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Research article

Ethnopharmacological investigation and antibacterial evaluation of the methanolic extract of *Asparagus racemosus* (Shatavari)

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Abstract: Plants are the base of sophisticated traditional medicine systems including Ayurvedic, Unani and Siddha. Phytochemical analysis of *Asparagus racemosus* revealed that numerous compounds of plants traditionally used for medicinal purposes have several therapeutical properties. The result of the phytochemical studies revealed the presence of saponins, tannins, alkaloids, steroids and other biochemicals. Saponins, tannins and alkaloids are well known for their antibacterial properties. Experiments demonstrating the pharmacological properties of saponins have aroused considerable clinical interest in these substances. The concentrations of the plant used were 25 mg ml^{-1}, 50 mg ml^{-1} and 100 mg ml^{-1} respectively. At these concentrations, the extract inhibited the growth of *Escherichia coli*, *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus* and produced percentage inhibition ranging from 72.4–86.5 %. The anti-bacterial activity demonstrated by the plant extract may due to the presence of the phytochemicals present in the plant.

Keywords: *Asparagus racemosus* - Phytochemical - Saponins - Antibacterial - Pharmacology.

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INTRODUCTION

Herbal medicine is a powerful method of disease treatment. Western drugs are usually used to control symptoms, but do not alter the disease process Citarasu (2010). Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity Harbone (1973). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious disease.

Plants have formed the base of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Green plants are the indispensable storehouse of many chemical metabolites which are grouped into two categories namely: primary and secondary metabolites. Secondary metabolites are the substances produced by plants as defence chemicals. They include alkaloids, flavonoids, essential oils, phenols, saponins etc. In India, different regions have specific features according to the climatic conditions (Kumaran & Citarasu 2015). These plants including medicinal plants are also used as a feeding for animals. They are indirectly shown by their effects by which animals do not suffer by any types of diseases. Growing plants are one of the cheapest sources of feeding for animals having crude proteins of 14–25% (Babu *et al.* 2011).

Saponins are secondary metabolites and play a role in the protection of plants against microorganism. Many saponins show strong antibacterial activities. As saponins are probably a part of plants' defence systems, they have been included in a group of protective molecules in plants called phyto-protectant (Francis *et al.* 2002). Saponins are used antioxidant, antimicrobial, and anti-inflammatory etc. according to medical field. It is a

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bioactive antibacterial agent of plants (Yoshiki 1998). The present study was designed to evaluate the fundamental phytochemical constituents and antimicrobial activities of the *Asparagus racemosus* (Shatavari).

MATERIALS AND METHODS

Collection and Extraction

Asparagus racemosus Willd. (Shatavari) roots were purchased from the commercial market at Nagercoil, kanayakumari district, Tamilnadu, India. Dried powder plant materials were boiled at above 100°C with two hour. After filtered the extracts, the supernatant was collected and the residue were discarded. The supernatant was condensed in the water bath and the condensate was extracted again by methanol. The methanolic extract was concentrated in rotatory evaporator under reduced pressure at the room temperature of 45°C to 50°C in order to avoid the evaporation of plant materials. Aqueous extract was concentrated using Lyophilizer and stored at 4°C as described by Andrea *et al.* (2012).

Phytochemical screening

The Phytochemical screening was determined by the method (Sofowora 1993; Trease 1989). This screening was carried out with the methanolic extracts using chemical methods and thin-layer chromatography (TLC) as per standard protocol (Wagner & Bladt 1996).

Saponin Estimation Procedure

Weigh accurately 1.5 to 2 gm of the material in a beaker add 50 ml of petroleum ether and gently heat to 40°C on a water bath for 5 minutes with regular shaking. Filter the petroleum ether repeat the operation with further 2×50 ml of petroleum ether. Discard petroleum ether and preserve the marc. Extract the marc obtained in the previous test with 4×60 ml of methanol with mild heating. Filter the methanol layer to another beaker. Concentrate the combined methanol layer to about 25 ml. Add 150 ml of dry acetone to precipitate the saponins. Filter the saponins through a filter paper and dry at 100°C for constant weight.

Calculation

$$\text{Percentage of total Saponin} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

Bioautography

A TLC Bioautographic method was used to detect active components. After application of the extract on a silica gel plate, thin layer chromatography (TLC) was developed using ethylacetate : methanol (9:1) as the eluent system for *Asparagus racemosus.* Observe the bands- the TLC plates were dried for complete removal of solvents. Then the fractions of TLC were spotted on already swabbed agar plates by bioautography method to evaluate the activity of the different essential compounds, and the plates were incubated at 35ºC for 24 hours. The activity of compound can detect by its zone formation.

Antibacterial Screening

Test Organisms

The test organisms were standard laboratory strains of *Escherichia coli, Pseudomonas aeruginosa* and *Vibrio parahaemolyticus.* The organisms were obtained from the Department of marine Science (CMST), Manonmaniam Sundaranar University, Rajakkamangalam, Kanyakumari district, Tamilnadu, India.

Antibacterial activity

Muller-Hinton agar was poured on to sterile Petri plates. When the media solidified, 0.1ml of inoculums with 0.5 OD was poured over feeder layer and spread evenly with a sterile spreader. A well of 6mm diameter was made by using a sterile cark borer. Each well-received the extract was tested in a different concentration (25 mg ml^{-1}, 50 mg ml^{-1} and 100 mg ml^{-1}. Distilled water was used as negative control while ampicillin was used as positive control. And the commercial antibiotics like as Ampicillin and Tetracycline tested against pathogens. They were incubated at 37ºC for 24 hours. After incubation, the diameter of the inhibition zone was measured.

RESULTS

Phytochemical Screening

The phytochemical screening of methanolic extracts showed the presence of different types of active constituents, namely alkaloids, anthraquinones, cardiac glycosides, flavonoids, terpenoids, tannins, saponins, sterols and triterpenes. These compounds were present in almost all the plants extracts. The details were given in

the (Table 1). The total percentage of saponin was estimated from the *Asparagus racemosus*, and it was found that 2 g of *Asparagus racemosus* contains 40% of saponin molecule.

Table 1. Summary of The Results Phytochemical Analysis of *Asparagus racemosus.*

S.No.	Phytochemical group	Result
1.	Alkaloids	+
2.	Saponins	+
3.	Flavonoids	+
4.	Tannins	+
5.	Steroids	+
6.	Terpenoides	+
7.	Titerpenoides	+
8.	Anthraquinones	+
9.	Cardiac glycosides	+

Note: +, Present.

TLC Studies on *Asparagus racemosus*

On TLC analysis for the hot water extract *Asparagus racemosus* was revealed that, the single spot were obtained, and it observed under UV-illuminator. The fraction obtained having the R_f values of 0.86 and it shows on figure 1.

Figure 1. Thin layer Chromatography (TLC) for the steroidal saponin from *Asparagus racemosus.*

Bioautography

Bioautography method was used to detect active components by its zone formation. The maximum zone of inhibition is measured in 6.1 mm in dm. The minimum zone of inhibition for the fraction of *Asparagus racemosus* is 1.8 mm against *Vibrio parahaemolyticus* (Table 2).

Table 2. Bioautography of the saponin activities of *Asparagus racemosus* against some pathogenic bacteria.

S. No.	Concentration ($g\ ml^{-1}$)	Zone of Inhibition in pathogenic bacteria (mm)		
		Escherichia coli	*Pseudomonas aeruginosa*	*Vibrio parahaemolyticus*
1	1.0	4.2	3.8	4.5
2	2.0	5.9	5.4	6.1
3	3.0	2.7	1.4	1.8

Antimicrobial activity

The antimicrobial activities of the plant extracts against the three bacteria strains examined were assessed by the presence or absence of inhibition zones. The aqueous extract of *Asparagus racemosus* exhibited moderate level antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus* the test organisms. Methanol extract of *Asparagus racemosus* was active against all the test organisms except *Pseudomonas aeruginosa*. On the other hand, it was found that the methanol extract of *Asparagus racemosus* exhibited high activity against Escherichia coli and *Vibrio parahaemolyticus* (Fig. 2)

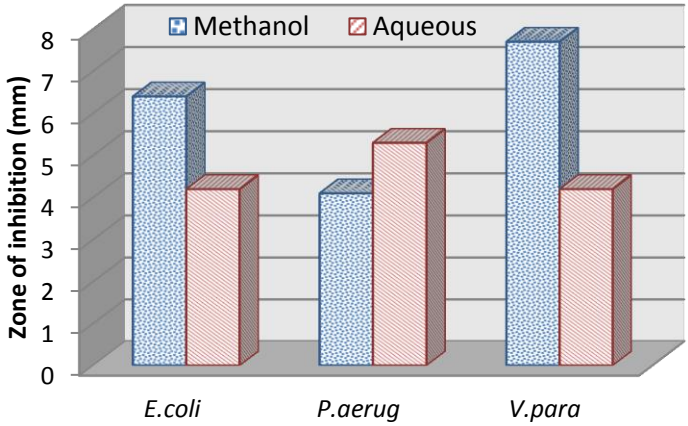

Figure 2. Antibacterial activity *Asparagus racemosus* against pathogenic microorganism (*E. coli* = *Escherichia coli; P. aerug* = *Pseudomonas aeruginosa; V. para* = *Vibrio parahaemolyticus*).

To screen the antibacterial activity against tested organisms, ampicillin and tetracycline were used as a standard. It was found that tetracycline (5 µg ml^{-1}) standard showed higher activity than ampicillin (30 µg ml^{-1}) standard against tested microorganisms (Fig. 3).

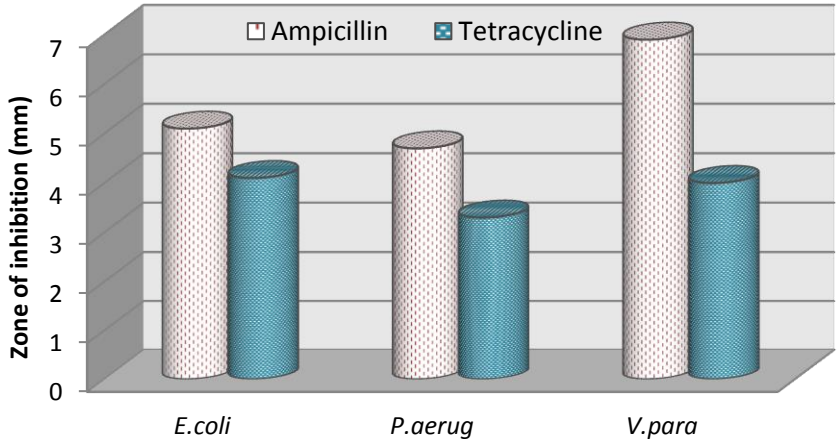

Figure 3. Antibacterial activity Ampicillin and Tetracycline against pathogenic microorganism.

DISCUSSION

Saponins may beconsidered a part of plants' defence systems, and as such have been included in a large group of protective molecules found in plants (Morrissey & Osbourn 1999). The present study focuses on both the phytochemical analysis and antimicrobial potential of *Asparagus racemosus*. In the present investigation, different extracts of *Asparagus racemosus* was evaluated for exploration of their antimicrobial activity against certain bacteria, which was regarded a pathogenic microorganism. Susceptibility of plant extract was tested by agar well diffusion method was determined.

The results of our studies have shown that *Asparagus racemosus* contains saponins, tannins, flavonoids, steroids, alkaloids and cardiac glycosides. The plant extract also showed antibacterial activity at concentrations of 25 mg ml^{-1}, 50 mg ml^{-1} and 100 mg ml^{-1} respectively. At these concentrations, the extract inhibited the growth of *Escherichia coli*, *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus* and produced percentage inhibition ranging from 72.4% to 86.5%. Therefore, the ethnomedical application of the plant in the treatment of bacterial infections is justified.

The antibacterial activity of aqueous extract (25 mg ml^{-1}, 50 mg ml^{-1} and 100 mg ml^{-1}) showed that the extract has activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus*. At 100 mg ml^{-1}, it produced the highest percentage inhibition of 80.8% on *Vibrio parahaemolyticus*; at 25 mg ml^{-1} and least percentage inhibition of 72.4% on *Vibrio parahaemolyticus*. The percentage inhibition of growth produced by the extract on the other bacterial strains also tested ranges from 65% to 75% (Table 3). It may be deduced that *Asparagus racemosus* showed a remarkable antibacterial activity (Table 2) when compared with Ampicillin

(positive control) which produced a percentage inhibition of 98.8% (Table 3). Therefore, the antibacterial activity exhibited by this plant extract may be due to the presence tannins, saponins and flavonoids in plant which have been reported to have antibacterial properties. In conclusion, of the present investigation *Asparagus racemosus* contain potential antimicrobial components that may be of great use for the development of pharmaceutical industries as a therapy against various diseases.

Table 3. Summary of results of antibacterial activities of the aqueous extract of *Asparagus racemosus*.

Test organisms	Percentage inhibition of growth (%)				
	Asparagus racemosus			Ampicillin	Distilled
	(25 mg ml^{-1})	(50 mg ml^{-1})	(100 mg ml^{-1})	(100 mg ml^{-1})	water
Escherichia coli	65.0	70.6	73.0	98.8	0.0
Pseudomonas aeruginosa	65.0	65.2	75.0	98.7	0.0
Vibrio parahaemolyticus	72.4	68.0	86.5	98.8	0.0

Note: Results are means of triplicate values.

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