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Phytochemical Profile and GC-MS-Identified Bioactive Compounds in *Pluchea ovalis* (Pers.) DC. Leaves

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ABSTRACT

Background: *Pluchea ovalis is a significant medicinal plant that belongs to the Asteraceae family. It is also known as "Woolly Camphor-Weed" and has traditionally been used in Ayurveda, Siddha, and Folk medicine for treating numerous ailments, particularly Jaundice, Piles, Bronchial Asthma, inflammation, and Carcinoma.*

Purpose: *Phytochemical Profile and GC-MS-Identified Bioactive Compounds in Pluchea ovalis.*

Research Design: *The phytochemical composition of P. ovalis leaves was screened using a Soxhlet apparatus after hot, continuous, and consecutive extraction. A variety of solvents were used for the qualitative assay. Different solvents were used in increasing polarity order to carry out the extraction operation. Total content of tannins, phenolic compounds, alkaloids (bromocresol green), and flavonoids (aluminum chloride) quantified (Folin-Ciocalteu). Additionally, quick and precise GC was used in conjunction with the Mass Hunter tool and mass spectrometry method to examine the absorbed components and determine the complete elements.*

Results: *The qualitative phytochemical screening revealed that alkaloids, flavonoids, saponins, steroids & terpenoids, phenols, tannins, glycosides, phytosterols, coumarins, quinones resins, cardiac glycosides, leuco anthocyanins, anthraquinones and fixed oils were found in all the extracts, the quantification of alkaloids in methanol, showcasing a remarkable concentration of 502 mg/g equivalent, while chloroform exhibits a substantial alkaloid content of 310 mg/g, flavonoids, yielding the highest concentration in methanol at 520 mg/g and ethyl acetate at 440 mg/g. Phenols and tannins in methanol 390 mg/g and 320 mg/g . Gas chromatography-mass spectrometry (GC-MS) analysis of P. ovalis leaf methanol extract revealed 50 peaks, identifying bioactive compounds based on retention time, molecular formula, structure, weight, and concentration (peak area%). The results indicated that the single major component, dodecanoic acid, 1,2,3-propanetriyl ester ($C_{39}H_{74}O_6$), had a peak area of 38.16%, a molecular weight of 638, and was reported to have hypercholesterolemic, anti-arthritic, nematocidal and hepatoprotective activity. 2-Ethoxyethylamine ($C_4H_{11}NO$), on the other hand, had the second-highest peak area, at 26.46%.*

Conclusion: *It is concluded from the current study. These findings highlight the rich phytochemical diversity and significant bioactive potential of Pluchea ovalis leaves, supporting its traditional use in various medicinal systems. The presence of potent bioactive compounds underscores its therapeutic potential and encourages further investigation into its pharmacological applications and mechanisms of action.*

Keywords: *Phytochemicals, GC-MS, Bioactive Compounds, Pluchea ovalis and Asteraceae*

Introduction

In recent decades, there has been a growing interest in scientific research on food and medicinal plants about the phytochemical profiles of plant materials [1]. Secondary metabolites including phenolics, terpenes, and organic acids are mostly produced by plants as a defense mechanism against environmental stressors. It's interesting to note that these substances also promote animal and human health [2-3]. Though their full potential is still understood, neglected and underutilized plants have gained importance in the food and pharmaceutical industries as possible substitutes for synthetic chemicals and nutraceuticals. To find novel sources of natural nutraceuticals, antioxidants, and added-value compounds, research on these underutilized plant resources is essential [3].

Since ancient times, people have utilized plants as traditional medicines for both preventive and therapeutic purposes due to their abundance of naturally occurring medicinal chemicals [4]. Plants are an excellent source for creating medications and other items since they are a repository of various naturally occurring substances [5].

Many chemical compounds found in plants are known to have biological activity, giving them pharmacological or therapeutic qualities as well as several other advantages for human health. Alkaloids, phenols, terpenoids, and flavonoids are the main types of phytochemicals that are present in plants [6]. Because of their unique biological functions and the potential, they hold to enhance health, produce new antioxidant and antibacterial compounds, and provide innovative treatments for a range of diseases like cancer, phytochemicals are currently the focus of much research [7]. Examples of natural materials that offer fresh possibilities for the creation of therapeutic medications are plant extracts. Almost 80% of people on the planet use herbal remedies, and most developing countries heavily rely on traditional medicine and medicinal plants as a standard method of preserving good health. This is because several chemicals present in plants can be used as remedies for both viral and chronic ailments [8]. While traditional herbal remedies are garnering a lot of attention due to their naturalness, environmental friendliness, and lack of side effects, modern synthetic and chemical medications are frequently investigated with hesitation due to their adverse effects [9].

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Because of this, despite the many advantages of contemporary synthetic medications, individuals continue to favor natural medications derived from plants over synthetic ones [10]. Because diverse useful phyto-constituents are present in different plant parts, the majority of medicinal plants are unique in their potential to treat and cure a variety of human disorders [11-13]. Since ancient times, India has employed various components of its 80,000+ species of medicinal plants as traditional remedies to cure a wide range of illnesses [14]. Medicinal plants that have been utilized as prescribed medicines have been found to have roughly 25% of the active ingredients [15]. According to some statistics, there are around 1.5 million practitioners of Indian folk and traditional medicine who prescribe over 25,000 plant-based medicines for preventative, persuasive, and therapeutic purposes [16]. To further expand on the significance of bioactive compounds derived from medicinal plants, it is essential to recognize their contribution to modern pharmacology. These compounds, such as alkaloids, flavonoids, terpenoids, and polyphenols, have been identified as pivotal in combating various diseases due to their diverse biochemical properties [17-18]. Research has demonstrated that these compounds not only offer therapeutic benefits but also enhance the efficacy of conventional drugs by acting synergistically [19], flavonoids are known for their potent antioxidant properties, which help in mitigating oxidative stress, a major factor in the pathogenesis of chronic diseases such as cancer and cardiovascular disorders [19]. Similarly, terpenoids have shown significant anti-inflammatory and anticancer activities, making them promising candidates for drug development [20]. This underscores the importance of employing advanced screening and extraction techniques to identify and isolate these compounds effectively [21]. The development of novel methodologies in phytochemistry is crucial for enhancing the discovery of new drugs from plant sources, thereby contributing to the advancement of medicine and public health, the formulation of more effective and targeted therapies, especially in the treatment of complex and resistant ailments. This highlights the need for continued research and innovation in the field of medicinal plant sciences [22].

Phytochemicals, despite being non-essential nutrients, play a crucial role in promoting health and preventing disease through their various bioactive properties. These naturally occurring compounds, found in an array of plant-based foods such as vegetables, fruits, grains, and herbs, contribute not only to the sensory characteristics of plants but also to their therapeutic potential [23]. The vibrant colors, distinct flavors, and aromatic profiles of plants are all attributed to these diverse compounds, which include flavonoids, carotenoids, alkaloids, and polyphenols, among others. Modern advancements in analytical techniques have significantly enhanced our ability to study these compounds. Spectrometric and chromatographic methods, such as FTIR and GC-MS, are now indispensable tools in the initial screening and analysis of medicinal plants. These methods provide a comprehensive understanding of the chemical makeup of plant extracts, allowing researchers to identify bioactive compounds that could serve as potential therapeutic agents [24].

Fourier-transform infrared (FTIR) spectroscopy, for example, is widely used for the detection of functional groups within complex mixtures, enabling the identification of key bioactive molecules. On the other hand, gas chromatography-mass spectrometry (GC-MS) stands out for its precision and efficiency in detecting a wide range of phytochemicals, including those with pharmacologically significant properties. The ability of GC-MS to analyze small volumes of plant extracts with high accuracy makes it an invaluable technique for secondary metabolite profiling, contributing to the discovery of novel compounds with potential health benefits [25-26], the application of these advanced techniques has broadened our understanding of the complex interactions between phytochemicals and their biological targets. This has led to the identification of new therapeutic compounds and the development of plant-based medicines that are both effective and sustainable [27]. The ongoing exploration and characterization of phytochemicals not only deepen our knowledge of plant-based nutrition but also pave the way for innovations in the treatment and prevention of various diseases, underscoring the vital role of plants in modern medicine.

The genus *Pluchea* in the Asteraceae family is known for its medicinal value, particularly the aerial parts of its plants [28]. This genus includes about 80 species, widely distributed across Africa, Asia, Australia, and the Americas [29]. Traditionally, *Pluchea* species have been used for their anti-inflammatory and antimicrobial properties, making them significant in various therapeutic applications. *Pluchea ovalis* (Pers.) DC. is an erect, woody sub-shrub, 1.2 m tall, stems striated, puberulous, commonly known as “Woolly Camphor Weed”, it is a tall shrub up to 2.5 m high, and distributed in throughout the world, in India Telangana Andhra Pradesh, Karnataka, Tamil Nadu and Kerala [30].

Reports indicate that *Pluchea ovalis* contains several key chemical components, including p-cymene, 2,5-dimethoxy-p-cymene, limonene, β-phellandrene, isocomene, β-maaliene, δ-cadinene, β-caryophyllene, and α-cadinol. These compounds contribute to its bioactive profile. *P. ovalis* has shown significant antifungal activity, as demonstrated by the agar well diffusion assay technique [31] reports that the four main constituents of the volatile compounds found in the pet-ether extracts of the aerial part of *P. ovalis* were a-selinene, b-eudesmol, humulene oxide, and dehydrosanssurea. These constituents were identified through GC/MS analysis and constitute dominant sesquiterpene hydrocarbons. The larvicidal action of *P. ovalis* is attributed to its rich source of sesquiterpenes in essential oil [32]. According to [33] reported on the essential oil composition of the aerial part of *P. ovalis*, the synthesis of silver nanoparticles, and the larvicidal activities against fall armyworms. Anti-fungal properties [34]. *P. ovalis* roots inhibit acetylcholine-induced bronchoconstriction observed in asthma.

Materials and methods

Selection criteria

The selection of the medicinal plant species under investigation in this study was based on their high frequency of reference recorded from three or more distinct ethnobotanical surveys that were published in the literature for their medicinal significance. After accomplishing an extensive literature review on the plant under consideration, it was discovered that there are currently very few published publications globally regarding the potential chemical components of "*Pluchea ovalis*." The goal of the current investigation was to ascertain the potential chemical components by first making a methanolic crude extract and then characterizing and identifying the compounds using GC-MS analysis.

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Plant material
The plant leaves from *Pluchea ovalis* (Pers.) DC.(Asteraceae) about 1kg, were collected from Kondurg Mandal, Ranga Reddy District, Telangana state, India, during August / September in the year 2023.

Authentication of *Pluchea ovalis*
The plant was taxonomically identified and authenticated by Botanical Survey of India, Deccan Regional Centre, Hyderabad, Telangana, India, (Voucher Number-.(BSI/DRC/2023-24/Admin./708)), and the specimen deposited at Herbarium, Hyderabadensis, Department of Botany, O.U, Hyderabad, Telangana, India.

Drying
The collected plants materials were washed with double distilled water. The plant leaves were air-dried at room temperature for at least 3 weeks and then pounded into fine powder.

Successive extraction using Soxhlet apparatus
To prepare the extracts of *Pluchea ovalis* leaves, fresh leaves have been obtained and thoroughly disinfected with water running through it to eliminate any soil or contamination. The leaves were extracted using an assortment of solvents, including as petroleum ether, chloroform, ethyl acetate, and methanol. The powder of dried leaves was prepared through the use of a mechanical grinder and then sieved to ensure that the powder had a consistent size. The leaf powder was successively extracted through the Soxhlet apparatus with petroleum ether at a temperature of 60° C chloroform at 61° C, ethyl acetate at 77° C, and methanol at 65° C. The extraction temperatures have been adjusted to correspond with the boiling points of the solvent, which allows a more rapid cycling of fresh solvent. Each solvent has been allocated an operating time of five hours for hot continuous and successive extraction. The extracts have been filtered through Whatman No.1 filter paper to obtain an extract without any of particles. The remaining substance was extracted two more times using a solvent and then filtered. The extracts undergo filtration and subsequent evaporation using a rotary evaporator to accomplish the volume that was needed. The extracted sample was placed in a desiccator to be analyzed qualitative, and quantitative phytochemicals and using GC-MS for the identification of various chemicals [35].

Calculation of percentage yield
The dried extracts obtained from each solvent were weighed, and the yield was calculated based on the air-dried weight of the plant material.

$$\text{Percentage Yield} = \frac{\text{Weight of the crude(mg/g)}}{\text{Weight of the plant material}} \times 100$$

Qualitative phytochemical examination of *Pluchea ovalis*
A screening procedure was conducted on the leaf extract in order to determine the presence of any secondary metabolites. A variety of tests were conducted using standard procedures in order to assess the presence or absence of many different bioactive substances including alkaloids, flavonoids, saponins, steroids and terpenoids, phenolic compounds, tannins, glycosides, cardiac glycosides, coumarins, phytosterols, lecuco anthocyanins, resins, fixed oils, anthraquinones, and quinones. The tests were undertaken qualitatively to ascertain the presence or absence of each component [36-37].

Test for alkaloids
A 50 mg sample of the solvent-free extract was stirred with 5 ml of dilute hydrochloric acid and then filtered. The resulting filtrate was carefully tested for the presence of alkaloids using specific reagents.

Mayer's Test: To 3 ml of the filtrate, a few drops of Mayer's reagent were added along the side of the test tube. The formation of a white or creamy precipitate indicated the presence of alkaloids.

Detection of flavonoids
To 1 ml of the extract, 1 ml of chloroform was added, followed by 2 to 3 ml of acetic anhydride and 1 to 2 drops of concentrated sulfuric acid. The development of a dark green color indicated the presence of steroids.

Shinodas test
To the test solution, a mixture of zinc dust and concentrated hydrochloric acid was added. The appearance of a magenta color after a few minutes indicated the presence of flavonoids.

Test for saponins
Approximately 0.5 g of the powdered drug was gently boiled for 2 minutes with 20 ml of water, then filtered while hot and allowed to cool. Five milliliters of the filtrate were diluted with water and shaken vigorously. The formation of froth indicated the presence of saponins.

Detection of Steroids and Terpenoids
Salkowki's test
Detection of Phenols
Ferric chloride test
Fifty milligrams of the extract were dissolved in 5 ml of distilled water. After adding a few drops of neutral 5% ferric chloride solution, a dark green color developed, indicating the presence of phenolic compounds.

Detection of Tannins
Potassium Dichromate test: To the test solution, 2% potassium dichromate solution was added. The appearance of a yellow precipitate confirmed the presence of tannins.

Detection of Glycosides
The test solution was prepared by dissolving the extract in alcohol or by boiling it with a hydro-alcoholic mixture.

a) Baljet's test
The test solution was treated with 2% sodium picrate. The appearance of yellow to
orange color indicated the presence of glycosides.

b) Legal's test
The test solution was treated with pyridine and made alkaline; the addition of 2% sodium nitroprusside gave pink to a red colour indicating the presence of glycosides.

c) Keller-Killiani test
The extract (100 mg) was dissolved in 1 ml of glacial acetic acid containing a drop of ferric chloride solution. It was then carefully layered with 1 ml of concentrated sulfuric acid. The presence of a brown ring at the interface indicated the presence of glycosides.

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Detection of Cardio glycosides: Two milliliters of the filtrate were mixed with 1 ml of glacial acetic acid, 1 ml of ferric chloride, and 1 ml of concentrated sulfuric acid. The appearance of a green-blue coloration indicated the presence of cardiac glycosides.

Detection of Coumarins

One milliliter of the extract was placed in a test tube, which was then covered with filter paper moistened with dilute sodium hydroxide solution. The test tube was heated in a water bath for several minutes. After removing the paper and exposing it to ultraviolet (UV) light, a green fluorescence on the paper indicated the presence of coumarins.

Test for phytosterols

A few drops of concentrated sulphuric acid were added to the extract solution, shaken well, and set aside. The lower chloroform layer of the solution turning red indicates the presence of phytosterols.

Detection of quinones

Dilute NaOH was added to 1 ml of the crude extract. The development of a blue-green or red color indicated the presence of quinones.

Detection of resins

To 2 ml of the extract, 5-10 drops of acetic anhydride were added and dissolved by gently heating. Then, 0.5 ml of sulfuric acid was added. The appearance of a bright purple color indicated the presence of resins.

Detection of leuco anthocyanins

One milliliter of crude extract was mixed with 1 ml of isoamyl alcohol. The appearance of a red color in the upper layer indicated the presence of leucoanthocyanins.

Detection of anthraquinone

To 1 g of powdered plant material, chloroform was added and shaken for 5 minutes. The contents were filtered, and 5 ml of ammonia solution was added to the filtrate. After gentle agitation, a bright pink color in the upper aqueous layer indicated the presence of anthraquinones.

Detection of fixed oils

A small quantity of the extract was pressed between two filter papers. The appearance of an oil stain on the paper indicated the presence of fixed oils.

Quantification of total content of alkaloids

One milligram of the plant extract was dissolved in dimethyl sulfoxide (DMSO) and mixed with 1 ml of 2N HCl, then filtered. The solution was transferred to a separating funnel, to which 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3, and 4 ml of chloroform, collected in a 10 ml volumetric flask, and diluted to volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80, and 100 µg/ml) was prepared similarly. The absorbance of the standard and test solutions was measured at 470 nm using a UV/Visible spectrophotometer, with the content of alkaloids expressed as mg of alkaloids equivalent per gram of plant extract [38].

Total content of flavonoids quantification.

A colorimetric assay was used to determine the total flavonoid content using aluminium chloride. In a 10 ml flask, 1 ml of plant extract and 4 ml of distilled water were combined. To this mixture, 0.30 ml of 5% sodium nitrite was added, followed by 0.30 ml of 10% aluminium chloride after 5 minutes. Five minutes later, 2 ml of 1M NaOH was added and the solution was diluted to 10 ml with distilled water. Standard solutions of quercetin (20, 40, 60, 80, and 100 µg/ml) were prepared similarly. The absorbance of both the test and standard solutions was measured at 510 nm using a UV-visible spectrophotometer, with the total flavonoid content expressed as mg of quercetin equivalent per gram of extract.

Quantification of tannin total content

The Folin-Ciocalteu method was used to quantify the total tannin content. In a 10 ml volumetric flask, 0.1 ml of plant extract was mixed with 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu phenol reagent, and 1 ml of 35% Na_2CO_3 solution, then diluted to 10 ml with distilled water. The mixture was shaken well and incubated at 30°C for 30 minutes. Standard solutions of gallic acid (20, 40, 60, 80, and 100 µg/ml) were prepared similarly. The absorbance of the standard and test solutions was measured at 725 nm using a UV-Visible spectrophotometer, with the total tannin content expressed as mg of gallic acid equivalent per gram of extract [39].

Quantification of total content of phenolic compounds

The concentration of phenolic compounds in the extract was quantified using the Folin-Ciocalteu method. In this procedure, 1 ml of plant extract was mixed with 9 ml of distilled water. One milliliter of Folin-Ciocalteu phenol reagent was added to the mixture and shaken well. After 5 minutes, 10 ml of 7% Na_2CO_3 solution was added, and the volume was adjusted to 25 ml. Standard solutions of gallic acid (20, 40, 60, 80, and 100 µg/ml) were prepared similarly. The mixture was incubated at 30°C for 90 minutes. The absorbance of the test and standard solutions was measured at 550 nm using a UV-Visible spectrophotometer, with the total phenolic content expressed as mg of gallic acid equivalent per gram of extract [40].

Gas chromatography and mass spectroscopy (GC–MS) analysis

Gas chromatography-mass spectrometry (GC-MS) was used to analyze the qualitative and quantitative identification of organic compounds in the methanolic leaf extracts of *Pluchea ovalis*. The analysis was conducted using a Shimadzu QP2010 GC-MS system. The GC employed a fused silica column (Elite-5 ms: 5% Diphenyl, 95% Dimethylpolysiloxane; 30 m × 0.25 mm × 0.25 µm df) with helium as the carrier gas at a constant flow rate of 1 ml/min. A 2 µl sample extract was injected into the instrument, and detection was performed using a Turbo Mass 5.2 software with a Turbo Gold mass detector. The GC conditions included an oven temperature of 110°C with a 2-minute hold, an injector temperature of 250°C, an inlet line temperature of 200°C, and a source temperature of 200°C. Mass spectra were recorded at 70 eV with a scan period of 0.5 seconds and fragments ranging from 45 to 450 Da. The MS detection was completed in 35 minutes. Interpretation of the mass spectra was conducted using the NIST and WILEY databases, which contain over 62,000 spectra. Unknown components were identified by comparing their spectra with those in the NIST and WILEY libraries [41-42].

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Results

Taxonomic classification of *Pluchea ovalis* (Pers.) DC.

Pluchea tomentosa DC. in Wt. Contrib. Bot. Ind. 16. 1834; FBI 3: 272. 1881; Gamble 2: 689. 1921, Hajra et al. in Flora of India 13: 155. 1995.

Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Asterales
Family	Asteraceae
Genus	*Pluchea*
Species	*Pluchea ovalis*

Morphological characters of *Pluchea ovalis*

Erect, woody sub-shrub, 1.2 m tall, stems striated, puberulous. Leaves simple, alternate, sessile, whitish tomentose below, glandular, elliptic or ovate-elliptic, 1.5-3.5 x 1-2.5 cm, auricled at base, serrate, acute to apiculate. Heads in compound corymbs, terminal and axillary, 6 mm, heterogamous, not rayed. Involucral bracts are multiseriate, pinkish-tinged, rigid, sparsely glandular, ovate, acute. Receptacle 2.5 mm across, slightly convex, fimbriate. Female florets filiform, tubular, glabrous, 3-lobed, exerted or inserted. Bisexual florets glabrous, 5-lobed. Stamens 5, anthers 2.5 mm, exerted, hood ovate, base sagittate. Pappus hairy, uniseriate, dry white, equal to those of bisexual florets, longer than the female florets. Achenes were long, ribbed, hairy. Rare in the stream banks of the forests. **Fl. & Fr.:** October – January (Fig: 1).

Fig: 1 A-D: Habitat of Pluchea ovalis

Qualitative phytochemical analysis of *Pluchea ovalis*

A qualitative assay of the leaf of *Averrhoa carambola* L revealed the presence of a diverse group of phytochemicals. The results are presented in the (Table-2 and figure 2) to enable their comparative study concerning the solvents used for extraction.

A phytochemical analysis approach detected several compounds in different solvent extracts. The methanol and chloroform extracts were found to include tannins, phenols, saponins, and alkaloids. Flavonoids were present in both the methanol and ethyl acetate extracts. The ethyl acetate and chloroform extracts were discovered to include coumarins, terpenoids, and steroids. All of the extracts included glycosides, except for the petroleum ether extract. The peroxide and chloroform extracts included phytosterols and fixed oils. Quinones were detected in all extracts, except for the ethyl acetate extract. Among all the extracts, only the methanol extract contained anthraquinones. Resins were found exclusively in the methanol and petroleum ether extracts. Both the ethyl acetate and methanol extracts were found to contain cardiac glycosides and leuco anthocyanins. The results differed according on the method of extraction. Numerous physiologically active phytochemicals were found in the extracts thanks to the method for extracts and screening tests (Table: 1 & fig: 2). The yields of petroleum ether, chloroform, ethyl acetate, and methanol crude extracts are 8.6%, 20.14%, 26.4%, and 38.18% respectively.

Table:2. Qualitative Phytochemical analysis of Pluchea ovalis

S. No	Phyto. Name	Pet. ether	Chloroform	Ethyl acetate	Methanol
1.	**Alkaloids** Mayer's	-	++	-	+++
2.	**Flavonoids** Lead acetate test	-	-	+++	+++
3.	**Saponins** Foam test	-	++	-	+++
4.	**Steroids & Terpenoids** Salkowki's test	-	++	+++	-
5.	**Phenols** Ferric chloride test	-	++	-	++
6.	**Tannins** Gelatin test	-	++	-	++
7.	**Glycosides** Borntrager's Test (Modified)	-	+++	++	+++
8.	**Coumarins** NaOH test	-	++	++	-
9.	**Phytosterols** Salkowski's test	++	+++	-	-
10.	**Quinones** Precipitate test	+	+	-	+++
11.	**Resins** Acetic anhydride test	+++	-	-	+++

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12.	**Cardiac Glycosides** Kellar - Kiliani	-	-	++	++
13.	**Leuco anthocyanins** Isoamyl alcohol test	-	-	++	+++
14.	**Anthraquinones** Borntrager's	-	-	-	+++
15.	**Fixed oils** Spot test/ Stain test	+++	+++	-	-

"+" = present; "-"= absent

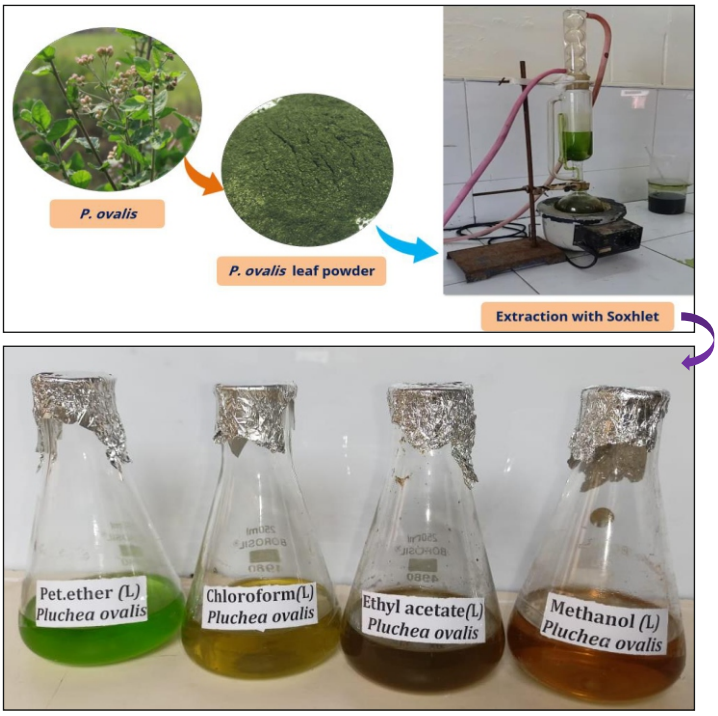

Fig:2. Phytochemical extractions

Quantitative phytochemical analysis of total alkaloids, flavonoids, phenols and tannins.

In order to gain a better understanding of the therapeutic potential of medicinal plants, phytochemical analysis is a crucial method. We take a close look at *Pluchea ovalis*, a plant that is well-known for the medicinal benefits it offers, in order to uncover the phytochemical components that it contains. An exhaustive investigation is carried out by the researchers in order to investigate the complexities of the leaf extract. As a result, they discover a complicated mixture of substances that contribute to the pharmacological significance of the leaf extract. When it comes to the intricate field of phytochemical analysis, alkaloids play an extremely important role. It is possible that there are particular solubility patterns within the plant matrix, as evidenced by the fact that they are not present in the petroleum ether and ethyl acetate fractions. The discovery of alkaloids in methanol, which displays a considerable concentration of 502 mg/g equivalent, highlights the usefulness of this solvent in extracting these biologically active molecules. Nevertheless, the discovery emphasizes the importance of methanol as a solvent. A considerable quantity of alkaloid content may be found in chloroform, specifically 310 mg/g equivalent that is present. The fact that it is useful in extracting phytochemicals is demonstrated by this.

There is a large variety of flavonoids found in *Pluchea ovalis*. Flavonoids are well-known for their antioxidant properties and have the potential to be used in medical applications. Flavonoids are more likely to be found in polar solvents, as evidenced by the fact that they are absent from the petroleum ether and chloroform fractions. It was discovered that methanol is an effective solvent, as it allows for the highest possible concentration of flavonoids to be produced, which is 520 mg/g equivalent. The fact that it is able to extract these components successfully is demonstrated with this. In spite of the fact that it is produced in substantially lower quantities, ethyl acetate nevertheless contains a considerable quantity of flavonoids, with a concentration of 440 mg/g equivalent. Therefore, this makes a contribution to the phytochemical profile of the extract as a whole. To a large extent, the medicinal efficacy of Pluchea ovalis can be linked to the major contribution of phenols and tannins, which are well-known for the wide variety of health-promoting qualities that they possess. It is important to note that the absence of these molecules in the petroleum ether and ethyl acetate fractions highlights the unique solubility qualities that they possess. Due to the fact that it has a considerable tannin content of 320 mg/g equivalent and a high concentration of 390 mg/g equivalent, methanol is the most popular choice for phenolic extraction.

In spite of the fact that it is present in lesser amounts, chloroform produces a significant enhancement of the phenolic and tannin reservoir, which in turn increases the extracts potential for theraptic use.

The changes in phytochemical content that have been observed shed insight on the intricate link that exists between the choice of solvent and the solubility of phytochemicals. In accordance with the particular polarity and affinity of each solvent, a distinct assortment of phytochemicals is selectively extracted, hence contributing to an increase in the total bioactive composition of the extract. Methanol is a highly effective solvent that can efficiently extract a variety of phytochemicals, including alkaloids, flavonoids, phenols, and tannins. This is because of the polar properties that methanol possesses. Chloroform, despite having a somewhat smaller capacity to extract, improves the extract by adding alkaloids, flavonoids, phenols, and tannins, even if these components may be present in slightly lower quantities in comparison to methanol. Not only does the comprehensive phytochemical study shed light on the intricate composition of the *Pluchea ovalis* leaf extract, but it also offers a glimpse into the medicinal potential that is contained within the botanical structure of the plant. The overall therapeutic efficacy of the extract is improved by the collaborative performance of each molecule, each of which possesses its unique pharmacological properties. As a result of scientific research that investigates the various intricacies of the chemical composition of *Pluchea ovalis*, a clearer grasp of its therapeutic qualities is emerging, which ultimately leads to the development of revolutionary treatments that are based on natural ingredients (Fig: 3).

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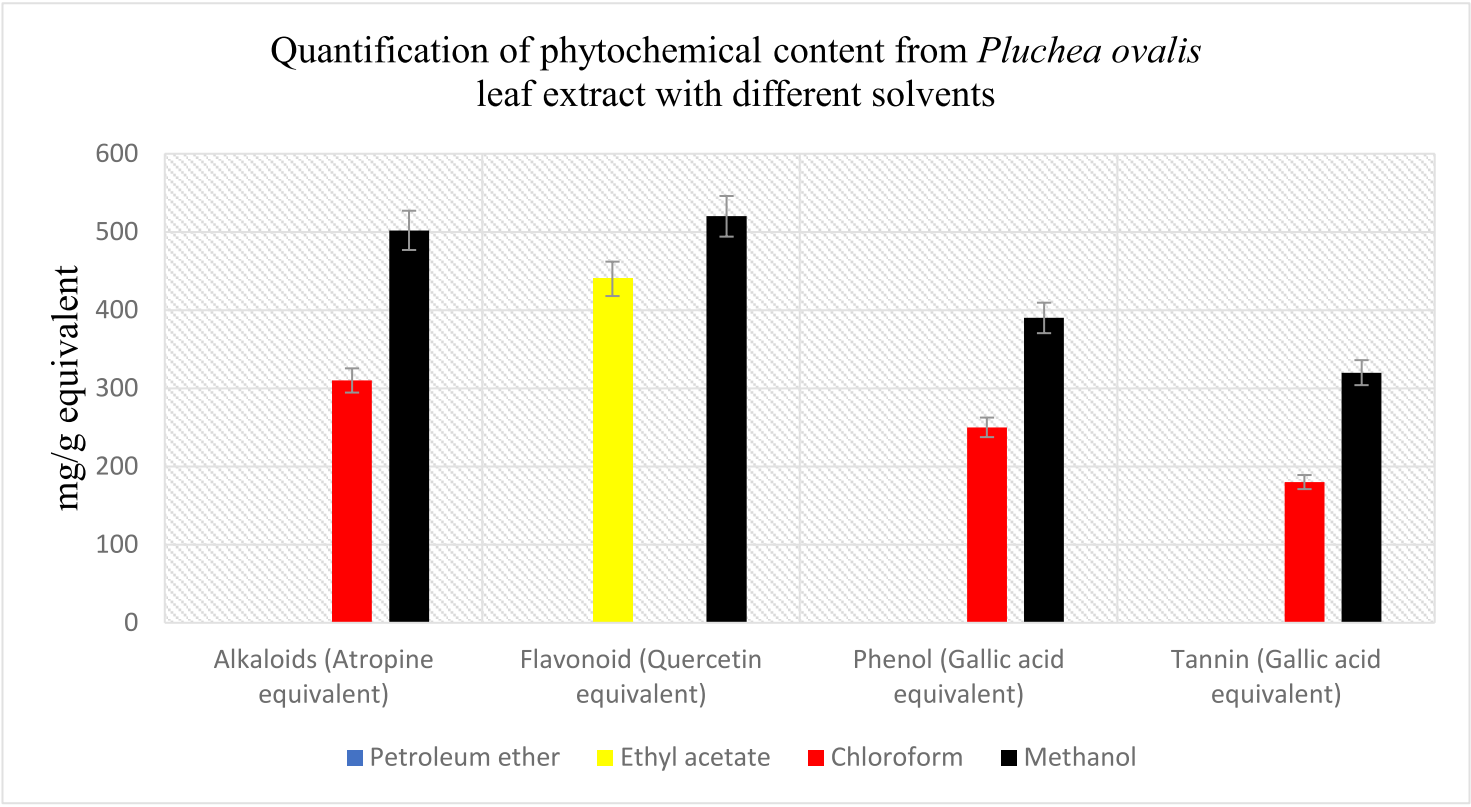
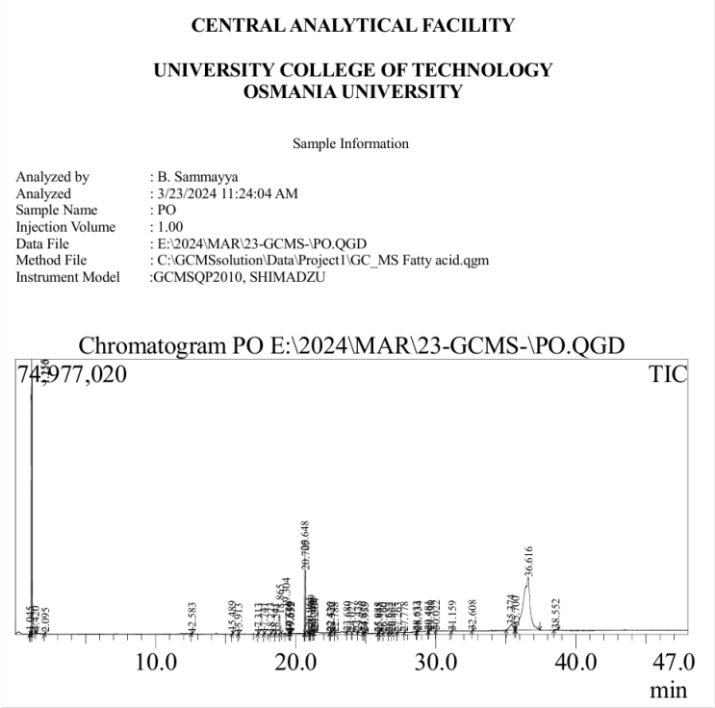


Fig: 3. Quantification of Phytochemicals of P. ovalis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of *Pluchea ovalis*

The methanol extract of the *Pluchea ovalis* leaf was analyzed using gas chromatography and mass spectrometry with a SHIMDZU QP-2020 Gas – Chromatography - Mass Spectrometry system. Table 3 presents the outcomes of the spectrum comparison conducted on chemical compounds that have not been discovered and those that have been recognized. The data exhibits 27 peaks that correspond to bioactive compounds with their biological activity. The results indicated that the single major component, dodecanoic acid, 1,2,3-propanetriyl ester ($C_{39}H_{74}O_6$), had a peak area of 38.16%, a molecular weight of 638, and was reported to have hypercholesterolemic, anti-arthritic, nematocidal and hepatoprotective activity. While 2-Ethoxyethylamine ($C_4H_{11}NO$), on the other hand, had the second-highest peak area, at 26.46%. not reported following by Tetraborane (10) (B_4H_{10}) peak area 19.61% with reported anti-microbial activity, 2-Ethoxyethylamine ($C_4H_{11}NO$); Tetraborane (10) (B_4H_{10}); Ethane, 1,1-diethoxy-($C_6H_{14}O_2$) ; Cyclooctasiloxane, hexadecamethyl- ($C_{16}H_{48}O_8Si_8$); Heptasiloxane, hexadecamethyl- ($C_{16}H_{48}O_6Si_7$); 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester ($C_{16}H_{22}O_4$); Octadecane ($C_{18}H_{38}$); Hexadecanoicacid, methylester ($C_{17}H_{34}O_2$); n-Hexadecanoicacid ($C_{16}H_{32}O_2$); Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl ($C_{16}H_{50}O_7Si_8$); 9-Octadecenoic acid, methyl ester($C_{19}H_{36}O_2$); 12-Methyl-E,E-2,13-octadecadien-1-ol($C_{19}H_{36}O$); 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-($C_{20}H_{34}O_2$); Eicosanoic acid, methyl ester ($C_{21}H_{42}O_2$); Docosanoic acid, methyl ester ($C_{23}H_{46}O_2$)
Tetracosanoic acid, methyl ester ($C_{25}H_{50}O_2$) Cyclohexane,1,1'-(1,5-pentanediyl) bis-($C_{17}H_{32}$);
2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene($C_{25}H_{42}$); Cyclononasiloxane, octadecamethyl-($C_{18}H_{54}O_9Si_9$); 2-Butenoicacid,2-methyl-,2(acetyloxy)1,1a,2,3,4,6,7,10,11,11a-decahydro-7,10-dihydrox($C_{27}H_{38}O_8$); Vitamin E acetate ($C_{31}H_{52}O_3$); Tetracosamethyl cyclododecasiloxane($C_{24}H_{72}O_{12}Si_{12}$); I-Stigmasterol($C_{29}H_{48}O$);gamma. Sitosterol($C_{29}H_{50}O$); Lup-20(29)-en-3-ol, acetate, (3.beta.)-($C_{32}H_{52}O_2$); Hexadecanoic acid, 2-[(1-oxododecyl)oxy]-1,3-propanediyl ester($C_{47}H_{90}O_6$);Dodecanoic acid, 1,2,3-propanetriyl ester($C_{39}H_{74}O_6$).this bioactive compounds biological activity reported in table


CENTRAL ANALYTICAL FACILITY

UNIVERSITY COLLEGE OF TECHNOLOGY
OSMANIA UNIVERSITY

Sample Information

Analyzed by	: B. Sammayya
Analyzed	: 3/23/2024 11:24:04 AM
Sample Name	: PO
Injection Volume	: 1.00
Data File	: E:\2024\MAR\23-GCMS-\PO.QGD
Method File	: C:\GCMSsolution\Data\Project1\GC_MS Fatty acid.qgm
Instrument Model	:GCMSQP2010, SHIMADZU

Chromatogram PO E:\2024\MAR\23-GCMS-\PO.QGD

Fig: 4. GC-MS analysis spectra of Pluchea ovalis

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Table:3. GC-MS analysis of Pluchea ovalis

Peak	R.Time	Area%	Height	Bioactive compound Name	Molecular formula & (Molecular weight)	Biological activity
1	1.155	26.46	74782975	2-Ethoxyethylamine	$C_4H_{11}NO$ 89	Anti-bacterial [43]
2	1.210	19.61	71680729	Tetraborane (10)	B_4H_{10} 54	Anti-microbial [44]
3	2.095	0.05	387784	Ethane, 1,1-diethoxy-	$C_6H_{14}O_2$ 118	Not reported
4	15.489	0.12	768801	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$ 592	Antimicrobial [45]
5	17.741	0.05	358406	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$ 532	Antibacterial [46]
6	18.223	0.06	304401	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$ 278	Antioxidant and Anti-inflammatory [47]
7	18.541	0.02	189875	Octadecane	$C_{18}H_{38}$ 254	Antibacterial [48]
8	18.865	0.82	5198647	Hexadecanoic acid, methyl ester	$C_17H_{34}O_2$ 270	Anticancer [49]
9	19.304	2.20	7009985	n-Hexadecanoic acid	$C_{16}H_{32}O_2$ 256	Antioxidant & anti-bacterial [50]
10	19.540	0.03	266493	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$ 578	Antimicrobial [51]
11	20.705	2.64	17145392	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$ 296	Not reported
12	21.160	0.52	1329025	12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$ 280	Antitumor [52]
13	21.364	0.36	1038441	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$ 306	Antileishmanial [53]
14	22.788	0.02	142411	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$ 326	Not reported
15	24.438	0.06	388248	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$ 354	Anticancer [54]
16	25.948	0.03	197157	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$ 382	Antibacterial [55]
17	26.260	0.02	126012	Cyclohexane, 1,1'-(1,5-pentanediyl)bis-	$C_{17}H_{32}$ 236	Not reported
18	26.652	0.04	229725	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	$C_{25}H_{42}$ 342	Not reported
19	26.862	0.04	223599	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$ 666	Antifungal [56]
20	27.263	0.04	216949	2-Butenoic acid, 2-methyl-, 2-(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-7,10-dihydrox	$C_{27}H_{38}O_8$ 490	Not reported
21	28.734	0.03	178119	Vitamin E acetate	$C_{31}H_{52}O_3$ 472	Wound healing [57]
22	29.481	0.02	157891	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$ 888	Hepatoprotective, antispasmodic, antirheumatic [58]
23	29.590	0.03	133058	I-Stigmasterol	$C_{29}H_{48}O$ 412	Apoptotic induction [59]
24	30.022	0.04	191547	.gamma.-Sitosterol	$C_{29}H_{50}O$ 414	Antibacterial [60]
25	32.608	0.05	225532	Lup-20(29)-en-3-ol, acetate, (3.beta.)-	$C_{32}H_{52}O_2$ 468	Not reported
26	35.700	0.26	931279	Hexadecanoic acid, 2-[(1-oxododecyl)oxy]-1,3-propanediyl ester	$C_{47}H_{90}O_6$ 750	Not reported
27	36.616	39.16	14374568	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$ 638	antiarthritic, nematocide, and hepatoprotective [61]

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Discussion

Pluchea ovalis (Pers.) DC. is erect, woody sub-shrub, 1.2 m tall, stems striated, puberulous, commonly known as "Woolly Camphor Weed", it is a tall shrub up to 2.5 m high, and distributed in throughout the world, in India Telangana Andhra Pradesh, Karnataka, Tamil Nadu and Kerala [62]. The plant leaves from *P. ovalis* (Asteraceae) about 1kg, were collected from Kondurg Mandal, Ranga Reddy District, Telangana state, India, during August / September in the year 2023.

According to reports, *P. ovalis* contains the main chemical components p-cymene, 2,5-dimethoxy-p-cymene, limonene, b-phellandrene, isocomene, b-maaliene, d-cadinene, b-caryophyllene, and a-cadinol. It also demonstrated significant growth inhibition of fungi using the agar well diffusion assay technique [63-64]. Presently we carried out the extraction process using Soxhlet apparatus with petrolueum ether chloroform ethyl acetate and methanol [66]; and the qualitative phytochemical analysis approach detected several compounds in different solvent extracts. The methanol and chloroform extracts contained tannins, phenols, saponins, and alkaloids. Both methanol and ethyl acetate extracts contained flavonoids. Coumarins, terpenoids, and steroids were found in ethyl acetate and chloroform extracts. All extracts except petroleum ether contained glycosides. Both peroxide and chloroform extracts included phytosterols and fixed oils. Quinones were found in all extracts except ethyl acetate. Methanol was the sole extract with anthraquinones. Only methanol and petroleum ether extracts included resins. Ethyl acetate and methanol extracts included cardiac glycosides and leuco anthocyanins. The highest yields methanol crude extracts are 38.18% and lower is petroleum ether, 8.6%.

Phenolic compounds and tannins, with over 8,000 known structures, play key roles in plant growth, reproduction, pigmentation, and protection from UV radiation and pathogens [69]. Flavonoids, the most common type of phenolic compounds, exhibit various pharmacological effects, including antibacterial, hepatoprotective, anticancer, antiviral, and anti-inflammatory activities [67-58]. Presently quantification of alkaloids in methanol at 502 mg/g equivalent shows its potential in extracting physiologically active compounds. However, the discovery highlights methanol's solvent value. Chloroform contains 310 mg/g equivalent alkaloids, while the flavonoids, with methanol producing the highest concentration [520 mg/g] and ethyl acetate producing the highest concentration is 440 mg/g. There are 390 mg/g of phenols and 320 mg/g of tannins in methanol, respectively.

GC-MS analysis has identified junenol as the major component of *Pluchea ovalis* essential oil, constituting 20.73% of the total composition, followed by α-cadinol (15.54%) and δ-cadinene (12.93%) among others [70]. The essential oil's chemical profile is crucial for studying its larvicidal activity, as the biological properties of phytochemicals depend on their structure and synergistic effects. Previous research also highlighted that sesquiterpenes are dominant in the essential oil, making up 52.85%, with oxygenated hydrocarbons at 40.88%, and selinene being a major component (33.40%) [71]. Present study confIrmed the methanolic leaf extract of *Pluchea ovalis* was identified by GC-MS analysis revealed 27 bioactive compounds were found out among these compound Dodecanoic acid, 1,2,3-propanediol ester was found to be the highest peak area of 39.16 % and possess potential antiarthritic, nematocide, and hepatoprotective [74]. The main constituents were 2-Ethoxyethylamine, (26.46%), with anti-bacterial activity reported by [75-76] while Tetraborane (10) (19.61%) with anti-microbial activity by [77].

Conclusions

Pluchea ovalis, a promising plant that is primarily responsible for a variety of biological characteristics, was found to have an abundance of phytochemicals in its methanolic extract, according to the findings of the study. When it comes to drug development, this study will prove to be helpful for researchers in the future when it comes to doing *in-silico* and cell-line studies for potential pharmacological lead molecules.

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