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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND IN-VITRO ANTIBACTERIAL ACTIVITY ON ASPARAGUS RACEMOSUS ROOT EXTRACT

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ABSTRACT

The purpose of present work is to study medicinally active substances present in ethanol-extract obtained from roots of *Asparagus racemosus*. Preliminary Phytochemical screening of the extracts revealed the presence of Alkaloids, Carbohydrates, Glycosides, Phenolic compounds, tannins, Saponins, Steroids and Flavonoids. The presence of these bioactive constituents is associated with the antibacterial activity of the plant. The root extracts of *Asparagus racemosus* solvented by ethanol, showed the spectrum of inhibition on *Staphylococcus aureus, Bacillus subtilis, Staphylococcus werneri, Pseudomonas putida, Pseudomonas aeruginosa and Proteus mirabilis* by Cylinder plate method. The observations revealed significant zone of inhibition and supports to antibacterial activity.

Keywords: *Asparagus racemosus*; Phytochemical; antibacterial activity.

INTRODUCTION

Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components[1].There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world[2-5]. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine[6]. Approximately 20% of the plants found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi-synthetic resources[7]. In the present study, medicinal plant ***Asparagus racemosus*** belonging to the family Asparagaceae was selected to assess antibacterial potential.

Asparagus racemosus (Shatavari) is recommended in traditional medicine for the prevention and treatment of gastric

ulcers, dyspepsia, diarrhea, nervous disorders[8]. Besides use in the treatment of and dysentery, the plant also has antioxidant, immunostimulant, anti-dyspepsia and antitussive effects[9]. Scanty work has reported an antimicrobial activity of whole plant[10]. *Asparagus racemosus* have been used for the treatment of the ulcers, depression, inflammation cancer, lithiasis, Hepatotoxicity, diabetes[11-30]. Thus study is aimed to demonstrate and determine the antibacterial effects of *Asparagus racemosus* root on various strains of bacteria.

MATERIALS AND METHODS

Plant material

Fresh plant roots were collected from local area of East Godavari district and the plants were identified by Botanist Dr. T. U. Ragharam. The plant was dried in shade, and then pulverized into powder.

Preparation of crude ethanol extract

The root powder was repeatedly macerated with 95% ethanol in a percolator. The combined filtrate was evaporated to dryness under reduced pressure at 40–50°C. The resulting crude ethanol extract was then stored at 10–15°C.

Test Organism Used

The various organisms like *Staphylococcus aureus ATCCBAA 1026, Bacillus subtilis ATCC 11774, Staphylococcus werneri ATCC 27836, Pseudomonas putida ATCC 700007, Pseudomonas aeruginosa ATCC 10662, Proteus mirabilis ATCC 14153 , Escherichia coli ATCC 10536 , Kleibsella pneumonia ATCC 33495* are procured from Microbes Speciality Lab Danavaipeta , Rajahmundry , East Godavari District 533103 , Andhra Pradesh , India.

Antimicrobial Agent

The reference standard Gentamycin was procured from Pradeep Organics and chemicals Pvt. Ltd , Hyderabad.

Phytochemical screening

The powdered root was evaluated for qualitative determination of major phytoconstituents *i.e.* Alkaloids, Carbohydrates, Glycosides, Phenolic compounds, tannins, Saponins, Steroids, Flavonoids; which were further confirmed by thin layer chromatography.

Qualitative screening

Alkaloid detection was carried out by extracting 1 g powdered sample with 5 ml methanol and 5 ml of 2N HCl; and then treating the filtrate with Mayer's and Wagner's reagents. The samples were scored positive on the basis of Reddish brown or cream precipitation. Flavonoids were tested by heating 1 g powdered sample with 10 ml ethyl acetate over a steam bath (40–50°C) for 5 min; filtrate was treated with 1 ml dilute ammonia. A yellow coloration demonstrated positive test for Flavonoids. Saponins content was determined by boiling 1 g powdered sample in 10 ml distilled water for 15 min and after cooling, the extract was shaken vigorously to observe froth formation. Cardiac glycosides were identified by **Borntrager's test.** Ammonical layer turning to pink was indicative of cardenolides/cardiac glycosides[31].

Thin layer chromatography (TLC)

TLC plates were prepared by using silica gel G for TLC, were left overnight for air drying. These plates were activated by hot air oven at 100°c for 1hr.Cold alcoholic extract was plotted on TLC plates[32]. The plates were dried and developed in suitable solvents for rapid screening. Pure ethyl acetate, 50% chloroform/methanol, 1:1 ethyl acetate/methanol. The plates were run in the above solvent systems and dried at room temperature. Derivatisation of TLC plates was done by spraying 10% H_2SO_4 in methanol. Different bands were observed and corresponding Rf values are determined. R_f value of each spot was calculated as

R_f = Distance travelled by the solute / Distance travelled by the solvent.

Antibacterial Assay

Root extract of A*sparagus racemosus* was evaluated for antibacterial activity against several Gram Positive and Gram Negative organisms.

The antibacterial activity of ethanolic extract was performed using Agar cup-plate

method. 20ml of sterile nutrient agar medium was poured into sterile Petri-dishes and allowed to solidify. The Petri dishes were incubated at 37°C for 24 hours to check for sterility. The medium was seeded with the organisms by pour plate method using sterile top agar (4 ml) contained 1 ml culture. Bores were made on the medium using sterile borer. Dried ethanolic extract of roots of A*sparagus racemosus* was dissolved in water to obtain different concentrations (100 , 300 and 500mg/ml) and sterilized by filtration through a Whattman filter paper no. 1, and 0.05 ml of the different concentrations of extract were added to the respective bores. 0.05ml of Gentamycin at a concentration of (25 µg/ml) was taken as standard reference. All the plates were kept in a refrigerator at 2 to 8 ° c for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37°c for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The diameter of the zone of inhibition was measured and recorded.

RESULTS AND DISCUSSION

Preliminary Phytochemical screening

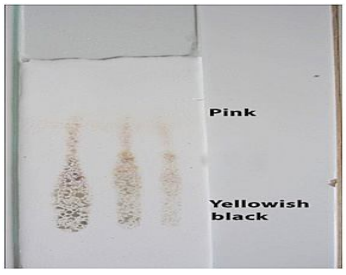
Phytochemical screening of the extracts of A*sparagus racemosus* revealed, the presence of Alkaloids, Carbohydrates, Glycosides, Phenolic compounds, tannins, Saponins, Steroids, and Flavonoids. (Table 1).

Table1: Preliminary Phytochemical analysis of Asparagus racemosus root extract

Components	Asparagus racemosus {ethanolic root extract}
Alkaloids	+
Carbohydrates	+
Glycosides	+
Phenolic compounds and tannins	+
Proteins and amino acids	-
Saponins	+
Steroids	+
Flavonoids	+
	+

Thin layer chromatography

The presence of phytoconstituents was further confirmed by thin layer chromatography and their R_f values have been presented as 0.7 (Figure 1). The components were best resolved in screening system using pure ethyl acetate, 50% chloroform/methanol, 1:1 ethyl acetate/methanol.



Yellow Colour Indicates Presence of Tannins, Flavonoids.

Pink Colour Indicates Presence of Steroids.

Fig. 1: TLC of Asparagus racemosus

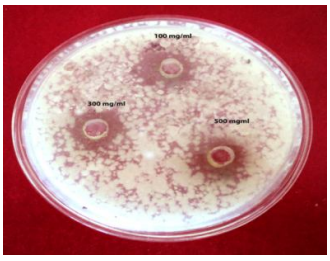
Antibacterial Activity

The root extract of A*sparagus racemosus* was studied for antibacterial activity employing standard cylinder method. Microbes used were *Bacillus subtilis, Staphylococcus aureus, Staphylococcus werneri, Pseudomonas aeruginosa and*

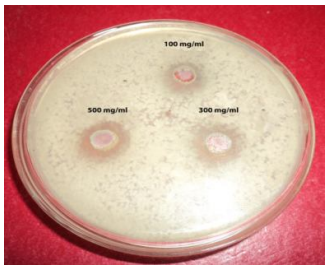
Escherichia coli, Proteus mirabilis, Kleibsella pneumonia, *Pseudomonas putida*. Both gram-positive and gram-negative bacteria were sensitive to the extract. The zone of inhibition recorded for various organisms was found *Staphylococcus aureus* (18mm) , *Bacillus subtilis* (13mm), *Staphylococcus werneri* (14mm), *Pseudomonas putida* (17mm), *Pseudomonas aeruginosa* (15mm), *Proteus mirabilis* (15mm). Activity of ethanolic extract of the plant was comparable to that of Reference Standard drug Gentamycin disc (25µg). *Asparagus racemosus* root extract exhibited good antimicrobial activity and results were tabulated along with figures (Table 2; figure 2,3&4).

Table 2: Antibacterial activity of Asparagus racemosus root extract

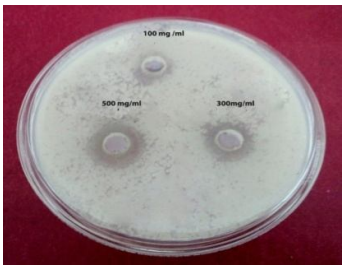
Zone of inhibition(mm)				
Microorganism	**100mg/ml**	**300mg/ml**	**500mg/ml**	**Gentamycin 25µg/ml**
Gram positive				
S.aureus	**16 ± 0.21**	**17 ± 0.36**	**18 ± 0.15**	**12 ± 0.12**
B.subtilis	**10 ± 0.62**	**12 ± 0.69**	**13 ± 0.36**	**14 ± 0.32**
S.werneri	**12 ± 0.48**	**14 ± 0.48**	**14 ± 052**	**13 ± 0.28**
Gram negative				
P.putida	**13 ± 0.26**	**13 ± 0.52**	**17 ± 0.25**	**15 ± 0.21**
P.aerugenosa	**12 ± 0.36**	**13 ± 0.39**	**15 ± 0.53**	**16 ± 0.12**
Proteus mirabilis	**13 ± 0.14**	**14 ± 0.28**	**15 ± 0.63**	**13 ± 0.36**
Escherichia coli	**8±0.39**	**9±0.35**	**9±0.24**	**13±0.28**
Klebsiella pneumoniae	**7±0.16**	**8±0.26**	**9±0.52**	**14±0.32**



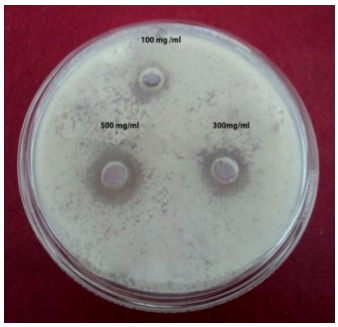
Staphylococcus aureus



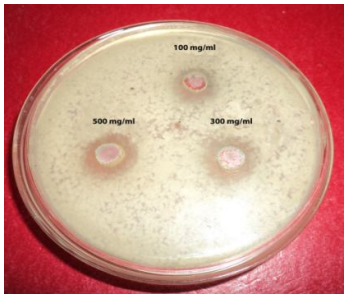
Bacillus subtilis



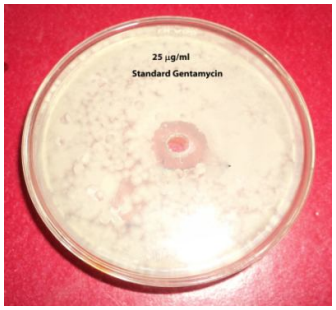
Staphylococcus werneri



Pseudomonas aeruginosa



Pseudomonas putida



standard

Fig. 2: Zone Of Inhibition of *Asparagus racemosus root extract*

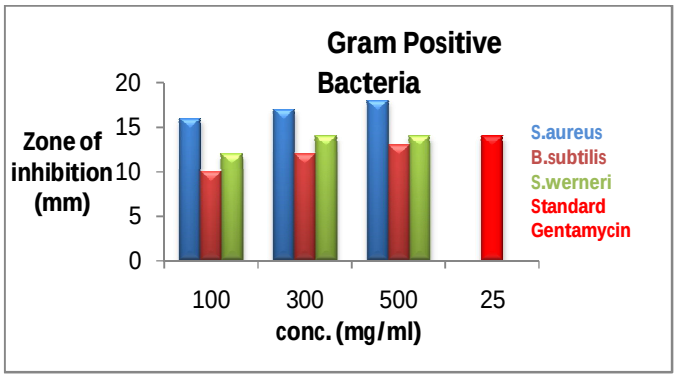


Fig. 3: Inhibition Zone of Asparagus *racemosus Root Extract against* Gram Positive Organisms

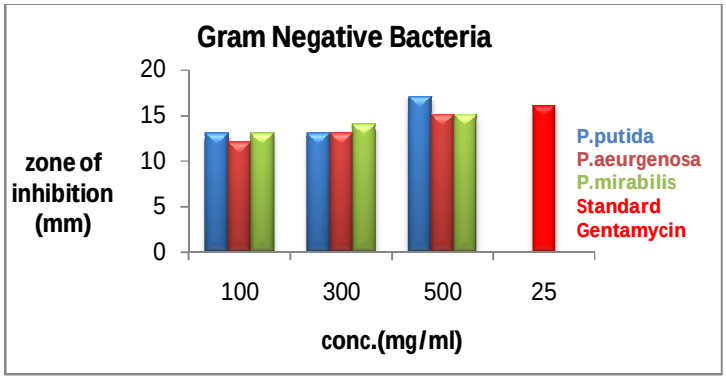


Fig. 4: Inhibition Zone of *Asparagus racemosus* Root Extract against Gram negative Organisms

CONCLUSION

The scientific paper establishes that *Asparagus racemosus* root extract has good significant antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Staphylococcus werneri, Pseudomonas putida, Pseudomonas aeruginosa, Proteus mirabilis* .The antibacterial activity of root extract with different concentrations 100 , 300 and 500mg/ml was very well compared with standard reference drug Gentamycin 25 µg/ml .The maximum zone of inhibition with root extract of *Asparagus racemosus at various concentrations like* 100 , 300 and 500mg/ml are 16,17 and 18 mm.Further investigations can be carried out in order to isolate new compounds from the plant root extract and to evaluate the bioactivities as it is necessary to introduce new biologically safe phytochemical compounds which are necessary to suppress the growth of the microorganisms.

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