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Phytochemical and Antimicrobial Activity of Leaf Extract of *Asparagus racemosus* Willd.

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INTRODUCTION

Plants, the first medicines of human being, have played a remarkable role in healthcare since the ancient times. Traditional plant-based medicines still exert a great deal of importance to the people living in developing countries and also lead to discovery of new drug candidates for a variety of diseases that threaten human health. *Asparagus* is the name of a genus of plants, a member of the family Asparagaceae (formerly placed in the Liliaceae). The *Asparagus* genus is considered to be of medicinal importance because of the presence of steroidal saponins and sapogenins in various parts of the plant.[1] *Asparagus* is the Greek word for "stalk" or "shoot". About 300 species of *Asparagus* are known to occur in the world in many countries in both hemispheres and throughout temperate and tropical regions. Some of the European species are *A. officinalis, A. sprengeri and A. acutifolius. A. officinalis* is reported

ABSTRACT: The aim of the present study was to explore the leaf extract of *Asparagus racemosus* Willd. belonging to family Asparagaceae for its antimicrobial activity. The *in vitro* antimicrobial activity of the leaf extract (ethanol-EE) of *Asparagus racemosus* and its fractions (hexane- HE and chloroform-CE) were assayed using the agar plate diffusion and nutrient broth dilution methods. Test microorganisms studied were *Bacillus pumilis*, *Bacillus subtilis, Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus, Aspergillus nigerand Candida albicans.* EE (300 mg/ml) inhibited the growth of all the test organisms and the maximum zone of inhibition against gram positive organism is *S. aureus* (14.3 ± 0.2 mm); against gram negative is *E. coli* (14.0 mm); and against fungal organism is *C. albicans* (16.2 ± 0.2 mm). EE and CE showed minimum inhibitory concentration (MIC) of 12.5 and 25 mg/ml respectively against the *B. pumilis, S. aureus, E. coli and C. albicans*. The minimum bactericidal concentration (MBC) of EE was found to be 12.5 mg/ml, against *S. aureus* and *E . coli* where as for CE , the MBC was 50 mg/ml against *S. aureus*, *E. coli, B. pumilis* and *C. albicans* . The EE exhibited antimicrobial activities followed by CE. HE exhibited least antimicrobial activity. The preliminary phytochemical screening of EE, CE and HE revealed the presence of sterols, flavonoids, tannins and carbohydrates, determined by utilizing standard methods of analysis. Preliminary phytochemical screening as well thin layer chromatography of the EE, CE and HE revealed that the leaves of *A. racemosus* contain flavonoids which might possibly be responsible for the antimicrobial activity of the extracts.

Keywords : *Asparagus racemosus*; Asparagaceae; Antimicrobial; MIC; MBC; Flavonoids.

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TABLE 1: Preliminary phytochemical screening.

S. NO	NAME OF THE TEST	ETHANOL EXTRACT	HEXANE FRACTION	CHLOROFORM FRACTION	RESIDUAL ETHANOLIC FRACTION
1	Leibermann-Burchard test (Sterols)	+ ve	+ ve	+ ve	- ve
2.	Shinoda test (Flavonoids)	+ ve	+ ve	+ ve	- ve
3.	Foam test (Saponins)	+ ve	+ ve	+ ve	+ ve
4.	Neutral FeCl3 test (Phenolic compounds)	+ ve	- ve	+ ve	- ve
5.	Mayer's, Dragendorff's test (Alkaloids)	- ve	- ve	- ve	- ve
6.	Molisch's test (Carbohydrates)	+ ve	- ve	- ve	+ ve

to be a popular vegetable consumed in many parts of the world.[2] Out of several species of *'Asparagus'* grown in India, *A. racemosus*, *A. gonaclades* and *A. adsendens* are most commonly used in indigenous medicine.[3] *A. racemosus* is commonly mentioned as a rasayana in the Ayurveda. Rasayanas are those plant drugs which promote general well being of an individual by increasing cellular vitality or resistance.

A study of literature survey claimed some therapeutic attributes for the leaves of *A. racemosus* (Sanskrit:-Shatavari) and has been used in treatment of boils[4], as antiseptic[4] and pesticide.[4] *A. racemosus* leaves are also used for various traditional uses in India like tonic[4], heel cracks[5], scabies[6], stomachache[7], urinary disorders[7] and high blood pressure[8], as galactogogue and aphrodisiac.[9] However no scientific proof, justifying all the above uses of the leaves of *A. racemosus* is available so far. Based on the traditional uses of *A. racemosus*, the present study was undertaken to evaluate antimicrobial effect of the ethanol leaf extract.

MATERIALS AND METHODS

Plant material

The plant material *(A. racemosus)* was collected from Chikkala Village, near Nidadavolu, West Godavari Dist., A.P., India in September 2002. The identification of the plant sample was carried out by Dr. M.Venkaiah, Associate Professor, Department of Botany, A.U., Visakhapatnam. The specimen (voucher No. BMK/BGR-10/2003) was kept in the Herbarium of the Phytochemistry and Pharmacognosy specialization, Andhra University, Visakhapatnam, Andhra Pradesh, India.

Chemicals

All solvents (Hexane, chloroform and ethanol) were procured from Ranbaxy (India). Test reagents?? Media ingredients?? TLC solvent, sorbent, plate etc.??

Preparation of the extracts

The plant material of *A. racemosus* was dried under shade, ground mechanically to fine powder in a grinder and weighed accurately as 450.86 g. The powdered material was subjected to successive solvent extraction with ethanol using soxhlet apparatus. The extract was concentrated under vacuum (50°C), dried completely and weighed (76 g). Ethanol extract was then fractionated with hexane and then with chloroform until the solvent used for extraction becomes colorless. The hexane, chloroform and residual ethanolic fractions were concentrated under vacuum (50°C), dried completely and weighed. Their weights were : hexane fraction (25 g), chloroform fraction (20 g) and residual ethanolic fraction (16 g).

Phytochemical screening

The ethanol extract (EE) and its fractions (HE , CE and residual ethanolic fraction) were tested by the Libermann Burchard, Shinoda , Foam, Ferric chloride, Mayer's and Dragendorff's, and Molisch's tests to determine the presence of sterols, flavonoids, saponins, phenolic compounds, alkaloids and carbohydrates respectively.

the extracts (EE, CE and HE) were subjected to TLC analysis for the presence of flavonoids. Silica gel G, (mesh size 100–200, Acme) absorbent was used for plate preparation. The spots were made with capillary tube, and n-butanol, acetic acid and water (4: 1 : 5, upper layer v/v) was used as solvent system. The visualization of spots was carried out by spraying with $FeCl_3$: K_3FeCN_6 (1% aq.sol 1:1)[10] and Rf values of the extract were calculated.[11,12] The spots were also identified by observation under UV light and by exposure to ammonium vapour.

Organisms

The test microorganisms used for the antimicrobial activity screening were *Bacillus pumilis (B. pumilis),*

Bacillus subtilis (B. subtilis), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Proteus vulgaris (P. vulgaris), Staphylococcus aureus (S. aureus), Aspergillus niger (A. niger), Candida albicans (C. albicans). All the organisms were obtained from Department of Biotechnology, University college of Pharmaceutical Sciences, Andhra University, India.

Antimicrobial Tests

Antimicrobial activity of the EE, HE and CE was determined using agar plate diffusion technique.[13,14] The dried plant extract and fractions were dissolved in 1% aqueous dimethylsulfoxide (DMSO) to a final concentration of 100 and 300 mg/ml. The microorganisms were maintained on agar slants. The inocula were prepared by inoculating the test organisms i.e. bacteria in nutrient broth and incubating them for 24 hours at 37°C while for fungi, sabouraud dextrose broth was used which was incubated for 48 hours. The final inoculum size was adjusted to 1 x 10^6 cfu/ml for bacteria or 1 x 10^8 cfu/ml for fungi. One milliliter of the diluted cultures was inoculated into sterile molten nutrient agar (48°C) and poured into sterile petri dishes. Similarly, 1 ml of the diluted fungal suspensions was poured into sterile sabourand dextrose agar plates. These were gently swirled and allowed to solidify. Afterwards, 6 mm wells were bored into the solidified and inoculated agar plates using sterile borer. The wells were filled with 100 µl of 100 and 300 mg/ml of each extract/fraction. penicillin G (10 U), ampicillin (25 µg), ciprofloxacin (5 µg), Gentamycin (10 µg) and nystatin (10 µg) standard discs were placed on the agar plate. Plates were left for 2 hours in a refrigerator for the extract to diffuse into the agar. Plates were then incubated overnight at 25°C and 37°C for fungi and bacterial strains respectively. At the end of the incubation period, inhibition zones were recorded in millimetres as the diameter of growth free zones around the bored holes using a transparent metre rule. Each extract and standard antibiotics were independently tested in triplicate.

Minimum Inhibitory Concentration

MIC was determined using the broth dilution technique.[15,16] The minimum inhibitory concentration value was determined for the microorganisms that were sensitive to the extracts/fractions under study (EE, HE and CE). The microorganisms were prepared as described earlier. A two-fold serial dilution of each extracts was made to a concentration ranging from 0.098–100 mg/ml using nutrient broth. Each dilution was seeded with 200 µl of test micro-organisms to the standard concentration (1 x 10^6 cfu/ml). MIC is defined

TABLE 2: TLC analysis of *A. racemosus* leaf extracts.

EXTRACT	DISTANCE RUN BY SOLVENT (cm)	DISTANCE RUN BY SOLUTE (cm)	R_f VALUE
EE	5.6	2.9	0.52
HE	5.4	2.6	0.48
CE	5.7	2.9	0.51

EE–Ethanol extract, HE–Hexane extract, CE–chloroform extract.

as the lowest concentration where no visible turbidity was observed in the test tubes.

Minimum bactericidal concentration (MBC)

MBC were determined by using the broth dilution technique[16] by assaying the test tubes resulting from MIC determinations. A 1oopful of the content of each test tube was independently inoculated by streaking on a solidified nutrient agar plate incubated at 37°C for 24 hours and then observed for bacterial growth. The lowest concentration of the subculture with no growth was considered the minimum bactericidal concentration.

Determination of MIC index value

MIC index value is calculated using the following mathematical equation Provide reference? Use equation option to insert equation

RESULTS AND DISCUSSION

The results of the preliminary phytochemical screening of ethanol extracts and it fractions of *A. racemosus* are presented in Table 1. The preliminary phytochemical screening of the ethanol extract (EE) and its fractions (HE and CE) revealed the presence of sterols, flavonoids, saponins, phenolic compounds and carbohydrates. The results of the thin layer chromatography (TLC) of EE, HE and CE of *A. racemosus* are presented in Table 2. TLC plates when placed in a chamber saturated with ammonia vapours, showed yellow color. The plates when placed under UV light showed fluorescent spots. The developed plates when sprayed with $FeCl_3$: K_3FeCN_6 (1% aq.sol 1:1) developed an orange color spot. Rf values of the ethanol extract (EE) and its fractions (HE and CE) were as 0.52, 0.48, 0.51 respectively. The results of TLC revealed the presence of flavonoids in the extracts.

The antimicrobial screenings are recorded in Table 3 expressing the zones of inhibition of bacterial and fungal growth. The extracts showed considerable amount of

TABLE 3 : Susceptibility study of the extracts of *Asparagus racemosus* against test microorganisms.

EXTRACTS/ STANDARDS	CONC. (mg/ml)	UNITS	INHIBITION ZONE (mm)							
			B.P	B.S	S.A	E.C	P.V	P.A	A.N	C.A
EE	300	mg/ml	13.8 ± 0.2	14.0 ± 0.0	14.3 ± 0.2	14.0 ± 0.0	12.0 ± 0.0	12.2 ± 0.2	15.8 ± 0.2	16.2 ± 0.2
	100	mg/ml	12.0 ± 0.0	12.0 ± 0.0	10.2 ± 0.2	10.0 ± 0.0	10.0 ± 0.3	10.0 ± 0.0	13.8 ± 0.2	14.0 ± 0.0
HE	300	mg/ml	10.2 ± 0.2	10.2 ± 0.2	10.0 ± 0.0	10.0 ± 0.0	9.3 ± 0.2	10.0 ± 0.0	10.0 ± 0.0	12.2 ± 0.2
	100	mg/ml	8.0 ± 0.0	9.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	9.2 ± 0.2
CE	300	mg/ml	11.2 ± 0.4	10.3 ± 0.2	12.3 ± 0.3	11.3 ± 0.7	10.2 ± 0.2	10.7 ± 0.2	12.0 ± 0.0	13.0 ± 0.0
	100	mg/ml	8.0 ± 0.0	8.0 ± 0.0	10.0 ± 0.0	10.2 ± 0.2	8.0 ± 0.0	9.0 ± 0.0	9.0 ± 0.0	10.2 ± 0.2
PB	10	µg/disc	17.2 ± 0.2	16.2 ± 0.2	13.3 ± 0.3	16.5 ± 0.3	16.0 ± 0.0	8.0 ± 0.0	-	-
AM	25	µg/disc	14.0 ± 0.0	14.2 ± 0.2	10.3 ± 0.3	14.0 ± 0.0	14.0 ± 0.0	13.2 ± 0.2	-	-
CP	5	µg/disc	22.0 ± 0.0	23.7 ± 0.3	23.0 ± 0.0	22.0 ± 0.0	ND	22.0 ± 0.0	-	-
GM	10	µg/disc	18.2 ± 0.2	21.0 ± 0.6	22.0 ± 0.0	14.0 ± 0.0	ND	14.0 ± 0.0	-	-
NY	10	µg/disc	-	-	-	-	-	-	19.5 ± 0.3	20.2 ± 0.2
DMSO	100	%	-	-	-	-	-	-	-	-

Key: EE = Ethanolic extract, HE = Hexane extract, CE = Chloroform extract, PB = Benzyl Pencillin, AM = Ampicillin, CP = Ciprofloxacin, GM = Gentamycin, NY = Nystatin, DMSO = Dimethylsulfoxide, B.P = Bacillus pumilus, B.S = Bacillus subtilis, S.A = Staphylococcus aureus, E.C = Escherichia coli, P.V = Proteus vulgaris, P.A = Pseudomonas aeuroginosa, A.N = Aspergillus niger, C.A = Candida albicans,ND = No disc, - = no inhibition. Values are expressed in Mean ± S.E.M

TABLE 4 : Minimum inhibitory concentrations (MIC) of *A. racemosus* leaf extracts.

EXTRACTS	ORGANISMS	CONCENTRATION (mg/ml)										
		0.098	0.195	0.391	0.781	1.563	3.125	6.250	12.500	25.000	50.000	100.000
EE	B.P	+	+	+	+	+	+	+	–*	–	–	–
HE		+	+	+	+	+	+	+	+	+	–*	–
CE		+	+	+	+	+	+	+	+	–*	–	–
EE	B.S	+	+	+	+	+	+	+	+	–*	–	–
HE		+	+	+	+	+	+	+	+	+	–*	–
CE		+	+	+	+	+	+	+	+	+	–*	–
EE	S.A	+	+	+	+	+	+	+	–*	–	–	–
HE		+	+	+	+	+	+	+	+	+	–*	–
CE		+	+	+	+	+	+	+	+	–*	–	–
EE	E.C	+	+	+	+	+	+	+	–*	–	–	–
HE		+	+	+	+	+	+	+	+	+	–*	–
CE		+	+	+	+	+	+	+	+	–*	–	–
EE	P.V	+	+	+	+	+	+	+	+	–*	–	–
HE		+	+	+	+	+	+	+	+	+	–*	–
CE		+	+	+	+	+	+	+	+	+	–*	–
EE	P.A	+	+	+	+	+	+	+	+	–*	–	–
HE		+	+	+	+	+	+	+	+	+	–*	–
CE		+	+	+	+	+	+	+	+	+	–*	–
EE	C.A	+	+	+	+	+	+	+	–*	–	–	–
HE		+	+	+	+	+	+	+	+	+	–*	–
CE		+	+	+	+	+	+	+	+	–*	–	–
EE	A.N	+	+	+	+	+	+	+	+	–*	–	–
HE		+	+	+	+	+	+	+	+	+	–*	–
CE		+	+	+	+	+	+	+	+	+	–*	–

Key: EE = Ethanolic extract, HE = Hexane extract, CE = Chloroform extract, PB = Benzyl Pencillin, AM = Ampicillin, B.P = Bacillus pumilus, B.S = Bacillus subtilis, S.A = Staphylococcus aureus, E.C = Escherichia coli, P.V = Proteus vulgaris, P.A = Pseudomonas aeuroginosa, A.N = Aspergillus niger, C.A = Candida albicans,+ = growth, – = no growth. – = MIC.*

inhibition against *B. pumilis*, *B. subtilis, S. aureus, E. coli, P. vulgaris, P. aeuroginosa*, *A. niger* and *C. albicans.* EE (300 mg/ml) inhibited the growth of all the test organisms and the maximum zone of inhibition was found to be against gram positive organism *S. aureus* (14.3 ± 0.2 mm); gram negative organism *E. coli* (14.0 mm); and fungal organism *C. albicans* (16.2 ± 0.2 mm). The standard antibiotic discs used in this study inhibited the growth of the test bacterial and fungal organisms. The zone of inhibition produced by penicillin G disc against *P. aeuroginosa* was found to be smaller than those produced by some extracts especially EE, HE and CE (300 mg/ml).

From the results of the MIC and MBC presented in Tables 4 and 5 respectively, it can be surmise that the active constituents responsible for the widest activities in this plant were residing in the CE fraction of the ethanol extract compared with HE; this was basically because the EE and CE fraction notably exhibited minimal inhibitory concentration of 12.5 and 25 mg/ml respectively against the *B. pumilis*, *S. aureus, E. coli* and *C. albicans*. HE fraction had an MIC of 50 mg/ml against *B. pumilis*, *B. subtilis, S. aureus, E. coli, P. vulgaris* and *P. aeuroginosa.*

The minimum bactericidal concentration of EE was found to be 12.5 mg/ml against *S. aureus* and

TABLE 5: Minimum bactericidal concentrations (MBC) of *A. racemosus* leaf extracts.

EXTRACTS	ORGANISMS	CONCENTRATION (mg/ml)										
		0.098	0.195	0.391	0.781	1.563	3.125	6.250	12.500	25.000	50.000	100.000
EE	B.P	+	+	+	+	+	+	+	+	–*	–	–
HE		+	+	+	+	+	+	+	+	+	+	–*
CE		+	+	+	+	+	+	+	+	+	–*	–
EE	B.S	+	+	+	+	+	+	+	+	+	–*	–
HE		+	+	+	+	+	+	+	+	+	+	–*
CE		+	+	+	+	+	+	+	+	+	+	–*
EE	S.A	+	+	+	+	+	+	+	–*	–	–	–
HE		+	+	+	+	+	+	+	+	+	+	–*
CE		+	+	+	+	+	+	+	+	+	–*	–
EE	E.C	+	+	+	+	+	+	+	–*	–	–	–
HE		+	+	+	+	+	+	+	+	+	+	–*
CE		+	+	+	+	+	+	+	+	+	–*	–
EE	P.V	+	+	+	+	+	+	+	+	+	–*	–
HE		+	+	+	+	+	+	+	+	+	+	–*
CE		+	+	+	+	+	+	+	+	+	+	–*
EE	P.A	+	+	+	+	+	+	+	+	+	–*	–
HE		+	+	+	+	+	+	+	+	+	+	–*
CE		+	+	+	+	+	+	+	+	+	+	–*
EE	C.A	+	+	+	+	+	+	+	+	–*	–	–
HE		+	+	+	+	+	+	+	+	+	+	–*
CE		+	+	+	+	+	+	+	+	+	–*	–
EE	A.N	+	+	+	+	+	+	+	+	+	–*	–
HE		+	+	+	+	+	+	+	+	+	+	–*
CE		+	+	+	+	+	+	+	+	+	+	–*

Key: EE = Ethanolic extract, HE = Hexane extract, CE = Chloroform extract, B.P = Bacillus pumilus, B.S = Bacillus subtilis, S.A = Staphylococcus aureus, E.C = Escherichia coli, P.V = Proteus vulgaris, P.A = Pseudomonas aeuroginosa, A.N = Aspergillus niger, C.A = Candida albicans,+ = growth, – = no growth. – = MBC.*

E. coli. For CE fraction, the MBC was 50 mg/ml against *S. aureus* , *E. coli*, *B. pumilis* and *C. albicans* and for HE fraction; the MBC was 100 mg/ml towards *B. pumilis*, *B. subtilis*, *S. aureus*, *E. coli*, *P. vulgaris* and *P. aeuroginosa*.

From the results of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) presented; it was observed that the broadest activity of the extract was against *E. coli* and *S. aureus.* MIC index results are presented in Table 6.

The EE exhibited antimicrobial activities followed by CE fraction. HE fraction exhibited least antimicrobial activity. Ethanol extract (EE) and its fractions (CE and HE) exhibited appreciable activity against *S. aureus,* a pyogenic bacterium known to play a significant role in invasive skin diseases including superficial and deep follicular lesion.[17] It also showed appreciable activity against *E. coli*. The broad-spectrum antibacterial activity exhibited by the EE and CE could be related with the concentrations of sterols, flavonoids, saponins, phenolic compounds and carbohydrates in these extracts. These classes of compounds are known to show curative activity against several pathogens[18] and may explain some of its antimicrobial actions since antimicrobial actions of most of these phytochemical substances have been documented.[19-21]

The literature survey of plant reported the presence of flavonoids[22] and preliminary phytochemical screening as well as thin layer chromatography of the

TABLE 6: MIC index of ***A. racemosus*** leaf extracts.

EXTRACTS	ORGANISMS	MIC (mg/ml)	MBC (mg/ml)	MIC INDEX
EE	B.P	12.500	25.000	2
HE		50.000	100.000	2
CE		25.000	50.000	2
EE	B.S	25.000	50.000	2
HE		50.000	100.000	2
CE		50.000	100.000	2
EE	S.A	12.500	12.500	1
HE		50.000	100.000	2
CE		25.000	50.000	2
EE	E.C	12.500	12.500	1
HE		50.000	100.000	2
CE		25.000	50.000	2
EE	P.V	25.000	50.000	2
HE		50.000	100.000	2
CE		50.000	100.000	2
EE	P.A	25.000	50.000	2
HE		50.000	100.000	2
CE		50.000	100.000	2
EE	C.A	12.500	25.000	2
HE		50.000	100.000	2
CE		25.000	50.000	2
EE	A.N	25.000	50.000	2
HE		50.000	100.000	2
CE		50.000	100.000	2

Key: EE = Ethanolic extract, HE = Hexane extract, CE = Chloroform extract, B.P = Bacillus pumilus, B.S = Bacillus subtilis, S.A = Staphylococcus aureus, E.C = Escherichia coli, P.V = Proteus vulgaris, P.A = Pseudomonas aeuroginosa, A.N = Aspergillus niger,C.A = Candida albicans.

extracts revealed that the leaves of *A. racemosus* contain flavonoids. Several flavonoids isolated from the medicinal plants have been discovered to possess significant antimicrobial activity.[21,23]

This study confirmed that the ethanol extract (EE) and its hexane (HE) and chloroform (CE) fractions of leaves of *A. racemosus* exhibit antimicrobial activity and the effects observed are attributable due to the presence of flavonoids in the plant.

In conclusion, the fact that the extracts (EE and CE) produced inhibitory activities but less when compared to reference drugs against almost all the test bacteria and fungi provides some scientific basis for some of the uses in traditional medicine like treatment of boils and scabies and as antiseptic. We therefore, suggest the isolation and possible characterization of the active constituent(s) from the extracts of this plant species as possible antibacterial agents.

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REFERENCES

1. Oketch Rabah HA. Phytochemical Constituents of the Genus *Asparagus* and their biological activities. *Hamdard*. 1998; 41**:** 33–43.
2. Shao YU, Poobsasert O, Kennelly EJ, Chin CK, Ho CT, Huang MT, et al. Steroidal saponins from *Asparagus officinalis* and their cytotoxic activity. *Planta Medica.* 1997; 63**:** 258–62.
3. Rao SB. Saponins (Sapogenins) from Indian Medicinal Plants:- Part I Sapogenins from *Asparagus*. *Indian J Pharmacy*. 1952; 14**:** 131–132.
4. Sharma GK. Medical ethnobotany in the Shivalik Range of the Himalayas. *Journal of the Tennessee Academy of Science*. 2004; 79**:** 67.
5. Ignacimuthu S, Ayyanar M , Sivaraman KS. Ethnobotanical investigations among tribes in Madurai District of Tamil Nadu (India). *Journal of Ethnobiology and Ethnomedicine*. 2006; 2**:** 25.
6. Yesodharan K, Sujana KA. Status of ethnomedicinal plants in the parambikulam wildlife sanctuary, kerala, south india. *Ann. For.* 2007; 15**:** 322–334.
7. Sajem AL, Gosai K. Traditional use of medicinal plants by the Jaintia tribes in North Cachar Hills district of Assam, northeast India. *Journal of Ethnobiology and Ethnomedicine*. 2006; 2**:** 33.
8. Bhutani KK. *Herbal Wealth of North-East India, Database and Appraisal,* Azad Hind Stores, Chandigarh, 2008.
9. Sumeet D, Shefali K. Ethnomedicinal uses of some plant species by ethnic and rural peoples of indore district of madhya pradesh, India. *Pharm. Review*. 2008; 6**:** 2.
10. Markham K. *Isolation Techniques for Flavonoids.* Academic press, New York, 1975.
11. Harbone JB. *Phytochemical Methods,* Reinhold publication, London, 1973.
12. Bobitt J M. *Thin Layer Chromatography*, Reinhold publication, London, 1966.
13. Odama IE, Shok M, Olurinola PF. *Ceiba Petandra* (Silk Cotton Tree). *The State of Medicinal Plant Research in Nigeria*. Ile-Ife, Univ. Press, Nigeria, 1986.
14. Chung KT, Thomson WR, Wu-Yan CD. Growth Inhibition of Selected Food-Borne Bacteria by Plant Extracts. *J. Appl. Microbiol*. 1990; 71**:** 398–401.
15. Sidney MF, William JM, Elvyn GS. *Bailey and Scott's Diagnostic Microbiology*. C.V. Mosby, St. Louis, 1978.
16. Vollekovà A, Kòst'àlovà D, Sochorovà R. Isoquinoline Alkaloids from *Mahonia aquifolium* Stem bark is active against Malassezia spp. *Folia Microbiol*. 2001; 46**:** 107–110.
17. Srinivasan D, Nathan S, Suresh T, Perumalsamy PZ. Antimicrobial Activity of Certain India Medicinal Plant used in Folkloric Medicine. *J. Ethnopharmacol*. 2001; 74**:** 217–220.
18. Eloff JN. Quantifying the bioactivity of plant extracts during screening and Bio-assay guided fractionation. *Phytomedicine*. 2004; 11 **:** 370–377.
19. Pereira JA, Pereira APG, Ferreira ICFR, Valentão P, Andrade PB, Seabra R, et al. Table olives from Portugal: phenolic compounds, antioxidant potential and antimicrobial activity. *J. Agric. Food Chem*. 2006; 54**:** 8425–8431.
20. Proestos C, Chorianopoulos N, Nychas GJE, Komaitis M. RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity. *J. Agric. Food Chem.* 2005; 53**:** 1190–1195.
21. Rauha J P, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala T, et al. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *Int. J. Food Microbiol.* 2000; 56**:** 3–12.
22. Velavan S, Nagulendran KR, Mahesh R, Hazeena Begum V. The Chemistry, Pharmacological and Therapeutic Applications of *Asparagus racemosus*- A Review. *Phcog Rev.* 2007; 1**:** 350–360.
23. Xu HX, Lee SF. Activity of plant flavonoids against antibiotic-resistant bacteria. *Phytother. Res.* 2001; 15**:** 39–43.