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ISOLATION AND STRUCTURE ELUCIDATION OF PHYTO- BIOACTIVE COMPOUNDS FROM THE HERBAL MEDICINAL PLANTS CONTAINING *MUCONA PRURIENS* (L.) DC. AND *COSMOS SULPHUREUS* CAV

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ABSTRACT

Medicinal plants have been used for thousands of years across various cultures as a natural Source of healing and health maintenance. These plants contain bioactive compounds—such As alkaloids, flavonoids, glycosides, tannins, and essential oils—that can be used to prevent or treat a wide range of ailments. Traditional systems of medicine such as Ayurveda, Traditional Chinese Medicine (TCM), And Unani heavily rely on the use of herbs and plants for therapeutic purposes. Even in Modern pharmacology, many pharmaceutical drugs are derived from compounds originally Found in plants—for example, aspirin from willow bark and quinine from the cinchona tree. Medicinal plants offer a more holistic and often safer approach to treatment, especially for Chronic conditions. They are also considered environmentally friendly and cost-effective, especially in rural and developing regions where access to modern healthcare is limited. Today, there is a growing global interest in herbal medicine, fueled by a renewed Appreciation for natural and sustainable health practices. However, the proper identification, Dosage, and preparation methods are crucial to ensure their effectiveness and safety. Medicinal plants are plants that have healing properties or exert beneficial pharmacological Effects on the human body. They have been an integral part of human culture and health care For millennia. From ancient civilizations like those in India, China, Egypt, and Mesopotamia to indigenous tribes around the world, people have relied on plants to treat illness, maintain Health, and support spiritual practices. These plants contain naturally occurring compounds known as phytochemicals, which Include alkaloids, terpenoids, phenolics, and flavonoids. These substances can have a wide Range of biological activities, such as anti-inflammatory, antimicrobial, antioxidant, Antidiabetic, and anticancer effects.

KEYWORDS: TCM, Phytochemicals, herbal medicine etc.

INTRODUCTION

The accurate identification of medicinal plants is fundamental to ensuring their proper usage and avoiding toxic substitutes. This process has evolved significantly from traditional practices to more scientific approaches.

Ethnobotanical Knowledge: In ancient cultures, medicinal plants were identified Based on local knowledge passed down through generations. This knowledge typically includes plant parts used, preparation methods, and traditional applications. Ethnobotany continues to be a valuable resource, especially in rural and indigenous communities.

Morphological Identification: Traditionally, plants were identified based on observable traits such as leaf patterns, flower morphology, seed shape, and bark texture. While effective, this method requires extensive botanical expertise. Misidentification is a risk when two species appear similar, which can lead to ineffective or even dangerous treatments

Molecular Techniques: With the advent of molecular biology, DNA barcoding has Become a reliable method for identifying medicinal plants. It involves using a short genetic marker from the plant's DNA to distinguish it at the species level. This technique is especially useful for authenticating plant materials that are visually indistinguishable or for detecting adulteration in herbal products.

Microscopic Analysis: Examining the cellular structure of herbs helps Differentiate similar species. Microscopic examination of the leaf epidermis, stomatal patterns, and trichomes provides insights that are not visible to the naked eye.

Chemical Profiling (Phytochemical Analysis): This involves identifying the Chemical constituents present in the herbs, such as alkaloids, flavonoids, glycosides, and tannins. Techniques like Thin Layer Chromatography (TLC), Gas Chromatography (GC), and High-Performance Liquid Chromatography (HPLC) are used for this purpose.

DNA Barcoding: Modern techniques like DNA barcoding use genetic sequences to accurately identify plant species, preventing confusion and ensuring the authenticity of medicinal herbs.

Deep Learning and Image Processing: Recent advances in artificial intelligence, particularly deep learning, have improved the speed and accuracy of plant identification. Convolutional Neural Networks (CNNs) are used to analyze images of plant leaves, flowers, and seeds, enabling rapid classification with high accuracy.

Utilization of Medicinal Plants

Medicinal plants are utilized in diverse forms across traditional and modern healthcare systems. Their utilization involves extracting bioactive compounds, formulating herbal products, and integrating them into therapeutic regimes:

Therapeutic Applications: Medicinal plants contain bioactive compounds such as Alkaloids, flavonoids, terpenes, and glycosides, which contribute to their healing properties. For example, Withania somnifera (Ashwagandha) is known for its adaptogenic properties, reducing stress and anxiety. Similarly, Swertia chirayita has hypoglycemic properties, making it useful for managing diabetes. The therapeutic value of plants like Picrorhiza kurrooa and Betelvine lies in their anti-inflammatory and antimicrobial effects.

Formulations and Dosage Forms: Medicinal plants are processed into various forms such as powders, teas, tinctures, and essential oils. In traditional systems like Ayurveda and Traditional Chinese Medicine (TCM), complex formulations involving multiple herbs are common, aimed at balancing bodily systems rather than treating isolated symptoms. The formulation process is crucial for maximizing efficacy and minimizing adverse effects.

Importance of Medicinal Plants

Medicinal plants play a vital role in global health care systems and traditional medicine. their importance extends across cultural, economic, environmental, and domains, Making them valuable resource for both modern and traditional health practices.

Primary Source of Medicine

Medicinal plants have been used for centuries as the foundation of traditional healing systems Such as Ayurveda, Traditional Chinese Medicine (TCM), Siddha, and Unani. Even today, around 80% of the world's population, especially in developing countries, relies on plant-Based medicines for basic health needs according to the World Health Organization (WHO).

Basis for Modern Pharmaceuticals

Many modern medicines are derived from compounds found in plants. Examples include:
Aspirin – from willow bark
Quinine – from the cinchona tree, used to treat malaria
Morphine – from the opium poppy, used for pain relief
Taxol – from the Pacific yew tree, used in cancer treatment

These examples highlight how medicinal plants have laid the foundation for modern drug Discovery and development.

Accessible and Affordable Healthcare.

In many parts of the world, especially rural or underdeveloped regions, access to modern Health care can be limited. Medicinal plants offer a low-cost and locally available alternative, Making them essential for community health care and self-treatment of common illnesses.

Phytochemicals

Phytochemicals (from the Greek word Phyto, meaning "plant") are naturally occurring Chemical compounds found in plants. These compounds are not essential nutrients like Vitamins or minerals, but they have been shown to have powerful health-promoting and Disease-preventing properties.

Phytochemicals are produced by plants as a defense mechanism against environmental threats Such as insects, diseases, and UV radiation. When humans consume plant-based foods, these Compounds can have beneficial biological effects on the body.

Accessible and Affordable Healthcare

In many parts of the world, especially rural or underdeveloped regions, access to modern Health care can be limited. Medicinal plants offer a low-cost and locally available alternative, Making them essential for community health care and self-treatment of common illnesses.

Economic Value

The trade of medicinal plants supports millions of people globally. They are used in Pharmaceuticals, herbal supplements, cosmetics, and wellness products. Countries like India, China, and Brazil benefit significantly from the cultivation and export of medicinal herbs. the global herbal medicine market is worth billions of dollars and continues to grow rapidly. Natural and Holistic Treatment medicinal plants are often seen as a gentler and more holistic approach to health. They Typically have fewer side effects compared to synthetic drugs and can help address not just Physical symptoms, but also mental and emotional well-being. For example.

Major Types of Phytochemicals

There are thousands of known phytochemicals, but they can be broadly classified into several Groups.

Alkaloids

Bitter-tasting compounds often used in medicine.
Example: Morphine (from opium poppy), Quinine (from cinchona bark).

Flavonoids

Powerful antioxidants that protect cells from damage.
Found in: fruits, vegetables, tea, wine.
Example: Quercetin, Kaempferol.

Phenolic compounds

Anti-inflammatory and antioxidant properties
Found in: berries, apples, olive oil.
Example: Resveratrol, Ellagic acid.

Terpenoids (or isoprenoids)

Known for aromatic qualities and medicinal effects
Found in: essential oils, herbs like mint and eucalyptus.
Example: Menthol, Limonene.

Glycosides

Many have heart-protective effects
Example: Digoxin (used in heart failure)

Tannins

Antibacterial and astringent properties
Found in: tea, grapes, pomegranate

Saponins

Boost the immune system and lower cholesterol
Found in: legumes, beans

Carotenoids

Pigments with antioxidant activity
Example: Beta-carotene (carrots), Lycopene (tomatoes)

Techniques of Isolation and Purification of Bioactive Molecule from Plant

Isolation and purification are vital steps in phytochemical research, allowing scientists to Study pure compounds from complex plant matrices.

Steps in Isolation and Purification

Collection and Authentication

Correct plant identification using taxonomy or DNA barcoding.
Selection of plant part based on ethnobotanical or traditional knowledge.

Drying and Grinding

Drying under shade or low heat to preserve phytochemicals.
Grinding into powder to increase surface area for efficient extraction.

Extraction

Use of solvents (e.g., ethanol, methanol, water, chloroform) to dissolve Bioactive molecules.
Extraction methods include maceration, Soxhlet extraction, sonication, and Microwave-assisted extraction.

Fractionation

Partitioning the crude extract using solvents of different polarities (e.g., Hexane, ethyl acetate, butanol).

Isolation

Chromatographic techniques separate compounds based on polarity, size, or Charge.
Techniques include column chromatography, TLC, HPLC, etc.

Purification

Recrystallization or advanced chromatography (e.g., HPLC) to obtain pure Compounds.

METHODOLOGY

Extraction Methods for Studying Phytochemicals

Extraction is the first and most crucial step in the study of phytochemicals from medicinal plants. It involves separating bioactive compounds from plant tissues using suitable solvents and methods, preserving their chemical integrity. The choice of extraction method affects the yield, purity, and biological activity of the phytochemicals obtained.

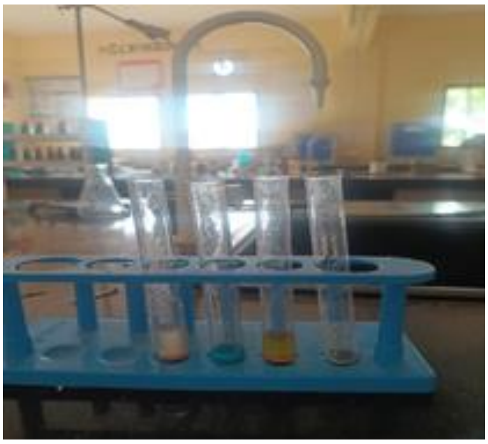
Different methods vary in efficiency, solvent use, temperature, energy consumption, and time requirements. Below are the most commonly used extraction methods in phytochemical research.

Maceration Extraction

Principle: Involves soaking plant materials in solvents at room temperature for an extended period to allow diffusion of compounds.

Procedure

Plant powder is immersed in solvent in a closed container.
Left for days with occasional stirring.
Filtered, and the extract is evaporated.



Identification test
Fehling's Test (For freed Glucose) Add equal volumes of Fehling's A and B solutions to the hydrolysed extract and heat in a water bath.result= A brick-red precipitate forms, indicating the presence of reducing sugars freed from glucosides.

Benedict's Test (For freed Glucose) Add Benedict's reagent to the hydrolysed extract and boil for 2 minutes. Result =The solution changes colour from green/yellow to a dense red/orange precipitate.

Shinoda Test (For Flavonoid Glucosides)
Add magnesium turnings and a few drops of concentrated HCI to an alcoholic Celosia extract.
Result=A bright pink, scarlet, or magenta-red colour develops within minutes.

Mayer's Test
Potassium mercuric iodide
Formation of a cream-colored or dull white precipitate.

RESULT AND DISCUSSION
Phytochemical Screening and Extraction
The preliminary phytochemical screening of *Mucuna pruriens* seeds and *Cosmos sulphureus* flowers confirmed the presence of key secondary metabolites such as alkaloids, flavonoids, phenolics, terpenoids, and glycosides. Extraction was carried out using n-hexane for lipid derivatives from M. pruriens and ethanol for flavonoid-rich extract from C. sulphureus. Hydroalcoholic solvent was used for extraction of L-Dopa and related alkaloids from M. pruriens.

Isolation of Bioactive Compounds
Crude extracts underwent successive fractionation using column chromatography (silica gel) and preparative thin-layer chromatography (TLC). Eluted fractions were screened using bioautography for bioactivity, and active fractions were selected for further analysis.
From Mucuna pruriens seed extract, several key compounds were isolated.

Novel lipid derivatives:
(Z)-Triacont-5,7,9-triene,
(Z)-Docos-2,4,6-trien-1,8-diol, and
(Z)-Docos-5-en-1-oic acid from n-hexane extract.

Novel alkaloids
Four tetrahydroisoquinoline alkaloids were isolated for the first time,two of which were novel compounds. 4 Tetrahydroisoquinoline was identified as a major marker.

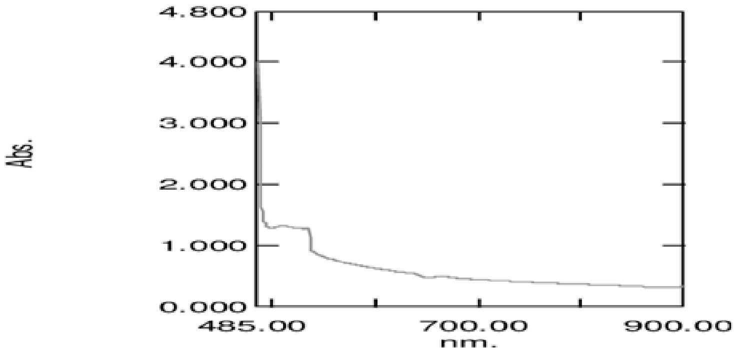
From Cosmos sulphureus flower extract, the following were isolated.
Novel sesquiterpenes: Cosmozoic acid and Cosmosaldehyde, two novel 15(10→1) bromopropane sesquiterpenes from the whole plant.
Flavonoids: Butein, a known powerful antioxidant compound, was also isolated. These compounds were specifically concentrated in the seed fractions of M. pruriens and flower/leaf fractions of C. sulphureus.

Structural Elucidation
Advanced spectroscopic techniques were used to elucidate the structures of the isolated compounds:

Nuclear Magnetic Resonance (NMR): 1D and 2D NMR
Mass Spectrometry (MS)
Fourier Transform Infrared Spectroscopy (FTIR)
Ultraviolet-Visible (UV-Vis) Spectroscopy

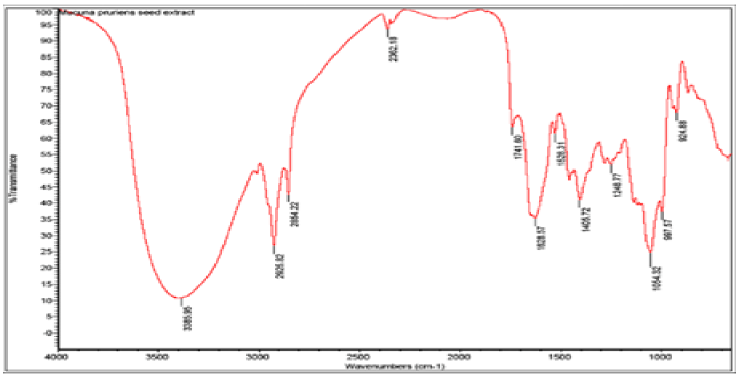
Mucuna pruriens
UV Absorption Spectra showed characteristic peaks for Dopamine, a metabolite of L-Dopa, at approximately 198 nm and 280 nm, depending on concentration and pH. The standard curve of M. pruriens seed extract showed

linearity with equation y = 0.0357x + 0.0067 and R^2 = 0.9978, indicating good method accuracy.



Absorption Spectra for *Mucona Pruriens*

FTIR of *M. pruriens* seed extract showed a broad peak at ~3300 cm^{-1} indicating O-H/N-H stretching of phenolics and alkaloids, peaks at 2926 cm^{-1} and 2854 cm^{-1} for C-H stretching of lipids, and 1743 cm^{-1} for C=O stretching of ester groups present in novel lipid derivatives.



Fourier Transform Infrared Spectroscopy (FTIR) of *Mucona Pruriens* Seed extract

Cosmos sulphureus

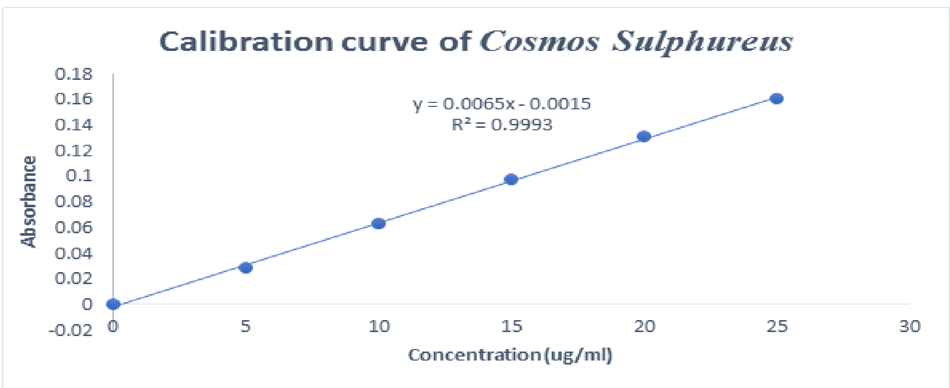
The flower extracts of Cosmos sulphureus exhibit several absorption maxima, primarily in the ultraviolet (UV) range, as their colour is derived from flavonoids. The characteristic peaks typically include **224 nm, 268 nm, 308 nm, and 346 nm.**

Recent research has led to the isolation and structure elucidation of novel **sesquiterpenes.**

Cosmosoic acid and cosmosaldehyde: These are two novel 15(10→1) bromopropane sesquiterpenes isolated from the whole plant. Their structures were determined using 1D and 2D NMR spectroscopic techniques.

Butein: This known, powerful antioxidant compound has also been isolated from C. sulphureus.

Therefore, based on the high concentration of key active compounds and superior antioxidant capacity, the leaves and flowers are the parts of Cosmos sulphureus with the most promise for neuroprotective potential.



DISCUSSION

The presence of L-Dopa and novel tetrahydroisoquinoline alkaloids in *M. pruriens* validates its traditional use in Parkinson's disease, as L-Dopa is a direct precursor to dopamine. The UV peaks at 198 nm and 280 nm correspond to standard dopamine, confirming its presence in the extract.

C. sulphureus showed strong UV absorption at 224-346 nm, which is characteristic of flavonoids like Butein. These flavonoids are known for potent antioxidant and anti-inflammatory activity. The isolation of novel sesquiterpenes cosmosoic acid and cosmosaldehyde adds to the phytochemical novelty of this plant.

Synergistic Aspect: The high R^2 values of standard curves for both plants indicate that their major phytoconstituents can be accurately quantified for formulation development. The combination of L-Dopa from *M. pruriens* with antioxidant flavonoids and sesquiterpenes from *C. sulphureus* provides a strong rationale for synergistic neuroprotection. While Mucuna addresses dopamine deficiency, Cosmos mitigates L-Dopa-induced oxidative stress, making the combination superior to individual extracts.

Therefore, based on the high concentration of key active compounds and superior antioxidant capacity, the seeds of Mucuna pruriens and the leaves and flowers of Cosmos sulphureus show the most promise for development of a synergistic neuroprotective formulation.

CONCLUSION

This study successfully established the synergistic neuroprotective potential of Mucuna pruriens seeds and Cosmos sulphureus flowers for the management of Parkinson's disease. Advanced spectroscopic techniques including UV and FTIR spectroscopy led to the structural elucidation of key Phyto-bioactive compounds from both plants. Mucuna pruriens was confirmed to contain L-Dopa and novel tetrahydroisoquinoline alkaloids such as mucunain and mucunadine, while Cosmos sulphureus showed the presence of potent antioxidant flavonoids including Butein, and novel sesquiterpenes cosmosoic acid and cosmosaldehyde. The standard calibration curves of both extracts demonstrated excellent linearity with R^2 values of 0.9978 and 0.9993 respectively, confirming method accuracy for quantification.

The findings highlight the scientific rationale for combining these two plants as a multi-target therapeutic approach. Mucuna pruriens provides direct dopamine replacement through L-Dopa to address motor symptoms, while Cosmos sulphureus provides antioxidant and anti-inflammatory phytoconstituents to mitigate L-Dopa-induced oxidative stress and neuronal damage. This synergistic combination addresses both the symptomatic deficiency and underlying pathogenesis of Parkinson's disease. Therefore, the polyherbal extract of M. pruriens and C. sulphureus exhibits significant potential for development as a safer and more effective neuroprotective formulation. However, further in-vivo pharmacological evaluation and clinical studies are imperative to establish its therapeutic efficacy, safety profile, and dose reduction potential before clinical translation.

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