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Antimicrobial Investigation of *Artemisia annua* Leaf Extract against Human Pathogenic Microorganisms

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Abstract* – An extract of *Artemisia annua*were screened for phytochemical analysis and antimicrobial activity against different pathogenic microorganisms. The solvent used for extraction is methanol. Phytochemical analysis of *Artemisia annua* leaf extract shows presence of carbohydrate, protein alkaloids, tannin, flavonoids, steroids and saponins. The results showed that methanol leaf extracts of *Artemisia annua* inhibited growth of some microorganism. Agar well diffusion method was used for study of antimicrobial activity of leaf extract. Standard antibiotic (Erythromycin) and 5% DMSO were used as a positive and negative control respectively. In result *Artemisia annua* leaf extract shows strong antibacterial activity against *Klebsiella pneumonia, Staphylococcus aureus, Bacillus cereus* and *Bacillus licheniformis.

***Keywords* – *Artemisia annua,* Antimicrobial Activity, Methanol, Agar Well Diffusion Method.**

I. INTRODUCTION

The medicinal plant can be used for production of secondary metabolites which generally have medicinal properties. Secondary metabolites with antimicrobial activity have potential to be use as a drug in place of chemical drugs. The overuse of chemical drugs results in the tolerance of the body to that particular drug.The use of herbal extract to treat disease is ancient practice which have potential to replace chemical drugs.(1)

Artemisia annua is also known as sweet wormwood, sweet annie, sweet fern, sweet sagewart or annual warmwood. The plant extract have antimicrobial activity against different pathogenic microorganisms.*Artemisia annua* is a perennial herb originally native to Asia but now grows naturally worldwide. It has long been recognized by traditional Chinese medicine as a useful plant and Chinese handbook of prescription for emergency treatments recommended a tea made from the dried leaves as a treatment for fever.(2)

II. MATERIALS AND METHODS

Plant material-

Fresh leaves of *Artemisia annua* were collected fromVillholi farm of K. K. Wagh College of Agricultural Biotechnology, Nashik, India. These fresh leaves were washed thoroughly with tap water and then by distilled water and then shade dried for 6-7 days. The dried leaves were powdered with the help of mixer and grinder, and 10 gm of powder used for the extract preparation.

Preparation of Extract-

For the preparation of plant extract 10 gm of leaf powder was extracted in a Soxhlet Apparatus using 100 ml of methanol solvent. The extract were concentrated using rotary evaporator. The extract obtained were weighed and stored at 4°C. 75 mg of solvent residue was dissolved in 5 ml of DMSO (5%) as a solvent and were used as the test extracts for antimicrobial activity.(3)

Phytochemical analysis-

Thephytochemical analysisof *Artemisia annua*leaf extract was out using standard procedures to identify the phytochemical constituents.(4)

a)Alkaloids- 0.5 g of each sample were dissolved with 5 ml of 2 N HCl and filtered. Filtrate was treated with Dragendroff's reagent. Formation of red precipitate indicates the presence of alkaloid.

b) Phenols- Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

c) Carbohydrates- To 1 ml of the filtrate, 5 ml of Benedict's reagent were added. The mixture was heated. Appearance of red precipitate indicated the presence of reducing sugar.(5)

d) Glycosides- 0.5 g of each extract was stirred with 10 ml of boiling distilled water. This was filtered and 2 ml of the filtrate hydrolyzed with a few drops of concentrated HCL and the solution rendered alkaline with a 5 drops of ammonia solution. 5 drops of this solution was added to 2 ml of Benedict's qualitative reagent and boiled. Appearance of reddish brown precipitate showed the presence of glycosides.

e) Terpenoids- 5 ml of extract was mixed in 2 ml of chloroform and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown colour formation shows positive results for the presence of terpenoids.(6)

f) Steroids- The extract was mixed with 2 ml of chloroform and concentrated sulphuric acid was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids.

g) Proteins (Millon's test)- Small portion of the extract when mixed with 2 ml of Millon's reagent, white precipitation appeared which turned red upon gentle heating that confirmed the presence of protein.(7)

Maintenance of pathogen-

Bacterial cultures of *Klebsiella pneumonia, Staphylococcus aureus, Bacillus cereus* and *Bacillus licheniformis* were procured from Government medical college, Nashik. All the cultures were stored at 4°C.

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Antimicrobial Assay-

Antimicrobial activity of *Artemisia annua leaf* extract was determined by Agar Well Diffusion Method on Nutrient Agar medium.[8] A well with diameter 5mm was made using sterile cork borerand inoculums of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50mg each of solvent extracts were poured in the wells of the inoculated plates. The plates were incubated for 24hrs. at 37°C and zone of inhibition if any around the wells were measured in mm

III. Result

After extraction of *Artemisia annua* leaf extract in Soxhlet using methanol as a solvent, extract was subjected to phytochemical analysis result of phytochemical analysis is summarized in Table 1. The zone of inhibition producedby Artemisia annua leaf extract against beneficial microorganisms and plant pathogenic microorganisms is observed and noted in Table 2. Standard antibiotic (Erythromycin) was used as a positive control and solvent preservative DMSO (5%) was used as a negative control.

Table 1: Phytochemical analysis of methanol extract of Artemisia annua leaf extract

S.No	Organic Solvent / Compounds	Methanol Extract
1	Protein	+
2	Carbohydrates	+
3	Alkaloids	+
4	Tannins	+
5	Flavonoids	+
6	Steroids	-
7	Saponins	+

Table 2: Antimicrobial activity of Artemisia annuamethanolicleaf extract.

S. No.	Organisms	Measurement of Zone of Inhibition *Artemisia annua*leaf extract (in mm)	Measurement of Zone of Inhibition of Erythromycin (in mm)
1	*Klebsiella pneumonia*	16mm	18mm
2	*Staphylococcus aureus*	17mm	16mm
3	*Bacillus cereus*	12mm	14mm
4	*Bacillus licheniformis*	18mm	19mm

IV. Discussion

In the present study, we have carried out the phytochemical analysis of methanol extract of *Artemisia annua*leaves. Alkaloids, Phenols, Carbohydrates, Terpenoids, flavonoids, Saponins and proteins are present in extracts all these biochemical compounds are biologically active compounds. In antimicrobial study we have investigated that *Artemisia annua*leaf extract shows antimicrobial activity against human pathogenic microorganisms like *Klebsiella pneumonia, Staphylococcus aureus, Bacillus cereus* and *Bacillus licheniformis* by forming zone of inhibition of 16mm, 17mm, 12mm and 18mm respectively.

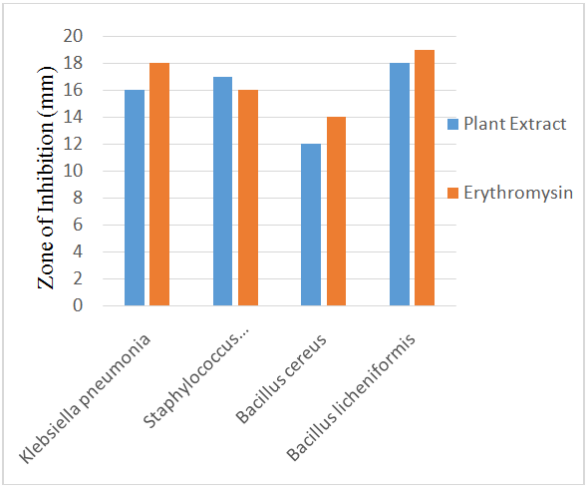

Fig.1. Antimicrobial activity of *Artemisia annua*methanolic leaf extract against human pathogenic bacteria

Fromall these study we canconclude that *Artemisia annua*is medicinal plant and leaves of *Artemisia annua* can be used to cure human diseases like fever, headache and dysentery.

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