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Pharmacological investigation, medicinal uses and antibacterial studies of the root extract *asparagus racemosus*

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Abstract
Medicinal plants are the richest bio resource of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Phytochemical analysis of *Asparagus racemosus* has revealed that numerous compounds in plants traditionally used for medicinal purposes have many therapeutical properties. The result of the phytochemical studies revealed the presence of Saponins, Tannins, Alkaloids, Steroids and numerous other chemicals. Saponins, Tannins and alkaloids are chemicals that are known to have anti-bacterial properties. The concentrations of the plant used were 25 mg/ml, 50 mg/ml and 100mg/ml respectively. At these concentrations, the extract inhibited the growth of *Escherichia coli*, *Pseudomonas aeruginosa* and *V. parahaemolyticus* and produced percentage inhibition ranging between 72.4% to 86.5%. The may due to the presence of the phytochemicals present in the plant. The results suggest that the phytochemical properties and anti-bacterial activity demostrated by the plant extract for curing various diseases and leads to the isolation of new and novel compounds.

Keywords: asparagus racemosus, phytochemical, saponins, antibacterial, V. parahaemolyticus

1. Introduction
Many plants have been investigated in recent times and found to contain active substances that are medically useful, whereas many more are yet to be scientifically investigated. Herbal medicinal products are virtually known to contain phytochemicals. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. Recent studies have showed that plants with medicinal values with antibiotic resistant bacteria can be used in drug discovery which need of an hour phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening (Sonalinigam and shrivastava, 2013) [9]

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) [5]. 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. Phytochemical constituents such as alkaloids, flavonoids, tannins, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms. In India, different regions have specific features according to the climatic conditions (Kumaran & Citarasu 2015) [6]. 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 Shankar *et al.*, 2011) [1].

Saponins are secondary metabolites and play a role in the protection of plants against microorganism. Many saponins show strong antibacterial activities. Assaponins 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) [4]. Saponins are used antioxidant, antimicrobial, and anti-inflammatory etc. according to medical field. It is a bioactive antibacterial agent of plants Yoshiki (1998) [12]. The present study was designed to evaluate the fundamental phytochemical constituents and antimicrobial activities of the *Asparagus racemosus*.

2. Materials and methods
2.1 Collection and Extraction
Asparagus racemosus were collected from the Centre for marine science and technology campus at Rajakkamangalam, Nagercoil, kanayakumari district, Tamil nadu, India. Shadow dried root 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.

2.2 Phytochemical screening (Sofowora, 1993; Trease, 1989)
This screening was carried out with the methanolic extracts using chemical methods and thin-layer chromatography

(TLC) according to the methodology given in Wagner and Bladt 1996.

2.3 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 40^0C on a water bath for 5 minutes with regular shaking. Filter the petroleum ether repeat the operation with further 2 X 50 ml of petroleum ether. Discard petroleum ether and preserve the marc. Extract the marc obtained in the previous test with 4 X 60 ml of methanol with mild heating. Filter the methanol layer to another beaker. Concentrate the combined methanol layer to about25ml. Add 150 ml of dry acetoneto precipitate the saponins. Filter the saponins through a filter paper and dry at 100^0C for constant weight.

a. Calculation

$$\text{Percentage of total saponins} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \text{ X } 100$$

2.4 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.

2.5 Antibacterial Screening

a. Test Organisms

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

b. Antibacterial activity

Muller-Hinton agar were 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 cork borer. Each well-received the extract was tested in a different concentration (25 mg/ml, 50 mg/ml and 100 mg/ml. 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.

3. Results

3.1 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: Phytochemical Analysis of *Asparagus racemosus* plant material extract.

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

Note: + = Present

3.2. 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 Fig (1).

Fig 1: Thin layer Chromatography (TLC) for the steroidal saponin from *Asparagus racemosus*

3.3. 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 *V. parahaemolyticus* (Table 2).

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

S. No	Concentration (g/ml)	Pathogenic bacteria Zone of Inhibition(mm)		
		E. coli	*P. aeruginosa*	*V.parahaemolyticus*
1	1.0 gram	4.2mm	3.8mm	4.5mm
2	2.0 gram	5.9mm	5.4mm	6.1mm
3	3.0 gram	2.7mm	1.4mm	1.8mm

3.4. 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 *V. 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 *V. parahaemolyticus* (Fig. 2)

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

Table 3: Summary of The Results of Antibacterial Activities of the aqueous Extract of *Asparagus racemosus*

Test organisms	Percentage inhibition of growth (%)				
	***Asparagus racemosus* (25mg/ml)**	***Asparagus racemosus* (50mg/ml)**	***Asparagus racemosus* (100mg/ml)**	**Ampicillin (100 mg/ml)**	**Distilled 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
V.parahaemolyticus	72.4	68.0	86.5	98.8	0.0

Note: Results are means of triplicate values.

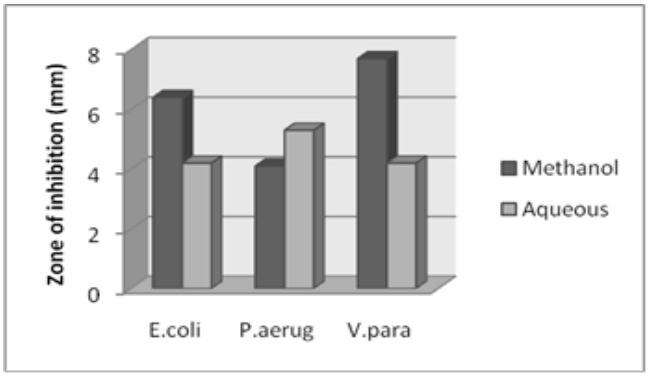

Fig 2: Antibacterial activity *Asparagus racemosus* against pathogenic microorganism

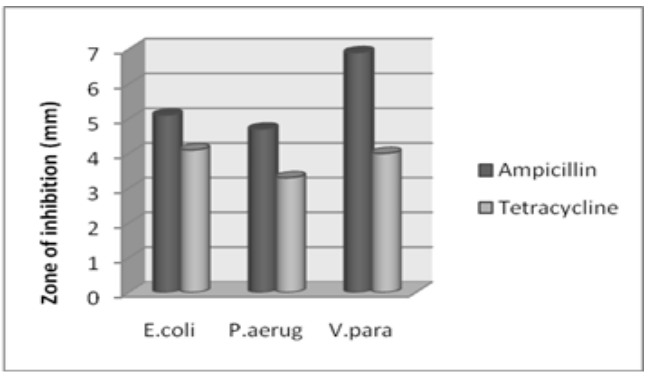

Fig 3: Antibacterial activity Ampicillin and Tetracycline against pathogenic microorganism

4. Discussion

Plants are the storehouses and rich sources of safer and cheaper chemical compounds. These natural plant products have been reported to have various activities like anti stress, growth promoters, appetiser, tonic, immunostimulants and antimicrobials (Citarasu *et al.,* 2002) [2]. Moreover, the substances are obtained from natural sources, besides possessing other interesting properties like non-toxic, biodegradable and biocompatible (Citarasu *et al.,* 2003) [3]

Saponins may be considered a part of plants' defence systems, and as such have been included in a large group of protective molecules found in plants (Morrissey and Osbourn, 1999) [7]. 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, 50 mg/ml and 100mg/ml respectively. At these concentrations, the extract inhibited the growth of *Escherichia coli*, *Pseudomonas aeruginosa* and *V. parahaemolyticus* and produced percentage inhibition ranging between 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, 50 mg/ml, and 100 mg/ml) showed that the extract has activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *V. parahaemolyticus* (Table. 3). At 100 mg/ml, the extract produced the highest percentage inhibition of 80.8% on *V. parahaemolyticus*. At 25 mg/ml, the extract produced least percentage inhibition of 72.4% on *V. parahaemolyticus* (Table 3). Also, the percentage inhibition of growth produced by the extract on the other bacterial strains tested ranges between 65% to 75% (see Table 3).

By observing Table 2, it can be deduced that *A. racemosus* showed a remarkable antibacterial activity when compared with Ampicillin (positive control) which produced a percentage inhibition of 98.8% (Table 3). Similar results were reported Kumaran & Citarasu 2015. *A. racemosus* contain potential antimicrobial components that may be of great use for the development of pharmaceutical industries as a therapy against various diseases. In conclusion, 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.

5. References

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