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Original Research Article

Phytochemical Screening and Antimicrobial Activity of Artemisia Absinthium

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ABSTRACT

Extracts of *Artemisia absinthium* (wormwood) (Asteraceae) were screened for their antimicrobial activity by well diffusion method and phytochemical screening. The antimicrobial activity of water, methanol, ethanol and acetone extract of the plant were studied using *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhii*, *Streptococcus pneumoniae* as test microorganisms. The results reveal that the plant has shown significant activity against *Staphylococcus aureus*, *Escherichia Coli*, *Salmonella typhii*, *Streptococcus pneumonia*. Similarly the methanol and water extract of plant has shown good inhibitory activity against all test microorganisms indicating that the plant can fight these organisms effectively.

Keywords: *Artemisia absinthium*, antimicrobial activity, phytochemical screening, herbal extracts.

INTRODUCTION

Artemisia absinthium is a plant of subfamily *Asteroideae Artemisi absinthium* (wormwood) is an aromatic, perennial small shrub. *A. absinthium* is commonly used in food industry in the preparation of aperitives, bitters and spirits. Wormwood has been naturalized in Northeastern North America, North and West Asia, Africa and known to possess biological properties such as anthelmintic activity, [1] antimicrobial activity [2] antioxidant activity. [3] Plant's essential oil and bitter principles underlie its medicinal and commercial significance. It shows antimicrobial and antioxidants activities, [4] the characteristic bitterness of wormwood is due to the presence of sesquiterpene lactones such as absinthin, anabsinthin. The essential oil from shade dried leaves was found to contain alpha thujone, p-cymene, 1, 8-cineol, methyl heptenone, beta phlanderene, caryophylleneoxide, terpineol thujyl alcohol, geraniol, thujyl acetate. [5] *A. absinthium* has been used since ancient times for medical purposes. Overdoses of essential oil cause irritation in central nervous system that ultimately leads to unconsciousness or death. Thujone rich chemotypes of wormwood are not prefered due to their toxic effects.

Present work was carried out with the objective to investigate antimicrobial activity of *A. absinthium* against different microbial cultures and also to identify important phytochemicals present in *A. absinthium.*

MATERIALS AND METHODS

Collection of plant material and extraction

Leaves of *Artemisia absinthium* were collected from Kashmir, (India) durings winters. Leaves were collected, dried and coarsely powdered with pestle motar. Powder was subjected to extraction using soxhlet apparatus with methanol,

ethanol, acetone, water separately. 5g dried powder of *A. absinthium* loaded into main chamber of soxhlet extractor into which glass wool was placed. The temperature of distillation port was set to boiling point of the solvent used. Repeated cycles were allowed till the colored extraction mixture changes to colorless. Liquid extract was evaporated using water bath to get dried extract. Extract was weighed and dissolved in solvent to get a solution. Plant extract was used for antimicrobial activity and phytochemical analysis.

Test organisms

Cultures of four microbial strains *Staphylococcus aureus, Salmonella typhii, Escherichia coli, and Streptococcus pneumonia* were used. The mentioned bacterial isolates were grown in nutrient agar at 37°C for 24 h and subculture into nutrient broth by a picking off technique for 24 hours before use. Different extracts of *Artemisia absinthium* were tested for phytochemical screening and for antimicrobial activity against test organisms.

Antimicrobial agents Susceptibility test

Susceptibility to antimicrobial agents was determined by well diffusion method of Kirby Bauer on Mueller Hinton Agar as described by Clinical and Laboratory Standard Institute. Muller Hinton agar media was prepared and plates were swabbed for 24h with cultures of respective bacteria grown in nutrient broth over night. Agar plate wells were made using sterile cork borer and extract, with different concentrations put into wells. Plates were then incubated at 37°C for 24h. After incubation plates were observed for zone of inhibition and zone was measured with inhibition zone scale.

Phytochemical Screening of A. absinthium Extract:

Biochemical analysis: Phytochemicals were evaluated using the methodology described by Farnswoth. [6]

1. **Test for Steroids:** To 1 ml of extract, 1 ml of glacial acetic acid and 1 ml of acetic anhydride and 2 drops of conc. H_2SO_4 were added. If red then blue and finally bluish green color appears it shows the presence of steroids.
2. **Test for Saponins:** To 1 ml of extract, 5 ml of water was added and tube was shaken vigorously. Copious lather formation indicates the presence of saponins in sample.
3. **Test for Phenols:** To extract few drops of 10% aqueous ferric chloride was added. If blue or green color appears which indicates the presence of phenols.
4. **Test for Flavonoids:**
   Shinoda test: To extract, few magnesium turnings and 1-2 drops of conc. Hcl was added. Flavonoids are present if red color appears.
5. **Test for alkaloids:**
   Hager's test: To extract 3 ml of Hager's reagent was added. Formation of yellow precipitate indicates presence of alkaloids.
6. **Test for tannins:** To extract ferric chloride was added. Dark blue or greenish black color indicates the presence of tannins.
7. **Test for proteins:** To the extract, 1 ml of 40% sodium hydroxide solution and 2 drops of 1% copper sulphate solution was added. Violet color shows the presence of proteins.
8. **Test for amino acids:** Two drops of ninhydrin solution was added to the plant extract in order to show the presence of amino acid in the plant extract.
9. **Test for carbohydrates:**
   Fehling's test: To the extract, equal quantities of Fehling's solution A and B were added and on heating if brick red color appears, it shows the presence of carbohydrates.
10. **Test for quinones:** To 1 ml of extract, 1 ml of conc. Sulfuric acid was added. Appearance of red shows the presence of quinones.

High performance liquid chromatography (HPLC)

HPLC is a chromatographic technique used to separate a mixture of

compounds in analytical chemistry and biochemistry with the purpose of identifying, quantifying or purifying individual components of mixture. 0.1 gram of sample (*Artemisia absinthium*) was taken and dissolved in 10 ml of solvent. Sample was sonicated for 10 minutes in an ultra sonicator. Sample was then filtered using whattman filter paper and diluted to 1000 ppm. HPLC analysis of sample was performed in C18 column which was observed at 216 nm, 1000ppm of sample was loaded after getting baseline. Quercetin, rutin, gallic acid and kaempferol were used as standards. Phenolic acids present in each sample were identified by comparing chromatographic peaks with the retention time (Rt) of individual standards and further confirmed by co- injection with isolated standards. The amount of each phenolic acid is expressed as micrograms per gram of fresh weight.

RESULTS AND DISCUSSION

Effectiveness of different extracts is determined by the size of the control organism growth inhibition zone around the well (diameter of zone in mm).

In Table 1 methanol extract showed larger inhibition zone against *Escherichia coli* as compared to *Staphylococcus aureus, Salmonella typhii, Streptococcus pneumoniae.* It showed highest inhibition zone of 35mm in concentration of 23mg/150µl. In table 2 ethanol extract showed larger inhibition zone against *Escherichia coli.* as compared to *Staphylococcus aureus, Salmonella typhii, Streptococcus pneumoniae.* It showed highest inhibition zone of 29mm in concentration of 30mg/150µl. In table 3 acetone extract showed larger inhibition zone against *Escherichia coli.* as compared to *Staphylococcus aureus, Salmonella typhii, Streptococcus pneumoniae.* It showed highest inhibition zone of 29mm in 27mg/150µl. In table 4 water extract showed larger inhibiton zone against *Salmonella typhii* as compared to *Staphylococcus aureus, Streptococcus pneumonia, Escherichia coli.* It showed highest inhibition zone of 20mm in 139mg/150µl. Phytochemical screening of *A. absinthium* showed the presence of constituents like saponins, flavonoids, phenols, tannins, quinones, steroids and absence of constituents like alkaloids, carbohydrates, amino acids and proteins in both methanol and ethanol extract. Acetone extract showed the presence of phenols, tannins, quinones, alkaloids, carbohydrates and absence of constituents like saponins, flavonoids, amino acids, proteins, steroids. Water extract showed the presence of saponins, phenols, tannins, quinones, carbohydrates, Steroids and absence of constituents like flavonoids, alkaloids, amino acids and proteins.

HPLC analysis

The HPLC analysis of plant extract of *A. absinthium* showed four types of Flavonoids i.e. quercetin, rutin, gallic acid, kaempferol that are present in varying amounts in the different solvent extracts (Table 5 and Figure 3, 4).

The extracts showed apparent effect in methanol extract and ethanol extract and moderate effect against water and lesser effect with acetone solvent .The phytochemical evaluation of plant is achieved through biochemical testing and HPLC analysis.

Through biochemical testing the important constituents present in plant extract are Flavonoids, phenols, Saponins, Tannins, Alkaloids, and Quinones and through HPLC analysis the important Flavonoids present in plant extract are Quercentin, Rutin, Kaempferol, and Gallic acid. [7] Due to presence of these important phytochemicals the plant (*Artemisia absinthium*) possess the activity like antimicrobial, antioxidant and antimalarial. As the plant posses such important activities the herbal extract of plant may be used as medicine against microbial infection.

Table 1: Table shows antimicrobial activity of *A. absinthium* extract in methanol solvent

Name of micro organism	Inhibition Zones at concentration (20mg)	Inhibition Zones at concentration (30mg)
Staphylococcus aureus	12mm	13mm
Salmonella typhii.	10mm	15mm
Escherichia coli.	32mm	35mm
Streptococcus pneumoniae.	Nil	Nil

Table 2: Antimicrobial activity of *A. absinthium* extract in ethanol solvent

Name of micro organism	Inhibition Zones at concentration (20mg)	Inhibition Zones at concentration (30mg)
Staphylococcus aureus	18mm	21mm
Salmonella typhii.	10mm	15mm
Escherichia coli.	12mm	16mm
Streptococcus pneumoniae.	Nil	Nil

Table 3: Antimicrobial activity of *A. absinthium* extract in acetone solvent

Name of micro organism	Inhibition Zones at concentration (20mg)	Inhibition Zones at concentration (30mg)
Staphylococcus aureus	10mm	12mm
Salmonella typhii.	NIL	NIL
Escherichia coli.	14mm	15mm
Streptococcus pneumoniae.	16mm	18mm

Table 4: Table shows antimicrobial activity of *A. absinthium* extract in water solvent

Name of micro organism	Inhibition Zones at concentration (20mg)	Inhibition Zones at concentration (30mg)
Staphylococcus aureus	14mm	16mm
Salmonella typhii.	NIL	NIL
Escherichia coli.	18mm	20mm
Streptococcus pneumoniae.	15mm	17mm

Table 5: Table shows Flavonoids extract in *A. absinthium*.

Plant extract in solvent	Flavonoids (mg/g of dry weight)			
	Quercetin	Rutin	Gallic acid	Kaempferol
Methanol	0.003	0.390	36	0.160
Ethanol	0.009	0.110	2	0.630

Figure 1: Antimicrobial susceptibility test: showing the resistances of *A. absinthum* against herbal extract

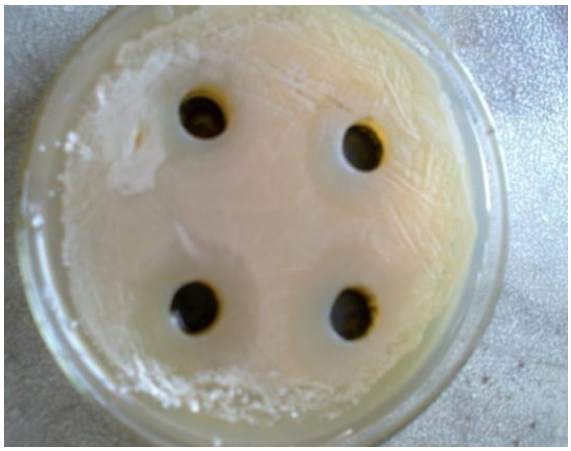

Figure 2: Antimicrobial susceptibility test: showing sensitivity of *A. absinthum* against herbal extract

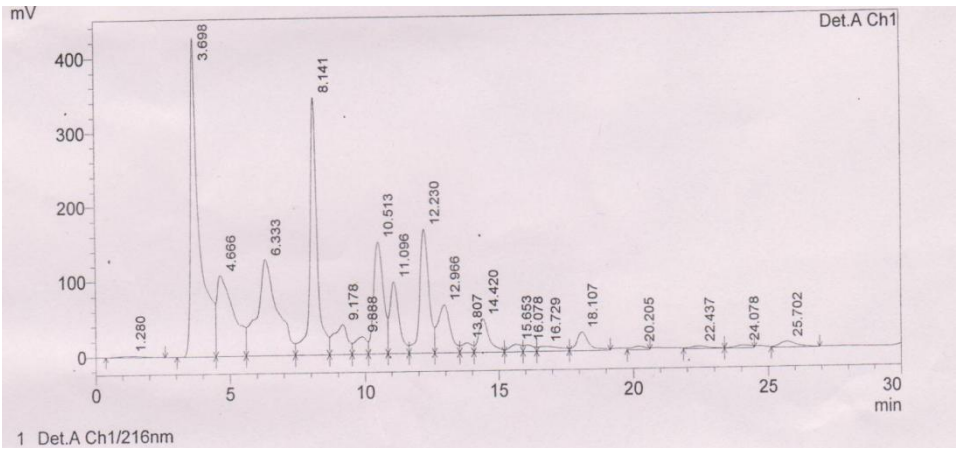

Figure 3: HPLC chromatogram of *Artemisia absinthium* in methanol

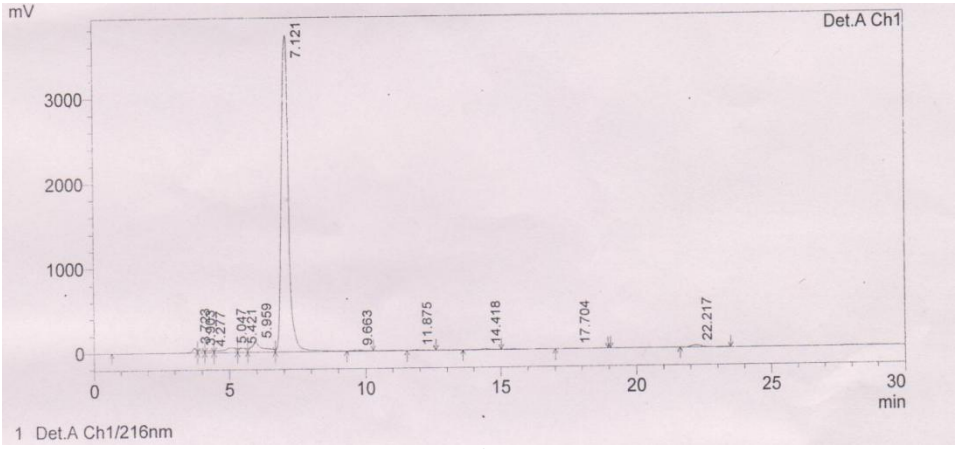

Figure 4: HPLC chromatogram of *Artemisia absinthium* in ethanol.

CONCLUSION

It can be concluded from the results that *Artemisia absinthium* plant leaves posses antimicrobial activity against test micro organism and also possess important phytochemicals. This means that the compound responsible for antimicrobial activity is present in each extract at different concentrations. The chance to find antimicrobial activity was apparent in methanol and ethanol extracts. The phytochemicals present in the extracts may be responsible for antimicrobial activity.

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