

Asian Journal of Phytomedicine and Clinical Research

Journal home page: www.ajpcrjournal.com



PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF ROOTS OF *ASPARAGUS RACEMOSUS* WILLD

R. Ganesan^{*1}, T. Latha², S. Mangai²

^{*1}Department of Biochemistry, Siddha Central Research Institute, Arumbakkam, Chennai, Tamilnadu, India.

²Department of Biochemistry Bhakthavatsalam Colleges for Women, Korattur, Chennai, Tamilnadu, India.

ABSTRACT

The aim of the present study is to explore the Physico-chemical parameter, Preliminary phytochemical analysis, TLC Photo documentation, HPTLC fingerprinting, Heavy metal analysis of Lead, Cadmium, Mercury and Arsenic and antibacterial activity of methanolic extract of the roots of *Asparagus racemosus* Willd.(Fam:Asparagaceae). The physico-chemical analysis was carried out and loss on drying of the sample, total ash content, acid-insoluble ash, water and alcohol soluble extractive values of the sample were estimated. By preliminary phytochemical screening the presence of alkaloids, aminoacids, flavonoids, glycosides, phenol, steroid, tannin and saponin were confirmed. TLC photo documentation showed 9 and 11 visible spots under 254nm, 366nm respectively and 8 spots after derivatization. HPTLC fingerprint profile showed 10peaks at 254 nm. Heavy metals such as Cadmium, Mercury and Arsenic were not detected and Lead content was within the permissible limit. The antibacterial activity of methanolic extract of root of *Asparagus racemosus* was carried out against gram positive and gram negative bacteria. The antibacterial activity studied is expressed in terms of zone of inhibition measured in mm. A significant growth inhibition was shown by many of the organisms tested indicating the profound potency of the plant drug. This study scientifically supports the usage of root as a remedy for various superficial bacterial infections in traditional medicine.

KEYWORDS

Asparagus racemosus Willd, Heavy metal analysis, TLC, HPTLC and Antibacterial activity.

Author for Correspondence:

Ganesan R,
Department of Biochemistry,
Siddha Central Research Institute,
Arumbakkam, Chennai, Tamilnadu, India.

Email: ganeshbiochem@yahoo.co.in

INTRODUCTION

Herbal medicines are the major remedy in traditional system of medicine for thousand years and have made great contribution to maintain human health in many parts of the world in rural areas of developing countries as primary source¹. The genus *Asparagushas* been recently moved from the subfamily Asparagaein the family Liliaceae to a newly created family Asparagaceae². *Asparagus racemosus* Willd is an important monocot

medicinal plant of tropical and subtropical countries like India³.

The genus *Asparagus* is considered to be of medicinal importance because of the presence of steroidal saponins and sapogenins in various parts of the plant³. Roots of *Asparagus racemosus* (Family - Asparagaceae) is commonly known as than neervittankizhangu in Tamil and Shatavari in Sanskrit. Its medicinal usage has been reported in the Indian and British Pharmacopoeia and in traditional system of medicine, such as Ayurveda, Siddha and Unani. It is recommended in traditional medicine for the prevention and treatment of gastric ulcers, dyspepsia, diarrhoea and nervous disorders⁵. The plants also possess antioxidant, immunostimulant, antidyspepsia and antitussive properly⁶. Scanty work has been reported on antimicrobial activity of the root of this plant⁷.

It is one of the best rejuvenative drugs of classical Ayurveda. Its pharmacological applications, particularly from the root extracts, have recently been found to possess a phytoestrogenic, antidiarrhoeal and effects on neurodegenerative disorders⁸.

MATERIALS AND METHODS

The fresh roots were collected from Mettur, Salem Dt., Tamilnadu and authenticated by Dr.Sorna Subramaniyan, Research Officer (Botany), SMPG, Mettur Dam. The roots were cleaned and shade dried at room temperature. The dried material was pulverized mechanically into coarse powder. The coarse powders were used for the various studies. Coarse powder was extracted with methanol by cold percolation method (48 hrs). The extract was filtered through Whatman No.1 filter paper, concentrated on a water bath and finally dried in vacuum.

Physico-chemical parameters

Physico-chemical parameters were carried out as per WHO guidelines⁹.

Estimation of Heavy metals

Heavy metals like Lead, Cadmium, Mercury and Arsenic were estimated as per the procedure given in WHO¹⁰.

TLC and HPTLC fingerprint profile

TLC and HPTLC finger print profile of the methanol extract of the root of *Asparagus racemosus* was carried out by adopting standard procedure. The plate was developed in a solvent system of toluene: ethyl acetate (1:1) for the methanol extract. Then the plate was photo scanned under UV 254 nm, 366 nm and in white light using CAMAG visualizaer, with Cats 4.05 version software using Deuterium lamp at UV 254 nm. Finally the plates were dipped in vanillin-sulphuric acid. Allowed to dry and then heated at 105°C till the development of coloured spots of the separated compounds^{10,11}.

Preliminary phytochemical studies

Preliminary phytochemical analysis was carried out as per the procedure^{12,13}.

Antibacterial Activity

Collection of microorganism

To evaluate the antibacterial studies, the cultures were procured from various laboratories in and around Chennai. The organisms used were *Escherichia coli*, *Staphylococcus aureus*, *Enterobacteraerogens*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Proteus vulgaris* and *Klebsiella pneumoniae*. All the organisms were confirmed using specific biochemical tests¹⁴.

Inoculum Preparation

A uniform suspension of the organisms listed above were prepared in 6ml of saline, and compared with the McFarland's standards¹⁵. Each microbial suspension was diluted with the saline to a density visually equivalent to the Barium sulphate standard, 0.5 McFarland's unit. The plates were inoculated within 15 minutes of the preparation of the suspension to avoid changes in the density of the cultures.

Preparation of plates and Inoculation of microbial cultures

The required quantities of the Muller Hinton agar were prepared. The pH of medium was adjusted to 7.2. Each plate was poured with 20ml of the media and was allowed to solidify. The tubes containing 0.5 McFarland's unit equivalent microbial cultures

were dipped with sterile cotton swabs, and excess of the fluid was removed by gently rotating the swabs against the sides of the test tube. The dipped swabs were swabbed over the Muller Hinton agar plates covering the entire surface of the plate by rotating the plates in all the directions. After solidification wells of 6 mm diameter were punched in agar plates. Plates were then allowed to set for few minutes.

Drug concentration

500mg of plant extract was accurately weighed and dissolved in 2.5ml of DMSO solvent¹⁶ to make the stock solution containing 0.2mg/ml concentration. A series of dilutions were made from the stock solution to obtain 100µg/µl, 50µg/µl, 25µg/µl, 12.5µg/µl, 6.25µg/µl, 3.1µg/µl, 1.5µg/µl, 0.7 µg/µl and 0.3µg/µl ml for determination of MIC.

Antibacterial assay and Determination of MIC

Antibacterial activity was assayed in duplicates by agar well diffusion method¹⁷ using the above mentioned test organisms. The well was loaded with 80µl of the drug (0.2mg/ml conc.). The commercially available drug Ampicillin (10mcg/disc) was used as control. The plates were incubated at 37°C for 24 hrs. The diameter of the clearing zones were measured in mm using the calipers.

The precise assessment of the effectiveness of the extract against the susceptible bacteria was achieved by determining the MIC with varying concentration ranging from 6.25µg/µl to 200 µg/µl by agar well diffusion method. The plates were incubated at 37°C for 24hrs and were observed for the MIC, which was read as the lowest concentration of the drug required to completely inhibit the growth of the organism.

RESULTS AND DISCUSSION

The physico-chemical data of the dry powder of the roots (80mesh) is given in Table No.1. The root contains 11.14 % moisture content. The root contains 2.44 % of silicates as shown by the acid insoluble ash, but contains considerable amount of inorganic materials as shown by the higher ash value (12.16%). The alcohol soluble extractive

value 15.88 shows the polar constituents of the plant and water soluble extractive values 48.47 % showed the polar and inorganic constituents of the plant.

Heavy metal analysis

The amounts of various heavy metals found in the root are given in Table No.2. The heavy metal contents of lead, cadmium, mercury and arsenic as per WHO and Ayurvedic Pharmacopoeia India guidelines showed that cadmium, mercury and arsenic were not detected and lead was found to be within the permissible limit.

Preliminary phytochemical test

Preliminary phytochemical test of the methanol extract was carried out and tabulated in Table No.3. It showed the presence of alkaloid, amino acid, flavonoid, glycoside/sugar, phenol, steroid, tannin and saponin and absence of coumarin, quinone, triterpenoid, furanoid, lignan and carboxylic acid.

TLC and HPTLC finger printing profile

The R_f values of various spots of methanol extract in the TLC chromatogram are given in Table No.4. 9 spots and 11 spots were observed at 254 nm and 366 nm respectively. Then the plate was dipped in vanillin-sulphuric acid reagent and heated at 105°C. The TLC chromatogram of the methanol extract is shown in Figure No.1.

HPTLC FINGER PRINT ANALYSIS OF METHANOL EXTRACT

The HPTLC finger print profile was recorded at 254 nm (Figure No.2). It showed 10 peaks of which 4 peaks R_f 0.19, 0.51, 0.70 and 0.89 were major peaks and others moderately smaller peaks, R_f values are shown in Table No.5.

Antibacterial Activity

The result of the antibacterial activity of methanolic extract of the plant was observed. The antibacterial activities of the extract were studied against gram positive and gram negative bacteria. The antibacterial activity studied is expressed in terms of zone of inhibition measured in mm. A significant growth inhibition was shown by many of the organisms tested indicating the profound potency of

the plant drug. Among the tested organism *Salmonella typhimurium* and *Bacillus subtilis* were found to be the most sensitive organisms followed by *Escherichia coli* with maximum zone of diameter ranging from 27mm to 12 mm (Figure No.3). The organisms *Pseudomonas aeruginosa*,

Klebsiella pneumonia, *Staphylococcus aureus*, *Enterobacteraerogens* and *Proteus vulgaris* did not exhibit any activity. The result observed with the minimal inhibitory concentration is depicted in Table No.6.

Table No.1: Physico-chemical parameters of the roots of *Asparagus racemosus*

S.No	Parameter	Results
1	Loss on drying at 105°C	11.01 %; 11.14%; 11.27 %
2	Ash values	12.04 %; 12.17 %; 12.28 %
	a. Total ash b. Acid insoluble ash	2.28 %; 2.36 %; 2.69 %
3	Extract values	15.80 %; 15.88 %; 15.96 %
	a. Alcohol b. Water	48.36 %; 48.49 %; 48.56 %

Table No.2: Analysis of Heavy Metals of The Roots of *Asparagus racemosus*

S.No	Name of the Element	Result	Permissible Limit
1	Lead	0.0154 ppm	10 ppm (WHO)
2	Cadmium	Not detected	0.3 ppm (WHO)
3	Mercury	Not detected	1 ppm (API, 2008)
4	Arsenic	Not detected	3 ppm (API, 2008)

Table No.3: Preliminary Phytochemical analysis of the Roots of *Asparagus racemosus*

S.No	Test	Methanol extract
1	Alkaloid	+
2	Amino acid	+
3	Coumarin	-
4	Flavonoid	+
5	Glycoside/Sugar	+
6	Phenol	+
7	Quinone	-
8	Steroid	+
9	Tannin	+
10	Triterpenoid	-
11	Saponin	+
12	Furanoid	-
13	Lignan	-
15	Carboxylic acid	-

Table No.4: R_f values for the methanol extract of the roots of *Asparagus racemosus*

S.No	Solvent System	UV-254 nm	UV-366 nm	Visible light (Vanillin –Sulphuric acid reagent)
1	Toluene: Ethyl acetate (1:1)	0.84 Green	0.95 Fluorescent blue	0.94 Grey
		0.77 Blue	0.89 Fluorescent blue	0.88 Violet
		0.62 Green	0.80 Blue	0.78 Violet
		0.49 Green	0.73 Blue	0.61 Brown
		0.45 Green	0.66 Blue	0.39 Violet
		0.32 Green	0.43 Blue	0.29 Grey
		0.28 Green	0.35 Fluorescent blue	0.18 Violet
		0.18 Green	0.32 Fluorescent blue	0.13 Grey
		0.11 Green	0.19 Blue	
			0.14 Blue	
	0.10 Blue			

Table No.5: R_f Table for the HPTLC finger print

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.08 Rf	2.6 AU	0.11 Rf	32.3 AU	2.90 %	0.13 Rf	0.3 AU	479.9 AU	1.34 %
2	0.13 Rf	0.0 AU	0.19 Rf	180.5 AU	16.22 %	0.23 Rf	7.3 AU	4685.8 AU	13.10 %
3	0.27 Rf	2.7 AU	0.32 Rf	104.9 AU	9.43 %	0.34 Rf	72.2 AU	2626.1 AU	7.34 %
4	0.34 Rf	72.6 AU	0.35 Rf	77.2 AU	6.93 %	0.41 Rf	1.8 AU	1526.4 AU	4.27 %
5	0.41 Rf	1.9 AU	0.45 Rf	23.3 AU	2.09 %	0.46 Rf	19.4 AU	505.9 AU	1.41 %
6	0.46 Rf	18.9 AU	0.51 Rf	140.6 AU	12.63 %	0.59 Rf	0.2 AU	6407.4 AU	17.92 %
7	0.62 Rf	0.6 AU	0.70 Rf	252.6 AU	22.69 %	0.75 Rf	22.3 AU	7481.1 AU	20.92 %
8	0.76 Rf	23.4 AU	0.76 Rf	23.4 AU	2.10 %	0.79 Rf	15.1 AU	457.3 AU	1.28 %
9	0.80 Rf	12.2 AU	0.89 Rf	171.8 AU	15.44 %	0.94 Rf	77.3 AU	8886.3 AU	24.85 %
10	0.94 Rf	77.4 AU	0.96 Rf	106.6 AU	9.57 %	1.00 Rf	4.0 AU	2704.6 AU	7.56 %

Table No.6: Antibacterial activity of the roots of *Asparagus racemosus*

S.No	Organism	200 µg/µl	100 µg/µl	50 µg/µl	25 µg/µl	12.5 µg/µl	6.2 µg/µl	Standard Amp
1	<i>Proteus vulgaris</i>	-	-	-	-	-	-	+
2	<i>Enterobacteraerogens</i>	-	-	-	-	-	-	-
3	<i>Escherichia coli</i>	27	20	19	14	12	-	+
4	<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	+
5	<i>Klebsiella pneumonia</i>	-	-	-	-	-	-	-
6	<i>Salmonella typhimurium</i>	25	23	19	15	8	-	+
7	<i>Staphylococcus aureus</i>	-	-	-	-	-	-	+
8	<i>Bacillus subtilis</i>	15	11	7	-	-	-	+

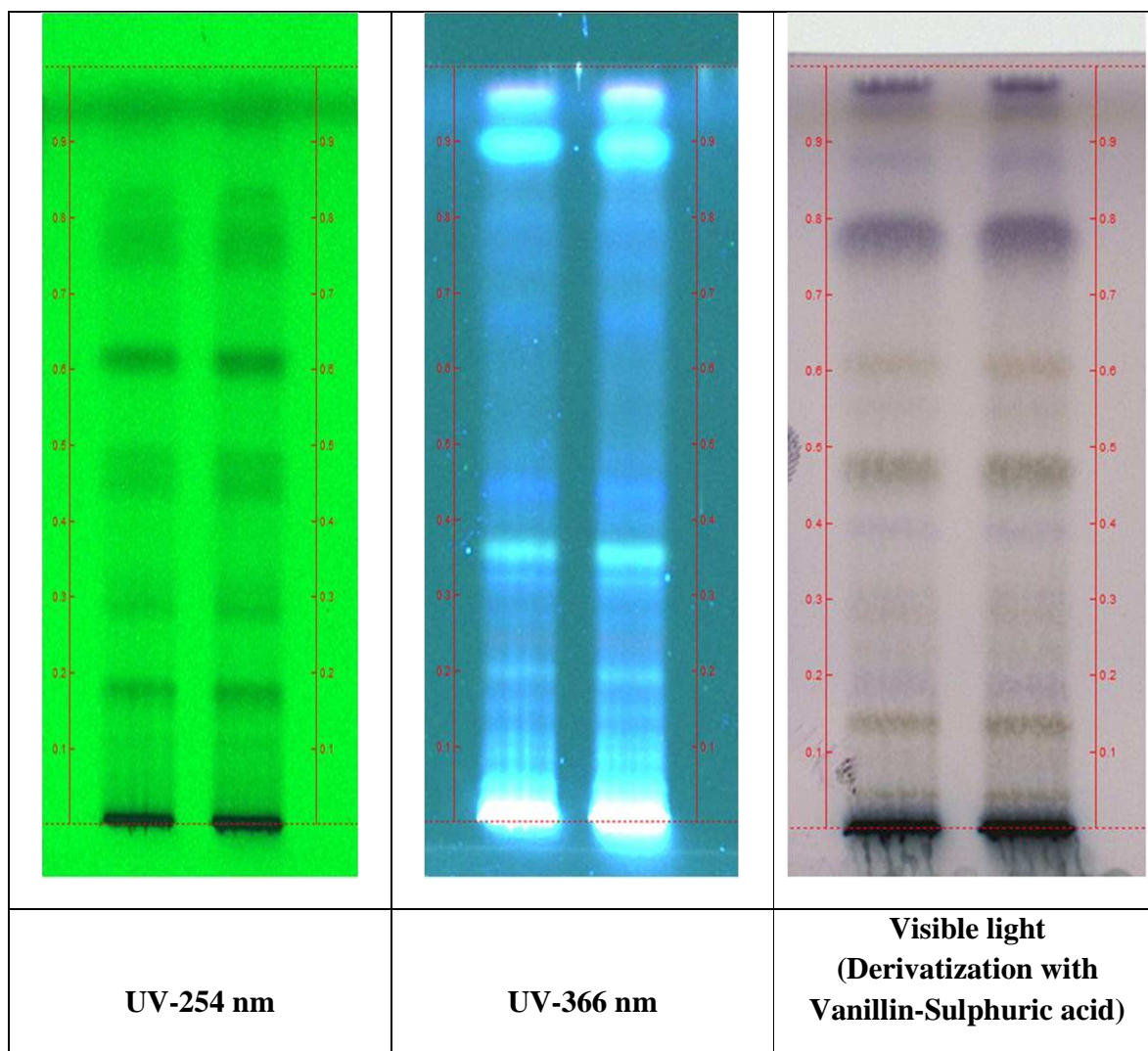


Figure No.1: TLC Chromatogram of the Methanol extract of the roots of *Asparagus racemosus*

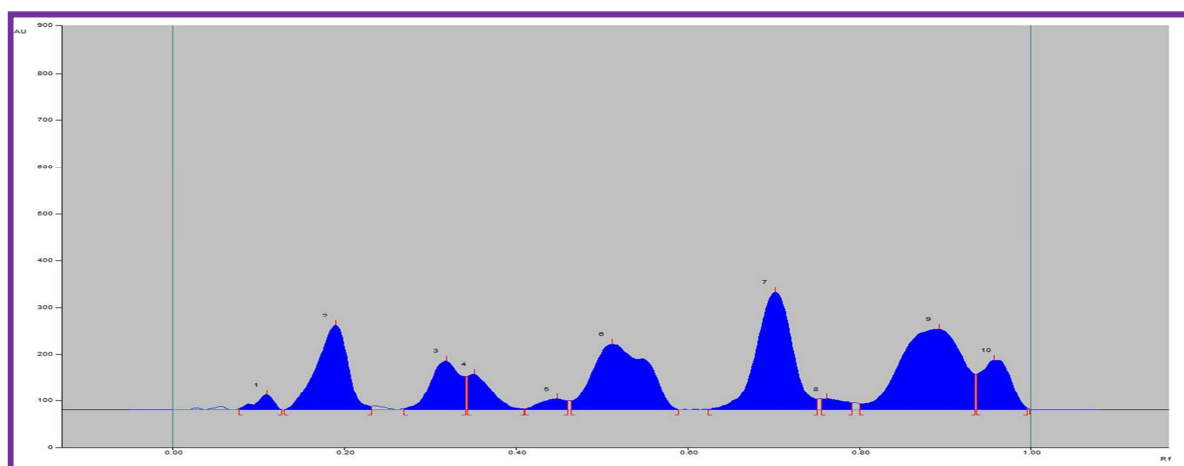


Figure No.2: HPTLC finger print of methanol extract of the roots of *Asparagus racemosus*

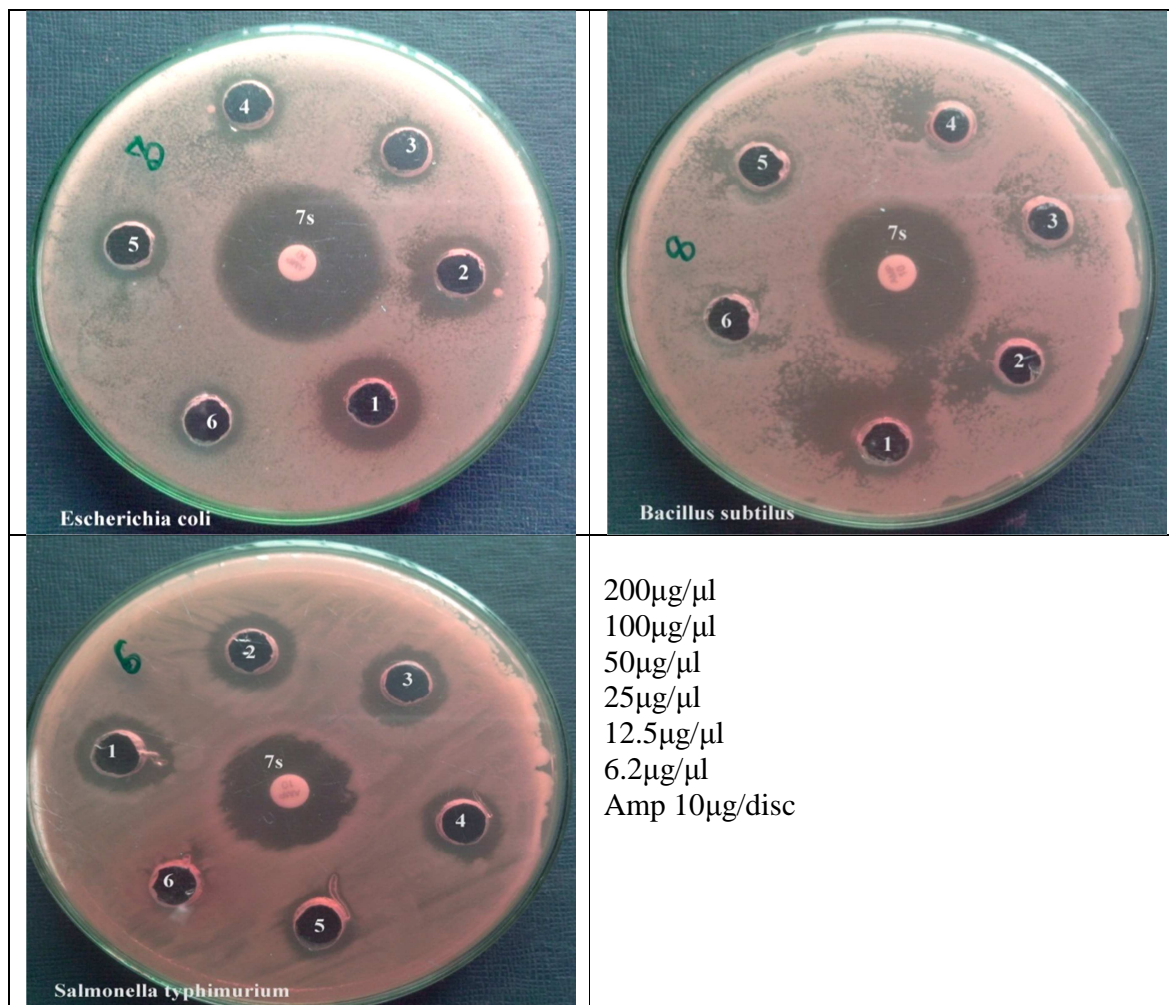


Figure No.3: Antibacterial activity of the methanol extract of the roots of *Asparagus racemosus*

CONCLUSION

Preliminary phytochemical analysis of the methanol extract revealed the presence of alkaloid, amino acid, flavonoid, glycoside/Sugar, phenol, steroid, tannin, and saponin. The methanol extract of root of *Asparagus racemosus* willd. Has a great potential as antibacterial agent against tested pathogenic bacteria viz. *Basillus subtilus*, *Escherichia coli* and *Salmonella typhimurium*. Physico-chemical parameters such as loss on drying, total ash, acid insoluble ash, alcohol and water soluble extractive would be helpful for fixing pharmacopoeial standards for the root of *Asparagus racemosus*. Heavy metal analysis showed that mercury, cadmium and arsenic were not detected and lead

was below the permissible limit. Thus showing this drug is safe for internal use. TLC Photo documentation and HPTLC finger print profile of the roots of *Asparagus racemosus* were evaluated and these will be useful in identification of the species. TLC photo documentation showed 9 and 11 visible spots under 254nm, 366nm respectively and 8 spots after derivatization. HPTLC fingerprint profile showed of 10peaks at 254 nm.

ACKNOWLEDGEMENT

The authors are sincerely thankful to Dr. Sasikala Ethirajulu, Research Officer (Pharmacognosy)-Scientist-2 for helping the paper writing of this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Anonymous. Research guidelines for evaluating the safety and efficacy of herbal medicines, *World Health Organisation, Regional Office for Western Pacific, Manila*, 1993, 1-3.
2. Madhavan V, Tijare R D, Mythreyi R, Gurudeva M R, Yoganarasimhan. Pharmacognostical studies on the root tubers of *Asparagus gonocladus* Baker, Alternate source for the Ayurvedic drug satavari, *Indian. J. Nature. Resour*, 1(1), 2010, 57-62.
3. Gomase V S and Sherkhane A S. Isolation, structure elucidation and biotransformation studies on secondary metabolites from *Asparagus racemosus*, *Inter. J. Microbio*, 2(4), 2010, 7-9.
4. Oketch-Rabah H A. Phytochemical Constituents of the Genus *Asparagus* and their biological activities, *Hamdard*, 41(4), 1998, 33-43.
5. Goyal R K, Singh J, Lal H. *Asparagus racemosus*, *Indian Journal of Medical Sciences*, 57(9), 2003, 408-14.
6. Bopana N, Saxena S. *Asparagus racemosus* ethnopharmacological evaluation and conservation needs, *Journal of Ethnopharmacology*, 110(1), 2007, 1-15.
7. Mandal S C, Nandy A, Pal M, Saha B P. Evaluation of antibacterial activity of *Asparagus racemosus* Willd root, *Phytotherapy Research*, 14(2), 2000, 118-9.
8. Venkatesan N, Thiyagarajan V, Narayanan S. Antidiarrhoeal potential of *Asparagus racemosus* Willd root extracts in laboratory animals, *J Pharm Sci*, 8(3), 2005, 39-46.
9. Anonymous. Quality Control Methods for Medicinal Plant Materials, *World Health Organisation, Geneva*, 10-31, 1998, 61-63.
10. Horwitz W, Latimer G W. Official Methods of Analysis of AOAC International, *Maryland*, Chapter, 18, 2005, 17-23.
11. Wagner H, Bladt S. Plant Drug Analysis A Thin Layer Chromatography Atlas, *Springer-Verlag, Germany*, 2nd Edition, 1996.
12. Sethi P D. High Performance Thin Layer Chromatography, *New Delhi, CBS publishers and Distributers*, 1(10), 1996, 1-56.
13. Harborne J B. Phyto-chemical Methods, A guide of modern techniques of plant analysis, London, *Chapman and Hall*, 2nd Edition, 1984.
14. Overton K H. Isolation, purification and preliminary observation in elucidation of structures by physical and chemical methods, Bently KH (Ed.), *New York, Inter Science.Pub*, 34, 1963.
15. Mackie, McCartney. Practical Medical Microbiology, *Churchill Livingston Publishers*, 14, 1996, 131-149.
16. Divakar C M, Nair G R N. Antiulcer, Antibacterial and Spermicidal activities of Salanin, *Indian Drugs*, 38(2), 2001, 629-932.
17. Vasudha Pai, Thangjam Rubee Chanu, Rituparna Chakraborty, Bangar Raju, Richard Lobo, Mamatha Ballal. Evaluation of the antimicrobial activity of *Punicagranatum* peel against the enteric pathogens *invitro* study, *Asian Journal of Plant Science and Research*, 1(2), 2011, 57-62.

Please cite this article in press as: Ganesan R, et al. Phytochemical analysis and antibacterial activity of roots of *asparagus racemosus* willd. *Asian Journal of Phytomedicine and Clinical Research*, 3(4), 2015, 132 - 139.