Conventional Hydrogen Bond	N:UNK1:H - A:VAL851:O	2.93
							Conventional Hydrogen Bond	N:UNK1:H - A:ASP933:OD1	2.07
							Carbon Hydrogen Bond	N:UNK1:C - A:SER854:O	2.74
							Pi-Sulfur	A:MET922:SD - N:UNK1	3.89
							Pi-Pi T-shaped	A:TYR836 - N:UNK1	5.44
							Pi-Alkyl	N:UNK1 - A:ILE848	5.49
							Pi-Alkyl	N:UNK1 - A:ILE932	4.00
							Pi-Alkyl	N:UNK1 - A:VAL850	4.95
							Pi-Alkyl	N:UNK1 - A:ILE932	5.29
							Pi-Alkyl	N:UNK1 - A:ILE848	4.28789
							Pi-Alkyl	N:UNK1 - A:ILE932	4.52762

S.N.	Ligand	PubChem ID	Target (PDB ID)	Binding affinity (kcal/mol)	Hydrogen bonds	Total no. of bonds	Type of Bond	Interacting residues	Bond Length
4.	Trametinib (FDA-approved Drug)	11707110	3ZIM	8.6	6	10	Conventional Hydrogen Bond	A:LYS271:HZ2 - N:UNK1:O	2.10096
							Conventional Hydrogen Bond	A:ASN756:HD22 - N:UNK1:O	2.24711
							Conventional Hydrogen Bond	A:ARG818:HH11 - N:UNK1:O	2.92073
							Conventional Hydrogen Bond	N:UNK1:H - A:LEU755:O	2.50251
							Carbon Hydrogen Bond	A:SER275:HB1 - N:UNK1:F	2.63923
							Carbon Hydrogen Bond	A:ARG818:HD1 - N:UNK1:O	2.82403
							Alkyl	N:UNK1:I - A:LEU834	4.26245
							Alkyl	N:UNK1:I - A:-PRO835	5.15128
							Pi-Alkyl	N:UNK1 - A:LYS271	5.28647
							Pi-Alkyl	N:UNK1 - A:LYS271	4.63378

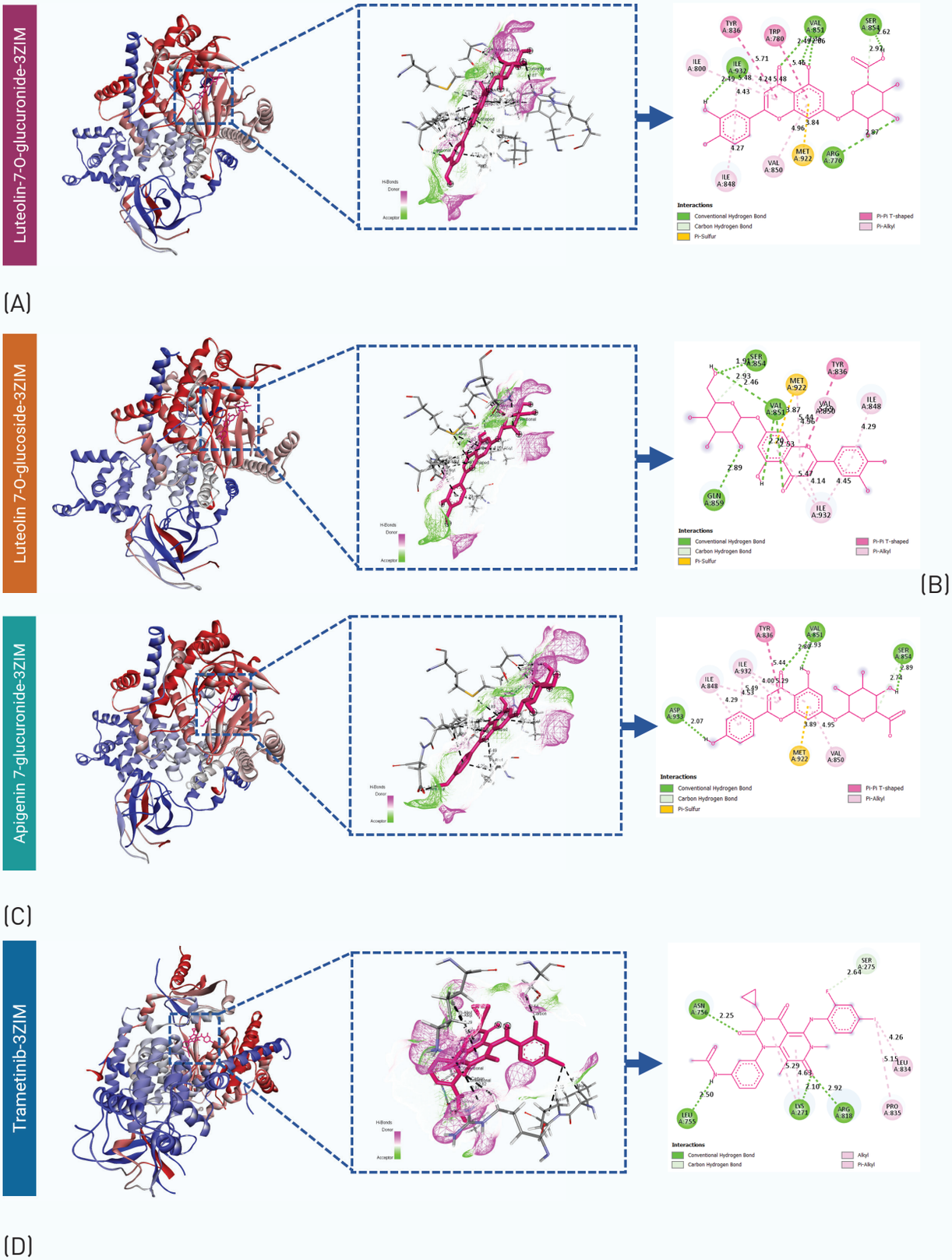


Fig. 3 (A-D) 3D and 2D structures of targeted protein docked with different phytochemicals. (A) Luteolin-7-O-glucuronide with the target protein 3ZIM (B) Luteolin 7-O-glucoside with the target protein 3ZIM (C) Apigenin 7-glucuronide with the target protein 3ZIM (D) Trametinib (FDA-approved drug) with the protein 3ZIM

Overall, these findings emphasized the promise of these compounds as therapeutic agents targeting specific disease pathways. Previous studies have shown that the PI3K/AKT signalling pathway is an essential node in cancer cells that controls cell growth, migration, proliferation, and metabolism, and targeting the oncogenic PI3K/AKT signalling pathway is currently thought to be an extremely promising strategy for melanoma intervention (Shen et al., 2022; Vasan and Cantley, 2022). Moreover, suppression of the PI3K/AKT pathway induces cell apoptosis *via* a variety of mechanisms, including regulation of Bcl-2 family members' activities and activation of members of the caspase family of proteases (Kircher et al., 2019; Braicu et al., 2022). In another study, sanguinarine treatment targets the PI3K-AKT pathway, inhibiting proliferation, migration, and invasion of melanoma cells and reducing key protein levels (Qi et al., 2023). A similar study reported that 'Cortex Mori', a traditional Chinese medicine, exhibited anti-melanoma activity through signalling pathway PI3K-AKT signalling inhibition (Hu et al., 2022b). A study focusing on 'Triphala' showed its ability to induce apoptosis and inhibit cell activity, migration, invasion, and proliferation in oral squamous cell carcinoma, primarily *via* inactivating the PI3K/Akt signalling pathway, suggesting its potential as an adjunctive therapeutic agent for oral cancer treatment. (Hu et al., 2024). Our results suggested that *R. sceleratus* derived phytochemicals may inhibit melanoma cell proliferation and migration while inducing apoptosis via inhibition of the PI3K/Akt signalling pathway, providing a mechanistic approach for exploiting these compounds in melanoma treatment.

Drug Profiling of Lead Compounds

The comprehensive assessment of lead compounds for drug development, including luteolin-7-O-glucuronide, luteolin 7-O-glucoside, and apigenin 7-glucuronide, needed examining their PubChem ID, radar chart, 2D structure, bioavailability, and drug-likeness. Drug profiling involves evaluating potential lead compounds based on various pharmacokinetic and pharmacodynamic properties to predict their suitability as drugs. The key parameters analyzed are bioavailability, which measures the fraction of an administered drug that reaches systemic circulation, and drug-likeness, which evaluated a compound's adherence to rules predicting its behaviour in the body. Luteolin-7-O-glucuronide shows a bioavailability of 0.41 and a drug-likeness score of 0.71, indicating moderate bioavailability and a relatively high drug-likeness, making it a promising candidate for further development. Luteolin 7-O-glucoside, with a bioavailability of 0.37 and a drug-likeness score of 0.6, is less favourable but still within a reasonable range for consideration as a lead compound. Apigenin 7-glucuronide exhibits similar bioavailability to luteolin-7-O-glucuronide at 0.41 and a drug-likeness score of 0.67, making it another strong candidate for drug development (Table 4). These leading compounds also exhibited values below the moderate threshold for various ADMET parameters, including Pgp-inh, Pgp-sub, BBB, Fu, CYP1A2-inh, CYP1A2-sub, CYP2D6-inh, CYP2D6-sub, CL, Ames, ROA, SkinSen, carcinogenicity and hematotoxicity (Table 5).

Table 4 Profiling of lead compounds for drug development

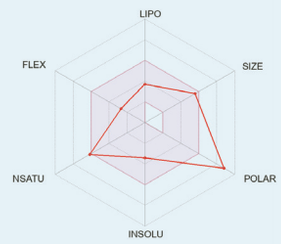
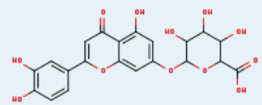
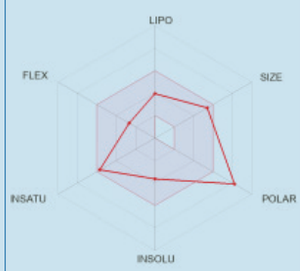
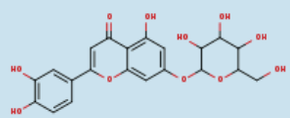
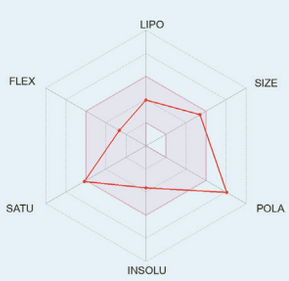
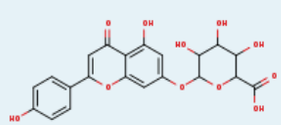
S.N.	Ligand	PubChem ID	Radar Chart	2D Structure	Bioavailability	Drug-likeness
1	Luteolin-7-O-glucuronide	13607752			0.41	0.71
2	Luteolin 7-O-glucoside	5280637			0.37	0.60
3	Apigenin 7-glucuronide	5319484			0.41	0.67

Table 5 Characterization of lead compounds through ADMET profiling

Category	Parameters	Luteolin-7-O-glucuronide	Luteolin 7-O-glucoside	Apigenin 7-glucuronide
Absorption	pgp_inh	0.00	0.00	0.00
	pgp_sub	0.48	0.40	0.70
Distribution	BCRP	0.11	0.90	0.05
	BBB	0.00	0.00	0.00
	Fu	19.81	19.35	18.65
Metabolism	CYP1A2-inh	0.00	0.00	0.00
	CYP1A2-sub	0.00	0.00	0.00
	CYP2D6-inh	0.00	0.00	0.00
	CYP2D6-sub	0.00	0.01	0.00
Excretion	cl-plasma	1.66	3.78	1.10
Toxicity	Ames	0.45	0.69	0.41
	ROA	0.39	0.31	0.40
	SkinSen	0.43	0.65	0.10
	Carcinogenicity	0.20	0.38	0.30
	Hematotoxicity	0.02	0.02	0.03

Structural Stability Analysis

Molecular dynamics simulations were utilized to examine interactions between compounds and proteins at a molecular level. This study focused on the most significant interactions, specifically the binding of luteolin-7-O-glucuronide, luteolin 7-O-glucoside, and apigenin 7-glucuronide with phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PDB ID: 3ZIM). Over a simulation period of 300 nanoseconds (ns), 3000 frames were recorded to evaluate the structural stability of the complexes luteolin-7-O-glucuronide-3ZIM, luteolin 7-O-glucoside-3ZIM, and apigenin 7-glucuronide-3ZIM. Various parameters such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), solvent accessible surface area (SASA), and hydrogen bonds (HB) were used in the assessment.

Root Mean Square Deviation (RMSD)

The backbone RMSD values for the complexes of luteolin-7-O-glucuronide, luteolin 7-O-glucoside, and apigenin 7-glucuronide with phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) indicated that all three complexes exhibit similar stability. The average RMSD values were found 0.43

nm, 0.39 nm, and 0.38 nm respectively, suggesting slight variations in stability, with luteolin-7-O-glucuronide showing the highest average. Minimum RMSD values of 0.19 nm, 0.09 nm, and 0.06 nm for the respective complexes indicated that luteolin 7-O-glucoside and apigenin 7-glucuronide form more stable interactions at their most stable points compared to luteolin7-O-glucuronide. The maximum RMSD values are 0.61 nm, 0.73 nm, and 0.64 nm, showing that luteolin 7-O-glucoside experiences the greatest conformational change. Standard deviations of 0.08 nm, 0.13 nm, and 0.14 nm revealed greater conformational variability for luteolin 7-O-glucoside and apigenin 7-glucuronide compared to luteolin-7-O-glucuronide. Overall, the data suggest that while all complexes are relatively stable, luteolin-7-O-glucuronide is slightly more stable on average, whereas the other two exhibit more flexibility(Fig. 4A).

Root Mean Square Fluctuation (RMSF)

The backbone RMSF values for luteolin-7-O-glucuronide-3ZIM, luteolin 7-O-glucoside-3ZIM, and apigenin 7-glucuronide-3ZIM showed similar average backbone flexibility with values of 0.1 for all 3compounds. The minimum RMSF values were also found consistent at 0.1 across the board. However, luteolin-7-O-glucuronide-3ZIM exhibited the highest maximum fluctuation at 1.4, indicating significant flexibility in certain regions, followed by luteolin 7-O-glucoside-3ZIM at 1.2 nm, and apigenin 7-glucuronide-3ZIM at 0.7 nm. The standard deviation further revealed that luteolin-7-O-glucuronide-3ZIM has the most variable flexibility (0.14), compared to luteolin 7-O-glucoside-3ZIM (0.12 nm) and apigenin 7-glucuronide-3ZIM (0.08 nm). While all 3compounds have a similar average level of flexibility, luteolin-7-O-glucuronide-3ZIM showed greater dynamic movement, potentially affecting its molecular interactions and stability (Fig. 4B).

Radius of Gyration (Rg)

The radius of gyration values for luteolin-7-O-glucuronide-3ZIM, luteolin 7-O-glucoside-3ZIM, and apigenin 7-glucuronide-3ZIM indicated consistent levels of molecular compactness. All 3compounds had an average Rg of 2.4 nm, demonstrating similar overall structural dimensions. While luteolin-7-O-glucuronide-3ZIM maintained a minimum Rg of 2.4 nm, luteolin 7-O-glucoside-3ZIM and apigenin 7-glucuronide-3ZIM could compact slightly more at 2.3 nm, although maximum Rg values were found 2.4 nm for each compound. The standard deviation of 0.01 nm for each compound indicated minimal variability in their compactness across different conformations. Therefore, these molecules exhibited stable and uniform structural characteristics with subtle differences in their ability to compact under specific conditions (Fig. 4C).

Solvent Accessible Surface Area (SASA)

The solvent accessible surface area for luteolin-7-O-glucuronide-3ZIM, luteolin 7-O-glucoside-3ZIM, and apigenin

7-glucuronide-3ZIM indicated their surface areas accessible to solvent molecules. Luteolin-7-O-glucuronide-3ZIM showed the highest average SASA at 235.2 nm\S2\N with a range from 223.2 to 250.0 nm\S2\N. Luteolin 7-O-glucoside-3ZIM and apigenin 7-glucuronide-3ZIM had slightly lower average SASA values of 229.9 and 230.4 nm\S2\N, respectively, with narrower ranges. Standard deviations of 4.33, 3.14, and 3.53 nm\S2\N indicated variability in SASA within each compound, with luteolin-7-O-glucuronide-3ZIM showing the highest variability. The SASA values indicate minimal and nonsignificant differences among the 3 compounds, possibly suggesting consistent structural conformations or similar levels of surface accessibility across the compounds (Fig. 4 D).

Hydrogen Bonds (HB)

Hydrogen bonds (HB) play a crucial role in molecular interactions, affecting the structure, stability, and function of biological molecules. Luteolin-7-O-glucuronide with 3ZIM formed a total of 2950 hydrogen bonds. It peaked at 844 instances for three-HB interactions, indicating a tendency for moderate HB formations. Other distinguished data points showed that 1, 2, 4, 5, 6, 7, and 8HBs were found in 800, 664, 404, 159, 67, 11, and 1 instance, respectively, during the entire 300-nanosecond MD simulation reaction (Fig. 4 E). As the number of HBs increased, the frequency of interactions decreased, showing a preference for fewer but significant HB formations. On the other hand, luteolin 7-O-glucoside with 3ZIM exhibited a total of 2975 hydrogen bonds, peaking at 904 instances for two-HB interactions. Moreover, this compound showed a higher capacity for multiple HB formations, with other significant values being 477 instances for one HB, 628 for three HBs, 437 for four HBs, 298 for five HBs, 161 for six HBs, 52 for seven HBs, 14 for eight HBs, 3 for nine HBs, and 1 for ten HBs (Fig. 4 F). These finding suggested that the ability to establish complex molecular interaction networks, potentially enhancing its stability and biological activity. Furthermore, apigenin 7-glucuronide-3ZIM formed a total of 2972 hydrogen bonds, with a peak at 946 bonds for three-HB interactions. Specifically, it formed 381 bonds involving a single hydrogen bond (HB), 853 bonds with 2hydrogen bonds, 585 bonds with 4hydrogen bonds, 173 bonds with 5hydrogen bonds, and 34 bonds with 6hydrogen bonds (Fig. 4 G). Overall, all 3compounds exhibited a similar pattern of interaction with the 3ZIM receptor, as evidenced by the comparable number of total hydrogen bonds formed by each compound.

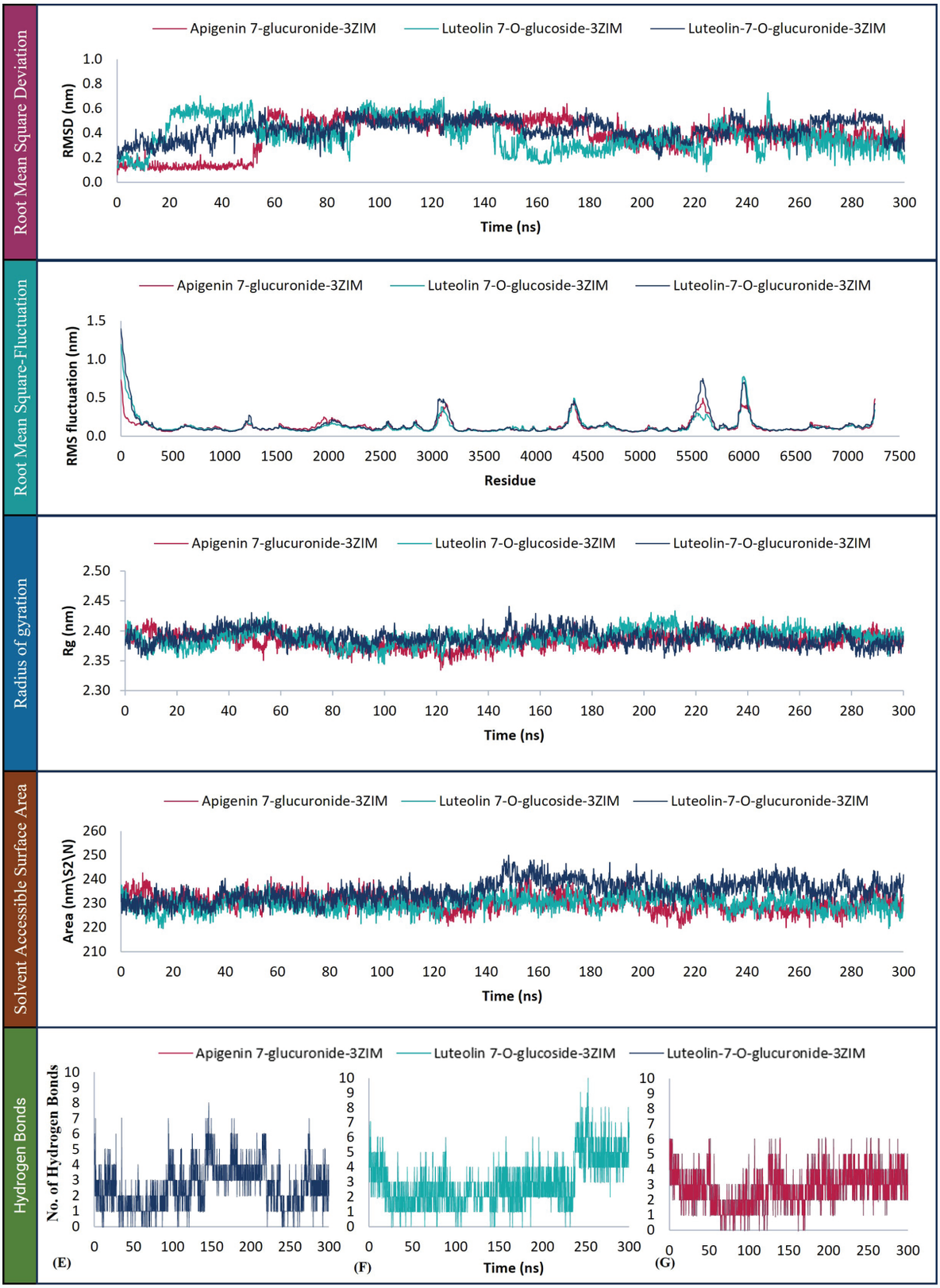


Fig. 4 (A-G) Molecular dynamics simulations endpoints as (A) Root Mean Square Deviation, (B) Root Mean Square Fluctuation, (C) Radius of Gyration (total and around axes), (D) Solvent Accessible Surface, (E) Hydrogen bond formed in Luteolin-7-O-glucuronide-3ZIM (F) Hydrogen bond formed in Luteolin 7-O-glucoside-3ZIM, and (G) Hydrogen bond formed in Apigenin 7-glucuronide-3ZIM complex

These findings were found in align with a previous study that employed *insilico* analyses to explore apigenin derivatives as potent inhibitors against human papillomavirus-associated cervical cancer and DNA polymerase theta, revealing strong binding interactions and stability assessed through molecular docking, dynamics simulations, and comprehensive screenings (Akash et al., 2023). In another study, luteolin was found as a potential anti-cancer agent against human lung cancer, through structural analysis, drug-likeness prediction, molecular docking, and dynamics simulations, aiming to establish its efficacy as an inhibitor, affirming alignment with the present study's findings (Lakhera et al., 2022).

Interaction Energy Analysis

The binding free energy (BFE) trajectory analysis utilizing MMGBSA was conducted for luteolin-7-O-glucuronide-3ZIM, luteolin 7-O-glucoside-3ZIM, and apigenin 7-glucuronide-3ZIM, highlighting their interactions with the 3ZIM receptor. Luteolin-7-O-glucoside-3ZIM showed the strongest average binding affinity with a BFE of -31.90 kcal/mol, followed by luteolin 7-O-glucuronide-3ZIM at -28.92 kcal/mol, and apigenin 7-glucuronide-3ZIM at -28.25kcal/mol. For the most favourable binding scenarios (minimum BFE), luteolin-7-O-glucuronide-3ZIM again showed the highest affinity with a minimum BFE of -62.23 kcal/mol, followed by apigenin 7-glucuronide-3ZIM at -53.01 kcal/mol, and luteolin 7-O-glucoside-3ZIM at -52.05 kcal/mol. Moreover, for the least favourable scenarios (maximum BFE), apigenin 7-glucuronide-3ZIM exhibited the weakest binding with a maximum BFE of -11.09 kcal/mol, followed by luteolin 7-O-glucoside-3ZIM at -11.74 kcal/mol, and luteolin-7-O-glucuronide-3ZIM at -11.28 kcal/mol. The variability in binding affinity across different conditions was reflected in the standard deviation (SD) of BFE values: luteolin 7-O-glucoside-3ZIM showed the least variability (5.68 kcal/mol), followed by apigenin 7-glucuronide-3ZIM (7.81 kcal/mol), and luteolin-7-O-glucuronide-3ZIM (8.93 kcal/mol).Despite having similar average BFE values, all three compounds demonstrated equivalent interaction with the receptor protein (Fig. 5A).

In the binding free energy components analysis for luteolin-7-O-glucuronide with 3ZIM, the van der Waals interactions (ΔVDWAALS) contributed -38.20±4.54 kcal/mol, indicating a significant interaction. Electrostatic interactions (ΔEEL) contributed -35.37±20.67 kcal/mol, also indicating strong interactions but with significant variability. The electrostatic solvation energy (ΔEGB) was 51.00±12.50 kcal/mol, indicating a weaker interaction, while the non-polar solvation energy (ΔESURF) was -5.68±0.46 kcal/mol, indicating a strong interaction. The gas-

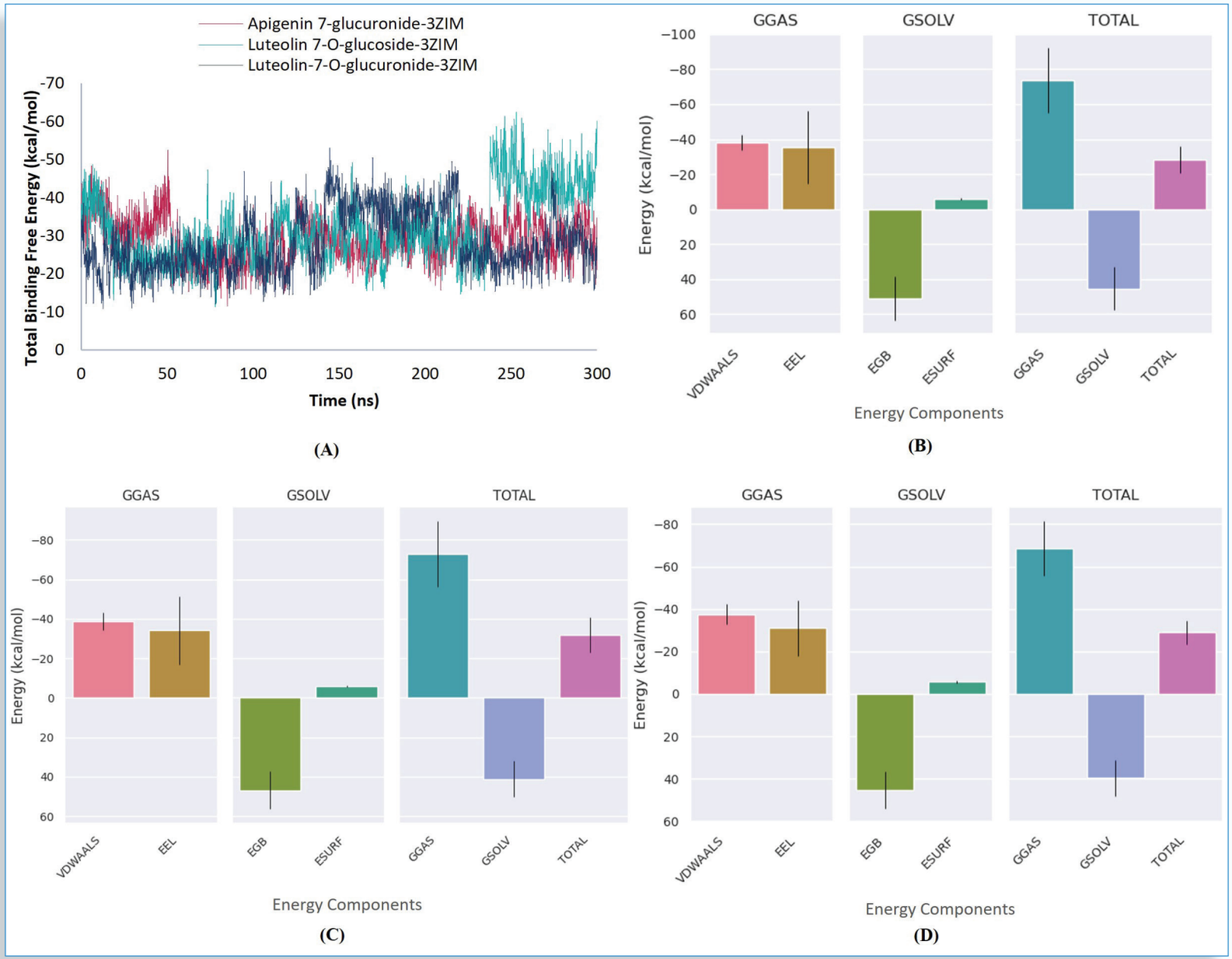


Fig. 5 **(A)** BFE trajectory of Luteolin-7-O-glucuronide -3ZIM, Luteolin 7-O-glucoside-3ZIM, and Apigenin 7-glucuronide-3ZIM complexes. Binding FreeEnergy components of **(B)**Luteolin-7-O-glucuronide-3ZIM **(C)** Luteolin 7-O-glucoside-3ZIM, **(D)** and Apigenin 7-glucuronide-3ZIM

phase interactions (ΔGGAS) were strongly attractive at -73.57±18.64 kcal/mol, while the solvation energy (ΔGSOLV) was weaker at 45.32±12.44 kcal/mol. The total binding free energy (ΔTOTAL) was -28.25±7.81 kcal/mol, indicating a moderately strong binding interaction. (Fig. 5B). Moreover, luteolin 7-O-glucoside-3ZIM showed strong van der Waals interactions at -38.89±4.43 kcal/mol. The ΔEEL was -34.09±17.22 kcal/mol, indicating strong electrostatic interactions with some variability. The ΔEGB was 46.83±9.51 kcal/mol, indicating weaker interactions, while ΔESURF was -5.75±0.47 kcal/mol, indicating a strong interaction. The ΔGGAS was -72.98±16.62 kcal/mol, indicating strong attractive gas-phase interactions, and ΔGSOLV was 41.08±9.28 kcal/mol, indicating weaker interactions. The ΔTOTAL was -31.90±8.92 kcal/mol, indicating a moderately strong binding interaction, slightly more so than luteolin-7-O-glucuronide-3ZIM (Fig. 5C). Furthermore, forapigenin 7-glucuronide-3ZIM, the ΔVDWAALS was -37.49±4.79 kcal/mol, indicating strong van der Waals interactions. The ΔEEL was -31.05±13.03 kcal/mol, suggesting strong electrostatic interactions with variability. The ΔEGB was 45.25±8.68 kcal/mol, indicating weaker interactions, while ΔESURF was -5.63±0.52 kcal/mol, indicating a strong interaction. The ΔGGAS was -68.54±12.82 kcal/mol, indicating strong attractive gas-phase interactions, and ΔGSOLV was 39.62±8.51 kcal/mol, indicating weaker interactions. The ΔTOTAL was -28.92±5.67 kcal/mol, similar to luteolin-7-O-glucuronide-3ZIM, indicating a moderately strong binding interaction (Fig. 5D). Overall, the main contributors to the binding free energy for all 3compounds were van der Waals interactions, electrostatic interactions, and solvation energies. Despite variations in individual components, all compounds exhibited moderately strong overall binding free energies, with luteolin 7-O-glucoside-3ZIM showing slightly stronger binding energy than the others.

The binding free energy was further divided into 3distinct components: ΔG complex, ΔG receptor, and ΔG ligand, where ΔG total encompassed the overall binding free energy. For luteolin-7-O-glucuronide-3ZIM, the ΔG complex (-8499.51± 68.37 kcal/mol) was influenced by the conjugation of the receptor (-8603.64±68.16 kcal/mol) and ligand (132.38±5.85 kcal/mol), resulting in a ΔG Total of -28.25±7.81 kcal/mol that implied a favourable binding interaction. Similarly, luteolin 7-O-glucoside-3ZIM had ΔG values of -8517.15±75.46 kcal/mol (ΔG complex), -8638.08±73.35 kcal/mol (ΔG receptor), 152.82 ± 5.65 kcal/mol (ΔG ligand), and -31.9±8.92 kcal/mol (ΔG total). This compound also showed a favourable binding interaction with a slightly higher stabilization energy compared to luteolin-7-O-glucuronide-3ZIM. For apigenin 7-glucuronide with 3ZIM, ΔG values were -8539.71±68.16 kcal/mol (ΔG complex), -8631.23±67.9 kcal/mol (ΔG receptor), 120.44±4.95 kcal/mol (ΔG ligand), and -28.92±5.67 kcal/mol (ΔG total), indicating

a strong binding interaction (ΔG complex) and a similar stabilization energy to luteolin-7-O-glucuronide-3ZIM (Table 6). Even though the average binding free energy (BFE) values were similar across all 3compounds, indicating comparable overall strength of binding, each compound showed an equivalent level of interaction with the receptor protein 3ZIM.

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

Complex	ΔG Complex	ΔG Receptor	ΔG Ligand	ΔG Total
Luteolin-7-O-glucuronide-3ZIM	-8499.51±68.37	-8603.64±68.16	132.38±5.85	-28.25±7.81
Luteolin 7-O-glucoside-3ZIM	-8517.15±75.46	-8638.08±73.35	152.82±5.65	-31.9±8.92
Apigenin 7-glucuronide-3ZIM	-8539.71±68.16	-8631.23±67.9	120.44±4.95	-28.92±5.67

The findings of present study suggested that energetic favourability of binding may be alike among the compounds, their individual molecular interactions with the receptor were similarly effective in forming stable complexes.A similar prior study on Aberrant class I histone deacetylase (HDAC) activity in cancer emphasized the quest for safer natural inhibitors, with apigenin showing stronger binding to class I HDAC isoforms than luteolin in molecular docking and MMGBSA analyses, both compounds demonstrating stable interactions in molecular dynamics simulations, promising for HDAC-targeted cancer therapy due to the adverse effects of synthetic HDAC inhibitors like atrial fibrillation and QT prolongation (Ganai et al., 2018). Another study employed static and dynamic docking simulations, molecular dynamics (MD) simulations, MMGBSA analysis, clustering, and ADMET filtering to screen 103 phytochemicals derived from *Ocimum gratissimum* against five anti-apoptotic Bcl-2 proteins. This effort identified five lead phytochemicals (ursolic acid, beta-sitosterol, luteolin, basilimoside, and apigenin 7,4',dimethyl ether) demonstrating robust binding affinities and favorable ADMET properties, suggesting their potential as natural inhibitors for cancers characterized by Bcl-2 family protein overexpression (Gyebi et al., 2022).Overall, the current study explored *R. sceleratus* for treating melanoma *via* molecular docking, ADMET analysis, and molecular dynamics simulations, identifying 17 phytochemicals that adhered to Lipinski's rule of five and ADMET criteria. Luteolin-7-O-glucuronide showed the highest binding affinity and stable interaction with the PIK3CA target, while luteolin 7-O-glucoside and apigenin 7-glucuronide also exhibited significant interactions with the 3ZIM receptor.

Conclusion

The study emphasized the unexplored therapeutic potential of *R. sceleratus* in addressing melanoma through molecular docking, ADMET analysis, and molecular dynamics simulations. The research provided valuable insights into how the bioactive components of *R. sceleratus* could alleviate melanoma pathogenesis. Among 75 phytochemicals, rigorous screened based on Lipinski's rule of five and ADMET parameters 17 phytochemicals were collected. These were subjected to molecular docking analysis against protein structures of key genes. In molecular docking analysis,luteolin-7-O-glucuronide represented as the most promising candidate, exhibiting the highest binding affinity and significant interaction stability with the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha target. Both luteolin 7-O-glucoside and apigenin 7-glucuronide showed significant interactions with the 3ZIM receptor, although luteolin 7-O-glucoside exhibited a higher binding affinity. The molecular dynamics simulations and binding free energy analysis highlighted that luteolin-7-O-glucuronide, luteolin 7-O-glucoside, and apigenin 7-glucuronide form stable and effective interactions with the 3ZIM receptor. Despite minor differences in stability and flexibility, each compound established equivalent interaction strength and stability, suggesting their potential as effective inhibitors for the PIK3CA. These findings provide valuable insights for further exploration and optimization of these compounds in therapeutic applications.

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CHAPTER

07

Reported Pharmacological Profile

INTRODUCTION

Ranunculus sceleratus L. (Ranunculaceae) is found throughout the world, although it is particularly prevalent in sluggish streams, ditches, and shallow, muddy-bottomed ponds with mineral-rich water. When the plant is new, all of its components are dangerous; however, the poisons are removed when it is cooked or dried. In the first century, Dioscorides referred to the striking effects of this species, writing that when consumed by humans, it provoked a characteristic effect on the muscles of the mouth, causing the lips to be pulled out and up over the teeth into a grimace defined as a mixture of smile and snarl (Font-Quer, 1982). Secondary metabolites found in *R. sceleratus* include flavonoids, steroids including ß-sitosterol, and γ-lactone glycosides, particularly ranunculin (Saber et al., 1968). *R. sceleratus* includes a variety of bioactive components, including protoanemonin, ranunculin, saponins, tannins, flavonoids, alkaloids, and essential oils, which contribute to its therapeutic qualities. This species has an extensive pharmacological history, including traditional use in skin care, respiratory health, and possess antibacterial property. Its bioactive components have the potential for anti-inflammatory and cytotoxic actions; however, caution is advised owing to its toxicity if not handled properly. As a result, this chapter summarizes current research on the biological activities of extracts and isolated compounds from this species.

Pre-clinical Study

Pre-clinical studies on *R. sceleratus* focus on its pharmacological characteristics and possible therapeutic use in laboratory settings before progressing to human clinical trials. These studies give useful information on its modes of action, safety profile, and effectiveness. Animal models are utilized to determine the acute and chronic toxicity of plant extracts. This involves looking for indicators of toxicity, evaluating organ function, and finding the highest tolerable dosage. Animal models of various diseases or conditions (for example, inflammatory disorders, infections, and cancer) are used to evaluate the therapeutic effectiveness of plant extracts. The disease progression, biomarker levels, histological alterations, and survival rates are all assessed. Pre-clinical research also assess plant's underlying mechanisms of action.

Anti-adipogenic Activity

Kim et al. (2022) investigated the anti-adipogenic effect of ethanol extract (50, 100 and 200 µg/ml) of *R. sceleratus* on adipogenesis in 3T3-L1 preadipocytes. The extract did not show any significant effect on viability after 24, 48, and 72 hrs. The treatment with extract reduced the amount of lipid droplets in 3T3-L1 preadipocytes by 27.83, 32.77, and 43.45%. In addition, the expression levels of key adipogenic transcription factors, such as CCAAT/enhancer-binding proteins-α (C/EBP-α) and peroxisome proliferator-activated receptors-γ (PPAR-γ) were also reduced by the extract. Further, the extract increased AMP-activated kinase (AMPK) phosphorylation and, decreased sterol regulatory element-binding protein-1 expression.

Anti-bacterial Activity

Shahid et al. (2015) evaluated the anti-bacterial activity of n-hexane, ethyl acetate, chloroform, n-butanol and aqueous soluble fractions of *R. sceleratus* against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa* by 96-wells microplate assay. Among tested fractions, the aqueous soluble fraction showed highest anti-bacterial effect with MIC of 5.3 µg/well, followed by n-butanol (5.6 µg/well), chloroform (7.1 µg/well), ethyl acetate (7.8 µg/well) and n-hexane soluble fractions (34.3%), but it was less active than ciprofloxacin (10.0 µg/well; 92.3%) used as standard.

Anti-dermatophytic Activity

Sharma et al. (2012) investigated the anti-dermatophytic activity of chloroform, methanol and water extracts (100 to 0.156 mg/ml) of *R. sceleratus* leaves against strains of dermatophytes *viz*., *Trichophyton rubrum, T. mentagrophytes, T. tonsurans, Microsporum gypseum* and *M. fulvum* by agar well diffusion method. The chloroform extract showed highest MIC of 1.25 to 2.50 mg/ml against *T. mentagrophytes* and *M. fulvum*, followed by *T. rubrum, T. tonsurans* and *M. gypseum* (2.5 to 5 mg/ml). Moreover, methanol extract exhibit MIC values in between 2.5 to 10 mg/ml, while water extract showed >10.0 mg/ml, against all tested pathogens.

Anti-inflammatory Activity

Marrelli et al. (2021) demonstrated the anti-inflammatory potential of ethanol extracts (70%) of aerial parts and roots of *R. sceleratus* against LPS-stimulated RAW 264.7 macrophage cell line. The aerial parts and root extracts suppressed nitric oxide production in LPS-stimulated RAW 264.7 cell line in a concentration-dependent manner, with IC_{50} values of 22.08 and 115.10 µg/ml, which was lower than indomethacin (53.00 µg/ml) and L-NAME (45.86 µg/ml), used as positive controls.

Antioxidant Activity

Neag et al. (2017) assessed the antioxidant activity of hydroalcoholic and glycerol-ethanol extracts of *R. sceleratus* using DPPH, TEAC

(trolox equivalent antioxidant capacity), FRAP, CUPRAC (cupric reducing antioxidant capacity) and SNP (silver nanoparticle) assays. The hydroalcoholic extract showed highest DPPH and trolox equivalent effects with IC_{50} values of 872.1 and 186.7 µl, followed by glycerol-ethanol extract with 988.4 and 250.7 µl, respectively. Moreover, the hydroalcoholic extract also exhibited higher FRAP, CUPRAC and SNP effects of 103, 61 and 297 µM ET/100 ml extract, respectively, than glycerol-ethanol extract with 60, 49 and 161 µM ET/100 ml extract, respectively.

Shahid et al. (2015) investigated the antioxidant activity of n-hexane (1000, 500, 250 and 120 µg/ml), ethyl acetate (30, 15 and 8 µg/ml), chloroform (500, 250 and 120 µg/ml), n-butanol (60, 30 and 15 µg/ml) and aqueous soluble fractions (1000, 500, 250 and 120 µg/ml) of *R. sceleratus* using FRAP, DPPH, ferric thiocyanate (FTC) and total antioxidant assays. Ascorbic acid (150, 60 and 30 µg/ml) and butylated hydroxytoluene (BHT) were used as positive controls. The n-hexane fraction showed highest percentage inhibition of 84.6% at 1000 µg/ml in DPPH assay, followed by ethyl acetate (80.9 at 30 µg/ml), n-butanol (80.8% at 60 µg/ml), chloroform (77.9 at 500 µg/ml) and aqueous soluble fractions (77.1% at 1000 µg/ml) when compared with ascorbic acid (79.4% at 150 µg/ml). Among tested fractions, n-butanol fraction showed good effect with IC_{50} of 44.1 µg/ml, when compared with ascorbic acid (58.9 µg/ml). Also, the ethyl acetate soluble fraction exerts highest FRAP value of 238.5±1.1 TE µM, followed by chloroform (158.0 µg/ml), n-butanol (148.0 µg/ml) and aqueous fractions (46.0 µg/ml), when compared with ascorbic acid and BHT. In ferric thiocyanate assay, the ethyl acetate and n-butanol soluble fractions attained highest inhibition of 53.7 and 48.0% lower than BHT (62.5%). Meanwhile, the n-hexane, chloroform and aqueous fractions showed weak inhibitions (11.0, 21.6 and 18.2%). Ethyl acetate and n-butanol soluble fractions showed highest total antioxidant activity 1.0 when compared to other fractions as chloroform (0.8), n-hexane (0.6) and aqueous (0.6) and BHT (0.8) µg/ml, respectively.

Anti-viral Activity

Li et al. (2005) reported the anti-viral activity of isoscopoletin and protocatechuic aldehyde (100, 20 and 4 µg/ml) isolated from *R. sceleratus* against hepatitis B virus (HBV), herpes simplex virus type-1 (HSV-1), HBsAg and HBeAg secretion from HepG2.2.15 cells. Acyclovir and lamivudine (100, 20 and 4 µg/ml) were used as positive controls. Apigenin 4'-O-alpha-rhamnopyranoside, apigenin 7-O-beta-glucopyranosyl-4'-O-alpha-rhamnopyranoside, tricin 7-O-beta-glucopyranoside, tricin, and isoscopoletin exerted inhibitory effect towards HBV replication. Isoscopoletin showed a significant ($p<0.05$) inhibitory effect on HBsAg and HBeAg secretion from HepG2.2.15 cells. Further, protocatechuic aldehyde exhibited anti-viral effect with IC_{50} value of 17.34 µg/ml for HSV-1, but it was less active than acyclovir (1.5 µg/ml). However, protocatechuic aldehyde attained CC_{50} of >200 µg/ml.

Cytotoxicity

Schinella et al. (2002) evaluated the cytotoxicity of methanolic extract (250 µg/ml) of aerial parts of *R. sceleratus* on rat polymorphonuclear cells using LDH and MTT assays. Chlorpromazine (200 µM) was used as positive control. The extract showed LDH value of 18.7 units/l, while percentage of cell viability was 108% in MTT assay but was less potent than chlorpromazine with 40.3 units/l and 69%, respectively for polymorphonuclear rat cells.

Enzyme Inhibitory Activity

Shahid et al. (2015) investigated the enzyme inhibitory activity of n-hexane, ethyl acetate, chloroform, n-butanol and aqueous soluble fractions (0.1 mg/ml) of *R. sceleratus* against α-glucosidase, butyrylcholinesterase, acetylcholinesterase and lipoxygenase enzymes. Quercetin, baicalein and eserine (0.5 mM/well) were used as positive controls. The n-butanol fraction showed highest percentage inhibition of 77.5%, with IC_{50} value of 35.7 µg/ml for α-glucosidase. However, the ethyl acetate fraction exhibit inhibitory effect of 38.6% for acetylcholinesterase. On the other hand, the ethyl acetate fraction showed 72.2% inhibition in lipoxygenase assay which was less active than that of quercetin (16.5 µg/ml; α-glucosidase) and baicalein (22.7 µg/ml; LOX). Also, the chloroform fraction attained effectiveness of 31.4 µg/ml, followed by ethyl acetate fraction (35.4 µg/ml) which was less active than eserine (0.9 µg/ml) towards BchE assay. Lastly, the other extracts exert inhibition between 15.5 to 32.1% for α-glucosidase, 9.5 to 67.5% for BchE, 5.1 to 23.5% for AchE and, 14.1 to 68.1% for LOX.

Hepatoprotective Activity

Zhang et al. (2020) demonstrated the hepatoprotective effect of TianJiu (TJ) therapy using fresh *R. sceleratus* (RS) on α-naphthyl isothiocyanate-induced intrahepatic cholestasis in Sprague-Dawley rats. The TJ treatment with fresh RS significantly ($p<0.01$) decreased the levels of serum aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TBIL), direct bilirubin (DBIL), total bile acid (TBA), as well as hepatic malondialdehyde and nitric monoxide levels at 108 h. The hepatic body ratio, bile flow and hepatic pathological changes of cholestatic rats at 108 h in TJ group were restored as compared with model group.

Trypanocidal Activity

Schinella et al. (2002) studied the trypanocidal activity of methanolic extract (250 µg/ml) of aerial parts of *R. sceleratus* against *Trypanosoma cruzi.* The extract showed highest percentage inhibition of 97% with IC_{50} value of 10.7 µg/ml when compared with allopurinol used as positive control (78%).

Summary and Future Scope

R. sceleratus, often known as "celery-leaved buttercup" or "cursed buttercup", is a plant with possible therapeutic benefits. This species has long been utilized in traditional medicine for a variety of purposes, including its analgesic, anti-inflammatory, and antibacterial qualities. The plant includes bioactive chemicals including alkaloids, saponins, flavonoids, and terpenoids, all of which contribute to its pharmacological properties. According to studies, the extracts of *R. sceleratus* may have analgesic effects, making them a promising alternative as a pain relief. Also, experiments suggest that plant extracts demonstrated anti-inflammatory properties, indicating a potential role in the treatment of inflammatory disorders. Some study reveals that *R. sceleratus* extracts exhibit antibacterial activity against specific bacteria and fungi, indicating that they might be used as an antimicrobial agent. Despite its medicinal promise, *R. sceleratus* is poisonous, especially when fresh, which is hazardous if not properly processed. Therefore, future research should focus on isolating and identifying the precise bioactive chemicals responsible for the plant's pharmacological effects in order to better understand its mechanisms of action. Clinical investigations evaluating the safety and efficacy of plant extracts or purified substances in humans might give important insights into their medicinal potential. Creating safe and standardized formulations, such as topical creams or oral supplements, may improve the plant's utility in healthcare. Strategies for reducing plant toxicity while keeping its therapeutic characteristics intact should be investigated to guarantee its safe usage in medicine. Additionally, investigating possible synergies with other herbal extracts or conventional drugs may result in combination treatments with better therapeutic effects. Addressing regulatory hurdles and developing standards for the cultivation, processing, and distribution of plant-based products is critical to their inclusion into mainstream healthcare. Overall, while plant shows promise in pharmacology, more extensive study, safety evaluations, and regulatory frameworks are required to fully realise its medical potential.

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