Phytochemical Screening and Antimicrobial Activity of *Artemisia annua* leaves

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Abstract- Extracts of *Artemisia annua* 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, Escheri-chia coli, Salmonella typhii, Streptococcus pneu-moniae* 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 annua, Asteraceae,* antimicrobial activity, phytochemical screening, herbal extracts.

Introduction

Traditional remedies have been used for thousand years for the prevention and treatment of infectious diseases, particularly in developing countries. Of growing interest, the plant *Artemisia annua*, known for its malarial properties, has been studied for its numerous biological activities including metabolic, anti-tumour, anti-microbial and immune modulatory properties. *Artemisia annua* is very rich in secondary metabolites such as mono terpenes, sesquiterpenes and phenolic compounds, of which the biological properties have been extensively studied. The purpose of this review is to gather and describe the data concerning the main chemical components produced by *Arte-misia annua* and to describe the state of the art about the biological activities reported for this plant and its compounds beyond malaria. The family *Asteraceae* comprises a wide number of genera, of which the genus *Artemisia* is one of the largest and most widely distributed worldwide[1]. *Artemisia annua* is a perennial herb growing up to 50-130 centimetres. The rootstock is thick, woody, and has a strong smell. The leaves are clustered at the rounded apex. The leaf blade is spatulate and oblong-obovate to broadly spatulate or flabellate. The achenes are brown and obovoid.

Artemisia annua has been used in traditional medicine for many years in Asia and Africa for

the treatment of malaria and fever, in the form of tea or pressed juice[2,3]. *Artemisia annua* is also described to have anti-hyperlipidemic, anti-plasm-odial, anti-convulsant, anti-inflammatory, anti-microbial, anti-cholesterolemic and antiviral properties[4,5,6]. *Artemisia ann-ua* would also have important pharmacological activities such as anti-inflammatory, antitumor and anti-obesity activities that contribute to the therapeutic effects of the plant[7,8,9].

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

Materials and Method

The plant material was collected from Khirshu Pauri Garhwal (India) during September 2024, dried in shade 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. annua* 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 coloured extraction mixture changes to colourless. 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 42°C for 18 hrs and sub culture into nutrient broth by a picking off technique for 18 hours before use. Different extracts of *Artemisia annua* 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 24hr with cultures of respective bacteria grown in nutrient broth overnight. Agar plate wells were made using sterile cork borer and extract, with different concentrations put into wells. Plates were then incubated at 42°C for 18hrs. After incubation plates were observed for zone of inhibition and zone was measured with inhibition zone scale.

Phytochemical Screening of *A. annua* Extract

Biochemical analysis: Phytochemicals were evaluated using the methodology described by Farnsworth[10].

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 colour appears it shows the presence of steroids.
2. **Test for alkaloids (Hager's test)**- To extract 3 ml of Hager's reagent was added. Formation of yellow precipitate indi-cates presence of alkaloids.
3. **Test for tannins**- To extract ferric chloride was added. Dark blue or greenish black colour indicates the presence of tannins.
4. **Test for proteins**- To the extract, 1 ml of 40% sodium hydroxide solution and 2 drops of 1%

copper sulphate solution was added. Violet colour shows the presence of proteins.

5. **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.
6. **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 colour appears, it shows the presence of carbohydrates.
7. **Test for quinones**- To 1 ml of extract, 1 ml of conc. Sulfuric acid was added. Appearance of red shows the presence of quinones
8. **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.
9. **Test for Phenols**-To extract few drops of 10% aqueous ferric chloride was added. If blue or green colour appears which indicates the presence of phenols.
10. **Test for Flavonoids-** Shinoda test: To extract, few magnesium turnings and 1-2 drops of conc. HCl was added. Flavonoids are present if red colour appears.

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 38 mm in concentration of 25mg/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 27mm in concentration of 25mg/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 31 mm 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 20 mm in 139mg/150µl. Phytochemical screening of *A. annua* 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.

Table-1 Table shows antimicrobial activity of *A. annua* extract in MeOH

S.No.	Name of micro-organism	Inhibition Zones at concentration (25mg)	Inhibition Zones at concentration (30mg)
1.	*Staphylococcus aureus*	13	15
2.	Salmonella typhii.	11	16
3.	Escherichia coli	38	35
4.	Streptococcus pneumoniae	Nil	Nil

Table-2 Table shows antimicrobial activity of *A. annua* extract in EtOH

S.No.	Name of micro-organism	Inhibition Zones at concentration (25mg)	Inhibition Zones at concentration (30mg)
1.	*Staphylococcus aureus*	18	15
2.	Salmonella typhii.	11	16
3.	Escherichia coli	27	35
4.	Streptococcus pneumoniae	Nil	Nil

Table-3 Table shows antimicrobial activity of *A. annua* extract in Acetone

S.No.	Name of micro-organism	Inhibition Zones at concentration (25mg)	Inhibition Zones at concentration (30mg)
1.	*Staphylococcus aureus*	13	12
2.	Salmonella typhii.	Nil	Nil
3.	Escherichia coli	15	15
4.	Streptococcus pneumoniae	17	18

Table-4 Table shows antimicrobial activity of *A. annua* extract in Water

S.No.	Name of micro-organism	Inhibition Zones at concentration (25mg)	Inhibition Zones at concentration (30mg)
1.	*Staphylococcus aureus*	14	15
2.	Salmonella typhii.	Nil	Nil
3.	Escherichia coli	15	20
4.	Streptococcus pneumoniae	19	17

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 annua*) possesses the activity like antimicrobial, antioxidant and antimalarial. As the plant possesses such important activities the herbal extract of plant may be used as medicine against microbial infection

Conclusions

It can be concluded from the results that *Artemisia annua* plant leaves possess antimicrobial activity against test microorganism 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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Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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