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Research and Reviews in Plant Science
Volume IV

Editors:
Dr. Devangi Chachad
Dr. Shakun Mishra
Dr. Parinitha Mahishi
Dr. Alok Ranjan Sahu

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Editors

Dr. Devangi Chachad

Department of Botany,

Jai Hind College (Empowered Autonomous),

Churchgate, Mumbai

Dr. Shakun Mishra

P. G. Department of Botany,

Govt. Shri Nilkantheshwar PG College,

Khandwa, M. P.

Dr. Parinitha Mahishi

Department of Botany,

NMKRV College for Women,

Bengaluru, Karnataka

Dr. Alok Ranjan Sahu

Department of Botany,

Vikash Degree College,

Bargarh, Odisha

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PREFACE

In the vast landscape of scientific inquiry, plant science stands as a cornerstone of our understanding of life on Earth. From the intricacies of photosynthesis to the complexities of plant-microbe interactions, the field encompasses a rich tapestry of research that continues to unravel the mysteries of the botanical world.

This inaugural volume of Research and Reviews in Plant Science represents a dedication to the pursuit of knowledge and innovation within the realm of plant biology. Our aim with this publication is to provide a platform for the dissemination of cutting-edge research, insightful reviews, and thought-provoking perspectives that advance our understanding of plants and their significance to the broader ecosystem.

The diverse array of topics covered within this volume reflects the breadth and depth of contemporary plant science. From the molecular mechanisms underlying plant development to the ecological dynamics shaping plant communities, each contribution offers a unique lens through which to explore the wonders of the botanical realm.

As editors, we are deeply grateful to the authors whose scholarly endeavors have enriched this volume with their expertise and dedication. Their commitment to advancing the frontiers of plant science is evident in the quality and rigor of their work, and we commend them for their contributions to the field.

We also extend our appreciation to the reviewers whose thoughtful feedback and constructive criticism have helped to ensure the integrity and excellence of the manuscripts presented herein. Their expertise and insights have been invaluable in shaping the content of this volume and maintaining the highest standards of scholarly inquiry.

As we embark on this journey of exploration and discovery, we invite you to join us in celebrating the marvels of plant science and the boundless opportunities it presents for understanding and stewarding the natural world.

Editors

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A REVIEW ON EFFECT OF GLOBAL CLIMATE ON ORCHIDS AND ITS CONSERVATION STRATEGIES

Samart Dutta and Anjalika Roy*
Department of Botany (UGC DRS (SAP-II) DST-FIST),
Visva-Bharati, Santiniketan, West Bengal
*Corresponding author E-mail: anjalika.roy@visva-bharati.ac.in

Abstract:

The Orchids are horticultural important with attractive flowers endangered plants. Some plants have medicinal value displaying diverse life forms maintaining ecological balance in nature. Orchids, the most highly evolved family among monocotyledons with near about 1,000 genera and 25,000-35,0000 species with predominance distribution in Northeast India. Increasing atmospheric temperatures may result in vegetational zones of orchids gradually moving vertically up mountainsides with phonological shifting according temperature and moisture. Different plan and strategies have been adopted for orchid conservation like synthetic seed *in situ* conservation by medium term storage, exsitu conservation by living collection OSSU seed bank etc. The orchid seed banking has been shown to be an invaluable tool for conserving the maximum amount of genetic diversity in the minimum space and has the potential to enable the conservation of valuable material for possible reintroduction and habitat restoration programmes in the future.

Keywords: Orchids, Global Warming, Seed Bank, Conservation

Introduction:

The family Orchidaceae is probably the largest among all angiosperms, with an estimated 28,000 species (Willis, 2017, WFO 2022). Orchids are well known for the great diversity displayed in their life forms and growth habits, in the contrivances of pollination and ecological adaptabilities. They have a worldwide distribution, occurring in different ecological habitat as saprophytes, terrestrial or epiphytes. According to Atwood (1986) more than 70% of Orchid species are epiphytic that mostly inhabit in the tropics. In India alone about 1100 species have been found that are distributed in 163 genera (Hegde, 1996). Among them more than 300 species are endemic. The major orchid rich phytogeographical regions of India are Peninsular India, Northeastern India and the Eastern and Western Himalayas. The flowers of orchids have always fascinated botanists and horticulturists for its various shapes, sizes and colors. However Benzing (1986) emphasized that the floral structure of orchids is rather stereotyped in so far as the number and organization of floral part are concerned, but the real diversity is to be found in the size, shape and other structural details.

Due to their attractive flowers the orchids comprise a major group of horticultural crops (Zhang, 2018). Apart from this ornamental value, a large number of species are also medicinally important. The essence of vanilla which is obtained from seed capsule of climbing orchid *Vanilla* is also well known. However, in spite of their manifold utility, for a long time the cultivation of orchids for commercial purposes was considered very difficult, since most orchids show extremely slow rate of vegetative multiplication. The breakthrough in orchid propagation came many years ago, when first Noel Bernard (1904, 1906, 1909) and later Lewis Knudson (1921) demonstrated that orchid seeds could be germinated in large numbers in artificial culture conditions.

Occurrence and distribution

Traditional knowledge has been used for centuries by indigenous and local communities in their culture and health care. It is an important factor for sustainability of natural genetic resource management. Orchids, the most highly evolved family among monocotyledons with near about 1,000 genera and 25,000-35,0000 species exhibit an incredible range of diversity in size, shape and colour of their flowers (Willis, 2017, WFO 2022). India is considered as a rich orchid heritage and recognized as a significant producer of wild orchids in the world. It is estimated that near about 1,600 species of orchids are found in India which constitutes almost 10% of the world orchid flora with Himalayas as their main home. Among India, the Northeastern region (located between 87°32'E to 97°52'E latitude and 21° 34' N to 29°50'N latitude), comprised of the 8 states, i.e. Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, and Tripura is considered as the most important biodiversity hot spot of the Indian subcontinent. High humidity and low temperature accompanied by good rainfall makes entire Northeastern region of the country a hot spot of orchids also. This region has about 876 orchid species in 151 genera which constitutes nearly 70% of total orchid flora of our country (Table 1). A large number of ornamentals, rare, endangered and threatened orchid species are available in this region. Some promising ornamental orchids of the region are- *Paphiopedilum fairieanum*, *Paphiopedilum insigne*, *Paphiopedilum villosum*, *Paphiopedilum spicerianum*, *Paphiopedilum hirsutissimum*, *Paphiopedilum venustum*, *Anoectochilus sikkimensis*, *Vanda coerulea*, *Vanda teres*, *Renanthera imschootiana*, *Rhynchostylis retusa*, *Pleione maculata*, *Pleione praecox*, *Pleione humilis*, *Cymbidium eburneum*, *Dendrobium hookerianum*, *Dendrobium densiflorum*, *Dendrobium devonianum*, *Dendrobium thrysiflorum* and *Thunia marshalliana*. Many of these species utilized for the production of modern commercial hybrids which play a significant role in the international floriculture trade as cut flowers. Among the Northeastern states, Arunachal Pradesh and Sikkim have the largest number of orchid species and recognized as the paradise of orchids. The rich orchid diversity in this region has provided an initial advantage to its inhabitants for observing and scrutinizing the orchid flora for

developing their own traditional knowledge for importance of valuable orchid diversity and their conservation. The important characters of traditional knowledge systems are-effective conservation of biodiversity through cooperation and collective action of the people, intergenerational transmission of knowledge, skills and strategies concern for the well being of the future generations, reliance on the local resources, restraint in resource exploitation, respect and gratitude for their nature mother, management, conservation and sustainable use of biodiversity and ultimately transfer of useful species among the households, villages and larger area. The people of Northeastern region maintain this traditional knowledge system with great care and attention. In India, of the total 427 tribal communities reported, more than 130 major tribal communities live in the Northeastern region. The major tribal communities of the Northeastern region have been categorized into sub-tribes and if these sub-tribes are taken into consideration the total number of tribal groups reach up to 300. In general, the tribes of Northeastern India have been categorized into two broad ethnic communities, such as the Khasi and the Jaintia tribes of Meghalaya, who belong to Monkhemar culture of Austoic dialect, and the rest of the tribal groups are basically Mongoloid, who belongs to Tibeto-Burman subfamily of Tibeto-Chinese group. Tribal communities are mainly the forest dwellers who have accumulated a rich knowledge on the uses of various forests and forest products over the centuries. Their dependence on nature had developed knowledge which ultimately is reflected in their traditional culture, religion, belief, folklore.

Table 1: Orchid distribution under forest cover in North East (Kataki *et al.* 1984)

State	**Area**	**Dense Forest**	**% Forest Cover**	**Orchid**	
	000 km2			Genera	Species
Arunachal Pradesh	83,743	54,542	65.13	130	600
Assam	78,438	15,842	20.19	74	182
Manipur	22,327	5,309	23.77	67	207
Meghalaya	22,429	3,305	14.73	98	352
Mizoram	21,081	4,279	20.29	74	249
Nagaland	16,579	3,531	21.29	64	241
Sikkim	7,096	2,403	38.86	132	540
Tripura	10,488	1,825	17.40	37	66

Effect of global warming on ecosystem

The world will be a very different place by the end of this century, if climate continue to change due to global warming occur on the scale that has been predicted by many experts. According to Ramanathan & Feng, 2008, increase in the concentration of greenhouse gases since

the pre-industrial era has probably committed the world to a warming of 2.4°C. The climate change interacts with habitat loss and fragmentation of introduced and invasive species and population growth therefore ecosystems are likely to undergo radical modification. It is generally accepted in the scientific community that global climate change due to global warming is already taking place and this is due to increased greenhouse gas emissions resulting from human activities. According to a report of the Intergovernmental Panel on Climate Change (IPCC, 2007), "Warming of the climate system is unequivocal ..." and "... many natural systems are being affected by regional climate change, particularly temperature increases." In Asia climate change is, "projected to compound the pressures on natural resources and the environment associated with rapid urbanization, industrialization and economic development." In Latin America there is, "a risk of significant biodiversity loss through species extinction in many areas" and "by mid-century increase in temperature and associated decreases in soil water are expected to lead to gradual replacement of tropical forests in eastern Amazonia. Semi-arid vegetation will tend to be replaced by arid land vegetation" (Solomon *et al.,* 2009). So the outlook for the future of ecosystem is disturbing and alarming.

Effects of global climate change on orchid populations

Orchidaceae is one of the largest and most diverse families of flowering plants, with more than 28,000 accepted species spanning 763 genera (Christenhusz and Byng, 2016).Long-term monitoring programs and population demographic models have shown that the population dynamics of orchids are to a large extent dependent on prevailing weather conditions, suggesting that the changes in climatic conditions can have far reaching effects on the population dynamics and hence the distribution of orchids (Evans *et al.*,2020).The potential effects of climate change on orchids are difficult to predict and some ecosystems are likely to be more vulnerable to climate change than others. There is evidence that vegetational zoning on tropical mountains is strongly controlled by temperature (Primack & Corlett, 2011). Increasing atmospheric temperatures may result in vegetational zones gradually moving vertically up mountainsides, both permitting lowland species to migrate upwards and eliminating species in the highest zones (Foster, 2001). Orchid populations on or close to the tops of limestone mountains in the Yachang Reserve in Guangxi, China, may be similarly vulnerable to climatic warming (Liu *et al.,* 2014). Not only may orchids in forest canopies be exceptionally sensitive to desiccation (Benzing, 2004), they may also be affected indirectly. Orchids are one component in a complex web of interactions with other epiphytes in the canopy which in turn may be affected in different ways by changes in the availability of light, nutrients and moisture. Neither do we know whether any changes in plant phenology associated with changes in temperature and precipitation will be synchronous with phonological shifts of orchid pollinators (Liu *et al.,* 2014). Resource needs of pollinators can be subtle and complex (Vereecken *et al.,* 2010). Climate change is a major threat

to pollination services and networks and there is a need to conserve the plant communities in which orchids live and survive (Pemberton, 2010). Numbers of European terrestrial orchids have continued to decline dramatically over the past 30 years or more due to a combination of factors, including habitat loss and fragmentation (Seaton, *et al.,* 2010, Wraith and Pickering, 2019, Phillips *et al.,* 2020). However, in the UK, *Himantoglossum hircinum* has been increasing. It has been suggested that the increase in *H. hircinum* may be due to climate change (Kull *et al.,* 2006). Neither should we forget the potential effects of climate change on the orchids' fungal partners. As just one example of the level of complexity that may occur between orchids and other members of its environment, the underground orchid *Rhizanthella gardneri* of Western Australia has an obligate mycorrhizal relationship with *Melaleuca uncinata*, a fungus and a termite as specialist pollinators and a specialist marsupial dispersal agent of their berrylike indehiscent seeds. The orchids long-term survival is threatened by droughts that are occurring because of climate change that are causing the demise of the *Melaleuca* sp. (Swarts *et al.,* 2009). Not all orchids will react to global warming in the same way, and some may be better adapted to be able to cope with future changes. A comparison of species belonging to the closely related genera *Paphiopedilum* sp. and *Cypripedium* sp. in China reveals that they have different physiological adaptations and survival strategies. The evergreen *Paphiopedilum* are adapted to lower resource environments, with a lower rate of photosynthesis and growth, whereas the deciduous *Cypripedium* species have higher rates of photosynthesis and show more rapid growth during the active period.

Plan and strategies for orchid conservation

Has the conservation of orchids progressed until recent years after the first International Orchid Conservation Congress in 2001? Certainly, orchid science has grown from less than 100 published works from 1900-1920 period to almost 3200 publication up to period of 2005. With knowledge of orchids spanning an astonishing array of disciplines it is therefore surprising that there remains a significant gap between orchid science and orchid conservation practice. This is no more telling than in the resolutions of the plenary session of the first IOCC to adopt for orchids four targets out of the 16 targets set for Plant Conservation from the Global Strategy (http://www.bgci.org/worldwide/gspc/) – that by 2010. Since than 90% of threatened orchids are secure in ex situ conservation collections; 50% of these threatened orchids are in active recovery programs; no orchids are threatened by unsustainable harvesting; and, every child aware of plant diversity (including orchids). How have we tracked on delivering these four targets? Besides a growth in botanic gardens to almost 2500 worldwide, the proportion of orchids in ex situ conservation collections, particularly rare and threatened taxa, has barely changed in past years. The need of science and technology to achieve this target has to well established yet. Equally the pace of the development of orchid recovery plans is outstripped by the annual increase in orchid

taxa being listed as rare. Indeed the most basic of information is often lacking in orchid conservation projects from knowledge of the causal factors of orchid rarity to whether research outcomes and management plans are being converted to successes in the field? An important criterion for recovery projects should be 'will it be possible to implement the results of the research – is the funding available and what are my cost-effective alternatives?' Some areas such as defining sustainability for the wild harvesting of orchids remains a complex and difficult issue often tied to local economy and cultural identity. The slow maturation of orchids means that any wild exploitation, unless carefully managed on scientific grounds, is bound to lead to a decline in the orchid. Finally, though the final target falls outside of the expertise of conservation scientists is in the long term, it is perhaps the most critical of all conservation actions for the long term conservation of orchids. It is much easier/ preferable/more fun to do research, write and field trip than to ensure that k-12 educational needs include sound conservation messages. Ultimately our ability to deliver effective conservation is controlled by funding bound to political processes that in themselves rely on awareness and education from an early age.

With the long term goal of greater community awareness and funding of conservation, as researchers we can maximize our conservation outcomes in the short term through the approach we take to research and the questions we ask. When attempting to conserve a particular species, establishing which aspect of the life cycle or human interaction is driving species rarity is a critical step. A key to success in orchid reintroduction is the need for integration of knowledge gaps –how many orchid reintroductions adopt a multidisciplinary approach? The majority of published works in orchid introductions rely on single principles as the basis for the reintroduction, often with a heavy emphasis on propagation/establishment techniques. However, pollination biology, mycorrhizal ecology, habitat requirements, changing habitat condition, habitat clearing and wild collecting are all potential causes of rarity that need to be considered for developing a multi-disciplinary and more sustainable conservation outcome for orchids. An obvious division within orchid conservation biology is between the terrestrial and epiphytic life forms and the practical implications for conservation programs.

Seed culture and Synthetic seed production

Currently, the main method used by orchid breeders to germinate orchid seeds is the asymbiotic method. In this method, seeds are cultured aseptically on a nutrient medium supplemented with s simple carbon source like sucrose. This asymbiotic germination of orchid seeds has been successful for many species. During seed germination the embryo first forms tuberose protocorm PLBs, from which the complete plant develops. Development of artificial seed production technology is currently considered as an effective and alternate method of propagation in several commercially important agronomic and horticultural crops. It has been suggested as a powerful tool for mass propagation of elite plant species with high commercial

value (Saiprasad, 2008). It is the most effective technique for the propagation of plant species that have problems in seed propagation and plants that produce non-viable seeds (Daud et al.,2008). Currently, systems of artificial seed production have progressed substantially, the most advanced being in seeding under *ex vitro* or field conditions, obtaining high percentages of conversion to plants (Nieves *et al.,* 2003). As the orchid industry is reliant on micropropagation as a major source of planting material, orchid synthetic seeds are indispensable as they could be delivered easily like true seeds from commercial tissue culture laboratories to growers(Hew *et al.*,1997). Synthetic seeds of orchids are produced by encapsulation of Protocorm Like Bodies (PLBs) in an alginate matrix. This system serves as a low-cost, high-volume propagation system (Saiprasad *et al.,* 2003). Developing a synthetic seed system by encapsulating protocorms for orchids can obviate the routine high cost propagation. Since, encapsulated PLBs can be directly sown in soil bypassing *in vitro* steps, synthetic seed system can revolutionize propagation and transportation of orchid germplasm (Singh, 2006). If encapsulated PLBs can be stored for a long duration and at different temperatures, it will greatly enhance the efficiency of micropropagation by this system. Storing suggests a new means of cryopreservation (Surenciski *et al*, 2007). Until now, synthetic seed production by encapsulating PLBs has been achieved in only a few orchids like artificial seeds in *Dendrobium densiflorum*, as a measure of *in vitro* conservation (Mohanty *et al.,* 2013).

An *Ex-situ* action plan

Although *ex situ* conservation techniques should not be viewed as a substitute for effective *in situ* programmes, considering the likely increase in risks to *in situ* populations and the fact that conservation through reserves alone is unlikely to be able to provide adequate protection to many orchid species, *ex situ* conservation and the storage of germplasm clearly has a role to play and is an indispensable component in the conservation tool box (Cribb *et al.,* 2003, Swarts *et al.,* 2009). The Gran Canaria Declaration II on Climate Change and Plant Conservation in 2006 (http://www.bgci.org/ourwork/gcdccpc/) asserts that, "*ex situ* collections have a key role to play in securing the conservation of wild plant species as natural resources, as an insurance policy for the future, as a basis for restoration and reintroduction programs and as support for adaptation of livelihoods to climate change and shifting climate zones".

The scientific orchid community produced a Conservation Action Plan more than many years ago (Hágsater *et al.,* 1996) which recognized the value of *ex situ* conservation techniques. The important role that *ex situ* conservation techniques are likely to have in the future has also been recognized in Target 8 of the Global Strategy for Plant Conservation (GSPC) which was unanimously agreed by all the parties to the UN Convention on Biological Diversity (CBD) in 2002 (www.bcgi.org/worldwide/gspc/). This is to have 60% of threatened plant species in accessible *ex situ* collections (preferably in the country of origin) by 2010, with 10% included

in recovery and restoration programs. At the first International Orchid Conservation Congress (IOCC) the orchid community agreed that by 2010 90% of threatened orchids should be held in secure *ex situ* collections, with 50% of these in active recovery programs (Dixon *et al.*, 2007). So far as we are aware, it is unlikely that this target will be reached globally, although The Orchid Seed Bank Challenge in Western Australia has achieved seed storage and mycorrhizal selection for three quarters of the 408 native terrestrial species in the southwestern Australian biodiversity hotspot (Swarts *et al.*, 2009) and the global Darwin Initiative project Orchid Seed Stores for Sustainable Use (OSSSU) already exceeded its initial 3 year target of 240 species by a considerable margin (Seaton *et al.*, unpublished data) and a new interim target of 1,000 species has been set.

Seed banking and OSSSU

More than 20 years ago, at the 1984 World Orchid Conference (WOC) held in Miami, it was proposed that the orchid community should begin banking orchid seed as an insurance against possible losses of species from their habitats in the wild (Anonymous, 1985). However, despite Knudson's report of 30 years earlier (Knudson, 1954) that dry seeds of at least some orchid species could be stored for at least 20 years at refrigerator temperatures, there remained a need for further data. Detailed research over the last 20 years has revealed that, although some orchid seeds are relatively short-lived, the benefits of seed drying are quantifiably similar to those of crops seeds and the principle of drying is as pertinent and valuable for orchids as for many other non-orchid species. Thus it is likely that the vast majority of species belonging to the Orchidaceae are capable of tolerating dry storage, probably for many decades when stored at −20°C (Seaton *et al.*, 2003). Furthermore, because orchid seeds are small in size (0.05–6 mm) and weight (0.31–24 μg) (Roberts *et al.*, 2008) large numbers can easily be stored in a small volume, making them ideal subjects for seed banking without the need for large facilities. Indeed, a domestic freezer will have sufficient capacity to store seed representing the orchid flora of an individual country. The Darwin Initiative project, 'Orchid Seed Stores for Sustainable Use' (OSSSU) is funded by DEFRA (the UK Government's Department for Environment, Food and Rural Affairs). The aim of the Darwin Initiative (http://darwin.defra.gov.uk/) is to fund joint projects between UK institutions and partner institutions which are rich in biological resources and would benefit from some additional financial input and UK expertise. OSSSU is a 3 year partnership between the Royal Botanic Gardens, Kew's Seed Conservation Department at Wakehurst Place and partner countries located initially in Asia and Latin America. The aim is to establish a global network of orchid seed banks, with an initial focus on countries with high orchid biodiversity in Asia and Latin America (Seaton *et al.*, 2008).

Living collections

One could argue that an orchid species cannot be said to be safe unless it has been taken into cultivation. However, although some orchids in living collections in botanical gardens may be long-lived, some dating as far back as the nineteenth century, the majority are not. Plants growing in living collections are an important resource for educational and research purposes in botanical gardens, but cultivation specifically for conservation is demanding and only rarely practised effectively (Ramsay *et al.,* 2003). A potential problem with living collections that needs to be addressed is the restricted size of the gene pool of most species in cultivation. Botanical gardens (as well as amateur/hobbyist growers) tend to have either one specimen, or a very small number of clones of any one species. There is a tendency to cultivate the so-called "best" forms. From the perspective of conservation, it would be more useful to grow large populations of at least some species, thereby providing a better representation of the wider gene pool and an insight into within species diversity as well as the diversity of orchid species.

More recently, the need to involve the wider orchid growing community has been recognised. Members of orchid societies have the potential to make an important contribution to orchid conservation. They are often a reservoir of largely untapped expertise which needs to be passed on to each generation. This knowledge base is vital to ensure that plants survive in cultivation. Collections are a dynamic resource. Even within the best maintained collections plants have a limited lifespan being subject to ageing, disease and accidental death. The assertion that, "A very small or very local population is ... set up for near-instant demise from any storm, flood, wildfire, drought or other natural disaster that comes along" (Wilson, 2002) could equally apply to an individual orchid collection. In the same way that small wild populations are vulnerable, so are living collections, plants may be lost due to heating failures in temperate climates (or indeed cooling in tropical climates), pests or disease, or poor culture. Thus there is a need to continually propagate species from seed within living collections. In order to minimise such losses there is a strong case to be made for increasing support for botanical gardens in the countries of origin and within each country to establish botanical gardens at different altitudes to accommodate the differing cultural requirements of individual species and there is no need for energy inputs for either cooling or heating. There is an opportunity for botanical gardens to co-ordinate their activities through organisations such as Botanical Gardens Conservation International (BGCI) and to begin to exchange information about what plants reside in their collections, and to exchange pollen of endangered species.

Conclusion:

A range of complimentary conservation strategies have been discussed. The orchid seed banking has been shown to be an invaluable tool for conserving the maximum amount of genetic diversity in the minimum space and has the potential to enable the conservation of valuable

material for possible reintroduction and habitat restoration programmes in the future. Although the scientific literature on global warming makes sobering reading as we face the prospect of a changing world, the outlook for orchid conservation is not all bleak. There is a growing awareness of the need to take prompt action and an increasing number of *ex situ* conservation projects are being set up around the world. The establishment of OSSSU has induced a positive response and has the potential to be expanded into a key global facility. Living collections are currently under-utilised as a conservation tool, and there is a need to do more to include members of the wider orchid community. There remains an urgent need to identify populations which are particularly vulnerable and at imminent risk of extinction in the wild so that they can be brought into cultivation.

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EXPLORING DISPARITIES IN PHYTOCHEMICAL PROPERTIES OF *CLITORIA TERNATEA* L: MARKETED PRODUCT VERSES FRESH HARVEST

Manasawala Ruqayya and Chachad Devangi*

Department of Botany, Jai Hind College (Autonomous), Mumbai, India.

*Corresponding author E-mail: devangi.chachad@jaihindcollege.edu.in

Abstract:

Clitoria ternatea L. or the butterfly pea is an important flowering climber of the Fabaceae family. *Clitoria* flowers have been traditionally used in agricultural practices as forage crop and Ayurvedic medicine to cure infertility, constipation, reduce stress, improve memory, in menstrual and uterine contractions etc. This plant is packed with phytochemicals ranging from polyphenols to alkaloids, flavonoids, glycosides, anthocyanins, and tocopherols contributing to its high antioxidant potential. Hence, this plant has gained importance in modern science and medicinal practices. The dried flowers are sold commercially as herbal tea in healthcare. The present study aimed at investigating the phytochemical richness of commercially available *Clitoria* flowers and freshly harvested flowers in terms of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant potential. Optimal extraction technique paves way for better phytochemical utilization. Hence, this study also compared two popular extraction methods viz. Hot and Cold maceration techniques. The antioxidant potential was measured in terms of 1,1 -diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and Ferric Reducing Antioxidant Power (FRAP). The study reported higher TPC and TFC along with better antioxidant potential in freshly harvested *Clitoria* flowers as against the commercially available flowers. The study also confirmed that the optimum extraction of phytochemicals occurs in Hot maceration procedure for fresh *Clitoria* flowers whereas the commercially available *Clitoria* flowers reported optimum extraction and higher antioxidant potential in the Cold Maceration.

Keywords: Butterfly Pea, Total Phenolic Content, Total Flavonoid Content, Antioxidant Potential

Introduction:

Clitoria ternatea Linn. well known as butterfly pea, blue pea, or Aparajita, is a perennial herbaceous climber, a member of the Fabaceae family. It is classified in the kingdom Plantae, phylum Tracheophyta, class of Magnoliopsida. (Jamil *et al.* 2018). *Clitoria* is widely distributed across tropical and subtropical areas of Southern and Eastern Africa, Madagaskar, India, China, and islands of Western Indian Ocean (Oguis *et al.* 2019). The vivid blue colored flower of *Clitoria* has been widely used as a natural food coloring agent and cultivated as an ornamental plant. It has been cultivated as a forage and fodder crop in arid and semi-arid regions with low

annual rainfall, as this plant was drought resilient and adaptable to harsh environmental conditions. (Abreu *et al.* 2019). Apart from traditional agricultural applications, *Clitoria* has been popular in Ancient Ayurvedic, Chinese and Cuban medicine. In Ayurvedic medicine, *Clitoria* has been described to be a nootropic herb due to its cognitive and memory enhancing abilities. Root decoctions were used for treatment of constipation, fever, indigestion, arthritis, skin diseases, sore throat, and eye ailments. The seeds have been used as laxatives and for treatment of colic and joint swellings. The Cuban traditional medicine made use of root decoctions in combination with flowers to induce uterine contractions, promote menstruation and as treatment for liver and intestinal disorders (Mukherjee *et al.* 2008). The pharmacological activities can be attributed to its phytoconstituents. Ternatin anthocyanins and various flavonol glycosides of quercetin, kaempferol, and myricetin, sitosterols, stigmasterol and tocopherols have been found in the flowers of *Clitoria*. (Jeyaraj *et al.* 2021). Hence, *Clitoria* gained a lot of popularity due to its potential health benefits. A few recent studies reported antidiabetic, antioxidant, antimicrobial, and antiproliferative activity of *Clitoria* (Jeyaraj *et al.* 2021). Successful use of plant phytochemicals in medicine, health care or cosmetics mainly depends on the stability and optimum yield of phytochemicals obtained from the employed extraction methods. Various conventional and non-conventional extraction methods have been in use, however careful selection of optimum method and assessment parameters of phytoconstituents is necessary. The current research aimed at comparing two conventional extraction protocols viz Soxhlet extraction and Cold maceration techniques for identification of optimum phytochemical extraction method for *Clitoria* flowers. These flowers have gained a lot of importance as an herb in stress relief and cosmetics leading to commercial sale of dried flowers. Consumption of commercially available flowers has become popular with the masses in the form of herbal tea with companies banking on its phytochemical richness and antioxidant activity (Kar & Barman, 2023). This study further aimed at assessing the phytoconstituents such as total phenols, total flavonoids, and antioxidant potential of freshly harvested and dried *Clitoria* flowers, against the dried and packaged market variety.

Material and Methods:

1. Collection and authentication: *C. ternatea* flowers were collected from South Mumbai and authenticated from Blatter's Herbarium, St. Xaviers College, Mumbai. The specimen matched with Blatter Herbarium specimen number Shah-1425 of G. L. Shah. The commercially packed variety was procured from a local supermarket.

2. Extraction: The freshly harvested flowers were dried for 3-4 days at 50º C in the Hot Air Oven. The oven dried material and commercially available flowers were ground separately to fine powder and subjected to extraction protocols. Hot maceration was carried out using Soxhlet apparatus for 20 cycles with 50 % Methanol. Cold maceration was carried out by keeping the

flower powder in contact with 50% Methanol for 8-hour on a Rotary shaker and 16-hour in static condition. (Sankeshwari *et al.,* 2018)

3. Determination of total phenolic and total flavonoid content

A. Determination of total phenolic content: The total phenolic content in methanolic extracts was determined by the Folin- Ciocalteu method (Mukherjee, 2019) with some modifications. To 0.1 ml sample, 0.1 ml of Folin-Ciocalteu phenol reagent was added followed by 1.0 ml of 7% Na2CO3 (Sodium carbonate) along with 3.4 ml D/W and mixed thoroughly. Post 30-minute incubation, the absorbance was read at 760 nm on UV-Vis Spectrophotometer (SHIMADZU UV VIS Spectrophotometer Model No 1780). A calibration curve for standard Gallic acid solution ranging from 0.25 to 0.1 mg/ml was plotted. The total phenolic content was reported as mg Gallic acid equivalents per gram, calculated using the formula TPC= C x V/m; where C is the concentration obtained from the Gallic acid standard curve, V is the volume of the sample used and m is the mass of the sample.

B. Determination of total flavonoid content: The total flavonoid content in methanolic flower extracts were determined using $AlCl_3$ Colorimetric method (Mukerjee, 2019) with some modifications. 2.0 ml of plant extract was added to 2.0 ml of 2 % $AlCl_3$ reagent and mixed thoroughly. Post 30-minute incubation, the absorbance was read at 415 nm on the UV-Vis Spectrophotometer (SHIMADZU UV VIS Spectrophotometer Model No 1780). Calibration curve of standard Rutin solution ranging from 0.01 to 0.05 mg/ml was plotted. The total flavonoid content was reported as mg Rutin equivalents per gram, calculated using the formula TFC= C x V/m; where C is the concentration obtained from the Rutin standard curve, V is the volume of the sample used and m is the mass of the sample.

4. Determination of antioxidant potential

A. DPPH assay: The antioxidant potential of extracts was measured in terms of their DPPH radical scavenging power (Fang *et al.,* 2017). 0.1 mM DPPH solution was prepared using 50% methanol to which 2.0 ml of sample was added. The contents were mixed and incubated for 30-minutes. Ascorbic acid (100 µg/ml) was used as standard. The absorbance of extracts were read at 517 nm on UV-Vis Spectrophotometer (SHIMADZU UV VIS Spectrophotometer Model No 1780) and % Radical Scavenging Activity was calculated using the formula: %RSA = (Abs of Control - Abs of Test) / Abs of control x 100.

B. FRAP assay: The Ferric reducing power of extracts was measured in terms of their ferric-tripyridyltriazine reduction potential at low pH, adapted from Benzic and Stain (1996) with some modifications. The working FRAP reagent was prepared as follows: 25 ml of 300 mM Acetate Buffer (pH 3.6) mixed with 2.5 ml TPTZ solution prepared in 40mM HCl and 2.5 ml of 20 mM Ferric Chloride solution. 100 µl of sample was added to 3.9 ml working FRAP reagent. A standard curve was prepared with Ferrous Sulphate (0.2-1.0 mM). The absorbance was read at

593 nm in a UV-Vis Spectrophotometer (SHIMADZU UV VIS Spectrophotometer Model No 1780). The results obtained were expressed in mM $FeSO_4$ equivalents.

Results:

1.Total phenolic and flavonoid content determination:

A. Total phenolic content: The TPC of the flower extracts was calculated from standard Gallic Acid curve (y = 0.3001x; R^2 = 0.9776) as shown in figure 1. The TPC of the flower extract was reported as mg GAE/g as shown in Table 1.

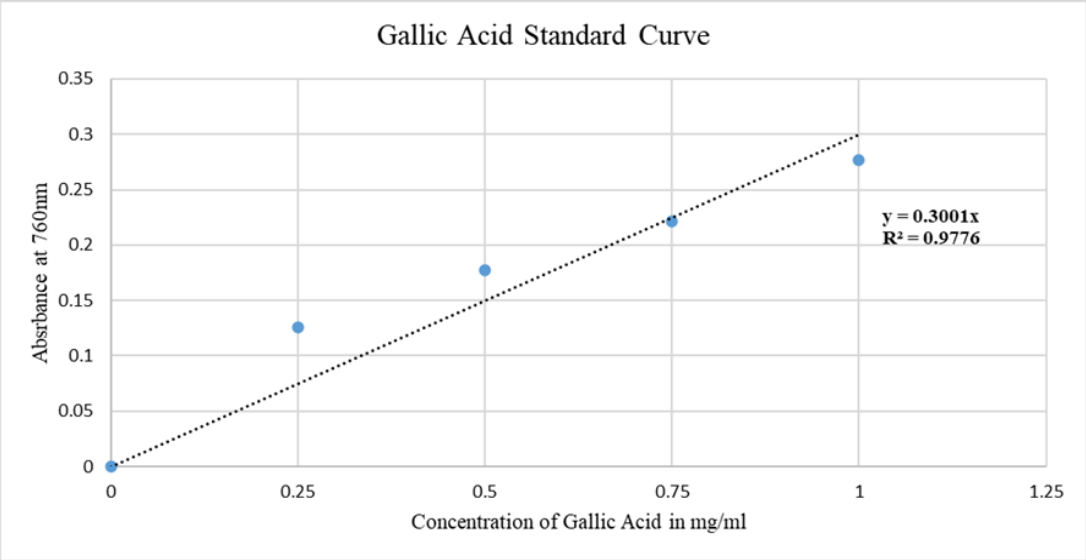

Figure 1: Standard Gallic Acid Calibration Curve

Table 1: Results of total phenolic acid content

Flower	TPC of Soxhlet extract in mg GAE/g ± SD	TPC of Cold Maceration extract in mg GAE/g ± SD
Fresh harvest	36.66 ± 0.007	8.69 ± 0.001
Marketed variety	33.24 ± 0.003	9.76 ± 0.001

B. Total flavonoid content: The TFC of the flower extracts was calculated from standard Rutin curve (y = 18.421x; R^2 = 0.9974) as shown in figure 2. The TFC of the flowers was reported as mg RE/g as shown in Table 2.

Table 2: Results of total flavonoid content

Flower	TFC of Soxhlet extract in mg RE/g ± SD	TFC of Cold Maceration extract in mg RE/g ± SD
Fresh harvest	1.012 ± 0.011	0.794 ± 0.046
Marketed variety	0.833 ± 0.005	0.900 ± 0.007

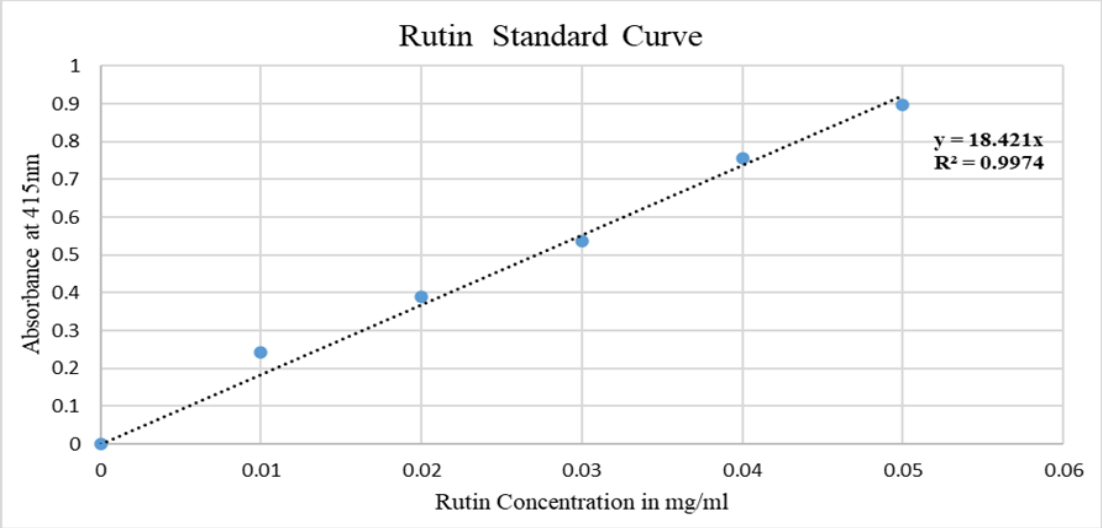

Figure 2: Standard Rutin Calibration Curve

2. Antioxidant potential

A. DPPH assay: The antioxidant activity of the extracts were calculated in terms of percent DPPH radical scavenging activity and compared with that of Ascorbic acid. %RSA of 100 µg/ml of flower extract is shown in Table 3. The %RSA of standard ascorbic acid of (100 µg/ml) was reported as 72.41± 0.002%.

Table 3: Percent RSA calculated for *Clitoria* flowers

Flower	%RSA of Soxhlet extract ± SD	%RSA of Cold Maceration ± SD
Fresh harvest	60.34 ± 0.0012	59.91± 0.0028
Marketed variety	53.74± 0.0017	57.90± 0.0012

B. FRAP assay: Free radical reducing power of extracts were calculated from Standard Ferrous sulphate curve (y=0.1936x; R^2 = 0.9964) as in figure 3. The FRAP value of flower extracts was reported in terms of mM equivalents of $FeSO_4$ in table 4.

Table 4: Result for Ferric reducing antioxidant power

Flower	mM $FeSO_4$ eq of Soxhlet extract ± SD	mM $FeSO_4$ eq of Cold Maceration ± SD
Fresh harvest	22.35 ± 0.0021	8.44 ± 0.0038
Marketed variety	17.25 ± 0.0391	13.12 ± 0.0035

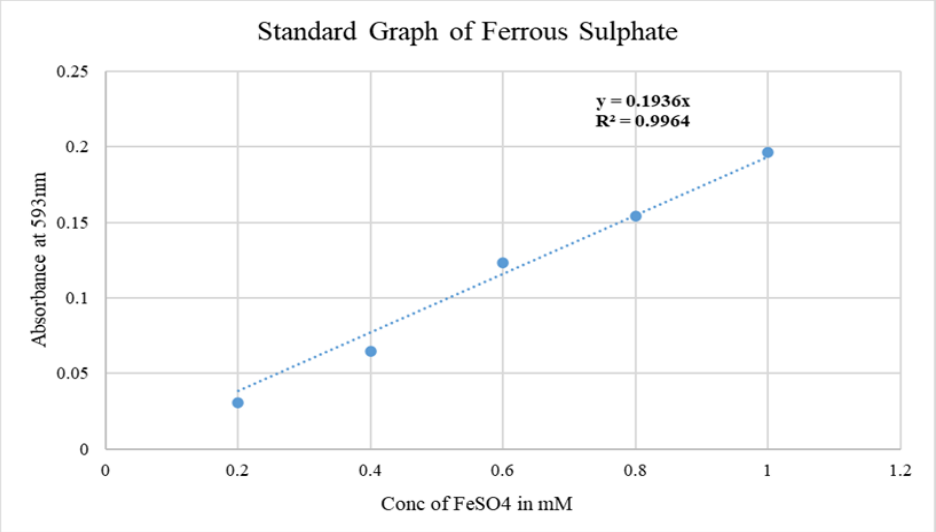

Figure 3: Standard Curve of Ferrous Sulphate

Discussion:

Solvents play a major role in optimum extraction of plant phytochemicals. In this study, TPC and TFC in hot macerated fresh *Clitoria* flowers was reported as 36.66 ± 0.007 mg GAE/g and 1.012 ± 0.011 mg RE/g respectively, 8.69 ± 0.001 mg GAE/g and 0.794 ± 0.046 mg RE/g respectively in cold macerated extract when 50% methanol was used as extraction solvent. A study by Budhika *et al.,* 2021, reported a TPC and TFC of 24.11 mg GAE/g and 14.06 mg RE/g respectively when 99% methanol was used as solvent in cold maceration technique. Similarly, a study by Escher *et al.,* 2020 reported a TPC of 5.94 to 6.92 mg GAE/g in aqueous *Clitoria* flower extract. Study by Prado *et al.,*2019 reported highest TPC in 50% methanol (14.1 mg GAE/g) compared to aqueous (13.8 mg GAE/g) and 100% methanol (5.72 mg GAE/g) extracts from petals of *Clitoria* flowers using cold maceration protocol. The current study is in tune with previous studies on *Clitoria* flowers thereby proposing 50% Methanol as an appropriate solvent for total phenol extraction. Apart from the solvent, parameters such as extraction temperatures also play a major role in optimum phytochemical extraction. A recent study by Thuy *et al.,* 2021 have reported the effect of temperature on anthocyanin extraction from *Clitoria* flowers. The anthocyanin content extracted increases considerably as extraction temperatures increase from 60 to 80 ℃. Hence, this study aligns with the findings of Thuy *et al.,* 2021 as higher TPC and TFC value was recorded in the Soxhlet extracts of freshly dried *Clitoria* flowers as compared to cold macerated extract. Easy diffusibility rate and solubility of phenols in the solvent may be the reason for optimum phenolic and flavonoid extraction at higher temperatures. TPC and TFC content in the commercially available dried *Clitoria* flowers was found to be 33.24 ± 0.003 mg GAE/g and 0.833 ± 0.005 mg RE/g respectively in the Soxhlet extract, 9.76 ± 0.001 mg GAE/g, 0.900 ± 0.007 mg RE/g respectively in the Cold macerated extracts. These results clearly indicated presence of higher TPC and TFC in the freshly harvested and dried *Clitoria* flowers

compared to the commercially available dried *Clitoria* flowers. The same correlation was observed in antioxidant potential with freshly harvested *Clitoria* flowers reporting a better radical scavenging activity, 60.34 % ± 0.0012, 59.91%± 0.0028 in Soxhlet and cold macerated extracts respectively as compared to 53.74 %± 0.0017, 57.90% ± 0.0012 in Soxhlet and cold macerated extracts respectively of commercial *Clitoria* flowers. The ferric reduction potential assay further confirmed the findings as freshly harvested and dried *Clitoria* flowers reported 22.35 ± 0.0021 mM eq of $FeSO_4$, 8.44 ± 0.0038 mM eq of $FeSO_4$ in Soxhlet and cold macerated extracts respectively, whereas the commercially available *Clitoria* flowers reported 17.25 ± 0.0391 mM eq of $FeSO_4$, 13.12 ± 0.0035 mM eq of $FeSO_4$ in Soxhlet and cold macerated extracts respectively. The results clearly indicate deterioration of certain phenols and flavonoids during the commercial drying and packaging process of *Clitoria* flowers thereby reducing their antioxidant potential. In case of freshly harvested and dried *Clitoria* flowers, optimum extraction of phenols and flavonoids was recorded in the Hot maceration technique whereas the Cold maceration technique was reported to be more suitable for flavonoid extraction from commercially dried *Clitoria* flowers.

Conclusion:

Optimum phytochemical extraction is necessary for its better utilization in commercial products. Hence, it is important to select the most suitable solvent and optimize extraction parameters such as temperature for the same. This study shows that an aqueous methanol (50%) as a solvent can be used for optimal extraction of total phenolic and flavonoid content from *Clitoria* flowers. Correct drying temperature for flowers influences the phenol and flavonoid stability. Further, it was found that hot maceration is well suited for phenol and flavonoid extraction from freshly harvested *Clitoria* flowers. The increased demand for *Clitoria* flowers has led to increased commercial availability of the flowers in the market. For enhanced shelf-life of flowers, they are often sold in the dried form. However, this commercial processing and drying of the flowers leads to reduced phenolic and flavonoid content leading to reduction in the antioxidant activity of the flowers.

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ENLISTING SOME THERAPEUTICAL PLANTS USED IN THE CURE OF SKIN DISEASES FROM THE WARDHA DISTRICT. MAHARASHTRA, INDIA

Ajay B. Jadhao

Department of Botany,

Arts and Science College Pulgaon, District Wardha, Maharashtra, India.

Corresponding author E-mail: cyrusajay@gmail.com

Abstract:

Skin diseases are the most common form of infections occurring in people of all ages. The aim of the study is not only to prescribe remedies for skin diseases in human beings but also to draw attention to the need for a detailed study of medicinal plants, which could provide novel remedies for other dreadful diseases. This review has highlighted the role and utilities of some medicinal plants on different skin diseases.

Introduction:

According to figures from the World Health Organization (WHO), more than 80% of people in poor nations receive their primary medical care from traditional practitioners (Shankar *et al.*, 1993). India is a world leader in the production of medicinal herbs and is aptly referred to as the botanical paradise of the globe due to its wealth of documented and customarily applied knowledge of herbal medicine. Approximately 17,000 species of higher plants in India and approximately 7,500 species worldwide are known to have medicinal potential; in other nations, this percentage is estimated to be between 7% and 13%. (Anad *et al.*, 2010). India's rural communities are aware of over 25,000 potent plant-based remedies that are utilized in traditional medicine. Because they are inexpensive and nontoxic, medicinal plants are essential for pharmacological research, drug discovery, and the direct application of plant ingredients as therapeutic agents and building blocks for drug synthesi. (Masood E. 1997).

In the present paper, an attempt has been made to document the list of ethnomedicinal plants used to cure skin diseases.

Materials and Methods

To gather information about the ethnomedicinal plants that the tribal people employ to treat skin conditions, the authors carried out a thorough field survey in the tribal area and other inner village areas. During the fieldwork, real-world examples of using plant parts to treat skin conditions were also seen. The plant species are listed alphabetically by family, then their tribal name, the part of the plant that is used, and how it is administered. Plants species were identified by using some flora such as Kamble and Pradhan (1988), Naik (1998), Deore (2009), and Ugemuge (1986).

Results and Discussion:

The study that a total of 22 species were identified. For each species, the botanical name, family name, plant part used, and usage were recorded. In the present study was used for the treatment of skin diseases. The results of the present study provide evidence that medicinal plants continue to play an important role.

Sr. No.	Botanical name	Family	Use
1	*Abrus precatorius*	Fabaceae	Seeds are used in skin diseases
2	*Achyranthes aspera*	Amaranthaceae	Leaves are crushed and applied directly for skin diseases.
3	*Aloe Vera*	Liliaceae	The leaf juice is used for skin diseases
4	*Argemone Mexicana*	Papaveraceae	Roots are given for skin diseases
5	*Aristolochia indica*	Aristolochiaceae	Leaves are made into a paste and then boiled with coconut oil and it can be applied externally.
6	*Azadirachta indica*	Meliaceae	Leaves, bark, and oil from seeds are used in the treatment of skin diseases
7	*Carica papaya*	Caricaceae	Latex is useful in skin diseases
8	*Cassia fistula*	Caesalpiniaceae	Roots are ground with water made into a paste and applied to cure skin diseases. Leaf juice and flowers are also useful in skin diseases.
9	*Catheranthus roses*	Apocyanaceae	Leaves paste is applied externally as a cure for pimples
10	*Centella Asiatica*	Apiaceae	Crushed leaves are applied orally for skin diseases.
11	*Clerodendum viscosum*	Verbenaceae	Decoction of the leaves is useful in skin diseases.
12	*Clitoria ternatea*	Fabaceae	Fresh leaves are pounded and made into a paste and applied to cure skin diseases.
13	*Cyperus rotundus*	Cyperaceae	Rhizome is made into a paste with water and applied externally.
14	*Dalbergia sissoo*	Fabaceae	Bark and heartwood are useful in skin diseases.

15	*Ficus benghalensis*	Moraceae	Bark and latex is useful in skin diseases.
16	*Ficus religiosa*	Moraceae	Paste of powdered bark is good for skin diseases.
17	*Marsilea quadrifolia*	Marsileaceae	Whole plant is useful in skin diseases.
18	*Ricinus communis*	Euphorbiaceae	Oil extracted from the seeds is used in children for skin diseases.
19	*Solanum nigrum*	Solanaceae	Leaves are used for skin diseases.
20	*Solanum suratense*	Solanaceae	Whole plant is used.
21	*Tactona grandis*	Verbenaceae	Leaves extract and Bark is used for skin diseases.
22	*Tephrosia purpurea*	Fabaceae	Roots and seeds are used as a remedy for skin diseases.

Conclusion:

To gather information about the ethnomedicinal plants that the tribal people employ to treat skin conditions, the authors carried out a thorough field survey in the tribal area and other inner village areas. During the fieldwork, real-world examples of using plant parts to treat skin conditions were also seen. The plant species are listed alphabetically by family, then their tribal name, the part of the plant that is used, and how it is administered.

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ANALYSIS OF NUTRITIVE VALUE OF EDIBLE SPOON FROM WHEAT FLOUR AND PUMPKIN SEEDS FLOUR

Swathy Saravanan* and Dhanalakshmi Nagaraj

Department of Nutrition & Dietetics,

K.S.R College of Arts and Science for Women,

Tiruchengode- 637215, Namakkal, Tamilnadu, India

*Corresponding author E-mail: swathysaravanan17@gmail.com

Abstract:

A brand-new and cutting-edge type of silverware manufactured from edible materials are edible spoons. They can also be manufactured from other plant-based materials, such as fruit and vegetable peels, but are commonly made from flours like wheat, rice, or sorghum. Due to their compostability and biodegradability, edible spoons are a sustainable and environmentally friendly alternative to plastic silverware. Even though research on edible spoons is still in its early stages, a growing body of evidence points to a variety of possible advantages. For instance, using edible spoons might lessen the quantity of plastic trash produced. A basic diet for billions of people worldwide is wheat flour. Numerous items, such as bread, pasta, biscuits, and cakes, are made with it. The goal of wheat flour research is to create new and creative uses for the grain while also enhancing its quality and nutritional worth. A nutrient-rich food product called pumpkin seed powder is created by finely powdering dried pumpkin seeds. It is a wonderful source of minerals, vitamins, healthy fats, fiber, protein, and minerals. All things considered, pumpkin seed powder is a nutrient-dense food item with a variety of possible health advantages. It can also be utilized in a variety of ways because it is a versatile substance.

Keywords: Wheat Flour, Edible Spoon, Pumpkin Seeds Powder, Antioxidants.

Introduction:

Wheat flour is a powder made from the grinding of wheat used for human consumption. Wheat varieties are called “soft” or “weak” if gluten content is low, and are called “hard” or “strong” if they have high gluten content. Hard flour, or bread flour, is high in gluten, with 12% to 14% gluten content, and its dough has elastic toughness that holds its shape well once baked. Soft flour is comparatively low in gluten and thus results in a loaf with a finer, crumbly Pumpkins come in a range of sizes, shapes and colours. It is available in two color options: orange and yellow.Furthermore, some variations can be brown, white, red, gray, or dark to light green.Pumpkin seeds and pulp are found within the thick shel.Fruit pulp ranges in color from goldenyellow to orange.Pumpkins are produced all year round in Vietnam because they thrive in hot, humid tropical settings.Pumpkin flour, sometimes referred to as pumpkin fruit flour, is a

kind of flour prepared from dried pumpkin flesh, either with or without the rind and seeds included. The pumpkin flesh is removed from the stem and leaves.Because pumpkin manufacturing is very inexpensive, there has been interest in both commercial and scientific aspects of pumpkin products. Moreover, pumpkin flour is free of gluten.

Figure 1: Edible Spoon

Objectives:

- To provide a nutritious and sustainable alternative to plastic spoons.
- To uses for boosting bone health, sexual wellbeing, and the immune system patients.
- To create a new product for eadiblSpoon in pumpkin seed flour.

Methodology:

The methodology pertaining to the present study wasas follows:

1. Selection of raw material:

To make edible spoons, raw resources such as water, wheat flour, and pumpkin seed flour are needed. Creating a dough out of all the basic elements is the first step in the manufacturing process. To help the gluten settle, the dough is rested for five to ten minutes. The dough is then formed into thin sheets. After being put on spoon molds, the sheets are baked. The manufactured spoons are put into a box after being baked and allowed to cool for five to ten minutes. For the manufacturing process, three machines are needed:

1. A machine for kneading dough is necessary to combine the ingredients into a dough.
2. Dough sheeter: Needed to roll out the dough into thin layers.
3. Baking machine and mold – After the dough sheets are placed on the molds, they are baked.

2. Ingredients:

Measure 150g of wheat flour and rinse it well, drain water. Spread on a plate and dry it completely. Then grind it into fine powder. Sieve it, discard the coarse mixture. Transfer the flour to a clean dry container. Add 70g of wheat flour then add 30g of Pumpkin seed powder.

Variations	Expansion
WPSV1	Pumpkin seed flour Wheat flour V1-10P:90W
WPSV2	Pumpkin seed flour Wheat flour V2-20P:80W
WPSV3	Pumpkin seed flour Wheat flour V3-30P:70W

WPES- Wheat flour, Pumpkin seed flour, Eadibl Spoon

Figure 2: Ingredients

3. Mixing:

In order to create gluten, which is the result of an improved interaction between dispersed and hydrated gluten-forming proteins, wheat flour and water are mixed together during the dough-making process. Because of the variations in their various formulations, particularly the ratio of liquid to dry ingredients, it differs significantly from batter mixing.

4. Addition of water:

A dough with ideal viscoelasticity qualities can be created with the appropriate amount of water, resulting in excellent gluten. In the end, this gluten holds the fermentation gas, allowing the dough to develop and provide the ideal volume.

5. Kneading:

The process of kneading dough involves rubbing it together before baking. Stretching the gluten fibers in the dough during kneading permits more expansion during fermentation.

6. Moulding:

The viscous material that needs to be molded is put into the proper mold. The substance hardens and solidifies during the molding process, eventually taking on a permanent shape.

7. Baking:

Baking is the process of cooking, usually in an oven, using dry heat. It's most likely the oldest cooking technique. The flour or meal used to make bakery goods, such as bread, rolls,

cookies, pies, pastries, and muffins, is often made from grains. In the human diet, bread, which was already a common staple in prehistoric times, supplies numerous nutrients.

7. Packing:

A coordinated system of preparing food for transportation, distribution, storage, retailing, and end-use to meet the highest standards of food safety is known as food packagingCustomer at the best possible price. Modern civilization would not be possible without food packaging; without it, commercially prepared food could not be handled and transported in a safe or effective manner.

Here the nutritive analysis of edible spoon was calculated using the standards values given by NIN (National Institute of Nutrition).

8. Flow chart:

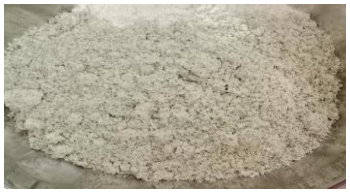
Selection of raw materials

↓

Wheat flour and pumpkin seed flour


↓

Mixing of all ingredients



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Addition of water and oil

↓

Knead to make smooth dough

↓

Fill into the spoon mould

↓

Bake the spoons at 160ºC for 20 mins in microwave oven

↓

Allow to cool

↓

Packing in airtight container

↓

Store in dry place

Results and Discussion:

Making educated judgments about food product creation, quality assurance, and marketing can be aided by the findings of sensory evaluations. For instance, a producer can change the recipe if they discover that customers like a new product that tastes sweeter. The sensory assessment takes notice of human characteristics such as taste, texture, and appearance. The product's nutritional content is provided by the Pumpkin seed flour formulation. Consequently, the product can enhance the recipe for different kinds of edible spoons.

Nutritive analysis for WPSES

Table 1: Nutriitive analysis of variation 1

Ingredients	**Quantity**	**Energy**	**Protein**	**Carbo.**	**Fat**	**Fibre**
Wheat flour	90g	313.2	9.9	66.51	0.81	0.27
Pumpkin seed flour	10g	57.4	1.9	5.4	4.9	0.66

The analysis for single edible spoon is Energy-**370.6kcal**, Protein-**11.8g,** Carbohydrate**71.91g,** Fat-**5.71g**, Fibre-**0.93g** respectively.

Table 2: Nutriitive analysis of variation 2

Ingredients	Quantity	Energy	Protein	Carbo.	Fat	Fibre
Wheat flour	80g	278.4	8.8	59.12	0.72	0.24
Pumpkin seed flour	20g	114.8	3.8	10.8	9.8	1.32

The analysis for single edible spoon is Energy-**393.2kcal**, Protein-1**2.6g**, Carbohydrate**69.92g**, Fat-**10.52g**, Fibre-**1.56g** respectively.

Table 3: Nutriitive analysis of variation 3

Ingredients	Quantity	Energy	Protein	Carbo.	Fat	Fibre
Wheat flour	70g	243.6	7.7	51.73	0.63	0.21
Pumpkin seed flour	30g	172.2	5.7	16.2	14.7	1.98

The analysis for single edible spoon is Energy-**415.8kcal**, Protein-**13.4g**, Carbohydrate**67.93g**, Fat-**15.33g,** Fibre-**2.19g** respectively.

Sensory evaluation:

Table 4: Sensory evaluation variation-1

Panels Name	Appearance	Texture	Taste	Flavour	Overall Acceptability
Trained Panels					
N. Indra	5	4	4	4	4
S. Logeshwari	5	5	5	5	5
S. Swathy	5	5	4	5	5
Semi Trained Panels	**Appearance**	**Texture**	**Taste**	**Flavour**	**Overall Acceptability**
K. R Anitha	5	5	5	4	5
N.M Jeeva	5	4	5	4	4
P. Vedhavithya	5	4	4	5	4
M. Yuvasri	5	5	5	4	4
M.Pavithra	5	5	5	5	5

Table 5: Sensory evaluation variation-2

Panels Name	**Appearance**	**Texture**	**Taste**	**Flavour**	**Overall Acceptability**
Trained Panels					
N. Indra	4	5	4	4	4
S. Logeshwari	5	5	5	4	5
S. Swathy	5	4	5	5	5
Semi Trained Panels	**Appearance**	**Texture**	**Taste**	**Flavour**	**Overall Acceptability**
K. R Anitha	4	4	4	5	4
N. M Jeeva	4	5	5	5	5
P. Vedhavithya	5	4	4	4	4
M. Yuvasri	5	5	5	4	5
M. Pavithra	5	4	5	5	5

Table 6: Sensory evaluation variation-3

Panels Name	**Appearance**	**Texture**	**Taste**	**Flavour**	**Overall Acceptability**
Trained Panels					
N. Indra	5	5	4	4	5
S. Logeshwari	5	4	5	4	5
S. Swathy	5	4	4	5	5
Semi Trained Panels	**Appearance**	**Texture**	**Taste**	**Flavour**	**Overall Acceptability**
K. R Anitha	5	5	5	4	5
N. M Jeeva	5	4	5	4	4
P. Vedhavithya	5	4	4	5	4
M. Yuvasri	5	5	5	4	4
M. Pavithra	5	4	5	5	5

Conclusion:

A wholesome and environmentally friendly substitute for conventional plastic spoons are edible spoons manufactured from wheat flour and pumpkinseeds.They are a good source of fiber, vitaminC, potasiumantioxident.They are minimal in fat and calories as well.pumpkin seeds are rich in fiber and vitaminC.It is a fantastic source of antioxident as well.Iron, calcium, and

phosphorus are among the vitamins and minerals that are abundant in pumpkin seeds.Thus, this edible spoon aids in controlling blood glucose levels.It is advised for those with diabetes.These edible spoonecan be used to create healthier ones.

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ANALYSIS OF NUTRITIVE VALUE OF EDIBLE SPOON FROM WHEAT FLOUR AND JAMUN SEED FLOUR

Swathy Saravanan* and Pavithra Marimuthu

Department of Nutrition & Dietetics, K.S.R College of Arts and Science for Women, Tiruchengode- 637215, Namakkal, Tamilnadu, India

*Corresponding author E-mail: swathysaravanan17@gmail.com

Abstract:

Wheat (*Triticum* spp.) is cereal crop that belong to the family Poaceae (order Poales). Wheat is a staple source of nutrients for around 40% of the world's population. Wheat has already been cultivated for millennia. Wheat was among the first cereals crop to be farmed, and that has been a staple diet throughout Europe, Western Asia, and Northern Africa for over 8000 years. This is most likely due to wheat's agricultural versatility, convenience of grain storage, and simplicity of flour conversion for a variety of cuisines. Wheat is probably the most frequently produced crop in the world, with over 218 million hectares under cultivation, and its global trade exceeds that of all other crops collectively. About a third of all food is wasted, according to the United Nations Food and Agriculture Organization (FAO). Food is wasted at every stage of production, from farming to distribution to retail to consumption. Among the causes are cooking losses, intentional food waste, losses from pests, mold, or insufficient climate management." For the edible portion of this, there were 1.3 billion tonnes of food wasted altogether. Meals can be served or consumed as a meal using edible cutlery, a plant-based product. Given that the product is composed of a variety of flours, it is typically recognized as being EBO (eco-friendly, biodegradable, and organic). Diabetes, allergies, viral infections, inflammation, and gastric ulcers are all treated with jamun seeds. Additionally, it has hypothermic, diuretic, antinociceptive, chemoprotective, and cardioprotective effects. Jamun seeds are very useful and good for human health since they include anti-bacterial, anti-inflammatory, anti-oxidant, and antidiabetic properties.The edible spoon trends in India are made of wheat flour with jamun seed flour and are 100 percent natural and do not contain artificial preservatives.Edible spoon is a fast moving product around the world. Edible spoons are consumable and biodegradable. They can be eaten without serving anything in it. Edible spoon is considered to be very healthy.

Keywords: Wheat Flour, Dietary Fiber, Jamun Seed, Edible Spoon

Introduction:

Wheat is essential for the health of people due to having a large number of diet contents and nutritional value. Its important can be guessed to see the developed countries that can use

only bread, noodle, cakes, pastry, and lactogen. Carbohydrates 55% and 20% of food calories are present in the wheat grains. Carbohydrates 78%, protein 14%, fat 2%, minerals 2.5%, and vitamins such as thiamine and vitamin B, as well as minerals such as zinc and iron, selenium, and magnesium make up a small percentage of the diet. Wheat has pericarp that is classified as true seed. Protein is stored in the endosperm; the protein contents are about 72%. Wheat grains are also rich in pantothenic acid, riboflavin and some minerals, sugars, etc. The barn, which consists of pericarp Testa and aleurone, is also a dietary source for fiber, potassium, phosphorus, magnesium, calcium, and niacin in small quantities. Jamun (*Syzygium cumini* L. Skeels) is highly perishable with a very short shelf life, hence, jamun fruit is either consumed fresh as soon as it is harvested or converted to value-added products such as jam, wine, juice, and jellies. The processing of jamun fruit generates a large quantity of seeds as the primary waste. Jamun seeds are a rich source of macronutrients such as carbohydrates, proteins, lipids, minerals, and vitamins, thus making them an important ingredient in the food industry. Along with its beneficial nutritional profile, the review also throws light upon the safety aspects associated with jamun seed consumption along with its acceptable daily intake. Jamun seeds with array of nutritional benefits can be an important functional ingredient; however, further extensive research is necessary to find suitable levels of application of jamun seed in food products for harnessing its nutritional potential without affecting the products' sensory palatability.

Figure 1: Edible Spoon

Objectives:

- To provide a nutritious and sustainable alternative to plastic spoons.
- To control the blood glucose level in diabetes patients.
- To promote weight loss.
- To create a new product that is both functional and delicious.

Methodology:

The methodology pertaining to the present study was as follows:

1. Selection of raw material:

Four raw materials are required to manufacture edible spoons, i.e., jamun seed flour, wheat flour, and water. Manufacturing process begins with mixing all the raw materials into a dough. The dough is rested for about 6–10 minutes to settle the gluten. Afterward, the dough is

converted into thin sheets. The sheets are placed on spoon molds and sent for baking process. After baking, the manufactured spoons are cooled for 5–10 minutes and then packed into a box.

2. Ingredient:

Measure 150g of wheat flour and rinse it well, drain water. Spread on a plate and dry it completely. Then grind it into fine powder. Sieve it, discard the coarse mixture. Transfer the flour to a clean dry container. Add 70g of wheat flour then add 30g of Jamun seed powder.

Variations	**Expansions**
JWV1	J-Jamun Seed W-Wheat Flour V1-10J:90W
JWV2	J-Jamun Seed W-Wheat Flour V2-20J:80W
JWV3	J-Jamun Seed W-Wheat Flour V3-30J:70W

3. Mixing:

Dough mixing is a process in wheat flour and water are mixed until gluten is developed, a result of the enhanced interaction between dispersed and hydrated gluten-forming proteins. It's quite different from batter mixing due to differences in their respective formulations specifically, the proportion between dry and liquid ingredients.

Figure 2: Mixing

4. Addition of water:

The addition of water in the right amount can form a dough with optimum viscoelasticity properties so that the resulting gluten is also optimal This gluten ultimately hold the fermentation gas so that the dough is developed so that the optimum volume is generated.

Figure 3: Addition of water

5. Kneading:

Kneading is the process of working a dough mixture to form a smooth and cohesive mass. It can be done by hand or mechanically. Proper kneading is essential for the formation of dough with adequate viscoelastic properties including: Gas retention capacity and Breads with fine grain, texture and crumb.

Figure 4: Kneading

6. Moulding:

The material to be moulded is in a viscous form and is fed into the appropriate mould. As the moulding process progresses the material becomes firmer and solidifies, up to the point that it becomes a fixed shape.

Figure 5: Moulding

7. Baking:

Baking, process of cooking by dry heat, especially in some kind of oven. It is probably the oldest cooking method. Bakery products, which include bread, rolls, cookies, pies, pastries, and muffins, are usually prepared from flour or meal derived from some form of grain. Bread, already a common staple in prehistoric times, provides many nutrients in the human diet**.**

Figure 6: Baking

8. Packaging:

Food packaging is defined as a co-ordinated system of preparing food for transport, distribution, storage, retailing, and end-use to satisfy the ultimate consumer with optimal cost. Food packaging is an essential part of modern society; commercially processed food could not be handled and distributed safely and efficiently without packaging.

Figure 7: Packaging

Here the nutritive analysis of edible spoon was calculated using the standards values given by NIN(National Institute of Nutrition).

Flow chart:

Flow chart:1

Preparation method

Selection of raw material

↓

Wheat flour and Jamun seed flour

↓

Mixing of all ingredients

↓

Addition of water/ oil/milk

↓

Knead to make smooth dough

↓

Fill into the spoon mould

↓

Bake the spoons at 160ºC for 20 mins in microwave oven

↓

Allow to cool

↓

Packing in airtight container

↓

Store in dry place

Results and Discussion:

The results of sensory evaluations can be used to make informed decisions about food product development, quality control, and marketing. For example, if a manufacturer finds that consumers prefer a new product with a sweeter taste, they can adjust the formulation accordingly. Sensory evaluation notes the human attires like taste, texture, flavour, appearance of the humans.The formulation of jamun seed flour provides nutritious value of the product. Thus the product can improve the formulation of various types of edible spoon**.** The overall acceptability of edible spoon (variation-3) were accepted by all age groups.

Nutritive analysis for WJSES:

Table 1: Nutritive analysis of variation-1

Ingredients	Quantity	Energy	Protein	Carbo.	Fat	Fibre
Wheat flour	90g	306.9	10.89	62.46	1.53	1.71
Jamun seed flour	10g	25.1	0.85	4.14	0.096	1.69

The analysis for single edible spoon is Energy-**70.5kcal**, Protein-**1.805g**, Carbohydrate-**18.1g**, Fat**0.01g**, Fibre-**1.76g** respectively.

Table 2: Nutritive analysis of variation-2

Ingredients	Quantity	Energy	Protein	Carbo.	Fat	Fibre
Wheat flour	80g	272.8	9.68	55.52	1.36	1.52
Jamun seed flour	20g	50.2	1.7	8.28	0.19	3.38

The analysis for single edible spoon is Energy-**64.5kcal**, Protein-**1.605g**, Carbohydrate-**11.1g**, Fat **0.25g**, Fibre-**1.66g** respectively.

Table 3: Nutritive analysis of variation-3

Ingredients	Quantity	Energy	Protein	Carbo.	Fat	Fibre
Wheat flour	70g	238.7	8.47	48.58	1.19	1.33
Jamun seed flour	30g	75.3	2.55	12.42	0.2905	5.07

The analysis for single edible spoon is Energy-**50.5kcal**, Protein-**1.505g**, Carbohydrate-**10.1g**, Fat**0.21g**, Fibre-**1.46g** respectively.

Sensory evaluation:

Table 4: Sensory evaluation for variation-1

Panels Name	Appearance	Texture	Taste	Flavour	Overall Acceptability
Trained Panels					
N. Indra	5	5	4	4	5
S. Logeshwari	5	4	5	5	5
S. Swathy	5	5	4	5	5
Semi Trained Panels					
K. R Anitha	5	4	5	4	5
N. M. Jeeva	5	4	5	4	4
S. Kaizer	4	5	4	4	4
A. Raveena	5	5	4	4	5

Table 5: Sensory evaluation for variation-2

Panels Name	Appearance	Texture	Taste	Flavour	Overall Acceptability
Trained Panels					
N. Indra	5	5	4	4	5
S. Logeshwari	5	4	5	4	4
S. Swathy	5	4	4	5	4
Semi Trained Panels					
K. R Anitha	5	4	5	4	5
N. M. Jeeva	5	4	5	4	4
S. Kaizer	4	5	4	5	4
A. Raveena	5	5	4	4	5

Table 6: Sensory evaluation for variation-3

Panels Name	Appearance	Texture	Taste	Flavour	Overall Acceptability
Trained Panels					
N. Indra	5	5	5	4	5
S. Logeshwari	5	4	5	5	5
S. Swathy	5	5	4	5	5
Semi Trained Panels					
K. R Anitha	5	4	5	4	5
N. M. Jeeva	5	5	5	4	5
S. Kaizer	4	5	5	5	5
A. Raveena	5	5	5	4	5

Conclusion:

Edible spoons made from wheat flour and jamun seeds are a nutritious and sustainable alternative to traditional plastic spoons. They are a good source of carbohydrates, protein, fiber, vitamins, and minerals. They are also low in calories and fat. Jamun seeds is high in nutrients and fiber. It is also a good source of protein. Jamun seeds are a good source of vitamins and minerals, including iron, calcium, and phosphorus. So this Edible spoon helps to keep the Blood glucose level in control.It is recommended for Diabetic patients.These Edible spoon to make the as a Healtheir one.

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ANALYSIS OF NUTRITIVE VALUE OF EDIBLE SPOON FROM WHEAT FLOUR AND WATERMELON SEED FLOUR

Swathy Saravanan* and Vedhavithya Palanisamy
Department of Nutrition & Dietetics
K.S.R College of Arts and Science for Women,
Tiruchengode- 637215, Namakkal, Tamilnadu, India
*Corresponding author E-mail: swathysaravanan17@gmail.com

Abstract:

The pulverized endosperm of wheat grains is used to make wheat flour, a powder. It serves as the main component of many common dishes found all throughout the world, like as bread, pasta, and pastries. A good source of fiber, protein, and carbs, as well as a number of vitamins and minerals, is wheat flour. The seeds of watermelon (Citrullus lanatus) are a goodsource of vitamins, minerals, fatty acids, protein, and fiber. Many cultures have long ingested them, and they are currently becoming more well-liked as a nutritious snack or an ingredient in other dishes. There are several potential health advantages of eating watermelon seeds, according to a new comprehensive assessment of the literature.A novel and cutting-edge idea called edible spoons provides a long-term and environmentally responsible replacement for conventional plastic silverware. Edible spoons can be eaten after usage and are made from a range of edible substances, including sorghum, wheat bran, and rice flour. A by-product of the watermelon industry known as watermelon seed powder (WSP) is normally thrown away. However, WSP is an excellent source of vitamins, minerals, and nutrients like protein and fiber. Additionally, it possesses anti-inflammatory and antioxidant properties. Recent studies have looked into the production of edible spoons using WSP. Alternatives to single-use plastic spoons that are sustainable include edible spoons. They are an effective approach to cut down on food waste.

Keywords: Wheat Flour, Edible Spoon, Watermelon Seed Powder, Antioxidant Properties.

Introduction:

Watermelon seed is one of the underexplored and unutilized sources of flour containingvitaminE, minerals and also have anti-oxidant activity. The objective of this study is to aware people about the properties of watermelon seed flour and the potential benefits of the flour. According to most of the researchers' watermelon seed flour has positive impact on growth and it has cardioprotective, hepatoprotective and anti-diabetic effects. Watermelon seeds are known to be highly nutritional; they are rich sources of protein, vitamins B, minerals (such as magnesium, potassium, phosphorous, sodium, iron, zinc, manganese and copper) and fat among

others as well asphytochemicals. The seeds of watermelons are known to have economic benefits especially in countries where cultivation is on the increase. The seeds are for instance used to prepare snacks, milled into flour and used for sauces. Oil from the seeds is used in cooking and incorporated into the production of cosmetics. Wheat flour is composed of almost 100% amylopectin and is suitable for use as shortening in baking. The addition of 10–30% waxy durum wheat flour retarded bread going stale. Possibly, the flour slowed down water transfer from gluten to starch, leaving more water to create plasticizer in the dough system. Edible spoons or cups can be made of millet,rice,wheat or other plant based raw materials.If waste products are used,as is the case for ALDI then the ecobalance is improved even more.If we make packaging and cutlery edible, we need to make sure they taste good too! If nibbling on cutlery for you rest assured the natural material disintegerates anyway in four to five days.

Figure 1: Edible Spoon

Objectives:

- Edible cutlery is environmentally friendly and sustainable.
- Edible cutlery is safe to eat- nutritious & healthy.
- Edible cutlery is strong and durable.
- It’s easy to make the switch to edible cutlery.

Methodology:

The methodology pertaining to the present study was as follows:

1. Selection of raw materials

Four raw materials are required to manufacture edible spoons, i.e., watermelon seed flour, wheat flour, and water. Manufacturing process begins with mixing all the raw materials into a dough. The dough is rested for about 6–10 minutes to settle the gluten. Afterward, the dough is converted into thin sheets. The sheets are placed on spoon molds and sent for baking process. After baking, the manufactured spoons are cooled for 5–10 minutes and then packed into a box.

2. Ingredient

Measure 150g of wheat flour and rinse it well, drain water. Spread on a plate and dry it completely. Then grind it into fine powder. Sieve it, discard the coarse mixture. Transfer the flour to a clean dry container. Add 70g of wheat flour then add 30g of Watermelon seed powder.

Variations	**Expansion**
WWV1	Watermelon seed flour Wheat flour V1- 10W:90 W
WWV2	Watermelon seed flour Wheat flour V2 - 20W:80W
WWV3	Watermelon seed flour Wheat flour V3-30W:70 W

WWSES – Wheat flour, Watermelon seed flour, Edible spoon

Figure 2: Ingredients

3. Mixing

Dough mixing is a process in wheat flour and water are mixed until gluten is developed, a result of the enhanced interaction between dispersed and hydrated gluten-forming proteins. It's quite different from batter mixing due to differences in their respective formulations specifically, the proportion between dry and liquid ingredients.

Figure 3: Mixing

4. Addition of water

The addition of water in the right amount can form a dough with optimum viscoelasticity properties so that the resulting gluten is also optimal This gluten ultimately holds the fermentation gas so that the dough is developed so that the optimum volume is generated.

Figure 4: Addition of Water

5. Kneading

Kneading is the massaging of dough before baking. Kneading stretches the strands of gluten in the dough, allowing for more expansion during fermentation.

Figure 5: Kneading

6. Moulding

The material to be moulded is in a viscous form and is fed into the appropriate mould. As the moulding process progresses the material becomes firmer and solidifies, up to the point that it becomes a fixed shape.

Figure 6: Moulding

7. Baking

Baking, process of cooking by dry heat, especially in some kind of oven. It is probably the oldest cooking method. Bakery products, which include bread, rolls, cookies, pies, pastries, and muffins, are usually prepared from flour or meal derived from some form of grain. Bread, already a common staple in prehistoric times, provides many nutrients in the human diet.

Figure 7: Baking

8. Packaging

Food packaging is defined as a co-ordinated system of preparing food for transport, distribution, storage, retailing, and end-use to satisfy the ultimate consumer with optimal cost. Food packaging is an essential part of modern society; commercially processed food could not be handled and distributed safely and efficiently without packaging.

Figure 8: Packaging

Here the nutritive analysis of edible spoon was calculated using the standards values given by NIN(National Institute of Nutrition).

Flow chart:

Preparation method

Selection of raw materials

↓

Wheat flour and watermelon seed flour

↓

Mixing all ingredients

↓

Addition of water/oil/milk

↓

Knead to make a smooth dough

↓

Fill in the spoon mould

↓

Bake the spoons at 160º C for 20 mins in microwave oven

↓

Allow to cool

↓

Packed in airtight container

↓

Store in dry place

Result and Discussion:

The results of sensory evaluations can be used to make informed decisions about food product development, quality control, and marketing. For example, if a manufacturer finds that

consumers prefer a new product with a sweeter taste, they can adjust the formulation accordingly. Sensory evaluation notes the human attires like taste, texture, flavour, appearance of the humans.The formulation of watermelon seed flour provides nutritious value of the product. Thus the product can improve the formulation of various Types of edible spoon. The overall acceptability of edible spoon (variation-3) were accepted by all age groups.

Nutritive analysis for WWSES:

Table 1: Nutritive analysis of variation-1

Ingredients	Quantity	Energy	Protein	Carbo.	Fat	Fibre
Wheat flour	90g	306.9	10.89	62.46	1.53	1.71
Watermelon seed flour	10g	62.8	3.41	0.45	5.26	0.08

The analysis for single edible spoon is Energy-**36.97kcal**,
Protein-**1.435g**, Carbohydrate**6.291g**, Fat-**6.79g**, Fibre-**0.179g** respectively.

Table 2: Nutritive analysis of variation-2

Ingredients	Quantity	Energy	Protein	Carbo.	Fat	Fibre
Wheat flour	80g	272.8	9.68	55.52	1.36	1.52
Watermelon seed flour	20g	125.6	6.82	0.9	10.52	0.16

The analysis for single edible spoon is Energy-**39.84kcal**,
Protein-**1.65g**, Carbohydrate**5.642g**, Fat-**1.18g**, Fibre-**0.168g** respectively.

Table:3 Nutritive analysis of variation-3

Ingredients	Quantity	Energy	Protein	Carbo.	Fat	Fibre
Wheat flour	70g	238.7	8.47	48.58	1.19	1.33
Watermelon seed flour	30g	188.4	10.23	1.35	15.78	0.24

The analysis for single edible spoon is Energy-**42.71kcal**, Protein-**1.87g**,
Carbohydrate-**4.99g**, Fat**1.697g**, Fibre-**0.157g** respectively.

Sensory evaluation:

Table 4: Sensory evaluation for variation-1

Panels Name	Appearance	Texture	Taste	Flavour	Overall Acceptability
Trained Panels					
N. Indra	5	5	4	4	5
S. Logeshwari	5	4	5	5	5
S. Swathy	5	5	4	5	5
Semi Trained Panels					
K. R Anitha	5	4	5	4	5
N. M. Jeeva	5	4	5	4	4
S. Kaizer	4	5	4	4	4
A. Raveena	5	5	4	4	5

Table 5: Sensory evaluation forvariation-2

Panels Name	Appearance	Texture	Taste	Flavour	Overall Acceptability
Trained Panels					
N. Indra	5	5	4	4	5
S. Logeshwari	5	4	5	4	4
S. Swathy	5	4	4	5	4
Semi Trained Panels					
K. R Anitha	5	4	5	4	5
N. M. Jeeva	5	4	5	4	4
S. Kaizer	4	5	4	5	4
A. Raveena	5	5	4	4	5

Table 6: Sensory evaluation forvariation-3

Panels Name	Appearance	Texture	Taste	Flavour	Overall Acceptability
Trained Panels					
N. Indra	5	5	5	4	5
S. Logeshwari	5	4	5	5	5
S. Swathy	5	5	4	5	5
Semi Trained Panels					
K. R Anitha	5	4	5	4	5
N. M. Jeeva	5	5	5	4	5
S. Kaizer	4	5	5	5	5
A. Raveena	5	5	5	4	5

Conclusion:

Edible spoons made from wheat flour and watermelon seeds are a nutritious and sustainable alternative to traditional plastic spoons. They are a good source of carbohydrates, protein, fiber, vitamins, and minerals. They are also high in calories and watermelon seeds is low in nutrients and fiber. It is also a good source of protein.

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UNDERSTANDING PLANT ELECTROPHYSIOLOGY: ACTION POTENTIALS, VARIATION POTENTIALS, AND SIGNAL PERCEPTION

Thabitha Zelin Rachel V*, Abish Carmel D, Loganathan N,
Mohamed Rashid K H and Thamizhmaran D
Department of Food Technology,
Paavai Engineering College, Namakkal, India
*Corresponding author E-mail: thabithavincentpec@paavai.edu.in

Abstract:

In certain higher plants and algae, electrical excitability and signalling are well recognised to be linked to quick reactions to external stimuli. Both animal and plant cells have been shown to produce electrical impulses called action potentials (AP), which suggests that plant cells also use ion channels to send information across great distances. Given the explosive advancements in plant biology over the last ten years, it has become clear that electrical signals are responsible for both the physiological processes and rapid leaf motions of common plants, as well as 'sensitive' plants like Dionaea muscipula and Mimosa pudica. The current analysis will summarise recent developments in the field of electrical signalling in plants and concentrate on the production and transmission of diverse electrical signals, their mechanisms of transmission inside the body of the plant and different physiological impacts.

Introduction:

The physiology of plants depends heavily on electrical signals, which control a range of functions including growth and development and reaction to external stimuli. Although they are frequently linked to the nervous systems of animals, plants also have an advanced electrical network that allows for quick coordination and communication between their tissues. This investigation delves into the intriguing domain of electrical signals in plants, revealing their workings, modes of propagation, and physiological importance in influencing plant behaviour and environmental adaptability. Gaining an understanding of these complex electrical dynamics will help you better understand the complex realm of plant biology and open up new possibilities for improving environmental sustainability and agricultural operations. Electrical signals play a crucial role in orchestrating physiological responses and adaptive behaviours in the complex field of plant biology. Plants have developed complex mechanisms to generate, transmit, and interpret electrical impulses despite not having an animal-like nervous system. These signals act as messengers, transmitting important data about stressors, internal states, and external cues to every part of the organism. Electrical signalling plays a wide range of vital functions in plants, from quick cell-to-cell contact to the synchronisation of growth and defence processes. As we

explore the nuances of electrical impulses in plants, we hope to shed light on the physiological significance of these signals as well as the amazing adaptations that enable plants to flourish in a variety of environments. Plants have an electrical activity that is concealed from view and shapes how they react to their constantly shifting surroundings. In contrast to mammals' intricate nervous systems, plants use an electrical signal network for communication, adaptation, and survival. These signals, produced by specialised cells and ion channels, act as channels for information transfer, coordinating defence, development, and resource distribution. Electrical signalling is fundamental to the dynamic physiology of plants, enabling processes like the coordinated expansion of roots towards water and nutrients and the quick closure of stomata in response to drought. In this investigation, we explore the fascinating nuances of plant electrophysiology, revealing the physiological importance of electrical signals and their significant influence on the resilience and function of plants. Electrical signals surging through plant tissues orchestrate a silent but complex performance in the symphony of life. Plants have a decentralised network of cells that can generate and transmit electrical impulses, in contrast to animals that lack a central nervous system.

Types of signals

In the context of this article, a stimulus is defined as anything that causes a reaction in a plant (referred to as a "stimulus-response coupling" in animal literature), and a signal is anything that the plant produces and sends out in response to that stimulus. As a result, although the signal needs to be produced inside the plants, the stimulus may be applied from the outside. Furthermore, when a signal reaches its target and elicits a response of its own, it turns into a stimulus in and of itself. As a perfect example to further clarify an electrical signal known as an action potential, or AP, can be generated in plants by applying voltage (a process known as electrical stimulation) and then propagating throughout the plant. It is quite likely that the cytoplasmic calcium influx is followed by potassium and chloride outflow in this travelling AP. When these AP signal sub-components, like elevated cytosolic calcium, cause reactions farther down the line, like phospholipase C activation, they turn into stimuli. When phospholipase C is present in close proximity to the plasma membrane, it functions as a signal triggers the release of inositol phosphate metabolites. Micro-electrodes have been inserted inside NITELLA cells to record APs. Following the initial intracellular recording of an AP in animal cells. Additionally, it was able to establish that MIMOSA PUDICA'S electrical signal propagation exhibited traits resembling APs in nerves. In plants, the term "electrical" has been used to describe a variety of signals, such as action potential (AP), which include spontaneous action potentials (SAPS); variation potentials (VP), also known as slow wave signals (SW); voltage transients (VT) or voltage spikes (VS); and rhythmical electrical activity (REA). We defined a "signal" as having some feature of transmission; it can be intracellular, intercellular, or systemic, i.e., throughout

the plant (which is by definition long distance), depending on the distance transmitted. Here, we'll focus on the two main long-distance signals, AP and VP, particularly in "normal" vascular plants that is, those without clear, fast visible manifestations. Non-vascular plants, notably algae with enormous cells, which are responsible for the majority of the early intracellular recordings, and plants with rapid movements will receive less attention. Plants have evolved electrical signals pathways, most likely due to their need to react quickly to outside stimuli like stressful circumstances. Recent research has demonstrated that various environmental stimuli cause distinct reactions in live cells that have the ability to send an electrical signal to the responding region.

Action potentials

APs are fast-propagating electrical messages that are widely known in animals. They move quickly along the nervous system's axons and over the surface of various muscle and glandular cells. Axons are short, travel at constant velocity, and retain a constant amplitude. They often have an all-or-nothing attitude. That is, once a stimulus has reached a specific threshold (which causes membrane depolarization), increasing its strength has no effect on its amplitude or shape. The reaction is an all-or-none depolarization that passively spreads from the stimulated part of the membrane to the adjacent, non-excited region. Thus, APs propagate electronically, with depolarization acting as a trigger for passive propagation. While the ionic mechanism of APs animals' axons depends on inward-flowing Na+ ions (depolarization) and outward-flowing K+ ions (repolarization), excitation of plant cells depends on during the repolarization phase, an outward current is generated. Plants' voltage-dependent anion channels require increased cytoplasmic Ca2+ levels for activation. Ca2+-permeable channels are thought to be a first step in an AP. Research has shown that many stressors can cause a rise in intracellular calcium. In CHARACEAE INTERNODAL cells, Ca2+ entrance trigger a C1-channels, halting cytoplasmic streaming. Ca2+-dependent anion channels were found in the CHARAPLASMALEMMA, 17, and 38 PS. Using the manganese quench technique, researchers discovered that Ca2+ is primarily released from internal storage (perhaps the endoplasmic reticulum) during AP. "MIMOSA PUDICA" produce electrical signals. (a) When the tip of a leaf pinna is triggered by spontaneous ice freezing. An AP is evoked and transferred by water or mechanical touch. Basipetally within the RHACHIS at a speed of 20-30 mm per second. The AP activates the tertiary pulvini at the base of the leaflets, creating ion and water fluxes that cause leaf movement. This type of signal terminates at the base of the pinna and is not transmitted farther. (b) When the leaf is cut, a basipetally moving VP is produced in the RHACHIS, which is uneven in shape and lasts a long time. It is slower (5-6 mm/s) than that of the AP; nonetheless, it is able to pass through secondary pulvini at the base of the pinna and induces leaflet movement.

The process advances rapidly until all accessible ion channels are open, resulting in a significant increase in membrane potential. The quick influx of sodium ions causes the plasma membrane's polarity to reverse, and the ion channels to rapidly deactivate. When the sodium channels close, sodium ions can no longer enter the neuron and are actively transported back out of the plasma membrane. The potassium channels are then activated, resulting in an outward current of potassium ions that returns the electrochemical gradient to its resting state. After an action potential, a temporary negative shift known as after hyperpolarization occurs.

In animal cells, action potentials are classified into two categories. Voltage-gated sodium channels form one type, whereas voltage-gated calcium channels produce the other type. Sodium-based action potentials typically last less than one millisecond, whereas calcium-based action potentials might last 100 milliseconds or more. Slow calcium spikes in some neurons serve as the driving power for a protracted burst of rapidly produced sodium spikes. In cardiac muscle cells, on the other hand, an initial fast sodium spike serves as a "primer" to trigger the rapid commencement of a calcium spike, which causes muscular contraction.

Variation potential

The electrical signals that slow wave potentials, or VPs, propagate also include a temporary alteration in membrane potential. Less variance and longer, delayed repolarizations are the key differences with APs. This non-self-sustaining signal fluctuates in response to the stimulus's strength and seems to represent a local shift in either a hydraulic pressure wave or chemicals carried via the dead xylem. Many plant species, including cucumber and pea seedlings, as well as woody species like vitis vinifera, have been researched for this phenomenon, which can be produced by wounding, organ excision, or fire. When the VP moves farther away from the site of injury, its amplitudes and velocity diminish. The capacity of the VP to pass through areas of dead tissue, its dependence on xylem tension, and amplitudes and speeds that diminish with increasing distance from the damaged site are its distinguishing features. VPs won't form under saturated humidity, when xylem tension is low. Because of their delayed repolarization phase, some authors refer to VPs as slow wave potentials as well. They have a different ionic mechanism than AP's; suggest that it involves a temporary shutdown of a P-type H+ ATP in the plasma membrane. Cutting the tip of a leaf pinna in Mimosa Pudica produced a VP in the rhachis that was longer-lasting and of irregular form than an AP. Changes in pressure/tension and chemical transfer are the two primary contenders for the xylem-transmitted component. There is strong evidence that both primary signal kinds exist, and their existence is most likely. MSC must underpin the former explanation of pressure/tension changes in VP (since it is dependent on ion channels and not VGC), whereas ligand-activated channels (LAC) must underpin the latter explanation of chemicals in the xylem. When a single leaf is subjected to heat shock, the entire stem essentially relaxes instantly, and the MP changes as one gets farther away from the site of the shock. These changes in MP and stem relaxation can be simulated by

applying brief or continuous mild pressure to a single leaf. Similar changes were also observed in wheat leaves, who at the time suggested a mechanic sensory explanation. More recently, however, Malone and colleagues have come to support a role for chemicals transmitted in the xylem. Hydraulic, variation potential propagation is achieved by rapidly increasing pressure and creating an axial pressure gradient in the xylem. As one moves farther away, this gradient changes into progressively longer lag phases for the pressure-induced depolarization of the epidermal cells. This enables communication along the plant's axis that can travel in both directions between the leaf and stem. One argument that could support or refute the involvement of ion channels in VP formation is the existence or lack of changes in the conductivity of the plasma membrane. An evidence in favour of the absence of ion channel involvement in VP generation was provided by Stahlberg *et al.*'s54 observation that VP development did not cause changes in plasma membrane conductivity in pea. A study conducted on wheat leaves, however, revealed that increased plasma membrane conductivity accompanied VP generation83. This finding suggests that ions channels are activated during VP creation. Because total plasma membrane conductivity involves both H+-ATPase conductivity and ion channel conductivities, it makes sense to explain these contradicting facts. Because of this, an increase in ion channel conductivities (ions) can somewhat alter total conductivity.

Difference between AP and VP

The shape, velocity, and reliability of these signals represent the most efficient ways of distinguishing between them. The VP, as its name implies, is significantly more variable, typically appearing as a quick rise followed by a protracted decrease, often with spikes superimposed and/or interspersed. In contrast, the AP typically consist of a sharp rise, a brief peak, and a sharp return to near baseline. The “all-or-none” nature of the AP is reflected in its nearly constant rate and magnitude of transmission. On the other hand, the VP changes in characteristics depending on the intensity and distance from the stimulus site, and its amplitude decrease with increasing distance from the site of formation. These signals can be identified not only by their electrical characteristics but also by how they relate to the stems deformation (elongation or contraction). Furthermore, as the VP gets smaller and “slower” as it moves through the plants, the AP practically stays the same. Not the VP itself travels; instead, it might be molecules in the xylem or a reduction of tension (hydraulic signal). Each plant experienced this brief contraction to a different extent. It depended on the water status of the plant as well as the transducer’s location along the stem. While the contraction of the two primary electrical signal types often fell between 10µm and 100µm for the AP, it occasionally exceeded 150µm for the VP, which is a necessary step in identifying which µm. The hydraulic component, or changes in tissue dimension, can cause an electrical reaction in the form of a voltage potential (VP) since these changes in tissue dimension occurs before changes in electrical potential, or the electrical component. The variation potential is a reflection of membrane depolarization, which once the

threshold is crossed, can in and of itself cause an action potential. By contrast, during the AP, the decrease in stem elongation either happens concurrently with the electrical change or very closely follows the electrical change. Therefore, the reason for the reduced stem elongation could be the electrical signal (AP). In order to differentiate the AP from the VP, more research would be required. This research would need to use a variety of techniques, such as stripping the bark, phloem-girdling and/or applying a cold block to prevent phloem AP, and/or high humidity to likely lower the VP. A study of this kind would be a valuable contribution to the body of research, but us aware of any such "complete" report. As any change in MP, including one prompted by a VP, can elicit an AP, there is a cross-talk between the VP and AP. Therefore, the tests will need to be conducted carefully. In fact, pressure can be transduce to produce an AP even though it is typically sensed by MSC and causes a VP.

Measure of AP and VP

To measure changes in MP, an electrode in contract with the plant must be linked to a recording device. Small electrical changes require amplification and recording. A high impedance device is required to prevent the plant's electrical output from driving the recording device and causing the signal to "disappear". Extracellular electrodes, such as surface contact or wire piercing, are the most basic options for application. Surface contact electrodes, like the lie detector electrodes, avoid causing tissue damage, making them ideal for researching wound reactions. However, the presence of KCL in these electrodes causes them to dry out, leading to alterations.

The approach for vascular plants is limited to big, accessible cells, such as root hairs or pollen tubes. To measure tissue, organs, or complete plants, they must be immersed in the bathing media, which can only be done on small seeding. Aphid's capacity to probe phloem sieve tubes has lately been used by numerous workers. Aphids are typically permitted to enter the phloem sieve tube, then their heads are severed, leaving their mouthparts in the phloem. Electrodes are then gently put into the aphid's mouthparts, providing direct access to the sieve tube.

This permits the AP travels in the phloem sieve tube; it may also pass via other tissues. The vibrating probe electrode can also measure changes in MP. However, it is also necessary to use a bathing medium. These approaches can measure particular ions in addition to MP, allowing for identification of ion fluxes in AP and VP in accessible cell types. Traditionally, a chart recorder was used to translate the signal from the electrode system into a visible representation. These were satisfactory for most application has been identified.

Extracellular recording

Extracellular measures are commonly used in animal electrophysiology to assess bioelectrical activity in large groups of cells. For ECGs and EEGs are commonly utilized in medicine. Higher plants can measure extracellular potential in two ways: surface recordings and

with metal electrodes implanted. To avoid wound responses when inserting electrodes, thin metal wires (e.g., AG/AGCl-wires 0.4-1.0 mm in diameter) must be used. When electrodes are inserted into shoot or leaf veins, they come into contact with tissue that contains larger cell groupings. For example, recordings are done in the cambial region of several tree species.

When neurons receive appropriate stimuli from neighbouring cells, their membranes depolarize and create ionic current. Currents will circulate in the surrounding cytoplasm. An appropriate electrode near an activated neuron can monitor the voltage drop associated with the extracellular current, or action potential. An extracellular action potential ranges in amplitude from 50 to 500 µV and frequency from 100 Hz to 10 kHz. To capture these neuronal impulses, the electrode must pass through the extracellular space to reach the activated neuron without harming the neuron or surrounding cells. The recording electrode should be tiny and non-invasive. Glass micropipettes are one form of recording electrode. To make these devices, a 1- to 2-mm diameter glass capillary is heated and pulled into two parts. Commercial pipette pullers provide temperature control. The force used to pull the capillary determines the taper of the final tip, which can reach 0.1 µm in diameter. Bevelling the tip can help define its dimensions and impedance more accurately. To create a conductive link with the tissue, a drawn pipette is filled with an electrolyte solution like KCL. A large-area reversible electrode is then introduced from the top of the pipette to couple to the external world. Glass electrodes offer unique electrical characteristics.

Inracellular recording

Glass microelectrodes with tip sizes of less than 1 um are typically used for intracellular electrical signal and membrane potential measurements. These electrodes are coupled to a high-input impedance amplifier, clamped in AG/AGC1 pellet holders, and filled with KCI. Using micro-manipulators, one microelectrode is carefully placed into the cytoplasm (or vacuole) of a cell once the amplifier has been zeroed with both electrodes outside the cell. While the solution surrounding the cell is in contact with the reference electrode. The recorded potential changes negatively, and the amplifier records this information when the microelectrode breaks through the cell membrane. This is the resting membrane potentials, usually with values between -80 and -200mV in the plants. The detection of signals with high velocities depends on intracellular recordings or undamaged sieve elements because phloem cells' very low resistance connections (sieve holes) enable long-distance electrical signalling. It is challenging to place microelectrodes since the phloem is housed inside the plant's body.

As microscopic examinations during the experiment reveal, the microelectrode up is frequently not put correctly into the phloem, making recordings in conjunction with dyes injected into the cell after acquiring electrical signals a time-consuming technique. Utilizing the "aphid technique," however, makes it possible to identify changes in the membrane potential or sieve tubes that mimic plant behaviour. Aphids are moved to fully grown leaves and left there for the

night. A laser pulse is used the next day to cut an aphid off from its stylet. The tip of a micro-electrode is linked to the sieve tube sap that the stylet stump exudes.

The electrical resistance of the stylet can be approximately calculated using the diameters of its feeding canal. If the canal were filled with 100 mm KCI and had an average area of 6, its resistance would be approximately 2.6 x 10.0° S. This result is still within the range of the electrometer that was used, even though it is around three times higher than the average resistance of a microelectrode (input impedance > 1012 2). Furthermore, the hardened saliva that surrounds the stylet acts as electrical insulation. An electrode that is in touch with the plant and connected to a recording device is necessary to measure any changes in MP. Small electrical changes typically require signals to be amplified, and a high impedance recording device; otherwise, the signal "disappears" since the recording device is driven by the plant's electrical output. Extracellular electrodes are the easiest to employ; these can be wire electrodes or surface contact electrodes. Surface contact electrodes provide the benefit of not causing tissue damage, which is crucial for researching how wounds react. These electrodes can only be used for brief (a few hours) recordings since they contain KCL and have a tendency to dry up, which alters the ionic condition of the region being examined. The drawback of piercing electrodes (silver, platinum) is that they can cause harm, although this issue can be avoided by letting the tissue heal from the wound. They have the benefit of being reusable for several days or even weeks, and they don't significantly change the tissue's ionic state. We have observed nearly identical recordings with both types of electrodes placed close together on the same plant. Both electrodes measure the concentration of apoplectic ions in the vicinity of the electrode. An additional electrode is required in order for the circuit to be completed. This may be a reference electrode at another location on the plant, or it could be a real ground electrode—that is, one in the soil. When using a reference electrode at a different location on the plant, one measures the difference in MP between what is happening at the reference and what is happening at the measuring electrode. With a ground electrode, one measures the change in MP at the measuring electrode compared with (presumably) no change in the ground electrode.

Perception of signals

Environmental stimuli like temperature changes, contact, or injury can produce electrical impulses at any point along the simplistic continuum. It was recently discovered that action potentials in soybeans are also induced by acid rain, in addition to irradiation at different wavelengths. The action potentials have duration times and amplitudes of roughly 0.3 Ms and 60 mV, respectively. This process, which results in an electrical replica of the stimulus that lasts for the duration that the stimulus is present, is called receptor potential creation. An AP is produced when the stimulus is strong enough to depolarize the membrane below a predetermined threshold. Electrical signals can spread to adjacent symplast cells through plasmodesmata after being perceived. Nonetheless, light stimulation can also cause AP’s. In terms of hurting or

igniting, a hydraulic wave that travels through the xylem typically induces so-called VPs. It is unclear how the hydraulic wave is able to trigger a local electrical reaction in the cells next to it. Large water inflows into live cells likely cause membranes to stretch, which may impact mechanosensitive ion channels or, in the case of chemical transport, ligand-activated channels.

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ECO-TILLING – A PROMISING TOOL TO DISCOVER SUPERIOR NATURAL VARIANTS IN PLANT GENETIC RESOURCES

Mithraa T

ICAR – National Bureau of Plant Genetic Resources, New Delhi.

Corresponding author E-mail: mithuagri@gmail.com

Abstract:

Allele mining is a systematic process of identifying and exploring genetic variations within a species, particularly focusing on alleles that exhibit beneficial traits. It helps in the development of climate-resilient, high-yielding, nutrient-rich plant varieties in addition to tracing the evolution of alleles, discovering new haplotypes, and constructing allele-specific markers irrespective of plant's genome size, ploidy level, or reproductive system. In the realm of functional genomics, TILLING and Eco-TILLING function as reverse genetics approaches. While TILLING primarily relies on induced mutagenized populations, Eco-TILLING explores the natural genetic variations present across diverse geographical origins. The Eco-TILLING platform's efficiency and promptness can significantly expedite the process of breeding programs for crop species by identifying an extensive range of desirable alleles. In this chapter, it is discussed about the Eco-TILLING strategy, its application in crop improvement, advantages, and challenges.

Introduction:

The global population is projected to be 9.1 billion by 2050 and so the overall food production requires about 50-60 percent raise in between 2019 and 2050. To meet the demand, collection, conservation, and utilization of plant and animal germplasm becomes essential. The crop domestication and current agricultural practices have led to genetic uniformity, genetic drift, decline in genetic potential, and loss of minor alleles rendering them highly susceptible to novel biotic and abiotic stresses. Conservation and utilization of genetic resources allows the breeders to combine the resistant or tolerant genes, to withstand adverse climatic conditions or special quality traits including colour, flavour, shape, size, and nutritional composition in crops. Genebanks serves as an asset for the scientific community by providing a source to fill genetic gaps, increase the genetic variability, and /or parental material for the crop improvement programme. To attain this goal, isolation of induced or natural SNP polymorphism for the specific trait of interest can be done by employing two approaches namely, Forward genetics and Reverse genetics. Conventionally, forward genetics approach (phenotype to genotype), initially screens for the phenotype of a large scale of germplasm accessions and the gene sequence is deduced. However, it is a tedious process due to its effort and time taken to identify the

gene/SNP coding for a particular phenotype. While, Reverse genetics (genotype to phenotype) approach requires less time than forward genetics. It involves the known gene sequence and mutants screened initially to identify individuals with trait of interest. The reverse genetics strategies include homologous recombination, transposon tagging, insertional mutagenesis, chemical mutagenesis, RNA interference and Post Transcriptional Gene Silencing (PTGS). In the era of next generation sequencing (NGS), the widespread access of sequencing data allows the researchers to promptly design the reverse genetic strategy in determining gene function.

Allele mining

Allele mining is the process of systematically exploring and identifying genetic variation within a species, with a specific focus on alleles that exhibit desirable traits. This involves screening diverse germplasm collections, such as landraces or wild relatives, to identify novel alleles associated with traits of interest, including disease resistance, abiotic stress tolerance, or improved agronomic traits. Advancement in next-generation sequencing (NGS), in parallel with the techniques such as TILLING, Eco-TILLING, and association mapping (AM), have streamlined allele mining approaches, making them more practical, less burdensome, and relatively cost-effective. Among them TILLING and Eco-TILLING remains substantial in their role. TILLING, which stands for Targeting Induced Local Lesions IN Genomes was developed by Claire McCallum, along with collaborators from the Fred Hutchinson Cancer Research Center and the Howard Hughes Medical Institute in the late 1990's while investigation the chromomethylase genes function in *Arabidopsis*. The primary stages of TILLING are as follows: first, the development of a mutant population; second, the isolation and pooling of DNA from the mutant population; third, the identification of desired genes and the design of primers for amplifying gene regions containing anticipated SNPs; and fourth, the detection of mutations through the screening of heteroduplexes formed by SNPs (Tadele (2016)). TILLING by Sequencing method employs high-throughput next-generation sequencing techniques to simultaneously screen multiple mutations (Tsai *et al.* (2011)).

Eco-TILLING in plants

Eco-TILLING, also known as Ecotype- Targeting Induced Local Lesions IN Genomes, is a molecular technique like TILLING, with the main difference being its focus on uncovering natural genetic variation rather than induced mutations. As many species are not suitable for chemical mutagenesis, Eco-TILLING provides a valuable means of discovering natural variants and their potential gene function (Comai *et al.* (2004); Zhang *et al.* (2018)). It is a valuable approach for identifying naturally occurring single nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs) in known genes from diverse geographical origins, leading to the development of variant populations with a broad genetic base. This technique was introduced by Comai and colleagues in 2004 to identify polymorphisms within the natural population of

Arabidopsis ecotypes and was later designated as "Eco-TILLING" (Comai *et al.* (2004)). The first study on Eco-TILLING identified a range of polymorphisms in five targeted genes of a screened natural population of *Arabidopsis* (Comai *et al.* (2004)). The method employed entailed combining each ecotype with the standard Columbia ecotype in a 1:1 ratio. A total of 192 accessions were examined, revealing 55 haplotypes across five genes of approximately 1 Kb in length. A significant portion of the variation was identified in the introns. The study demonstrated that CEL I could detect SNPs, INDELS, and polymorphisms in microsatellite repeats. Notably, the enzyme also detected and cleaved a 21 bp deletion. In general, Eco-TILLING was proven to be an effective method for haplotyping individuals without requiring sequencing of all the individuals involved in the study. In addition to this, this technique enables rapid screening of multiple samples containing a gene of interest to identify naturally occurring SNPs and small INDELS, making it a useful tool for plant breeding and genetics research.

Table 1: List of Bioinformatic tools useful for Eco-TILLING

Name of the tool	Application	URL
GenBank	Targeted gene sequence	https://www.ncbi.nlm.nih.gov/genbank/
Primer3	Primer design	https://primer3.ut.ee/
ClustalW2	Multiple sequence alignment	https://www.genome.jp/tools-bin/clustalw
PlantPromDB	Plant promoter database	http://www.softberry.com/
CODDLE	Codons Optimized to Discover Deleterious Lesions - Determine the regions of the target gene in which G/C to A/T transitions are most likely to cause deleterious effects on the protein.	http://www.proweb.org/coddle/
PARSESNP	Project Aligned Related Sequences and Evaluate SNPs - Presents the locations of polymorphisms in a gene or genes in a graphical format.	http://www.proweb.org/parsesnp/
SIFT	Sorting Intolerant from Tolerant - predicts mutation effect on the protein	https://provean.jcvi.org/
MEGA	Molecular Evolutionary Genetics Analysis and Sequence alignment	https://www.megasoftware.net/
TRANSFAC	Transcription factor (TF) and TF binding motifs Database	https://genexplain.com/transfac/
EPD	Eukaryotic promoter database	https://epd.expasy.org/epd/

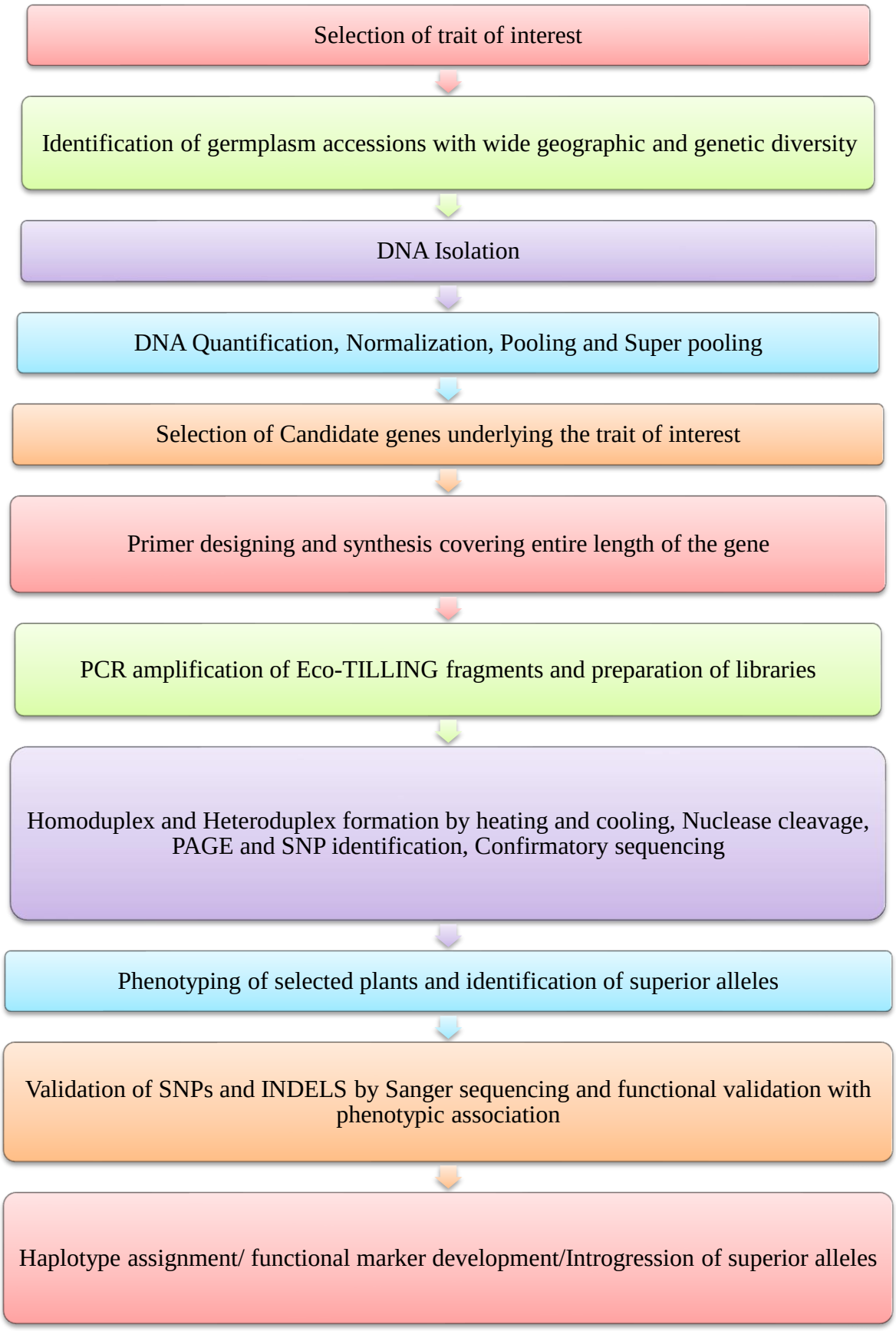

Figure 1: Steps involved in Eco-TILLING assays

Eco-TILLING in germplasm characterisation

The application of Eco-TILLING for the characterization of germplasm and population genetics was initially explored in studies on the western black cottonwood (*Populus trichocarpa*) (Gilchrist *et al.* (2006)). After the initial development and application Eco-TILLING for genotyping black cottonwood, multiple research groups have since applied the technique for characterizing germplasm in various plant species. For example, Rakshit *et al.* (2007) utilized Eco-TILLING to estimate linkage disequilibrium in rice, while Barkley and colleagues developed an Eco-TILLING platform for characterizing *Vigna radiata* (mung bean) germplasm held by the USDA-ARS Plant Genetic Resources Conservation Unit (Barkley and Wang (2008)). Low-throughput and cost-effective methods, like traditional Eco-TILLING, will continue to serve as valuable tools for smaller-scale projects where resources are scarce, or outsourcing is not practical. These low-cost techniques, like agarose gel Eco-TILLING, will likely remain essential in developing countries with abundant local germplasm that is largely uncharacterized.

Table 2: Application of Eco-TILLING in crop improvement

S.No.	Crop	Targeted Gene	Reference
1	Rice	Gelatinization temperature: ALK gene - Alkali degeneration gene	Kadaru *et al.* (2006)
2	Rice	Amylose synthesis: ECQ gene - Waxy gene	Kadaru *et al.* (2006)
3	Rice	Amylose biosynthesis and amylopectin synthesis: GBSS I, SS I, SS IIa, SS IIIa, SBE Ia, SBE IIb	Raja *et al.* (2017)
4	Rice	Salt stress tolerance: OsCPK17 - Calcium-dependent protein kinase, and salt-tolerant genes, OsHKT1;5 and OsRMC	Negrão *et al.* (2011)
5	Rice	Drought tolerance: OsALF11, OsGRF5, OsAE115, OsGRF8, OsNFYB12, OsAE25, OsAE128, and OsZIM14	Yu *et al.* (2012)
6	Wheat	Grain hardness: Puro-indoline proteins Pina and Pinb	Hourston *et al.* (2017)
7	Wheat	Vernalization: VRN1	Chen *et al.* (2011)
8	Wheat	Starch synthase: TaSSIV	Irshad *et al.* (2019)
9	Barley	Environmental stress tolerance: HSP17.8	Xia *et al.* (2013)
10	Barley	Powdery Mildew resistance: mlo and Mla	Mejlhede *et al.* (2006)
11	Barley	Light harvesting: chlorophyll A/B-binding protein: Lhcb1	Xia *et al.* (2012)

12	Mung Bean	Intron spanning targets	Barkley *et al.* (2008)
13	Common Bean	virus resistance: SR2	Galeano *et al.* (2009)
14	Chickpea	100 Seed weight: bZIP, SBP, Zinc finger-domain containing protein, NAC bHLH, AP2-EREBP, ARF, and mTERF	Bajaj *et al.* (2016)
15	Cotton	Sucrose synthase: GhSus	Zeng *et al.* (2016)
16	Brassica	Low erucic acid content: FAE1	Wang *et al.* (2010)
17	Soybean	soybean cyst nematode resistance: Rhg4 locus LRR-RLK	Liu *et al.* (2011)
18	Melon	Virus infection: eIF4E	Nieto *et al.* (2007)
19	Capsicum	Virus resistance: eIF4E and eIF(iso)4E	Ibiza *et al.* (2010)
20	Tomato	Virus resistance: SIeIF4E	Rigola *et al.* (2009)
21	Tomato	Brix content: Tiv1, lin7, SUS3, and Frk2	Bauchet (2010)
22	Beet	Flowering time: BvBTC1, BvFL1, and BvFT1	Frerichmann *et al.* (2013)
23	Banana	Hypocotyl length: NPH3	Till *et al.* (2010)
24	Banana	Nutrition: PSY and LYB	Mmeka *et al.* (2013)

Advantages of Eco-TILLING

- Eco-TILLING is a versatile approach that can be utilized for any organism, regardless of their reproductive system, genome size, ploidy level, or whether they are wild or cultivated.
- Without employing mutagenesis, it is ideally suited for detecting natural variation within populations or even natural mutations among germplasm.
- Single nucleotide polymorphisms (SNPs), small insertions and deletions (InDels), and variations in microsatellite (SSR) repeat number can all be employed to identify genetic variations.
- It has the potential to benefit a wide variety of crop species by allowing for the simultaneous screening of numerous individuals at a single genetic locus. These projects can identify rare haplotypes and enhance the genetic diversity of heterozygous populations.
- It enables rapid and cost-effective identification of all possible variations in coding and regulatory regions.
- In the context of functional genomics, it serves as a valuable tool that can aid in deciphering the functions of numerous newly discovered genes.

- It is a non-transgenic method in reverse genetics, particularly for the purpose of allele identification.

Difficulties faced in Eco-TILLING

- One potential drawback in the identification of polymorphisms is the possibility of false negatives resulting from background noise generated by mispriming or weaker fluorescence, (Frerichmann *et al.* (2013)).
- Determining the appropriate germplasm and selecting the optimal and precise phenotyping techniques to characterize the chosen germplasm.
- Delineating and identifying promoters in non-coding regions is an extremely challenging task.
- Large-scale genetic resources stored in gene banks necessitate interdisciplinary expertise in their management.
- Developing core collection of germplasm to be 'mined' is a pressing issue.

Conclusion:

In summary, the Eco-TILLING method presents a practical and efficient approach for the development of crop plants with superior alleles. This method involves the identification of superior variant alleles through marker development and haplotype assignment, followed by the introgression of these alleles into desirable germplasm through classical breeding or CRISPR/Cas9 genome editing. The Eco-TILLING approach is non-transgenic, which enables rapid and cost-effective development of new varieties with free regulatory paradigm. Therefore, Eco-TILLING platform takes advantage of the natural genetic diversity and create novel superior alleles for crop plants.

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IMPORTANT ASPECTS OF ALGAE AND ITS AGRICULTURAL USES: A MINI REVIEW

Vilas V. Thakare*[1], Atish R. Mehetre[2], Sanjay M. Pawar[3] and Rajendra S. Gosavi[4]

[1] Department of Botany,

S.S.S.P.M's Arts, Science and Commerce College, Rahuri, Dist. Ahmednagar (MH)

[2]Department of Chemistry,

Shivaji Arts, Commerce and Science College, Kannad, Dist.- Chh. Sambhajinagar (MH)

[3]UG, PG and Research Centre, Dept. of Botany, Shivaji Arts, Commerce and Science College, Kannad, Dist.- Chh. Sambhajinagar (MH)

[4]Department of Electronic Science,

S.S.S.P.M's Arts, Science and Commerce College, Rahuri, Dist. Ahmednagar (MH)

*Corresponding author E-mail: vilasthakare11@gmail.com

Abstract:

Algae are a diverse group of photosynthetic microorganisms. They play a crucial role in agriculture as biofertilizers and soil stabilizers that increase the soil fertility. Algae are cultivated all around the world for fertilizers and food supplements also. The milk producing cattles has been fed with the seaweeds to increase the iodine level in the milk. The algae feed additives also increased the egg laying rate in hens. The yield of paddy is increased by using nitrogen fixing blue-green algae like *Anabaena oryzae*, *Auloxira fertilissima*, *Nostoc commune*, *Tolipothrix tenius*, *Calothrix canfervicola* etc. The seaweeds extract is very rich in minerals, vitamins, amino acids, plant growth regulators which are particularly used in organic farming. The role of algae is an interesting matter regarding with increasing soil fertility, promoting plant growth, development and increasing yield of various crops hence we discussed the most important aspects of algae and its agricultural uses in this article. This review article gives an overview of the research work that has been done regarding the use of algae in agriculture.

Keywords: Algae, Agriculture, Biofertizers, Nitrogen Fixation

Introduction:

The science that deals with the study of algae is known as algology. The Greek word for algae is *Phycos* (meaning seaweed) hence the study of algae is also called as Phycology. Algae are a large and diverse group of microorganisms that carried out photosynthesis. The thallus size of algae may range from 1 mm to over 50 meters (Vymazal, 1995). They can found almost in every habitable environmental condition on the earth including fresh water, marine water, brakish water, soil, ice, snow fields, hot springs. They are major primary producers of all aquatic ecosystems. They play an important role in soil fertility, soil reclamation, bio-controlling of

agricultural pests, agricultural waste water treatment and recycling of treated water (Abdel-Raouf *et al.,* 2012).

Agriculture is facing a pressure to meet the increasing food demands of the increasing world population. Traditional fertilizers are obtained from declining nonrenewable resources. (Alice *et al.,* 2023). The crop productivity has been affected by the developing pests and diseases, increase of water scarcity, intensive use of synthetic fertilizers (Chatterjee *et al.,* 2017). The organic farming practices appears as a solution to increasing demand for healthy foods that promotes the use of natural bioproducts like biofertilizers, biostimulants and biopesticides (Clavo *et al.,*2014; Bulgari *et al.,* 2015; Colla and Rouphael, 2015; Chatterjee *et al.,* 2017; Win *et al.,* 2018).

The success of agriculture depends on the soil fertility. Algae found in different soil types can help the soil to improve its characteristics like carbon content, texture, aeration (Ibraheem, 2007) and nitrogen fixation also (Hamed, 2007). Blue-green algae are used as biofertilizers in rice based cropping systems and serve as the cheapest sources of natural biofertilizers (Omar, 2000).

Previous work revealed that various researches carried out their research with respect to importance of algae in agriculture including Aboul-Fadl *et al.* (1967), Bailey *et al.* (1973), Banerjee and Kumar (1992), Adam (1999), Haroun and Hossein (2003), Nisha *et al.* (2007), Calvo *et al.* (2014), Bulgari *et al.* (2015), Chatterjee *et al.* (2017), Akgul (2019), Atzori *et al.* (2020), Bao *et al.* (2021), Viegas *et al.*(2021a), Alice *et al.* (2023) etc.

Algae as fertilizers:

Seaweeds like *Aland, Ecuonia, Limnothamnion, Macrocystis, Phymatopthon* and all other drift seaweeds are used as fertilizers. Seaweed fertilizers are rich in potassium, phosphorous and trace elements such as cobalt, manganese and boron which are required for the plant growth and are applied directly or in the form of compost (Awasthi,2022).

Algae as biofertilizers:

Blue-green algae or Cyanobacteria has been used as biofertilizer in the paddy fields of major rice producing countries like China, Japan, Thailand, Philippines and India. The paddy fields are inoculated with the BGA like *Anabaena, Nostoc* and *Tolypothrix* at the time of crop planting which increased crop yield by around 15% (Awasthi,2022).

Algae as nitrogen fixer:

Some heterocystous as well as non-heterocystous BGA are having capacity to fix the atmospheric nitrogen into soluble form of nitrogen like nitrates, nitrites and ammonia required for the growth and development of plants that includes *Nostoc, Anabaena, Tolypothrix, Aulosira, Calothrix, Oscillatoria, Anabaenopsis, Gloeocapsa, Scytonema* etc. (Awasthi,2022).

Algae as source of growth hormones:

Some BGA like *Phormidium foveolarum*, *P. tenue* and *P. frigidium* have been reported to have growth hormones that promotes the yield of paddy crop. BGA secretes growth promoting compounds like IAA, IBA, NAA, amino acids, proteins and vitamins which are required by the plants for their growth and development (Awasthi,2022).

Conclusion:

In this review article, an attempt was made to discuss the various beneficial roles of algae in agriculture with respect to the relationship of algae with crop plants. They are major primary producers of all aquatic ecosystems. The important aspects of algae are to increase soil fertility, soil reclamation, bio-controlling of agricultural pests, agricultural waste water treatment and recycling of treated water, The success of agriculture depends on the soil fertility. Algae found in different soil types can help the soil to improve its characteristics like carbon content, texture, aeration and nitrogen fixation also. The yield of paddy is increased by using nitrogen fixing blue-green algae like *Anabaena oryzae*, *Auloxira fertilissima*, *Nostoc commune, Tolipothrix tenius, Calothrix canfervicola* etc. The seaweeds extract are very rich in minerals, vitamins, amino acids, plant growth regulators which are particularly used in organic farming. The algae also play an important role in promoting plant growth, development and increasing yield of various crops hence the most important aspects of algae and its agricultural uses are discussed in this review article.

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A MINI-REVIEW ON PHYTOCHEMICAL SCREENING, BIOLOGICAL ACTIVITY, AND THERAPEUTIC CAPABILITY OF HIBISCUS: AN ORNAMENTAL PLANT SPECIES

Alok Ranjan Sahu*[1], Nihar Ranjan Nayak[2] and Nirius Jenan Ekka[3]

[1]Department of Botany, Vikash Degree College, Bargarh, Odisha

[2]Department of Botany,

Maa Manikeshwari University, Manikya Vihar, Bhawanipatna, Odisha

[3]School of Life Sciences, Sambalpur University, Jyoti Vihar, Odisha

*Corresponding author E-mail: alok.btgene@gmail.com

Abstract:

Hibiscus rosa-sinensis, commonly known as China rose belongs to family Malvaceae, is a prominent flower throughout the Asian subcontinent's tropical regions. It is rich in subtropical and tropical locations, and it is generally grown as an ornamental plant. This review explicates the distribution and morphological description of the *Hibiscus* plant, along with its phytochemical as well as various bioactive chemical composition and their structures, the ethno-medicinal and botanical benefits on human health, and toxicity research. Altogether, present study affords an all-inclusive understanding of the plant *Hibiscus*, including its physical, chemical, and structural characteristics.

Keywords: *Hibiscus rosa-sinensis*, Phytochemistry, Bioactive Compounds, Ethno-Medicinal Properties

Introduction:

Hibiscus rosa-sinensis, commonly known as China rose or the "Queen of Tropics," is mainly found in southeast China and certain Pacific and Indian Ocean islands. This flower symbolizes both Malaysia and Haiti's national flower, thus making it unique (Yuan *et al.*, 2016). These flowering species are well-known for their big, colourful flowers, which are commonly referred to as "*Hibiscus*" or, on occasion, rose mallow. Other names for this plant include hardy *Hibiscus*, rose of Sharon, and tropical *Hibiscus*. In some areas, the common garden *Hibiscus*, scientifically known as *Hibiscus syriacus*, is also known as the "rose of Althea" or "rose of Sharon" (Akpan, 2007). In tropical and subtropical regions, the Chinese Hibiscus (*H. rosa-sinensis*) with its several huge hybrids is still the most common choice. Hawaii has a specific fondness for the Hibiscus, which is considered one of their most prized national flowers, and they normally wear it in their hair for cultural events. Carolus Linnaeus first named the plant (Linnaeus, 1753). This plant blooms in subtropical and tropical climates and is extensively grown for ornamental purposes. It has large flowers on its dense hedges, which are

deep crimson and lack scent. This plant belongs to the subkingdom Magnoliophyta and the class Magnoliopsida, which means it is a vascular plant that reproduces through seeds. There are more than 300 species under the genus *Hibiscus*, which belongs to the Malvaceae family. Besides, the extract composed from the leaves and flowers has long been employed as a natural medicine for many diseases and painful symptoms, in addition to in herbal cosmetics such flaccid (Jadhav *et al.,* 2009; Missoum, 2018).

The leaves are simple oblong or ovate-lanceolate, with coarse teeth at the apex. Flowers feature pedicels and are made up of five crimson petals. In Nigeria's Akwa Ibom State, the young leaves are commonly consumed as a vegetable and are reputed to have therapeutic benefits. *Hibiscus rosa-sinensis* has played an important role in human health because it contains unique biologically active chemical components (Al-Snafi, 2018). Over 50% of all modern clinical medications are derived from natural products. *Hibiscus* has traditionally been utilized as an anti-asthmatic agent, analgesic, anti-inflammatory, antipyretic, anti-tumor, and anticonvulsant (Vincenta and Patel, 2016). The rise of antibiotic-resistant strains of bacteria and fungi is common, necessitating the search for and use of alternate sources of antimicrobial agents against the majority of human pathogens and microorganisms accomplished of causing disease in humans. Many investigations have found antibacterial activities in the blooms of *Hibiscus rosa-sinensis*. The leaf extracts of *H. rosa-sinensis* L. comprise variable quantities of alkaloids, tannins, saponins, flavonoids, cardiac glycosides, anthraquinones, and phlorotannin, that enhances their therapeutic potentiality (Tiwari *et al.*, 2015; Al-Snafi, 2018; Vastrad and Byadgi, 2018).

Review of literature:

Distribution and description

India has a wealth of traditional medicinal systems and rich biodiversity to sustain the herbal rations of the treatment provided by its traditional medical systems. Ayurveda, Siddha, and Unani are the known Indian systems of medicine, utilizing herbs and minerals in their formulations. In India, across 15 agro-climatic zones, 4700 plant species of which 15000 are reported to have medicinal properties varying degrees. *Hibiscus rosa-sinensis* is cultivated in tropical and subtropical regions and the region of tropical Asia. It *is* a bushy evergreen shrub of 2.5-5 m (8-16 ft) tall and 1.5-3 m (5-10 ft) wide. It has glossy leaves. The colour of the flowers varies from red, brilliant red, white, pink, orange, peach, to purple and yellow; and generally, flowering occurs in summer and autumn. The flower is five-petaled and is 10 cm in diameter, with a red tip anther. At the bottom of every *Hibiscus* bud, green colour calyx is present and the ends of the points are called sepals. It is generally grown as an ornamental plant (Mohamed *et al.,* 2014; Singh and Khan 2017).

The flower of *Hibiscus* comprises the ovary and other female parts within the pistil, a long tubular structure. A single flower possesses both male and female apparatuses, with pollen being assembled at the top of the stigma and the style in the middle, allowing pollen to reach the ovary. The ovary is located at the base of the blossom, and has a single superior ovary. Additionally, the *Hibiscus* boasts many stamens. The stem is aerial, green, cylindrical, and branched, while the leaf is simple with alternate phyllotaxy and a petiole. The leaf is ovate, with an acute tip and serrated margins. These herbs are not only profitable but also easily available associated with modern alternative pharmaceutical agents (Jadhav *et al.,* 2009).

Chemical configuration

The preliminary phytochemical investigation showed that *Hibiscus rosa-sinensis* was found to have a variety of compounds such as tannins, anthraquinones, quinines, phenols, flavonoids, alkaloids, terpenoids, saponins, cardiac glycosides, protein, free amino acids, carbohydrates, reducing sugars, mucilage, essential oils, and steroids. Additionally, it also contained cyclopropanoids, methyl sterculate, methyl-2-hydroxy sterculate, 2-hydroxysterculate, malvalate, and beta-sitosterol. The primary anthocyanin present in the flower was cyanidin 3-sophoroside, while the flowers were found to contain four different types of flavonoids: rutin, quercetin, kaempferol, and myricetin. The flowers also contained substantial quantities of proanthocyanidins and anthocyanins (Patel *et al.,* 2012; Garg *et al.,* 2012; Mukhopadhyay *et al.,* 2018). Other compounds found in *Hibiscus rosa-sinensis* are campesterol, stigmasterol, cholesterol, taraxeryl acetate, beta-sitosterol fructose, glucose, and flavonoids. Hibiscetin, cyanin glucosides and alkanes are also present. This plant was reported to contain proteins, carbohydrates, fats, and fibre contents. It also contains appreciable amounts of vitamins, iron, β-carotene, and calcium. The stem and leaves contain stigma sterol, taraxeryl acetate, β-sitosterol, and three cyclopropane compounds. The flowers are abundant in Quercetin-3-diglucoside, cyanidin-3-sophoroside-5-glucoside, kaempferol-3-xylosylglucoside, cyanidin-3, 5-diglucoside, and 3, 7-diglucoside. Plant extracts serve as a source of numerous potential antioxidants and anticancer compounds, including quercetin, glycosides, riboflavin, niacin, carotene, malvalic acid, gentisic acid, margaric acid, and lauric acid. The roots are particularly rich in tannins, mucilage, flavonoids, and saponins. Saponins are useful for patients of hypercholesterolemia as they bind with cholesterol, form insoluble complexes, and excrete through bile, to lower blood pressure (Agarwal *et al.,* 2017; Khristi and Patel, 2016).

Chief bioactive ingredients

The plant consists of plant acids, including citric acid, malic acid, tartaric acids, and also-hydroxy citric acid lactone, commonly known as hibiscus acid. These acids make up approximately 15%-30% of the plant's composition and are unique to this plant species. Hibiscus leaves contain carotene, riboflavin, anthocyanins, ascorbic acid, niacin, calcium, iron and

vitamin-C. Several studies reported that *H. rosa-sinensis* contains flavonoids, cyanidin, quercetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin, ascorbic, citric, tartaric, and oxalic acid. Recently, four new phytoconstituents (n -hexacosa-3-one-20, 21-diol, n - triacontane n -triacontan-15-one and n - hentriacontane) have been isolated from the alcoholic extracts of leaf and flower. The leaf extract displayed notable antioxidant and anticancer properties because of elevated levels of flavonoids and terpenoids. The phytochemical analyses revealed that the components identified (flavonoids, terpenoids, saponins, tannins, and glycosides) contribute to the observed pharmacological effects. Flowers have been reported to contain cyaniding diglucoside, flavonoids and vitamins - thiamine, riboflavin, niacin and ascorbic acid; leaves contained beta-sitosterol, sigma sterol, taraxerol, acetate and three cyclopropane compounds and their derivatives Pharmacologically, leaves, stem and root of *H. rosa-sinensis* contain a remarkable quantity of flavonoids which are associated with antioxidant, fever-reducing, pain-relieving and spasm-inhibiting activities and the flower has soothing properties which are used to relieve menstrual cramps and relax spasms and general cramping and treating inflammations (Olumuyiwa *et al.,* 2009; Vasudeva and Sharma 2008; Sivaraman and Saju, 2021).

Molecular diversity

The RAPD (Random Amplified Polymorphic) method is dependable and full of potential for the assessment of the Hibiscus germplasm (Crawford *et al.*, 1993). Utilizing molecular markers for plant characterization is a valuable approach to the preservation of plant genetic diversity. Molecular characterization aids in identifying the breeding patterns of species, individual reproductive achievements, and the presence of gene flow, which involves the transfer of alleles among populations of the same or closely related species, along with its implications. DNA extraction can be performed by the CTAB method. Spectrophotometric analysis was carried out to determine the concentration and purity of the DNA (Bassam *et al.*, 1991). The research was conducted to identify and analyze genetic differences among four varieties of *Hibiscus rosa-sinensis* L. with varying colors (red, pink, orange, and white) using ISSR (Inter-Simple Sequence Repeat) and isozyme analysis. The ISSR findings revealed distinct banding patterns that were measurable. The study was to find out the genetic relationship within the nine varieties of *Hibiscus rosa-sinensis* through RAPD markers. The research indicates a high level of genetic diversity. The genetic similarity was found to be significant among the various types. By utilizing RAPD analysis alongside morphological traits, it is possible to distinguish and assess the genetic differences among different types and species of *Hibiscus*. The RAPD method is dependable and encouraging for the classification of *Hibiscus* germplasm, allowing for the creation of SCAR (Sequence Characterized Amplified Regions) primers for numerous *Hibiscus* types and species. Consequently, these RAPD markers hold promise for recognizing and

characterizing genetic diversity within the various types of species. This may also be helpful in *Hibiscus* breeding programs and provides a major input into conservation biology (Lai *et al.*, 2001; Nybom, 2001).

Ethno-medicinal uses

The stem bark and flower bud of *Hibiscus rosa-sinensis* were used for abortion, contraceptive by the native of Bargarh district, Odisha (Sahu *et al.*, 2010; Sahu *et al.*, 2013). Small stems of the same plant were used as tooth brush to clean the teeth and tongue cleaner by the local community of Kalahandi district and Bargarh district, Odisha (Sahu *et al.*, 2020; Sahu and Sahu, 2020). This plant is well-known for its antibacterial properties. *Hibiscus* plants have the potential to provide compounds that enhance the effectiveness of antibiotics against bacterial diseases. The roots of *H. rosa-sinensis* contain aqueous and alcohol extracts that exhibit notable antiulcer activity. The extract of *H. rosa-sinensis* was found to have hypolipidemic potential. It is also helpful in wound healing. Aqueous extract and ethanolic extract of the flowers and leaves have anti-diabetic effects. The antithetic potential of the aqueous extract from *H. rosa-sinensis* flowers was assessed *in-vitro*. *H. rosa-sinensis* is used as an effective treatment for leucorrhoea, chronic cough, urinary diseases, and psychiatric ailments, offering a cost-effective solution without any adverse effects (Nithya, 2011; Raduan and Hakim, 2013; Tomar *et al.*, 2010; Sankaran and Vadivel 2011).

Table 1: Table showing Ethno-medicinal benefits of *Hibiscus*

Parts of the Plant	**Benefits**
Roots	Antifungal, Anti-diabetic, pyretic, and Neuroprotective, In the treatment of venereal diseases, treatment of coughs and colds
Stem	Gastroprotective, Good quality fiber can be obtained
Leaves	Anti-bacterial, Antifungal, antioxidant, anti-cancer, and diabetic, hair growth promoting activity, wound healing activity, anti-inflammatory, cardioprotective, antipyretic, Helps in digestion Hepatoprotective, anti-asthmatic
Flower	Antihyperlipidemic, gastroprotective, cardioprotective, anti-inflammatory, improves immune response, wound healing activity, anti-fertility, hair growth promoting activity, For painful menstruation, cystitis, venereal diseases, feverish illnesses, bronchial catarrh, coughs. Hibiscus flower extracts have demonstrated the ability to impede the proliferation of various cancer cells, address hypertension, and regulate cholesterol synthesis. It has phytochemical and pharmacological benefits., anti-tumor, convulsive

With special mention, *Hibiscus* tea is found to be rich in vitamin-C. It also has the property of analgesic (Singh *et al.,* 2018). It may be due to the presence of alkaloids which produce narcotic analgesic activity mediated through the opioidergic receptor. The flower has antioxidant properties and is considered a natural antioxidant (Mak *et al.,* 2013). *Hibiscus rosa-sinensis* has wound-healing properties too. The flowers and leaves of *H. rosa-sinensis* contain important constituents that confer its antibacterial activity and may be used in treating pathological conditions caused particularly by isolates (*P. aeruginosa*, *Serratia*, *Micrococcus*, *Enterobacter*, and *Salmonella*). As per the study *in-vitro*, the antibacterial activity of *H. rosa-sinensis* flower extract against human pathogens has been studied. The other therapeutic benefits obtained from the *H. rosa-sinensis* plants were shown in Table-1 (Ruban *et al.,* 2012; Vijayakumar *et al.,* 2018).

Toxicological study

Hibiscus rosa-sinensis is not a toxic plant; it is found to be safe in the recommended dosage. It has abortifacient properties and should not be used by a pregnant woman or by infants. It causes abortion in pregnant women. For infants, it should be avoided as it contains alcoholic constituents and persons who are undergoing detoxification should avoid it. The oil also has hallucinogenic effects. The administration of *H. rosa-sinensis* flower extract at doses ranging from 100 to 800 mg/Kg did not elicit any noteworthy alterations in behaviour, skin, respiration, defecation, posture, food intake, water consumption, or hair related issues (Onyenekwe *et al.,* 1999). But a dose of 1600 mg/kg showed 20% mortality. *Hibiscus rosa-sinensis* exhibits notable medicinal characteristics. Numerous prior investigations have demonstrated the plant's beneficial anti-fertility properties, anti-ovulatory, anti-spermatogenic, androgenic, analgesic, anti-inflammatory, wound healing, and antidiabetic agent. Some *in-vitro* studies also described that *Hibiscus rosa-sinensis* shows notable antioxidant activity (Odigie *et al.,* 2003).

Conclusion:

Based on data gathered from earlier research, *Hibiscus rosa-sinensis* demonstrated a variety of pharmacological properties, including anti-inflammatory, antioxidant, anti-microbial, and anti-diabetic effects, contingent upon the specific extracts employed and the plant portion examined. According to phytochemical analysis, every portion of the plant is made up of a diverse spectrum of chemical elements, some of which are responsible for the pharmacological effect shown in the studies. More research is needed to turn this plant into a medicinal formulation, however extracts of *H. rosa-sinensis* flowers and leaves are already sold in the market as conventional therapies for a variety of illnesses.

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AN ACCOUNTABLE ROLE OF INDIAN TRADITIONAL AYURVEDIC HERBAL SYSTEM IN WOUND HEALING- A SHORT REVIEW

Gayathri Sundar[1,2], Annie John[1,2] and Annie Abraham*[1]

[1]Department of Biochemistry, University of Kerala, India

[2]Centre of Excellence in Stem Cell Research, Division of Biotechnology,

Karunya Institute of Technology & Sciences, Karunya Nagar, Coimbatore, Tamil Nadu, India

*Corresponding author E-mail: annieab@keralauniversity.ac.in

Introduction:

According to recent studies, the global people have been facing a leading problem is chronic wound injuries. Worldwide wound statistics estimated that per year around 13 million people suffer from chronic wounds. The Indian community-based epidemiological study of wounds reported that around 4.48 per 1000 are affected chronically, and 10.55 per 1000 are acute wounds (Gupta N *et al.*, 2004). Chronic wound is one of the serious tissue damages has typically has failed to proceed through the regular cascade of wound healing in an orderly and timely manner to repair and restore the functional and anatomical integrity of tissues. Complete recovery of wounded area is depended up on the nature of the wound and it may prolong to more than 3 months affected by major injuries such as trauma, diabetes, vascular disease, infection, pressure, or radiation (Jarbrink K *et al.*, 2016). Wound assessment and management is a significant process. It requires a healthy understanding, detailed evaluation and proper implementation of action with the combined knowledge of treatment materials available in the market. In the current scenario, innumerable products, including synthetic and natural, both small and large, are available in the market to manage chronic wounds. The emerging trend in the wound management system is to provide a blending concept of traditional and modern knowledge. An ideal wound dressing product should offer a foundation for proper healing of wounds with good vascularization, free of cell debridement, protection from infections and inflammations, and maintaining moisture content in the wound environment. It should also consider properties like biocompatibility, duration of healing, quality and cost-effectiveness and eco-friendly nature of the product (Bowler PG *et al.*, 2001). Based on the etiology of wounds, a recent study held in 2020 among 265 patients treated at a wound care clinic, the data revealed that 45.66% of patients had venous leg ulcers, 23.77% of patients with surgical and traumatic wounds, 11.70% with diabetic foot ulcers, 9.06% with pressure ulcers, 6.42% with arterial injuries, 2.26% with a pilonidal cyst, 0.75% with skin cancer and 0.38% with burns [Figure. 1] (Zhou K *et al.*, 2020).

Keywords: Wound Healing, Plant Compounds, Tissue Engineering, Traditional Medicine

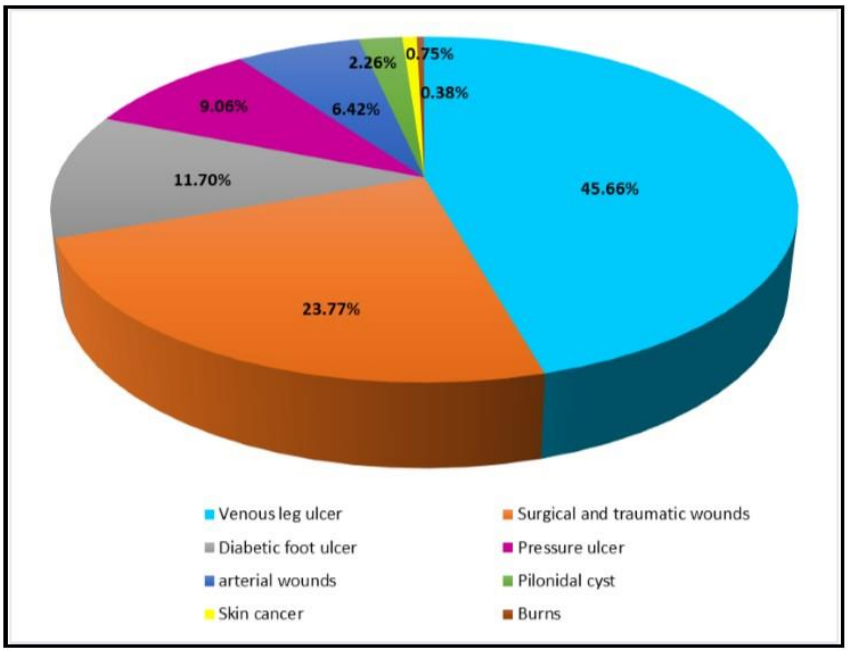

Figure 1: Etiology of causes of wounds

Short outline of wound healing mechanism

The wound healing mechanism is a complex process, requiring significant contributions from various specialized cells like platelets, macrophages, fibroblasts, and epithelial and endothelial cells. In order to the activation and interaction of these specialized cells lead to the triggering of many healing related receptors, proteins and glycoproteins such as cytokines, chemokine, growth factors, inhibitors etc. The collective action of those molecules with several stages of healing makes the complex process to simple healing process. Usually, wound healing efficiency progress through four major predictable phases haemostasis, inflammation, proliferation and remodelling of tissues. Usually, acute wounds are easily healed through this systematic process, but in the case of chronic wounds, it may progresses and prolong the phases of inflammatory, proliferative, or remodelling stages. Occasionally, these types of wounds ensue tissue fibrosis and non-healing ulcers (Maxson S*et al.*, 2012).

Haemostasis: The first phase of the wound healing process appears to prevent exsanguination, vasoconstriction, platelet activation, adhesion and aggregation. Platelets become activated when exposed to extravascular collagen. Cell surface receptors mediated all cellular interactions with the extracellular matrix, especially via specific integrin receptors. And finally, fibrin mesh formation leads to a thrombus or clot that keeps the platelets and blood cells trapped in the wound area.

Inflammation: The next stage of wound healing says to control bleeding and prevent infections by the action of neutrophils, monocytes, and macrophages. They help to clean the wound area by removing the damaged cells, pathogens, and bacteria. The specific indication of inflammation, the rubor (redness), calor (heat), tumor (swelling) and dolor (pain) is generated by the action of white blood cells, growth factors, nutrients and enzymes.

Proliferation: In this phase, the wound area is rebuilt with a new matrix of collagen fibres, proteoglycans, and fibronectin to maintain the structure and function of the tissue. Healthy angiogenesis occurs in this phase, and it helps to provide sufficient oxygen and nutrients for newly developed granulation tissue and support epithelialization mechanisms to resurface the injured area.

Remodeling: Remodeling represents the final phase of wound healing mechanisms. Here, the granulation tissue matures into scar and tissue tensile strength increases due to the remodelling of collagen from type III to type I. (Schultz GS *et al.,* 2011).

Existing concepts and practices in wound management

Wound management encompasses a broad spectrum of concepts and practices aimed at promoting wound healing and preventing complications. Assessing the wound site, size, and depth is a critical step in the initial phase of wound management. Following this assessment, thorough cleaning of the wound and removal of debris through debridement are essential to initiate the healing process effectively. Creating an optimal wound environment is pivotal for successful wound healing. Controlled moisture content plays a vital role in enhancing wound healing and reducing the risk of infection. Therefore, in most cases, moisture-based dressings such as hydrogels, hydrocolloids, foams, or films are utilized to maintain an appropriate wound environment. Treatment strategies are tailored based on the depth and characteristics of the wound. Topical agents and advanced therapies may be employed to promote healing, manage infection, and facilitate tissue regeneration. Additionally, providing adequate cell nourishment support is crucial for promoting cell proliferation and accelerating the healing process.

Furthermore, effective pain relief management is imperative in wound treatment to ensure the patient's comfort and psychological well-being. Employing strategies for pain relief helps stabilize the patient's mental state and improve their overall experience during the healing process. Thus, a comprehensive approach that addresses wound assessment, cleaning, moisture management, tailored treatment modalities, cell nourishment support, and pain relief management is essential for successful wound management and optimal patient outcomes.

In the realm of modern wound management, a diverse array of wound dressings exists, each categorized into various forms tailored to specific wound types and conditions.

Films/Membranes: Film dressings are a convenient and widely-used option for managing superficial injuries. These dressings, such as Tegaderm, Cutifilm, Blisterfilm, and Bioclusive, consist of semi-permeable adhesive sheets that create a barrier against infection while remaining flexible to conform to the wound site (Bhoyar SD *et al.,* 2023).

Non-adherent dressings: Non-adherent dressings encompass materials like Tulle Gras Dressing, which includes gauze impregnated with paraffin or similar substances. Some non-adherent dressings may also contain antiseptics or antibiotics for added wound protection. Dry perforated plastic film materials coating absorbent pads, as seen in products like Melolin, Melolite, and Tricose, are also part of this category.

Silver dressings: Silver dressings incorporate silver as a broad-spectrum antimicrobial agent effective against various pathogens, including bacteria, viruses, fungi, and yeast. Examples include ACTICOAT® Flex, Sorbsan® Silver, SILVERCEL®, AQUACEL® Ag, and Mepilex® Ag.

Hydrogels: Hydrogel dressings consist of three-dimensional networks of hydrophilic polymers, fostering a moist wound environment conducive to tissue regeneration through granulation and re-epithelialization.

Additional wound dressing categories include Hydroconductive dressings, Silicone dressings, Hydrocolloids, Larval therapy and leech therapy, Calcium alginate dressings, Composite dressings, Hydro fibers, Negative pressure wound therapy (NPWT), Polyhexamethylene biguanide and honey dressings, Foams, Charcoal dressings, Hypertonic dressings, Hydrophilic fibers, Antimicrobial dressings, Other specialized devices

Each of these dressing categories offers unique benefits and applications in wound management, catering to the diverse needs of patients and healthcare providers alike.

Exploiting an Indian traditional herbal knowledge in wound healing

Traditional medicine also referred to as indigenous or folk medicine encompasses a wealth of knowledge that has evolved over generations within diverse societies predating the advent of modern medical practices. Exploiting traditional herbal knowledge in wound healing, particularly from the rich Indian traditional medicine systems like Ayurveda, Siddha, and Unani, holds significant promise. These systems have a long history of utilizing various herbs and natural substances for promoting wound healing and managing various skin ailments.

Traditional Indian herbal plants have been extensively utilized for wound healing throughout literature, showcasing their significant contribution to modern medicine. Plant extracts, as well as nano forms and scaffolds incorporating plant materials, represent innovative approaches in regenerative medicine. These advancements leverage the therapeutic properties of plants to promote tissue regeneration and healing. Plant extracts contain bioactive compounds that can stimulate cell growth, modulate inflammation, and enhance wound healing processes. Furthermore, nano forms of plant extracts offer increased bioavailability and targeted delivery, improving their efficacy in regenerative applications. Additionally, scaffolds incorporating plant materials provide a three-dimensional framework for cell adhesion, proliferation, and differentiation, facilitating tissue regeneration. By harnessing the potential of plant-based therapies, regenerative medicine aims to develop novel strategies for treating injuries and diseases, ultimately improving patient outcomes.

Some common magic plants like Turmeric, Neem, Aloe Vera, Indian Gooseberry, and Indian Ginseng possess remarkable traditional wound healing properties. Turmeric reduces inflammation and fights infection, while Neem provides antibacterial and anti-inflammatory effects. Aloe Vera soothes and moisturizes while protecting against contaminants, and Indian Gooseberry aids collagen synthesis. Indian Ginseng promotes tissue regeneration. Together, these natural remedies offer effective wound management options. Many studies have revealed

that *Aegle marmelos*, commonly known as Koovalam, aids in wound healing by facilitating the formation of connective tissue and boosting antioxidant levels. This helps to reduce the presence of free radicals and myeloperoxidase, which can otherwise cause tissue damage (Gautam MK *et al.,* 2014).

*Butea monosperma (*Chamata*)* treated wounds heal much faster as indicated by improved rates of epithelialization and wound contraction (Sumitra M *et al.,* 2005)**.** The tuber extract of *Mirabilis jalapa*, also known as Nalumanipoovu, has demonstrated remarkable efficacy in promoting wound healing (Alerico GC *et al.,* 2015). Its potent healing properties accelerate the regeneration of damaged tissues, enhance collagen synthesis, and stimulate angiogenesis, thereby facilitating the closure of wounds and the restoration of skin integrity. Additionally, the extract exhibits anti-inflammatory and antimicrobial effects, which contribute to reducing the risk of infection and inflammation at the wound site. These combined actions make *Mirabilis jalapa* tuber extract a promising natural remedy for promoting the effective healing of wounds. Our research group has found that the silver nanoform of *Mirabilis jalapa* provides effective wound recovery *in vitro* experiments (Sundar G *et al.,* 2023). Another team within our department has reported on the efficacy of *Cuminum cyminum* (jeera) in nano formulations, demonstrating its ability to promote fibroblast proliferation and its potential role in tenocyte proliferation. (Amrutha D.S *et al.,* 2021). In another research group within our laboratory, we have conducted studies investigating the potential role of incorporating *Calotropis gigantea*, a medicinal plant known for its therapeutic properties, into alginate dialdehyde-gelatin hydrogels. These hydrogels, which are composed of alginate and gelatin, have been explored for their suitability in promoting cartilage tissue regeneration in the context of osteoarthritis, a degenerative joint disease. Our findings suggest that the inclusion of *Calotropis gigantea* in these hydrogels may offer promising therapeutic benefits for the treatment of osteoarthritis by facilitating cartilage repair and regeneration (Aswathy J *et al.,* 2023). Several research studies on curcumin delivery at the wound site have reported the effectiveness of curcumin in eradicating reactive oxygen species. Curcumin, derived from *Curcuma longa* (Manjal), and its nano formulations have shown promising results in wound healing (Kumari A *et al.,* 2022).

Advanced approach of tissue engineered scaffolds in wound management

Advanced approaches in tissue-engineered scaffolds have revolutionized wound management by offering innovative solutions for tissue regeneration and repair. These scaffolds composed of biocompatible materials such as polymers, ceramics, or natural extracellular matrix components, provide a supportive framework for cell attachment, proliferation, and differentiation. Incorporating bioactive molecules, growth factors, or stem cells into these scaffolds enhances their therapeutic potential by promoting specific cellular responses crucial for wound healing. Furthermore, advanced techniques such as 3D bioprinting enable precise control over scaffold architecture and composition, facilitating the customization of scaffolds tailored to individual patient needs. With their ability to mimic the native tissue microenvironment and stimulate tissue regeneration, these advanced tissue-engineered scaffolds represent a promising

frontier in wound management, offering hope for improved outcomes and enhanced quality of life for patients with chronic or severe wounds.

Future perspectives:

In the future, our laboratory aims to further advance tissue engineering applications through the development and utilization of phytochemical or phyto nano material incorporated matrices. These matrices hold immense potential for various tissue engineering applications, ranging from wound healing to regenerative medicine. Our research endeavors will focus on elucidating detailed wound healing mechanisms by exploring the intricate interactions between these matrices and the cellular environment. Specifically, we will delve into the specific roles of plant compounds in promoting wound healing and investigate the underlying cellular pathways involved in this process. By unraveling these mechanisms, we can enhance our understanding of how phytochemicals contribute to tissue regeneration and tailor our approaches for more effective wound management strategies.

Moreover, our future research will involve the exploration of novel delivery systems for plant-derived drugs. We recognize the importance of developing efficient and convenient methods to deliver these therapeutic compounds into the body system. Thus, we will investigate various matrix formulations that offer easy modes of drug delivery while ensuring optimal therapeutic outcomes. By leveraging advanced techniques and interdisciplinary approaches, we aspire to pave the way for the development of innovative strategies that harness the healing potential of plant-based compounds for improved wound care and overall well-being.

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A COMPREHENSIVE REVIEW *TUSSILAGO FARFARA* LINN. TAXONOMICAL, MORPHOLOGICAL CLASSIFICATION AND ITS PHARMACOLOGICAL ACTIVITIES

Rajeswar Das[1,2] and Durgaprasad Kemisetti*[1]

[1]Faculty of Pharmaceutical Science, Assam down town University,
Sankar MadhabPath, Gandhi Nagar, Panikhaiti, Guwahati, Assam, India, Pin- 781026

[2]School of Pharmacy, The Neotia University, Dimond Harbour Road, West Bengal, India.

*Corresponding author E-mail: kdp251999@gmail.com

Abstract:

Tussilago farfara L., a perennial species, is a medicinal herb used in traditional medicine, mainly for the treatment of respiratory tract-related pathology. In traditional Chinese medicine, flower buds are preferred; in Europe, the leaves are used; and in some parts of India, the whole plant is utilized. This preferential usage of the plant organs might be based on differences in the chemical composition due to environmental conditions, along with preferred traditional and cultural approaches. In this article, the impact of pedoclimatic growth conditions on the morpho-anatomical development and phytochemical profile of the plant were studied on T. farfara in the vegetative state, collected from two different locations in the Romanian spontaneous flora, revealing significant variations. Furthermore, the antioxidant profile of the specific extracts from the aerial and subterranean plant parts is also in accordance with these discrepancies. The plant anatomy was assessed histologically by optical microscopy, while the analytical chemistry evaluation was based on LC/MS and spectral methods for the evaluation of the antioxidant and enzyme inhibitory activity. To our knowledge, this is the first comparative analysis contextually reporting on the histology, phenolic profile, antioxidant capacity, and geographical location of the vegetative form of T. farfara.

Keywords: *Tussilago farfara* Linn, Ethnopharmacology, Phytochemistry, Pharmacology, Medicine.

Introduction:

Tussilago farfara L. (coltsfoot), a perennial plant, is the only species within the Tussilago genus (Composite). As an outstanding lung herb, in the USA the root of coltsfoot has medicinal values for debilitated coughs, whooping cough and humid forms of asthma (Tobyn *et al.*, 2011). In Norway, the dried and cut leaves of coltsfoot, as folk medicines, are sold and used in teas for the relief of coughs and chest complaints (Xue *et al.*, 2012). In Europe, the leaves are used to treat bronchial infections while in China the flower buds are preferred (Adamczak *et al.*, 2013). Moreover, in traditional Chinese medicines (TCMs), the flower bud of coltsfoot is often used the

form of processed honey-fry, which showed a detoxifying effect. It also has been used as a dietary supplement and health tea in many countries (Kang *et al.,* 2016; Tobyn *et al.,* 2011). Traditionally, leaves of the plant especially in European countries, are widely used by indigenous people against a wide range of ailments, including gastrointestinal, wounds, burns, urinary, injury's inflammation within the eye and mainly the relief of respiratory complaints (Jaric *et al.,* 2018; Rigat *et al.,* 2015). As an important folk medicine, coltsfoot has been studied for its pharmacological activities, including anti-inflammatory (Cheon *et al.,* 2018), anti-oxidative (Kim *et al.,* 2006; Qin, K. *et al.,* 2014), anti-microbial (Uysal *et al.,* 2018), anti-diabetic (Gao *et al.,* 2008), neuro-protection, (Lee *et al.,* 2018), platelet anti-aggregation (Hwang *et al.,* 1987), and anti-cancer (Li *et al.,* 2014) etc. As well as, several investigations have evaluated the phytochemistry of coltsfoot. Approximately 150 compounds have been identified, including sesquiterpenoids (Qin *et al.,* 2014), triterpenoids (Yaoita *et al.,* 2012), flavonoids (Kim *et al.,* 2006), phenolic acids (Kuroda *et al.,* 2016; Wang *et al.,* 2019), chromones (Sun *et al.,* 2019), pyrrolizidine alkaloids (Nedelcheva *et al.,* 2015) and others. Some of them have been deemed to possess biological activities, and this is notably the case with TSL (13). Most notably, the concentration of PAs in coltsfoot varies widely (Adamczak *et al.,* 2013) and cultivation of a PAs-free variety is being developed in Austria, (Wawrosch *et al.,* Journal Pre-proof 8 2000). Moreover, Pfeiffer et al (2008) highlights the correlation of vegetative multiplication in coltsfoot, as an important pioneer species, which has been achieved rapidly via repeated clonal growth and subsequent clonal reproduction (Pfeiffer *et al.,* 2008). As coltsfoot is widely consumed as a vegetable, it is imperative to evaluate its biological attributes to support it prospective uses as functional foods (Uysal *et al.,* 2018). This review addresses specifically on the ethnobotanical value, phytochemistry, pharmacology, toxicity and quality control coltsfoot, and to analyses critically the reported studies, with the purpose of providing the theoretical basis for the clinical application of *Tussilago farfara* L.

Botanical description, distribution and habitat:

- **Taxonomy and morphology**

In the *Chinese Pharmacopoeia* (2015), one species of the genus *Tussilago* has been registered as ‘Kuan-Dong-Hua’, denoting *Tussilago farfara* L., which is a flowering perennial plant. *Tussilago farfara* L. is known as ‘podbel’ (bottom is white) in Bulgaria (Nedelcheva *et al.,* 2015), ‘Kantoka’in Japan. According to “Plant of world online”, *Tussilago farfara* L. is the only accepted name for the plant, with other 10 synonyms. The synonyms with the highest confidence levels, include *Cineraria farfara* (L.) Bernh., *Tussilago alpestris* Hegetschw., *Tussilago radiata* Gilib. And *Tussilago umbertina* Borbás. However, the value of positive identification is of great importance prior to exploring functional phytochemicals and potential health benefits from botanicals.

In terms of morphology, the principal botanical characteristics of coltsfoot, including the whole plant, leaves, flower buds, and flowers are highlighted here. Above ground, the plants reach 5–15 cm height, although up to 30 cm during fruitdispersal (Norani *et al.,* 2019). The flower buds are in the shape of a long round rodand single or 2 to 3 consecutive bases, with a length of 12.5 cm and a diameter of 0.5to 1 cm (*Chinese Pharmacopoeia* 2015). It also owns upper thick, lower tapering orwith short pedicel, outer covered with many fish-scale bracts and fragrant smell. The flower buds of coltsfoot precede the leaves and appear early (February–April) in the year, bearing bright yellow and dandelion-like flowers (Norani *et al.,* 2019; Zhi *et al.,*2012). The large, round, heart-shaped Leaves with long petioles, 3-12 cm long and 4-14 cm wide, and have radial veins and crinkly, slightly toothed edges, palmately reticulated veins. They have a thick white downy covering underneath (Tobyn et al). However, there are few reports on chemical components in roots of coltsfoot. Thus, chemical studies on the components from this need to be strengthened.

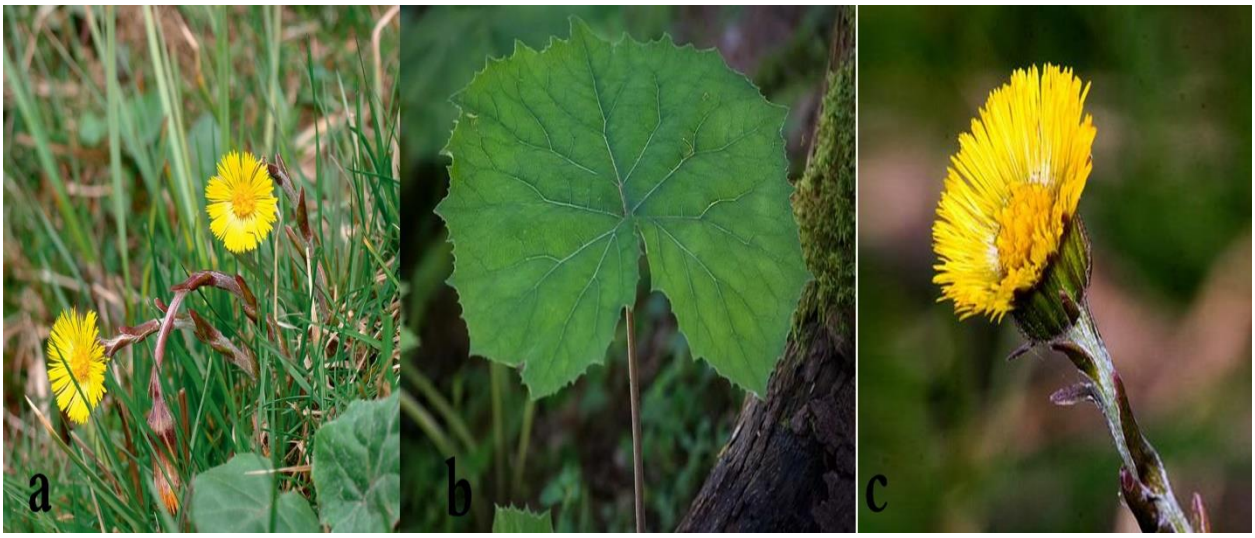


Figure 1: Images of *Tussilago farfara* L. (coltsfoot) (a) whole-plant of coltsfoot picture adapted from (Tobyn *et al.,* 2011); (b) leaves of coltsfoot; (c) flowers of coltsfoot.

- **Distribution and habitat**

Although coltsfoot occurs naturally and indigenous to the temperate Eurasia to N. Africa and Nepal, it is currently distributed in up to 46 countries around the world. In China, the wild resources are present in at least 10 provinces, particularly in the Yellow River basin area of provinces. Coltsfoot has become naturalized in tropical and temperate areas where it grows wild as a weed in riverbanks, roadsides, wastelands, and crop fields, cultivated or uncultivated. In introduced areas, coltsfoot can quickly form dense stands that aggressively invade well-established cultivated lands, with some regarding it as being naturalized as a causal weed in American (Sparks *et al.,* 2020). Hence, it may be of particular interest for the sustainable

development of new drugs or other derived products, since adequate amounts of raw material are always available.

Figure 2: Distribution map of coltsfoot Native Introduction reproduced

Local and conventional medicinal uses:

Coltsfoot is listed as a "Middle grade" drug in the Divine Farmers Materia Medica (Han Dynasty, A.D. 25-220), the oldest book on Chinese medicine. It was also recorded in many ancient classic traditional Chinese medicine books such as the Compendium of Materia Medica (Ming Dynasty), written by Li Shi Zhen, extensively described the function of coltsfoot, which can be a remedy for chronic cough, phlegm syndromes with blood and chancre the mouth. The Collective Notes to Canon of Materia Medica (Nan Dynasty, written by Tao Hongjing, A.D. 456─536, published in A.D. 502-557) describes the flower of coltsfoot as possessing a pungent, wen, non-toxic nature. It can primarily treat cough inverse of breath, sore throat, epilepsy induced by terror, chills and fever and evil. As well as, it can be applied for treating diabetes and gasping respiration. According to the Amplification on Materia Medica (Song Dynasty, A.D. 960-1279, published in A.D. 1116) written by Kou Zongshi, spring into or when mining to vegetables, as medicine this herb is good to see the flower slightly. Moreover, coltsfoot and its ten Chinese patent medicines (Ju Hong Tablets/Pills/granula and capsule, Qingfei Huatan Pills, Runfei Zhisou Pills, Zhisou, Huatan Pills, Ermu Ansou Pills, Jiegong Donghua Pills and Chuanbei Xueli Gao) were recorded in Chinese pharmacopoeia (Pharmacopoeia Committee of China, 2015). Coltsfoot has widespread cultural uses in many countries. Several ethnomedicinal survey studies were conducted and highlighted the customary uses of coltsfoot. The most common focus on its leaves and flower buds.

Yakammaoto, as a classic formula of traditional Chinese medicines, is used in the treatment of asthma, flu-like symptoms, and cough with wheezing in the throat in China and

Japan and is a formulation comprising nine various herbs as follows: Ephedra sinica, Pinellia ternate, Zingiber officinale, *Tussilago farfara*, Aster tataricus, Ziziphus jujube, Belamcanda chinensis, Asarum sieboldii, and Schisandra chinensis. The initial description of this prescription can be traced back to the prescriptions Synopsis of the Golden Chamber (Eastern Han Dynasty, written by Zhang Zhongjing A.D. 152-219), which reveals that it can be treatment of the early stage of acute asthma. To assess the effect of Yakammaoto on asthma, an experiment was performed in the OVA-induced asthma mouse model in which Yakammaoto was administered to two groups with corresponding daily doses of 2.52 and 0.63 gml-1 through gavage. The positive control group received dexamethasone intraperitoneally injected. The results indicated that Yakammaoto can attenuate asthmatic airway hyperresponsiveness. The mechanism of the prescription may be via hindering Th2/Th17 differentiation, promoting CD4+FoxP3+Treg generation, and suppressing mTOR and NF-κB activities (Lin et al., 2020). Additionally, it has been proved that Yakammaoto is not only against flu-like symptoms but against cellular injuries in airway mucosa and renal tubular epithelia cause by Coxsackievirus B 4. An experiment was carried out in HEp-2, A549, and HK-2 cells, administering Yakammaoto (10, 30, 100, 300 μgml-1) dose-dependently inhibited viral attachment, internalization, and replication while compared to the positive control (Yen et al., 2014). Antiviral activity against enterovirus 71 infection have also been reported (Yeh et al., 2015). Globally, the world is scrambling to cope with the COVID-19 pandemic, which caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Recently, a paper report on lung cleansing and detoxifying decoction (LCDD) widely was used in treating COVID-19 patients in China. LCDD is based on four formulae described in the classic TCMs text treatise on cold pathogenic and Miscellaneous Diseases by Zhang Zhongjing (AD 150–219) (Weng, 2020). Fan et al., (2020) further investigated the buds of coltsfoot mechanism in LCDD mechanism against COVID-19 by network pharmacology and molecular docking. The resulted indicated that 14 compounds in coltsfoot may combined with SARS-CoV-2 3CL hydrolase and ACE2, thereby acting on many targets to regulate multiple signaling pathways, thus exerting the therapeutic effect on COVID-19 (Jian-xin *et al.*, 2020).

Ethnobotanical knowledge of anti-cancer medicinal plants was collected by Katrin *et al.* (2014) in Estonian folk medicine. Coltsfoot was reported to be used for the management of cancer as herb tea (Sak *et al.,* 2014). Additionally, Jerusalem Balsam, an herbal formulations, may be prepared using some raw materials which are thujones, estragole and *Tussilago farfara* suspected of anti- cancer activities due to their chemical constituents. Although possible formulations were reported, rawmaterial used in this formulation was not known (Łyczko *et al.,* 2020).

An ethnobotanical study of vulnerary medicinal plant was collected by Jan *et al.,* (2018) in a Catalan district. The fresh leaves of coltsfoot were reported to be used for the management of

festering wounds (Rigat *et al.,* 2015). Similarly, people of the Balkan region reported the use of coltsfoot leaves for the treatment of wound healing (Jaric *et al.,* 2018).

Phytochemical aspect:

To date, approximately 150 phytochemicals have been isolated from the coltsfoot. previous studies of coltsfoot have identified the presence of chemical constituents such as sesquiterpenes, triterpenoid, flavonoids, phenolic compounds, chromones and its derivatives, alkaloids, and other phytochemicals. The phytochemicals present in coltsfoot.

- Previous phytochemical investigations revealed that sesquiterpenoids containedoplopane and bisabolane, the skeletons of which are substituted withdiverse ester derivatives (Song *et al.,* 2019). Recently, some novel sesquiterpenoidshave been reported such as bisabolane-type tussfararins A–F (Qin *etal.*,2014),farfaroneB-AandfarfaroneD(Xu*etal.*,2017),anoplopane-type sesquiterpene skeleton. In addition, three novel sesquiterpenoids wereseparated by Song *et al.* (2019), bearing an unreported substituent for the first time.Among these compounds, altaicalarin C was first reported from *Ligularia altaica*and first seen in nature (Song *et al.,* 2019). As well as, eudesmane skeletonsand a bicyclic norsesquiterpenoid were also identified, including, (-)-spathuleno,ligucyperonol, and tussfarfarin A, one new norsesquiterpenoid. Additionally,total seven substituents have been reported on several studies (Jang *et al.,* 2016; Li *etal*., 2012; Liu *et al.,* 2011; Park *et al.,* 2008; Qin *et al.,* 2014; Song *et al.,* 2019; Xu *etal*.,2017).
- After isolating arnidiol and faradiol from the flower buds of coltsfoot (Santer and Stevenson, 1962) , bauer-7-ene-3□,16□- diol, a bauerane-type triterpenoid has also been isolated from this plant, along with bauerenol and isobauerenol (Yaoita *et al.,* 2012). However, in recent years researches were carried out extensively the review of sesquiterpenes but few triterpenoids.
- The phenolic compounds, phenylmethane derivatives, phenylpropane derivatives and esters of phenylpropanoic acids, have also been identified in coltsfoot. The aerial parts of coltsfoot from Kastamonu, Turkey afforded benzoic acid p- Hydoxybenzoic acid, syringic acid and gallic acid using reversed-phase high performance liquid chromatography technique (Uysal *et al.,* 2018). However, the authors did not isolate and elucidate those compounds by chromatographic, mass spectrometric and NMR spectroscopic techniques. Additionally, The MeOH extract of the dried flower buds of coltsfoot afforded two phenylmethane derivatives by comparison of their physical and spectroscopic data with the present literature.
- Norani *et al.*（2019）have been identified and quantified essential oil from seven major regions of Iran. The results indicated that coltsfoot has a relatively low yield of volatile oils, especially in leaves (Norani *et al.,* 2019). Additionally, Liu *et al.* (2006) identified the chemical constituents of the essential oil from the buds of coltsfoot from China (Liu *et al*.,

2006). Sixty-five components were characterized in the essential oil, representing 84. 62% of the total volatile oil (Liu *et al*., 2006).

- Other metabolites belonging to coltsfoot, including sterols, amino acids, organicacid (Zhi *et al*., 2012) and polysaccharides have also been documented in the coltsfoot (Safonova *et al*., 2018b). A chemical investigation of the MeOH extracts of coltsfootleaves by enzyme assay-guided fractionation of the extract resulted in the isolation oftwo glucosinates, for the first time (Kuroda *et al*., 2016). Of particularinterest is the noteworthy this plant contained significant levels of trace metals (suchas Zn, Mg and Se) which are likely to be responsible for their activities (Ravipati *et al*., 2012; Wechtler *et al*., 2019).

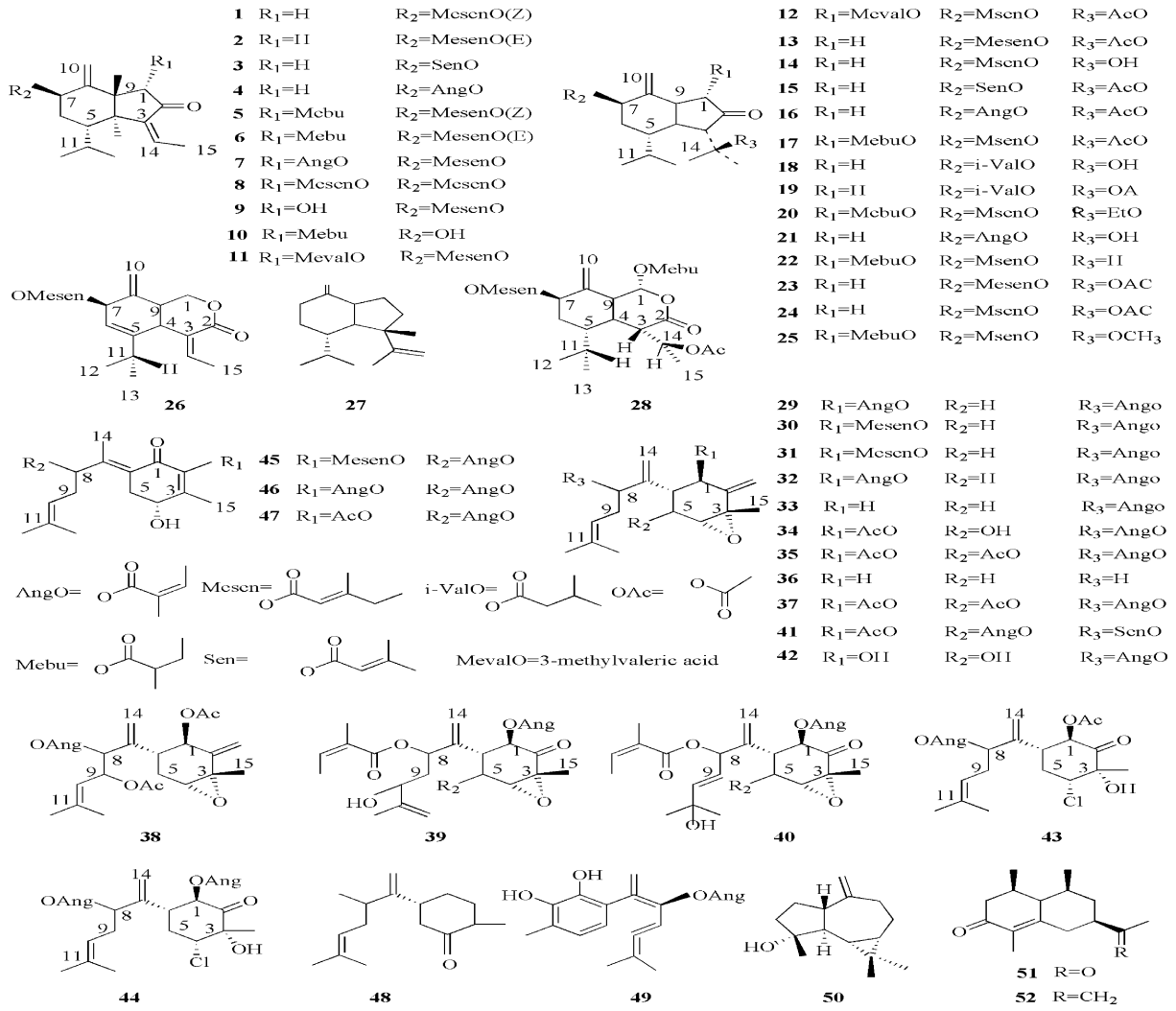


Figure 3: The sesquiterpenoid compounds isolated from coltsfoot

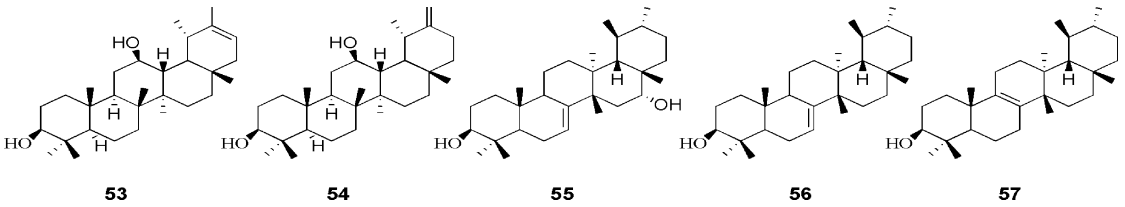
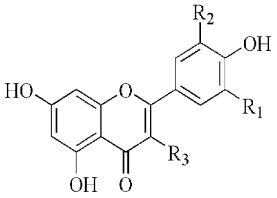
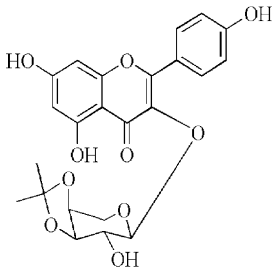


Figure 4: The triterpenoid isolated from coltsfoot

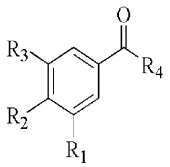


58	R_1=H	R_2=H	R_3=H
59	R_1=H	R_2=OH	R_3=H
60	R_1=OH	R_2=H	R_3=H
61	R_1=H	R_2=OH	R_3=OAra
62	R_1=H	R_2=OH	R_3=OGlc
63	R_1=H	R_2=OH	R_3=OGal
64	R_1=H	R_2=OH	R_3=ORut
65	R_1=H	R_2=H	R_3=OH
66	R_1=H	R_2=H	R_3=OAra
67	R_1=H	R_2=H	R_3=OGlc
68	R_1=H	R_2=H	R_3=OGal
70	R_1=H	R_2=H	R_3=ORut

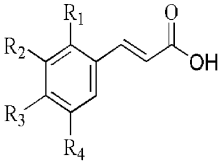


69

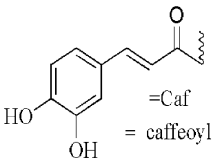
Figure 5: The flavonoids and flavonoid glycosides isolated from coltsfoot



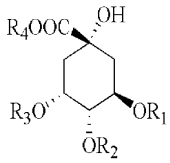
	R_1	R_2	R_3	R_4
71	H	H	H	OH
72	H	OH	H	OH
73	OCH_3	OH	OCH_3	OH
74	OH	OH	OH	OH
75	OH	H	H	OH
76	OH	H	H	CH_3

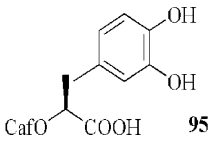


	R_1	R_2	R_3	R_4
77	H	H	H	H
78	H	OH	OCH_3	H
79	OH	OCH_3	H	H
80	H	H	OH	H
81	H	OCH_3	OH	OCH_3
82	H	H	OH	OH



=Caf
= caffeoyl

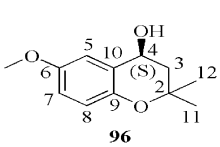




95

	R_1	R_2	R_3	R_4
83	Caf	H	H	H
84	H	Caf	H	H
85	H	H	Caf	H
86	Caf	H	H	Me
87	H	Caf	H	Me
88	Caf	Caf	H	H
89	Caf	Caf	H	Me
90	Caf	H	Caf	H
91	Caf	H	Caf	Me
92	H	Caf	Caf	H
93	H	Caf	Caf	Me
94	H	Caf	Caf	But

Figure 6: The phenolic compounds isolated from coltsfoot



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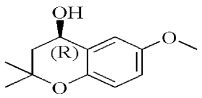

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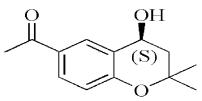

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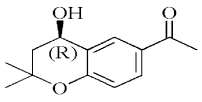

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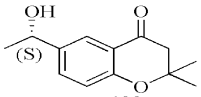

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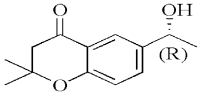

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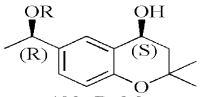
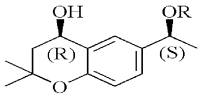


102 R=Me
104 R=Et



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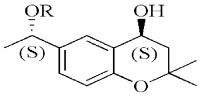
105 R=Et



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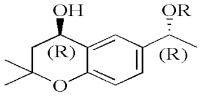
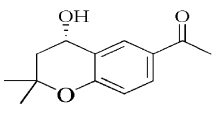
108 R=Et



107 R=Me
109 R=Et



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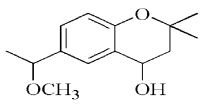

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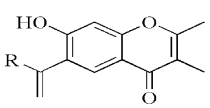
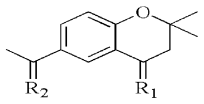
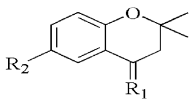


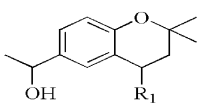
110 R=H
111 R=OH



112 R_1=H,β-OH, R_2=O



113 R_1=O R_2=OH
114 R_1=OH R_2=Ac
117 R_1=O R_2=Ac



115 R_1=O
116 R_1=OH

Figure 7: The chromones and its derivatives isolated from coltsfoot

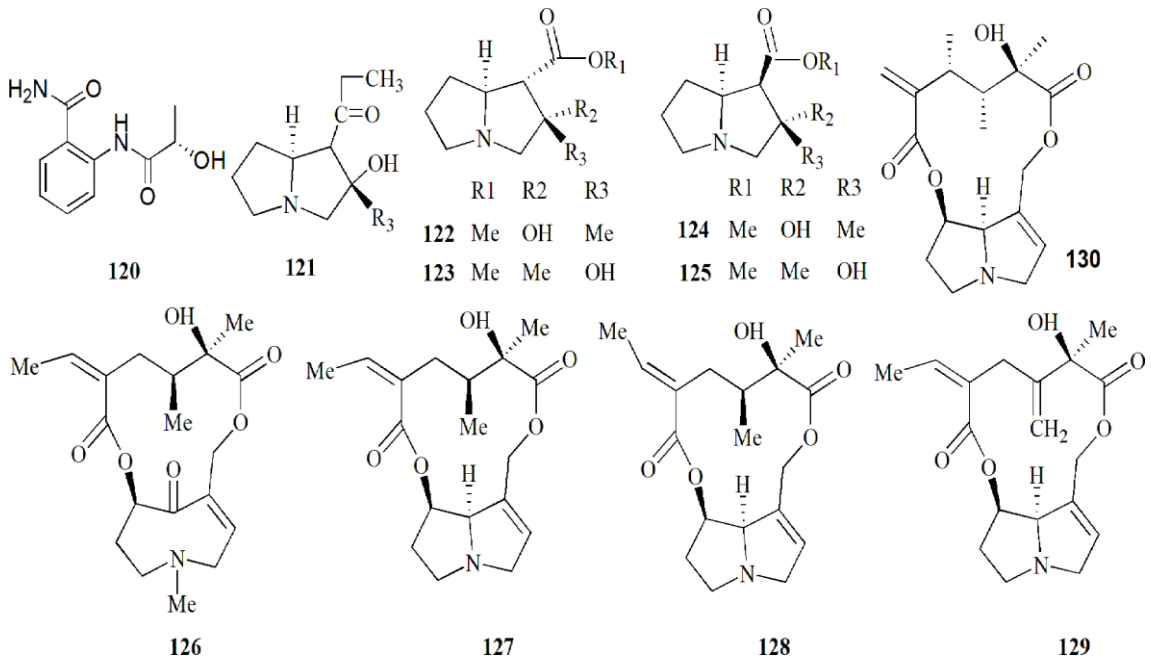


Figure 8: The alkaloids isolated from coltsfoot

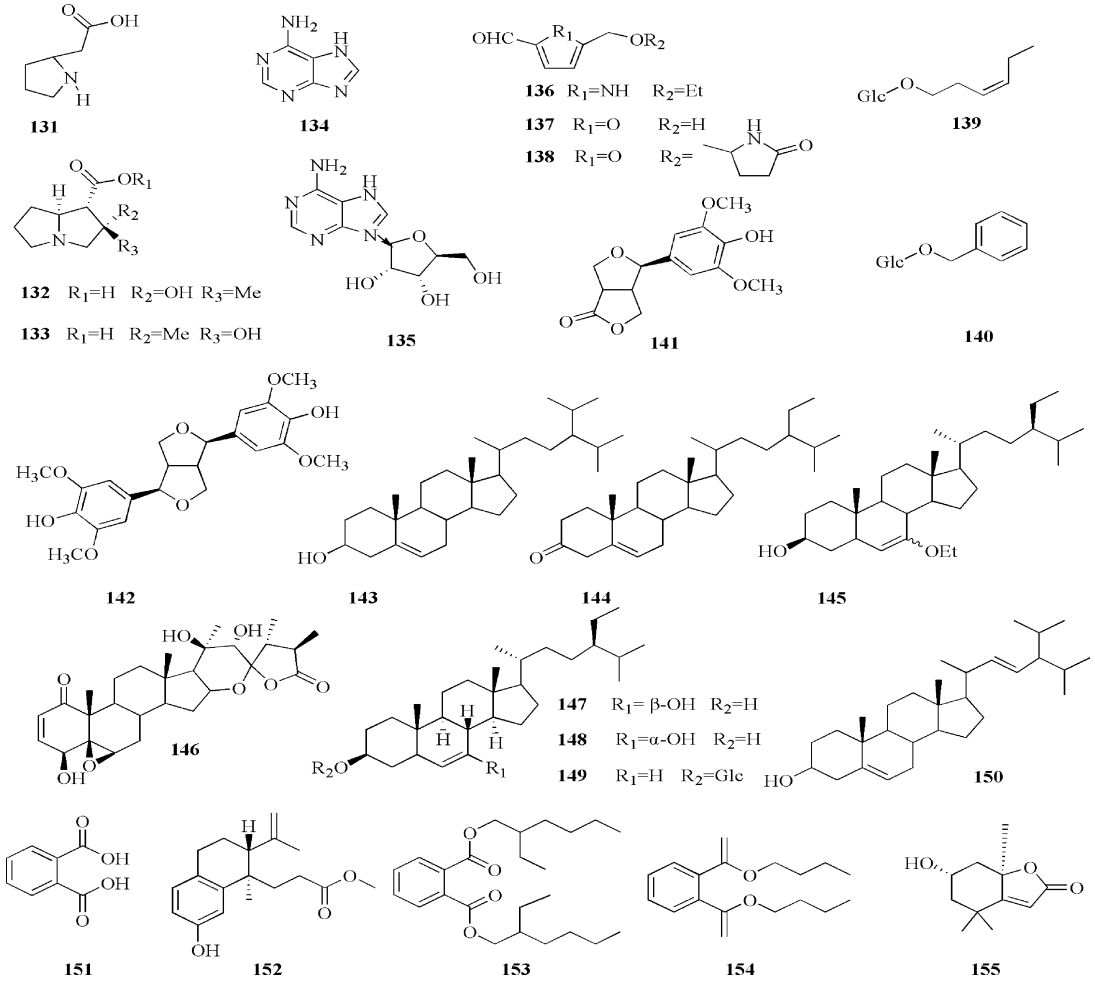


Figure 9: The other phytochemical compounds isolated from coltsfoot

Pharmacology:

There are some biological activities in the extracts or compounds of coltsfoot. Anti-inflammatory, anti-microbial, antiviral and anti-cancer are similar with traditional uses. In addition, many new biological activities have been discovered in modern research, such as anti-diabetic, neuro-protective activities, immune stimulating activities, anti-oxidant activity and cardiovascular.

- **Anti-inflammation**

  The biological analysis showed that 41 sesquiterpenoids inhibited NO production in LPS-stimulated RAW 264.7 cells with IC50 values rang 3.5 μM from 60.29 μM (Jang *et al.,* 2016; Li *et al.,* 2012; Qin *et al.,* 2014). Further mechanism studies indicated that TSLand its allied were evaluated for the anti-inflammation activity, with in vivo/vitro model, such as a CLP-induced mouse model of sepsis, having been used. Moreover, Wu *et al.* (2016) investigated a mixture of four compounds isolated from flower buds, (83:90: 88:92) (5:28:41:26), for its anti-inflammation, antitussive and expectorant activities in mice with the ammonia liquor-induced and the phenol red secretion. Subsequently, the authors suggested that the compound 4,5-di-O-caffeoylquinic acid showed the strongest effect to inhibit the leucocytosis by 49.7%, and they may act in a collective and synergistic way. However, the mechanism of the action requests further investigation (Wu *et al.,* 2016).

- **Neuro-protective activity**

  Cho *et al.* (2015) examined the effects of different concentrations (0.1-30 μg ml-1) of the EtOAc fraction from coltsfoot, which has shown to significantly inhibit various types of neuronal cell damage in cortical cells, including NO-induced, Aβ (25—35)-induced, excitotoxic induced by glutamate or oxidative stress-induced by measuring the `cell viability. It will be interesting to investigate whether other compound exhibit neuro-protective activities (Cho *et al.,* 2005). Subsequently, ECN administration (5 mg kg−1/day) can significantly ameliorate movement impairments and dopaminergic neuronal damage induced by 6-hydroxydopamine in mice. As well as, ECN (5, 10 μM) increasing cell viability of up to 80.7 % and 87%, respectively. The result indicated that ECN can activate the Nrf2/HO-1 signaling pathway both in vivo and vitro (Lee *et al.,* 2018). However, a single dose was used throughout in vivo studies, which failed to reflect the dose-dependent response and enhance understanding of its function on diseases. Furthermore, Hwang *et al.* (2018) investigated that flower buds extracts of coltsfoot (300 mg kg−1, p.o.) had a neuro-protective effect (Hwang *et al.,* 2018). However, further research should undergo to screen dominant compounds, which might be valuable for treating neurodegenerative illness.

- **Cytotoxicity and anti-cancer activity**

  Notably, Lee *et al.* (2014) findings first indicate that MeOH fraction of leaves and stems from coltsfoot. It could be used as a novel TRAIL sensitizer, having been activity in TRAIL-resistant Huh7 cells. Of particular interest, is the noteworthy anti-cancer activities demonstrated by sesquiterpenoids from the flower buds of coltsfoot for the eco-friendly synthesis of silver and gold nanoparticles (Lee *et al.,* 2019).

  In another study, TSLisolated from coltsfoot was evaluated for the anti-cancer/anti-proliferation, with colon cancer cell (SW 480 and HCT116). The results revealed that TSL may be held responsible for therapeutic new target and regard as potential scaffolds to treat angiogenesis dependent diseases (Li *et al.,* 2019). An in vitro study showed that TFPB1 (0 to 1000 μg ml-1), a homogeneous polysaccharide with a molecular weight of 37.8 kDa, dose-dependently induced apoptosis and inhibited proliferation. Interestingly, TFPB1 (1 mg ml-1) could expansively arrest cell cycle in G2-M phase, hence it is worth noting that the underlying mechanism of different structural polysaccharides to further study on cell cycle (Qu *et al.,* 2018).

- **Anti-microbialandanti-viraleffects**

  Turker and Usta (2008) highlighted the key role of the type of solvent used forextracting the plant materials, and although Kokoska *et al.* (2002) studied MeOHextracts of coltsfoot aerial parts did not show antimicrobial activity against *E. coli* (Kokoska *et al.,* 2002; Uysal *et al.,* 2018), Unlike the author's experiment, which showed inhibited bacterial growth (Turker and Usta, 2008). However, the diameter of the inhibition zone which established about the tested extracts was measured instead of determination of minimum inhibitory activity (Uysal *et al.,* 2018) investigated the antimicrobial activity of the EtOAc, MeOH, water extracts of coltsfoot. The authors suggested that the highest antimicrobial activity exhibited may be due to synergistic effect. However, the results were presented by the authors, which fail to mention the dose range used of the different extracts and the duration of cultivation of microorganisms with test ingredients (Uysal *et al.,* 2018).

- **Anti-oxidantactivity**

  Dragicevic *et al.* (2019) firstly, investigated the anti-oxidative properties of coltsfoot leaf water extracts in vitro, in human cell lines. Treatment of human bronchial epithelial cell lines with preparing plant extracts exhibited a significant anti-oxidative effect when oxidative stress was induced by hydrogen peroxide (Dragicevic *et al.,* 2019). However, their potential under in vivo, conditions and underlying mechanisms need to be investigated for these effects to be considered clinically relevant.

- **Cardiovascular**

  Li and Wang (1988) evaluated the cardiovascular effects of TSL. The authors indicated that the treatment of anesthetized rats, cats and dogs injected with TSL 0.4-4, 0.02-0.5, 0.02-0.3 mg kg^{-1}, respectively. Four animals produced an instant and dosedependent pressor effect within six minimums, similar to that of dopamine (0.01-0.03mgkg^{-1}). Unfortunately, tachyphylaxis was not observed. It can be seen that dose-related decrease in the heart rate of anesthetized dogs, as well as the acute injection LD50 in mice of TSL was 28.9 mg kg^{-1}. Other studies suggested that the mechanism of cardiovascular effect of TSL was peripheral, which was totally different from that of norepinephrine (Li and Wang, 1988).

Conclusions:

This review major provided a summary of the pharmacology, phytochemistry of coltsfoot, in addition to the application of coltsfoot in folk medicine. This plant is distributed extremely worldwide, and it is markedly used in traditional medicine. The medicinal uses depend on the location and the part, but similarities can be noticed. Indeed, in many countries, coltsfoot, as a functional food, are employed against respiratory disease, for the management of cough, sputum and pneumonia and against skin diseases.

Available pharmacological studies on constituents and crude extracts were shown extensive biological activities of coltsfoot demonstrating anti-inflammatory, neuro-protective, anti-microbial, anti-diabetes, anti-cancer, and cardiovascular etc. Additionally, sesquiterpenoids are considered as the main bioactive constituents, in particular, TSL, thus numerous bioactivities of TSL have been reported while other constituents, such as TNG and ECN have also been reported to be of prominent pharmacologic activities and are worth to be given more attention. Moreover, polysaccharide from coltsfoot has been identified as a very strong anti-proliferative agent and protective effects on bone marrow cells and small intestinal epithelium. However, all the above-described bioactivity they have not yet undergone clinical trials currently.

Subsequently, the neuro-protective, anti-diabetic and anti-obesity. and anti-cancer effects of coltsfoot showed that the plant could be a natural source to discovery promising, cost-effective and with minimal adverse side effect’s guide compounds for Alzheimer's disease, obesity, type 2 diabetes and cancer.

In addition, added identification and isolation can be done on extracts with reported bioactivities (e.g. Chromane enantiomers) to discover new active phytochemicals, and elucidate their structure-relationships and possible synergetic effects.

Moreover, quality control is poorly researched, and no direct clinical evidence has been reported. Well-developed methods should be established to ensure the consistency, safety, and efficacy of the coltsfoot.

Nevertheless, reports on the toxicity and safety evaluation are limits to provide guidance for clinical applications. Thus, firstly, the role of primary compounds in the therapeutic action

and in vivo experiments and systematically studies of coltsfoot should be further investigated. Secondly, the comprehensive evaluation of quality control, and long-term in vivo toxicity requires further detailed studies. Thirdly, the simple metabolic profile of the PAs content in the extracts needs to be define and should be carefully used with monitoring of liver function.

Based on this, we hope to highlight the importance of coltsfoot and provide some new research directions for this ethnomedicine.

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STANDARDIZATION METHODS: EUGENOL AND URSOLIC ACID

Faruk Alam*[1], Josef Yakin[1], Surabhi Mandal[2], Mukinur Hussain[3], Mohidul Islam[1], Bhupendra Shrestha[2] and Honey Jajo[2]

[1]Faculty of Pharmaceutical Science,

Assam down town University, Panikhaiti, Guwahati, Assam, India, 781026.

[2]Himalayan Pharmacy Institute, Majhitar, Rangpoo, East Sikkim, 737136.

[3]NEF College of Pharmaceutical Education and Research, Nagaon, Assam,78200.

*Corresponding author E-mail: faruk_2007a@rediffmail.com

Introduction:

Since the earliest humans realized they needed to rely on nature to live a healthy existence, people have relied on various plant resources for food, clothing, shelter, and medicinal purposes to treat a wide range of illnesses. Natural products have shown to be the most successful source of medications for us. A vast array of intricate and unusual chemical compounds, the structures of which would normally remain beyond human comprehension, may be synthesized by any plant [Nikam *et al.* (2012)], and distinct phytochemicals have been found to act as modulators of numerous metabolic and regulatory processes [Batra and Sastry (2014)]. The chemical phytoconstituent composition of the finished product determines the qualities of herbal medicine. A system for establishing quality control criteria for ayurvedic formulations currently needs to be developed because of the variability and sophistication of chemical constituents found in herbal plant-based medicines. In general, all medicines, whether synthetic or of plant origin, should fulfill the basic requirements of being safe and effective [Shailajan and Menon (2011)].

The literature of ancient Ayurveda has references to the standardization and quality control of drugs. Rigid standardization criteria are nomenclature used to describe the physical, chemical, and therapeutic characteristics of several herbs [Deogade *et al.* (2014)]. Despite the great effectiveness of Ayurvedic medicine, much remains unclear about the pharmacology, pharmacokinetics, and pharmacovigilance of several critical Ayurvedic pharmaceuticals. Moreover, a systematic comprehension of the underlying philosophies of Ayurveda is not adequate from a scientific standpoint due to a lack of data. It is vital to validate the fundamental ideas and medications employed in the ayurvedic medical system using cutting-edge research methodology, especially in light of the fact that the Western medical system has nearly achieved the pinnacle of contemporary medicine thanks to validated research and sophisticated methodologies.

Therefore, advancements in current research techniques are essential to the continued growth of Ayurveda [Chauhan *et al.* (2015)]. The world over, people have been using polyherbal formulation due to its beneficial and healing properties. To achieve the enticing healing effects, individual plants' dynamic phytochemical elements are insufficient. When different spices are

combined in a careful ratio in a polyherbal and herbo-mineral composition, the beneficial effects are enhanced and the adverse effects are decreased. Certain benefits, such as synergism, that are not present in single herbal formulae are bestowed by polyherbalism. At a healthy high dosage, polyherbal preparations show excellent efficacy in a number of disorders [Karole *et al.* (2019)].

Various active ingredients can be found in polyherbal formulations, such as tannins, camphor, flavonoids (such as luteolin, orientin, vicenin, triterpene, urolic acid, zinc, manganese, and sodium), volatile oil (eugenol, linalool, estragol, methyl chaviol, methyl cinnamate, and cileole), etc. According to the US National Library of Medicine (October 16, 2021), eugenol is an allyl chain-substituted guaiacol that belongs to the allylbenzene class of chemical compounds. It can help regulate blood cholesterol levels. Ursolic acid, a pentacyclic triterpenoid that was discovered in apple peels as early as 1920 and is commonly found in fruit peels, as well as in herbs and spices like thyme and rosemary, is found to be the active antiviral ingredients derived from plants, such as Tulsi (Ocimum sanctum).

Using molecular docking and molecular dynamics simulation analysis, the aforementioned active phytoconstituents were tested against the major protease (Mpro), the most often used macromolecular target of SARS-CoV-2, which is revealed to be accountable for the virus's proteolytic cleavage-mediated proliferation. With few to no negative consequences, blocking the primary protease with natural phytoconstituents would act as a checkpoint for viral entry and prevent further replication and propagation [Shree *et al.* (2022)]. Macroscopic, microscopic, and physicochemical examinations are examples of observational experiments that are necessary for achieving maximum quality control of herbal ingredients. Standardization techniques employing nonconventional analytical practices are therefore necessary for the authenticity of herbal or polyherbal Ayurvedic medicines [Kaur *et al.* (2020)]. Therefore, our goal is to list the standardization procedure for active ingredients such as ursolic acid and eugenol that are found in plant-based or polyherbal preparations. The type of chemicals included in a certain plant's extract can be determined through analysis of the medicinal plant. Additionally, it serves to discover the bioactive substances, as well as to standardize their effects. As models for the synthesis of novel medications, they are frequently useful [Pattanayak *et al.* (2010)].

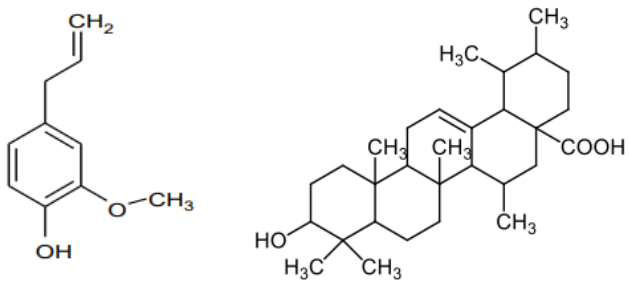

Figure 1: Chemical Structure of Eugenol and Ursolic Acid

Methods and Materials

1. Collection and authentication

The study's therapeutic herbs were acquired from several sources. Only freshly picked, shade-dried portions or the entire plant must be verified by the Botanical Survey of India.

2. Preparation of extract

The entire or selected portions of the harvested polyherbal plant were dried in the shade, cleaned, and ground using a machine. Each chosen plant's 1000 g of crushed or powdered material was added to distilled water until it was completely exhausted. Using Whatman filter paper that was concentrated at the proper temperature (400C) on a rotary evaporator, successive extracts were independently filtered before being dried in a freeze drier. The final dried ingredients were kept in a closed container at a cool temperature, and the percentage yield was computed (w/w) accordingly [Agnihotri and Singh (2014)].

3. Characterization of extract

The physiologically potent dried extract of polyherbals represents a range of micrometric and physical features. Because it is made up of discrete particles with varying sizes and shapes sporadically strewn with air spaces, the dried extract is heterogeneous. This complexity increases when it contains polyherbal [Nagar *et al.* (2011), Tiwari and Sharma (2017)]. For every formulation, measurements were made three times and the results were given as the average ± standard deviation (SD).

4. **Identification of eugenol content by TLC from eugenol tablet [Shah and Patil (2019), Mane and Manish (2018)]**

Eugenol tablets will be taken and ground into a powder. The powdered material will then be extracted in a Soxhlet extractor at 45–500C for 48 hours along with a few milliliters of methanol. The extracted material will be evaporated and transferred to Petri plates, with the leftover medication being gathered.

4a. Standard Preparation [Saran *et al.* (2013)]

In a dry and clean test tube, a few milliliters of 99% pure eugenol will be taken, and the standard stock solution will be made by dissolving it in methanol to yield a stock solution with 100 µg/mL of eugenol. On the Merck aluminum plate precoated silica gel F254 with a thickness of 0.2 mm, the standard eugenol will be visible.

4b. Preparation of Sample Solution of Eugenol [Sundaram *et al.* (2011)]

The dosage for the Tulsi tablet's extracted solution is 10 mg/10 ml. Since it was in methanol, which has a 100µg/ml concentration, the volume will be modified by methanol up to 10 ml.

4c. Preparation of mobile phase [Joshi *et al.* (2015)]

Using an ultrasonicator, 95% methanol and 5% chloroform were combined to create the mobile phase, which was then placed into a chromatographic chamber in an amount of about 100 ml. To prevent the edge effect, the chamber will be flooded for thirty minutes.

4d. Procedure [Shah and Patil (2019)]

The spotted plates will be inserted into the chamber following chamber saturation. Next, the mobile phase will permit running, and the retardation factor (Rf) of the current eugenol at the location will be determined by observing its movement in accordance with its affinity towards the mobile phase.

5. Identification of Ursolic Acid content by TLC [Naumoska *et al.* (2013)]

Three triterpene acids (ursolic, oleanolic, and betulinic acid) were separated using a variety of sorbents for thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) (silica gel 60, C2 RP, and C18 RP). Several developing solvents were used, with methyl acetate, ethyl acetate, and ethyl propionate serving as selectors. The diluent n-hexane was used as a non-polar diluent.

5a. Preparation of sample solution of Ursolic Acid from tablet [Rout *et al.* (2012, Sundaram *et al.* (2011)]

The tablets containing ursolic acid will be crushed into a powder, and the powder will be extracted using a microwave-assisted extraction technique in 100 ml closed vessel units with methanol serving as the solvent. The microwave power (450 W), time (4 min), and solvent (20 ml) will be the parameters for the microwave extraction process. The extracted material will be allowed to cool, then filtered using Whatman no. 1 filter paper and dried in a rotating evaporator. To obtain clear extracts, the tubes will be centrifuged for 5 minutes at 7500 rpm (40C) after the final amount of each extract has been made with methanol (10 mg/ml). For a subsequent phytochemical examination, the produced extracts will be stored in a refrigerator at 40 degrees Celsius.

5b. Preparation of standard solution of Ursolic Acid

Accurately weigh out 10 mg of the standard ursolic acid. Then, make standard solutions by adding 10 mL of methanol to get a concentration of standards of 1 mg/mL.

5c. Preparation of mobile phase

Using ultrasonication, n-hexane, ethyl acetate, and acetone will be combined in a ratio of 16.4: 3.6: 0.2 v/v to create the mobile phase. To prevent edge effect, a 100 mL mobile phase will be collected from this and placed in a chromatographic chamber. The chamber will then be allowed to saturate for 30 minutes.

5d. Method

The spotted plates will be inserted into the chamber following chamber saturation. After allowing the mobile phase to run, the amount of ursolic acid that is currently present in the area will shift under its affinity for the mobile phase, and its retardation factor (Rf) will be determined.

6. HPLC analysis of Eugenol

By precisely weighing 10 mg of eugenol standard in a 10 mL volumetric flask, a stock solution of 1000 parts per million (ppm) will be created. It will then be further diluted using HPLC-grade methanol until the desired concentration is reached. For 10 seconds, the solution will be vortexed [Yun *et al.* (2010), Patra and Kumar (2010)].

6a. Preparation of sample solution

A rotary shaker will be used to carry out the solvent extraction process after a few grams of the powdered formulation are taken and mixed with a few milliliters of methanol for a full day. Whatman filter paper no. 41 will be used to filter the solution after the tubes are centrifuged for ten minutes at a speed of 4000 revolutions per minute (rpm). The filtrate will be injected into the HPLC apparatus after being suitably diluted in the mobile phase.

6b. Chromatographic conditions

A flow rate of 1 mL/min will be used to pump the mobile phase. Before being used, the mobile phase will be degassed in an ultrasonic bath and filtered through a 0.45 μm nylon membrane filter. The injection volume was 30μL, and a chromatographic peak will be seen at 215 nm at a flow rate of 1.0 mL/min.

7. UV-Spectroscopic analysis of Eugenol

7a. Determination of λ_{Max}

Using appropriate sonication, 95 parts methanol and 5 parts chloroform will be combined to create the solvent. Using this solvent, a standard eugenol solution containing 100 μg/mL of eugenol is created. To obtain 10 μg/mL of eugenol, 1 mL will be extracted from this solution and dissolved once more up to 10 mL of solvent. To obtain 1 μg/mL of eugenol, 1 mL of this solution will be extracted and dissolved in up to 10 mL of solvent. To find λ max, the three produced eugenol concentrations (1 μg/mL, 10 μg/mL, and 100 μg/mL) will be analyzed [Pramod *et al.* (2013)].

7b. Preparation of standard curve

A stock solution of ethanol with a concentration of 1000μg/mL will be made. Various aliquots containing 1, 2, 3, 4, 8, 10, 20, and 25 μg/mL will be generated and scanned in UV spectroscopy using that stock solution. We'll record the matching absorbances. Following that, a calibration curve will be drawn [Omkar *et al.* (2021)].

7c. Analysis of eugenol

The band of eugenol obtained from the extract of *Ocimum sanctum* tablet will be identified and scrubbed away by comparing it to the Rf value of standard eugenol from thin layer chromatography. After fully combining the scrub silica gel with solvent, the eugenol-containing material will be filtered. Subsequently, the solution will be extracted for UV spectroscopy examination.

8. HPLC precedure for Ursolic Acid

Nutraceuticals found in highly beneficial herbs are ursolic and betulinic acids. It is quite difficult to quantify them from the solvent extracts of the herbs. Since each of the chromatographic techniques currently in use for the identification and quantification of these acids is distinct from the others, a unique technique would be needed for each acid. To separate betulinic acid and ursolic acid from their methanol extract of *Vitex negundo* Linn leaves, the reverse phase (RP-HPLC) method was devised [Taralkar and Chattopadhyay (2012)].

8a. Preparation of standard solution

Accurately weighing 10 mg of ursolic acid reference standards into 100 ml volumetric flasks and using sonication to dissolve in acetonitrile, water, and methanol (90:5:5) will yield stock standard solutions with a concentration of 1 mg/ml. After serial diluting the solution to obtain 100 µg/ml, it will be filtered using Whatman filter paper.

8b. Preparation of sample solution

The ethanolic sample will be precisely weighed into a volumetric flask, and sonication will be used for 10 minutes to extract the ursolic acid using acetonitrile, water, and methanol (90:5:5 v/v/v). After centrifuging the mixture for five minutes at 4500 rpm, the supernatant will be poured into a volumetric flask. The remaining solid will be extracted again using a 90:5:5 v/v/v mixture of acetonitrile, water, and methanol for ursolic acid. It will then be sonicated for five minutes and centrifuged as previously described. The supernatants will be mixed with water to make a volume of 100 ml. Prior to being injected for HPLC analysis, every sample will be centrifuged for 10 minutes at 13,000 rpm.

9. Analytical conditions for HPLC [Shailajan *et al.* (2012)]

A C18 reverse phase column will be used for the analysis, and the temperature will be maintained at 200C. The mobile phase will flow at a rate of 1.0 milliliter per minute. The injection volume of the sample will be 10 µl. For ursolic acid, the ideal wavelength for detection is 261 nm. Three injections of the extract are planned. By contrasting the retention time and UV spectra of the ursolic acid with those of their reference standards, the chromatographic peaks of the acid will be verified. Concentration versus area will be plotted to create standard plots.

10. Estimation of Eugenol in formulation

By applying the appropriate volume of 10 µl of the test solution and 10 µl of the reference solution, respectively, the content of eugenol was ascertained. The suggested chromatographic conditions were followed in developing and scanning the plate. The linear regression equation yielded the concentration.

10a. Method validation

i. Accuracy

Based on recovery studies carried out using the conventional addition method (spiking), the accuracy of the suggested approach was determined. On the sample tracks of the TLC plate, a

precisely measured quantity of standard eugenol was put in triplicate, subsequently increasing the concentration by 8 ng/spot. Following the suggested chromatographic conditions, the chromatogram was created and scanned. It was computed what proportion of standard eugenol was recovered using the suggested procedure.

ii. Precision

***Intra-Day*:** Three analyses of Eugenol standard solutions in the 24-64 ng/band were performed on the same day to determine the intraday precision. The percentage RSD was then computed. ***Inter-Day*:** %RSD was computed after the same solution was analyzed three times in a week to evaluate inter-day precision.

iii. Repeatability

A TLC plate (n = 6) was spotted with 10 µL containing 40 ng/spot of standard ethanol to evaluate the sample application's repeatability. The suggested chromatographic conditions were followed in developing and scanning the plate. Peak area was measured and its average, standard deviation (S.D.), and percentage relative standard deviation (% RSD) were determined.

iv. Specificity

By evaluating standards and formulation and applying them concurrently on the same plate, the specificity of the procedure was investigated. The presence of eugenol spots in the formulation was verified by contrasting the Rf values with those of the benchmark. By comparing spectra at the spot's peak start, peak apex, and peak end positions, the peak purity of each standard in the sample track was evaluated. The formulation, reference standard, and their overlaid spectrum chromatograms are displayed, in that order.

v. Ruggedness

The method's robustness was tested at concentrations of 40 ng/spot of a working standard eugenol solution. The method's ruggedness was indicated by the % RSD readings being less than two(<2).

Discussion:

Temperature, storage, packing, drying, and other variables have an impact on the quality of phototherapeutic agents as well as the therapy effectiveness of plant elements. As such, method validation in addition to standardization is becoming more and more crucial for regular raw material quality control analyses as well as for evaluating the quality of marker compounds whose active principle is unknown. Our knowledge regarding the quantification of phytochemicals from commercial ayurvedic formulations to set quality specifications, stability profiles, and chemical analysis of analyte of interest is largely unknown, despite the number of studies published on the standardization of in-house and marketed herbal medicinal formulations. This is primarily due to the lack of simple, reliable, and sensitive validated analytical methods.

Conclusion:

According to the International Council for Harmonisation (4-ICH) guidelines Step 4, an analytical technique must be created for marketed herbal formulations containing ursolic acid and eugenol and verified by UV method. As far as we are aware, a titrimetric technique was created to measure the amount of vitamin C in the commercial product. Thus, the newly developed analytical method for phytochemicals was robust, linear, and accurate, and it can be used to determine ursolic acid and eugenol in pharmaceutical dosage forms as well as bulk. Several analytical instruments are now in use, and it has been noted that the proposed procedures may be utilized for routine analysis of ursolic acid and eugenol semisolid dosage forms. These validation characteristics, which include linearity, accuracy, and ruggedness, demonstrate this. Every result that was produced by utilizing a variety of analytical techniques ought to adhere to BEER'S law and fulfill the ICH criteria. estimating and measuring the product's active ingredient content, such as ursolic acid and eugenol, using analytical techniques including UV, TLC, and HPLC to verify the product's legitimacy.

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EXPLORATION OF PHARMACOGNOSTIC AND PHARMACOLOGICAL ACTIVES ON *LANTANA CAMARA*

Gowri Sankar Chitapalli[1,2] and Durgaprasad Kemisetti*[1]

[1]Faculty of Pharmaceutical Science, Assam down town University,
Sankar MadhabPath, Gandhi Nagar, Panikhaiti, Guwahati, Assam, India, Pin- 781026.

[2]School of Pharmacy, Arka Jain University, Jharkhand, India.

*Corresponding author E-mail: kdp251999@gmail.com

Abstract

Worldwide interest in plant research has grown recently, and a substantial amount of data has accumulated to demonstrate the enormous potential of medicinal plants utilised in a variety of traditional systems. The majority of the treatments in conventional medical systems were derived from plants, and they were shown to be effective. *Lantana camara* L, a member of the Verbenaceae family, is a herb that has been traditionally used to cure a number of illnesses, including asthma, rheumatism, fevers, coughs, tetanus, and malaria. Numerous studies on phytochemistry have been conducted with this plant. Numerous chemicals, including triterpenoids, proteins, carbohydrates, lactones, furfural, flavonoids, amino acids, alkaloids, saponins, glycosides, tannins, and steroids, have been reported to be present in the plant. Numerous traditional uses have been validated by scientific research. This review discusses the pharmacology, phytochemistry, and ethnopharmacology of *Lantana camara* L.

Keywords: *Lantana camara* L, Phytochemistry, Phytoconstituents.

Introduction:

Seven species, six from South America and one from Ethiopia, made up the genus Lantana (Verbenaceae) when it was first described by Linnaeus in 1753. While certain species of Lantana are native to tropical Asia and Africa, most are endemic to subtropical and tropical America. Currently, it is found in about 50 countries, where numerous species are grown under hundreds of cultivar names. *Lantana camara* L. (Verbenaceae), also referred to as wild or red sage, is the most widely distributed species in this genus and is valued as an ornamental garden plant as well as a renowned weed. Tropical, subtropical, and temperate climates are home to *Lantana camara*, which grows lushly up to 2000 meters above sea level. It is a straggling, woody plant that has red, pink, white, yellow, and violet flowers [1,2].

A. FRESH PLANT B. DRIED PLANT

Figure 1: Plant of *Lantana camara*

Tropical and subtropical America is home to the plant. In the late 1600s, Dutch explorers transported seeds from Brazil to the Netherlands, and subsequently, explorers from other nations brought the seeds to Europe, Great Britain, and North America. It was brought as a garden flower to Hawaii and quickly expanded throughout the islands of the Pacific, Australia, and southern Asia. Nurserymen commercialized and popularized various colourful variants in the 18th and 19th centuries, and it is today grown as an ornamental plant all over the world. The L. camara complex is linked to most of the 650 cultivar names in the genus. In many tropical and subtropical climates, the plant an aggressive, obligate out-breeder weed has overtaken large tracts of pastures, orchards, and forest areas. It is considered to be among the top ten noxious weeds worldwide [3,4].

In addition to being a well-liked garden plant, L. camara is also claimed to make a helpful hedge and to be an excellent crop preparation by mulching the ground with fine leaves. It enriches the soil, prevents soil erosion, helps to hold onto humus in deforested areas, and increases the fertility of rocky, grave, or hard laterite soils. In India, Lantana leaves and twigs are frequently utilised as green mulch. The ash's high manganese and potassium content is beneficial for manuring coconut trees. Ripe blue-black berries are consumed in tropical regions, however consuming the green fruit has killed people. Both humans and livestock are poisoned by L. camara **[5, 6].** Both humans and livestock are poisoned by L. camara. When grazing animals consume leaves from L. camara, it can lead to photodermatitis, jaundice, liver damage, and even death **[7].** The pharmacology, phytochemistry, ethnopharmacology, and plant biography of Lantana camara L. are examined in this review. This data serves as a foundation for assessing Lantana camara as a valuable renewable resource.

About the *Lantana Camara*

- It's also referred to as giant sage, angel lips, ach man, and so on.
- *Lantana Camara* is a wild, evergreen, and perennial foreign weed.
- It was originally brought to India in 1809 from Sri Lanka.

- If there is any injury anywhere in the body, its leaves can heal it.
- It is used to cure malaria.
- It is also use to cures joint pain.
- If someone has a problem with his teeth then it cures that also.

Pharmacognostic studies of *Lantana camara*

1. Biological source

Lantana camara is an attractive ornamental shrub [10].

2. Geographical source

Native to the Caribbean, Central and Northern South America, and other tropical regions, lantanas are tropical plants. Today, Lantana is grown in around 60 nations.The plant is widely distributed throughout India's northeastern states, Himachal Pradesh, Uttarakhand, and Uttar Pradesh [11,12].

3. Morphology of *Lantana camara*

Brief morphological characterstics of *Lantana camara* shown in Fig. 2.

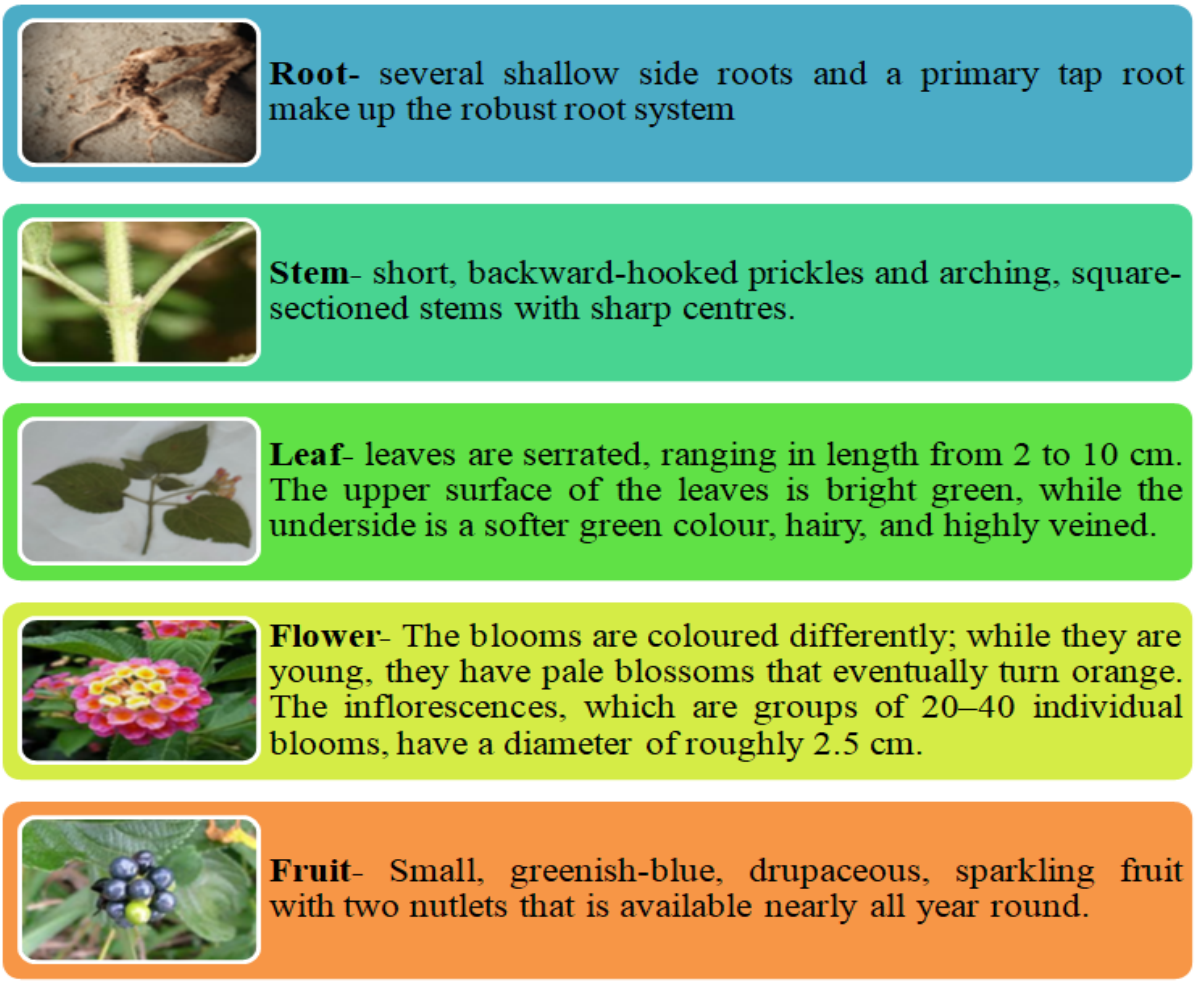

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Figure 2: Morphological characterstics of *Lantana camara*

5. Cultivation

Lantana camara is a robust shrub that grows to a height of 2 to 4 meters. It is low and upright or subscandent. The leaves are oppositely paired, ovate or ovate oblong, 2–10 cm long, and 2–6 cm broad. The leaves have a strong smell when crushed and are brilliant green, tough, and coarsely hairy. They also have serrated edges[14,15]. In cultivated types, the stem is often free of thorns, while in weedy varieties, it bears recurved prickles. It has a square cross section, is woody, hairy when young, and becomes cylindrical and up to 15 cm thick as it ages. Lantana can reach a height of 15 meters when other vegetation provides support. Flower heads typically include 20 to 40 flowers, each measuring 2.5 cm in diameter. The flowers' colours range from orange, pink, purple, and red to white, cream, or yellow **[22,23].** August to March is when flowers appear, though they can bloom year-round if enough light and moisture are present. Thrips and lepidopteran species are examples of pollinators. The fruit has two nutlets and is greenish blue-black in colour. It is drupaceous, shiny, and has a diameter of 5 to 7 mm. From September to May, 1 to 20 seeds are set on each flower head. A mature plant can yield up to 12,000 seeds per year. When there is enough moisture in the air, seeds germinate; low light levels inhibit this process. With a major taproot and a mat of several shallow side roots, the root system is incredibly robust [25,26].

6. Chemical constituents

Lantana contain chemicals like Lantacin, camarin, camarinin, alphaphellandrene, germacrene D, limonene, betacaryophyllene, sabinene, elemene, veside, geniposide, 8-epiloganin, lamiridoside. Some more chemicals and biological activities are shown in Table1. [27,28,29]

Table 1: Biological activity of chemical compound present in *Lantana camara*

S.No.	Compound Name	Biological Activity
1.	B- pinene	Inhibiting the seed germination, growth and antibacterial activity.
2.	Caffeic acid	Suppress root- infecting fungi and root- knot nematode.
3.	Camaric acid	Nematicidal activity
4.	1,8- Cineole	Inhibiting the growth of plant
5.	Cinnamic acid	Inhibiting the activity of plasma H+ ATPase, PPase and inhibit the process of seed germination.
6.	Dipentene	Inhibiting the growth of plant
7.	Ferulic acid	Reduced chlorophyll contents of soyabean leaf and inhibit the process of seed germination
8.	Geniposide	Inhibited hepatoxicity and the DNA repair synthesis induced by aflatoxin B1 in rat primary hepatocytes.

9.	Icterogenic acid	Toxic to sheep, cattle, goats.
10.	Lantanilic acid	Nematicidal activity
11.	Lentadene A,B,C	Death of horses, cattle, sheep, goats and rabbits by failure of liver and other organs
12.	Linaroside	Antimicrobial and Nematicidal activity
13.	Lantanoside	Antimicrobial and Nematicidal activity
14.	linaroside	Antibacterial activity
15.	Myristic acid	Inhibiting the growth of plants
16.	Oleanolic acid	Hepatoprotective, Anti inflammatry, Antimicrobial, antiulcer, Antifertility, Antimicrobial and Nematicidal activity
17.	Oleanolic acid	Inhibiting the growth of mouse melanoma cells in cultures and herpes simplex virus type I and type II in vitro.
18.	Palmitic acid	Inhibiting the growth of vegetables
19.	p- Coumaric acid	Supress root- infecting fungi, root-knot mematode, inhibit the process of seed germination and inhibit the growth of morning glory
20.	**1.** Hydroxybenzoic acid	Inhibit the enzymatic activity, Nematicidal activity
21.	Ursonic acid	Inhibit the growth of mouse melanoma cells in cultures and Herpes simplex virus type I and type II in vitro.
22.	Ursolic acid	Inhibitors of human leucocyte elastase
23.	Verbasocide	Inhibit the enzy6matic activity

Pharmacological activities on L*antana camara*

The plant has been utilised to treat a wide range of illnesses in many different places of the world. The plant's leaves are cooked, much like tea, and the resulting infusion is used as a cough cure. The plant's decoction is used to treat rheumatism, malaria, tetanus, and abdominal viscera ataxia. A decoction of the leaves is applied as a wound lotion, and pulverised leaves are used to treat cuts, ulcers, and swellings. *Lantana camara* was used in traditional medicine to treat tumours and malignancies. The leaves and petals were used to make a tea that was used to treat fever, influenza, and stomachaches. The leaves were used as a poultice in Central and South America to cure chicken pox, measles, and ulcers. Preparations made from the plant were used to treat excessive blood pressure, rheumatisms, colds, and fevers. In Ghana, children were given the powdered root in milk to relieve stomachaches, and the entire plant was infused to treat

bronchitis **[**30,31]. A steroid from the leaves called lancamarone has allegedly been shown to have cardio tonic qualities. It has also historically been used as an insecticide, anthelmintic, tonic, and treatment for stomachaches [32]. Asian nations employed leaves to cure ulcers, rheumatism, and wounds [33]. Pharmacological activities are shown in Fig.3.

Figure 3: Pharmacological activities on *Lantana camara*

Antibacterial activity

Extracts of leaves and flowers made with ethyl acetate were tested for antibacterial properties by Deepak Ganjewala *et al. Bacillus subtillis* (1429), *Pseudomonas aeruginosa, Staphylococcus aureus* (MTCC96), and *Escherichia coli* (also known as E. COLI) were the test organisms used in this investigation. Significant antibacterial activity was demonstrated by leaf and flower ethyl acetate extracts against the tested bacteria, with values of zone of inhibition ranging from 10–21 and 9–15 mm, respectively. The zone of inhibition (mm) in flower and leaf ethyl acetate extracts ranged from 10–21 and 9–15 mm, respectively. Different L. camaras have different antibacterial properties. The type of bacterium employed in the study and the extracts' concentration both affect how effective they are [34,35]. Additionally, Mary Kensa V has investigated the antibacterial properties of several L. camara extracts. By using the disc diffusion method, extracts of leaves, stems, and roots from methanol, petroleum ether, water, and chloroform were evaluated against different pathogenic bacteria species, including *E. Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus saprophiticus*. On every tested bacterial strain, leaves demonstrated a significant inhibitory effect in contrast to stem and root extracts [36,37].

Antihelmintic activity

Three concentrations of each extract (10, 50, and 100 mg/ml) were utilised in the study to examine the anthelmintic activity of successive extracts from the leaves of *Lantana camara* Linn against *Pheretima posthuma*. The worm's time of paralysis and time of death were the parameters utilised to calculate the activity. Significant anthelmintic activity was shown by the ethanolic extract at the maximum concentration of 100 mg/ml. A standard reference of 10 mg/ml piperazine citrate was used, and a control of 1% gum acacia in normal saline was used. The hydroalcoholic extract of *Lantana camara* exhibited a greater anthelmintic efficacy than the ethanol extract [38].

Antiulcer activity

The antiulcerogenic efficacy of *Lantana camara* methanolic extract was assessed in models of cysteamine-induced duodenal ulcers, ethanol-induced gastric ulcers, and aspirin-induced gastric ulcerogenesis in pyloric ligated rats. Two separate oral dosages of 250 mg/kg and 500 mg/kg of the extract were given. Lipid peroxidation, ethanol-induced gastric ulcer model with decreased glutathione levels, and inhibitory zone diameter against *Helicobacter pylori* were also found. In models of aspirin + pylorus ligation caused ulcerogenesis and ethanol generated stomach ulcers, the L. camara extract considerably ($P < 0.01$) decreased ulcer index, total acidity, and significantly ($P < 0.01$) elevated gastric pH. Additionally, the extract considerably ($P < 0.01$) decreased the ulcer index of the duodenal ulcer caused by cysteamine. Lipid peroxidation was significantly ($P < 0.01$) decreased and reduced glutathione levels were increased in the L. camara. In rats, the methanolic extract of Lantana camara leaves has been found to heal stomach ulcers and to stop the growth of duodenal ulcers [14,39].

Termiticidal activity

The termiticidal properties of *Lantana camara* var. aculeata leaf extracts against adult termite workers were investigated. It was discovered that the 5% chloroform extract was highly efficient against termite labourers [20]. Activity that heals wounds Sprague Dawley rats were used to test the ethanol leaf extract of L. camara's ability to heal burn wounds. The rate of wound contraction and the length of epithelialization were used to measure healing. Wounds treated with extract recovered in roughly 21 days, not much longer than the controls. According to the evidence, L. camara does not appear to promote wound healing in burn wounds [40]. An excision wound model was used to test the L. camara aqueous extract's wound-healing potential, and the results were promising. The main metrics used to assess the wound healing activity were wound contraction, wound healing time, and collagen production [41].

Using antimitotic activity, pet ether, chloroform, ethanol, and aqueous extracts of *Lantana camara* were evaluated for potential anticancer action. The ethanol extract had superior antimitotic action, as evidenced by the decrease in mitotic index from 90.2% to 61.4% and 96.6

% to 49.5 % following a 1- and 3-hour treatment with an extract concentration of 10 mg/ml. The mitotic index of all the roots treated with extracts was considerably lower than the control's mitotic index. After a one-hour and three-hour treatment, respectively, the mitotic index of methotrexate was determined to be 56.0% and 55.8% [42].

Effect on red blood cells

An aqueous extract of *Lantana camara* was tested for its effects on RBC shape and osmotic fragility. The data revealed a significant ($p < 0.05$) increase in hemolysis and alterations to the shape of red blood cells when the extract was present. The potential pharmacological characteristics of the chemical components in the aqueous extract of *Lantana camara* may be linked to these actions [43].

Antimotility activity

The antimotility activity of *Lantana camara* leaf powder, Lantana camara methanol extract (LCME), lantadene A, neostigmine, and neostigmine + LCME was assessed in the intestinal tract of mice. A promotility agent was employed with neostigmine. The charcoal meal test was used to measure intestinal motility, and the gastrointestinal transit rate was calculated by dividing the length of the small intestine by the percentage of the distance the charcoal travelled. In normal mice, the intestinal transit of charcoal was entirely stopped by a higher dose of 1g/kg of LCME, while the intestinal transit at 500 mg/kg was 26.46%.At the same dosages, the intestinal transit percentages in the neostigmine pretreatment groups were 24 and 11, respectively. When mice were given plant extracts intraperitoneally at doses of 125 and 250 mg/kg, their faecal output was much lower than that of mice treated with castor oil. Higher dosages (500 and 1000 mg/kg) nearly totally ceased the production of faeces [44].

Cytotoxicity and antitubercular activity

Investigations into the antitubercular activity of *Lantana camara* on multiple-drug-resistant Mycobacterium were conducted among HIV-positive individuals in Nigeria. The potency of extracts was compared with conventional medications, and the well-in-agar-diffusion method was used to assess the minimal inhibitory concentration (MIC). The cytotoxicity of brine shrimp was ascertained. Drugs with MICs of 0.33 mg/ml, 0.25 mg/ml, and 0.20 mg/ml were rifampicin, streptomycin, and isoniazide, respectively.L. Camara's minimum inhibitory concentration (MIC) for *M. tuberculosis* and unknown *M. avium* complex was 0.89 mg/ml and 0.63 mg/ml, respectively. 32.6 ppm of *M. tuberculosis*, 55.9 ppm of *M. avium* complex, and 51.3 ppm of unknown species were found in the LC50 of L. camara. The extracts' demonstrated efficacy is in line with their traditional medicinal application in the management of Mycobacterium species [45,46].

Anti hyperglycemic activity

Extract of *Lantana camara* methanol Oral administration of linn fruits at dosages of 100 and 200 mg/kg was examined for its hypoglycemic effect in both normal and streptozotocin-induced diabetic rats. In both streptozotocin-induced diabetic rats and normal rats, the fasting blood glucose level was significantly reduced by the 200 mg/kg methanol extract of *Lantana camara* Linn fruit [47].

Effect on general reproductive performance and teratology

There have been reports on the effects of a hydroalcoholic extract from the leaves of *Lantana camara* var. aculeata on rat teratology, overall reproductive function, and fertility. The results demonstrated that, in the absence of any indications of maternal toxicity, the extract caused embryotoxicity as demonstrated by post-implantation loss and interfered with the frequency of foetal skeleton abnormalities in dams treated with it [48,49].

Some various herbal formulations of *Lantana camara* in India

The use of herbal medicine has grown in importance on a global scale for both medical and financial reasons. Even though the use of these herbal remedies has grown, both developed and developing nations have severe reservations about their efficacy, safety, and quality. Due to the absence of the common adverse effects associated with allopathic medications, patients are becoming more compliant with herbal therapies [50].

The goal of the current study is to manufacture of herbal gel that contains extract from the leaves of *Lantana camara*. Using Carbapol 940, propylene glycol, methyl and propyl parabens, *Lantana camara* leaf extract, and the necessary amount of distilled water, the gel formulation was created. Tri-ethanolamine was added drop by drop to preserve the skin pH (6.8–7). The formulations' physicochemical parameters (pH, spreadibility, stability, etc.) were ascertained. According to ICH requirements, stability investigations were conducted for three months at various humidity and temperature levels. The formulation with *Lantana camara* leaf extract demonstrated superior stability, according to the data. Additional formulations were tested for skin irritation using rats as the animal model, and the results indicated that there was none. The herbal gel made from *Lantana Camara* leaf extracts was not studied, as the literature review indicated; for this reason, the activities have been looked at in this study [51].

Importance of *Lantana camara* [52]

Traditional uses

- It can be applied as ground cover or as a border. It can be utilised for arid landscaping and is resistant to salt and drought.
- It endures the entire summer, is drought-resistant, and is simple to grow. The Lantana flower enhances the beauty of a scene.
- *Lantana camara* oil has cooling, balancing, and soothing properties.

- beneficial for menstruation health and respiratory treatments.
- Excellent for skin care, however stay away from it if you have sensitive skin
- If there is any injury on the body, its leaves can heal it.

Medicinal uses

- The decoction made from *L. camara* leaves is mostly used in herbal therapy to treat like Fever, Cough, Influenza, stomach aches, Malaria, wounds.
- Additionally, it has been noted that it can be used to treat ulcers, rheumatism, chickenpox, cancer, and measles.
- Leprosy, scabies, and skin irritation are all treated externally with the refined and tested Lantana essential oil.
- It also functions as a wound antiseptic. Diabetics have also utilized it.

Conclusion:

The market for natural medications is growing daily. Numerous chemical moieties with a range of pharmacological actions are found in plants. Many powerful and effective therapeutic compounds that are used to treat terrible illnesses have been identified in the plant kingdom. Thus, it is evident that researching medicinal plants is necessary to meet the needs of successful treatment. In many regions of the world, *Lantana camara* is regarded as a weed that is utilised in folk medicine. The plant is abundant in essential oils and devoid of diterpenoids, according to phytochemical investigations. It has been observed that *Lantana camara* contains steroids, iridoid glycosides, flavones, coumarin, triterpenes, and monoterpenes. The two most prevalent secondary metabolites in *Lantana camara* are flavones and triterpenes. The majority of pharmacological investigations were exploratory, conducted on animals, and insufficient to support the creation of a pharmaceutical product. However, in-depth preclinical and clinical research is still needed to assess the safety and effectiveness of these plant-based therapeutics.

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TARGETED MUTAGENASIS

Sourav Ranjan Nanda[1] and Priya Sharma*[2]

[1]Department of Genetics and Plant Breeding,

Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, 751003

[2]Genetics and Plant Breeding, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Himachal Pradesh, 176061

*Correspondence author E-mail: sharmapriya081995@gmail.com

Introduction:

A molecular biology approach called targetedmutagenesis is used to intentionally and precisely alter a gene's or any gene product's DNA sequence. Gene targeting is mostly achieved by homologous recombination or non-homologous end joining. For more than twenty years gene targeting has been used efficiently in bacteria, mice and yeast.

In this method DNA fragments are introduced into the cultured cells by homologous recombination by which means the DNA fragment is incorporated into the homologous locus. Numerous attempts have been made to attain gene targeting in higher plants over the last seventeen years. The gene is introduced into the cultured cells by direct or *Agrobacterium tumefacien* method and the gene is incorporated by non-homologous end joining. The efficiency of non-homologous end joining is better than homologous recombination for targeted mutagenesis.

Targeted mutagenesis is based on the DNA repair system by homologous recombination and non-homologous end joining. An undamaged homologous sister chromatid can be used as a template to repair a double-strand break in DNA that was induced by UV radiation or another chemical or physical mutagen. Foreign DNA comprising homologous regions is employed as a template for recombination during targeted mutagenesis. However, in plants, it is achieved by non-homologous end joining and results in the integration of foreign DNA in plant genome. Some strategies have been developed to enhance the homologous recombination by overexpression of RAD54, recA and ruvA proteins which are involved in homologous recombination.

Targeted mutagenesis has turned out to be an effective technique, used to edit the genome of plants by using the engineered nucleases and synthetic oligonucleotides at a specific location. Nucleases induce the double-strand break at the target site which is then repaired by homologous recombination or non-homologous end joining, depending on the type of mutation to be introduced (insertion or deletion).

There are three types of targeted mutagenesis techniques:

1. Zinc finger nucleases (ZFNs).
2. Transcription activator-like effector nucleases (TALENs).
3. CRISPR (clustered regularly interspaced short palindromic repeats)/Cas (CRISPR-associated) 9.

Zinc finger nucleases (ZFNs)

Zinc finger nuclease is consisted of two word – zinc finger and nuclease. Zinc finger is a structural domain/motif of protein. Amino acids are building blocks of protein which are join together to make alpha helix and beta plated which are secondary structures. The tertiary structure is formed by gradually reorganizing the secondary structures among themselves. Different types of domains that interact with one another are contained in the tertiary structure.

Zinc finger is discovered in Xenopus leavis (frog). In transcripotion factor iii, a Specific motifs/domain found which contains a zinc in the domain which interact with all the structure present in domain/motif. The domain contains all the amino acid which can bind to the DNA and RNA.The amino acid can modify in the zinc finger domain which cause the targeted mutagenesis.

Zinc-finger nucleases (ZFNs), which have separate DNA-binding and DNA-cleavage domains. These synthetic proteins originated in the natural type IIS restriction enzyme, FokI, has physically separable binding and cleavage activities. There appears to be no sequence specificity in the cleavage domain. The most useful of these was a set of Cys2His2 zinc fingers (ZFs) in which each unit of 30 amino acids bound a single atom of zinc. The crystal structure of a set of three fingers bound to DNA showed that each finger contacts primarily 3 bp of DNA in remarkably modular fashion

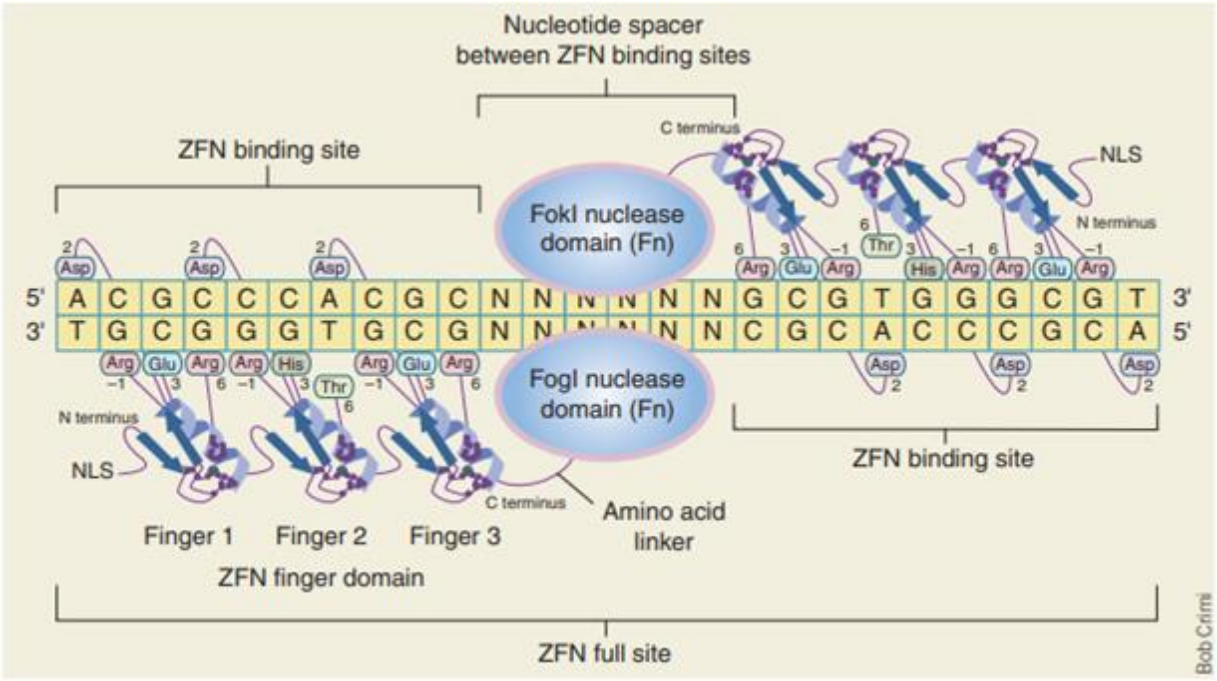
The DNA binding zinc finger motif is coupled to the non-specific DNA cleavage enzyme Fok1 in zinc finger nucleases, which are well-characterized designer nucleases. Two monomers, each with three or four zinc fingers, constitute ZFN Each zinc finger recognizes 3 nucleotide and 18 nucleotides by a whole zinc finger nuclease. Zinc finger domains are engineered to bind with specific DNA sequences and could be designed to identify every sequence in the plant genome. They could therefore be able to alter every gene in the plant's genome. ZFNs can be tailored to specific requirements using the distinct 18-nucleotide combination. ZFNs can be used as an effective vehicle for targeted mutagenesis in plant species. This tool has been successfully used for targeted mutagenesis in Arabidopsis thaliana and soybean by non-homologous end joining, as well as targeted mutagenesis by homologous recombination in Arabidopsis, tobacco and maize. Targeted mutagenesis using ZFNs is also applicable the perennial trees such as apple and fig.

Mechanism of action

Zinc-finger nucleases (ZFNs), which have separate DNA-binding and DNA-cleavage domains. These synthetic proteins originated in the natural type IIS restriction enzyme, FokI, has physically separable binding and cleavage activities. The cleavage domain has no apparent sequence specificity. The most useful of these was a set of Cys2His2 zinc fingers (ZFs) in which each unit of 30 amino acids bound a single atom of zinc.

The crystal structure of a trio of fingers bound to DNA revealed that each finger exhibits remarkably modular contact with three base pairs of DNA. It was first discovered that the FokI cleavage domain needs to dimerize in order to cut DNA. The best method to accomplish cleavage because of the weak dimer interface is to construct two sets of fingers that point towards adjacent sequences and connect them to a monomeric cleavage domain. High local concentration builds dimerization and cleavage when both sets of fingers bind to their respective recognition sequences. A number of studies have demonstrated that the best configuration places a short linker between the protein's domains and a spacer of five or six base pairs (though seven can also be effective) between binding sites that are oriented inverted.

Zinc finger nucleases cause a double-strand break in DNA and activate the repair machinery. When there's no donor template, the break is fixed by the prone to error non-homologous end join. Homologous recombination takes place when a homologous template is present, editing the gene. Instead of using non-homologous recombination, ZFNs are engineered to cause mutagenesis in plants through homologous recombination. ZFNs introduce targeted mutagenesis in plants with good efficiency.



Source: Porteus *et al.,* (2005)

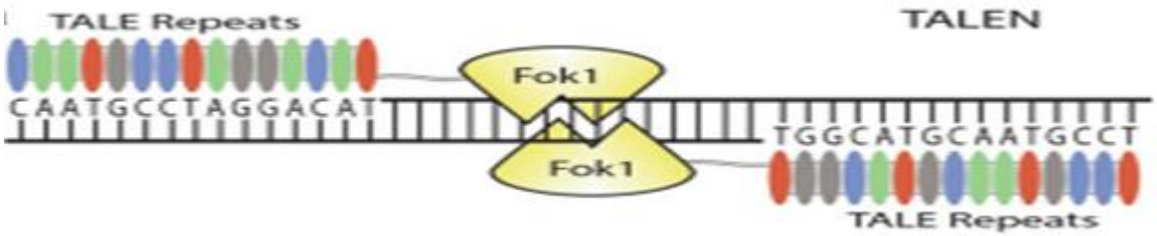
Transcription Activator-Like Effector Nucleases (TALEN)

TALEN is a newly developed technique for targeted mutagenesis. Transcription activator-like effector nucleases (TALENs) have rapidly emerged as an alternative to ZFNs for genome editing and introducing targeted DSBs. TALENs are similar to ZFNs and comprise a nonspecific FokI nuclease domain fused to a customizable DNA binding domain. This DNA-binding domain is composed of highly conserved repeats derived from transcription activator-like effectors (TALEs), which are proteins that are secreted by Xanthomonas spp. bacteria to alter gene transcription in host plant cells.

TALE DNA-binding domains.

Xanthomonas spp. (proteobacteria) encodes naturally occurring TALEs, which serve as the basis for the highly conserved repeat domain that is used to engineer the DNA-binding region of TALENs. Through the use of a type III secretion system, these TALEs are injected into host plant cells, where they bind to genomic DNA to change transcription and promote the colonization of pathogenic bacteria4. Arrays of highly conserved 33–35 amino acid repeats, with extra TALE-derived domains positioned at the array's carboxy and amino-terminal ends, mediate DNA binding. Two hyper variable residues, usually located at positions 12 and 13, help identify the single base of DNA that each individual TALE repeat in an array specifically binds to it.

The hyper variable residues at positions 12 and 13 are located in the DNA major groove, and individual repeats consist of two-helix v-shaped bundles that stack to form a super helix around the DNA, according to co-crystal structures of TALE DNA-binding domains bound to their cognate sites which were recently discoverd. While the residue at position 13 can make base-specific contacts with the DNA, the residues at positions eight and twelve within the same repeat interact with one another and may stabilise the domain's structure. For the recognition of guanine, adenine, cytosine, and thymine, respectively, nearly all engineered TALE repeat arrays published to date use four domains that contain the hyper variable residues NN, NI, HD, and NG. It has been reported that a different repeat with the hyper variable residues NK is more specific for guanine than the repeat containing NN; however, TALE repeat arrays containing the NK repeats exhibit lower activity than those containing the NN repeats. It is possible to create hybrid nucleases that are active in a yeast assay by utilizing the non-specific DNA cleavage domain from the end of the FokI endonuclease. Additionally active in both plant and animal cells are these reagents. In order to find sites in the target genome with the right orientation and spacing, the FokI domain dimer requires two constructs, each with a distinct DNA binding domain. To achieve high levels of activity, it appears that the number of bases between the two individual TALEN binding sites and the number of amino acid residues between the TALE DNA binding domain and the FokI cleavage domain are crucial parameters.



Source: Malzahn *et al.*, 2017

Mechanism:

By causing double-strand breaks (DSB), which cells respond to with repair mechanisms, TALEN can be used to alter genomes.DNA from either side of a double-strand break where there is little to no sequence overlap for annealing can be directly ligated by non-homologous end joining (NHEJ). Through chromosomal rearrangement, insertion, or deletion, this repair mechanism introduces errors into the genome that could make the gene products coding for that region non-functional.] When creating new systems, this activity should be kept an eye on since it can change based on the species, cell type, target gene, and nuclease employed. Alternatively, DNA can be introduced into a genome though NHEJ in the presence of exogenous double – standard DNA fragments. Homology directed repair can also introduce foreign DNA at the DSB as the transected double-stranded sequences are used as templates for the repair enzymes.

Cluster regularly interspaced short palindromic repeats/Cas 9 (CRISPR-associated)

CRISPR-Cas9 is a genome editing tool which is faster, cheaper and more accurate than previous techniques of editing DNA and has a wide range of potential applications.

What is CRISPR-Cas9?

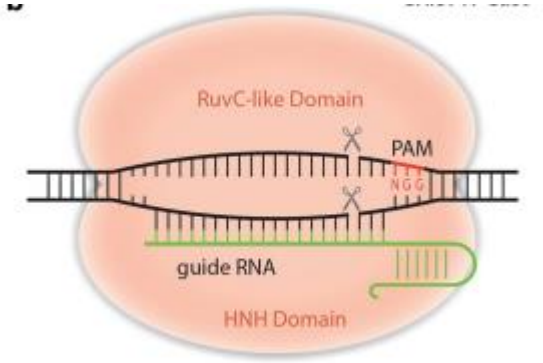
CRISPR-Cas9 is a unique technology that enables geneticists and medical researchers to edit parts of the genome by removing, adding or altering sections of the DNA sequence. It is currently the simplest, most versatile and precise method of genetic manipulation and is therefore causing a buzz.

How does it work?

Two essential molecules that cause a change (mutation) in DNA constitute the CRISPR-Cas9 system. These are an enzyme called Cas9. This functions as a set of "molecular scissors" that can split the two DNA strands at a specific location in the genome to allow for the addition or deletion of DNA segments.

Guide RNA (gRNA) is a segment of RNA. This is made up of a 20-base-long segment of pre-designed RNA sequence embedded in a larger RNA scaffold. The pre-designed sequence "guides" Cas9 to the appropriate region of the genome while the scaffold component binds to DNA. By doing this, the Cas9 enzyme is guaranteed to cut the DNA at the correct location.

The purpose of the guide RNA is to locate and attach to a particular DNA sequence. The RNA bases of the guide RNA are complementary to the target DNA sequence found in the genome. This indicates that the guide RNA will only, at least in theory, bind to the target sequence and not to any other parts of the genome. The Cas9 cuts through both strands of DNA at the same spot in the DNA sequence that the guide RNA points to. At this point, the cell attempts to repair the damaged DNA after realizing it has occurred.



How was it developed?

- Some bacteria have a similar, built-in, gene editing system to the CRISPR-Cas9 system that they use to respond to invading pathogens like viruses, much like an immune system.
- Using CRISPR the bacteria snip out parts of the virus DNA and keep a bit of it behind to help them recognize and defend against the virus next time it attacks.
- Scientists adapted this system so that it could be used in other cells from animals, including mice and humans.

What are the applications and implications?

CRISPR-Cas9 has a lot of potential as a tool for treating a range of medical conditions that have a genetic component, including cancer, hepatitis B or even high cholesterol. While there has been much discussion and interest in the possibility of editing germ line (reproductive) cells, many of the proposed applications involve editing the genomes of somatic (non-reproductive) cells. Any alterations made to germ line cells will be inherited by subsequent generations, which raises significant ethical concerns. On the other hand, there is no debate over the application of CRISPR-Cas9 and other gene editing technologies in somatic cells. Indeed, in a limited number of extraordinary and/or life-threatening cases, they have already been used to treat human disease.

Applications of targeted mutagenesis

Study functional genomics

Targeted mutagenesis has been used to achieve modification in the plant genomes via ZFNs, TALEN and CRISPR. Several modifications such as gene insertion, point mutation, substitution and deletion of a large fragment of a gene are made to introduce mutation in the

plant genome. The main purpose of targeted mutagenesis is to study the function of a specific gene in plants. Scientists use targeted mutagenesis to study the role of an individual gene, not only in a single cell but also in the whole organism. By studying functional genomics of crop plants with different targeted mutagenesis techniques, we can increase the molecular breeding of crop plants. Targeted mutagenesis can be used for single-gene knockout and for multiplex gene knockout to check the effect of multiple genes simultaneously. Large fragment deletion by introducing two double-strand breaks in the target DNA has become possible. Gene knockout in polyploid plants that was very difficult to achieve with traditional methods of mutagenesis, has become quite easy by using targeted mutagenesis. In *Triticum aestivum*, all six alleles of MLO gene have been mutated by using TALEN and CRISPR systems.

Enhanced yield

Enhancement of crop plant quantity and quality are the ultimate goals of targeted mutagenesis. The quantity and weight of the grains determine a crop's yield. Crop yield-related genes have been identified, and targeted mutagenesis is used to alter these genes. For instance, a few genes (GS3, DEP1, GS5, GW2, Gn1a, and TGW6) in rice plants have a negative effect on rice yield. These genes were altered through the use of the CRISPR technology, increasing rice yield in the process. Similar to this, CRISPR was used to eliminate the genes in wheat that negatively control kernel weight and width in order to increase yield.

Disease resistance

Through targeted mutagenesis in the DNA's TAL binding sequences, scientists were able to successfully produce disease-resistant rice in their quest to create disease-resistant plants. In order to create a vulnerable response between plants and pathogens, pathogens that attack plants translocate their virulence protein into the plant nucleus, bind to the promoter gene (S), and activate the gene expression. When TALEN or other target mutagenesis introducing tools mutate these promoter elements, they become unavailable for protein binding. Through targeted mutagenesis, wheat varieties resistant to powdery mildew have been created. When powdery mildew attacks wheat, it interferes with the wheat defense mechanism by targeting the MLO locus, which encodes the G-protein. The pathogen that is mutated using the TALEN method can no longer access the mildew-resistance locus (MLO) gene in TargetedMutagenesis in Plants 431, the powdery mildew resistant wheat.

Herbicide resistance

Herbicides are used to eradicate plants that interfere with crop growth in addition to killing the plants themselves. Acetolactate synthase (ALS) and 5'-enolpyruvylshikimate-3-phosphate synthase (EPSPS) are the genes whose products are targeted during the synthesis of herbicides. Since the products of both genes are involved in the biosynthesis of amino acids, the plants perish from starvation when the herbicide inhibits these enzymes. ZFNs that target

imidazolinon and sulfonylureas, triazolopyrimidines, pyrimidinyl oxybenzoates, and sulfonylamino carbonyl triazolinone herbicides have mutated the Acetolactate synthase (ALS) gene. TALEN and CRISPR have also been used to mutate this gene in rice, maize, and soybeans (Li *et al.,* 2016b). In the future, this technology could be used to develop plants resistant to herbicides. Glyphosate is a herbicide that inhibits the EPSPS gene product. To make the EPSPS genes of rice and flax plants resistant to the herbicide, oligonucleotide-directed mutagenesis and CRISPR have been used to modify the genes.

Oil content in seeds can be improved by targeted mutagenesis

The intended effect of zinc finger nucleases is to lower palmitic acid and increase C18. the improved gene-to-gene replication through b-ketoacyl ACP synthase II (KASII), wherein the transcriptional activator domain of VP16 was linked. By regulating the activity of the two-desaturase genes, FAD2 and FAD3, which convert oleic acid to linoleic acid, TALEN has been used to increase the amount of oleic acid in soybeans. Targeted mutagenesis has been used in other contexts besides the one described above. For example, aromatic rice has been developed, in which TALEN disrupts the betaine aldehyde dehydrogenase 2 (BADH2) gene and increases the amount of 2-acetyl-1-pyrroline (2AP), which gives the rice its fragrance. In maize, phosphorus is present in the form of phytic acid that is an anti-nutrient compound and inhibits the digestion of food. So, to reduce the phytic acid content of maize, ZFNs have been designed to modify the gene involved in the production of phytic acid.

Conclusion:

Mutation is very useful for crop improvement .it was found that use of different physical and chemical mutagen helps to create variation in genotype makeup of the plant. In physical mutagen the gamma rays use is more than any other physical mutagen. Combination of physical and chemical mutation can be used. Targeted mutagenesis of the plant genome is also very important for investigating the function of genes and to genetically modify crop plants for their trait improvement. Targeted mutagenesis could be used to improve the crop plants that lack the transgenic DNA. It has been found that targeted mutagenesis is preferable to random mutagenesis due to its precise and effective results on plant genomes to study plant biology. Targetedmutagenesis has become more efficient and easier with the engineered nucleases and guided RNA which are designed in such a way that they can induce mutation at the target site and the chance of off-target effects is reduced. Finally, Mutation and targeted mutagenesis will help us to identify new alleles and improve the quality and quantity of crops to deal with the upcoming challenges.

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About Editors

Dr. Devangi Chachad, born in August 1979, serves as an Associate Professor at Department of Botany, Jai Hind College (Empowered Autonomous) in Churchgate, Mumbai. With over 23 years of teaching experience, she earned her Bachelor's (1999) and Master's (2001) degrees in Botany from Mithibai College and obtained her Ph.D. from K.V. Pendharkar College in 2005. Dr. Chachad boasts a portfolio of more than 20 research papers published in journals indexed in Scopus, Web of Science, UGC CARE, and other peer-reviewed publications. Additionally, she has contributed chapters to various books. Her noteworthy achievements include the 2015 Joseph Lister Award in recognition of her work in ethnobotany. Acknowledged as a reputable Ph.D. mentor, she continues to guide students for their research work. Furthermore, she was awarded the fellowship from the Indian Botanical Society. Her research pursuits primarily focus on Ethnobotany, Phytochemistry, Pharmacognosy, and Pharmacology.

Dr. Shakun Mishra is working as Head Department of Botany, Govt. S. N.P.G.College, Khandwa (M.P.) INDIA. She is doing her research work for Ph. D. on the topic, "Ethnobotany of Korku, Gond and Nihal Tribes of East Nimar (M.P.)". She did her Ph. D. in the year 2011 from D.A.V.V. Indore (M.P.) India. She has 40 years of teaching experience at U. G. level and 30 years at P.G. level. She is actively engaged in research from two decades. Dr. Mishra published 70 research papers in various journals and proceedings, 08 book chapters and associate editor for eight books published by Bhumi Publishing, India. She attended and presented 60+ research papers in various national and international conferences and also guest lecturer and chairperson in two national seminars. She is member of editorial board for two journals. Presently, she is Fellow/Life Member of 10 National and International research societies. She received some prestigious awards like F.I.C.C.E., F.S.L.Sc. and F.E.S. She is completed one M.R.P. funded by U.G.C. and Ex-Chairman of BOS in subject of Botany, Soil Sc., Seed Tech. and Horticulture in D.A.V.V. Indore and also same in Central University Bhopal.

Dr. Parinitha Mahishi, based in Bangalore, India, was born and raised in rural Karnataka. Currently serving as an Assistant Professor in the Department of Botany at NMKRV College for Women, Bengaluru, she completed her PhD in Diseases Of Medicinal Herbs In Bhadra Wildlife Sanctuary from Kuvempu University. Before joining NMKRV in 2021, Dr Mahishi worked as a Botanist for mining companies and as an agricultural advisor for private organic agricultural start-ups, developing strategies for forest restoration, agricultural productivity enhancement, and socioeconomic upliftment in communities surrounding mining sites for over a decade. She also served as a Lecturer at Kuvempu University, with a research focus on medicinal plant pathology, ethnobotany, biotechnology, and more. Dr. Mahishi has published extensively in international and national journals, authored books, presented at conferences, undergone specialized training, and contributed as a co-principal investigator for ethnobotanical studies in Karnataka. Her research articles, edited books, and advisory roles for journals reflect her diverse skills and dedication to academia.

Dr. Alok Ranjan Sahu serves as an Assistant Professor in Botany at Vikash Degree College, Bargarh, Odisha. He received M.Sc., M.Phil. (Life Science) and Ph.D. in Biotechnology from the School of Life Sciences, Sambalpur University, Odisha, India. He has published more than 48 research papers in National and International Journals and Books. He also has obtained five National Patents to his credit. He is the sole author of two books and co-author of five books. He is a Fellow and Life member of Six reputed International and National Academic Societies. He is a Reviewer and Editorial Board Member of more than 32 numbers of National and International Journals, including Biology, Genes, Biomolecules, Sustainability, BABT, JAPS, JABB, etc. For his outstanding research contribution, he was awarded Research Excellence Award-2023, Academic Excellence Award (Botany/ Plant Biotechnology)-2022, Young Achiever Award-2022, Outstanding Scientist Award-2022, Best Faculty Award-2021, Young Plant Breeder Award-2021, Best Researcher Award-2020, Young Scientist Award-2014, Best Poster Presentation Award-2013. His current areas of research interest are Plant Molecular Biology & Genome mapping, Medicinal Plant Conservation, Natural products & therapeutics.

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