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Phytochemical investigation and antimicrobial activity of *Asparagus racemosus* Willd root against some pyogenic bacteria

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Abstract

Asparagus racemosus Willd belongs to family Asparagaceae is a common medicinal plant found in hilly and plane regions of India. The plant has got immense medicinal properties and because of that it has got special importance in ayurvedic system of medicine. In the present investigation attempts were made for study of phytochemical and antibacterial activity of ethanolic extract of root. The study showed presence of primary phytochemicals like steroids, alkaloids, saponins, carbohydrates, flavonoids, amino acids except tannins. Chromatographic analysis (HPTLC) showed presence of seven polyvalent phytochemical compounds with variable R_f value and concentration. The antimicrobial study of extract showed significant growth inhibition against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. The mixture of polyvalent compounds present in the extracts might be responsible for the antibacterial activity against the pyogenic bacteria. The results obtained support the application of *Asparagus racemosus* in several traditional ethnomedicinal applications. Furthermore HPTLC fingerprint developed may find its application in the correct taxonomical identification of *Asparagus racemosus* and in detecting adulterations in the crude plant drug.

Keywords: *Asparagus racemosus*, HPTLC, pyogenic bacteria

Introduction

Medicinally important plants present around us are of great importance in primary healthcare of individual and society in many developing countries (Vasundra and Divya, 2013) ^[1]. It is said that medicinal plants are nature's gift to human being to make life disease free and healthy. In present scenario world health organization is taking official interest in this to develop traditional system of healthcare where special attention has been given on folk medicine as safety for microbial and non-microbial diseases (WHO, 1978) ^[2]. Herbal medicines are the major remedy for thousands of years and have made great contribution to maintain human health in many parts of the world in the rural areas of developing countries as primary source (WHO, 1993) ^[3]. This genus has recently placed in family Asparagaceae from Liliaceae (Madhavan, et. al. 2010) ^[4]. *Asparagus racemosus* Willd is an important medicinal plant of tropical and subtropical countries like India (Gomase and Sherkhane, 2010) ^[5]. Now a day's substantial number of drugs are being developed from plants which are active against number of diseases. The majority of these involve the isolation of the active ingredients found in a particular medicinal plant and its subsequent modification. In the developing countries 25 percent of medicinal drugs are based on plants and their derivatives and the use of medicinal plants is well known among the indigenous peoples of many developing countries (Vasundra and Divya 2013) ^[1].

In Indian system of medicine *Asparagus racemosus* is an important medicinal plant. Its root paste or root juice has been used in various ailments and as health tonic (Kirtikar and Basu, 1975; Goyal, et. al. 2003) ^[6, 7]. *A. racemosus* is used to prevent ageing, increase longevity, impart immunity, improve mental function, nervous disorders, dyspepsia, tumours, inflammation, neuropathy and hematopathy (Sharma and Charaka, 2001) ^[8]. Review of literature showed that the root extract of *A. racemosus* has anti-ulcer activity (Sairam, et. al. 2003) ^[9], antioxidant, anti-diarrhoeal, antidiabetic, and immunomodulatory activities (Kamat, et. al. 2000) ^[10]. A study of ancient classical Ayurvedic literature claimed several therapeutic attributes for the root of *A. racemosus* and has been specially recommended in case of threatened abortion and as a galactagogue (Mishra and Verma, 2017) ^[11].

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Roots of *A. racemosus* has been referred as bitter sweet, emollient, cooling, nervine tonic, constipating, aphrodisiac, diuretic, rejuvenating, carminative, stomachic, antiseptic and as a tonic (Chopra, et. al. 1994) ^[12].

The plant is also known as Shatavari which is a climbing plant grows in low as well as high forest of Himalayan, sub-Himalayan and in planes of India. Because of its multiple applications it is one of the important medicinal plant which is used in many allopathically incurable diseases in ayurveda (Mishra and Verma, 2017) ^[11]. Though the plant carries lot of ayurvedic application but very little work has been reported on antimicrobial activity of root of this plant (Mondal, et. al. 2000) ^[13]. Therefore in present study we have made an attempt to study its antimicrobial activity against some pyogenic bacteria.

Standardization of natural crude drug plant is a difficult task because of their heterogeneous phytochemical composition (Ahmed, et. al. 2017) ^[14]. The first step towards ensuring quality of standardizing material is authentication. Thus in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance (Venkatesh, et. al. 2004) ^[15]. The evaluation of these drugs is primarily based on the phytochemical, pharmacological and allied approaches including various instrumental techniques such as microscopy, chromatography etc. Therefore in present study we have used phytochemical fingerprint developed by High Performance Thin Layer Chromatography (HPTLC) technique for authentication and identification of crude plant drug. HPTLC is very useful in analysis of different phytochemical compounds present in plant extract. In last two decades HPTLC has emerged as an important tool for the phytochemical analysis of herbal drugs. It combines fascinating art of chromatography with accuracy and quickness with better separation and resolution. Silica gel of fine particle is used as a stationary phase in HPTLC. The use of smaller particle size helps in greater resolution and sensitivity. HPTLC requires high concentrated solution as very less amount of sample is needed for application. The major advantage of this chromatography is that multiple samples can be analyzed simultaneously using small quantity of mobile phase (Ahmed, et. al. 2017) ^[14]. Now a days it is applied to obtain fingerprint patterns of plant species, plant drugs, herbal formulations, quantifications of active ingredients and also for detection of adulterations in herbal formulations.

Therefore in this work we have done preliminary phytochemical studies and developed HPTLC chromatogram of root extract of *A. racemosus* which will be helpful in further identification and authentication of plant drug.

Materials and Methods

Collection of Plant Material

The plant material of *Asparagus racemosus* Willd were collected from local medicinal plant garden maintained by department of botany. The botanical identity of the plant was confirmed by studying morphological characters and comparing them with characters mentioned in various floras. Roots were washed properly under running tap water to remove foreign mater like soil particle, sand etc. and kept it for shade drying at room temperature. After complete drying, it was powdered using electric grinder. The powder was then passed through mesh 40 and the fine powder thus obtained is used for extraction.

Preparation of ethanolic extract

20 gram fine powder was taken in 250 ml soxhlet extractor and extracted by using 70% ethanol for about six hours continuously. The obtained extract were vaporised under reducer pressure on vacuum evaporator. Finally the extract was stored at low temperature in refrigerator for further study.

Phytochemical studies

Primary phytochemical analysis of extract was carried out as per methods described by Arti Sharma and Vandana Sharma (2013) ^[16].

Test for Carbohydrates: For the test of carbohydrates, extract were dissolved in little distilled water and filtered. The filtrate obtained was used to test carbohydrates as follows.

1. Molisch's test: The extract first treated with Molisch's reagent and then concentrated sulphuric acid was added from the side of test tube to form a layer. The formation of reddish violet ring shows the presence of carbohydrates.
2. Benedict's test: The filtrate and 2 ml Benedict's reagent was boiled in water bath. green reddish brown precipitate formation shows presence of carbohydrates.

Test for Alkaloids: About 2ml of root extract were reacted with 10% NaOH solutions. The white precipitate was taken as positive test for alkaloid (Patil, et al. 2015) ^[18].

Test for Saponin: Foam test was performed for detection of saponin. Small quantity of extract was shaken with little quantity of water. If the foam produced and persists for about ten minutes; it indicates presence of saponin. The alcoholic extract showed positive result for saponin

Test for Steroids: To the extract few drops of concentrated sulphuric acid was added, shaken and allowed to stand. In the extract appearance of red colour indicates the presence of steroids.

Test for Tannins: For tannin ferric chloride test was performed. The extract was taken in test tube and few drops of 1% neutral ferric chloride solution was added, formation of blackish blue colour indicates the presence of tannins. In this case the test was negative.

Test for Flavonoids: In the alcoholic solution of extract few drops of sodium hydroxide solution were added. Intense yellow colour formed were disappeared after adding dil HCl (Patil, et al. 2015) ^[18].

Test for Amino Acids: About 3 ml of root extract were heated and 3 drops of ninhydrin solution was added in boiling water bath for 10 minutes. The purple colour developed showed presence of amino acids.

Antibacterial Activity: The ability to kill or inhibit the growth of pathogenic bacteria was evaluated by antibacterial screening of plant extract. This was performed by agar well diffusion method and the zone of inhibition (ZOI) was measured in mm for each bacteria. The different bacteria used in the study are *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. The activated bacterial cultures were seeded on sterile nutrient

media. Root extract were poured in the central well and the zone of inhibition were measured against the standard antibiotic ciprofloxacin (Ravishankar, *et al.* 2012) [19].

HPTLC fingerprinting of root extract

HPTLC fingerprinting of extract of *Asparagus racemosus* were carried out as per the method described by Ganeshan *et al.* (2015) [17]. Three microliters of the ethanolic extract was applied (band length 8.0 mm) on a precoated TLC aluminium sheets of silica gel G60 F254 of 200 µm thickness plate having size of 05 x10cm (Merck) using Linomat V TLC applicator (Camag, Muttenz, Switzerland) equipped with a 100µl syringe. Prior to application, the plate was pre-washed with methanol AR and dried at 60°C. TLC plates were developed using the mobile phase Ethyl acetate: Chloroform: ethanol (5:3:2) in a Camag HPTLC twin-trough chamber (10 x10cm). The chamber was saturated with filter paper for 15 minutes and plate equilibrium was carried out for 10 minutes. Plate was developed up to 85.0 mm and dried under stream of air. Separated bands were quantified by HPTLC densitometric scanning using Camag TLC Scanner IV in the absorption mode (multi wavelength scanning) operated by Win CATS software (version 1.4.8). After scanning the spectra and tables thus obtained were analyzed to interpret the results.

Results and Discussion

Table 1: Preliminary phytochemical analysis of root extract of *Asparagus racemosus* Willd.

S. No.	Compound	Present (+)/Absent (-)
1	Steroids	+
2	Alkaloids	+
3	Saponin	+
4	Carbohydrates	+
5	Flavonoids	+
6	Tannins	-
7	Amino Acids	+

The alcoholic extract of *Asparagus racemosus* root were subjected to qualitative phytochemical screening. The results of analysis are presented in table 1, which showed the presence of phytoconstituents like Steroids, Alkaloids, Saponin, Carbohydrates, Flavonoids and Amino Acids. The test performed for Tannin was found negative. The results obtained in this study shows slight similarity with the previous work of Ravishankar *et al.* (2012) [19] and Patil *et al.* (2015) [18].

Table 2: Antibacterial activity of *Asparagus racemosus* Willd root extract on nutrient agar.

S. No	Test Organism	Zone of Inhibition (in mm)	Standard Ciprofloxacin
1	<i>Escherichia coli</i>	12	19
2	<i>Staphylococcus aureus</i>	10	20
3	<i>Staphylococcus epidermidis</i>	15	20
4	<i>Pseudomonas aeruginosa</i>	12	20
5	<i>Streptococcus pyogenes</i>	15	21
6	<i>Streptococcus pneumoniae</i>	08	19

The results of antibacterial activity of *Asparagus racemosus* root extract on some pyogenic bacteria are presented in table 2. From the results it is clear that root extract has shown significant antibacterial activity in comparison with standard antibiotic ciprofloxacin. Maximum zone of inhibition (15 mm) were obtained in case of *Streptococcus pyogenes* and *Staphylococcus epidermidis* followed by *Escherichia coli* and *Pseudomonas aeruginosa* (12 mm) *Staphylococcus aureus* (10 mm) and *Streptococcus pneumoniae* (08 mm). From this it is clear that ethanolic extract of *Asparagus racemosus* root

has great potential as antibacterial agent against some common pyogenic bacteria. The study supports application of paste of root extract in curing different local pyogenic bacterial infections. The results justifies scientific traditional use of this plant in ayurvedic system of medicine. Our findings supports observations of earlier workers such as Patil *et al.* (2015) [18] and Ganeshan *et al.* (2015) [17]. The study throws light on usage of *A. racemosus* root as a remedy for various superficial infections in traditional medicine.

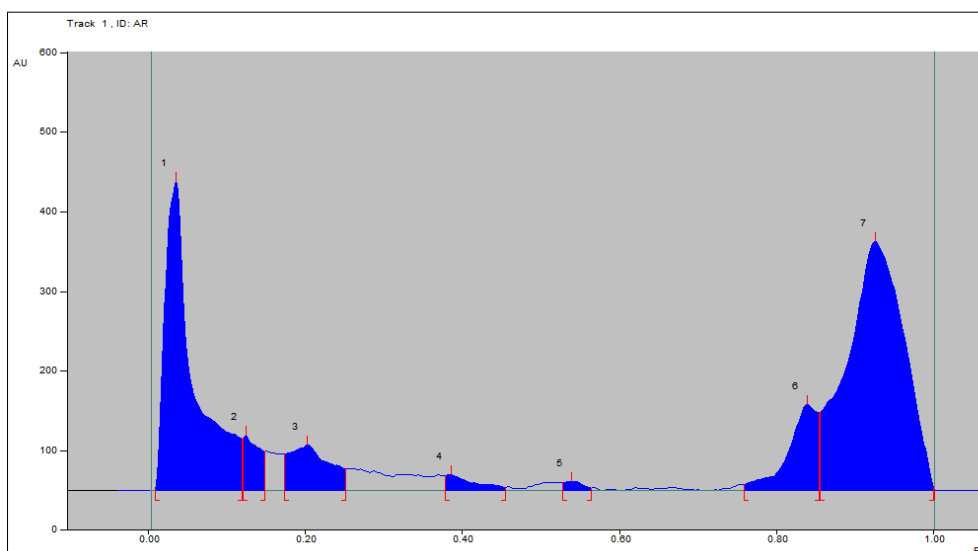


Fig 1: HPTLC chromatogram of *Asparagus racemosus* root extract

Results of HPTLC profile of *A. racemosus* root recorded at 254 nm showed presence of seven polyvalent compounds (Fig 1) with ascending Rf values 0.04 to 0.93. From the chromatogram and Rf table (Table 3) it is clear that the compound number first and seventh has highest concentration that is 40.15% and 32.36% with 0.04 and 0.93 respectively.

The antibacterial activity shown by root extract might be due to presence of some active ingredients in these polyvalent compounds. Further investigations can be carried out in order to isolate new compounds from the plant root extract and to evaluate the bioactivities as excess use of antibiotics in curing infections may develop resistance in some bacteria.

Table 3: Rf table for HPTLC Fingerprint

Track 1, ID: AR									
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	1.2 AU	0.04 Rf	388.0 AU	40.15 %	0.12 Rf	65.1 AU	12688.0 AU	30.72 %
2	0.12 Rf	65.6 AU	0.12 Rf	68.8 AU	7.12 %	0.15 Rf	48.8 AU	1285.0 AU	3.11 %
3	0.17 Rf	46.4 AU	0.20 Rf	57.1 AU	5.91 %	0.25 Rf	27.2 AU	2620.8 AU	6.35 %
4	0.38 Rf	19.1 AU	0.39 Rf	19.9 AU	2.06 %	0.46 Rf	4.0 AU	643.7 AU	1.56 %
5	0.53 Rf	9.4 AU	0.54 Rf	11.6 AU	1.20 %	0.56 Rf	3.1 AU	247.3 AU	0.60 %
6	0.76 Rf	7.5 AU	0.84 Rf	108.3 AU	11.21 %	0.86 Rf	98.1 AU	3598.8 AU	8.71 %
7	0.86 Rf	98.5 AU	0.93 Rf	312.8 AU	32.36 %	1.00 Rf	3.7 AU	20213.3 AU	48.95 %

The finding recorded in the current research confirms that *A. racemosus* root shows presence of several bioactive compounds like alkaloid, steroid, saponin, flavonoid, carbohydrates etc which could be responsible for versatile medicinal property of this plant. The data developed in HPTLC studies will serve as reference for quality control and finding adulterations in different ayurvedic medicine containing *A. racemosus* root. HPTLC fingerprint profile of root extract of *A. racemosus* will be useful in the correct identification of species before its use. From earlier research reports it is found that *A. racemosus* is widely used to cure different types of diseases. Some plants are of great importance due to their special constituents. These constituents have chemical substances that produces definite physiological action in human body when consumed in some fixed amount (Jadhav, 2018) [20]. The same fact may also applicable to *A. racemosus*.

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