ISBN: 978-93-95847-74-2

RESEARCH AND REVIEWS IN PLANT SCIENCE

VOLUME I

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BHUMI PUBLISHING, INDIA

FIRST EDITION: FEBRUARY 2024

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Bhumi Publishing

February, 2024

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Published by:

Bhumi Publishing

BHUMI PUBLISHING

Nigave Khalasa, Tal – Karveer, Dist – Kolhapur, Maharashtra, INDIA 416 207

E-mail: bhumipublishing@gmail.com

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.

Finally, we would like to express our gratitude to the readers of Research and Reviews in Plant Science. It is our sincere hope that this volume will serve as a source of inspiration and knowledge for students, researchers, and enthusiasts alike, fostering a deeper appreciation for the beauty and complexity of the plant kingdom.

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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USE OF MOLECULAR MARKER IN CROP IMPROVEMENT

Sourav Ranjan Nanda* and Shreya Singh

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Abstract:

With the advancement of technology, the molecular marker changes the fate of plant breeding programs. Various molecular markers have been developed over the decades and have accelerated progress in crop improvement programs. Markers like RFLP, RAPD, SSR, and SNPs have been developed. The progress in molecular plant breeding, gene editing, genetics, and genome selection has contributed to a comprehensive understanding of molecular markers. It provided a deeper understanding of the diversity present in crops. Different new technologies like Tilling, MAGIC, CRISPER, and association mapping have created a great revolution in molecular breeding, significantly decreasing the time and labor with high output. The discussion encompasses the types of markers and the utilization of molecular markers in crop improvement.

Introduction:

Plant breeding combines science, art, and technology that modify a plant's genetic makeup to fit human needs better. Although plant breeding has existed since about 10,000 years ago, modern plant breeding methods involve the scientific study of cytogenetics and genetics, which began only after the rediscovery of Mendel's paper by Carl Correns, Hugo Devries, Erich von Tschermark that was initially published in an experiment in plant hybridization 1866 (Mendel, 1866). Mendel's law of inheritance provided the basis knowledge accumulated in genetics through which it was established that the units of specific material (genes), which can be transferred from one generation to the next, determine the inheritance of traits. Since then, plant breeders have tried reorganizing these genes, which contain desirable traits in one variety, to make them more suitable for farmers' needs. Conventional plant breeding that crosses the best genotype with the other genotype with the superior traits (e.g., high yield or disease resistance) has helped achieve the objective. For this purpose, thousands of individual plants are selected and tested to develop a variety that can take up to 6-15 years in conventional breeding methods.

During the last few decades, there has been a severe change in the earth's climate condition, which frequently causes different types of biotic and abiotic stress in crops. Different genes are responsible for the cause of varying diseases and pest attacks. Modern biotechnology brings new

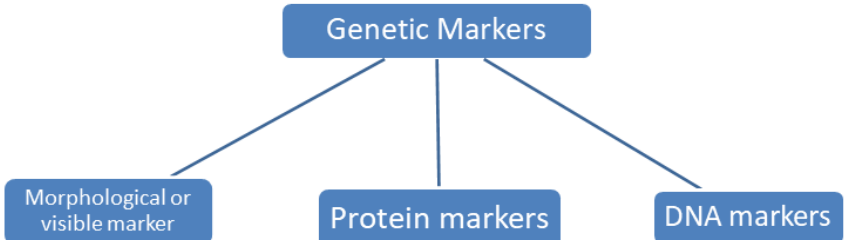
tools that can facilitate the development of improved plant breeding methods. The discovery of restriction enzymes by Smith and Wilcox and the polymerase chain reaction (PCR) by Kary Mullis created an opportunity to understand the composition of organisms at the DNA level and obtain genetic fingerprints. These studies involve separating DNA fragments on a gel resulting from selective digestion of DNA with enzymes or selective DNA amplification using PCR. DNA fragments produced in different gel patterns between samples or individuals are called polymorphic markers, with the visible differences in the gel resulting from variations at the DNA level. All markers are not identical; the information content depends on the method used to obtain the marker data and the population in which the markers were 'scored.' Advanced tools for retrieving marker data and subsequent analysis have been developed, allowing quick and reliable results in most plant species. DNA segments that are detectable by laboratory techniques are known as molecular markers. For the detection of markers, restriction enzymes or Polymerase Chain Reaction (PCR) or their combination are used to generate/amplify the DNA sequences that are linked to a heritable trait such as yield or disease resistance gene. With the advancement of biotechnology, marker-assisted selection (MAS), a new breeding tool, is now available to make more accurate and valuable selections in breeding populations. This section aims to introduce genetic terminologies and concepts associated with molecular markers.

What is a molecular marker?

A gene or DNA sequence in the genome that may be found and recognized in an organism is called a molecular marker. Varied plants within the same species may have a varied base composition at a given site in the genome due to genetic changes such as mutations, insertions, and deletions.

These differences in base composition in the genomic sequence are collectively called polymorphisms, which plant breeders can identify and map. Plant breeders always prefer to detect the gene of interest as the molecular marker, but this is only sometimes possible. There is an alternative to have markers that are closely associated with genes of interest and inherited together.

Genetic markers can be divided into Morphological or visible marker, Protein marker and DNA markers



Morphological or visible marker-

- It is the most accessible genetic marker used in scientific study.
- These traits are easily scorable by the naked eye, so-called naked eye polymorphism.
- Some examples of the markers are seed shape, flower color, seed color

Limitation of morphological marker

- The numbers of good morphological markers are limited
- Difficult to determine the phenotype of different traits in a single plant.
- Traits can be scored at a specific stage of development stage.
- Maintenance of genetic stock is essential for various molecular markers.

Protein-based markers

- These markers are generated to alter the amino acid sequences of the concerned proteins.
- Only a small amount of change can be visible.
- Detected from the seedling stage and even from seed
- Analysis is relatively easy.

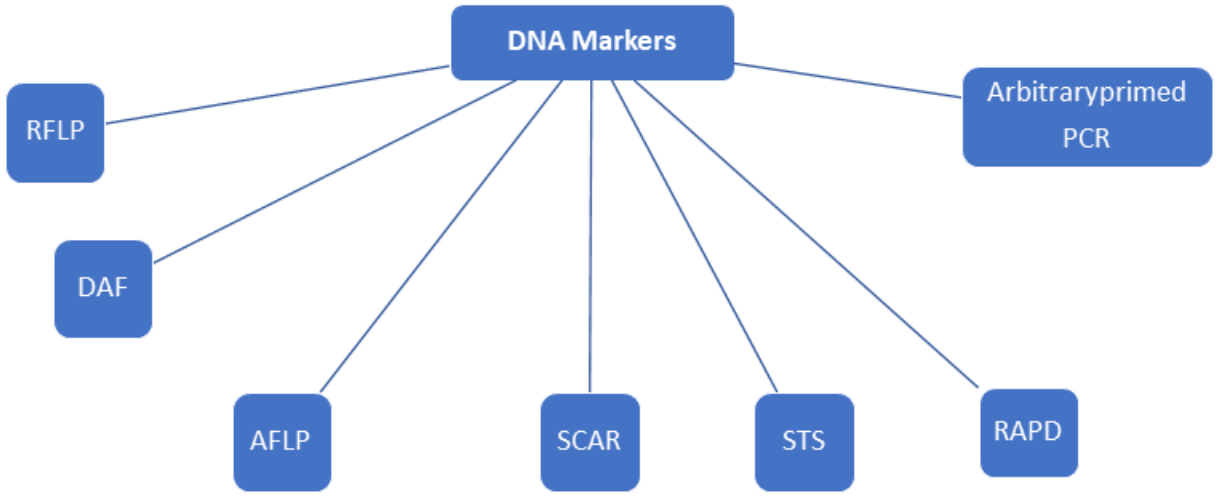
The limitation of protein marker

- Isozymes is that they represent only a small, non-random sample of the genes of an organism
- They detect only when mutations that produce a functional enzyme with changed electrophoretic mobility.
- It may vary with tissue, developmental stage, and environment.

DNA markers

Variation of the genomic sequence of different individuals is called DNA markers.

Types of DNA Markers



Characteristics	RFLP	RAPD	AFLP	ISSR	SSR	DArT	SNP
Co-dominant/Dominant	Co-dominant	Dominant	Dominant	Dominant	Co-dominant	Dominant	Co-dominant
Reproducibility	High	High	Intermediate	Medium–High	High	High	High
Polymorphism level	Medium	very high	High	High	High	High	High
Required DNA quality	High	High	High	Low	Low	High	High
Required DNA quantity	High	Medium	Low	Low	Low	Low	Low
Marker index	Low	High	Medium	Medium	Medium	High	High
Genome abundance	High	Very high	Very high	Medium	Medium	Very high	Very high
Cost	High	Less	High	High	High	Cheapest	Variable
Sequencing	Yes	No	No	No	Yes	Yes	Yes
Status	Past	Past	Past	Present	Present	Present	Present
PCR requirement	No	Yes	Yes	Yes	Yes	No	Yes
Visualization	Radioactive	Agarose gel	Agarose gel	Agarose gel	Agarose gel	Microarray	SNP-VISTA
Required DNA (ng)	10000	20	500–1000	50	50	50–100	50

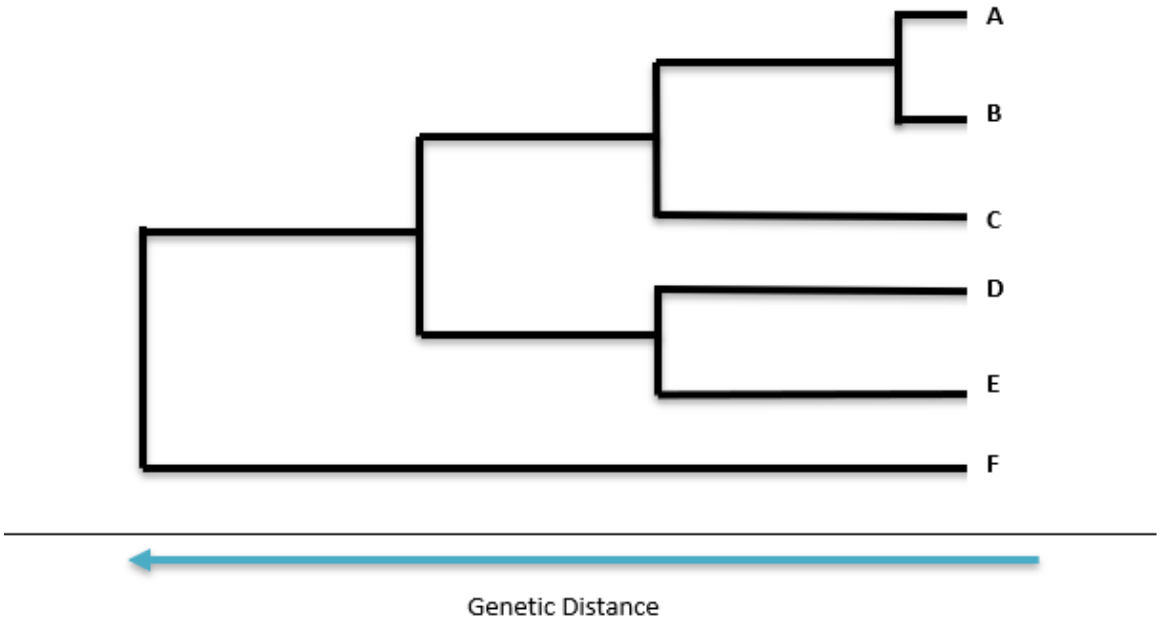
Uses of molecular markers in crop improvement

Evolutionary relationship

Evolution was dependent on both geological and morphological changes occurring among organisms. Due to the advancement of molecular techniques, molecular markers help find the evolutionary relationships between organisms. Molecular study on the Chloroplast genome sequence is mostly used to understand the phylogenetic relationships.

The diversity among genotypes assists the breeder in developing superior offspring. Traditionally, diversity evaluation was based on anatomy, morphology, physiology, and

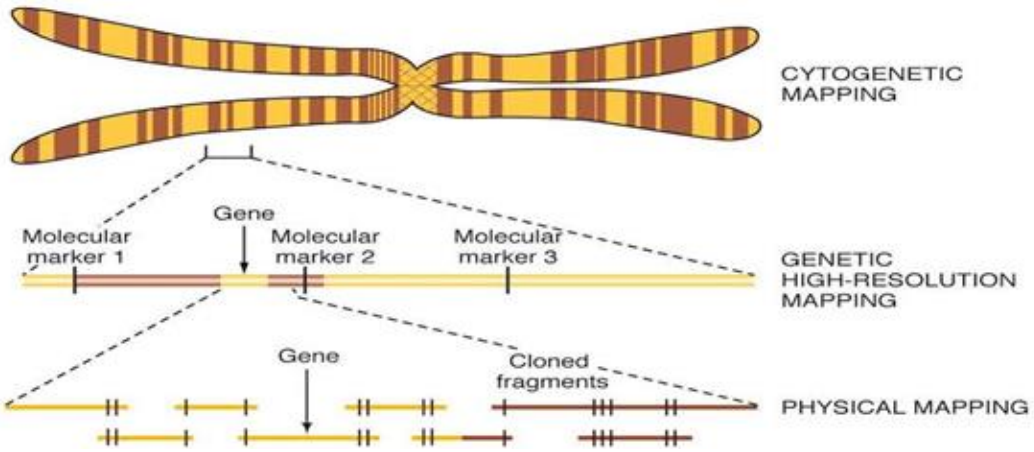
embryology, but nowadays, molecular markers based on genotyping involve the development of unique marker profiles for an individual. A DNA fingerprint is a unique technique for unambiguous patterns in crop varieties. DNA fingerprinting was first developed by Alec Jeffrey in 1985 in Human and was used in rice for the first time by Dallas for cultivar identification. Different molecular markers were used based on discriminatory power and reproducibility. Nowadays, microsatellite is used for varietal identification due to their abundance and high polymorphisms.



Heterosis breeding

The performance of progeny (F_1) over greater than its parent is called positive heterosis, while where the effect of F_1 lower than its parent is known as negative heterosis. Various studies have been conducted using molecular markers in crops like rice, mustard, and wheat. SSR markers were used to investigate the heterotic groups.

Genetic mapping



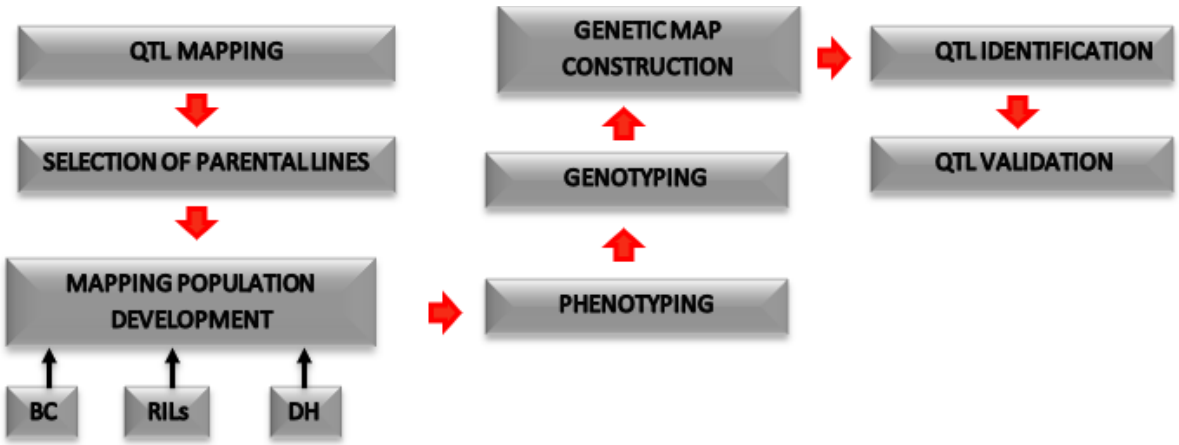
Determining locus and distance between the genes can be identified using various markers. Markers present close to the gene of interest on the same chromosome are known as linked markers. Genetic mapping is a major area of research using molecular markers nowadays.

Identification of variety and hybrids

DNA fingerprinting is a unique technique to identify varieties and hybrids by use of different molecular markers. Different molecular markers like AELP, STR, and SCAR are used for DNA fingerprinting, but SSR markers are widely used. Sometimes, marker data present some difficulties for the use of plant breeders' rights as some varieties are uniform in phenotype, but they show variation at the molecular level.

QTL mapping

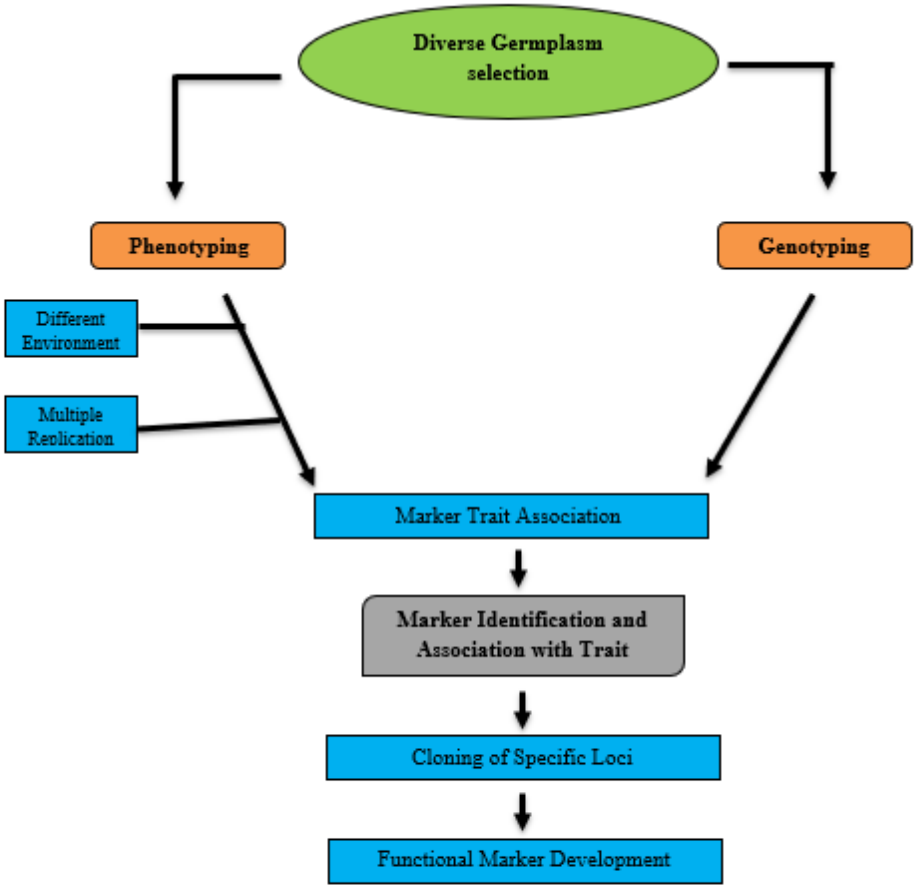
Genetic factors responsible for the observed phenotypic variation for a quantitative trait are called quantitative trait loci (QTL). The QTL term was coined by Gelderman (1919).QTL mapping is done by locating the gene with effects on quantitative traits using molecular markers analysis and is dependent on linkage disequilibrium mapping depending on the number of genes controlling the targeted traits and position of the traits, the heritability of genes, types, and size of mapping populations. Double haploid lines, Backcross population, F2 population, and Recombinant inbred lines are different mapping populations used in QTL.MAS, MABS, and MARS are used in the indirect selection of QTL, which requires less time, resources, labor, and space.



Association mapping

The association of molecular markers with phenotypic traits is called Association mapping. It is based on linkage disequilibrium. Non-random associations of alleles at different loci of a species are called Lineage disequilibrium. It shows an equal frequency of haplotypes in a population. Many factors, including selection, drift, and mating patterns, affect linkage disequilibrium.

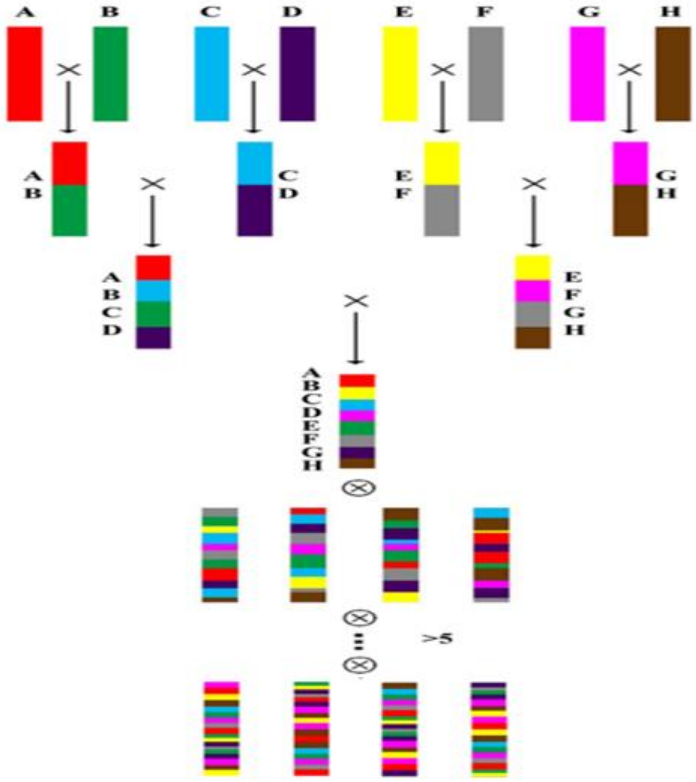
Step of association mapping



Multiparent advanced generation intercross populations

The multiparent advanced generation intercross populations are a collection of Recombinant inbred lines produced from a complex cross-population involving several parental lines. The parental lines may be inbred clones, lines, or individuals selected based on origin. MAGIC populations were proposed by Darvasi and Soller (1995); this concept was first used in mice as "heterogeneous stocks" and later extended to plants by Mackay and Powell (2007), who also proposed the name MAGIC. MAGIC populations can be used for both linkage and association analyses and can be developed appropriately during the intermating process to afford the desired mapping resolution. Since these populations are created from several parent lines, they are likely to show segregation for multiple QTLs for each trait and more than two alleles for individual QTLs. These are ideal for the construction of high-density maps, and they allow the modeling of cytoplasmic effects. The parents of a MAGIC population are selected to represent a large part of the variation present in the elite germplasm of a crop species. These populations can be used directly or indirectly for variety development by combining traits. These populations can be used as training populations for genomic selection.

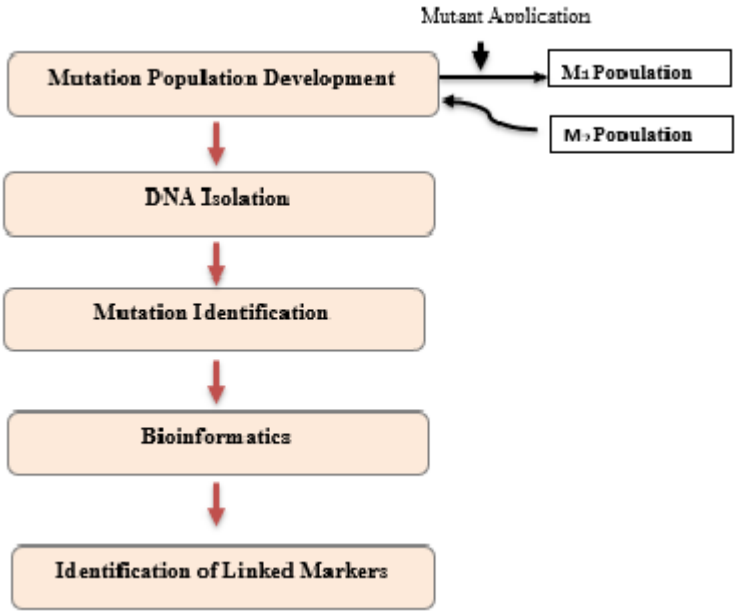
Construction of MAGIC population



Tilling

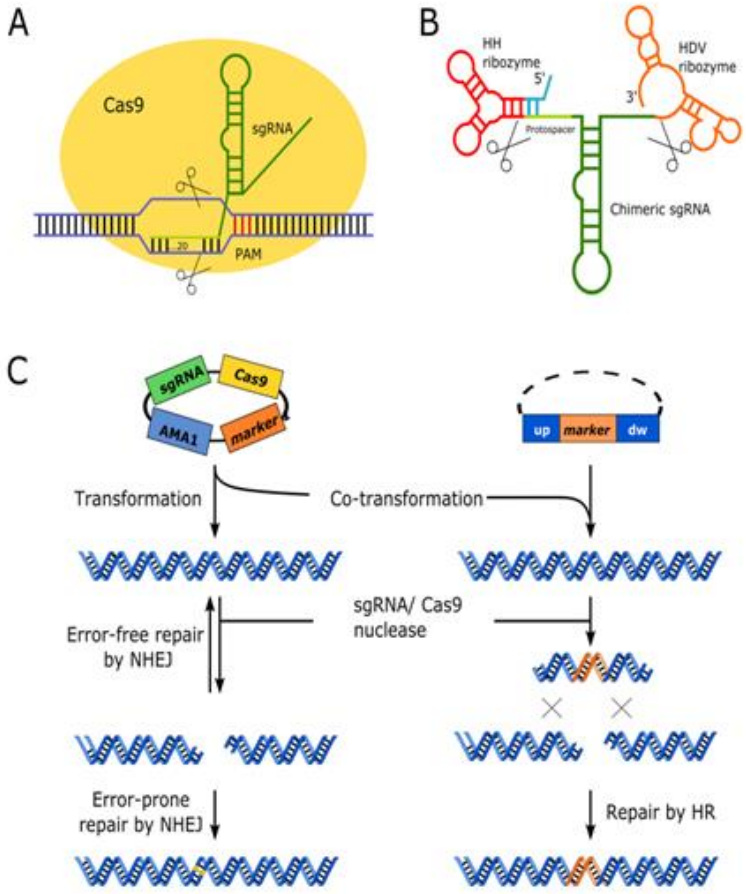
Targeting-induced local lesions in the genome were first developed by McCallum in late 1990 while working on the Arabidopsis. It is a non-transgenic technique and applies to most plants. It represents a reverse genetic strategy applicable to any species, irrespective of its genome size and ploidy level.

Step in tilling



Eco-tilling is a molecular technique similar to Tilling that reveals the natural genetic variation instead of inducing variation. It can used to discover natural variants and their gene action. It can used to determine heterozygosity levels within a gene fragment. It reduces time and effort for SNP discovery in identical genotypes.

CRISPER



Source: Nodvig *et al.* (2015)

CRISPR, also known as Clustered Regularly Interspaced Short Palindromic Repeats, represents an innovative and groundbreaking technology that empowers geneticists and medical researchers to modify specific parts of the genome, involving the deletion, addition, or alteration of sections within the DNA sequence. It stands out as the most straightforward, adaptable, and precise method of genetic manipulation. The CRISPR-Cas9 system comprises two essential components responsible for inducing genetic changes—Cas9, an enzyme functioning as "molecular scissors," capable of cutting both strands of DNA at a specified position in the genome, thereby allowing the addition or removal of DNA fragments, and guide RNA (gRNA), a piece of RNA. The guide RNA comprises a small, pre-designed RNA sequence approximately 20 bases long, embedded within a more extended RNA scaffold. The scaffold binds to the DNA,

and the pre-designed sequence guides Cas9 to the precise location in the genome sequence. The guide RNA is meticulously crafted to recognize and bind only to a specific sequence within the DNA. As its RNA bases complement those in the target DNA sequence, the guide RNA exclusively binds to the intended sequence. Cas9 then follows the guide RNA to the exact location in the DNA sequence, making a precise cut across both strands. Subsequently, the cell identifies the DNA damage and initiates repair mechanisms to address the modification.

Conclusion:

There has been continuous development in molecular markers technology over the last 35 years, from RFLP to SNPs and Diversity Array Technology. These advancements help reduce labor costs with high, precise output. Despite advancement, we still need to achieve our goals due to inaccurate phenotyping. A large number of markers were developed and have been applied to various aspects of plant breeding. CRISPR is a revolutionary technique that focuses on editing the genomes of economically important plants. In the coming years, there is a tendency for ongoing progress in molecular marker technology, aiming to enhance precision and cost-effectiveness for studying genes of interest in economically significant plants.

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HERBAL APPROACHES TO ASTHMA MANAGEMENT: EXPLORING POTENTIAL REMEDIES AND CONSIDERATIONS

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Abstract:

Asthma, a chronic respiratory condition characterized by airway inflammation and bronchoconstriction, often requires long-term management with conventional medications. However, the exploration of herbal remedies as complementary approaches has gained attention. This abstract reviews key herbal remedies, including Butterbur (*Petasites hybridus*), Boswellia (*Boswellia serrata*), Turmeric (*Curcuma longa*), Ginger (*Zingiber officinale*), Honey, and Garlic (*Allium sativum*), in the context of asthma treatment. These herbs exhibit potential mechanisms such as anti-inflammatory properties, leukotriene inhibition, and smooth muscle relaxation. While some studies suggest positive outcomes, robust evidence is lacking, and individual responses may vary. Safety considerations, dosage guidance, and cautions are crucial, emphasizing the importance of consulting healthcare providers before incorporating herbal remedies into asthma management. Future research is needed to establish the efficacy and safety of these herbal interventions.

Keywords: Asthma, Herbal remedies, Anti-inflammatory, Leukotriene inhibition, Smooth muscle relaxation, Complementary medicine, Safety considerations, Dosage guidance, Respiratory health, Healthcare consultation

Introduction:

Asthma, a chronic respiratory condition affecting millions worldwide, is characterized by inflammation and constriction of the airways, leading to symptoms such as wheezing, shortness of breath, and coughing. While conventional medical treatments play a central role in asthma management, there is growing interest in exploring complementary and alternative approaches, particularly herbal remedies.

Herbal remedies have been an integral part of traditional medicine for centuries, and their potential in alleviating respiratory symptoms has drawn attention from individuals seeking additional options for asthma care. This exploration is rooted in the belief that certain herbs may

harbor properties beneficial in mitigating inflammation, relaxing airway smooth muscles, and modulating immune responses—key factors in asthma pathophysiology.

This introduction delves into the mechanisms and potential benefits associated with select herbal remedies in asthma treatment. Butterbur (*Petasites hybridus*), Boswellia (*Boswellia serrata*), Turmeric (*Curcuma longa*), Ginger (*Zingiber officinale*), Honey, and Garlic (*Allium sativum*) are among the herbs under consideration. Understanding their properties, modes of action, and safety profiles is essential for informed decision-making by both healthcare professionals and individuals navigating asthma management.

While scientific investigation into the efficacy of herbal remedies for asthma is ongoing, this exploration highlights the importance of a comprehensive and personalized approach to asthma care, where conventional medications and herbal interventions may work synergistically to optimize respiratory health. It is essential to underscore, however, that any incorporation of herbal remedies into asthma management should be done under the guidance of healthcare professionals to ensure safety, efficacy, and compatibility with established treatment plans. As research advances, a nuanced understanding of the role of herbal remedies in asthma may contribute to more holistic and individualized approaches to respiratory health. [1]

Herbal remedies:

1. **Butterbur (*Petasites hybridus*):**

Butterbur, scientifically known as *Petasites hybridus*, is a perennial herb native to Europe, Asia, and North America. Traditionally, it has been employed for various medicinal purposes, including the treatment of respiratory conditions. This herb is recognized for containing active compounds, such as petasins, believed to contribute to its potential therapeutic effects. [2]

Mechanisms for asthma:

1. **Anti-inflammatory properties:** Butterbur is thought to possess anti-inflammatory effects, making it potentially valuable in managing asthma. Given that inflammation in the airways is a hallmark of asthma, substances with anti-inflammatory properties may assist in alleviating symptoms.
2. **Leukotriene inhibition:** Studies suggest that butterbur may inhibit the production of leukotrienes, inflammatory mediators involved in asthma-related bronchoconstriction. By reducing leukotriene production, butterbur may contribute to improved airway function.
3. **Smooth muscle relaxation:** Butterbur extracts have been explored for their potential to relax smooth muscles, including those in the airways. This relaxation may aid in widening the air passages, facilitating easier breathing for individuals with asthma.

Research studies: While there is some research on the potential benefits of butterbur for asthma, the evidence is not yet robust, and more studies are needed to establish its efficacy. Some studies have shown promising results, while others have not demonstrated significant benefits.

Safety considerations: Raw butterbur contains pyrrolizidine alkaloids, which can be toxic to the liver and may cause adverse effects. Therefore, it is crucial to use only commercially prepared butterbur products that have undergone a process to remove these harmful compounds.

Dosage and form: Individuals considering the use of butterbur for asthma should consult with a healthcare provider for guidance on the appropriate dosage and form. Butterbur supplements are available in various formulations, including capsules and extracts.

Cautions:

1. Pregnant or breastfeeding women should avoid using butterbur due to potential risks associated with pyrrolizidine alkaloids.
2. Individuals with liver conditions should exercise caution and consult with a healthcare professional before using butterbur.

2. Boswellia (*Boswellia serrata*):

Boswellia, derived from the resin of the *Boswellia serrata* tree, is an herbal remedy with a rich history in traditional medicine. Commonly known as Indian frankincense, Boswellia has garnered attention for its potential therapeutic properties, including anti-inflammatory effects, which make it a subject of interest in the context of asthma management. [3]

Mechanisms for asthma:

1. **Anti-Inflammatory properties:** One of the key components of Boswellia is boswellic acid, which is believed to have anti-inflammatory properties. This may be particularly relevant in asthma, where inflammation in the airways contributes significantly to symptoms.
2. **Leukotriene inhibition:** Boswellic acids have been studied for their ability to inhibit the formation of leukotrienes, inflammatory molecules involved in bronchoconstriction. By reducing leukotriene production, Boswellia may contribute to easing airway constriction in asthma.

Research studies: While research on Boswellia for asthma is ongoing, studies have suggested potential benefits. However, the evidence is not yet conclusive, and further research is needed to establish the efficacy and safety of Boswellia in asthma management.

Safety considerations: Boswellia is generally considered safe for short-term use, but long-term effects are not well-established. Individuals with certain medical conditions or those

taking medications should consult with a healthcare professional before incorporating Boswellia into their asthma management plan.

Dosage and form: The appropriate dosage and form of Boswellia may vary. It is available in various formulations, including capsules, extracts, and topical preparations. Consulting with a healthcare provider is recommended to determine the most suitable form and dosage for individual needs.

Cautions:

1. Pregnant or breastfeeding women and individuals with certain health conditions should exercise caution and seek medical advice before using Boswellia.

3. Turmeric (*Curcuma longa*):

Turmeric, derived from the rhizomes of the *Curcuma longa* plant, is a bright yellow spice known for its culinary uses and traditional medicinal properties. The active compound in turmeric, curcumin, has garnered attention for its potential anti-inflammatory and antioxidant effects, making it a subject of interest in the context of asthma management.

Mechanisms for Asthma:

1. **Anti-inflammatory properties:** Curcumin is renowned for its potent anti-inflammatory effects, which may be relevant in the context of asthma. Inflammation plays a significant role in the pathogenesis of asthma, and substances with anti-inflammatory properties may help alleviate symptoms.
2. **Antioxidant activity:** Turmeric's curcumin is also recognized for its antioxidant properties, which could contribute to reducing oxidative stress in the airways. Asthma is associated with increased oxidative stress, and antioxidants may help mitigate this aspect of the condition.

Research studies: While laboratory and animal studies suggest that turmeric may have potential benefits for asthma, clinical evidence in humans is limited and inconclusive. Further research is needed to establish the efficacy and safety of turmeric in the management of asthma symptoms.

Safety considerations: Turmeric is generally considered safe when used in moderate amounts in cooking. However, concentrated curcumin supplements may interact with certain medications or have side effects. Individuals should consult with a healthcare professional before incorporating turmeric supplements into their asthma management.

Dosage and form: Turmeric supplements are available in various forms, including capsules, powders, and extracts. Determining the appropriate dosage and form should be done in consultation with a healthcare provider based on individual health needs.

Cautions:

1. Turmeric supplements may interact with blood-thinning medications, and individuals taking these medications should exercise caution and seek medical advice before using turmeric supplements. [4]

4. Ginger (*Zingiber officinale*):

Ginger, derived from the rhizome of the *Zingiber officinale* plant, is a well-known spice with a history of use in traditional medicine. Beyond its culinary applications, ginger has been investigated for its potential health benefits, including anti-inflammatory properties, making it of interest in the management of conditions such as asthma. [5]

Mechanisms for asthma:

1. **Anti-inflammatory effects:** Ginger contains bioactive compounds, such as gingerol, known for their anti-inflammatory properties. In the context of asthma, where inflammation in the airways is a central feature, ginger's anti-inflammatory effects may contribute to symptom relief.
2. **Bronchodilator potential:** Some studies suggest that ginger may have a bronchodilator effect, helping to relax the smooth muscles of the airways. This relaxation could facilitate improved airflow, making it potentially beneficial for individuals with asthma.

Research studies: While there is some preliminary evidence supporting the anti-inflammatory and bronchodilator properties of ginger, more research is needed to establish its efficacy and safety in the specific context of asthma. Existing studies often involve small sample sizes, and larger, well-controlled trials are necessary for conclusive findings.

Safety considerations: Ginger is generally considered safe when consumed in moderate amounts, such as in culinary use or as a tea. However, concentrated ginger supplements may interact with certain medications, and individuals should consult with a healthcare professional before incorporating ginger supplements into their asthma management.

Dosage and form: Ginger can be consumed in various forms, including fresh or ground in cooking, as ginger tea, or in supplement form. Determining the appropriate dosage and form should be done in consultation with a healthcare provider, considering individual health needs.

Cautions:

1. Pregnant individuals and those with certain medical conditions, such as bleeding disorders, should exercise caution and seek medical advice before using ginger supplements.

5) Honey:

Honey, a natural sweet substance produced by bees from flower nectar, has been valued for its medicinal properties for centuries. While primarily recognized as a remedy for coughs and sore throats, honey is also explored for its potential benefits in managing respiratory conditions, including asthma. [6]

Mechanisms for asthma:

1. **Soothing properties:** Honey is known for its soothing effect on the throat and airways. It may help alleviate coughing and throat irritation, providing relief for individuals with asthma experiencing respiratory symptoms.
2. **Antioxidant and anti-inflammatory effects:** Honey contains antioxidants and exhibits anti-inflammatory properties. These characteristics may contribute to reducing inflammation in the airways, potentially offering relief from asthma symptoms.

Research studies: Research on honey for asthma is limited, and existing studies often focus on its impact on symptoms such as cough rather than asthma management specifically. While honey's soothing and anti-inflammatory properties are acknowledged, further research is needed to establish its role in comprehensive asthma care.

Safety considerations: Honey is generally considered safe for adults. However, caution should be exercised when giving honey to infants due to the risk of infant botulism. Individuals with pollen allergies should also be cautious, as honey may contain trace amounts of pollen.

Dosage and form: The consumption of honey for potential respiratory benefits can be done by adding it to warm water, herbal teas, or incorporating it into the diet. There is no standardized dosage for honey in the context of asthma, and individual preferences and tolerances may vary.

Cautions:

1. Children under one year of age should not consume honey due to the risk of botulism.
2. Individuals with pollen allergies should be cautious and may want to choose processed or filtered honey to reduce the risk of allergic reactions.

5. Garlic (*Allium sativum*):

Garlic, a member of the Allium family, has been utilized for both culinary and medicinal purposes throughout history. Known for its distinctive flavor, garlic also contains various bioactive compounds, including allicin, which contribute to its potential health benefits. While primarily recognized for its cardiovascular benefits, garlic has also been explored for its potential impact on respiratory health, including its role in managing asthma.

Mechanisms for asthma:

1. **Anti-inflammatory effects:** Garlic contains compounds with anti-inflammatory properties, which may help modulate the inflammatory response in the airways. This could be particularly relevant in asthma, where inflammation plays a central role in symptom development.
2. **Immunomodulatory effects:** The bioactive compounds in garlic may have immunomodulatory effects, influencing the immune system's response. This modulation could contribute to a balanced immune reaction in the context of asthma.

Research studies: While there is some evidence supporting the anti-inflammatory and immunomodulatory effects of garlic, research specifically focused on its role in asthma management is limited. Further studies are needed to establish the efficacy and safety of garlic as a complementary approach to asthma care.

Safety considerations: Garlic is generally safe when consumed in moderate amounts as part of the diet. However, concentrated garlic supplements may interact with certain medications or have side effects. Individuals considering garlic supplements for asthma management should consult with a healthcare professional.

Dosage and form: Garlic can be incorporated into the diet in various forms, including fresh, cooked, or as a supplement. Determining the appropriate dosage and form should be done in consultation with a healthcare provider based on individual health needs.

Cautions:

1. Individuals with bleeding disorders or those scheduled for surgery should exercise caution with garlic supplements due to its potential anticoagulant effects.
2. Some people may experience digestive discomfort or allergic reactions to garlic. It's important to monitor individual responses.[7]

Conclusion:

In the realm of asthma management, the exploration of herbal remedies offers a promising avenue for complementary approaches. Herbs such as Butterbur, Boswellia, Turmeric, Ginger, Honey, and Garlic have been studied for their potential anti-inflammatory, bronchodilator, and immune-modulating properties, presenting possible benefits in alleviating asthma symptoms.

While preliminary research shows promise, it's crucial to approach herbal remedies with caution and under the guidance of healthcare professionals. The evidence supporting the efficacy of these remedies in asthma management varies, and more robust clinical trials are needed to establish their safety and effectiveness conclusively.

Importantly, herbal remedies should not replace prescribed asthma medications but can be considered as supplementary components in a comprehensive treatment plan. Open communication with healthcare providers is essential to ensure proper integration, prevent potential interactions, and address individual health considerations.

As the field of herbal medicine continues to evolve, ongoing research will provide a clearer understanding of the role these remedies can play in asthma care. Until then, individuals are encouraged to engage in collaborative discussions with their healthcare team, fostering an informed and personalized approach to asthma management that combines the strengths of conventional medicine with the potential benefits of herbal interventions.

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HERBS IN PCOS MANAGEMENT: A HOLISTIC PERSPECTIVE AND THE NEED FOR FURTHER RESEARCH

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Abstract:

Polycystic Ovary Syndrome (PCOS) presents a significant health challenge for individuals with ovaries, manifesting in irregular menstrual cycles, ovarian cysts, and hormonal imbalances. The complex nature of PCOS extends to potential complications, such as insulin resistance, emphasizing the necessity for comprehensive management approaches. This chapter provides a concise overview of herbal treatments explored as potential complementary options for PCOS, spotlighting herbs like spearmint, cinnamon, liquorice root, fenugreek, turmeric, Chaste Tree, Saw Palmetto, and Milk Thistle. Despite the promising potential of these herbs in addressing hormonal imbalances and associated symptoms, the limited evidence in the current research landscape underscores the need for cautious consideration. The content delves into the specific properties of each herb, emphasizing the call for further research to establish standardized dosages and long-term safety data. Stressing the importance of consulting healthcare professionals, the abstract advocates for a holistic approach, integrating herbal treatments with lifestyle modifications and medical guidance. In conclusion, while herbal interventions offer intriguing possibilities for PCOS management, their incorporation into comprehensive care necessitates a nuanced understanding of both their potential benefits and limitations.

Keywords: Herbs, PCOS, Treatment, Potential

Introduction:

Polycystic Ovary Syndrome (PCOS) is a common hormonal disorder that affects individuals with ovaries. A range of symptoms, including irregular menstrual cycles, ovarian cysts, and hormonal imbalances, characterizes it. PCOS can also lead to complications such as insulin resistance, which may result in metabolic disturbances and an increased risk of type 2 diabetes. Herbal treatments have gained attention as potential complementary options for managing PCOS symptoms. Various herbs, such as spearmint, cinnamon, liquorice root, fenugreek, turmeric, Chaste Tree, Saw Palmetto, and Milk Thistle, have been studied for their potential benefits in addressing hormonal imbalances and related symptoms. However, it's

essential to approach herbal treatments with caution and seek guidance from healthcare professionals to ensure safety and effectiveness in the context of PCOS management [1].

Polycystic Ovary Syndrome (PCOS) is a hormonal disorder that affects people with ovaries. While herbal remedies may be considered complementary to conventional medical treatment, it's crucial to consult with a healthcare professional before using any herbs for PCOS. The following herbs have been traditionally used and studied for their potential benefits in managing PCOS symptoms:

1. Spearmint:

Spearmint tea may help reduce elevated levels of androgens (male hormones) in women with PCOS. It is believed to have anti-androgenic properties. Polycystic Ovary Syndrome (PCOS) is a hormonal disorder that affects people with ovaries, causing symptoms such as irregular periods, ovarian cysts, and hormonal imbalances. While spearmint tea has been studied for potential benefits in managing PCOS symptoms, research in this area is still limited, and individual responses may vary. Some studies suggest that spearmint tea may have anti-androgenic properties, reducing elevated levels of male hormones (androgens) associated with PCOS symptoms like hirsutism and acne. However, the research on spearmint and PCOS is not extensive, necessitating more studies to establish its effectiveness conclusively [2].

Considerations include the limited scope of research, potential symptom relief demonstrated in some studies, and the lack of well-established effective dosage and duration for spearmint tea consumption in PCOS. Before incorporating spearmint tea or any herbal remedy into a PCOS management plan, consulting with a healthcare professional is crucial for personalized advice based on individual health conditions.

Managing PCOS typically involves lifestyle changes such as a healthy diet, regular exercise, and weight maintenance. Spearmint tea is considered a potential complementary option rather than a standalone treatment. Taking a holistic approach under the guidance of a healthcare professional ensures a comprehensive plan tailored to individual needs, considering factors like overall health, lifestyle, and existing medical conditions [3].

2. Cinnamon

Cinnamon may help improve insulin sensitivity and regulate menstrual cycles in women with PCOS. It can be added to food or taken as a supplement. Cinnamon has gained attention for its potential role in

managing Polycystic Ovary Syndrome (PCOS), a hormonal disorder-affecting people with ovaries. While research is ongoing, some studies have explored the effects of cinnamon on PCOS symptoms. Cinnamon is known for its anti-inflammatory and antioxidant properties, which may have implications for PCOS management. Inflammation and oxidative stress are factors associated with PCOS, contributing to insulin resistance and hormonal imbalances. Cinnamon's anti-inflammatory properties may help mitigate these factors.

One of the key features of PCOS is insulin resistance, leading to elevated insulin levels. Cinnamon has been studied for its potential insulin-sensitizing effects, which could contribute to better blood sugar regulation. Improved insulin sensitivity may help address some metabolic aspects of PCOS and reduce the risk of associated conditions such as type 2 diabetes [4].

It's important to note that while some studies suggest potential benefits, the overall evidence on the efficacy of cinnamon for PCOS is limited, and more research is needed to establish clear recommendations. Additionally, individual responses to cinnamon supplementation may vary.

Before incorporating cinnamon or any other supplement into a PCOS management plan, it is advisable to consult with a healthcare professional. They can provide personalized guidance, taking into account individual health conditions, medication interactions, and overall treatment goals.

In conclusion, while cinnamon shows promise for its anti-inflammatory and insulin-sensitizing properties, further research is necessary to determine its effectiveness as a specific treatment for PCOS. A holistic approach to PCOS management, including lifestyle modifications and medical guidance, remains crucial for comprehensive care [5].

3. Liquorice root:

Liquorice root may have anti-androgenic effects and could help regulate hormonal imbalances. However, it should be used cautiously due to potential side effects, and consulting with a healthcare professional is advised. Liquorice root, derived from the Glycyrrhiza glabra plant, has been explored for its potential role in managing Polycystic Ovary Syndrome (PCOS), hormonal disorder-affecting individuals with ovaries. Some studies have investigated the impact of liquorice root on hormonal balance and other symptoms associated with PCOS.

Liquorice root contains compounds that may have anti-inflammatory and anti-androgenic properties. These properties are of interest in the context of PCOS, as elevated androgen levels contribute to symptoms such as hirsutism and acne. By potentially reducing androgen levels, liquorice root may address these specific manifestations of PCOS. Moreover, liquorice root may

influence cortisol metabolism. Cortisol is a stress hormone produced by the adrenal glands, and its dysregulation is associated with PCOS. Liquorice root's potential impact on cortisol metabolism may contribute to improved hormonal balance in individuals with PCOS.

Despite these potential benefits, it is crucial to approach the use of liquorice root cautiously. Prolonged or excessive consumption of liquorice root can lead to an increase in blood pressure and potassium loss, which may have adverse effects on cardiovascular health. Therefore, any consideration of liquorice root as a PCOS treatment should be done under the guidance of a healthcare professional, who can monitor its usage and potential side effects [6].

As with many natural remedies, the evidence on the efficacy of liquorice root for PCOS is still limited, and more research is needed to establish clear guidelines. Before incorporating liquorice root or any other herbal supplement into a PCOS management plan, consultation with a healthcare professional is advisable to ensure safety and appropriateness for individual health conditions. It's important to view liquorice root as a potential complementary option rather than a standalone treatment, with lifestyle modifications and medical guidance remaining integral components of a comprehensive PCOS management approach [7].

4. Fenugreek

Fenugreek seeds may help regulate menstrual cycles and improve insulin sensitivity. They can be used in cooking or taken as a supplement. Fenugreek, a herb commonly used in traditional medicine, has been investigated for its potential role in managing Polycystic Ovary Syndrome (PCOS). PCOS is a hormonal disorder characterized by various symptoms such as irregular periods, ovarian cysts, and hormonal imbalances. Fenugreek seeds contain compounds that may have potential benefits for individuals with PCOS. One area of interest is its potential impact on insulin sensitivity. Insulin resistance is a common feature of PCOS, leading to elevated insulin levels. Fenugreek may help improve insulin sensitivity, thereby contributing to better blood sugar regulation. This could be particularly relevant, as insulin resistance is often associated with weight gain and metabolic disturbances in individuals with PCOS.

Additionally, fenugreek may have anti-inflammatory properties, and inflammation is considered a contributing factor to the development and progression of PCOS. By reducing inflammation, fenugreek may help alleviate some symptoms associated with the condition [8].

Despite these potential benefits, it is important to note that research on fenugreek for PCOS is still in the early stages, and more studies are needed to establish its effectiveness and appropriate dosage. As with any herbal supplement, it is crucial to consult with a healthcare professional

before incorporating fenugreek into a PCOS management plan. They can provide personalized advice, considering individual health conditions, potential interactions with medications, and overall treatment goals.

In conclusion, fenugreek shows promise for its potential impact on insulin sensitivity and anti-inflammatory properties, making it a subject of interest in PCOS management. However, further research is needed to validate these effects and establish clear guidelines for its use. As with other natural remedies, fenugreek should be viewed as a potential complementary option alongside lifestyle modifications and medical guidance in the comprehensive management of PCOS [9].

5. Turmeric

Curcumin, the active compound in turmeric, has anti-inflammatory properties and may help regulate insulin levels. It can be added to food or taken as a supplement. Turmeric, a yellow spice derived from the *Curcuma longa* plant, has been studied for its potential therapeutic properties, including its role in managing Polycystic Ovary Syndrome (PCOS). PCOS is a hormonal disorder that affects individuals with ovaries, leading to various symptoms such as irregular periods, ovarian cysts, and hormonal imbalances.

Curcumin, the active compound in turmeric, is known for its anti-inflammatory and antioxidant properties. Chronic inflammation is often associated with PCOS and can contribute to insulin resistance, a common feature of the condition. Turmeric's anti-inflammatory effects may help alleviate some of the inflammatory processes associated with PCOS, potentially affecting insulin sensitivity. Moreover, curcumin has been investigated for its potential role in improving insulin resistance. Insulin resistance is a key factor in the pathophysiology of PCOS, contributing to metabolic disturbances and hormonal imbalances. Turmeric's ability to enhance insulin sensitivity may positively influence blood sugar regulation in individuals with PCOS.

While early research suggests promising effects, it is important to approach the use of turmeric for PCOS cautiously. The available evidence is still limited, and more research is needed to establish optimal dosage, duration, and long-term safety.

Before incorporating turmeric or curcumin supplements into a PCOS management plan, it is advisable to consult with a healthcare professional. They can provide personalized guidance, considering individual health conditions, potential interactions with medications, and overall treatment goals. Turmeric should be viewed as a potential complementary option rather than a standalone treatment, with lifestyle modifications and medical supervision remaining crucial components of a comprehensive PCOS management strategy.

In summary, turmeric, specifically its active compound curcumin, holds promise for its anti-inflammatory and insulin-sensitizing properties in the context of PCOS. However, further research is needed to establish its efficacy and safety, and individuals should exercise caution and seek professional advice before incorporating turmeric into their PCOS management plan.

6. Chaste tree (Vitex)

Chaste tree berry, also known as Vitex, is often used to help regulate menstrual cycles and balance hormones. It may take several months to see noticeable effects. Chaste Tree, also known as Vitex agnus-castus, is an herbal remedy that has been explored for its potential role in managing Polycystic Ovary Syndrome (PCOS). PCOS is a hormonal disorder characterized by symptoms such as irregular periods, ovarian cysts, and hormonal imbalances. Chaste Tree is believed to influence hormonal balance by acting on the pituitary gland, which plays a key role in regulating the menstrual cycle. It is thought to have a modulating effect on the hormones involved in the menstrual cycle, including luteinizing hormone (LH) and follicle-stimulating hormone (FSH). By affecting these hormones, Chaste Tree may help regulate the menstrual cycle in individuals with PCOS, promoting more regular ovulation [11]

Some studies and clinical trials have suggested potential benefits of Chaste Tree in improving certain symptoms associated with PCOS, such as irregular periods and hormone imbalances. However, it is important to note that the research on Chaste Tree for PCOS is still limited, and further studies are needed to establish its efficacy, optimal dosage, and long-term safety.

Before considering Chaste Tree as a part of PCOS management, it is crucial to consult with a healthcare professional. They can provide personalized advice, considering individual health conditions, potential interactions with medications, and overall treatment goals. Chaste Tree should be viewed as a potential complementary option rather than a standalone treatment, and its use should be integrated into a comprehensive PCOS management plan that includes lifestyle modifications and medical guidance.

In conclusion, Chaste Tree holds promise for its potential impact on hormonal balance and menstrual regularity in individuals with PCOS. However, more research is necessary to fully understand its effectiveness and safety in the context of PCOS treatment. Individuals should exercise caution and seek professional advice before incorporating Chaste Tree into their PCOS management plan [12].

7. Saw palmetto

Saw palmetto may have anti-androgenic effects and has been studied for its potential in managing PCOS symptoms. Saw Palmetto, derived from the Serenoa repens plant, is being investigated for its potential role in managing Polycystic Ovary Syndrome (PCOS), a hormonal disorder marked by irregular periods and elevated androgen levels. Known for its anti-androgenic properties, Saw Palmetto may inhibit the activity of 5-alpha-reductase, an enzyme involved in the conversion of testosterone to dihydrotestosterone (DHT). By potentially reducing DHT levels, Saw Palmetto could address symptoms associated with androgen excess in PCOS, such as hirsutism and acne. Despite some preliminary evidence suggesting positive effects, research on Saw Palmetto for PCOS is limited, and questions regarding optimal dosage and long-term safety remain unanswered [13].

Individuals considering the use of Saw Palmetto for PCOS should consult with a healthcare professional before incorporating it into their management plan. Personalized advice can address individual health conditions, potential interactions with medications, and overall treatment goals. Saw Palmetto should be viewed as a potential complementary option within a comprehensive PCOS management plan, incorporating lifestyle modifications and medical guidance. While its anti-androgenic properties hold promise, cautious and informed use is essential until further research establishes its efficacy and safety in the context of PCOS treatment [14].

8. Milk thistle

Milk thistle is known for its liver-supporting properties and may help improve liver function, which is important for hormone metabolism. Milk Thistle (Silybum marianum) is an herbal supplement that has gained attention for its potential benefits in managing Polycystic Ovary Syndrome (PCOS). PCOS is a hormonal disorder characterized by irregular periods, ovarian cysts, and often-elevated insulin levels. Milk Thistle contains a compound called silymarin, known for its antioxidant and anti-inflammatory properties. Some studies suggest that silymarin may have hepatoprotective effects and could potentially support liver health, which is important in PCOS management as the liver plays a role in metabolizing hormones.

While there is some evidence supporting the potential benefits of Milk Thistle in addressing aspects of PCOS, the overall research is limited, and more studies are needed to establish its efficacy and optimal dosage. Additionally, as PCOS is a complex condition with various contributing factors, it's crucial for individuals to consult with a healthcare professional before incorporating Milk Thistle or any herbal supplement into their PCOS management plan. A comprehensive approach, including lifestyle modifications and medical guidance, remains essential for managing PCOS effectively [15].

Conclusion:

Polycystic Ovary Syndrome (PCOS) presents a complex hormonal challenge, and while herbal remedies may offer complementary benefits, it is imperative to approach their usage with caution and consult with healthcare professionals. Spearmint, cinnamon, licorice root, fenugreek, turmeric, Chaste Tree, Saw Palmetto, and Milk Thistle have all been studied for potential benefits in managing PCOS symptoms, yet more research is needed to establish their efficacy, optimal dosages, and long-term safety. Each herb should be viewed as a potential part of a comprehensive PCOS management plan, integrated with lifestyle modifications and professional medical guidance. While promising, the role of herbs in PCOS treatment requires further exploration and individualized approaches to ensure safe and effective management of this complex hormonal disorder.

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HERBS USED IN WOUND HEALING

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Abstract:

The healing of a wound, defined as a disruption in the skin due to injury, involves a complex process of tissue repair. Throughout history, plants and their derivatives have been widely used to manage various types of wounds. In contemporary research, there's a focus on exploring different biopolymers to create cost-effective, sustainable, stable, and efficient delivery systems for wound treatments. This chapter aims to catalog medicinal plants known for their efficacy in treating wounds. It systematically discusses wound dressings derived from plant constituents, including patented formulations developed by various inventors. In conclusion, this compiled data serves to update researchers and scientists, offering them valuable insights into the significance and role of plant-based components in the treatment and care of wounds.

Keywords: Wound healing, Herbs, Traditional medicine, Therapeutic potential.

Introduction:

Wound healing is a fundamental biological process essential for the body's resilience and recovery from injuries, surgical incisions, or trauma. It is a finely orchestrated sequence of events involving an interplay of cells, proteins, growth factors, and signaling molecules to restore tissue integrity and functionality. When the body experiences tissue damage, whether from a cut, a surgical procedure, or an injury, a dynamic and complex series of events ensues. This process of wound healing is not merely a passive response but an active and precisely regulated mechanism aimed at restoring the structural and functional integrity of the injured tissue.

Mechanism of wound healing

Hemostasis

Following tissue injury, the body initiates hemostasis to control bleeding. Initially, blood vessels constrict to reduce blood flow. Platelets then aggregate at the injury site, forming a temporary plug and releasing chemicals that activate the clotting cascade. This results in the formation of a fibrin clot, halting bleeding and establishing a provisional matrix for subsequent repair processes.

Inflammation

Inflammation is a crucial phase characterized by the influx of immune cells to the wound site. Neutrophils arrive first, eliminating bacteria and debris through phagocytosis. Subsequently, macrophages, key regulators of the immune response, remove dead cells and debris while secreting growth factors and cytokines. These signaling molecules stimulate cell proliferation and migration for tissue repair.

Proliferation

During the proliferation phase, fibroblasts migrate to the wound area and synthesize collagen, a structural protein crucial for tissue strength. Angiogenesis, the formation of new blood vessels, occurs through endothelial cell migration and proliferation. Epithelial cells at the wound edges proliferate and migrate across the wound bed, eventually leading to wound closure.

Remodeling

The final phase, remodeling, involves the reorganization and strengthening of the newly formed tissue. Collagen fibers undergo remodeling, becoming more organized and gaining strength. This phase, lasting months to years, aims to restore tissue strength and function. However, it often results in scar tissue, which may lack the original tissue's characteristics.

Factors affecting wound healing

Age

Advanced age influences wound healing due to reduced cellular turnover, diminished immune response, and decreased growth factor production. Older individuals may have delayed healing and increased susceptibility to chronic wounds.

Chronic diseases

Conditions like diabetes, vascular diseases, and immunodeficiency disorders can impair wound healing. Diabetes affects blood circulation and impairs immune function, leading to chronic wounds. Vascular disorders compromise blood flow, hindering nutrient and oxygen delivery to the wound site.

Nutrition

Adequate nutrition, especially proteins, vitamins (like vitamin C and A), and minerals (like zinc), is crucial for cellular function and tissue repair. Deficiencies in these nutrients can delay healing by impeding cell proliferation and collagen synthesis.

Infection

Microbial contamination in wounds prolongs the inflammatory phase and disrupts the healing process. Infections can lead to chronic wounds and exacerbate tissue damage, impeding healing.

Medication and lifestyle

Certain medications, such as corticosteroids and immunosuppressants, can interfere with the immune response and collagen synthesis, affecting healing. Lifestyle factors like smoking, obesity, and poor wound care practices can hinder healing by impairing circulation, immune function, and tissue oxygenation.

Traditional use of herbs in wound healing

The traditional use of herbs in wound healing dates back centuries, reflecting a rich history of relying on nature's remedies to address injuries and promote tissue repair. Across diverse cultures and civilizations, indigenous communities have harnessed the therapeutic properties of various plants and botanical extracts to manage wounds effectively. Before the advent of modern medicine, traditional healers and herbalists curated a wealth of botanical knowledge, identifying specific plants known for their remarkable wound-healing properties. These natural remedies were often applied topically as poultices, salves, or infusions to accelerate healing, reduce infection risk, and alleviate pain associated with wounds. Herbs revered for their wound-healing attributes often possess diverse bioactive compounds, such as flavonoids, tannins, alkaloids, and essential oils, which exhibit antimicrobial, anti-inflammatory, and regenerative properties. Calendula, *Aloe vera,* comfrey, and lavender are among the many plants historically recognized for their efficacy in wound care. Ancient practices documented the use of these botanicals for diverse wound types, ranging from superficial cuts and abrasions to more severe injuries. Additionally, traditional medicine systems like Ayurveda, Traditional Chinese Medicine, and Indigenous healing modalities preserved valuable knowledge on plant-based remedies tailored to specific cultural contexts. The reliance on herbs for wound healing persisted across generations due to their perceived effectiveness and often minimal side effects compared to conventional treatments. While modern medicine has made significant advancements, there's a resurgence of interest in traditional herbal remedies. Contemporary research aims to validate and elucidate the scientific basis behind these traditional practices, exploring the mechanisms of action and identifying active compounds within these herbs. Understanding the historical context and the empirical knowledge embedded in traditional herbal practices provides a foundation for modern scientific inquiry. Integrating traditional wisdom with evidence-based research holds promise for identifying novel therapeutic agents, potentially contributing to the development of complementary or alternative approaches in contemporary wound care. Some examples of medicinal plants and their wound healing effects are listed below

Centella asiatica

Centella asiatica, also known as Asian pennywort, has a historical application in expediting wound healing, especially for chronic ulcers. Extracts from its aerial parts aid in healing chronic ulcers by influencing their distance, depth, and size. Asiaticoside, a compound isolated from *Centella asiatica*, demonstrates the ability to accelerate epithelialization and collagen production in wounds. Triterpenes derived from this plant also contribute to enhanced collagen remodeling and glycosaminoglycan synthesis. Studies indicate that oral administration of madecassoside, another compound from *Centella asiatica*, promotes collagen synthesis and angiogenesis at the site of injury.

Curcumin

Curcumin, derived from *Curcuma longa* or turmeric, has been a longstanding remedy and culinary spice. This compound, extensively used in various traditional medicine practices, exhibits a wide range of therapeutic properties. In Ayurvedic medicine, it is applied to manage conditions like asthma, liver disorders, diabetes, and skin injuries. Similarly, traditional Chinese medicine utilizes curcumin for addressing stomach ailments. This multifaceted molecule interacts with key cellular pathways at multiple levels, impacting inflammatory cytokines, cellular signaling molecules, and enzymatic processes. Research shows that curcumin's diverse effects include stimulating fibroblast proliferation, encouraging granulation tissue formation, and promoting collagen deposition in cutaneous wound healing by influencing pericellular and extracellular matrix components.

Sphagneticola trilobata

Sphagneticola trilobata, commonly known as Bay or Wedelia trilobata, was initially native to tropical Americas but has become invasive in tropical regions. Extracts from its leaves have been used traditionally to treat rheumatism, persistent wounds, and arthritic joints. Luteolin, a flavonoid found in the leaves, contributes to its medicinal properties, exhibiting neuroprotective, anti-cancer, antioxidant, and immune-modulatory effects. Studies confirm the use of Wedelia trilobata leaves by traditional healers to treat skin wounds. Luteolin in these leaves inhibits proinflammatory cytokines' expression linked to skin infections and psoriasis. Research validating this traditional use showed that specific subfractions from the plant's ethanolic extracts supported fibroblast viability, proliferation, migration, and exhibited activity against certain bacteria.

Aloe vera

Aloe vera, known for its numerous bioactive compounds, includes glycosides, polysaccharides, saponins, and anthraquinones. Acetone extracts from *Aloe vera* leaves

demonstrate antimicrobial activity, with compounds like saponins, acemannan, and anthraquinones exhibiting proven antimicrobial effects. Acemannan, a large polysaccharide from *Aloe vera*, stimulates macrophages and T cells, inducing proinflammatory gene transcription, promoting phagocytosis, and stabilizing secreted cytokines, growth factors, and other bioactives. Topical application of acemannan accelerates wound closure by influencing signaling pathways related to cell proliferation and skin repair.

Burdock

Burdock, also known as Arctium lappa, is a perennial weed used traditionally to treat sore throats, skin issues, and pathologies like boils and rashes. Clinical studies confirmed its antioxidant, antimicrobial, anti-inflammatory, anti-diabetic, antiviral, anti-cancer, and hepatoprotective effects. Arctium lappa root extract positively influences dermal ECM metabolism, glycosaminoglycan turnover, and gene expression in skin cells, impacting wound healing pathways. An ointment containing Arctium lappa was found to improve healing and reduce discomfort in burns and wounds in a pilot study.

Panax ginseng

Panax ginseng, a popular medicinal plant in several Asian regions, is believed to enhance immunity, physical stamina, and reduce fatigue. Ginsenosides, its active constituents, confer various properties, including antioxidant, anti-inflammatory, antibacterial, and anti-aging effects. Extracts from *Panax ginseng* root protect skin from UVB irradiation, improve wound healing, enhance collagen production, and promote keratinocyte migration. Ginsenoside Rb2 isolated from *Panax ginseng* stimulates epidermal growth factor and receptor expression, vital for wound healing, by influencing critical skin proteins.

Azadirachta indica

Azadirachta indica, commonly known as Neem, has been renowned for its diverse medicinal properties including anti-ulcer, antifungal, antibacterial, antiviral, anticancer, and antioxidant effects. Viji *et al.* assessed its wound healing potential by investigating its nitric oxide scavenging activity in RAW 264.7 cell lines. The incorporation of neem extract into collagen films exhibited promising nitric oxide scavenging and anti-inflammatory activity. Furthermore, these films, when tested for antioxidant activities and biocompatibility using RAW 264. cell lines, showed a significant increase in DPPH scavenging and cell viability, signifying their potential wound healing attributes. In another study, electrospun nanofibrous scaffolds incorporating neem extract, along with other plant extracts, were evaluated for skin tissue engineering. Results indicated increased cell proliferation, evident F-actin and collagen staining, and the secretion of extracellular matrix (ECM) by human dermal fibroblasts (HDFs) on the

nanofibrous scaffolds containing neem extract. Additionally, these nanofibers facilitated epidermal differentiation markers in human adipose-derived stem cells (ADSC), demonstrating their potential in supporting skin regeneration.

Chamomilla recutita

Chamomilla recutita, known as German chamomile, possesses specific phenolics and flavonoids, particularly apigenin, with notable wound healing effects. Electrospun nanofibrous membranes containing chamomile extract were studied for their wound healing properties. In vitro studies exhibited antibacterial and antifungal efficacy against microorganisms like S. aureus and *C. albicans*. Additionally, MTT assays demonstrated cell adhesion and viability of mesenchymal stem cells on these nanofibers. Rat wound models treated with nanofibers containing 15 percent chamomil extract showed significant wound closure and tissue regeneration, including reepithelization and collagen accumulation in dermal tissue, without any signs of necrosis.

Conclusion:

Wound healing, a persistent challenge in clinical settings since ancient times, involves intricate interactions among various cell types, the extracellular matrix, and signaling molecules like growth factors and cytokines. Substantial research efforts have focused on advancing wound care techniques, particularly in Ayurveda, where herbal therapies play a significant role. Researchers are actively exploring novel therapeutic approaches, including innovative formulations, dressings, and compositions derived from medicinal plants. The aim is to develop cost-effective, stable, and efficient delivery systems for treating and managing both acute and chronic wounds. The emergence of nanotechnology and the availability of new materials have significantly enhanced wound management strategies, leading to more effective and patient-centered approaches. These advancements are driving the development of wound treatment methods that are not only efficient but also tailored to meet individual patient needs.

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HERBAL REMEDIES FOR MALE INFERTILITY

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Abstract:

Male infertility can vary from hormonal disproportions and disruptions in reproductive tissues to issues with semen quality and quantity, as well as sexual behavioral concerns. The declining reproductive health in men globally is a significant worry, given that modern treatments for male infertility are costly, less manageable, require long-term commitment, and often come with various side effects. Conversely, herbal therapies offer more general avenues for enhancing male reproductive health. Within Ayurvedic medicine, a specific category of herbs known as vajikarana or aphrodisiacs is recognized for their ability to nourish and stimulate sexual tissues. This chapter focuses on the Ayurvedic approach to improving male reproductive health by highlighting scientifically tested herbs that have demonstrated the capacity to enhance male fertility through either stimulating or beneficial effects on the reproductive organs.

Keywords: Aphrodisiac, Erectile dysfunction, Male infertility, Testosterone, Hormonal imbalances.

Introduction:

Organisms have a natural drive to maintain their genetic lineage through successive generations, making humans highly successful in this regard. However, the current situation is concerning as both female and male reproductive health issues are on the rise worldwide. Sexual dysfunction, a serious medical concern affecting social and biological aspects, affects a significant percentage of both women (25%-63%) and men (10%-52%). Male sexual dysfunction encompasses difficulties achieving normal intercourse, responding to arousal, maintaining erections, delayed ejaculation, decreased libido, and irregular sexual behavior [1]. Numerous synthetic products flooding the global market promise quick remedies but often come with side effects and primarily suppress symptoms. Consequently, there's a renewed interest in herbal and Ayurvedic aphrodisiacs, which take a full method to address male reproductive issues by targeting their root causes. Aphrodisiacs, particularly those derived from herbs, have shown the

ability to directly stimulate male sexual desire, support reproductive functions, restore healthy tissue functioning, and assist in neuroendocrine regulation for achieving necessary sexual vigor with a balanced state of mind and body. This review aims to spotlight scientifically tested herbs known to enhance male fertility, emphasizing their potential as long-term remedies for male sexual problems [2]. Standard clinical and laboratory assessments often fall short in uncovering the underlying causes behind many cases of male infertility. This condition can stem from various issues such as hormonal or neuronal imbalances, disruptions in reproductive tissues, and the qualitative and quantitative decline of semen. A primary causative factor in male infertility is oxidative stress induced by reactive oxygen species (ROS). These ROS can cause damage to the polyunsaturated lipid membrane enclosing mature spermatozoa, leading to impaired spermatogenesis and reduced sperm quality, motility, and morphology. Male reproduction is mostly regulated by the hypothalamic-pituitary-gonadal (HPG) axis [3]. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are secreted by the anterior pituitary gland in response to gonadotropin-releasing hormone (GnRH), which is released by the brain. Then, to promote spermatogenesis and the synthesis of testosterone, FSH and LH operate on Sertoli cells and Leydig cells, respectively [4]. Spermatogenesis depends on the appropriate control of this axis, as well as intratesticular testosterone and other hormones. Changes in the HPG axis can have a major effect on semen parameters. When treating males with aberrant semen parameters, reproductive endocrinologists frequently turn to injectable drugs such as recombinant FSH, human menopausal gonadotropin (hMG), human chorionic gonadotropin (hCG), and gonadotropin-releasing hormone (GnRH) [5]. Another option is Clomiphene citrate, an oral medication that acts as an estrogen receptor antagonist to stimulate gonadotropin release from the pituitary. However, these treatments are costly, less available, require long-term commitment, and can have numerous adverse effects [6].

Herbal therapy and male reproductive health

Ayurveda holds that health hinges on the equilibrium among three fundamental bodily bio-elements or doshas—Vata, Pitta, and Kapha. Vata represents the airy element and embodies traits like dryness, coldness, lightness, minuteness, and mobility. Pitta signifies the fiery element, circulating through the liver and contributing to overall body warmth. Kapha embodies the watery element, characterized by heaviness, coldness, tenderness, sluggishness, lubrication, nutrient transportation, and nourishment. According to Ayurvedic medicine, vajikarana, a unique class of rasayana herbs, improves attractiveness and sex appeal while nourishing and stimulating sexual tissues. Another group of herbs known as shukrala is specifically designed to increase spermatogenesis. Selecting the right herbs to meet particular demands can be made easier by being aware of the possible causes and contributing factors of male infertility [7]. Both 'vaji' and

'karana', which translate to 'power or strength like that of a horse,' are Sanskrit words for these plants that are traditionally used as aphrodisiacs in the West. Vajikarana herbs function as stimulants or tonics to bolster male reproductive vitality. Stimulants, such as fenugreek, garlic, damiana, and onion, possess invigorating effects on male sexual organs. Tonics, like garlic, fenugreek, shilajit, and ashwagandha, offer enhanced nourishment to rejuvenate reproductive tissues, combining stimulating and nourishing properties [8].

Common ayurvedic herbs to improve male fertility

Ashwagandha *(Withania somnifera)*

Ashwagandha, which is called 'the scent of a horse' in Sanskrit because of the potent smell of its root, which is similar to horse urine, got its name from the notion that it gives men sexual energy like a horse. By stimulating spermatogenesis, improving blood flow to reproductive organs, and controlling endocrine processes, this herb's many advantageous properties help treat male reproductive diseases. Spermatorrhea, nocturnal emission, early ejaculation, and enlarged prostate are among the diseases it successfully treats. According to studies, *Withania somnifera* can prevent endocrine malfunctions in male reproduction and counteract stress-induced male infertility. Its aqueous extract elevates interstitial cell stimulating hormone, exhibits testosterone-mimicking effects, and induces nitric oxide synthase, thereby improving spermatogenesis. Ashwagandha root extract administration significantly enhances spermatogenic activity and increases serum hormone levels in oligospermic patients compared to placebos. Moreover, it combats oxidative stress by reducing lipid peroxidation, increasing sperm count and motility, and revitalizing antioxidant enzyme levels in seminal plasma. Administering *Withania somnifera* root powder to normozoospermic infertile men for three months notably reduces stress, improves antioxidants, enhances semen volume, elevates vitamins A, C, and E levels, and corrects fructose levels. Studies also demonstrate its impact on the HPG axis by significantly increasing serum testosterone and LH levels while reducing FSH and prolactin in infertile men. In Ayurvedic terms, Ashwagandha's grounding and nourishing qualities make it one of the best medicines for balancing vata. However, its warming energy can provoke pitta if used excessively, hence it's recommended to combine it with cooling vajikarana herbs like licorice, vidari, shatavari, and bala. With its stress-relieving, relaxing effects on the body and mind, and its ability to fortify all dhatus (body tissues), it's considered the best herb for addressing various factors associated with diminished sexual energy [9].

Kapikacchu *(Mucuna pruriens)*

Mucuna pruriens has a dense, oily texture and is noted for its sweet, sometimes bitter flavor. As too much of this plant tends to raise kapha and ama, its main usage is in vata and pitta balancing. The usual dosage range for *Mucuna pruriens* powder is 1.5–6 grams. Research on

alkaloids obtained from *M. pruriens* seeds has demonstrated that in male albino rats, there is a rise in the total weight of testicles and accessory glands as well as activation of spermatogenesis. Male rats who use this herb had increased mounting frequency, ejaculation delay, and higher intromission frequency, all of which are signs of increased sexual activity. *M. pruriens* effectively restored spermatogenic loss in male rats administered ethinyl estradiol in studies by reducing reactive oxygen species (ROS), controlling apoptosis, and elevating. L-DOPA, its main component, greatly adds to its pro-spermatogenic qualities. Studies on the seed extract of *M. pruriens* have shown significant enhancements in libido, sperm parameters, endocrine levels, and sexual potency. It also works well to balance lipid levels, including phospholipids, cholesterol, triglycerides, and vitamins A, C, and E. It also works to adjust fructose levels, which lowers oxidative stress-induced lipid peroxidation in seminal vesicles and raises antioxidant enzyme levels in seminal plasma. In terms of neuroendocrine function, *M. pruriens* causes infertile men to have lower levels of FSH and PRL and higher levels of testosterone, LH, dopamine, adrenaline, and noradrenaline. Steroidogenesis and semen quality have improved in infertile men treated with *M. pruriens* [10].

Shatavari *(Asparagus racemosus)*

Shatavari, a powerful male tonic with a bittersweet flavor, is well-known for its blood and liver-cooling and blood-purifying properties. It mostly affects the small intestine's pitta. Its cooling qualities go well with hot herbs like ashwagandha, garlic, and onion that increase sperm count. Shatavari has an important function in reducing sperm depletion caused by high pitta. Owing to its dense and filling qualities, it is advantageous for vata, particularly when paired with warming vajikarana herbs like bala or ashwagandha. It can be consumed on its own or in conjunction with other herbs, or made as a milk decoction using three to six grams of the plant. Since sexual and emotional needs are often entwined, using Shatavari together with calming nervine herbs might help in these situations. One of Ayurveda's greatest pitta pratyanika herbs, shatavari is frequently used in formulae to regulate pitta and vata, which in turn affects male reproductive systems. This herb may be used to treat ailments including urogenital infections, poor sexual energy, rage, stress, irritability, inflammation, and hyperacidity. Shatavari's normal dosage consists of 2 to 6 grams of crushed herb, taken twice or three times a day. However, if you have sinus or respiratory congestion, you should stay away from this plant [12].

Bala *(Sida cordifolia)*

The Sanskrit word bala, meaning "strength," is a revitalizing tonic herb for pitta and vata. It is applied to nourish and reinforce all body tissues, especially the bone marrow, muscles, plasma, and reproductive tissues. Bala has a mildly cooling quality and might enhance kapha due to its sweet, heavy, and greasy character. In Ayurveda, bala is highly recognized as an anti-vata

herb that works wonders for mental and physical ailments alike. It is particularly effective in treating chronic weariness, or balakshaya, and restoring general health when there is physical or mental depletion. It also functions as a tonic to improve male fertility, boost spermatogenesis, and restore sexual arousal. Bala can be consumed internally or used as a massage oil in combination with Ashwagandha for improving penile tone and preventing premature ejaculation. Its combination with herbs like gokshura, ashwagandha, vidarikandha, saw palmetto, and kapikacchu has demonstrated benefits for prostate health. The recommended dosage of Bala typically ranges from 2 to 6 grams, taken 2 to 3 times daily.

Vidarikandha *(Ipomoea digitata)*

Vidarikandha, a starchy tuber, proves highly effective in stimulating spermatogenesis, particularly when consumed as a milk decoction, showcasing faster action. Compared to shatavari and bala, it is lighter for kapha types. When combined with kapikacchu, it displays efficacy in treating enlarged prostate conditions. This sweet and cooling herb plays a significant role in promoting ojas (vitality), enhancing muscle tone, improving motor coordination, and counteracting sexual debility associated with nervousness and adrenal stress when formulated with ginseng, licorice, gokshura, and ashwagandha. Vidarikandha strikes a balance between being neither too warming nor too cooling, making it valuable as both a vata and pitta balancing herb.

Shilajit (*Asphaltum*, mineral pitch)

Shilajit holds immense significance in Ayurveda as it enhances virility and sexual stamina through its heating energy, eliminating excess kapha while maintaining normal genital tone. When addressing reproductive imbalances linked to kapha, it is often combined with Ashwagandha. In cases of enlarged prostate, effective herbal remedies involve combining Shilajit with saw palmetto, punarnava, gokshuradiguggulu, ashwagandha, or vidhari. The recommended dosage for this herb ranges from 250 to 500 mg taken twice daily.

Pippali *(Piper longum)*

Warming, invigorating, and kapha-reducing in nature, pippali is a revitalizing plant. Compared to other spicy spices and herbs, its pleasant post-digestive impact tends to be more calming to pitta, while its oily nature avoids dryness, making it appropriate for vata balance. Pippali helps to improve blood flow to the reproductive tissues when taken with Ashwagandha. According to Ayurveda, pippali is said to improve vitality and expel pollutants through breathing. In addition, it has been shown to lessen arthritic pain and improve respiratory, assimilation, digestion, and absorption. In complicated formulae, the usual dosage of Pippali is one to three parts, or 250 mg to 1.5 grams [13].

Tribulus terrestris

Tribulus terrestris Linn., commonly known as puncture vine, is a perennial creeping herb found globally and has been esteemed as an aphrodisiac since ancient times. Apart from its historical use, it has been employed to address various conditions such as inflammations, leucorrhoea, urinary infections, edema, and ascites. Studies administering T. terrestris to animals have demonstrated an enhancement in plasma testosterone levels and stimulated spermatogenesis. Additionally, it increases testosterone and luteinizing hormone levels and influences the activities of dehydroepiandrosterone, dihydrotestosterone, and dehydroepiandrosteronesulphate. In experiments conducted on New Zealand white rabbits, T. terrestris treatment showed a proerectile effect on corpus cavernosal tissues when subjected to various pharmacological agents and electrical field stimulation in vitro. This herb has been observed to increase sexual behavior by enhancing mount and intromission frequency while reducing mount and intromission latency, alongside an increase in prostate weight and intracavernosal pressure. Furthermore, its properties in increasing androgens like testosterone, dihydrotestosterone (DHT), and Ddehydroepiandrosterone (DHEA) have stimulatory effects on reproductive functions, making it suitable for mild to moderate cases of endocrine disruptions. Its ability to induce nitric oxide release might contribute to its reputed aphrodisiac properties [14].

Conclusion:

Herbs known as vajikirana or aphrodisiacs can be used as tonics or stimulants to improve the vigor of male reproduction. Heat-producing herbs are commonly used as stimulants to arouse the male genitalia, whilst herbal tonics provide better nutrition to the reproductive tissues, revitalizing both their number and quality. Certain plants are both nutritious and invigorating. Herbal aphrodisiacs are seeing a renaissance due to the health complications, poor effectiveness, and high cost of current therapy for male infertility. By improving sexual behavior, vitality, neuroendocrine regulation, and the structural and functional characteristics of reproductive tissues, they are exhibiting long-term effects on male reproductive health. They contribute to qualitative and quantitative improvements in semen, sperm morphology, and motility. This pursuit is critical as male infertility poses a significant threat to future generations, and understanding these mechanisms can lead to effective interventions.

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HERBAL PLANTS FOR THE TREATMENT OF URINARY TRACT INFECTION

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Abstract:

Since the beginning of civilization, medicinal plants have been an integral element of human society's efforts to treat illness. The World Health Organisation (WHO) estimates that approximately 80% of the world's population primarily uses plant-based traditional medicine, particularly for their primary healthcare needs. Traditional medicine has become more popular globally as a result of worries about more invasive, costly, and potentially harmful mainstream practices. Urinary tract infections can be treated using herbal medicines, among their many other medicinal applications. Traditional medical systems' treatments are less commonly shown to be safe and effective than mainstream pharmaceuticals since they do not need to undergo clinical testing or formal regulatory agency clearance before being sold. We provide an overview of the current research on domestically produced medications in this chapter, which are mostly used to treat urinary tract infections.

Keywords: Medicinal plants, Urinary tract infection, Traditional medicine, Therapeutic

Introduction:

Although cystitis and urinary tract infections (UTIs) are sometimes used interchangeably, an infection of any part of the urinary system, including the kidneys, ureters, bladder, and urethra, is referred to as a UTI. An enormous number of people are impacted by urinary tract infections every year. Urinary tract infections are the second most prevalent form of infections in the human body. About 80% of adult urinary tract infections are caused by Escherichia coli. A urinary tract infection (UTI) is caused by bacteria. An infection happens when germs from the rectal region travel via the urethra to the bladder, where they multiply in the urine. Bacteria frequently start their journey at the urethra. Bacteria may grow and cause an illness. Mycoplasma hominis, Chlamydia tracomatis, and Staphylococcus saprophyticus are other bacteria that can

result in urinary tract infections. The severity of the sensations can be overpowering, they can progress fast, and they can be severe. Urinary tract infections typically start with burning while urinating, frequent urination, and little amounts of urine—possibly including blood—being voided [1-4]. When creating a herbal remedy for a urinary tract infection (UTI), the main ingredients to take into account are immune-stimulating, demulcent, aquaretic/diuretic, antimicrobial/antiseptic, and anodyne/antispasmodic herbs. Both specific and non-specific immune-modulating and tonic herbs are valuable adjuvants.

Plant medicine: Across several civilizations, plant remedies have been utilised for thousands of years. Medicinal plants are becoming a more and more popular substitute for creating a healthy internal environment. Since ancient times, they have been crucial in the treatment of a variety of illnesses and conditions, including urinary tract infections [5]. As disinfectants, analgesics, diuretics, or opioids, they can have an impact on urinary tract infections. Certain substances have demonstrated antibacterial properties against bacteria that cause urinary tract infections (UTIs), including Escherichia coli. Programmes for treating UTIs can benefit greatly from the use of medicinal herbs [6].

Alternatives to medicinal plants: Around the world, medicinal plants are utilised to cure and prevent infectious illnesses. For primary healthcare, plant medicines are highly sought after in both developed and developing nations due to their broad range of biological and therapeutic activity, greater safety margin, and lower cost. When antimicrobial medications are used indiscriminately to treat infectious infections, multiple-drug resistance has emerged in recent years [7]. A reinvigorated search for antimicrobial drugs resistant to existing antibiotics is necessary in light of the resistance issue [8]. Furthermore, even though traditional antibiotics are potent medications that can save lives, improper administration of them can have the opposite effect of what is intended [9]. The World Health Organisation released its guidelines for research on the "safety and efficacy of herbal drugs" in 1993. They explicitly stated that, without scientific proof to the contrary, the historical usage of herbal drugs is a reliable indicator of their safety [10]. Herbal treatments used in traditional healthcare systems around the globe are a valuable source for the development of novel antimicrobials [11,12]. Many secondary metabolites, including tannins, alkaloids, terpenoids, and flavonoids, are abundant in plants and have been shown to have antibacterial qualities in vitro. These compounds may be used as safe, affordable, and effective substitutes for antibiotics for treating microbial infections [11].

Several significant herbs that are used to treat UTIs include:

- **Cranberry (*Vaccinium macrocarpon*):** Both doctors and herbalists have long advised cranberries (*Vaccinium macrocarpon*) as a means of preventing urinary tract infections [13].

Because it stops E. coli, the bacterium that causes the majority of UTIs, from adhering to the walls of the bladder, research has indicated that cranberries may be effective against UTIs [14–15]. But when it comes to treating acute UTIs, cranberries cannot replace medicines. Moreover, supplementing with cranberry juice did not lower the risk of infection in children whose UTIs are caused by neurogenic bladder [16]. Cocktails made with cranberry juice may provide defence against P-fimbriated E. Coli strains that are both sensitive and resistant [17].

- **Blueberry (*Vaccinium angustifolium*):** The blueberry, or *Vaccinium angustifolium*, naturally contains antioxidants. Herbal literature from the past mentioned the benefits of a blueberry (*Vaccinium angustifolium*) tea for urinary tract infections. Urinary tract infections have also traditionally been treated and prevented using blueberries. Blueberries, like cranberries, exhibit bioactive chemicals that prevent E. Coli from adhering to the bladder walls [18].
- **Buchu (*Agathosma betulina*):** Preparations made from the leaves of Buchu (*Agathosma betulina*) have long been used as a diuretic and urinary tract disinfectant in traditional herbal medicine [19]. Preparations made from buchu leaves have long been used as diuretics and urinary tract disinfectants in traditional herbal medicine [20]. The German Commission E monograph on buchu, however, comes to the conclusion that there is not enough data to justify its use in treating inflammation and UTIs in the present era. Its volatile oil is essentially eliminated by the kidneys and causes urine. It can be used effectively in cases of acidic urine, bladder infections, and persistent urges to pee without any discernible alleviation [21].
- **Goldenseal (*Hydrastis canadensis*):** Native Americans utilised goldenseal (*Hydrastis canadensis*) to alleviate irritation and inflammation of the mucous membranes of the digestive, respiratory, and urinary tracts [22].
- **Horseradish (*Cochlearia armoracia*):** Herbalists utilised horseradish (*Cochlearia armoracia*), which was thought to be a diuretic, to cure edoema and kidney stones [23].
- **Juniper berries (*Juniperus communis*):** Urinary tract disorders are managed using juniper berries (Juniperus communis). Juniperus communis berries have been used in traditional medicine to treat a wide range of ailments, including kidney and urinary tract disorders [24]. It has bitter ingredients that make pee flow more readily. Additionally, it stimulates the formation of digestive juices, which both reduce discomfort and aid in absorption. The fruit is frequently used as a stimulant, diuretic, and antibacterial. Although it is quite helpful for treating chronic cystitis, it is better to avoid using it in cases of acute inflammation since it

may irritate the bladder. It has aromatic components in it that make pee flow more readily. These plants are often consumed as tea [25].

- **Oregon grapes (*Berberis aquifolium*):** UTIs were among the conditions for which Oregon grapes were used as a remedy [26]. Oregon grape berberine may be useful in the treatment of urinary tract infections [27].
- **Sassafras (*Sassafras albidum*):** Native Americans utilised sassafras (*Sassafras albidum*) for a variety of ailments, but mostly for stomach issues and infections. It is most helpful when treating urinary tract infections [28].
- **Uva ursi (*Arctostaphylos uva-ursi*):** Due to its astringent or "drying" properties, ava ursi (*Arctostaphylos uva-ursi*) is widely used to treat infections in all areas of the body, especially UTIs [29]. In Europe and North America, traditional herbal medicine use uva ursi extract as a remedy for urinary tract infections [30]. Germany has authorised the use of this herb to treat bladder infections [31]. It works well against bladder-dwelling E. Coli. Strong, non-irritating diuretic uva ursi is an effective urinary antiseptic for kidney and bladder infections. It aids in the removal of kidney and bladder stones when mixed with marshmallows. It works well to cure blood in the urine and tones and strengthens the urinary passageways.
- **Garlic (*Allium sativum*):** *Allium sativum*, or pure garlic, is known as the "Heavy Weight" of herbal treatments due to its potent antibacterial qualities, which make it effective in treating a variety of illnesses, including urinary tract infections [32].
- **Corn silk (*Zea mays*):** *Zea mays*, or corn silk, is a calming, anti-inflammatory diuretic that lessens discomfort and swelling brought on by inflammation. It is a demulcent for the urine. It works particularly well for children's bladder discomfort and urine that is too alkaline [33].

Conclusion:

This chapter shows how various phytoconstituents found in herbs might be beneficial in treating urinary tract infections and may even serve as a source for future UTI-fighting pharmaceuticals. Herbal remedies are efficient, secure, cost-effective, and free of adverse effects when treating simple UTIs. Antimicrobial drugs are required for certain of the most complex UTIs that do not improve with herbal formulations. Therefore, it can be said that treating complex UTIs with a herbal formulation in addition to antibiotics is beneficial.

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PLANT PROFILE OF NUTGRASS, *CYPERUS ROTUNDUS* (CYPERACEAE) – A REVIEW

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Abstract:

Cyperus rotundus, commonly known as "nutgrass" and "purple nutsedge." is indigenous to tropical and subtropical regions of Asia and Africa and is a member of the Cyperaceae family. Due to its multiple therapeutic properties, the plant is used in traditional medicine to treat a wide range of conditions, including inflammation, fever, diarrhea, and dysentery. The antioxidant, anti-inflammatory, and antibacterial potential may be attributed to the presence of essential oils, alkaloids, flavonoids, and tannins. Due to its exceptional pharmacological action, *Cyperus rotundus* has been widely explored for its potential in herbal therapy.

Introduction:

Cyperus rotundus is a perennial, herbaceous plant that is commonly known as "nutgrass" or "purple nutsedge." It belongs to the family Cyperaceae and is native to tropical and subtropical regions of Africa and Asia. The plant has several medicinal properties and is used in traditional medicine for treating various ailments such as diarrhea, dysentery, fever, and inflammation. It is rich in essential oils, alkaloids, flavonoids, and tannins which have anti-inflammatory, antimicrobial, and antioxidant properties. Studies have shown that the plant extract has anticonvulsant, antidiabetic, and anti-inflammatory effects. It has also been found to have anticancer potential and can inhibit the growth of cancer cells [1]. *Cyperus rotundus* has been extensively researched for its potential in herbal medicine, because of its remarkable pharmacological activity.

Plant profile

Plant Name: *Cyperus rotundus* L.

Common Name: Nutgrass, Purple nutsedge, Cocograss

Family: Cyperaceae

Figure 1: *Cyperus rotundus* -Whole plant

Figure 2: *Cyperus rotundus* rhizomes

Vernacular name

Tamil: Korai kilangu

Telugu: Bhadra-tunga-mustalu

English: Coco grass, nutgrass, purple grass

Hindi: Nagar motha, motha

Taxonomical status

Domain: Eukaryota

Kingdom: Plantae

Subkingdom: Viridiplantae

Superdivision: Embryophyta

Division: Tracheophyta

Subdivision: Spermatophyta

Class: Magnoliopsida

Order: Poales

Family: Cyperaceae

Genus: *Cyperus*

Species: *rotundus*

Distribution and habitat

Mustaka has been present in Indian culture since ancient times. It had been cultivated on high levels in ancient India. It is indigenous to india but now found in various temperature, tropical and subtropical region [2].

Biography of plant

Habit

It is extensively distributed due to its ability to adapt to a wide range of soul types, temperature, soil pH, altitudes and moisture levels. This plant grows in small clumps up to 100

cm high. It is the native of India. Mustaka is found mostly all over the India, also in tropical and subtropical regions of Africa, southern Asia, Southern and Central Europe [2].

Plant description

Cyperus rotundus, also known as nut grass or coco grass, is a perennial herbaceous plant belonging to the family Cyperaceae. It is a common weed found in tropical and subtropical regions around the world, including Asia, Africa, and the Americas.

The plant has a slender stem that can grow up to 60 cm tall, with numerous underground rhizomes that give rise to new plants. The leaves are long and narrow, reaching up to 60 cm in length and 3-6 mm in width, with a V-shaped cross-section. The inflorescence is a compact, spherical or cylindrical cluster of spikelets that are yellowish-brown in color. *Cyperus rotundus* is known for its medicinal properties and has been used in traditional medicine for the treatment of various ailments, including fever, dysentery, and diarrhea. It contains several bioactive compounds such as flavonoids, alkaloids, and essential oils, which contribute to its therapeutic properties [3].

Morphology

Purple nutsedge is a grass-like weed in the sedge family (Cyperaceae) with top growth 4–30 inches tall an extensive underground network of basal bulbs, fibrous roots, thin wiry rhizomes and tubers borne in chains of 2–6 or more on rhizomes, with tubers spaced 2-10 inches apart. The leaves are mostly basal, dark green, 0.1–0.25 inches wide with a prominent midrib, and abruptly tapered at the tips. The purplish to red-brown inflorescence is borne on a culm (stem) that is triangular in cross section and usually taller than the foliage. The inflorescence itself consists of an umbel of spikes, some of which are sessile, and others are borne on stalks of unequal length. The subtending leaflike bracts are usually shorter than the longest spikes. Roots are fibrous, extensively branched, clothed with bent hairs. The plant is spread by an extensive, horizontal, slender network of rhizomes which are white and fleshy. They are 2-3 cm long and 1cm large, white and succulent when young turning fibrous brown or almost black with age [4].

Ethnobotanical uses

- Anti-inflammatory and analgesic properties
- Anti-diabetic properties
- Anti-cancer properties
- Culinary [5]

Pharmacognostical studies

Anand Prakash *et al.,* (2019) have reported the Pharmacognostical analysis of different parts of *Cyperus rotundus* L. [6]. Srishti Dhyani *et al.,* (2017) have studied the morphological

(2017)- have studied the morphological & microscopical features of leaf and stem of *Cyperus rotundus* Linn and *Cyperus procerus* Rottb: A comparative analysis [7]. Hongbo wei *et al.,* (2022) have studied the anatomical and histochemical structures of *Cyperus rotundus* L., facilitate success in amphibious environments [8]. John Adams *et al.,* (2013) have studied the Pharmacognostic and phytochemical studies on Ayurvedic drugs Ativisha and Musta [9]. Ajay singh *et al.,* (2011) – have studied the morphological, microscopical and physico- chemical investigations on the rhizomes of *Cyperus rotundus* Linn. [10].

Phytochemical studies

Kumar R *et al.,* (2010) have studied the standardization and preliminary phytochemical investigation on *Cyperus rotundus* Linn rhizome [11]. Salem Edrah *et al.,* (2019) have studied the phytochemical analysis and antibacterial activity of leaves and roots of *Cyperus rotundus* [12]. Alireza Ghannadi *et al.,* (2012) have studied the phytochemical screening and essential oil analysis of one of the persian sedges; *Cyperus rotundus* L. [13]. Kandikattu Hemanth Kumar *et al.,* (2014) have studied the phytochemical analysis and biological properties of *Cyperus rotundus* L. [14]. AR Kasarkar *et al.,* (2017) have studied the preliminary phytochemical investigation of the leaves of *Cynodon dactylon* (L.) Pers., *Cyperus rotundus* L. and *Typha angustifolia* L [15].

PHARMACOLOGICAL STUDIES

Uddin SJ *et al.,* (2006) have studied the antidiarrhoeal activity of *Cyperus rotundus* [16]. Nishikant A. Raut *et al.,* (2006)- have studied the antidiabetic activity of hydro- ethanolic extract of *Cyperus rotundus* in alloxan induced diabetes in rats [17]. A Puratchikody *et al.,* (2006)- have studied the wound healing activity of *Cyperus rotundus* [18]. Fernanda Grillo Rocha *et al.,* (2020)- have studied the preclinical study of the topical anti-inflammatory activity of *Cyperus rotundus* L. extracts (Cyperaceae) in models of skin inflammation [19]. Manivannan Rajamanickam *et al.,* (2016)- have studied the analgesic and anti inflammatory activity of the extracts from *Cyperus rotundus* Linn rhizomes [20].

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EXPLORING THE MEDICINAL POTENTIAL: *CADABA FRUTICOSA LEAVES*

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Introduction:

Forests and other natural environments are the source of more than 905 therapeutic plants. Ironically, a number of highly promising medicinal plant species have seen a severe fall in population due to the loss of their natural habitats. Moreover, the rapidly evolving lifestyle brought on by modernization is causing a rapid erosion of folkloric culture. Therefore, before it is lost forever, it is imperative that the traditional wisdom that exists among ethnic communities be documented. The development of new medications requires the scientific validation of traditional claims in order to meet the demands of the world's growing population. Indian medicinal plant has been an integral part of traditional medicine for centuries, contributing to the rich tapestry of country's culture & heritage. These medicinal plants with diverse properties and application have played a crucial role in addressing various health concerns. *Cadaba fruticose* ("Bhutaki" in Sanskrit "Viluthi" in Tamil) is versatile plant shrub that holds significant place in traditional medicine with a rich history dating back centuries. This plant has been a corner store in Ayurveda, an ancient system of medicine. It reflects its unique characteristics and widespread presence across the Indian subcontinent. The best plant for the sustainable use and development of herbal medications derived from local resources is *Cadaba fruticosa* (L.) Druce.Plants used for herbal medicine continue to be quite significant.

Taxonomy

Kingdom	Plantae
Clade	Angiosperms
Order	Brassicale
Family	Capparaceae
Genus	Cadaba
Species	*C.fruitcosa*

Description

Indian Cadaba is commonly distributed and found throughout the drier lowland regions of India. This Indian medicinal plant is essentially an unarmed, straggling and much-branched

shrub, reaching up to 3 metres in height, bearing cylindrical stems. As the plant grows older, it turns smooth and purplish, whereas, the younger variation is pubescent and yellowish-brown in colour. The leaves of Indian Cadaba vary between 1.2 centimetres to 3.5 centimetres in length and 0.8 centimetres to 1.8 centimetres in breadth. The shapes of the leaves appear to be ovate-oblong or ovate, together with additional features of being glabrous and entirely margined. The apex is framed at an angle that is acute or obtuse-mucronate, with the base being rounded and venation well-defined beneath. The petioles of Indian Cadaba measure within 0.2 centimetres to 0.4 centimetres in length. The flowers of this said Indian medicinal plant however bear an appearance of dirty-white, that are 1.5 centimetres.

Figure 1: *Cadaba fruticosa*

Medicinal properties

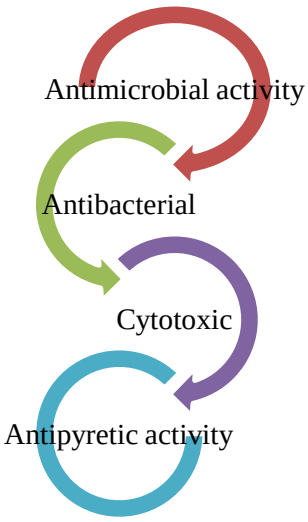
As an antidote to poisoning, stimulant, antiscorbutic, and to treat general body discomfort (Sandhya *et al.,* 2006). The leaves of Indian Cadaba is a long-acting medicinal plant used for treating a variety of chronic illnesses. *Cadaba fruticosa (L.),* is a member of the Capperidaceae family and is used medicinally. It is also referred to as "capper bush" in English and "vizhuthi" in Tamil. The Indian Subcontinent, including Bangladesh, India, Pakistan, Sri Lanka, and Indo-China (Myanmar), is home to this indigenous species. In native traditional medical systems, Leaf juice of *Cadaba fructicosa* is a regularly utilized and is taken internally for overall weakness, energy during diarrhoea. The roots are also prescribed as an emmenagogue, anthelmintic, and deobstruent. The leaves are used to treat eczema (Arokiyaraj *et al.,* 2008) and also to treat leucoderma (Chatterjee *et al.,* 1993). Moreover, it was reported to have the active constituents, like cadabicine, cadabicine diacetate (Viqar Uddin *et al.,* 1990), Capparisine and α – B – dihydroferulic acid (Aziz-Ur-Rehman *et al.,* 1990).

Ethnomedicobotanical data

1. In the event of conjunctivitis, a decoction of the fresh leaves (2–5 ml) is taken orally for three days (pain and reddening of the eyes).

2. Three days are spent injecting two to three drops of an aqueous leaf extract into the nostril to treat rhinitis (relieving cold).
3. The plant's aerial portion is air-dried, ground into a powder, and given orally for three days in cases of fever on doses of one to two grams with warm water or milk.
4. To relieve stomach issues, fresh leaves are chewed daily for approximately 15 days at a dose level of 0.5 to 1.5g.
5. To halt the white discharge, an aqueous extract of the roots and leaves is given at a dose of 1–1.5g and 0.5–1g with hot water once a day for 45 days.
6. When suffering from fever and dehydration, fresh juvenile Viluthi leaves are consumed as a side dish with rice gruel.
7. To reduce the increased blood glucose level, three to five fresh leaves, weighing around 0.3 to 0.6g, are chewed twice a day for approximately ninety days.

Pharmacological activity



a) Antimicrobial activity

The flower of *cadaba fruticosa* were collected. Chemicals like Ethanol, nutrient broth, potato dextrose agar, petroleum ether, chloroform, ethyl acetate are used for extraction. Powdered samples of wild plant leaf and tissue-cultured plant of *Cadaba fruticosa* (100 grams each) were placed into a Soxhlet apparatus thimble and extracted using petroleum ether, chloroform, ethanol, methanol, and ethyl acetate in that order. The microorganism used to test antimicrobial activity are *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli* and fungal pathogens like *Candida albicans* and *Trichoderma viride*. Bacterial cultures were maintained on a nutrient broth at 37 °C and fungus was maintained on potato dextrose agar at 28 °C. The agar disk diffusion assay is used to screen for antimicrobial activity of plant ethanol and methanol extracts. The extracts are dissolved in solvents, and sterile disks are impregnated with

different concentrations. The disks are incubated at 37°C for 24 hours, and the activity of bacterial and fungal pathogens is determined after 72 hours of incubation at 28°C.

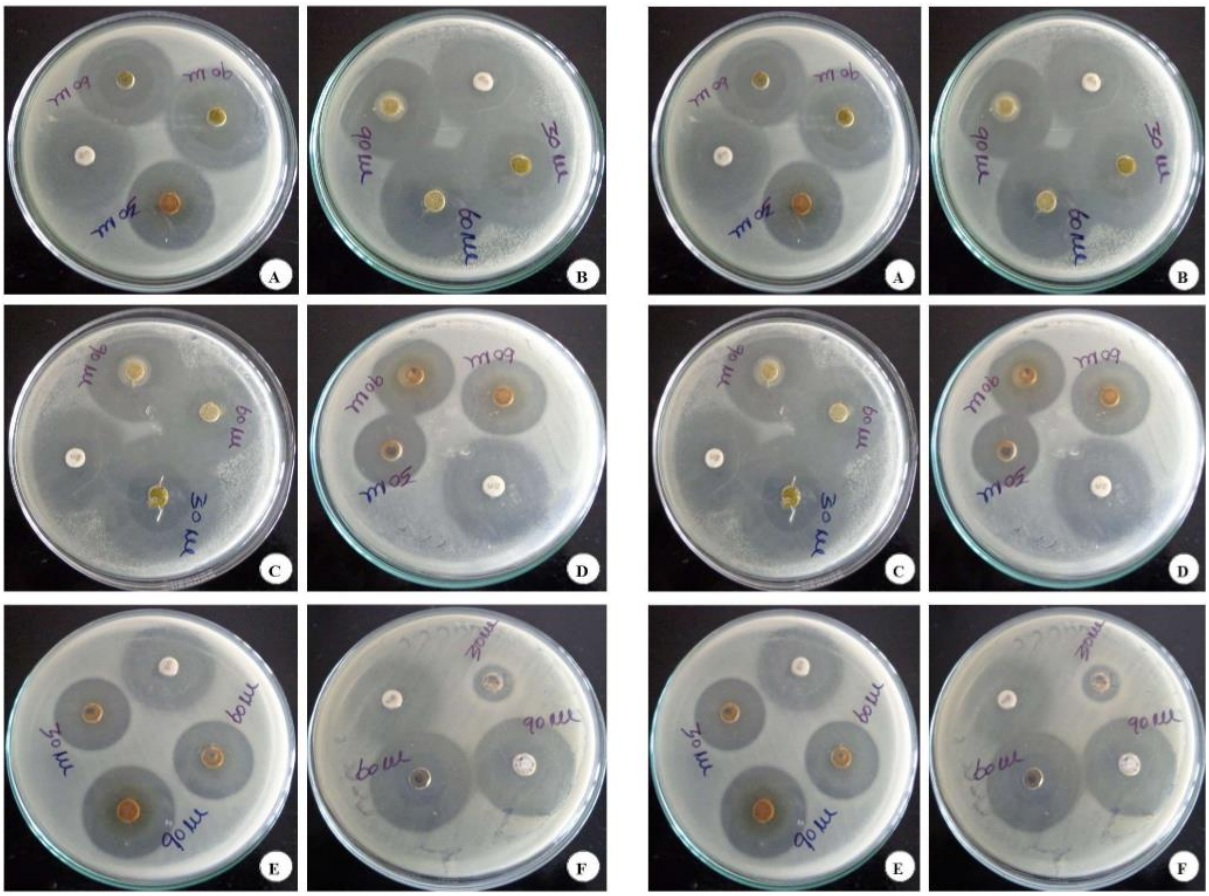


Figure 2: Antimicrobial activity of WCFE extract of *C. fruticosa*.

Figure 3: Antimicrobial activity of MCFE extract of *C. fruticosa*

The study analysed the antimicrobial activity of ethanol and methanol extracts from wild plant leaf and tissue cultured plant samples using the agar disc diffusion method. The highest zone of inhibition was observed in E. coli and 0.8±1.05 at 60 µL concentration of wild and tissue cultured plant ethanol extracts, respectively. The methanol extract of wild reported *C. fruticosa* showed significant antimicrobial activity against S. *aureus*, E. *coli*, K. *pneumonia, and C. albicans* T. *viride*. The tissue cultured plant methanol extract showed antibacterial potential against and with S. *aureus*, E. *coli*, K. *pneumonia*, and *C. albicans* T. *viride.*

b) Antibacterial activity

The ariel parts of *cadaba fruticosa* was dried. A grinder was used to grind the material.2g of sample in 25ml of methanol and filtered using filter paper, then filtrate was left to evaporate until it is completely dried. Then extract was again suspended in 2ml of methanol. The final extract had a concentration of 1g material/ml. Microorganism like (two gram positive) *Bacillus*

subtilis, Staphylococcus aureus and (three-gram negative bacteria) *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* were cultured in nutrient broth for 24hrs and inoculum was taken for test. The standard disk diffusion experiment, which was adapted from Taylor *et al.* (1995), was the bioassay utilized. To prepare the test disks, dip and saturate plant extract in a sterilized filter paper disk. The same amount of extract was absorbed by filter paper disks of the same size (6 mm diameter). Tetracycline paper disks were utilized as the positive control, and methanol paper disks, made by dipping the disk into methanol, were used as the negative control. The solution was made by combining 9.2 ml of methanol with 0.8 ml of tetracycline solution, which was made by dissolving 500 mg of tetracycline pills in 20 ml of methanol. Tetracycline's final concentration was 0.25 mg/ml. Sabouraud dextrose was used as the media for growth of bacteria. Fresh cultured plates of bacteria were transferred into tube containing 10 ml of liquid media and incubating over night at 37°C.The tubes were shaken to aerate and promote proper growth. To facilitate the transformation of bacteria, a swab dipped in a standard inoculum was added to the Petri dish containing nutritional agar. The germs were then dispersed across the media in a confluent lawn using the swab after it had been dipped. After that, prepared dried disks were moved using flame-sterilized forceps onto bacterial grass. After that, the Petri dishes were incubated at 37°C for 24 hours while upside down. According to Lennette *et al.* (1995), the bioassay observation was documented as the zone of inhibition's presence or absence. Test paper disks' surrounding inhibitory zone, which showed no bacterial growth, was classified as positive (growth inhibition was seen) and its absence as negative.

Table 1: Results of antibacterial test

SI. No.	**Plant**	**Part used in test**		**Bacteria and result**			
			B. subtilis Gram + ve	***S. aureus*** Gram +ve	***E. coli*** Gram - ve	***P. aeruginosa*** Gram - ve	***S. typhi*** Gram - ve
1.	*Cadaba fruticosa*	Leaf	+	-	-	+	-

c) Cytotoxic effect

The leaves of *cadaba fruticosa* was collected and dried at 25-30°C for 5 days and powdered. The powdered was extracted using alcohol and water using Soxhlet apparatus. Then it is evaporated to dryness to reduce pressure. The study used Vero (primary monkey kidney cell lines), RD (Rhabdo myosarcoma cell lines), and Hep 2 cell lines (Human epithelioma cell lines

of the larynx) to assess cytotoxicity using MTT-dye assay. Quercetin was used as a positive control drug. Cell lines were cultured in EMEM medium with a plating density of 2000 cells/well. Cell viability was determined colorimetrically after exposure to test samples. The final mixture used contained no more than 0.5% of the vehicle, showing no effect on cell growth. The IC50 value was calculated from dose-response curves plotting between percentage of inhibition and concentrations.

Table 2: Cytotoxicity of alcohol and aqueous extracts of *Cadaba fruticose*

Plant	Extract	Yield (%w/w)	Phytochemical screening	Vero IC_{50}[a] (µg/ml)	RD IC_{50}[b] (µg/ml)	Hep 2 IC_{50}[c] (µg/ml)
Quercetin	-	-	-	48.23	50.34	54.21
C.fruticosa	Aqueous	7.37	• Glycosides • Phenolic Compounds • tannins	198.03	196.21	248.56
	Alcohol	15.51	• Alkaloids • Glycosides • Phenolic • Compounds • Tannins • Flavonoids • Steroids • Triterpenoids	48.31	80.35	28.92

d) Antipyretic activity

The study evaluated the antipyretic activity of aqueous and ethanol extracts of *Cadaba fruticosa* Druce leaf on Wistar albino rats. Results showed that the extracts significantly reduced normal body temperature and yeast-induced pyrexia at 500 mg/kg body weights at 23 hours of administration of yeast compared to the standard antipyretic drug paracetamol. The dose of 100 mg/kg of both extracts produced less significant antipyretic effect. The rats were divided into six groups and their rectal temperature was recorded before, during and after the yeast injection. The rats were given Brewer's yeast, a dietary supplement, and a thermistor probe to record their basal rectal temperature. After nineteen hours, they were restrained and administered extracts, distilled

water, or paracetamol as a drug control. The rat's rectal temperature was recorded at 19 hours before, extract, and paracetamol administration. The extracts also reduced fever by increasing Brewer's yeast suspension.

Pharmacologically active compounds

The compounds that are responsible for its pharmacological activity was found using gas chromatography mass spectroscopy. GCMS of *C. fruticosa* with wild and micro propagated with ethanol extract exhibit the presence of various bioactive compounds with many bioactive principles. The WCFE extract GCMS spectrum exhibited 83 peaks. Among these 18 are known bioactive compounds. The main properties of bioactive compounds are antioxidant, anti-inflammatory, anticancer, antimicrobial, antiandrogenic and antitumor activities.

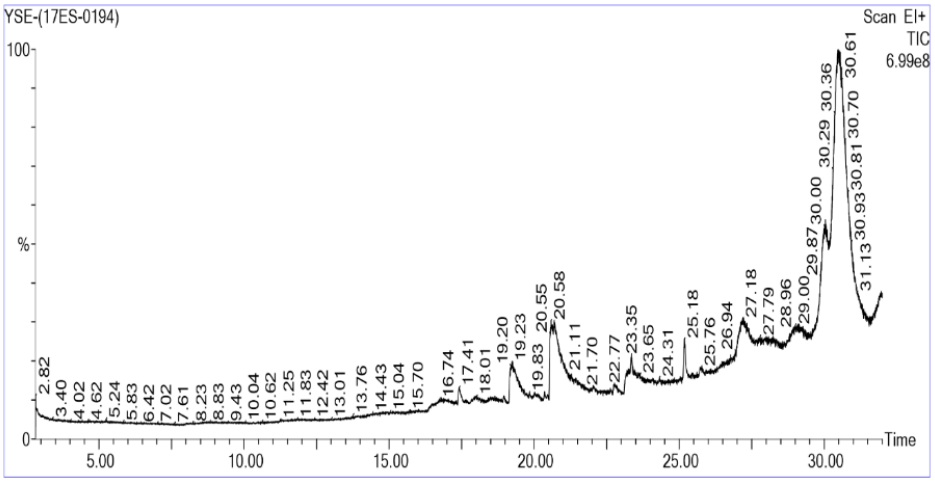


Figure 3: Gas chromatograph of WCFE extract of *Cadaba fruticose*

Table 3: GCMS analysis of WCFE extract of *Cadaba fruticose*

Sr. No.	Compound Name	Uses
1	N-Hexadecenoic acid	• antioxidant, pesticide, antiandrogenic flavor, (Kumar *et al.*, 2010), • Anti-inflammatory (Aparna *et al.*, 2012)
2	Octadecanoic acid	• Anti-inflammatory (Othman *et al.*, 2015)
3	Pentadecanoic acid	• Antioxidant activity (Vijisaral Elezabeth *et al.*, 2014)
4	Tetradecanoic acid	• Antioxidant, cancer preventive, cosmetic, Hypercholesterolemic, nematicide, lubricant (Amutha Iswarya Devi *et al.*, 2014)

5	Dodecanoic acid	• Antimicrobial, anti-inflammatory (Dinesh Kumar *et al.*, 2016)
6	Oleic acid	• Anemiagenic, insectifuge, antiandrogenic, cancer preventive, dermatitigenic (Vijisaral Elezabeth *et al.*, 2014)
7	Erucic acid	• Antimicrobial activity (Arumugham Suresh *et al.*, 2014)
8	Cis-10-nonadecenoic acid	• Antitumor (Fukuzawa *et al.*, 2008)
9	6-octadecenoic acid, (z)-	• Cancer preventive, insectifuge (Vijisaral Elezabeth *et al.*, 2014)
10	9-Eicosene, (E)-	• Anti-microbial and cytotoxic properties (Dalli *et al.*, 2007)
11	3-Eicosene, (E)-	• Antibacterial properties (Vinay Kumar *et al.*, 2011)
12	Cis-vaccenic acid	• Cosmetics (Santhosh *et al.*, 2014)
13	Cis-9-hexadecenoic acid	• Flavoring agent (Santhosh *et al.*, 2014)
14	Trans-13-octadecenoic acid	• Anti-inflammatory and cancer preventive characters (Karthika Krishnamoorthy *et al.*, 2014)
15	Cis-9-hexadecenal	• Antimicrobial (Dinesh Kumar *et al.*, 2016)
16	Lupeol	• Anti-inflammatory (Geetha et al.,2001)

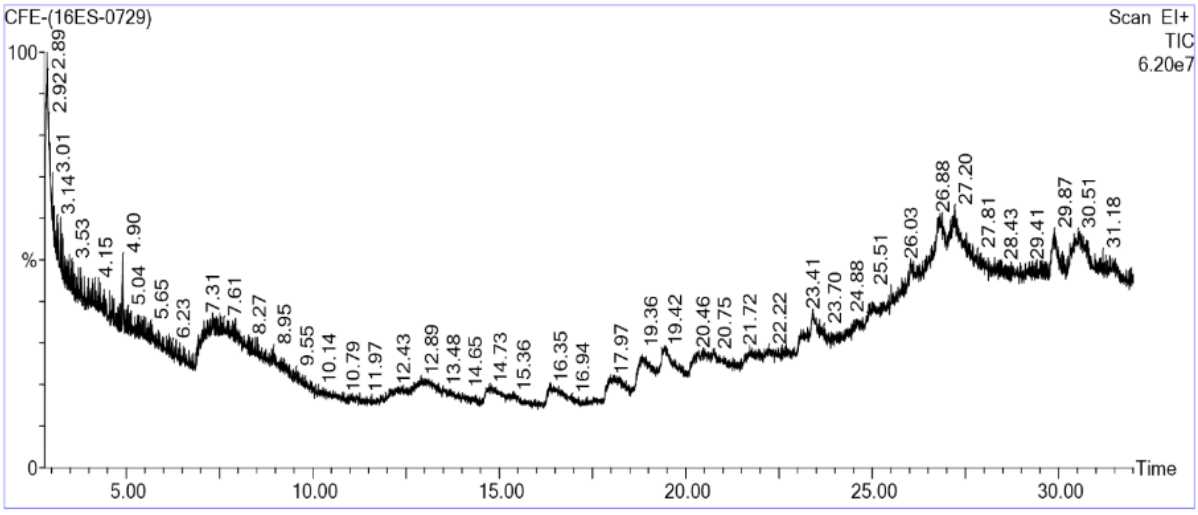


Figure 4: Gas chromatograph of MCFE extract of *Cadaba fruticosa*

Table 4: GCMS analysis of MCFE extract of *Cadaba fruticosa*

S. No	Compound name	Uses
1	Cyclotrisiloxane, hexamethyl-	• Antioxidant activity (Alok Prakash *et al.*, 2014)
2	Silicic acid, diethyl Bis (Trimethylsilyl) ester	• Antibacterial activity (Hema *et al.*, 2011).

Conclusion:

In conclusion, provides valuable information on the importance of preserving traditional knowledge about medicinal plants, focusing on *Cadaba fruticosa*. The plant's taxonomy, description, medicinal properties, and ethnomedicobotanical data are well-detailed. Additionally, the antimicrobial activity, antibacterial activity, cytotoxic effect, and antipyretic activity of *Cadaba fruticosa* are explored, backed by experimental methodologies and results. The inclusion of GCMS analysis further enhances the understanding of bioactive compounds present in the plant. Overall, the study highlights the potential therapeutic value of *Cadaba fruticosa* and emphasizes the need for conservation efforts.

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EXPLORING THE RICH CULINARY HERITAGE OF DISTRICT KINNAUR, HIMACHAL PRADESH

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Abstract:

Ethnobotany delves into the captivating connection between humans and plants, investigating how different societies have utilized plants across history for various purposes. Indigenous food plants are known to have a nutritive purpose and to offer health advantages. By virtue of their conventions, habits and traditions, these traditional food plants are those that rural or tribal societies embrace. These foods have been a staple diet that the people regard to be very healthful and well-liked. Documentation of such traditional knowledge is essential and necessary for the conservation and utilization of biological resources. The documentation exploring the rich culinary heritage of district Kinnaur of Himachal Pradesh will fully recognize the contribution of the local people who have been using this indigenous knowledge.

Keywords: Ethnobotany, Indigenous, Documentation, Traditional knowledge, Resources.

Introduction:

Ethnobotany delves into the captivating connection between humans and plants, investigating how different societies have utilized plants across history for various purposes (Jain, 2004). Over the past three decades, ethnobotany has gained popularity. Studies on ethnobotany have demonstrated its importance in the quest for novel herbal remedies, food and fodder, economic growth and for the preservation of cultural aspects and natural resources (Supriya *et al.*, 2022).

Indigenous food plants are known to have a nutritive purpose and to offer health advantages. In addition to being essential nutritional supplements, they are valuable sources of proteins, minerals, vitamins, and trace elements for those with limited resources. By virtue of their conventions, habits and traditions, these traditional food plants are those that rural or tribal societies embrace. People know how to prepare them for consumption because they have been accustomed to them since ancient times (Singh *et al.,* 2014). In tribal and hilly regions, a significant amount of the food consumed by the locals is made up of plants. The locals have amazing identification skills when it comes to these plants, as well as their products and

preparation. These traditional food plants meet cultural needs and are less harmful to the environment (Lata *et al.*, 2021).

Kinnaur is not impervious to the effects of the rise of industrialization as seen by the people growing interest in cash crops like apple and seasonal vegetable production. Few farmers are still growing these traditional crops and people are switching from growing them for personal use to commercial use. They ignore these wholesome food crops because they don't understand how important these traditional crops are (Savitri and Ballah, 2007). The majority of traditional foods are prepared and consumed on a regular schedule, but some are only prepared and consumed on special occasions like weddings and festivals. These foods have been a staple diet that the people regard to be very healthful and well-liked (Mehta *et al.*, 2010).

The knowledge about the wild edible plants has come from the age old experiences of ancient people and is transmitted orally from one generation to another. But the knowledge about these edible plants is declining due to various factors such as population migration to urban areas and declining of natural resources (Jhamta *et al.*, 2019). Thus documentation of such traditional knowledge is essential and necessary for the conservation and utilization of biological resources.

Study area

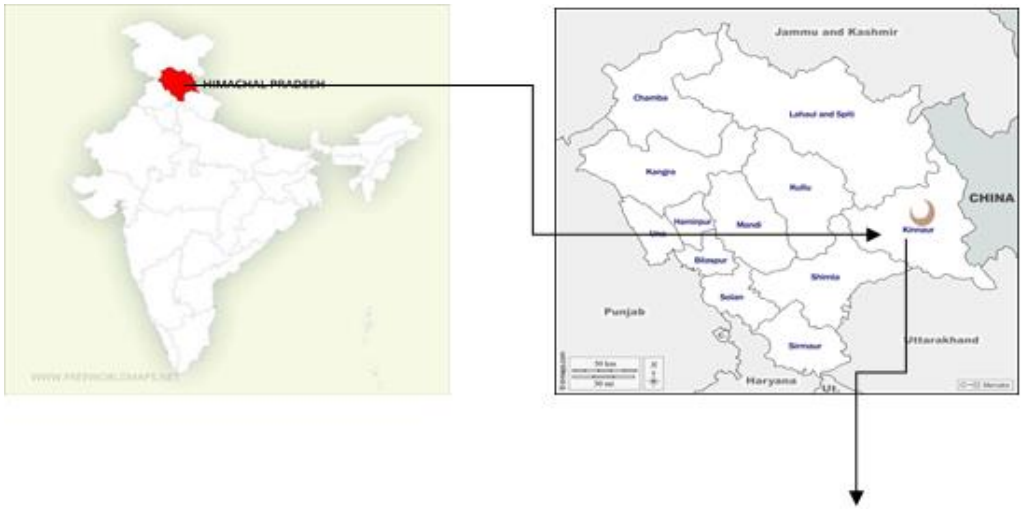

Figure 1: Location Map of Kinnaur District (Himachal Pradesh) (Source: https:d-maps.com)

The present study was undertaken in Kinnaur (30°22'40″ N to 33°12'40" N Latitude and 75°47'55" E to 79°04'20″ E Longitude), which spans over an area of over 6,400 km^2 in the western Himalaya. The district is bounded by Lahaul and Spiti district in the north, Kullu district in the northwest, Shimla district in the southwest, Uttarakhand state in the south and international border with China (Tibet) in the east. River Sutlej enters the region at an elevation of 3,050 m, and leaves it at an elevation of 1,320 m above mean sea level dividing the region into almost two equal halves.

Field study and data collection

Information on the traditional food plants of District Kinnaur was collected from 5 villages *i.e* Nichar, Chagaon, Bhaba, Bhabanagar and Jani. The personal observations, oral interviews, discussions with the villagers were the bases of collection of data about the uses of the plants (Jain and Rao, 1976).

They were assured that all information provided would be used only for education purposes and would be confidential. The interview was conducted in the local dialect ('kinnauri'), so as to facilitate communication to local people with low literacy. During field visits, when many plants were mentioned by the participants, where possible, the participant was encouraged to show a sample of the plant which was collected or photographed.

Traditional knowledge of plants is mainly transferred through oral means to younger generations. This documentation will fully recognize the contribution of the local people who have been using this indigenous knowledge.

Quantitative analysis of data:

Relative Frequency Citation (RFC):

Relative frequency citation was calculated by following Tardío and Pardo-De-Santayana, 2008. $RFC=FC/N*100$

Where FC=is the number of informants reporting the use of species divided by the total number of informants participating in the survey (N), without consideration of the use categories.

Use Value (UV):

Use value (UV) was calculated using the formula given below following Philips *et al.,* 1994: $UV = \sum U / N$

Where, 'U' is the number of plant uses cited for a given species by the informants and 'N' is the total number of informants quizzed.

Abbreviations: FC = Frequency of Citation, RFC = Relative Frequency of Citation and UV = Use Value.

Table1: Traditional Food Plants of District Kinnaur (Himachal Pradesh)

S.No.	Botanical name	Vern. Name	Family	Part/s Used	Ethnobotanical uses
1.	*Allium humile* Kunth	Shaudh	Alliaceae	Leaves	Leaves are used as vegetables.
2.	Amaranthus *caudatus* L.	Dhanker	Amaranthaceae	Seeds	Roasted seeds are consumed with milk to make the sweet dessert 'kheer' in marriages.
3	*Angelica glauca* Edgew	Safal	Amaranthaceae	Roots	Root powder is used as spices.
4	*Betula utilis* D.Don	Shag	Betulaceae	Bark	Powder of bark used for preparing 'Namkeen chai' in many festivals and ceremonies
5.	*Brassica juncea* (Linn.) Hook.f. & Thoms	Sarson	Brassicaceae	Leaves	Tender leaves are used as vegetables.
6.	*Bunium persicum (Boiss.) B. Fedtsch.*	Kala jeera	Apiaceae	Seeds	Seeds are used as a spice and as a condiment in marriages and temples for making 'puris' and 'chutney'.
7.	*Eleusine coracana (L.) Gaertn.*	Kodro	Poaceae	Seeds	Seed flour is used to prepare 'rottis' known as 'Khodoro Rotich', served with honey and ghee
8.	*Diplazium esculentum (Retz.) Sw*	Lemkut	Woodsiaceae	Leaves	Tender leaves are used as vegetable.
9.	*Fagopyrum esculentum Moench*	Olgo	Polygonaceae	Seeds	Seed flour used in preparation of 'rotti's' known as 'Ghashang hod' is served with any vegetable along with ghee and honey.
10.	*Fagopyrum tataricum (L.) Gaertn*	Bras	Polygonaceae	Seeds, Leaves	Seed flour is used in preparation of rottis locally known as 'Brasu hod'. Dried leaves are used as vegelable popularly known as 'Bras kan' served, with rice and apricot oil.

11.	*Glycine max (L.) Merr.*	Botang	Fabaceae	Seeds	Roasted seeds are eaten with dry fruits especially during "Bishu" festival.
12.	*Hordeum vulgare L.*	Chag	Poaceae	Seeds	Seed flour used in preparation of 'Sattu' locally called 'yudh' is eaten with salted tea and apricot oil.
13.	*Laportea terminalis Wight*	Chogya	Urticaceae	Leaves	Tender leaves are used as vegetables.
14.	*Pinus gerardiana Wall. ex D.Don*	Ree	Pinaceae	Seeds	The Chilgoza nuts are used for preparation of salted tea locally known as 'Namkeen cha'.
15.	*Prunus armeniaca L.*	Chuli	Rosaceae	Fruits	Fruits puree known as 'chulfhanting' is consumed empty stomach in the morning.

Table 2: Quantitative ethnobotanical parameters

S. No.	Botanical Name	FC	RFC	UV
1.	*Allium humile* Kunth	10	31.25	0.31
2.	*Amaranthus caudatus* L.	8	25.00	0.25
3.	*Angelica glauca* Edgew	6	18.75	0.18
4.	*Betula utilis* D.Don	11	34.37	0.34
5.	*Brassica juncea* (Linn.) Hook.f. & Thoms	13	40.62	0.40
6.	*Bunium persicum* (Boiss.) B.Fedtsch.	9	28.12	0.28
7.	*Eleusine coracana* (L.) Gaertn.	15	46.87	0.46
8.	*Diplazium esculentum* (Retz.) Sw	7	21.87	0.21
9.	*Fagopyrum esculentum* Moench	16	50.00	0.50
10.	*Fagopyrum tataricum* (L.) Gaertn	11	34.37	0.34
11.	*Glycine max* (L.) Merr.	19	59.37	0.59
12.	*Hordeum vulgare* L.	12	37.50	0.37
13.	*Laportea terminalis* Wight	8	25.00	0.25
14.	*Pinus gerardiana* Wall. ex D.Don	10	31.25	0.31
15.	*Prunus armeniaca* L.	11	34.37	0.34

Abbreviations: FC = Frequency of Citation, RFC = Relative Frequency of Citation and UV = Use Value.

Results and Discussion:

A total of 32 informants were interviewed with ages ranging from 50 to 80 years. Out of total, 22 were male and 10 were female. From the study area, a total of 15 plant species belonging to 12 families and 14 genera were collected for their traditional uses. All the documented plant species are indicated in Table 1 along with their botanical name, vernacular name, family, part used, ethnobotanical uses and the calculated quantitative indices *i.e.* Frequency of Citation, Relative Frequency of Citation and Use Value are mentioned in Table-2. The dominant plant families are Amaranthaceae, Poaceae and Polygonaceae (2spp). The remaining families contributed for single plant species. The most exploited plant parts were seeds (8 spp), followed by leaves (5 spp), roots, bark and fruits (1 spp each) as shown in (Fig. 2). Herbs (11spp) were used predominantly followed by trees (3spp) and one fern as shown in Fig. 3.

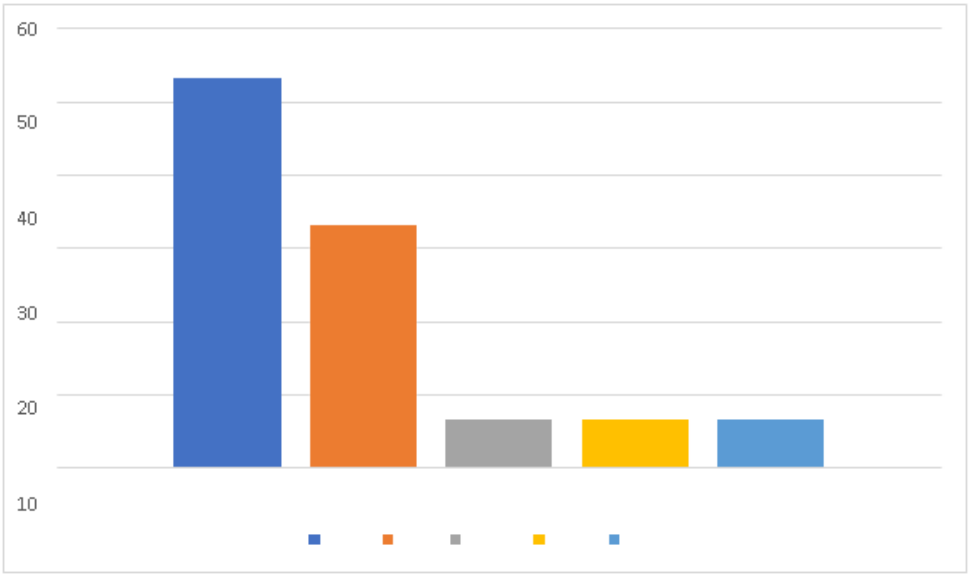

Figure 2: Catalog of plant parts used as traditional food

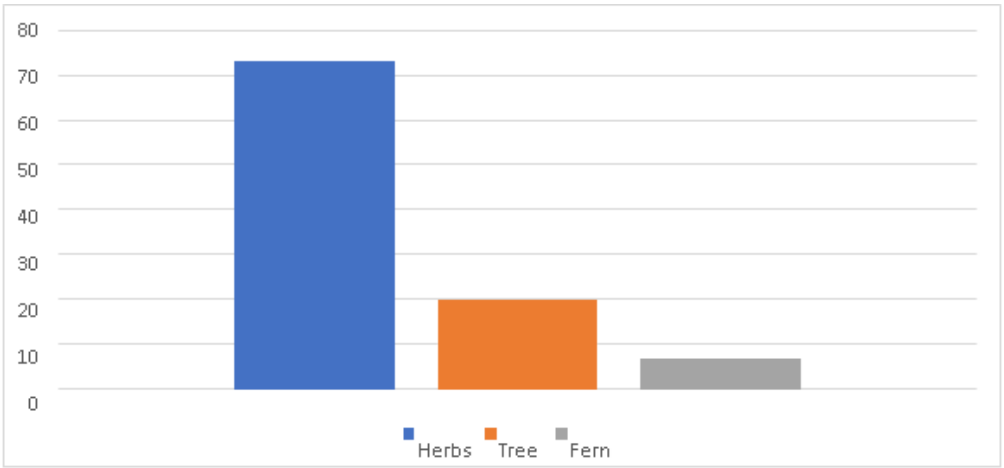

Figure 3: Habit wise distribution of traditional food plants

The relative frequency index shows that the highest relative frequency of citation was for *Glycine max* (59.37) followed by *Fagopyrum esculentum* (50.00), *Eleusine coracana* (46.87) and *Brassica juncea* (40.62). The lowest relative frequency of citation was calculated for *Angelica glauca* (18.75). Use value of plant was calculated following Phillips & Gentry, 1993 in order to assess the importance of plants in the studied area. A highest use value was recorded for *Glycine max* (0.59) followed by *Fagopyrum esculentum* (0.50) and *Eleusine coracana* (0.46). *Angelica glauca* attained the lowest use value of (0.18) (Table 2). It means that all the above mentioned plant species are most commonly and frequently used by the local rural populace of the region.

Conclusion:

The present study reveals that the tribal inhabitants of Kinnaur have a vast knowledge of traditional plants. This ethnobotanical study serves as a valuable resource for conservation efforts and the promotion of sustainable practices that honour the ancestral wisdom embedded in Kinnaur's traditional food culture. This extensive knowledge has been accumulated over generations through exploration and adaptations, becoming an intrinsic part of their cultural identity and daily life. This traditional knowledge is at risk of fading away as it's primarily retained by the elderly and traditional healers, lacking interest among the younger generation. Documenting this wealth of traditional knowledge not only validates the efficacy of these age-old practices but also highlights the importance of respecting and preserving the cultural heritage of these tribal communities for the benefit of future generations and the advancement of medical science.

Aknowledgement:

The authors are highly thankful to the people of Bhaba valley for sharing their valuable traditional knowledge and UGC-Delhi for providing financial assistance in the form of Junior Research Fellowship (JRF).

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THE PHARMACOLOGICAL EXPLORATION OF *JUSTICIA ADHATODA*

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Introduction:

Pharmacological exploration is a methodical examination and analysis of how different drugs and substances affect biological systems. This process includes assessing properties like therapeutic benefits, side effects, and interactions to gain a comprehensive understanding of how a substance interacts with the body, revealing its potential applications in the medical field.

India, known as the botanical garden of the world, leads in medicinal herb production. Plants synthesize diverse secondary metabolites with unique structures, offering applications in pharmaceuticals, insecticides, dyes, and more. *Justicia adhatoda*, a shrub found in India, particularly in the lower Himalayan regions, is referred to interchangeably as Vasaka or Malabar nut and is valued in traditional medicine for treating both chronic and infectious diseases. Widely esteemed in Indian traditional medicine, *Justicia adhatoda* is a valued medicinal plant used to treat respiratory conditions such as asthma, cough, bronchitis, and tuberculosis. Its flowers, leaves, and roots possess antispasmodic properties, with reported effectiveness against tuberculosis. The plant's leaves are a primary source for formulating the herbal drug 'vasaka,' renowned for its health benefits, especially in managing bronchitis. In the indigenous system of medicine, various parts of the plant are utilized for treating ailments like joint pain, cough, malaria, and venereal diseases (Gricilda Shoba *et al.*, 2015).

The current investigation involved the qualitative phytochemical characterization of extracts from *J. adhatoda* leaves, exploring their antimicrobial, antioxidant, and anti-inflammatory properties. Additionally, cytotoxicity was assessed through a brine shrimp lethality bioassay, and essential oils were extracted for antimicrobial evaluation. Extraction yields for water, methanol, ethanol, and n-hexane were 35.76%, 17.58%, 7.54%, and 2.6%, respectively. The extracts and essential oils exhibited promising antimicrobial effects against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas fluorescens. Inhibitory activity against soybean lipoxygenase mirrored that of the standard vanillin, with n-hexane > water > methanol > ethanol.

The lethality on brine shrimp nauplii followed the order: n-hexane > methanol > ethanol > water. These findings suggest the presence of bioactive metabolites, indicating potential for drug development studies (Anju Betty Jose *et al.*, 2023).

Figure 1: *Justicia adhatoda* flower with leaves

Scientific classification

Table 1: Scientific classification of Malabar nut

Kingdom	Plantae
Order	Lamiales
Family	Acanthaceae
Class	Magnoliopsida
Genus	Justicia
Species	*Justicia adhatoda*
Botanical Name	*Justicia adhatoda*

Botanical description

Justicia adhatoda, commonly known as Vasaka or Malabar nut, is a perennial shrub belonging to the Acanthaceae family. It typically grows up to 2-4 meters in height and has lance-shaped leaves with a serrated margin. The leaves are opposite, about 10-15 cm in length, and have a rough texture. The plant produces tubular, white to blue-violet flowers arranged in spikes. The fruits are small, capsule-like structures containing numerous tiny seeds. Known for its medicinal properties, *Justicia adhatoda* is often used in traditional medicine for respiratory ailments due to its bronchodilator and expectorant properties. Its stem is woody, and the plant is often found in tropical and subtropical regions.

The plant *Justicia adhatoda Linn*. It exhibits a wide range of therapeutic, it demonstrates antioxidant, hepatoprotective, sedative, antispasmodic, anthelmintic, antimicrobial, antidiabetic, and wound-healing properties. Furthermore, it shows activity against infertility, ulcers, and bacteria, as well as antihistaminic effects. The plant also displays moderate hypotensive and

thrombopoietic activities, along with cardiac depressant, uterotonic, and abortifacient effects. Notably, the leaves activate the digestive enzyme trypsin.

Vernacular names

Table 2: Vernacular names of Malabar nut

English	Malabar nut
Tamil	Adhathodai
Hindi	Arusha, Adusa, Bansa
Sanskrit	Vasaka
Telugu	Vasa, Addasaramu
Bengal	Adusa, Bakash,Vasok
Konkani	Adusoge
Gujarati	Araduso, Adaluso

Microscopical study

Analyzing *Justicia adhatoda* microscopically entails examining diverse plant tissues and structures under a microscope to comprehend their anatomical characteristics. Notably, the leaves showcase unique features, with the epidermis revealing a layer of cells containing stomata that facilitate gas exchange.

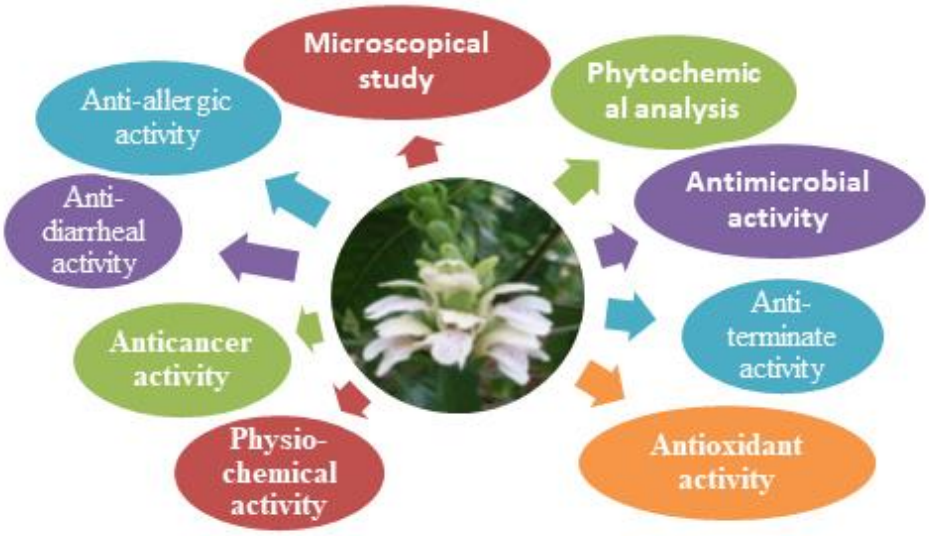

Figure 2: Activities of *Justicia adhatoda*

The mesophyll encompasses palisade and spongy parenchyma cells, essential for photosynthesis. The leaf's midrib exhibits observable vascular bundles, consisting of the xylem and phloem. Trichomes, both glandular and non-glandular, may be evident on the leaf surface. The study extends to the stem, exploring the arrangement of vascular tissues like collenchyma and sclerenchyma cells, which contribute to structural support.

Examination of *Justicia adhatoda* flowers unveils reproductive structures such as the pistil, stamen, and pollen grains. Similarly, observing root sections involves scrutiny of the endodermis, epicycle, and the organization of vascular tissues.

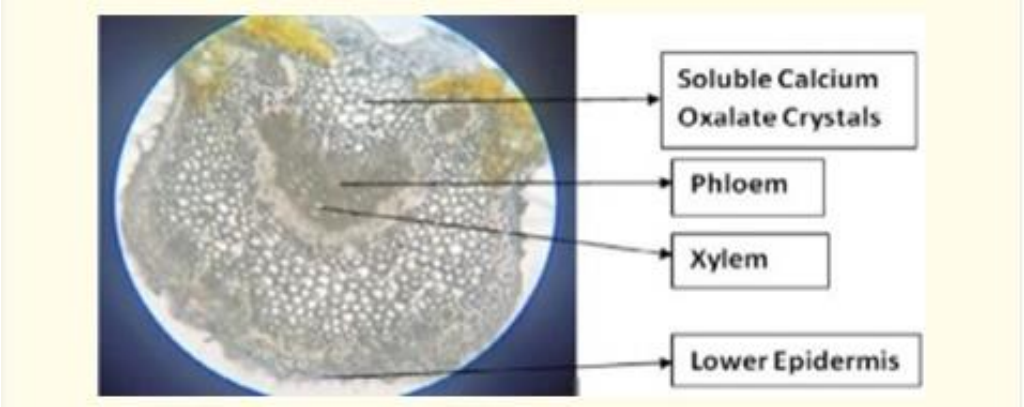

Figure 3: *Justicia adhatoda* leaves (Dukre *et al.,* 2023).

Phloem- Carries food downward from the leaves to the roots.

Xylem - Distribute the water and minerals from the roots.

Lower epidermis - Single layer of cells on the lower surface of the leaf

Table 3: Micro-chemical tests of *Justicia adhatoda* leaves

Test No	Test	Observation	Inferences
1.	Phloroglucinol + Conc. HCl (1:1).	Pink	Xylem, Phloem (V.B.).
2.	Sudan Red III	Red	Epidermis, Oil Globules
3.	Dil.Sulphuric Acid	Soluble	Calcium Oxalate Crystal
4.	Conc.Hydrochloric Acid	Soluble	Calcium Oxalate Crystal

Phytochemical analysis

Phytochemical analysis was carried out using a Camag HPTLC system, which included a sample applicator and a Camag TLC scanner operating at wavelengths 254nm and 366nm.

The data were filtered using Savitsky-Goyal 7. For sample application through the Camag automatic TLC sampler, three conditions were maintained: nitrogen (N2). as the spray gas, methanol as the sample solvent, and a filling speed of 15µl/second. Pre-coated silica gel 60G F254 TLC aluminum plates (10x10cm, 0.2mm thick).

Analytical grade toluene, ethyl acetate, methanol, chloroform, glacial acetic acid, diethyl amine, and formic acid. Table 4 provides an overview of the various phytochemicals screened along with the respective solvent system and derivatizing agent (Rahul Chavan *et al.,* 2014).

Table 4: List of phytochemicals, solvent systems, and derivatizing agents used in HPTLC analysis

Phytochemicals	Solvent system	Derivatizing agent
Alkaloids	Toluene: ethylacetate: diethylamine (7:2:1).	Draagendroffs reagent
Saponins	Chloroform: acetic acid:methanol: water (6.4:3.2:1.2:0.8).	Anisaldehyde, sulphuric acid
Flavanoids	Ethyl acetate: formic acid: glacial acetic acid: water (10:0.5:0.5:1.3).	Anisaldehyde solution
Tannins	Toluene: ethyl acetate: formic acid (6:4:0.3).	Dragendroffs reagent

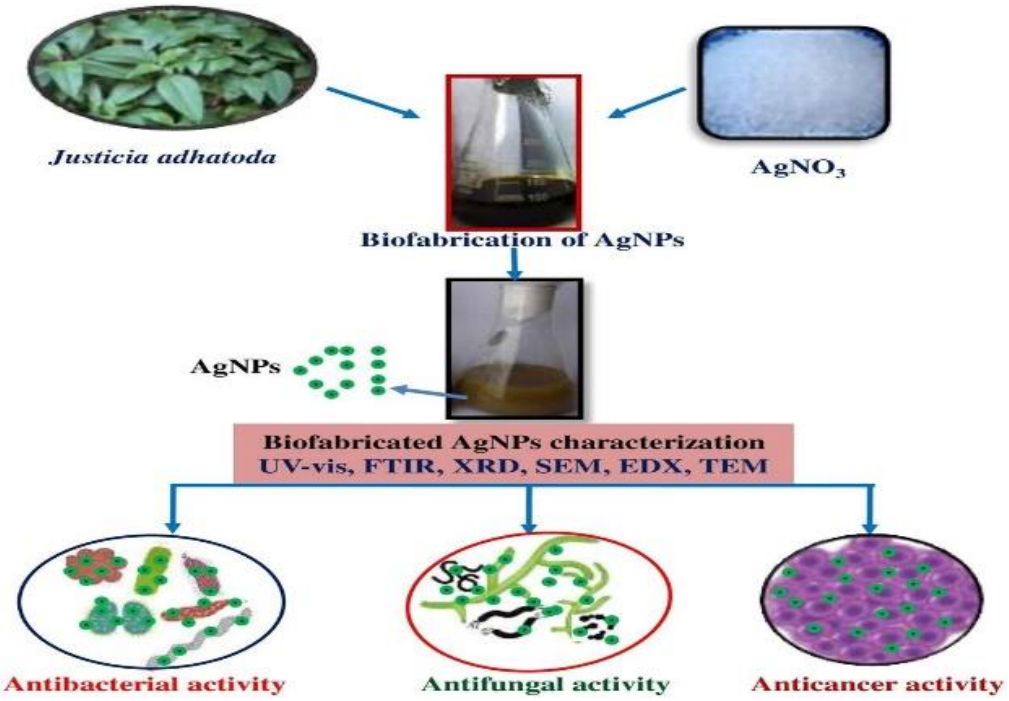

Figure 4: Biofabricated AgNPs characterization

Antimicrobial activity

The methods of *J. adhatoda* were investigated for their potential anti-bacterial and anti-fungal activities. The research utilized the following bacterial species*: Staphylococcus aureus, Pseudomonas aeruginosa, Morganella morganii, Methicillin-resistant Staphylococcus aureus*, and *Acinetobacter baumannii.* The antibacterial activity was conducted according to the method outlined by Ahmad *et al.* in 2009, utilizing sterile Nutrient agar and broth media for the antibacterial assays. Ciprofloxacin (10mcg/disc). and ketoconazole (10mcg/disc). exhibited effective inhibition against the tested microorganisms. The methanolic extract demonstrated notable activity against *Staphylococcus aureus, Klebsiella pneumonia*, and *Candida albicans,*

while no antibacterial activities were observed against *Pseudomonas aeruginosa* and *Proteus vulgaris.* The alcoholic extract from the roots and leaves of *Justicia adhatoda L*. exhibited antibacterial effects against both *Escherichia coli* and *Staphylococcus aureus*. In contrast, the water extract of the plant demonstrated antibacterial activity specifically against *Staphylococcus aureus* (Gricida Shoba *et al.*, 2015).

Table 5: Antimicrobial activity of *Justicia adathoda*

Micro-organisms	**Zone of inhibition(mm).**	
	Std.	**Std.**
Klebsiella pneumonia	24	18
Pseudomonas aeruginosa	23	NS
Proteus vulgaris	30	NS
Staphylococcus aureus	30	20
Escherichia coli	25	09
Candida albicans	22	14

- Indicates Bacterial Strains; -Indicates fungal strain;

Std. – Standard; ME – Methanol extract; NS – Not specified.

Anticancer activity

The cell line, sourced was cultured in Eagles Minimum Essential Medium supplemented with 10% fetal bovine serum (FBS). The cultivation conditions were rigorously controlled, maintaining a temperature of approximately 37°C, an atmosphere of 5% CO2, and 95% air, with 100% relative humidity to sustain optimal cell growth conditions. Cultures were carefully managed by weekly passaging, and the culture medium was refreshed twice each week to ensure the provision of essential nutrients and maintain the viability and health of the cell cultures. In the MTT assay, various parameters such as growth inhibition, cell viability, and morphological changes were assessed by comparing untreated control cells with those treated with different concentrations (18.75µg, 37.5µg, 75µg, 150µg, 300µg). of ethyl acetate extract obtained from *Justicia adathoda*.

The metabolic activity of *Justicia adathoda* led to a decreased cell viability, as evidenced by significant reductions in cell viability at concentrations of 75µg/ml and 150µg/ml, relative to various concentrations of the extracts. It's noteworthy that the percentage of viability exhibited a less pronounced effect compared to the robust growth inhibitory activity observed. The half-maximal inhibitory concentration (IC50). was determined to be 176µg/ml, indicating the concentration at which 50% inhibition of cell growth occurred. The growth inhibition

percentages of cells treated with different doses *of Justicia adathoda* are detailed values provided in Table 6. These findings collectively highlight the potential cytotoxic effects of the ethyl acetate extract of *Justicia adathoda*, indicating its capability to inhibit cell growth and reduce viability. Further exploration of these outcomes may contribute to understanding the extract's potential implications for therapeutic applications (Susmitha Sudevan *et al.*, 2019).

Table 5: Growth inhibition percentages of cells treated with different doses *of Justicia adathoda*

Conc (µg/ml).	**% Cell viability**
18.75	92.15
37.5	91.88
75	82.97
150	67.32
300	44.75

Physiochemical activity

Precisely measure an amount of the test sample equivalent to 2 to 4 g of the air-dried material and place it in a pre-weighed crucible. Proceed to incinerate the sample gradually, starting with gentle heating and gradually increasing the temperature to 675 ± 25 ºC, ensuring complete removal of carbon. Record the weight of the resulting ash.

Heat the ash, acquired as instructed in the Total Ash procedure above, by boiling it with 25 ml of 3 N hydrochloric acid for 5 minutes. Gather the insoluble components using a pre-weighed filtering crucible or an ash-less filter, wash them with hot water, then ignite and measure the weight. Calculate the percentage of acid-insoluble ash based on the initial weight of the drug.

Heat the ash, following the Total Ash instructions, by boiling it with 25 ml of water for 5 minutes. Gather the insoluble components in a sintered-glass crucible or on ash-less filter paper. Rinse with hot water and ignite for 15 minutes, ensuring the temperature does not exceed 450 ºC. Deduct the weight of this residue (in mg). obtained under Total Ash and compute the percentage of water-soluble ash relative to the sample's weight determined under Total Ash.

Disperse the sample in a thin layer and manually remove foreign organic matter as thoroughly as possible. Weigh the separated material and ascertain the percentage of foreign organic matter with the initial weight of the drug. Utilize the hot extraction method for moisture content under alcohol-soluble extractives, substituting water for alcohol, and apply the cold extraction method for alcohol-soluble extractives, replacing alcohol with water.

Table 7: Physicochemical Screening of *J. adhatoda* (Dukre *et al.*, 2023).

Parameters	Values
Total ash value	16.66%
Acid insoluble ash value	2.33%
Water soluble ash value	3.0%
Foreign organic matter	0.0%
Moisture content	12.30%
Water soluble extractive value	0.76gm
Alcohol soluble extractive value	0.24gm

Antioxidant activity

J. adhatoda's impact on antioxidants, including reduced glutathione (GSH)., Glutathione S-transferases (GST)., and catalase, was assessed in the context of Carrageenan-induced paw edema. The extracts' ability to scavenge free radicals was evaluated using 1, 1-diphenyl-2-picryl hydroxyl (DPPH). and was examined using spectrophotometric analysis. *J. adhatoda* was estimated using different in vitro assays viz. 2-2-diphenyl 1-picryl hydroxyl (DPPH). radical scavenging potential, Fe3+–Fe2+ transformation ability, reduction in cupric ions (Cu2+)., superoxide anion scavenging ability (Arvinder Kaur., *et al.*2015).

A stock solution of 10 mg extract in 1 ml methanol was prepared and further diluted into five concentrations (100-500 µg/ml). Similar dilutions were made for the ascorbic acid standard. Each concentration (1 ml). was mixed with freshly prepared DPPH solution and incubated for 10 minutes in the dark at room temperature, and the absorbance at 517 nm was measured to assess antioxidant activity (Ibrar Khan., *et al.*2018). The scavenging capacity was compared to a control (1 ml methanol + 2 ml DPPH)., and the percent scavenging activity was determined using the equation:

$$\text{Percent (\%). inhibition of DPPH activity} = ((Ab - As). / Ab).). \times 100$$

Where Ab is the absorbance of the control and as is the absorbance of the test sample. A concentration curve was plotted, and the EC50 was determined at the concentration where scavenging reached 50%.

Anti-terminate activity

Termites play a crucial role in recycling plant materials and enhancing soil aeration through their tunneling activities. They thrive in tropical and subtropical regions, yet their wood-destructive behavior poses significant economic challenges by damaging wooden products and infrastructure. The anti-termite activity of different fractions of *J. adhatoda*, including Crd. Met.

Ext (crude methanolic extract)., n-hexane, CHCl3, EtOAc, and aqueous fractions, were assessed against H. indicola. Results presented in Table 6 indicate the limited effectiveness of Crd. Met. Ext, requiring an extended two-day experiment. Notably, the n-hexane fraction took two days to eliminate all termites, with 7 termites dead on the first day and complete elimination on the second day. CHCl3 and EtOAc fractions achieved complete termite eradication within 24 hours. The aqueous fraction experiment spanned three days, resulting in an average of 6 termites dead on day 1, 9 on day 2, and none surviving by the third day. All experiments were conducted in triplicate for reliable results (Bashir Ahmad *et al*., 2018).

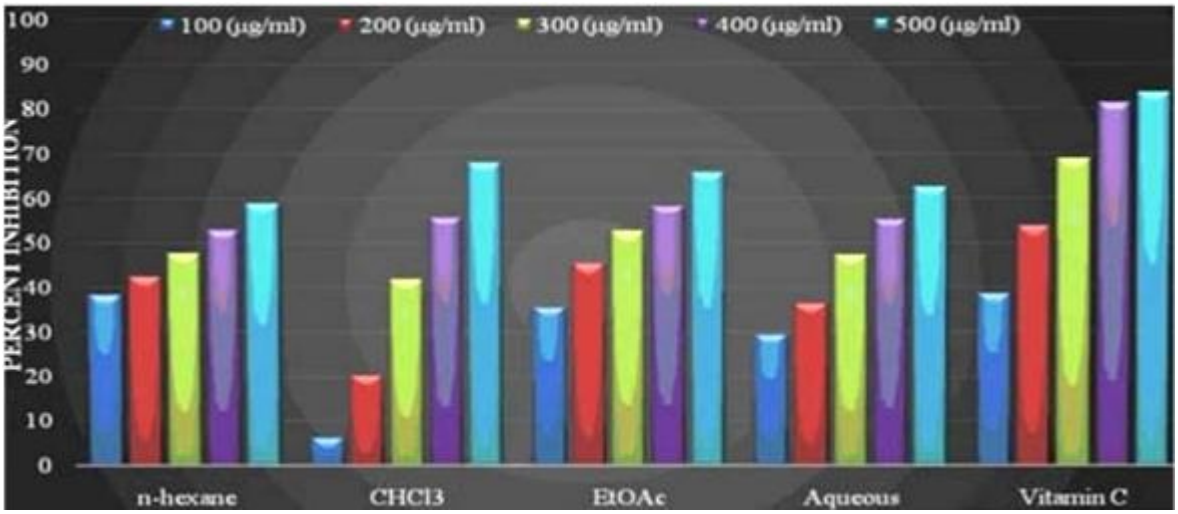

Figure 5: DPPH radical scavenging activity in various fractions of *J. adhatoda*

Table 8: Examining the effectiveness of Crd. Met. Ext and different fractions obtained from *Justicia adhatoda* against termite activity

Sample	No. of termites	Day	Average Termites killed
Crd. Met. Ext	10	1	9
		2	10
n-hexane		1	7
		2	10
$CHCl_3$		1	10
EtOAc		1	10
Aqueous		1	6
		2	9
		3	10

Anti-allergic activity

Compound 73/602 (AA)., a structural analog of vasicinone present in both the leaves and roots of *Justicia adhatoda* L., displays potent antiallergic properties in mice, rats, and guinea pigs. The study investigated the collaborative potential of combining *Justicia adhatoda* L. with *Glycyrrhiza glabra* and *Solanum xanthocarpum* to counteract egg albumin-induced degranulation and mast cell stabilization induced by compound 48/80. Mast cells, pivotal in generating inflammatory mediators leading to allergic responses, were effectively shielded by various combinations of extracts against compound 48/80 and egg albumin-induced degranulation in a dose-dependent manner. This underscores the synergistic effects of licorice extract when combined with *Justicia adhatoda* L. and *Solanum xanthocarpum*, providing noteworthy protection for mast cells (Mehak Jamwa *et al.*, 2022).

Table 9: Ethnopharmacological and therapeutic uses of *Justicia adhatoda* L.

Part used	Administration	Treatment
Whole plant	Decoction	Asthma, cough, bronchitis, fever, Joint pain, malaria, rheumatism, lumber pain, eczema
Whole plant	Preparation of syrup along with Tulsi and ginger	Bleeding piles
Leaves	Mature leaves fried in mustard oil	Indigestion, cold, cough
Leaves	Chewing of leaf buds alone or in combination with ginger root	Clearing of the respiratory passage
Leaves	Various preparations	Bleeding hemorrhage, wounds, skin diseases, headache
Leaves	Powder, decoction, juice	Relieve acidity, antipyretic
Leaves	Decoction, infusion, poultice	Rheumatic and painful inflammatory swellings
Roots	Paste, powder, decoction	Malarial fever, diphtheria, tuberculosis
Flowers	Various preparations	Phthisis, bronchitis, asthma, cough, cold, gonorrhea, antiseptic
Fruits	Various preparations	Cold, jaundice, dysentery, diarrhea

Anti-diarrheal activity

In this study, the researchers aimed to investigate the anti-diarrheal potential of the methanolic extract obtained from the roots of *Justicia adhatoda* L. The assessment was

conducted on Swiss albino mice of both sexes, and diarrhea was induced using castor oil. The choice of castor oil as an inducer is a common method in experimental setups to induce diarrhea in rodents. The results revealed that *Justicia adhatoda* L. exhibited a 29.31% inhibition in defecation. In the context of this study, inhibition of defecation is considered a positive outcome, indicating a reduction in the frequency or severity of diarrhea. The comparison with the standard drug loperamide, which is a well-known anti-diarrheal medication, showed that loperamide achieved a more significant inhibition of 58.62%. The findings suggest that *Justicia adhatoda* L. possesses noteworthy anti-diarrheal properties, although its efficacy appears to be slightly lower when compared to the established standard, loperamide. It's important to note that such studies contribute to our understanding of the potential therapeutic applications of natural extracts and herbs, in this case, *Justicia adhatoda* L., in managing conditions like diarrhea. Further research and exploration may be warranted to delve into the mechanisms behind this anti-diarrheal activity and optimize its potential use in clinical or preventive settings (Muhammad Gulfraz *et al*., 2011).

Conclusion:

In conclusion, the comprehensive pharmacological exploration of *Justicia adhatoda* highlights its multifaceted medicinal properties, paving the way for further research and development in the field of herbal medicine and drug discovery. The plant's rich phytochemical profile and diverse pharmacological activities underscore its significance in traditional and modern healthcare systems. The major biochemicals present in *Justicia adhatoda* L. build up its strong pharmacological profile viz., Physio-chemical activity, Microscopical study, Anti-allergic activity, Anti-diarrheal activity, Antimicrobial activity, Anti-terminate activity, Anticancer activity, Antioxidant activity.

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IN-VITRO CULTURE TECHNIQUES OF PLANT CELLS

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Introduction:

The conventional breeding methods are the most widely used for crop movement. During the last two decades plant cell, tissue and organ culture have developed rapidly and become a major biotechnological tool in agriculture, horticulture, forestry and industry. But in certain situations, these methods have to be supplemented with plant tissue culture techniques either to increase their efficiency or to be able to achieve the objective, which is not possible through the conventional methods. Those problem which is not feasible through conventional techniques, now have been solved via these techniques for example, inter- and intra- specific crosses, micropropagation, somaclonal variation, encapsulated seeds etc. Production of pure lines or inbreeds involves six to seven generation of selfing. Production of haploids through distant crosses or using pollen, anther or ovary culture, followed by chromosome doubling, reduces this to two generation. This represents saving of 4 to 6 years. The other example is the transfer of useful bacterial gene say, cry (crystal protein) gene from Bacillius thuringeinsis, into a plant cell and ultimately, regeneration of whole plant containing and expressing this gene (transgenic plant). This can be achieved only combination of tissue culture and genetic engineering; none of the conventional breeding approaches can ever produce such a plant.

The term tissue culture is commonly used in a very wide sense to include in vitro culture of plant cell, tissues as well as organs. But in strict sense, tissue culture denotes the in vitro cultivation of plant cell in an unorganized mass. E.g., callus culture. Another term cell culture is used for in vitro culture of a single or relatively small group of plant cell. E.g., suspension cultures. But in general, the term tissue culture is applied to both callus and suspension cultures, and cell cultureis often used for callus culture as well. When organized structures like root tips, shoot tips, embryos etc. are culture In vitro to obtain their development as organized structures, it is called organ cutlture.

History of tissue culture techniques

Historically Henri –Louis Duhamel du Moncaeu (1756) pioneered the experiments on wound healing in plants through spontaneous callus (unorganized mass of cells) formation on decorticated region of elm plants. But the science of cell and tissue culture could be advanced after propounding the cell theory by Schleiden and Schwan (1839). Trecul (1853) observed callus formation in a no. of plants. Vochting (1878) suggested the presence of polarity as a key feature that guide the developed of fragments. He observed that the upper portion of a piece of a stem always produced buds and the basal region produced callus or roots.

During 19th century the idea of development of callus from isolated stem fragments and root apices came into existence. Callus could also be developed from buds, and root and shoot fragments of about 1.5mm in size without using nutrient medium.

The term 'Tissue Culture' can be applied to any multicellular culture growing on a solid medium (or attached to substratum and nurtured with a liquid medium) that consist of many cells in protoplasmic continuity. But in organ culture (e.g., excised roots) the cultured plant material vitro of the parent plants (Doods and Robers, 1985).

When fragments of callus are transferred into a liquid medium and aerated on a shaker, it gives a suspension of a single and aggregate of cells. A cell can be propagated by subculturing . Muir(1953) developed a successful technique for the single isolated cells which is commonly known as paper- raft nurse technique (placing a single cell on filter paper kept on an actively growing nurse tissue). Later on, attempts were also made for single cell culture by humping drop and agar plate method. During this period phenomenon of totipotency was fully developed by demonstrating that a single isolated cell can divide and regenerate a whole plant (Vasil and Hilderbrant, 1965).

In 1952, the Pfizer Inc., New York (USA) got the US Patent and started producing industrially the secondary metabolites of plants. The first commercial production of a neutral product **shikonin** by cell suspension culture was obtained.

In India work on tissue culture was started during mid 1950s at the Dept of Botany (university of Delhi) by Panchanan Maheshwari who is regarded as **Father of Embrology** in India. Different tissue culture methodologies were involved for morphogenesis studies involving ovary, embryo, endosperm ovules, etc. At the University of Delhi, Sipra Guha Mukherjee and S.C. Maheshwari (1964-67) for the first time developed the haploid through anther and pollen culture. Discovery of haploid production was a land mark in the development of plant tissue culture.

The in vitro technique were developed initially to demonstrate the totipotency of plant cell predicted by Haberlandt in 1902. Totipotency is the ability of plant cell to perform all the

functions of development, which are characteristics of zygote, i.e., its ability to develop into a complete plant. In 1902, Haberlandt reported culture of isolated single palisade cells from leaves in knop's salt solution enriched with sucrose. The cells remain alive for up to one Month, increased in size, accumulated starch, but failed to divide. Efforts to demonstrate totipotency lead to the development of techniques for cultivation of plant cells under defined condition. This was made possible by the brilliant contributions from R.J. Gautheret in France and P.R. white in USA during the third and the fourth decades of 28th century. Most of the modern tissue culture medium have been derived from the work of skoog and co-workers during 1950s and 1960s.

The first embryo culture although crude was carried out by Hanning in 1904; he cultured nearly mature embryos of certain crucifers and grew them to maturity. The technique was utilised by Laibach in 1925, to recover hybrid progeny from an interspecific cross in linum. subsequently, contributions from several workers have lead to the considerable refinements in of this technique.

Haploid plants from Pollen grain were first produced by Maheshwari and Guha in 1964 by culturing the anthers of Datura. This marked the beginning of anther culture or pollen culture for the production of haploid plants.The technique has been further developed by many workers, more notably by G.P. Nitch and co-workers. These workers showed that isolated microspores of tobacco produce complete plants.

Plant protoplast are naked cells from which cell wall has been removed. In 1960 cocking produced large quantities of protoplasts by using cell wall degrading enzymes. The techniques of protoplast production have now been considerablely refined. It is now possible to regenerate whole plant from protoplast and also to fuse protoplasts of different plants species. In 1972 Carlson and Coworkers produced the first somatic hybrid plant by fusing the protoplast of Nicotiana glauca and longsdorfii. Since then many divergent somatic hybrids have been produced.

Principle and scope

The explant inoculated over culture media are can undergo either mere cell division form to form an unorganised mass of cell called callus are the cells me divide and differentiate to a specific cell type. It is possible to divert the weight of the explant as per our need by manipulating the culture condition to form either or a differentiated organ. Sales in calories are least specialized and relatively Homogeneous. thus they can be used to study the process of cell differentiation. The cell wall of these cells is thin and cellulosic (parenchymatas). Although totipotency is deemed to the inherent property of the living cell; but it is expressed only under specific conditions. In this plant growth regulators (phytoharmones) play an important role. More

precisely auxin and cytokinin (both endogenous and exogenous) are the major determinants during organogenesis.

The culture system are designed to promote cell growth and their differentiation before becoming quiescent. Tissue cultures frequently arise from apparently quiescent cells. the senescent cells show no de - differentiation. The fate of quiescent cell is dependent upon the degree of autolytic activity (degradative process of the cell initiated by the hydrolytic enzyme in the cell. The hydrolytic enzymes are present in the vacuoles, lysosomes, spherosomes and space between plasma membrane and cell wall. If the enzymes are abundantly present it leads to callusing. However if these enzymes are meagre it leads to xylogenesis.

The cyto differentiation of plant cells is not irreversible and hence the differentiated cell of explant can be induced to de differentiate rejuvenation of mature cells for proliferation and also to redifferentiate and can form cells of cambial type. Such cells can be brought into primary meristematic state. Young companion cells perivascular elements medullary ray parenchyma and secretary cells can be readily stimulated for division rather than the pith parenchyma. Moreover these cells have potential for organogenesis. This they develop organs like roots, buds etc. Similarly young lignified cells like fibres thick walled collenchymatous cells and immature tracheary & sieve elements can be partially de- differentiated. However vascular elements with walls of more than 2 micron thickness have only potential of histogenesis. During histogenesis typically cell types like fibres, sieve tubes and vessels are formed in some and product biosynthesis and ofcourse plant genetic engineering.

The advancement made in a cell and tissue culture technology are due to the development in composition of culture media. Based on the success of plant culture techniques many recent advances have been done in the area of propagation, production of secondary metabolites and pathogen – free plants, genetic transformation and production of transgenic plants.

Requirement for in vitro cultures

Laboratory space

In general, space for the following is needed:

1) Washing, drying and storage of vessels
2) Preparation, sterilization and storage of media
3) Asceptic handling of explants and cultures
4) Maintenance of cultures
5) Observation of cultures

In the modest laboratory provisions for activities 1 and 2 can be made in a single room. While the remaining work can be done in another room. In such situation, the following precaution should be taken.

1) The washing area should be physically separated.
2) The weighing balances should be kept in a separate enclosed space.
3) Autoclave, refridgerator, deep freeze and incubators may be commonly used.

Culture room

The culture room should have following facilities:

1) Controlled temperature, with the help of airconditioners and room heaters.
2) Culture rack fitted with light generated by fluroscent tubes.
3) A shaker for agitation of liquid culture.

Sterilization

All the materials, e.g., vessels, instruments, medium, plant material, etc., used in a culture work must be free from microbes. This is achieved by following approaches:

i) Dry heat
ii) Flame sterilization
iii) Autoclaving
iv) Filter sterilization
v) Wiping with 70% ethanol
vi) Surface sterilization

Nutrient medium

Virtually all tissue culture media are synthetic or chemically defined; only a few of them use complex organics, e.g., potato extract, as their normal constituent. A synthetic medium consists of only chemically defined compounds.

Inorganic nutrients: In addition to C, H, and O all media provide the 2 elements essential for plant growth, viz., N, P, k, Ca, S, Mg (these are called Micronutrients, and are needed in concentrations >0.5 mM) Fe, Zn, Mn, Cu, B and Mo (these are knows as micronutrients, and are required in concentrations < 0..5 mM). The different tissue culture media provide different concentration of the inorganic nutrients, the White's medium being rather poor, and B5 and Ms media being quite rich, especially for and N. The media also provide Na and Cl bit their necessity is not established. Generally iron is provided as iron. EDTA complex to keep it available at higher (>5.8) pH. Nitrate is superior to ammonium as the sole N source, but use of NH+ checks the drifts of pH towards alkalinity.

Vitamins: For optium callus growth, the following vitamins are required; inositol, thiamine, pyridoxine and nicotinic acid of which thiamine is essential and the rest are promotory.

Carbon source: Sucrose (20 – 50g) is the most commonly used carbon source for all cultured plant matrials, including even green shoots.

Growth regulators: The following growth regulators are used in plant tissue culture. **Auxins**, e.g., IAA (indole acetic acid, IBA (indole butyric acid), NAA (napthalene acetic acid), NOA (napthoxy acetic acid), 2 4 D(2, 4- dichlorophenoxy acetic acid) etc., are commonly used to support cell division and callus growth . **Cytokinins** like kinetin (furfuryl amino purine), BAP (benzyl amino purine), Zeatin, ip (isopopentenyl adenine), TDZ (thidiazuron, a compound having cytokinin activity) are employed to to promote cell division, regenetation of shoots, to enhance proliferation and growth of axial buds. Abcsisic acid (ABA) promotes SE and shoot bud regeneration in many species and markedly improves SE maturation. GA3 is almost exclusively used; it promotes shoot elongation and SE germination.

Complex organic additives: Complex additives like yeast extract, coconut milk, casein hydrolysate, corn milk, malt extract and tomato juice were used to support plant tissue growth.

Amino acid: Although nitrogen sources are present in the inorganic salts, yet various amino acids and amides are used in plant tissue culture media as given by Huang and Murashige (1977). The most widely used amino acids are L- asparagin, L- glutamic acid, L-gutamine and L-arginine.

Solidify agent: Most commonly agar (obtained from red alga, Gelidium amansii) is used as a solidifying agent.

pH: Optimum pH between 5.0 to 6.0 is required for growth and development of cultured tisues. Therefore optimum pH of the medium should be maintained before sterilization of the medium.

Callus induction

Callus culture

Callus is an unorganized mass of cells growing over solid media. It is parenchymatous tissue formed by vigorous proliferation by cell division showing no polarity. The callus culture starts with an explant like embryonal tissues, fleshy storage organs, shoot buds, apical meristems, pith, segments of root and stem, floral parts, young inflorescences, nucellus, vascular cambia of tress, root pericycle, petiole, leaf mesophyll, endosperm, mesophyll, ovular tissues, seedling hypocotyl, immature embryos, leaves, cotyledons, pollen, epidermis, fern sporeling, nodal region etc. Quiescent types of parenchyma and the cells in meristem, cambia and embryonic tissues are ‘undetermined’ and can be manipulated to switch over to different pathways of development depending upon the microenvironment in which they grow. Thus, they can be de-differentiated to produce callus.

A callus mass may show differences in its texture and physical properties. While some are hard and compact, others are friable. Such friable calli are suitable for suspension cultures. A compact callus may be made friable but not vice versa. High concentrations of yeast extract and 2,4-D in the medium induce friability in pea callus. There is anatomical difference between a

compact and friable callus. While friable callus has several meristematic centres distributed over its surface; the compact callus has enlarged, vacuolated and compactly arranged cells. Further, the cells of compact callus are high in total cell wall polysaccharide content with more pectic substances and hemicelluloses as compared to friable callus. In a callus individual cells or groups of cells of smaller dimensions may form small nests of tissue scattered throughout the callus. These are called as 'meristemoids', which may form centres of further cell proliferation. However, the regenerative capacity of each callus differs. A callus culture may appear homogenous but portions of the callus may segregate to form a cell mass differing in morphology and pigmentation from rest of the tissue. Not only this the calli may differ in terms of synthesis and storage of various compounds like alkaloids, anthocyanins, naphthaquinones etc.

Procedure of callus initiation

The explant to be used for callus initiation has be initially surface sterilized to remove the microflora present on its surface. The sterilizing agent to be used depends on the sensitivity of the material and its degree of contamination. The commonly used sterilants are 2% sodium hypochlorite, 0.1% mercuric chloride, 70% alcohol etc. The time period of treatment with these sterilants varies again with the sensitivity of the material and its degree of contamination. The size of explant is also important as explants of size lesser than critical size may not respond. Explant consisting of uniform parenchyma cells may not be beneficial for callus initiation. It is observed that explant consisting of several types of cells, particularly vascular tissues, are favourable for callusing. A well-defined medium is preferred for callus initiation over semi-synthetic medium containing extracts of plants. However, in each case suitable alterations can be done to achieve the desired results. Mostly, these alterations related to change in PGR concentration and type, which varies from species to species.

Cell growth

The callus development occurs through three sequential processes viz. induction, regressive change (cell division) and differentiation. The induction phase is characterized by changes in cell size, cell structure and metabolic state. Cell divisions are confined to the peripheral cell layers of the explant. This results in decrease on cell size and whole of the outer region de-differentiate and revert to a meristematic state. This region encloses a core of non-dividing tissues within. The initiation of cell division may occur as a result of a number of interacting factors like, leptohormones (wound hormones), other endogenous substances, availability of nutrients and conditions of incubation in a basal medium with sucrose alone.

Subculture

The callus needs to be transferred to fresh medium after a particular period. The process of transferring the callus from old medium to freshly prepared medium is called as 'subculturing'. The period after which callus is subcultured depends on the rate of callus growth. Usually the callus is subcultured after 3 to 6 weeks. Subculturing is necessary as with the growth of callus the nutrients in the medium are depleted, toxic products of metabolism are accumulated and the media dries up. The callus may cease to grow over such medium and eventually die. For subculturing the callus is aseptically split into small pieces of 2-3cm. and inoculated over the fresh medium. The callus may be preserved for short or long durations. The subculturing of callus for long shows cytological changes and selection pressure to a dominant cell type. Genetic instability of its cells is reflected in changes in ploidy level and chromosomal abnormalities. The heteroploidy in pea root callus has been shown to be caused form exogenous plant extracts, yeast extract and 2,4-D present in the culture medium.

Cell suspension culture

Cell suspension is prepared by transferring a fragment callus (about 500 mg) to the liquid medium (500 ml) and agitating them aseptically to make them cells free. It is difficult to have suspension of single cell. However, the suspension includes single cell, cell aggregates (varied number of cells), residual inoculum and dead cells (Dodds and Roberts, 1985). King (1980) has described that a good and suspension consists of a high proportion of single cells the small cluster of cells. It is more difficult to have a good suspension than to find optimum environmental factors for cell separation. King and Street (1977) described the techniques of cell separation by changing the nutritional composition of medium. No standard technique for separation of cells from callus has to be recommended. When cells are transferred into a suitable medium they divide after lag phase cell division) and linearly increases their population. After some time, based on rate of cell division decelerates until it comes to stationary phase.

At this stage to keep the cells viable, it is essential to subculture the cells. By using plate technique, cell line over medium. Further, growth of nutrient level, depends on cell density. Street (1977) suggested that cell density should be determined before subculturing Grow of culture depends on a critical cell density below which culture will not grow. Cell culture systems have been employed in numerous morphological analyses by varying the origin of cell and physicochemical factors (White, 1963; Street, 1973).

Kurz and Constabel (1979) have described the properties of cultured plant cell suspensions in common with culture of microorganisms as (1) they grow in sterile environment, (ii) they homogeneous in size, (iii) they have a doubling time which is longer than that of microorganisms but considerably shorter than cells in situ, and (iv) they can be grown on a large

scale. The cell suspension cultures are used: (i) induction of somatic embryos and shoots, (ii) in vivo mutagenesis and selection of mutants, (iii) genetic transformation studies, (iv) production of secondary metabolites.

Benifits from cell culture:

Cell suspension cultures have many advantages over callus cultures as below:

(i) The suspension can be pipette.

(ii) They are less heterogeneous and cell differentiation is less pronounced.

(iii) They can be cultured in volumes upto 1,500 litres.

(iv) They can be subjected to more stringent environmental controls.

(v) They can be manipulated for production of natural products by feeding precursors (Ku and Constabel, 1979).

Somatic embryogenesis

A somatic embryo (SE) is an embryo derived from a somatic cell, other than zygote, and obtained usually on culture of the somatic cells in vitro; this process is known as somatic embryogenesis contrast, embryos developing from zygotes are called zygotic embryos or often simply embryo or, who those derived from pollen are known as pollen embryos or androgenetic embryos. By 1978, some embryogenesis was reported from 80 species belonging to 33 families; the list has expand considerably since then (over 100 species by 1993).

Developmental pattern

SEs generally originate from single cells, which divide form a group of meristematic cells. Usually, this multicellular group becomes isolated by breaking cytoplasmic connections with the other cells around it and subsequently by cutinization of the outer walls of this differentiating cell mass. The cells of this meristematic mass continue to divide to give rise to globular (round ball shaped), heart-shaped, torpedo and cotyeledonary stages of SEs.

Somatic embryos are bipolar structures in that they have a radicle and a plumule. The radicular end is always oriented toward the centre of callus or cell mass, while the plumular end always sticks out from the cell mass. SEs often show abnormal developmental features, e.g., three or more cotyledons, bell-shape cotyledon, larger embryo size, etc.; these problems are often overcome by the presence of ABA or suitable concentration of mannitol. The SEs regenerating from an explant or a callus are termed as primary somatic embryos. many cases, SEs regenerate from the tissues of other SEs or the parts of germinating SEs; such SEs are secondary somatic embryos. Ordinarily SEs originate from cells at the surface of callus.

Growth regulators

In most species an auxin (generally 2, 4-D at 0.5-5 mg/l) is essential for somatic embryogenesis. The auxin causes dedifferentiation of a proportion of cells of the explant, which

begin to divide. In carrot, these small, compact cells divide asymmetrically, and their daughter stick together to produce cell masses called proembryogenic masses or embryogenic clumps. In the presence of auxin, the ECs grow and break up into smaller cell masses, which again produce ECs. But when the auxin is either removed or reduced (0.01-0.1 mg/l) and cell density is lowered, each EC gives rise to few to several SEs; each SE is believed to develop from a single superficial cell of the EC. The ability to regenerate SEs, ie., totipotency, is acquired by cells during differentiation in response to the high auxin treatment, but the precise mechanism is not well known. Auxin at high concentration prevents its own polar transport. Auxins also promote hypermethylation DNA, which may have a role in totipotency acquisition. ln many species like carrot, coffee; alfalfa, etc., somatic embryogenesis is a two step process: (i) SE induction occurs on high auxin (upto 40-60 mg/l 2, 4-D), and (ii) SE development takes place low auxin or GR-free medium. In the SE induction phase, explant cells dedifferentiate, became totipotent and, in many species, form embryogenic clumps (ECs). In different cell lines of carrot, ECS develope to different stages (from BCs only to globular stage SEs) on the induction medium before reverting to the EC stage. The cell masses from the EC stage to the globular stage appear to be insensitive to auxin, and they do not synthesize auxin. The globular stage SEs, however, become sensitive to and synthesize auxin. Cells can be maintained in embryogenic stage on the induction medium for prolonged periods (over 10 years in the case of carrot).

Organogenesis

Root, shoot and leaves (but not embryo) are the organs that are induced in plant tissue culture. Since embryo is an independent structure and does not have vascular supply, it is not supposed to be the plant organ. Organogenesis (i.e. development of organs) starts with stimulation caused by the chemicals of medium, substances carried over from the original explants and endogenous compounds produced by the culture (Thomas and Davey, 1975).

Skoog (1944) for the first time indicated that the organogenesis could be chemically controlled. He observed root initiation (rhizogenesis) and shoot inhibition (caulogenesis) after addition of auxin to the medium. Further, Skoog and co-workers gave the concept of regulation of organogenesis by a balance between cytokinin and auxin. Skoog and Miller (1957) demonstrated that a high ratio of auxin: cytokinin stimulated the formation of root in tobacco callus, but a low ratio of the same induced shoot formation. The hypothesis of organogenesis was advanced by Torrey (1966) who propounded that organogenesis in callus starts with the development of a group meristematic cells i.e. meristemoids that can respond to the factors within the system to initiate primordium which, depending on kinds of factors, induces either root, shoot or embryoid.

Somatic hybridization

Production of hybrid plants through the fusion of protoplasts of two different plant species/varieties is called somatic hybridization and such hybrids are known as somatic hybrids. The technique of somatic hybridization involves the following four steps: (i) isolation of protoplasts, (i) fusion of the protoplasts of desired species/varieties, (iii) selection of hybrid cells, and (iv) culture of the hybrid tells and regeneration of hybrid plants from them.

Protoplast isolation

Isolation of protoplasts is readily achieved by treating cells/tissues with a suitable mixture of cell wall degrading enzymes. Usually, a mixture of pectinase or macerozyme (0.1-1.9%) and cellulase (1-2%) is appropriate for most plant materials. Hemicellulase may be necessary for some tissues. Generally, crude commercial preparations of enzymes are used. The pH of enzyme solution is adjusted between 4.7 and 8.0 and the temperature is kept at 25-30°C. The osmotic concentration of enzyme mixture and of subsequent media is elevated (usually, by adding 500-800 m mol 1^{-1} sorbit mannitol) to stabilize the protoplasts and to prevent them from bursting. Usually, 50-100 m mol 1^{-1} CaC_2, is added to the osmoticum as it improves plasma membrane stability. The cells and tissues are incubated in the enzyme mixture for few to several (generally, 16-18) hours; naked protoplast devoid of cell wall are gradually released in the enzyme mixture.

Protoplasts have been isolated from virtually all plant parts, but leaf mesophyll is the men preferred tissue, at least in case of dicots, for this purpose. In general, fully expanded leaves surface-sterilized, their lower epidermis is peeled off with a pair of forceps and the peeled areas are cut into small (Ca. 1 cm^2) pieces with a scalpel and suspended in the enzyme mixture. When epiderm cannot be peeled, leaf may be cut into Ca. I mm^2 pieces and treated with the enzyme mixture vacuum infiltration may be used to facilitate the entry of enzymes into the tissues. After the period of incubation, protoplasts are washed with a suitable washing medium in order to remove the enzymes and the debris. The protoplasts may be cultured in a suitable medium in a variety of ways: (i) Bergmann's plating technique (in agar medium), (ii) in a thin layer of liquid medium or (ii) in small microdrops of 50-100 ul. Protoplasts readily regenerate cell wall (within 2-4 days) and undergo mitosis to form macroscopic colonies, which can be induced to regenerate whole plants. The conditions for isolation and culture of protoplasts and regeneration of complete plants has been standardized a large number of plant species, but cereals still present some problems.

Generally, MS and BS media, and their modifications are used for protoplast culture. The media are supplemented with a suitable osmoticum and, almost always, with an auxin and a cytokinin, their concentrations depending on the plant species. After 7 – 10 days of culture, protoplasts regenerate cell wall, and the osmolarity of medium is gradually reduced to that of

normal medium. The macroscopic colonies are transferred onto normal tissue culture media. Protoplasts are very sensitive to light ; therefore they are cultured in diffuse light or dark for the first 4 – 7 days.

Protoplast fusion

The techniques for protoplast fusion are pretty well refined and highly effective for almost all the systems. Protoplasts of desired strains/species are mixed in almost equal proportion; generally, they are mixed while still suspended in the enzyme mixture. The protoplast mixture is then subjected to high PH (10.5) and high Ca^{2+} concentration (50 m mol 1-^{1}) at 37°C for about 30 min. This technique is quite suitable for some species, while for some others it may be toxic.

Polyethylene glycol (PEG) induced protoplast fusion is the most commonly used as it induces reproducible high frequency fusion accompanied with low toxicity to most cell types. The protoplast mixture is treated with 28-50% PEG (MW 1,500-6,000) for 15-30 min, followed by gradual washing of the protoplasts to remove PEG; protoplast fusion occurs during the washing. The washing medium may be alkaline (pH 9-10) and contain a high Ca^{2+}ion concentration (50 m mol 1^{-1}); this approach is combination of PEG and high pH high Ca^{2+} treatments, and is usually more effective than either treatment alone. PEG is negatively charged and may bind to cation like Ca, which in turn may bind to the negatively charged molecules present in plasma lemma; they can also bind to cationic molecules of plasma membrane. During the washing process PEG molecule may pull out the plasma lemma bound to them. This would disturb plasma lemma organisation and may lead to fusion of protoplasts located close to each other.

Cybrids

Cybrids or cytoplasmic hybrids are cells or plants containing nucleus of one species but from both the parental species. They are produced in variable frequencies in normal protoplast fusion experiment due to one of the following: (i) fusion of a normal protoplast of one species with an enucleated protoplast or a protoplast having an inactivated nucleus of the species, (ii) elimination of the nucleus of one species from a normal heterokaryon, or (iii) gradual elimination of the chromosomes of one species from a hybrid cell during the subsequent mitotic division . cybrids may be produced in relatively high frequency by (i) irradiating (with X-rays or gamma rays) the protoplasts of one species prior to fusion in order to inactivate their nuclei, or (ii) by preparing enucleate protoplast (cytoplasts) of one species and fusing them with normal protoplasts of the other species.

The objective of cybrid production is to combine the cytoplasmic genes of one species with the nuclear and cytoplasmic genes of another species. But the mitotic segregation of

plasmagenes, evidenced by the distribution of chloroplasts, leads to the recovery of plants having plasmagenes of one or the other species only; only a small proportion of the plants remain 'cybrid', which would further segregate into the two parental types.

Cybrids provide the following unique opportunities: (i) transfer of plasmagenes of one species into the nuclear background of another species in a single generation, and even in (ii) sexually incompatible combinations, (iii) recovery of recombinants between the parental mitochondrial or chloroplast DNAs (genomes), and (iv) production of a wide variety of combinations of the parental and recombinant chloroplasts with the parental or recombinant mitochondria. When cybrids are produced by irradiating the protoplasts of one species prior to fusion, they provide the additional opportunity for (v) the recovery of chromosome segment introgressions from the lost genome in combination with variations in the plasmon. The cybrid approach has been used for the transfer of cytoplasmic male sterility from N. tabucum to N. sylvestris, from P. hybrida to P. axillaris, etc. (vi) In addition, mitochondria from one parental species may be combined with the chloroplasts of the other parental species.

Cryopreservation

Plant tissues and on organ can be frozen and stored in liquid nitrogen (LN) at -196°C for long-term storage of germplasm. This would be of great value in the conservation of germplasm of those crops, which normally do not produce seeds, e. g., root and tuber crops, produce recalcitrant seeds, or where it may not be desirable to store seeds. The preservation of cells, tissues and organs in liquid nitrogen is called cryopreservation, and the science pertaining to this activity is known as cryobiology. Many studies have been carried out on cryopreservation of plant cells and organs, and the approach appears have considerable promise in germplasm conservation.

At-196°C, since at this temperature, all metabolic processes and growth are suppressed, and the occurrence of genetic, karyotypic, morphological and biochemical changes are also prevented. Cryopreservation has proved to be the most reliable method for long-term preservation of cell cultures. Calli, cell suspensions, protoplasts, pollen shoot-tips and embryos have all been successfully preserved, A serious drawback of the technique is that a general protocol applicable to all species and explants is not available. In addition, survival tends to decline with storage period in most of the cases, most likely due to injuries sustained by cells during the freezing step.

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THE REVIEW OF INDIAN HERB '*CATHARANTHUS ROSEUS*' EFFECTIVENESS IN CANCER TREATMENT

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Abstract:

Cancer is one of the main challenge diseases which has been recorded as second largest cause of death. Though there are several therapies to treat but there are severe adversities and pain like chemotherapy. Other alternatives for these adversities gives the lot of side effects. More interestingly the natural products can give the effective alternatives in the long usage. *Catharanthus roseus* is a medicinal plant provides anticancer activities distributed in many countries. It has attracted due to their high range of phytochemicals with various biological activities such as anticancer, antimicrobial, antibiotic, antibacterial etc. Importantly, Vinblastine and vincristine isolated from this plant which were the anticancer agent used in clinical purpose. Hence *Catharanthus roseus* has numerous pharmacological impacts. It has revealed that *Catharanthus roseus* contains more than 70 different types of alkaloids and chemotherapeutic agents that are effective in treating various types of cancers, breast cancer, lung cancer, uterine cancer, melanomas, and Hodgkin's and non-Hodgkin's lymphoma**.** The plant (roots, shoots, and leaves) extracts the plant parts extricates are being utilized against a few infections like loose bowels, Alzheimer's illness, asthma, hacks, throat infections etc. This study reveals about the *C. roseus* their bioactive components how effectively against the cancer and other usage of C. roseus.

Introduction:

India has a wide and interesting variety of different bio diversity of beneficial plant sources that are still yet to be investigated totally. Because of the cost and higher optional impacts connected with the misleadingly manufactured prescriptions, the necessity for novel medication stuffs out by the plant achieved an uncommon notice in the current investigation. As per the research *c. roseus* can effectively against the cancer. Our aim is to review the research about the *Catharanthus roseus* and people should know about the effectiveness of this plant. According to the Indian Council of Indian Research (ICMR) one in nine Indians develop cancer during lifetime. In India reported the highest number in the year. The reports states that the one in 68 men will develop lung cancer and one in 29 women will develop breast cancer. With

around 2.10 lakh new cancer cases in 2023. Cancer can be defined as a mode of cell death which results in erasing healthy cells from the normal tissues and losing their function. The apoptosis are induced by oncogenic factors/mutations which facilitates the uncontrolled cell proliferation or tumour initiation (metastasis). More surprisingly, the treatment of tumour with cytotoxic anticancer agents has been reported as inducer of malignant cells. The most used therapy to treat cancer is exploited by using chemotherapy which indeed has been observed with apostatic cell death on nearby subset of tissues that suggested as contributing toxicity across normal cells. There are different types of cancer treatment to treat the patients such as Surgery, chemotherapy, Radiation therapy, Targeted therapy, Immunotherapy, Stem cell or Bone Marrow Transplant, Hormone therapy. Therefore, most of the cancer treatment was done by the targeted therapy, anticancer agents are now in use developed by empirical screen designed to identify the tumour cells to kill. Amongst various form of cancer, breast cancer is progressively increasing which has been correlated with involvement of certain types of food such as rich animal fat. At present, there has just been a restricted information presence for the cytotoxic impacts of organically combined separates towards particular human breast cancer cells.

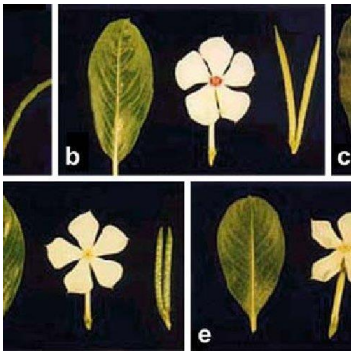

Figure 1: Parts of croesus

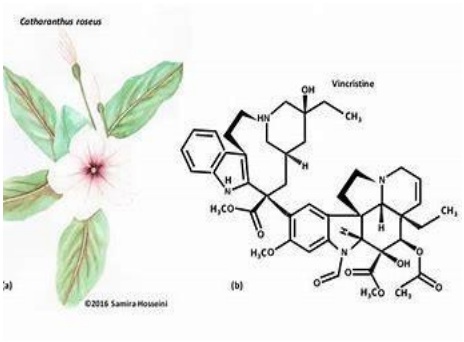

Figure 2: Vincristine in croesus

We have made an investigation of the *C. roseus* plant for its capacity to biosynthesize the methanolic leaf extractions. A major aim of this study is to assess the cytotoxic impact of biosynthesized methanolic leaf extracts against cancer cell lines. Our gathering has endeavoured to investigate the bio genic synthesis of methanolic leaf extracts from *C. roseus* plants for biomedical practices. With such an objective, an undertaking has been to join the methanolic extracts exploiting usually existing ordinary things by a applying the C. roseus plant leaves. The leaves of *C. roseus* show supportive and automatic activities because of the occurrence of pharmacological and phytochemical activities [15, 43]. Consequently, current examination shows antimicrobial and apoptotic exercises of the isolated *C. roesus* plant leaves extractions (Carpel) against the bacterial strains - *Escherichia coli* - ATCC 8739, and Staphylococcus aureus - ATCC 6538; Aspergillus Niger - ATCC 6275 and Candida albicans - ATCC 10231. Further, the cytotoxic impacts of a methanolic method of the *C. roseus* plant leaf extractions (Carpel)

were tried against MDA-MB-231 cell lines. The current examination opens an innovative opening to utilize this strategy for an eco-approachable for the planning and biomedical applications of Carpel. And also, Researchers investigated that *C. roseus* contain alkaloid that has potential uses in cancer treatment. More than 400 alkaloid present the plant which are used by pharmaceuticals, flavours, agrochemicals, fragrance, food additives, pesticides etc..,

Taxonomy

Scientific classification

Botanical Name(s): Vinca Rosea (*Catharanthus roseus*)

Family Name: Polynucleate

Kingdom: Plantae

Division- Magnoliophyte (Flowering plants)

Class- Magnoliopsida (Dicotyledons)

Order- Gentian ales

Family- *Apocynaceae*

Genus- Catharanthus

Species: *Catharanthus roseus*

Bioactive components

Products from plants – phytochemicals – provide simple, eco-friendly, low-cost, fast, and less toxic remedies when compared to conventional treatment methods [1]. Many plants in the Apocynaceae family have been used for medicinal purposes, for example, *Catharanthus roseus* and Vinca minor [2]. These plants are linked to a number of beneficial characteristics, such as anti-diabetics, bactericides, and antihypertensive activities. In *C. roseus* contains various bioactive phytochemicals such as phenols, flavonoids, aldehyde, fatty acids, ketones and indole alkaloids. The main point of interest is focused on the terpenoid indole alkaloids, of which more than 130 have been discovered. The bioactive components perform various activities.

Alkaloids: *C. roseus* is renowned for its alkaloid content. Alkaloids are nitrogen-containing organic compounds that play significant roles in various biological activities. Notable alkaloids found in *C. roseus* include vinblastine and vincristine, which exhibit potent anticancer properties.

Phenols: Phenolic compounds are abundant in *C. roseus*. These include catechin and caffeic acid, which contribute to antioxidant and antimicrobial effects.

Flavonoids: Flavonoids are another group of bioactive compounds present in this plant. They have diverse functions, including antidiarrheal, antidiabetic, and antioxidant activities.

Indole alkaloids: *C. roseus* contains indole alkaloids such as ajmalicine, serpentine, and reserpine. These compounds have been studied for their potential in treating various health conditions.

Fatty acids: Although less discussed, fatty acids are essential components of *C. roseus.* They contribute to overall plant health.

C. roseus has been found to contain a range of alkaloids possessing anticancer activity including vinblastine, vincristine, vindoline, vinzolidines, indolizine, vindoline and vindogentianine. They mainly inhibit the cell proliferation through the microtubular dynamics that is give the fascinating structure within the cell that exhibit highly dynamic behaviour. Of these, vinblastine and vincristine were the first plant-derived anticancer agents deployed for clinical use. This plant has been found to possess a number of important bioactive components that greatly contribute to the herbal medicine industry; however their amounts present in the plant are often low.

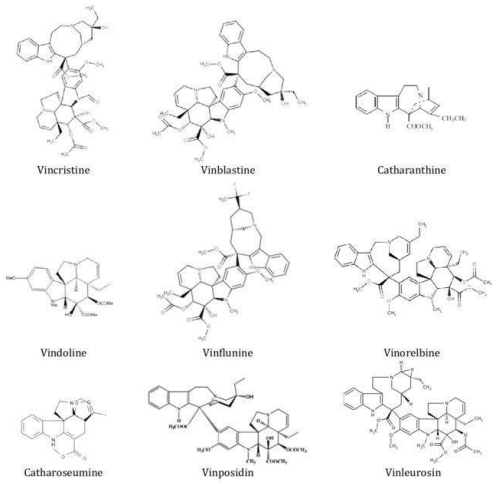

Figure 3: Bioactive alkolids

Materials and Methods:

The leaves or other parts of *C. roseus* were collected. The leaves were dried under shade and grind well. For Microwave Assited Extraction (MAE) method, take 1g of powder with 10ml of solvent and also added 0.1ml of HCl were closed with polyethylene bag to avoid solvent evporation. The time ranges should be 30 to 90 sec. Ethanol and water wre used seperatly for extraction of alkaloid. The extract was placed in the pre-weighed each time and placed under placed for drying. Dry extract was placed in refrigerated at 4℃. Then the 30g of leaves powder was loaded in a soxhlet appartus, it can be done carefully to ensure the maximum possible contant with plant an solvent. The time of extraction should be 5 to 15hrs, ethonal was used for the extraction. The final extraction was done by rotary evaporater and used pre-weighter to dry completely. Extracted was weighed accurately in each process.

Several phytochemical compounds obtained from *C. roseus* using advances technology to develop anti-cancer property (Table 1).

Table 1: Phytochemicals extracts and fractions obtained from *C. roseus*.

S.No	Bioactive phytochemicals	Extraction / Determination method	References
1.	Vindoline, vinblastine, catharanthine,	vincristine Ultraviolet-c treated CMCs extraction	Moon *et al.*, 2018
2.	Vincristine, vinblastine, ajmalicine, atharanthine, serpentine, vindoline	Ultra HPLC-quadrupole time-of-flight (UPLC-Q-TOF) mass spectrometry method	Jeong *et al.*, 2018
3.	Vinblastine, vincristine	Modular transcriptional control of MIA (monoterpoid indole alkaloid) biosynthesis	Schweizer *et al.*, 2018
4.	Vincristine, vinblastine, vindesine, ajmalicine, ajmaline, reserpine, vindoline	Ultra HPLC – tandem mass spectrometry	Kumar *et al*.,2018
5.	Vinblastine, vincristine.,	Yeast extract elicitation	Maqsood *et al*., 2017
6.	Catharanthine, vinblastine, vincristine, vindoline	HPLC and qualification reverse – transcription polymerase chain reaction qtr.-PCR ultraviolet-C	Moon *et al*., 2017
7.	Vincristine, vinblastine, Vindoline, catharanthine, yohimbine	HPLC with diode array detector (HPLC-DAD)	Liu *et al.*, 2016
8.	Catharanthine and vindoline	Centrifugal partition chromatography	Kotlin *et al.*, 2016
9.	Chaconne, dimeric indole alkaloids	Nuclear factor kappa-light chain-enhancer of activated B cells($NFKB$) and c-Jun N terminal kinase (JNK), HPLC	Wang *et al.*, 2016
10.	Ajmalicine, Serpentine, Catharanthine, vindoline, vindoline, vincristine, vinblastine, anhydro vinblastine	HPLC –DAD method simultaneous analysis of terpenoid indole alkaloids (TIAs)	Pan *et al.*, 2016
11.	Vincristine, vinblastine and catharanthine	Agilent HPLC, C18 column, HPLC with UV	Hanafy *et al.*, 2016
12.	Vindoline, vindolidine, vindolinine, vindolicine, serpentine and perivine	NMR, MS, UV and IR	Tiong *et al.*, 2015

Phytochemical screening of antibacterial and antifungal activity

Antibacterial activity, the plant extracts were prepared in dimethyl sulfoxide (DMSO). The methanol extract of *C. roseus* showed antibacterial activity against S. aureus, B. subtilis, P. aeruginosa, s. typhi. It showed maximum inhibition zones. Among different concentration, 20µl.,1:1 & 1:2 of plant extracts of *C. roseus* tested. The highest inhibition zones occurs in *S. aureus, B. subtilis, S. typhi*. The maximum inhibitory effect in terms of the area of the zone of inhibition was S. aureus 1:1 (20mm), *B. subtilis* 1:1 (19mm), *S. typhi* 1:2 (19mm) in the extract of *C. roseus*. The growth inhibition exhibited by plant extract and DMSO. The ethyl acetate extract of *C. roseus* does not showed antibacterial activity against *S. aureus, B. subtilis, P. aeruginosa, S. typhi*.

Antifungal activity, the plant extracts prepared in dimethyl sulfoxide (DMSO). The methanol extract of *C. roseus* showed antifungal activity against *C. albicans*. It showed maximum inhibition zones. Among different concentration, 20µl.,1:1 & 1:2 of plant exracts of C. roseus tested. The growth inhibition exhibited by plant extract and DMSO. The ethyl acetate extract of *C. roseus* does not showed antifungal activity against *C. albicans*. The maximum area of the inhibition was 1:1 (18mm) in the extract of C. roseus against *C. albicans*.

Phytochemical screening test of *C. roseus* extracts such as tanin, saponins, alkaloids, protein, terpeniods, carbohydrates. Besides, alkaloid contains high amount. But steroid was completely absent in both solvent. Tannin is present in both extracts. In saponins the methanol is positive. In protein the ethyl acetate is positive. In carbohydrates both are positive.

Table 2: Phytochemical screening

Plant Extracts	Methanol	Ethyl Acetate
Test for alkloids	Positive	Positive
Test for Protien	Negative	Positive
Test for tannin	Positive	Positive
Test for Saponins	positive	Negative
Test for terponids	Positive	Negative
Test for steroids	Negative	Negative
Test for carbohydrates	Positive	Positive

Table 3: Antibacterial activity of methano extracts

Organism	Extracts	Zone of Inhibition	
		1:1	**1:2**
S. aureus	Methanol	20mm	19mm
P. aeruginosa	Methanol	13mm	15mm
B. subtilis	Methanol	19mm	15mm
S. typhi	Methanol	15mm	19mm

Table 4: Antifungal activity of methanol extracts

Organism	Extract	Zone of Inhibition	
		1:1	**1:2**
C. albicans	Methanol	18mm	14mm

Anticancer activity

Catharanthus roseus contains more than 120 terpenoid indole alkaloids (TIA's). A number of alkaloids are isolated from these plants for its medicinal use. Some of them are ajmalacinean anti-hypertensive alkaloid, and vincristine and vinblas-tine – the antineoplastic bisindole alkaloids. The leaves of *Catharanthus roseus* are used as an anticancer agent. The leaves are about 3cm oval and oblong. The anticancer compounds present in the plant makes it a lifesaver. Vinorelbine is active against treatment of breast cancer.

The anticancer activity of alkaloids vinblastine and vinocristine were derived from stem and leaves. These alkaloids had growth inhibition effect of some human tumur Vinblastine was use experimentally for treatment of neoplasmas and is recommended for Hodgkins disease, chorio carcinoma. Vincristine another alkaloids is used for leukemia in children. Different percentage of the methanolic crude extracts of *Catharanthus* was found to show the significant anticancer activity against numerous cell types in the in vitro condition and especially greatest activity was found against the multidrug resistant tumor types. Vinblastine is sold as Velban or Vincristine as oncovin.

Along with the studies on anticancer activity of individual alkaloids from *C. roseus*, effect of the entire crude extract on various cancer cell lines was also investigated. Recent findings found that *C. roseus* root and stem extract possessed strong in vitro cytotoxic activity against a panel of cancer cell lines. Vinflunine is one of the vinca alkaloids.

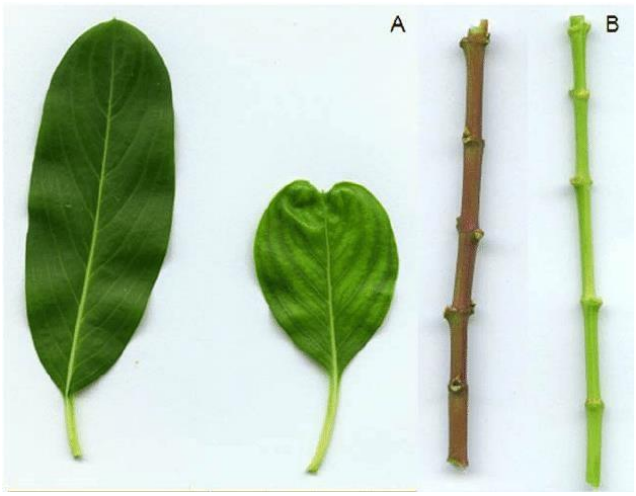

Figure 4: Leaf and stem of *C. roseus*

It has been studied for its effects on centromere dynamics:

At concentrations close to the IC50 (concentration inhibiting 50% of cell growth), vinflunine reduces centromere dynamicity by 44% and increases the time centromeres spend in a paused state by 63%.

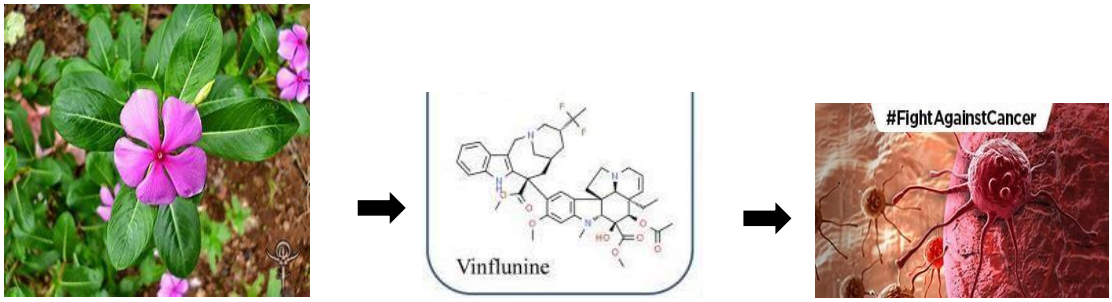

Figure 5: Anticancer activity of *C. roseus*

Other uses of *C. roseus*

Wound healing property

Rats treated with 100 mg /kg/day of the *Catharanthus roseus* ethanol extract had high rate of wound contraction significantly decreased epithelization period, significant increase in dry weight and hydroxyproline content of the granulation tissue when compared with the controls. Wound contraction together with increased tensile strength and hydroxyproline content support the use of *C. roseus* in the management of wound healing.

Anti-ulcer property

Vincamine and Vindoline alkaloids of the plant showed anti-ulcer property. The alkaloid vincamine, present in the plant leaves shows cerebrovasodilatory and neuroprotective activity. The plant leaves proved for anti-ulcer activity against experimentally induced gastric damage in rats.

Anti-helminthic activity

Helminthes infections are the chronic illness, affecting human beings and cattle. *Catharanthus roseus* was found to be used from the traditional period as an anti-helminthic agent. The anti-helminthic property of *C. roseus* has been evaluated by using *Phreesia posthuman* as an experimental model and with Piperazine citrate as the standard reference.

The ethanolic extract of the concentration of 250 mg/ml was found to show the significant anti helminthic activity.

Anti- diabetic agent

The juice from the leaf of *C. roseus* was reported to produce a dose-dependent reduction in blood glucose of both normal and diabetic rabbits. The whole Plant C. roseus methanolic extract displayed effective antihyperglycemic activity, correlating with improvement in body weight, lipid profile regeneration of β-cell of the pancreas in diabetic rats, with development of Vindoline, Vinzolidines, Vinzolidine, and Vindoline isolated from *C. roseus*

Conclusion:

C. roseus can be considered as a rich source of alkaloids and phenolics, which possess diverse biological properties, which possess diverse biological properties including anticancer, antidiabetic, antioxidant, antimicrobial and antihypertensive actives. Importantly, there leaf and stem play the major role in cancer treatment. Phytochemical screening of antifungal and antibacterial property was well explained with methanol extract. And also, *C. roseus* gives the properties such as wound healing, anti-ulcer, anti-diabetic, anti-helminthic etc...

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A REVIEW ON PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) AS BIOINOCULANTS FOR SUSTAINABLE AGRICULTURE

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Abstract:

Sustainable agriculture is crucially important in today's world because it offers the potential to meet the future agricultural demands. The use of plant growth-promoting rhizobacteria (PGPR) as bio-inoculants increases the availability of nutrient elements in soil, minimize the use of chemical fertilizers, reduces environmental pollution, and promotes sustainable agriculture. The use of PGPR is a potential alternative and promising technique compared to the old routinely used techniques that increases the burden of pollution to the soil in agriculture. PGPRs by various mechanisms protect plants from diseases and also help plants to grow healthy under different environmental stresses. In this chapter, some of the most important mechanisms and processes by which PGPR enhances the availability and uptake of nutrient elements by plant are reviewed.

Keywords: Bioinoculant, PGPR, sustainable agriculture, environmental stress.

Introduction:

The twenty-first century has been witnessing critical issues in global agroecosystems, leading to decreased productivity and degeneration of sustainable agroecosystem along with rapid growth in human population. The over use of chemical fertilizers could lead to soil acidification, nutritional imbalance, deterioration of the rhizosphere micro-ecological environment, water and air pollution.

The shrinkage of land, depletion of non-renewable resources and climate change due to increased human activities causes a threat to global food security and life subsistence. In the present circumstance, sustainable agriculture is essential as it offers the capacity to meet not only our present needs but also ensure a healthy future (Santoyo *et al.*, 2017).

Over the last decades, the global demand for food products has increased tremendously (Elferink and Schierhorn 2016). Global food demand is predicted to enhance by 59–98% by 2050. In developing nations, food demand is also increasing, where the expansion of crop lands

resources is limited. In this scenario, for enhancing food production from existing land is hard to contribute to meet such an essential requirement (Bargaz *et al.* 2018). In order to address this problem, there is a need to enhance agricultural production sustainably through the use of efficient agro-bioresources, whereas soil microbial diversity can play an important role and also help to mitigate many problems associated with the soil fertility, abiotic stress, insect pests, and diseases (Tilman *et al.* 2011; Utuk and Daniel 2015; Timmusk *et al.* 2017).

Plant Growth Promoting Rhizobacteria (PGPR):

Plant growth promoting rhizobacteria are the soil bacteria that inhabit around or on the root surface and are directly or indirectly involved in promoting plant growth and development via production and secretion of various regulatory chemicals in the vicinity of rhizosphere (Ahemad and Kiberat, 2013). PGPR are associated with several plants and offer various favorable functions including enhanced plant growth and decreased sensitivity to diseases caused by plant pathogens (Shahzadi *et al.* 2012). The enhanced root hair branches, efficient seed germination, more leaf area per plant, liberation of phytohormones, enhanced availability of nutrients to plants, and enhanced plant biomass are the mechanisms of PGPR in plant growth promotion (Podile and Kishore 2006).

PGPR include several genera such as *Arthrobacter, Azotobacter, Azospirillum, Bacillus, Enterobacter*, *Pseudomonas*, *Serratia,* and *Streptomyces spp.* (Ma *et al.* 2011a, b).

On the basis of relationship to plants, PGPR can be classified as extracellular PGPR (ePGPR) and intracellular (iPGPR) (Martínez-Viveros *et al.* 2010). The ePGPR reside in the rhizosphere or in the spaces between the root cortex whereas the iPGPR reach the nodular structures of roots. The bacteria known to be ePGPR are *Azotobacter, Azospirillum, Bacillus, Arthrobacter, Caulobacter, Erwinia, Flavobacterium*, *Micrococcus*, *Pseudomonas* and *Serratia*. Rhizobia are the well-known group belonging to the intracellular PGPR, and they produce nodules in legume plants. Some endophytic iPGPR, for example, *Rhizobia* (*Allorhizobium, Bradyrhizobium,* and *Mesorhizobium*) and *Frankia*, are known to fix atmospheric nitrogen (Bhattacharyya and Jha, 2012; Mondal *et al.,* 2020; Rai *et al.,* 2020). Due to the negative environmental impact of artificial fertilizers and their increasing costs, the use of beneficial microorganisms such as PGPR for sustainable and safe agriculture has increased globally. Significant increase in growth and yield of crops in response to inoculation with PGPR has been reported.

Mechanisms of action of PGPR:

Generally, PGPR promote plant growth by two mechanisms, i,e., direct mechanisms and indirect mechanisms. They promote plant growth directly by either facilitating resource

acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents (Glick, 2012).

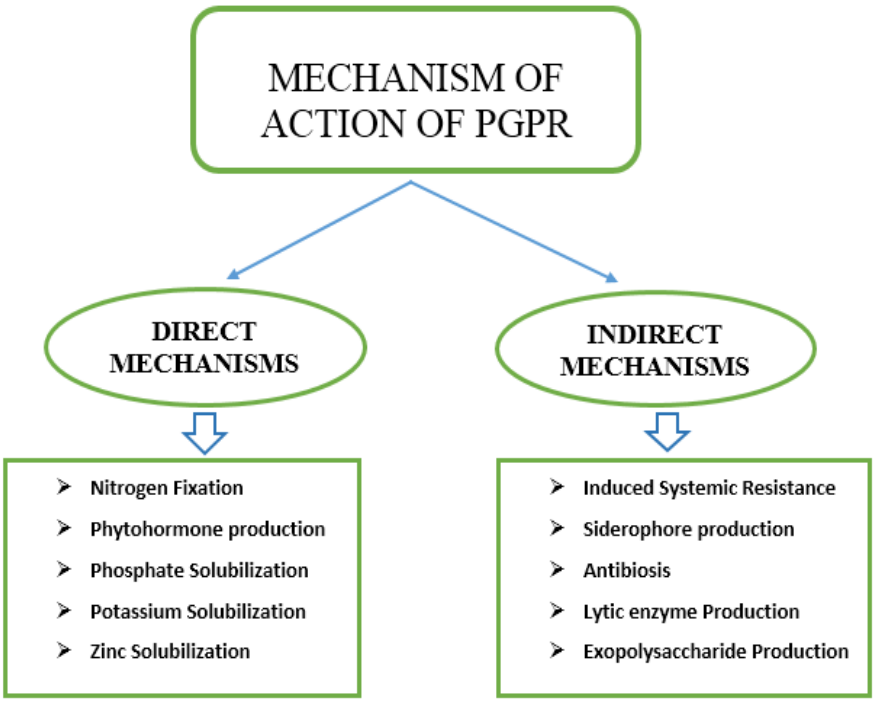

Figure 1: Direct and indirect mechanisms in relation to PGPR

Direct mechanisms:

Plant growth promoting rhizobacteria having direct mechanisms that facilitate nutrient uptake or increase nutrient availability are as follows.

Nitrogen fixation:

Nitrogen is an essential element for all forms of life and it is the most vital nutrient for plant growth and productivity. Although the nitrogen presents 78 % of the atmosphere, it remains unavailable to the plants. The biological fixation of molecular nitrogen (N2) by rhizobacteria appears among one of the most important mechanisms of plant association with its microbiota.

Plant growth promoting rhizobacteria have the ability to fix atmospheric nitrogen and provide it to plants by two mechanisms: symbiotic and non-symbiotic. Symbiotic nitrogen fixation is a mutualistic relationship between a microbe and the plant. The microbe first enters the root and later on form nodules in which nitrogen fixation occurs. Rhizobia are a vast group of rhizobacteria that have the ability to lay symbiotic interactions by the colonization and formation of root nodules with leguminous plants, where nitrogen is fixed to ammonia and make it available for the plant (Ahemad and Kibret, 2014).

The plant growth promoting rhizobacteria widely presented as symbionts are *Rhizobium*,

Bradyrhizobium, *Sinorhizobium*, and *Mesorhizobium* with leguminous plants, *Frankia* with non-leguminous trees and shrubs (Zahran, 2001).

The non-symbiotic nitrogen fixation is carried out by free living diazotrophs and this can stimulate non-legume plants growth such as radish and rice. Non-symbiotic Nitrogen fixing rhizospheric bacteria belonging to genera including *Azoarcus*, *Azotobacter*, *Acetobacter*, *Azospirillum*, *Burkholderia*, *Diazotrophicus*, *Enterobacter*, *Gluconacetobacter*, *Pseudomonas* and cyanobacteria for instance *Anabaena, Nostoc* (Bhattacharyya and Jha 2012; Vessey JK 2003).

Phosphate solubilization:

Phosphorus is the most important key element in the nutrition of plants, next to nitrogen (N). It plays an important role in virtually all major metabolic processes in plant including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis and respiration. It is abundantly available in soils in both organic and inorganic forms. Plants are unable to utilized phosphate because 95-99% phosphate present in the insoluble, immobilized, and precipitated form (Pandey and Maheshwari, 2007). Plants absorb phosphate only in two soluble forms, the monobasic (H2PO4) and the diabasic (HPO42-) ions (Bhattacharyya and Jha, 2012).

Plant growth promoting rhizobacteria present in the soil employ different strategies to make use of unavailable forms of phosphorus and in turn also help in making phosphorus available for plants to absorb. The main phosphate solubilization mechanisms employed by plant growth promoting rhizobacteria include: (1) release of complexing or mineral dissolving compounds e.g. organic acid anions, protons, hydroxyl ions, CO2, (2) liberation of extracellular enzymes (biochemical phosphate mineralization) and (3) the release of phosphate during substrate degradation (biological phosphate mineralization) (Sharma et. al., 2013).

Phosphate solubilizing PGPR included in the genera *Arthrobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium Pseudomonas*, *Rhizobium*, *Rhodococcus*, and *Serratia* have attracted the attention of agriculturists as soil inoculums to improve plant growth and yield (Bhattacharyya and Jha 2012).

Potassium solubilization:

Potassium (K) is the third major essential macronutrient for plant growth. The concentrations of soluble potassium in the soil are usually very low and more than 90% of potassium in the soil exists in the form of insoluble rocks and silicate minerals (Parmar and Sandhu, 2013). Plant growth promoting rhizobacteria are able to solubilize potassium rock

through production and secretion of organic acids which lower pH and help in potassium solubilization.

A wide range of bacteria namely *Pseudomonas, Burkholderia, Acidothiobacillus ferrooxidans, Bacillus mucilaginosus, Bacillus edaphicus, B. circulans* and *Paenibacillus* sp. has been reported to release potassium in accessible form from potassium-bearing minerals in soils (Sheng, 2005; Liu et. al., 2012).

Zinc solubilization:

Zinc is one of the imperative micronutrients required relatively in small concentrations (5–100mg kg−1) in tissues for healthy growth and reproduction of plants. Zinc deficiency in plants leads to reduced membrane integrity and synthesis of carbohydrates, auxins, nucleotides, cytochromes, and chlorophyll and develops susceptibility to heat stress (Singh, 2005). Insoluble Zn cannot be assimilated by crops, resulting in Zn deficiency; more Zn fertilizers are applied to crops to combat Zn deficiency. However, this technique is costly and can be harmful to both human health and the natural environment. Zinc solubilizing bacteria have the ability to improve crop quality. Bacterial genera, such as *Pseudomonas, Bacillus, Acinetobacter, Azotobacter, Azospirillum, Gluconacetobacter, Burkholderia,* and *Thiobacillus* have shown their ability to solubilize Zn (Bhakat *et al.* 2021).

Phytohormone production:

Plant growth and development involves a tight coordination of the spatial and temporal organization of cell division, cell expansion and cell differentiation. Orchestration of these events requires the exchange of signaling molecules between the root and shoot, which can be affected by both biotic and abiotic factors.

Proposed molecules for plant-growth promotion by PGPR include bacterial synthesis of the plant hormones indole-3-acetic acid, cytokinin, and gibberellin and breakdown of plant produced ethylene by bacterial production of 1- aminocyclopropane- 1-carboxylate deaminase.

Microbial synthesis of the phytohormone auxin (indole-3-acetic acid/indole acetic acid/IAA) has been known for a long time. It is reported that 80% of microorganisms isolated from the rhizosphere of various crops possess the ability to synthesize and release auxins as secondary metabolites (Patten and Glick, 1996). Generally, IAA secreted by rhizobacteria interferes with the many plant developmental processes because the endogenous pool of plant IAA may be altered by the acquisition of IAA that has been secreted by soil bacteria (Glick, 2012; Spaepen *et al.,* 2007). Cytokinins are purine derivatives that promote and maintain plant cell division in cultures and are also involved in various differentiation processes including shoot formation, primary root growth and callus formation. Plants continuously use cytokinins to

maintain the pools of totipotent stem cells in their shoot and root meristems (Leibfried *et al.* 2005).

Indirect mechanisms:

Induced systemic resistance (ISR), antibiosis, competition for nutrients, parasitism, production of metabolites (hydrogen cyanide, siderophores) suppressive to deleterious rhizobacteria are some of the mechanism that indirectly benefit plant growth (Jha C. K. and Saraf M 2015).

The major indirect mechanism of plant growth promotion in rhizobacteria is through acting as biocontrol agents (Glick, 2012). In general, competition for nutrients, niche exclusion, induced systemic resistance and antifungal metabolites production are the chief modes of biocontrol activity in PGPR (Lugtenberg and Kamilova, 2009). Many rhizobacteria have been reported to produce antifungal metabolites like, HCN, phenazines, pyrrolnitrin, 2,4-diacetylphloroglucinol, pyoluteorin, viscosinamide and tensin (Bhattacharyya and Jha, 2012).

Induced systemic resistance:

Interaction of some rhizobacteria with the plant roots can result in plant resistance against some pathogenic bacteria, fungi, and viruses. This phenomenon is called induced systemic resistance (ISR) (Lugtenberg and Kamilova, 2009). Moreover, ISR involves jasmonate and ethylene signaling within the plant and these hormones stimulate the host plant's defense responses against a variety of plant pathogens (Glick, 2012).

Siderophore production:

Siderophores, derived from a Greek word meanings "iron carrier" basically are the compounds with lower molecular weight with high iron affinity and these small iron chelating compounds are released by the beneficial microorganisms (Miller and Marvin, 2008).

Siderophore are low molecular weight compounds (400– 1,500 Dalton) preferentially chelate iron (Fe+++) and transport it into the cell across the cell membrane. Iron is an essetial mineral growth element for plants. Therefore, Fe3+ must first be reduced to Fe2+ before being absorbed by the plant. Once Fe3+ chelated at the root surface, the phytosiderophore-Fe complex is directly assimilated (Glick et. al., 1998). The plants capture these bacterial complexes and the latter can play a significant role in nutrition and growth (Loper and Schroth 1986). Siderophore-producing rhizobacteria improve plant health at various levels: they improve iron nutrition, inhibit growth of other microorganisms with release of their antibiotic molecule and hinder the growth of pathogens by limiting the iron available for the pathogen, generally fungi, which are unable to absorb the iron siderophore complex (Jha and Saraf 2015).

Antibiotics:

One of the most effective mechanism by which PGPR employ to prevent proliferation of phytopathogens is the synthesis of antibiotics. Antibiotics include a heterogeneous group of organic, low-molecular-weight compounds that are deleterious to the growth or metabolic activities of other microorganisms (Duffy, 2003). There are six classes of antibiotic compounds linked to the biocontrol of root diseases are, phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin, cyclic lipopeptides (all of which are diffusible) and hydrogen cyanide (HCN which is volatile) (Haas and Défago, 2005). The mechanism of action is to inhibit synthesis of pathogen cell walls, influence membrane structures of cells and inhibit the formation of initiation complexes on the small subunit of the ribosome (Maksimov *et al.*, 2011).

HCN production:

The production of volatile inhibitory substances can increase the survival rate of bacteria in soil, eliminating potential competitors for nutrients (McNeill and Unkovich 2007). Bacteria of Pseudomonas genus emit some volatile compounds, such as hydrogen cyanide (HCN). They have antibiotic effects, and play a role in protecting the host plant (Voisard et. al., 1989).

HCN is a broad-spectrum antimicrobial compound involved in the biological control of root diseases (Ramette et. al., 2003).

Production of 1-aminocyclopropane-1-carboxylase:

Plant growth promoting rhizobacteria (PGPR) contain a vital enzyme, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (EC 4.1.99.4), which regulates ethylene production by metabolizing ACC (an intermediate precursor of ethylene biosynthesis in higher plants) into a-ketobutyrate and ammonia (Shaharoona *et al.* 2007). The microbial enzyme 1-aminocyclopropane-1- carboxylate deaminase cleaves ACC irreversibly, this being the immediate precursor of ethylene in plants (Saraf *et al.* 2010). This enzyme facilitates plant growth as a consequence of the fact that it sequesters and cleaves plant produced ACC, thereby lowering the level of ethylene in the plant. In turn, decreased ethylene levels allow the plant to be more resistant to a wide variety of environmental stresses, all of which induce the plant to increase its endogenous level of ethylene; stress ethylene exacerbates the effects of various environmental stresses. The ACC deaminase-containing soil bacteria decrease a significant portion of the physiological damage to plants following environmental stresses including phytopathogen infection, exposure to extremes of temperature, high salt, flooding, drought, exposure to metals and organic contaminants, and insect predation (Jha C. K. and Saraf M 2015).

Cell wall-degrading enzymes:

The cell walls of insect pests and fungal pathogens of plants have polymers like lipids, glucan, chitin, cellulose, and proteins. The ability of PGP microbes to generate cell wall-degrading enzymes is well known. Insect pests and pathogens have their cell walls disrupted by these enzymes, which cause cell lysis. The microbes for growth promotion use these as a defense mechanism against plant pathogens and insect pests. Plant promoting microbes produce hydrolytic enzymes such as peroxidase, chitinase, glucanase and protease (Chater, 2010).

Exopolysaccharide (EPS) production:

Exopolysaccharides are carbohydrate polymers that are secreted by a wide variety on plant growth promoting rhizobacteria. Thay can remain associated with the cell wall to form a bound capsule layer or they can be released in to cells surrounding as extracellular slime (Glick *et al.* 1999). EPS have vital roles in a variety of processes such as formation of biofilm (Bhaskar and Bhosle 2005), protection of bacterial cell from desication (Pal *et al.* 1999), for maintaining primary cellular functions and antibacterial activity against predators, gelling ability, pollutant degradation kinetics (Fusconi and Godinho 2002), bioremediation activity and plasma substituting capacity (Allison, 1998).

Conclusion:

With increasing concern about the natural environment and the understanding that the era of the large scale use of chemicals in the environment needs to come to an end, PGPR offer an attractive alternative that contains the possibility of developing more sustainable approaches to agriculture. PGPR are excellent model systems which can provide the biotechnologist with novel genetic constituents and bioactive chemicals having diverse uses in agriculture and environmental sustainability. PGPRs are considered an eco-friendly alternative to hazardous chemical fertilizers. The use of PGPRs as biofertilizers is a biological approach toward the sustainable intensification of agriculture.

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COMPARATIVE ANALYSIS: PLANT-BASED VS. NON-PLANT-BASED FOOD PRODUCTS

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Abstract:

Plant-based foods have become increasingly popular as alternatives to traditional meat and dairy products, driven by concerns for health, environmental sustainability, and animal welfare. This shift has led to the development of various plant-based products such as milk, meat alternatives, ice cream, and chocolate. These products offer a wide range of nutrients and health benefits, including weight management, improved digestion, and boosted immunity. However, they may have shorter shelf life and use different packaging materials compared to their conventional counterparts. This explores the types of plant-based food products available, including their ingredients, nutrients, health benefits, shelf life, packaging materials, and environmental implications. Plant-based milk options include soy, almond, coconut, and oat milk, offering nutrients such as protein, calcium, and vitamins. Meat alternatives are made from ingredients like tofu, lentils, and pea protein, providing benefits such as improved blood sugar control and lower cholesterol. Vegan ice cream and chocolate offer delicious dairy-free options, with ingredients like coconut milk and cocoa beans providing antioxidants and promoting heart health. While plant-based alternatives offer numerous health benefits and environmental advantages, they may also pose challenges such as shorter shelf life and the use of different packaging materials. Nevertheless, the growing popularity of plant-based foods signifies a positive shift towards more sustainable and ethical dietary choices.

Introduction:

In recent times, plant-based foods have gained widespread popularity as alternatives to traditional meat and dairy products. Innovations, exemplified by products like the Impossible Burger, utilize inventive combinations of ingredients such as textured wheat protein, coconut oil, potato protein, yeast, soy protein, and gums to replicate the taste and texture of ground beef. This shift extends beyond meat substitutes to milk alternatives derived from nuts, fruits, and grains, contributing to a decline in the consumption of dairy milk and impacting the dairy industry.

Legal disputes have arisen, with meat and dairy industry groups challenging the use of terms like "burger" and "milk" for these plant-based substitutes, prompting calls for intervention from the FDA. However, companies like Danone North America have embraced the transition

rather than engaging in litigation, thriving with a diverse portfolio that includes both dairy and plant-based offerings.

Despite the generally positive health perception associated with plant-based foods, not all of them possess robust nutritional profiles. Some substitutes may contain elevated levels of negative nutrients like saturated fats, sodium, and sugars compared to their traditional counterparts. Consumers are advised to carefully examine labels to make informed choices.

From a public health standpoint, initial affordability challenges for plant-based foods may diminish as their market share expands. The increasing production costs for dairy and beef could render plant-based alternatives more economically viable, potentially emerging as a significant protein source for a diverse range of demographics.

Plant-based foods can be categorized into various groups including**:**

- Fruits
- Vegetables
- Legumes
- Grains
- Nut
- Seeds.

Additionally, there are processed versions derived from these sources, such as

- Breads
- Pasta
- Breakfast cereals
- Cooked and fermented vegetables
- Legumes

Furthermore, plant-based ingredients encompass items like oils derived from**:**

- Oleaginous seeds.
- Sugars.
- Certain herbs and spices.

As well as **"Fruit purees, juices, and jams"**. Let us see about the types of plant-based food products and comparative of plant-based food products with normal products.

Types of plant-based food products

There are various types of plant-based products. These are some types**:**

1. Plant milk.
2. Meat alternatives.
3. Ice cream.
4. Chocolates.

Plant milk

Milk is a widely recognized term that has become an integral part of people's diets, spanning from infants to the elderly. Despite our historical reliance on traditional dairy sources, an increasing number of individuals are turning to alternatives like vegan or non-dairy milk. This shift is driven by factors such as lactose intolerance, ethical concerns related to animal exploitation, and a desire to reduce environmental impact, including carbon footprint. Although

non-dairy alternatives may resemble dairy milk, they differ significantly in composition. Unlike the natural emulsion found in dairy milk, non-dairy options are susceptible to sedimentation, prompting research efforts to enhance their stability and appeal to consumers.

To address these challenges and improve the overall quality of non-dairy milk, researchers have explored modern technologies, with ultrasonication emerging as a noteworthy preservation and processing technique for a variety of food products. This review seeks to illuminate the effects of ultrasonication on plant-based milk beverages, examining various aspects such as physical, functional, microbial, and organoleptic properties. Non-dairy milk alternatives, including those derived from peanuts, almonds, soy, and coconuts, are specifically analyzed in this exploration.

Plant-based milk serves as a substitute for individuals who either cannot or choose not to consume traditional dairy milk from cows, sheep, or goats. People turn to these alternatives due to factors such as allergies, lactose intolerance, or a commitment to a vegan lifestyle. The growing popularity of these dairy substitutes is reflected in the $21 billion in sales recorded between 2009 and 2015, coinciding with a 13% decrease in the consumption of regular cow's milk over the past five years.

Although plant-based milks are gaining traction in today's markets, they have historical origins in various cultures worldwide. Coconut milk, for example, has played a role in culinary and ceremonial practices in India and Southeast Asia for centuries. Similarly, soy milk has been documented in China as far back as 1365. While these alternatives aren't novel, their resurgence can be attributed to the diverse benefits they offer.

Meat alternatives

Plant-based meat substitutes are designed to meet consumer demands and address the imperative for sustainable future food sources. These alternatives feature nutritional profiles closely mirroring those of traditional animal-sourced meats. Embracing meat alternatives aids in mitigating the adverse impacts of livestock on both the environment and human health. Recent progress in product development and marketing initiatives has resulted in a notable upswing in the production of plant-based meat substitutes. Despite this positive trajectory, the industry is still in its nascent stages and grapples with various technological challenges.

Current innovations in processing technology and creative product formulations are specifically focused on enhancing the meat-like characteristics of these substitutes. However, the incorporation of various additives to achieve meat-like texture, juiciness, mouthfeel, and flavor raises concerns regarding nutrition, food safety, clean labeling, cost, and consumer confidence. This review provides a comprehensive assessment of the materials and processes associated with meat analogs, scrutinizing ongoing developments, market challenges, consumer perceptions, and identifying potential pathways for future growth.

Ice cream

Vegan ice cream offers a delectable dairy-free option for those seeking an alternative to traditional ice cream. Crafted without any animal products, it relies on plant-based ingredients like coconut, almond, or soy milk. This delightful dessert features natural sweeteners such as agave syrup and is flavored with ingredients like cocoa powder, nuts, and fruits. Enjoyable in the same way as regular ice cream, it provides a delicious treat without compromising your dietary choices.

Chocolate

Chocolate labeled as plant-based or vegan is meticulously crafted without any ingredients derived from animals. In contrast to conventional chocolate treats that commonly include cow's milk, plant-based chocolate embraces alternatives like oat milk, coconut milk, or various nut milks. While dark chocolate frequently aligns with vegan principles naturally, it's imperative to carefully examine labels to avoid potential traces of milk or other animal-derived components such as honey, whey, or specific types of caramels. It’s worth noting that some chocolates fall under the category of "accidentally vegan," signifying that, even though the manufacturer may not have initially intended to produce a plant-based product, the final outcome is entirely devoid of animal product.

Summary

Based on the provided information, here's a summary of various aspects related to plant-based food products compared to normal food products in terms of milk, ice cream, meat, and chocolate**:**

Milk

- Plant-based milk options include soy milk, rice milk, coconut milk, almond milk, tiger nut milk, peanut milk, cashew nut milk, oat milk, and hemp milk, offering various nutrients such as protein, calcium, Vitamin-D, B12, E, and antioxidants.
- Health benefits of plant-based milk include promoting weight loss, improving digestion, boosting the immune system, and lowering bad cholesterol.
- Plant-based milk typically has a shorter shelf life compared to normal milk, ranging from 1 day to 6 months depending on the type.
- Packaging materials for both types of milk include high-density polyethylene bottles and polyethylene terephthalate covers.

Ice cream

- Plant-based ice cream is made from soy milk, oat milk, rice milk, almond milk, walnut milk, and coconut milk, with added sugar, flavoring agents, silken tofu, and a pinch of sea salt.

- Health benefits of plant-based ice cream include being an alternative for those with lactose intolerance, reducing blood sugar, preventing high blood pressure, and aiding in weight loss.
- Plant-based ice cream typically has a shorter shelf life of 21 days when stored below 18°C, compared to 2 to 4 months for normal ice cream under the same conditions.
- Packaging materials for plant-based ice cream include bioplastic and poly lactic acid (paper board), while normal ice cream is packaged in plastic or paper wrappers.

Meat

- Plant-based meat alternatives are made from ingredients like tofu, soy seitan, wheat gluten, pea protein, lentils, beans, coconut oil, nuts, and vegetables.
- Health benefits of plant-based meat alternatives include improving blood sugar control, better heart health, decreasing inflammation, and lowering cholesterol.
- Plant-based meat alternatives typically have a shorter shelf life of 10 days or at least a week compared to 3 to 5 days for normal meat when refrigerated at 40°F.
- Packaging materials for plant-based meat alternatives include bio plastics made entirely from plant starches, while normal meat is packaged in plastics, more specifically laminates.

Chocolate

- Plant-based chocolate is made from cocoa beans, coconut milk, sugar or rice milk, cane sugar, rice syrup powder, cocoa butter and masa, chickpea protein concentrate, sunflower lecithin, nuts, and natural vanilla flavoring.
- Health benefits of plant-based chocolate include being an antioxidant, improving heart and brain function, protecting cells from damage, and helping regulate blood sugar levels for people with diabetes.
- Plant-based chocolate typically does not contain preservatives, unlike normal chocolate which may contain preservatives such as potassium sorbate, citric acid, sodium benzoate, sodium lignosulfonate, and sodium citrate.
- Packaging materials for plant-based chocolate include compostable packaging and renewable plant material, while normal chocolate is packaged in translucent glassine paper.

In summary, plant-based alternatives offer various health benefits and environmental advantages, but they may have shorter shelf life and different packaging materials compared to their normal counterparts.

Comparission

Context	Plant Based Food Products	Normal Food Products
Milk		
Ingredients	Soy milk, Rice milk, Coconut milk, Almond milk, Tiger nut milk, Peanut milk, Cashew nut milk, Oat milk, Hemp milk.	Cow milk, Buffalo milk.
Nutrients	Protein, Polysaccharides, Calcium, Sugar, Vitamin-D,B12,E, Antioxidant, Fatty acid, Alpha linolenic acid, Phosphorous, Magnesium, Selenium.	Protein, Vitamins, Calcium, Carbohydrates, Minerals, Sodium, Potassium, Water, Casein, Saturated fat and cholesterol
Health Benefits	Helps in lowering the bad cholesterol, Gut friendly, Promote weight loss, Reduce the risk of cancer, Improves digestion, Boost up the immune health.	Boost up immune system, Improves bone and teeth health, Aids in digestive health, Strengthens the heart health, Helps in lowering blood pressure, Prevent weight gain.
Shelf life	Almond milk-10 days at refrigeration. Canned coconut milk-2years. Carton coconut milk-6 to 12 month. Soya, Cashew, Hemp milks-7 to 10 days at refrigeration.	Fresh milk-1day. Pasteurized milk-12 to 14 days. Sterilized milk-6months.
Products	Cream soups, Sauces, Savory dishes, Tea, Coffee, Shakes, Dessert.	Cheese, Yogurt, Kefir, Ice cream, Butter, Panner, Curd, Ghee.
Packaging Material	High density polyethylene bottles, Polyethylene terephthalate covers.	High density polyethylene bottles, Polyethylene terephthalate covers.

Ice cream		
Ingredients	Soy milk, Oat milk, Rice milk, Almond milk, Walnut milk and Coconut milk, Sugar, Flavoring agents, Silken tofu, A pinch of sea salt.	Cow milk, Milk fat, Milk protein, Sugar, Cream, Mono and diglycerides, Non fat milk, Whey, Flavoring agents, Water.
Nutrients	Calories261, Total fat(12gms), Saturated fat(2.7gms), Cholesterol, Sodium, Protein, Vitamin, Calcium, Iron, Potassium.	Cholesterol, Sodium, Protein, Vitamin-C, B6,D, Calcium, Iron, Potassium, Total fat(11gms), Dietary fiber, Cobalamin, Calcium, Magnesium.
Health Benefits	An alternative choice for people who have lactose intolerance, Reduce blood sugar, Prevent high blood pressure, Reduce cancer risk, Aids in lose excess weight, Improves kidney function.	Boost up the immune system, Presence of calcium leads to strengthen the bones, Stimulates the brain function, Boosts our mood and help us to feel happy.
Side effects	May cause allergy for with nut allergies or intolerance to soy milk based options.	Weight gain, Cause Obesity, Increase in blood pressure.
Shelf life	21 days at below 18℃.	2 to 4 months at below 18℃.
Cost	High Cost compared to normal ice cream.	Low cost compared to vegan ice cream.
Products	Vegan chocolate ice cream, Vegan coconut ice cream, Vegan strawberry and vanilla ice cream, Vega hazelnut ice cream, Vegan raspberry sorbet.	Choco bar, Sandwich ice cream, Cotton candy ice cream, Cone and cup ice cream.
Packaging Material	Bio plastic, Poly lactic acid(paper board).	Plastic or Paper wrappers.

Meat		
Ingredients	Tofu, Soy seitan or wheat gluten, Potato starch, Pea protein, Lentils, Beans, Coconut oil, Seeds and Nuts, Vegetables, Almonds, Flax seeds, Coco butter, Peanuts, Rice brand oils, Canola oil.	Beef, Grass fed chicken, Free range chicken, Organic chicken, Turkey, Skinned chicken, Pork, Partidge, Meat stock, Keema, Mutton liver, Ham, Kidney meat, Crab, Chicken liver.
Nutrients	Calories, Total fat(saturated fat, trans fat, unsaturated fat), Cholesterol, Sodium, Total carbohydrate, Dietary fiber, Sugars and protein.	Protein, Moisture, Amino acid level, Fat, Carbohydrates, Minerals, Iron, Vitamin-B12,D.
Health Benefits	Improves blood sugar control, Better heart health, Decreasing inflammation, Improves digestion, Lowering cholesterol.	Cures insomnia, Good for memory, Muscle growth, Prevents aging.
Side effects	Cardiac vascular disease, Osteoporosis, Kidney disease, Stomach cancer.	Ischemic heart disease, Pneumonia, Diabetes, Colon polyps.
Shelf life	10 days or at least a week	3 to 5 days at refrigerator at 40°F.
Packaging Material	Bio plastics made entirely from plant starches.	Plastics more specially laminates.
Products	Burgers, Chicken, Sausage, Pepperoni, Ham, Bacon,Hot dogs, Ribs, Wings, Chicken nuggets, Salmon,Turkey, Roasts.	Fresh meat balls, Beef, Hamburger, Patties, Canned chicken meat.
Additives Used	.Carrageenan and Methylcellulose.	Potassium nitrate and Sodium nitrate.

Chocolate		
Ingredients	Cocoa beans, Coconut milk, Sugar / Rice milk, Cane sugar, Rice syrup powder, Cocoa butter and masa, Chickpea protein concentrate, Sunflower lecithin, Nuts, Natural vanilla flavoring.	Milk, Butter, Cocoa beans, Sugar, Milk powder, Lecithin phospholipids, Nuts, Other dairy products, Flavors like vanilla.
Nutrients	Vitamins, Minerals, Magnesium, Iron, Fiber, Potassium, Calcium, Copper, Vitamin-C.	Carbohydrate, Saturated fatty acids, Minerals, Potassium, Fat, Magnesium, Copper, Iron.
Health Benefits	Antioxidant, Improves heart and brain function, Protect cells from damage, Reduce inflammation, Helps people with diabetes to regulate blood sugar levels.	Prevents liver damage, Boost up the vision, Improves brain function, Skin benefits.
Preservatives	No preservatives.	Potassium sorbate, Citric acid, Sodium benzoate, Sodium lignosulfonate, Oxygen absorber, Sodium citrate.
Shelf life	Unopened **:** 2 – 4 months. Opened **:** 2 weeks.	Unopened **:** 2 years. Opened **:** 6 – 8 months.
Packaging Material	Compostable Packaging, Renewable Plant Material.	Translucent Glassine Paper.
Products	Heidi, Sofit, Ditch the guilt, Daarzel vegan & gluten free dark chocolate bars.	Dark chocolate, White chocolate, Milk chocolate.
Storage Conditions	Cool and dry place, Away from direct sunlight, heat sources & strong odours. 12℃ to 20℃.	Dry, cool and dark place. 15℃ to 18℃ (59℉ to 64℉).

Conclusion:

Plant-based alternatives like milk, ice cream, meat, and chocolate provide diverse nutrients and health benefits, including weight management, improved digestion, and boosted immunity. However, they often have shorter shelf life and use different packaging materials compared to conventional products. Plant-based options are also environmentally advantageous.

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EXPLORING THE INFLUENCE OF CHANGING RAINFALL PATTERNS ON RICE IN ASSAM

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Introduction:

Rice stands as the linchpin of Assam's agricultural identity, dominating a sprawling 2.54 million hectares within the state's expansive gross cropped area of 4.16 million hectares. This cereal crop is not merely a staple but a lifeline, contributing a staggering 96% to the state's total food grain production. What makes Assam particularly distinctive is its recognition for hosting a reservoir of rich rice genetic diversity. Over the years, the cultivation of rice across diverse agro-ecological conditions has facilitated the evolution of numerous strains, shaped by the forces of natural selection and the discernment of skilled farmers.

The unique physical attributes, geographical positioning, and historical complexities of Assam have transformed it into a haven of unparalleled diversity. Ethnic migrations and immigration over time have woven a tapestry of different rice genetic stocks, further enriching the agricultural landscape. This inherent diversity is underscored by the fact that Assam's indigenous rice germplasm contributes significantly, representing around 20 out of the 12,256 collections maintained on the National Rice Research Institute (NRRI).

However, the agricultural landscape in Assam is not immune to the pressing demands for increased production. Faced with the need to produce more, farmers are gradually transitioning from traditional rice varieties to modern, high-yielding alternatives. This shift is supported by concerted efforts from institutions such as the Assam Agricultural University and the Department of Agriculture. As a result, rice cultivation occupies a substantial 60% of the total cultivated area, amounting to approximately 2.35 million hectares in the agricultural year 2021-22.

Yet, this agricultural prosperity coexists with a poignant tale of environmental challenges. Assam, like many regions, grapples with the impacts of climate change, most notably manifested in severe floods. In 2022 alone, about 2.46 lakh hectares of land were affected, spanning across 33 districts, as a consequence of three distinct floods. These events cast a shadow on the agricultural community, particularly those engaged in paddy cultivation.

Northeast India, including Assam, has witnessed a gradual rise in temperature and an increase in the number of rainy days in recent years. These shifts in climate patterns pose a substantial risk to farmers heavily reliant on crop production. Studies conducted in Assam have been dedicated to understanding farmers' strategies to mitigate flood risks and assessing their vulnerability to these recurring events. Despite some criticisms regarding methodological rigor in these studies, they shed light on the adaptive measures employed by households in the flood-prone Brahmaputra plains.

In the face of these challenges, the farming communities in Assam exhibit resilience and adaptability. Despite the struggles posed by climate-induced adversities, the state has experienced an overall increase in productivity. This success is attributed to the introduction of high-yielding rice varieties over the years, signalling the ability of Assam's agricultural sector to navigate and overcome the complex interplay of environmental and socio-economic factors. Outlining the chapter's goals, this section sets the stage for a comprehensive exploration of how changing rainfall patterns influence various facets of rice cropping systems. The focus is on providing actionable insights for researchers, policymakers, and practitioners in the field.

Discussion:

Impact of rainfall over rice cultivation:

Rice, a staple crop requiring substantial water resources for cultivation, takes centre stage in Assam's agricultural landscape. The Sali season, encompassing over 25 lakh hectares, emerges as the primary rice growing period, crucial for the state's economy (Dutta *et al.,* 2023). This season predominantly relies on rainfed agriculture, amplifying its vulnerability to climate variations. Monocropping is a prevalent practice among farmers, concentrating their cultivation efforts during the Sali season.

The significance of the Sali season is not only agricultural but deeply intertwined with the socio-economic fabric of Assam. Variations in climate patterns, notably changes in rainfall, directly impact this crucial period of rice cultivation. The reliance on rainfed agriculture and the concentration of cultivation during a specific season make the crop particularly susceptible to climatic shifts. A discernible trend has been observed over the years, indicating variations in rainfall patterns during the Sali season. This observation is corroborated by numerous studies, such as the work conducted by Kakoti *et. al.* in 2023.

The recognition of these climate-induced variations in the Sali season necessitates a proactive response. It underscores the imperative to adapt agricultural practices to the evolving climate to safeguard both crop yields and the economic stability of Assam. The need for necessary changes becomes paramount as farmers predominantly engage in monocropping

during this season. This evolving understanding of the intricate relationship between changing climatic conditions and the Sali season rice cultivation lays the groundwork for informed decision-making and adaptive measures in the agricultural sector.

Crop response to altered rainfall:

Drought and flooding events in India have significantly contributed to substantial reductions in crop yields, mirroring the challenges faced by the United States. The changing precipitation patterns bring forth additional hurdles, resulting in crop damage and off-farm loss of soil and nutrients due to excessive water. Severe precipitation events in India, similar to those in the Midwestern United States, have led to soil waterlogging, a critical factor alongside floods, causing significant losses in crop production.

The adverse impact of excessive rainfall on crop yields is evident in historical instances in India, such as devastating floods, where saturated soil conditions caused considerable crop losses. Instances like poorly-drained soil during crucial growth stages of crops showcased reductions in yields. For example, waterlogging during the critical growth stages led to a reduction in grain yields for crops like rice and wheat. Reports from various states underscored yield losses for major crops due to soil waterlogging, highlighting the complexity of challenges faced by the agricultural sector in India.

Furthermore, soybean seed yields saw notable reductions due to soil waterlogging at different growth stages, with reports from states like Punjab, Haryana, and Uttar Pradesh indicating significant yield losses for various crops. Flooding events in India present their unique challenges, including the deposition of mud and crop residues on plants, sand deposition, soil crusting, and physical damage due to strong flow rates, posing further threats to crop growth. The impact of flooding stress is not limited to physical harm, as it induces wilting within hours to a few days, affecting crops like rice, wheat, and pulses. The intricate interplay of these factors underscores the complex challenges faced by the agricultural sector in India, with varying impacts based on regional climatic and economic conditions, soil types, plant species and age, and management differences.

Varietal adaptation:

In the early stages of international climate change research, the primary focus centred on mitigating the impending impacts of climate change and understanding the multifaceted consequences that could unfold. However, a notable paradigm shift has occurred in recent times, marked by an increasing emphasis on adaptation strategies. This transformative trajectory indicates a broader acknowledgment that frames climate change as a pervasive and mainstream

issue, weaving together the comprehension of vulnerabilities and the imperative for adaptation with concerted efforts aimed at poverty reduction.

This evolving perspective highlights the intricate connections between climate change challenges and the broader tapestry of developmental goals, emphasizing the need for a comprehensive and integrated approach. Ongoing discourse stresses the pivotal importance of seamlessly integrating adaptation measures into diverse developmental initiatives, recognizing the complex interplay of challenges arising from climate change.

A tangible embodiment of this conceptual evolution is the Resilience project implemented in the districts of Sivasagar and Golaghat. This project emerged as a proactive response to counteract the adverse impacts of climate change. In practical terms, it introduced climate-resilient rice varieties, specifically the Ranjit Sub-1 and Bahadur Sub-1. These varieties, known for their extended growth duration, high yield, and remarkable ability to withstand complete submergence for 10-12 days, were strategically introduced to empower farmers. The overarching objective was to equip them with the means to proactively adapt to potential alterations in the crop season without disrupting established cropping patterns.

To assess the effectiveness of these resilient crops, the project unfolded a comprehensive analysis of their intrinsic value and an evaluation of the losses experienced by farmers due to climate change. This meticulous approach aimed to provide actionable insights into the practicality and sustainability of climate-resilient agricultural practices. In doing so, the Resilience project substantially contributed to the broader discourse on climate change adaptation and sustainable development.

In essence, the resilience project serves as a practical model for integrating adaptation measures into a local context, offering tangible solutions to address the complex challenges posed by climate change at the intersection of agriculture, poverty reduction, and broader developmental initiatives. It stands as a testament to the growing acknowledgment that a holistic and interconnected approach is essential to successfully navigate the intricacies of our changing climate.

Conclusion:

Assam's agricultural landscape, deeply rooted in the cultivation of rice, faces a dynamic interplay of challenges and opportunities. The intrinsic significance of rice in the state's agricultural identity and its vital role in sustaining the economy underscore the need for a nuanced understanding of the evolving climate patterns and their impact on rice cultivation. The Sali season, pivotal for rice cultivation, relies heavily on rainfed agriculture, making it susceptible to variations in climate, particularly changes in rainfall patterns. The observed trend

of such variations over the years poses a direct threat to the socio-economic fabric of Assam, calling for adaptive measures to safeguard both crop yields and the economic stability of the region.

The discussion delves into the intricate relationship between rainfall patterns and rice cultivation, emphasizing the vulnerability of the agricultural sector to climatic shifts. It highlights the need for proactive responses and adaptive practices to address the evolving challenges posed by climate change, especially as farmers predominantly engage in monocropping during the critical Sali season. The analysis further explores the adverse effects of extreme precipitation events, such as floods and droughts, on crop yields, mirroring the challenges faced by the agricultural sector globally. The complex interplay of climatic factors, soil conditions, and regional variations presents multifaceted challenges that demand a comprehensive and integrated approach to sustainable agricultural practices. The introduction of the Resilience project in the districts of Sivasagar and Golaghat stands out as a practical model for integrating adaptation measures into the local context. By introducing climate-resilient rice varieties and assessing their effectiveness, the project contributes significantly to the discourse on climate change adaptation and sustainable development. The success of the project underscores the importance of a holistic and interconnected approach to navigate the intricacies of changing climates successfully.

In essence, the challenges faced by Assam's agricultural sector serve as a microcosm of the broader global struggle to adapt to climate change. The resilience and adaptability demonstrated by farming communities, coupled with innovative projects like Resilience, provide valuable insights for researchers, policymakers, and practitioners seeking to address the complex intersection of agriculture, poverty reduction, and sustainable development in the face of a changing climate. As Assam's agricultural sector evolves, the lessons learned from these experiences will undoubtedly play a crucial role in shaping resilient and sustainable practices for the future.

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Research and Reviews in Plant Science Volume I (ISBN: 978-93-95847-74-2)

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ISBN 978-93-95847-74-2