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ANTIBACTERIAL ACTIVITY OF *GOMPHRENA SERRATA* L. AGAINST HUMAN PATHOGENS

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Gomphrena serrata L. (Amaranthaceae) is a medicinally important plant used in India since long. The present investigation was carried out in order to assess the antibacterial property of the ethanolic and aqueous extracts of *Gomphrena serrata* L. on the common human pathogens *viz. Escherichia coli*, *Vibrio harveyi*, *Staphylococcus aureus* and *Bacillus cereus* by Kirby- Bauer disc diffusion assay. The antibacterial activity of the biosynthesized silver nanoparticles was also carried out. The preliminary phytochemical screening revealed the presence of alkaloids, tannins, phenols, glycosides, saponins, flavonoids, protein, carbohydrate, and terpenoids in both aqueous and ethanolic extracts. Both the extracts showed similar activities against all four strains of bacteria. Nanoparticle solution showed significant activity against *Escherichia coli* with an inhibition zone of 11mm. Maximum zone of inhibition was exhibited by the standard antibiotic drug Amoxycillin. Further characterisation and screening are essential for finding out the actual principle behind the antibacterial property.

Key words: Antibacterial, *Gomphrena serrata* L., Human Pathogen, Nanoparticle and Phytochemical screening.

Introduction

Plants are a rich source of bioactive constituents with diverse pharmacological properties and medicinal values. Medicinal plants have been playing a vital role in health and healing of man since many centuries. The extraction and characterization of phytochemicals from plants have resulted in the discovery of novel drug entities with high therapeutic value [1]. Medicinal plants are the sources of natural drugs that are potentially safe drugs and have been tested for biological, antimicrobial and hypoglycemic activity, and some have been effectively deployed in the modern medicine[2]. There are many plants which possess free radical scavenging activity and antioxidant potential[3].

Gomphrena serrata L. is such a plant with lot of medicinal values. It belongs to family Amaranthaceae. *Gomphrena serrata* is found in America, Australia, and Indo-

Malaysia and Brazil. In India it is commonly found in states of Assam, Bihar, Gujarat, Kerala, Karnataka, Odisha and Tamil Nadu. It is usually found growing in dry fields open places and along road sides. All parts of this plant are widely used as a folklore medicine for the treatment of various ailments by Indian traditional healers, such as respiratory diseases like asthma, gastrointestinal conditions like diarrhoea, gastric disturbances, piles, skin diseases like dermatitis, as antimicrobial, anticancer, antimalarial, analgesic, as tonics and carminatives and allergic conditions like hay fever etc. [1]. Traditionally, the plant is utilized in the remedy of bronchial asthma, diarrhoea, hay fever, pains, tonic, carminative, diabetes, dermatitis and piles [4, 6].

The present study focuses on the preliminary phytochemical analysis, antibacterial activity and silver nanoparticle assay of *Gomphrena serrata* L. The medicinal properties of the plants are due to the phytochemicals present in it; they are non-nutritive chemicals that protect human from various. Discovery of new phytochemicals with therapeutic value leads to the establishment of several pharmaceutical industries [7]. The phytochemical constituents play a significant role in the identification of crude drugs. Plants are also has been reported for its antimicrobial properties. Hence, the antibacterial activity against common human pathogens were carried out.

Nowadays plant extracts act as reducing as well as capping agents for the synthesis of nanoparticles which is more advantageous than chemical and microbial synthesis. Biological methods of nanoparticle synthesis by using microorganisms such as bacteria, fungus, algae, enzymes and plants both intra-cellularly and extra-cellularly is an eco-friendly process. Among the metal nanoparticles silver has been consumed largely due to their antimicrobial and pharmaceutical applications [8, 10].

Material and Methods

Collection and identification of plant materials

Whole plant of *Gomphrena serrata* L. were collected from Panampilly Nagar, Ernakulam, Kerala, India. The plant specimen was identified using standard authentic literature [1, 11].

Preparation of the plant extract

The whole plants collected were washed under running tap water to get rid of dust particles, cut into small pieces, shade dried and then homogenized to fine powder and stored in sterile air tight bottles for the experimental work. The aqueous and ethanolic extracts were prepared by weighing 20gm of each of the powdered samples and mixed thoroughly with 200ml of each solvent. They were allowed to soak in the solvent for 48 hours at room temperature. The extracts were then filtered through Whatman no. 1 filter paper. The ethanolic extract obtained was air dried and later in a water bath. The aqueous extract obtained was evaporated at 50°C in hot air oven. The extracts were then dissolved in known amount of distilled water for further studies.

Preliminary phytochemical screening

The plant extracts were tested for the presence of various phytochemicals by using standard methods [12].

Silver Nanoparticle Assay

Silver Nanoparticle assay was carried out in order to find out whether the plant biosynthesized silver nanoparticle or not. The powdered samples were used for the assay [8].

Preparation of silver nitrate solution

2 mM silver nitrate solution was prepared by adding 0.0339g of silver nitrate in 100 ml

of double distilled water. The solution was mixed thoroughly and stored in brown coloured bottle in order to prevent auto-oxidation of silver.

Preparation of the plant extract

25 gm of the powdered sample was taken in 250 ml beaker and boiled along with 100 ml distilled water. After 10 minutes of boiling solution was cooled to room temperature and filtered using Whatman's no. 1 filter paper. The collected extract was used for the synthesis of silver nanoparticles.

Synthesis of silver nanoparticles

10 ml of extract was added to 90 ml of 2 Mm aqueous silver nitrate solutions (1:9 ratio) and mixed thoroughly by manual shaking. The beaker was then placed under sun light for reduction into silver nitrate nanoparticle. After 10 minutes colour changes were noted. This indicates the initial confirmation of the formation of plant mediated silver nanoparticles.

Purification of silver nanoparticles

After 5 hours, grey nanoparticles started to settle at the bottom. The solution was centrifuged at 8000 rpm for 15 minutes, supernatant was discarded and the pellet containing nanoparticles were taken out on a petri plate and kept in hot air oven to dry at 50° C for 4-5 hours. The silver nanoparticles were then taken out on a glass slide and observed under 40 X resolution of the microscope (Biolinkz M2000 series) and photographs were taken.

Antibacterial Assay

Kirby- Bauer disc diffusion method was performed for the antibacterial assay [13]. Four different bacterial strains were used in the present study which belonged to gram-positive (*Staphylococcus aureus*, *Bacillus cereus*) and gram-negative categories (*Escherichia coli*, *Vibrio harveyi*). *Bacillus cereus* is a gram positive, rod shaped, facultatively anaerobic bacterium responsible for food borne illness like severe nausea and vomiting. *Staphylococcus aureus* are gram positive, facultatively anaerobic, round shaped bacterium responsible for common cause of skin infections including abscesses, respiratory infections such as sinusitis and food poisoning. *Escherichia coli* are gram negative, rod shaped, facultative anaerobes, commonly found in the lower intestine of warm-blooded organisms which sometimes cause food poisoning in their hosts. *Vibrio harveyi* are gram negative, bioluminescent, marine, rod shaped bacterium responsible for Luminous Vibriosis.

The pure cultures maintained in slants were collected from Microbiology laboratory, Post Graduate and Research Department of Botany, Maharaja's College, Ernakulam, Kerala, India for the study. Each strain was separately inoculated into 5 ml nutrient broth and was incubated at 37° C for 24 hours. Filter paper discs (Whatman filter paper No.1) were prepared using paper punch and sterilized. Lawn cultures of the test organisms were made on nutrient agar plates using a sterile cotton swab under aseptic conditions. The filter paper discs were loaded with plant extracts (aqueous and ethanolic) and nanoparticle solution using a micropipette under aseptic conditions. Discs impregnated with Amoxillin served as positive control (standard) and the filter paper disc soaked in solvents were used as negative control. The discs were placed on the surface of nutrient agar with flamed forceps and gently pressed down to ensure complete contact of the disc with the agar plate. The prepared plates were incubated at 37 °C for 24 hours. Inhibition zones were measured after incubation period.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations of the ethanolic extract as well as the biologically synthesized nanoparticles were carried out. Minimum inhibitory concentration (MIC) was determined by making decreasing concentrations (100 µg, 80 µg, 60 µg 40 µg, 20µg) of the stock extracts (ethanolic and nanoparticle) into nutrient broth using a pipette. Suspension of the bacterium was added to the test tubes containing the different concentrations of the extract aseptically and incubated to allow growth of the bacteria at 37° C for 24 hours. The test tube was examined for growth or turbidity in the samples [14].

Results and Discussion

Phytochemical Screening

Aqueous extract showed the presence of alkaloids, tannins and phenols, glycosides, saponins, flavonoids, carbohydrates and steroids. Ethanol extract also showed the presence of alkaloids, tannins and phenols, glycosides, flavonoids, carbohydrate. The obtained results are given in the Tab-1.

Silver Nanoparticle Assay

Green synthesis of silver nanoparticle was shown by *Gomphrena serrata* L. For the extract the colour change observed after adding silver nitrate solution was from pale brownish colour to dark reddish-brown colour. After 5 hours the nanoparticles started to settle down at the bottom and was centrifuged and washed. The intensity of colour change is representative of the amount of silver nanoparticle synthesized. Under 40X resolution of the microscope, the silver nanoparticle produced by the *Gomphrena* was found to be small in size and almost round in shape.

Antibacterial Assay

The ethanolic extract, aqueous extract and silver nanoparticle showed antibacterial activity against the strains of bacteria studied. Amoxycillin was used as the standard antibacterial drug. The zones of inhibition of aqueous extract was similar for all the strains of bacteria studied i.e., 8mm. For the ethanolic extract, the maximum zone of inhibition was against *Bacillus cereus* (9mm) and minimum zone of inhibition was observed against *Staphylococcus aureus* (6mm). The zone of inhibition of nanoparticle solution against *B. cereus* was the maximum i.e., 10mm, followed by 8mm against *Escherichia coli* and 7mm against *Staphylococcus aureus* and *Vibrio harveyi*. Compared to other extracts Amoxycillin; the standard antibiotic drug showed the maximum zones of inhibition. Against *Staphylococcus aureus* the zone of inhibition observed was 32mm, followed by 20mm against *Vibrio harveyi*, 9mm against Bacillus cereus and 7mm against *Escherichia coli*. The minimum inhibitory concentration (MIC) was observed at a concentration 40µg for the nanoparticle solution. For the plant extract MIC was observed at concentration 60µ. Obtained results are given below in Table-2.

Gomphrena serrata L., belongs to the family Amarantheceae. *Gomphrena* is a well-known traditional Folk medicine. A variety of compounds that are of plant origin are found to act against a wide range of microbes. Hence such compounds can be exploited as antimicrobial agents to treat a variety of diseases [5]. In the present study, preliminary phytochemical screening of *Gomphrena serrata* L. was carried out and the result showed that both aqueous and ethanolic extract contain chemical compounds like alkaloids, glycosides, saponins, flavonoids, carbohydrate, terpenoids, tannins and phenols. Earlier studies [15,13,8,16,14] reported that *Sida acuta* Burm. F., *Sida rhombifolia*, *Gomphrena serrata* L, and *Trianthema portulacastrum* Linn. possessed alkaloids which were found

S. No.	Phytoconstituents	Test	Aqueous Extract	Ethanol Extract
1.	**Alkaloids**	a. Mayer's test b. Wagner's test c. Hager's test	-ve +ve +ve	-ve +ve +ve
2.	**Tannins and phenols**	a. $FeCl_3$ test b. Lead acetate test	-ve +ve	+ve +ve
3.	**Glycosides**	a. Borntrager's test b. Keller Killiani's test	+ve -ve	-ve +ve
4.	**Saponins**	Foam test	+ve	-ve
5.	**Flavonoids**	Shinoda test	+ve	+ve
6.	**Protein**	Biuret test	-ve	-ve
7.	**Carbohydrate**	a. Molish's test b. Benedict's test	+ve -ve	+ve +ve

Table -1: Preliminary phytochemical screening of *Gomphrena serrata* L. extracts (aqueous and ethanolic)

Sample	Zones of inhibition of different bacteria (in mms)			
	B. cereus	***S. aureus***	***E. coli***	***V. harveyi***
Aqueous	8	8	8	8
Ethanol	9	6	7	8
Nanoparticle solution	10	7	8	7
Amoxycillin	9	32	7	20

Table-2: Antibacterial activity of aqueous and ethanolic extract of *Gomphrena serrata* L.

to be antimicrobial against bacteria such as *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa.*

In the present investigation, *Gomphrena serrata* L. showed the presence of alkaloids. As per the earlier reports, alkaloids are a promising source of antimicrobial agent. *Gomphrena serrata* L. also contain tannins and phenols which can also act against a variety of strains of bacteria[5,7,17]. Both aqueous and ethanolic extract showed significant antibacterial activity against *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Vibrio harveyi*. The standard antibiotic Amoxycillin was used to compare the antibacterial activity of *Gomphrena serrata* L. There are no previous works on the antibacterial activity of *Gomphrena serrata* L. A similar species *Gomphrena celosioides* have shown to possess antibacterial activity against various strains of bacteria like *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. In the present study, the antibacterial properties were studied against bacterial stains like *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Vibrio harveyi*. *Bacillus cereus* is a facultatively anaerobic bacterium responsible for food borne illness like severe nausea and vomiting. *Staphylococcus aureus* are round shaped bacterium responsible for common cause of skin infections including abscesses, respiratory infections such as sinusitis and food poisoning. *Escherichia coli* are coli form bacterium commonly found in the lower intestine of warm-

blooded organism which causes food poisoning in their hosts. *Vibrio harveyi* are bioluminescent, rod shaped bacterium responsible for Luminous Vibriosis. Hence, the present study revealed that *Gomphrena serrata* L. is a potential source of antibacterial components. However further fractionations and characterisations are required to identify the exact principle.

Silver nanoparticle synthesized with various plants showed antibacterial activity. In the present investigation, the antibacterial activity of the biosynthesized nanoparticles was tested. Silver nitrate solution was used as the control [8,18]. The percentage of inhibition (78%) was found to be more than that of aqueous and ethanolic extracts. This indicates that biosynthesized nanoparticles possessed a better antibacterial activity than the aqueous and ethanolic extracts.

Conclusion

The present study revealed that the antimicrobial property associated with *Gomphrena serrata* L. was promising for further investigation. Hence, further characterisation and screening is needed to ascertain the exact molecules behind the antibacterial property of *G. serrata* L.

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