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PHYTOCOMPOUND ISOLATION, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF ARTEMISIA PALLENS

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ABSTRACT

Current study focussed about the isolation and identification of phytochemical constituents of the plant *Artemisia pallens*. Among the various solvents, methanolic solvent showed high yield of extract. Phytochemical analysis revealed the presence of metabolites in the methanolic extracts of *A. pallens*. FTIR and GCMS spectral analysis revealed the presence of active biocompounds in the extracts. Antimicrobial assay revealed the microbial growth inhibition properties. Finally, this study concluded that *A. pallens* contains various bioactive compounds with an efficient antimicrobial activity.

Keywords: Artemisia pallens, antibacterial, Phytochemical

INTRODUCTION

India is the biodiversity country rich with numerous flora and fauna. India's ecosystem ranged between Himalayas (temperate) to South India (tropical) and also between Central India (dry) to Assam (wet). It is also considered as a bioethical garden due to their diverse agro favoured climatic conditions which enhances various aromatic and medical plant growth **[1, 2]**.

In India, 34 different species of Artemisia (Class: Eudicots, Family: Asteraceae) are reported and nearly 400

species are reported in South America and South Africa. Artemisia is small herb rich with aromatic essential volatile oils which are widely used as perfumery, stimulant and vermifuge. It is also used as an antioxidant, antispasmodic, antimicrobial and antihelminthes **[3, 4]**.

Phytocompounds contains bioactive compounds which contains the pharmacognostical properties **[7, 8]**. These compounds are used as adjuvant in metabolism triggering drugs against various human ailments **[9, 10]**. For the phytocompounds quantification and identification in the plant extracts, FTIR and GCMS are considered as ideal techniques.

MATERIALS AND METHODS

Experimental plants and solvent extraction

The experimental plants *Artemisia pallens* **(Figure 1)** are procured from the flower vendors from local markets (Coimbatore, Tamilnadu). The samples are transported into the laboratory and washed which removes the dirt from the plants and then air dried in a shady place. Plant leaves are collected and ground well by using mortar with pestle. Powdered plant products are dissolved by using various solvents such as water, methanol, diethyl ether, chloroform and n-butanol **(Figure 2)**. For phytochemical screening, standard test and procedures are performed for flavonoids, alkaloids, terpenoids, phenols, carbohydrates **[11-13]**.

Characterization of Phytocompound: FTIR and GCMS

For the functional groups identification, the plant extract is subjected to the Fourier Transform Infrared spectroscopy with the ranges of 400 to 4000cm^{-1}. Gas chromatography mass spectroscopy is used for the identification of active compounds by using Shimadzu GC-2010 & QP2010 along with 0.25µm film in silica capillary column ZB-5 MS (0.25 mm i.d., 30 m). Samples are kept in an oven for 3min at 50°C and temperature increased up to 250°C by increasing 8°C/min. EI mode of the instrument is 40 to 500amu scan range, 0.20sec scan rate and 70eV ionisation energy. Ion source, injector and interface are maintained at 220, 250 and 250°C respectively. As a carrier gas helium is flowed into the split injection at 1mL/min. By using homologous n-alkanes series, the retention indices are studied and finally with the help of computer supported spectral library, the spectral results are obtained **[5, 6]**.

Figure 1: Experimental plant *Artemisia pallens*

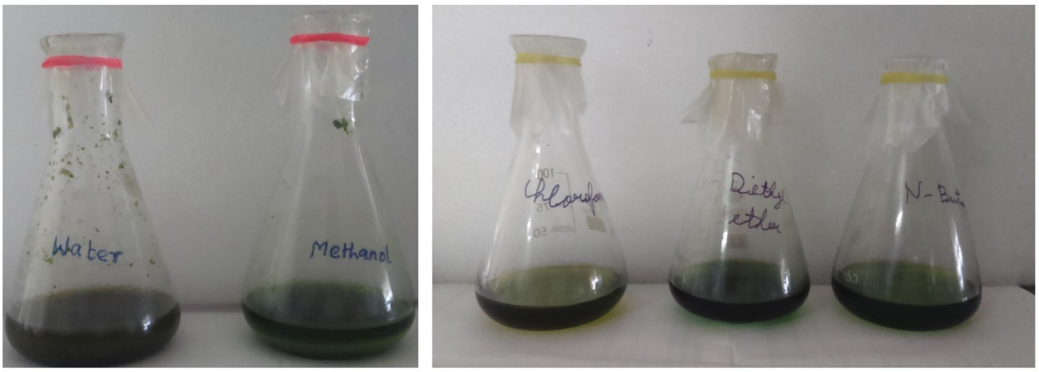

Figure 2: Different solvent (water, methanol, chloroform, diethyl ether, n-butanol) extractions

RESULTS AND DISCUSSION

Phytochemical identification

Five different solvents such as water, methanol, diethyl ether,chloroform and n-butanol are used to isolate the compound present in the *A. pallens*. By using standard procedures, ten different phytochemical compounds are identified. Among the five different solvents, methanol extracts showed positive results for six compounds. Among the ten compounds, Phenols and Tannins are observed in all the solvents except n-butanol **(Table 1)**.

Chloroform water, ethanol, chloroform and hexane solvents are used for the phytochemical analysis of the plants. The results showed the presence of saponins, tannin, phytosterols, carbohydrates, proteins, flavonoids and phenols. The isolated compounds showed pharmaceutical properties **[3]**.The phytochemical constituents of *Artemisia pallens* by using chloroform, chloroform water, ethanol and hexane extracts such as phytosterols, flavonoids, saponins, proteins, amino acid and carbohydrates**[14]**.

CHARACTERIZATION STUDIES

FTIR spectra showed various peaks with represents the active compounds in the methanolic extracts of *Artemisia pallens* **(Figure 3)**. High absorbance peaks are observed between 3800 to 3000cm^{-1}

represented the presence of hydroxyl groups whereas more number of peaks are observed between 1500 to 1000cm^{-1}represented the presence of C=C, C-O rings. Numerous peaks observed at 1100cm^{-1}represented the presence of primary and secondary metabolites. Peaks around 800-500cm^{-1}represented the presence of ester bonds. GCMS spectra showed the presence of active compound with high relative abundance at 19.92RT (Cyclopropane, 1-(1-hydroxy-1-heptyl)-2-methylene-3-pentyl-) **(Figure 4)**. The other sequential relative abundance and their respective RT are 34.80, 30.34, 27.08, 25.22, 22.70, 15.97 (Cinnamic acid, ethyl ester), 11.42 (3, 5-Octadien-2-ol), 9.06 (2(3H)-Furanone, 5-ethenyldihydro-5-methyl-) and 5.34 (Toulene) respectively.

The phytochemical analysis and characterization of medicinal plants by GCMS which concluded that the cinnamic acid and other derivatives showed antibacterial and antifungal properties was reported by **[16]**. Similar results are reported by **[15, 16]**.

Antimicrobial studies

Five microbes such as *B. subtilis*, *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus* are tested against water, methanol and chloroform extracts of *A. pallens* **(Figure 5)**. DMSO used as control which showed negative inhibition control. *Bacillus subtilis* growth is inhibited by all the solvent extracts of *A. pallens* **(Table 2)**. Among the tested five microbes, methanolic extracts showed high inhibition against *Bacillus subtilis*, *E. coli* and *K. pneumonia*.

In India, *A. parviflora* and *A. nilagirica* different extracts showed significant antimicrobial activities against various infectious microbes. Among the tested solvents, chloroform and ethanolic extracts of *A. nilagirica* showed increased inhibition activity **[17]**. The antimicrobial activities of *A. pallens* against various pathogens was also reported by **[18]**. Among the tested extracts, methanolic extracts showed high antibacterial activities against the tested the yeast and bacterial strains. This study supports our findings that *A. pallens* contains antimicrobial activity against human pathogens.

Table 1: Identification of phytochemicals in *Artemisia pallens*

S .No.	Tests	Water	Methanol	Diethyl Ether	N-Butanol	Chloroform
1	Alkaloids	+	+	-	+	+
2	Terpenoids	+	+	-	-	-
3	Phenol	+	+	+	+	-
4	Tannins	+	+	+	+	-
5	Sugar	+	-	-	-	-
6	Saponins	+	-	-	-	-
7	Flavonoids	+	-	-	-	-
8	Quinones	+	+	-	+	+
9	Protein	+	-	+	+	-
10	Steroids	-	+	-	-	+

+: presence; –: absence

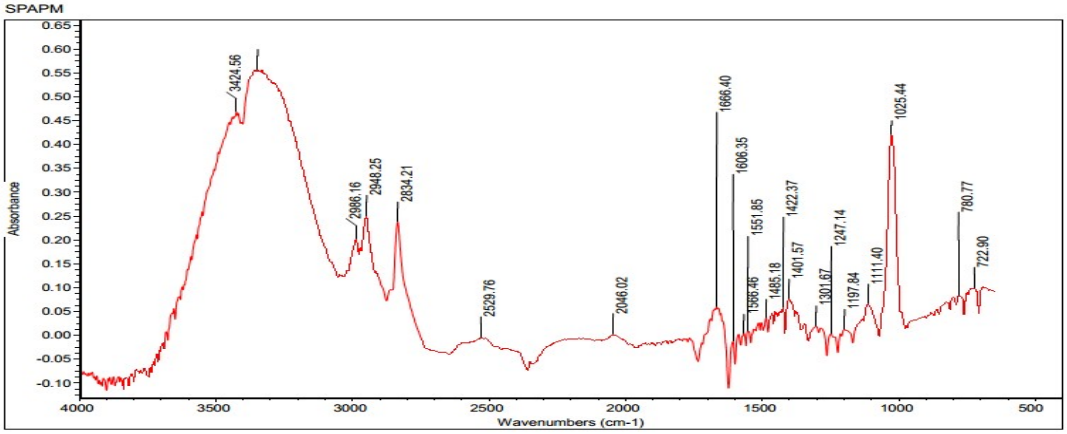

Figure 3: FTIR spectra of methanol extract of *A. pallens*

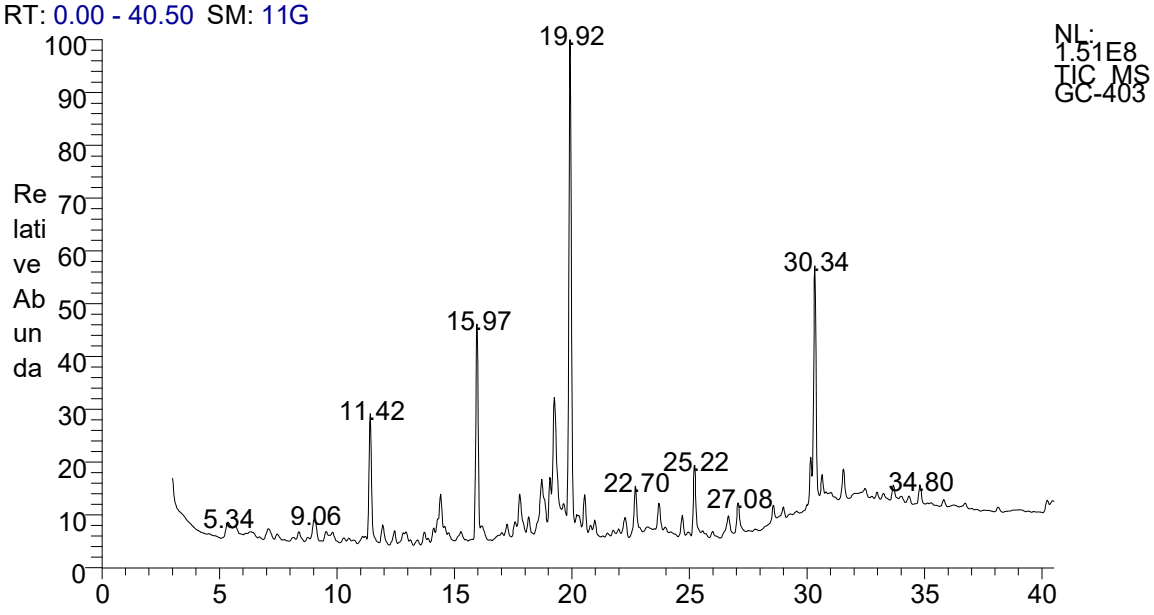

Figure 4: GCMS analysis of methanolic extracts of *A. pallens*

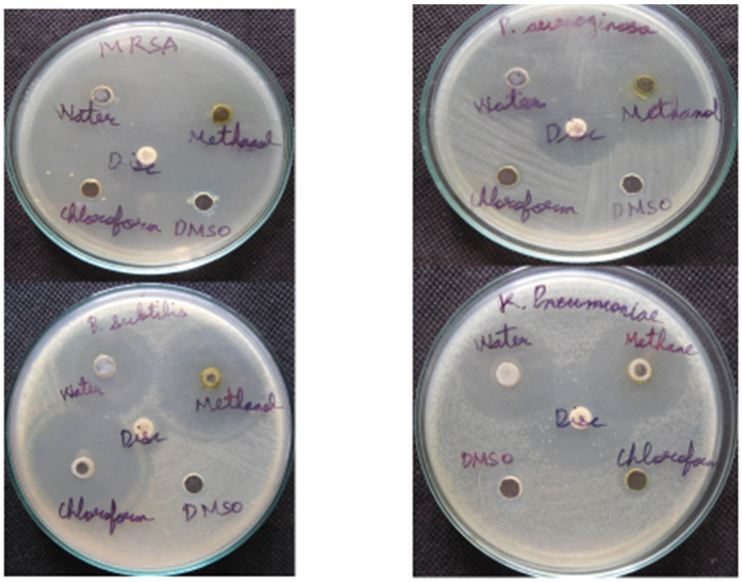

Figure 5: Antimicrobial analysis of *A. pallens*bydisc diffusion method

Table 2: Antimicrobial studies of *Artemisia pallens* extracts

Organism name	Inhibition zone (mm)				
	Disc	Water	Methanol	Chloroform	DMSO
Bacillus subtilis	4.1±0.1	14.6±0.3	15.2±0.4	18.5±0.8	Nil
E.coli	10.2±0.9	8.2±0.6	9.6±0.7	2.3±0.8	Nil
K. pneumoniae	12.4±0.2	11.4±0.8	12.3±0.5	3.7±0.3	Nil
Pseudomonas aeruginosa	8.5±0.7	10.2±0.6	5.9±0.3	8.3±0.9	Nil
Staphylococcus aureus	Nil	5.5±0.7	5.1±0.4	Nil	Nil

CONCLUSION

Our study concluded that methanolic extracts of Artemesia pallens contains various phytochemical components which are responsible for the primary and secondary metabolites. Characterization analysis revealed the presence of bioactive compounds which are responsible for many pharmacognostical activities. Antimicrobial studies evidenced the microbial growth inhibition of various pathogens.

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