

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *ASPARAGUS RACEMOSUS* WILLD. AND *ASPARAGUS CURILLUS* BUCH.-HAM. EX ROXB.

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ABSTRACT

Asparagus racemosus Willd. is an important medicinal plant of tropical and subtropical regions of Nepal and India. Its medicinal usage has been reported in the Indian and British Pharmacopoeias and in traditional systems of medicine such as Ayurveda, Unani, and Siddha. *Asparagus curillus* Buch-Ham.ex Roxb. is also one of the species found in higher altitude of Nepal. Its roots are used as substitute for *A. racemosus*. Phytochemical investigation was done for these two species of Nepalese Asparagus as per Methodology for Analysis of Vegetable Drugs by I. Ciulei. 1982. Phytochemical screening revealed the presence of coumarin, flavonoid, catecholic tannin, reducing compound in alcoholic extract of *A. racemosus* while its aqueous extract revealed polyuronoid, reducing compound, polyoses, saponin, gallic tannin, catecholic tannin, etc. Similarly, alcoholic extract of *A. curillus* revealed catecholic tannin, reducing compound and aqueous extract revealed polyuronoid polyoses, saponin, gallic tannin as main phytochemical compounds. Comparative antimicrobial activity of ethanolic extract of these two species has been evaluated using Kirby-Bauer Agar well diffusion method. The extracts were screened for their antimicrobial activity on nine different strains of human pathogenic microorganisms such as *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Saccharomyces cerevisiae* and *Candida albicans*. Among them *A. racemosus* has shown selected antimicrobial effects against *B. subtilis*, *E. coli*, *E. faecalis*, *S. cerevisiae* and *C. albicans* with zone of inhibition of 25 mm in an average. While *A. curillus* showed effects on *S. cerevisiae* and *C. albicans* only with zone of inhibition about 12 mm.

Keywords: medicinal plant, traditional medicine, alcoholic extract, phytochemical compounds, pathogenic microorganisms

INTRODUCTION

Plants are major source of potent drugs for traditional medicine since ancient period. Secondary metabolites present in these plants are known to exhibit numerous biological activities like antibacterial, anticancer, antifungal and antioxidant activities that promote positive health effects for humans and animals. Some secondary metabolites such as plant pigments, alkaloids and isoprenoids are responsible for color, flavor, and smell in plants and have been source for drugs,

fine chemicals, insecticides, dyes, flavors, and fragrances for human use. Similarly, steroidal glycoside in *Parmelia* seems to be responsible for its strong antibacterial activity (Karn, 2003).

Asparagus racemosus Willd. (Kurilo, Satavari) is an important medicinal plant of tropical and subtropical regions of Nepal and India mostly. Its medicinal usage has been reported in the Indian and British pharmacopoeias and in traditional systems of medicine such as Ayurveda, Unani, and Siddha. It is one of the top ten most traded high value medicinal plant species of Nepal having therapeutical as well as nutraceutical importance (Tiwari, 2002; Acharya, 2005). Steroidal saponin, Isoflavenoids and polysachharides are some of the major components found in this plant which are responsible for its antidiarrheal, antidysenteric, wound healing, antioxidant properties (Nagar *et al.*, 2015). Devkota & Dutta (2001) also studied and found antibacterial activity of *Asparagus recemosus*, against six bacteria (*Escherichai coli*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Vibrio cholerae*). But no research so far has been carried out on Nepalese *Asparagus* species.

Of the 300 species of *Asparagus* found worldwide, Nepal harbors 7 species so far viz. *Asparagus lycopodineas*, *Asparagus adscendens*, *Asparagus curillus*, *Asparagus filicinus* (var. *brevipes* & var. *filicinus.*), *Asparagus penicillatus* H. Hara (endemic species from Dolpa), *Asparagus racemosus* (var. *racemosus*, & var. *subacerosus*), and *Asparagus tibeticus* (new record from Mustang, in the wild. *Asparagus curillus* is also one of the species of Nepal found in higher altitude. It is also reported to have similar medicinal value and used as substitutes for wild asparagus (*Asparagus racemosus*). If its therapeutic value is proved to be equally effective it can contribute as alternative source for *A. racemosus*. The main objective of this study is to assess and compare phytochemical characteristics and antimicrobial properties of these two *Asparagus* species from different altitudes and habitat types and to know their potentialities of medicinal values.

MATERIALS AND METHOD

Study species

***Asparagus racemosus* Willd.:** It is slightly scrambling sub shrubs with a short rhizomatous rootstock having cluster of fusiform tuberous roots ca. 6-14 cm long. Stem 1-2.5m long with woody base, ca. 4-5cm thick and densely branched. Leaves are reduced to scales or spines. Instead cladodes are found in fascicle of 3-11, v-shaped in outline, 6-30x0.5-1mm, straight or curved, with acuminate tip. Spines are usually curved, 1.5cm long in main branch and smaller in upper branch lets. Flowers are white and produced in raceme or panicles. Fruits are berry; globose or lobed, 1-3 seeded; red when ripe. It is found around 100-2100m altitude in forest floor of Nepal, E Himalaya, S E Asia, Australia, and Africa.

***Asparagus curillus* Buch-Ham.ex Roxb.:** It is a sub-scandent shrub 1.5-3.5 1-3 m tall. Roots are long and slender, tuberous, whitish brown up to (8-)30-80cm long. Stem is light yellow in colour, armed with spine, smooth at base and ribbed distally. Spines are straight or curved, 0.5-2.5cm. Branches are many ribbed and spiny with branch lets spreading and angled. Cladodes are in fascicles of 3-8(-12) number, not equal in same fascicles, and are 3-6 (-8) X 0.5-0.8 mm long, linear flattened or sub triquetrous, straight or curved with acute tip. Inflorescence are simple or branched racemes usually with cladodes at top, 1-3 nate, axillary. Racemes are 1-8 cm long with 10-12 flowers. Pedicel are 3-6 mm long, jointed at middle. Bracts are lanceolate,

1.2--1.7 mm long. Flowers are white, bisexual, 2--3 mm in diam. Tepals are ca. 3 X 1 mm, cuneiform, spreading with obtuse tip, entire, companulate. Stamens are shorter than tepals, ca. 2 mm. Anthers are oblong, Gynoecium are clavate with style ca 2.2 mm, stigma 3 recurved lobes. Fruit is berry, 3-4 mm long, 3 lobed and red when ripe. Seeds are 2 mm in length and turbinate. It is distributed in west Himalaya, Nepal along the altitudinal range of 1000-2850m.

Plant material preparation

The root tubers of *Asparagus racemosus* were collected from Kitne VDC Daman and Manahari VDC Hetauda of Makawanpur district, while that of *A. curillus* were from Dhunche VDC of Rasuwa. The voucher specimen was identified from KATH herbarium Godawari. The collected plant materials were dried in sun for few days and then at room temperature. It was then powdered and the powdered drug was stored in cool and dry place for further studies.

Preparation of extracts

Forty grams (40g) of dried coarsely powdered samples was extracted with 50% ethanol by soxhlet method (Ciulei, 1982). All the extracts were completely dried keeping on water bath. Drying was done within a day. The dried extracts were then used for phytochemical analyses and antibacterial activity.

Physicochemical and phytochemical tests

The crude ethanol extract of *A. racemosus* and of *A. curillus* were subjected to preliminary phytochemical screening for the detection of major phytochemical constituents per the standard methods. Parameters such as percentage of total ash, acid-insoluble ash, water soluble ash, extractive values of water and alcohol were calculated as per the methods of Indian pharmacopoeia (Anonymous, 1996). The presence or absence of different phyto-constituents viz, triterpenoids, alkaloids, steroids, sugar, tannin, glycosides and flavonoids etc. were detected by usual prescribed methods (Culie, 1985) as follows:

Etheric extract test

Test for volatile oil: 2 ml extractive solution were placed in a capsule and allowed to evaporate to dryness. 0.5 ml of alcohol are added. Some drops from the concentrated alcoholic solution are placed on a filter paper. A spot formed after evaporation denotes presence of oil.

Test for alkaloids: 6 ml extractive solution are evaporated on water bath and residue obtained is dissolved in 1.5 ml of 2N HCL. 0.5 ml of this sol is reacted with 2-3 drops of Mayer's reagent (Mayer's test). Yellowish white precipitation indicates presence of alkaloids as bases.

Test for fatty acids and coumarins: 10-15 ml extractive solution are extracted 3 times each with 1.5 ml of 10% KOH in a separating funnel on gently shaking. One aqueous alkaline solution re-acidulated with conc. HCL then extracted 3 times each with 5 ml of ether in extraction funnel. 2 drops of this etheric solution are placed on filter paper. If the spot persists after evaporation it indicates presence of fatty acids. 2 ml of etheric solution were concentrated till the residue is obtained. It is then dissolved in hot water. After cooling the sol was divided in two tubes: one tube will serve as standard. Aqueous solution of second tube was made alkaline with 0.5 ml of 10% NH₄OH and observed under UV light. The occurrence of intense fluorescence under

UV light indicates presence of coumarins and derivatives.

Test for flavenoids: 2 ml of etheric solution were concentrated till residue was obtained. The residue was dissolved in 1-2 ml of 50% methanol by warming up with metal magnesium and 2-3 drops of conc. HCL was added. A red or orange color indicates the presence of flavenoids. 2 ml of etheric solution is concentrated till it gives a residue which is dissolved in 0.5 ml of acetic anhydride and 0.5 ml of chloroform. The solutions are transferred to a dry tube and, at its bottom added 1-2 ml of conc. H₂SO₄ (Liebermann-Brofad's reagent). At contact zone of two liquid violet ring is formed, superior layer becomes green gallic denoting presence of sterols and some triterpenes respectively.

Alcoholic extract test

Test for tannin: 0.5 ml of alcoholic extractive solution was diluted with 1 ml of water and 2-3 drops of diluted FeCl₃ (light yellow) are added. The occurrence of a blue-blackish precipitate shows presence of gallic tannins and a green-blackish color indicates catchcolic tannins.

Test for reducing compounds: 0.5 ml of alcoholic extractive solution are diluted with 1ml of water and 0.5 ml of Fehling (I and II) solution and warmed up. A red brick precipitate denotes the presence of reducing compounds.

Test for coumarin derivatives: 4 ml of etheric solution was concentrated till a residue remains. A residue obtained thus was dissolved in 1-2 ml water by heating if needed. The aqueous solution is divided in two equal volumes, in two tubes. To one of the tube 0.5 ml of 10% NH₄OH are added, the other tube serving as standard. The presence of blue, violet fluorescence under UV light, deeper for alkaline solution indicates the presence of coumarin.

Test for glycosides: To 10-15 ml of alcoholic extractive solution, an equal volume of 10% HCL added by refluxing and heated for 30 minutes. During hydrolysis, the solution becomes turbid due to precipitation of aglycones resulted by the division of the glycosides. After cooling the solution is extracted 3 times each in a separating funnel, with 6-8 ml of ether. The etheric extractive solutions are placed together (16-20 ml) and dehydrated with dry Na₂SO₄ thus resulting and etheric and aqueous solution. The etheric solution will serve to identify anthracenoside of coumarin, the flavonoids, glycosides, sterols and triterpenes by means of specific test to aglycones.

Test of anthracenoside: 4ml of etheric solution are concentrated till 2 ml remains, then 1-2 ml of 25% NH₄OH were added by shaking. A cherish red colour of the alkaline solution indicates the presence of emodine (aglycones of anthracenoside) in an oxidized form (Brontrager's reation)

Test of flavonic glycosides: 4 ml of an etheric solution are concentrated to residue which is then dissolved in 1-2 ml of 50% methanol y heating. Metal magnesium and 5-6 drops of conc. HCL (Shibata's reaction) were added. The red solution indicates presence flavonoid, orange indicates flavones and violet for flavonones.

Test for sterols: Liebermann-Burchard test: 4 ml of etheric solution are concentrated till a residue is obtained. It is dissolved in 0.5 ml of acetic anhydride and 0.5 ml of chloroform. By means of a pipette, 1-2 ml of conc. H₂SO₄ are placed at the bottom. At the separating level of the two liquids, a brown reddish or brown violet ring is formed, the superior layer being blue green (for sterol) or violet (often for triterpenes)

Test for saponin: Small amount of extracts was shaken with little quantity of water vigorously. The foam generation that persists for 10 minutes indicates the presence of saponin.

Test for alkaloids: 4.5 ml alcoholic extract solution are pipetted in a capsule and concentrated on the boiling water. To the residue, 1-2 ml of 2% HCl are added.

Antimicrobial activity

Comparative antimicrobial activity of ethanolic extract of these two species has been evaluated using Kirby-Bauer Agar well diffusion method. The extracts were screened for their antimicrobial activity on nine different strains of human pathogenic microorganisms such as *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Pseudomonas auruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Sachharomyces ceravisae* and *Candida albicans*. The latter two *S. ceravisae* and *C. albicans* are fungal pathogens. The bacterial and fungal cultures were provided by Microbiology section of Department of Plant Resources. All the bacterial organism was prepared in 10 ml of Muller Hinton agar (MHA) broth culture and incubated at 37°C for 24 h. Then it was inoculated in MHA agar plate by cotton swab ensuring uniform carpeting of organism to cover the media and dried in incubation for 1 h. After that wells of 6 mm diameter were punched in the medium using sterile borer and 50 µl of the extracts (100 mg/ml concentration) were poured to the respective wells and 50 µl of the solvent (50% ethanol) was poured to one well as control. All the plates were kept as it is for few minutes for effective diffusion of the extracts. Later they were incubated at 37°C for 24 h. Similarly, for fungal microorganisms same process was followed using SDA media and incubation at 27°C.

Antibacterial and anti-fungal activity was observed after 24 hrs. The diameter of the inhibition zones was measured. The average area of zone of inhibition was calculated and compared with that of the standards. Similarly, Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of effective plant extracts were evaluated by two fold serial dilution method (CLSI, 2006).

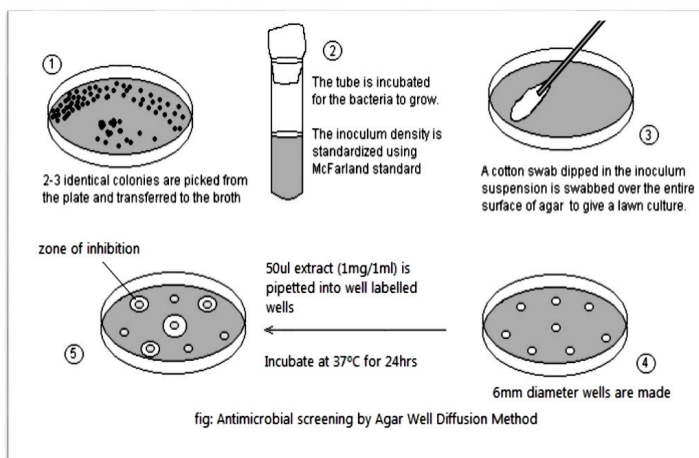


FIG. 1. Antimicrobial susceptibility test by Kirby Bauer Well Diffusion Method (CLSI, 2006).

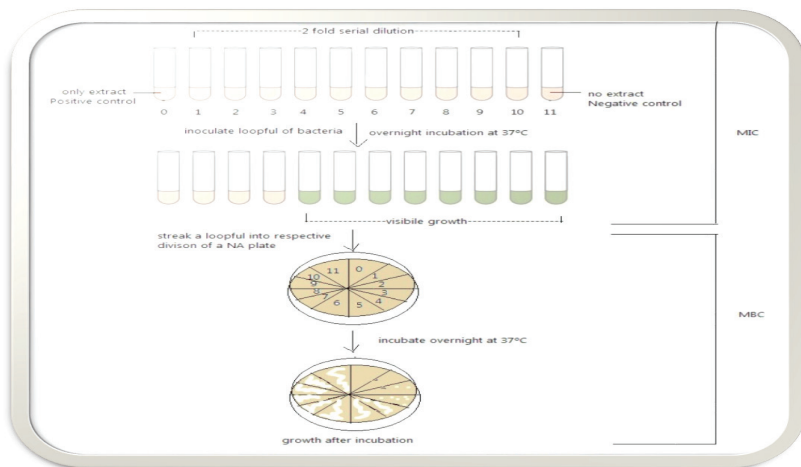


FIG. 2. MBC/MFC evaluation by two-fold serial dilution method (CLSI, 2006).

RESULTS AND DISCUSSION

Phytochemical screening of study species

Phytochemical screening revealed the presence of coumarin, flavonoid, catecholic tannin, reducing compound in alcoholic extract of *A. racemosus* while its aqueous extract revealed polyuronoid, reducing compound, polyoses, saponin, gallic tannin, catecholic tannin, etc. Similarly alcoholic extract of *A. curillus* revealed catecholic tannin, reducing compound and aqueous extract revealed polyuronoid polyoses, saponin, gallic tannin as main phytochemical compounds (table 1). Similar results were revealed showing the presence of alkaloids, glycosides, phenolic compounds, tannins, saponins, steroids, flavonoids and carbohydrates (Sharma & Sharma, 2013; Shevale *et al.*, 2015). *Asparagus racemosus* is one of the important medicinal plant used worldwide showing the properties to use for prevention and treatment of gastric ulcers, dyspepsia and as a galactotagogue. It is also used successfully for nervous disorders, inflammation, liver diseases and certain infectious diseases (Patel & Patel, 2013; Shevale *et al.*, 2016).

TABLE 1. Phytochemical constituents of study species.

Extract solvent	<i>Asparagus racemosus</i>	<i>Asparagus curillus</i>
Petroleum ether	Volatile oil	Steroids, fatty acids
Ethyl alcohol extract	Coumarin, flavenoid, Catecholic tanin, reducing compound	Catecholic tanin, reducing compound
Aqueous extract	Polyuronoid, reducing compound, polyoses, Saponin, Gallic tanin, catecholic tannin	Polyuronoid, reducing compound, polyoses, saponin, gallic tanin, catecholic tanin

Physico-chemical parameters and extractive values were in the range of standard value coated by the Indian pharmacopeia (table 2).

TABLE 2. Physicochemical characteristics.

SN	Name of species	Total ash (%) (wet basis)	Moisture (%)	Water soluble Ash (%)	Acid insoluble ash (%)
1	<i>Asparagus racemosus</i> (Makwanpur)	5.88	6.01	2.34	0.96
2	<i>Asparagus curillus</i> (Rasuwa)	3.52	10.17	1.24	0.66
3	Standard value (Gupta <i>et al.</i> , 2003)	<6	11	NA	<1

Antimicrobial susceptibility assay

The extracts were screened for their antimicrobial activity on nine different strains of human pathogenic microorganisms such as *E. coli*, *S. typhi*, *B. subtilis*, *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, *E. faecalis*, *S. cerevisiae* and *C. albicans*. Among them latter two are fungal pathogen and rest other are bacterial pathogen.

A. racemosus showed selected antimicrobial effects against *B. subtilis*, *E. coli*, *E. faecalis*, *P. aeruginosa*, *S. cerevisiae* and *C. albicans* with zone of inhibition of 25 mm in an average. While *A. curillus* showed effects on *S. cerevisiae* and *C. albicans* only with zone of inhibition about 12 (fig. 3, plate 1 & 2). MFC values of *A. curillus* for *C. albicans* was 12.5 mg/m and *S. cerevisiae* was 6.25 mg/ml while MFC of *A. racemosus* against *C. albicans* was 0.78mg/ml and *S. cerevisiae* was 1.56mg/ml. To compare with Gentamycin it was said to be resistant if it is less than 12 mm and susceptible if it is more than 15 mm (Hudzicki, 2009).

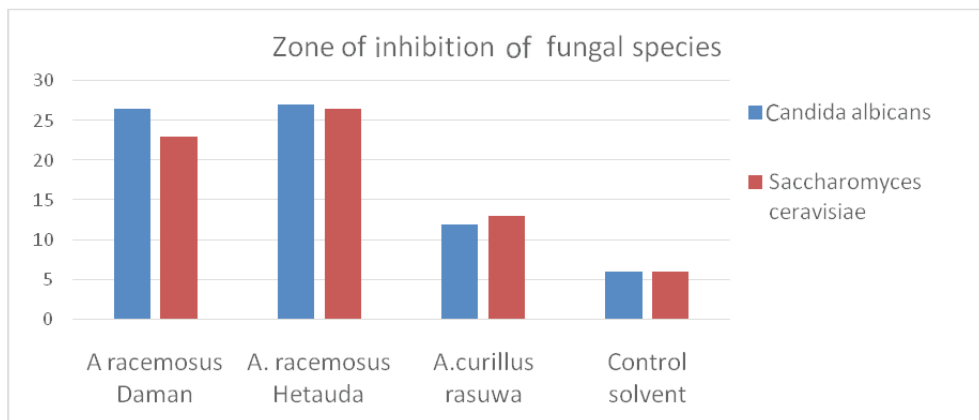
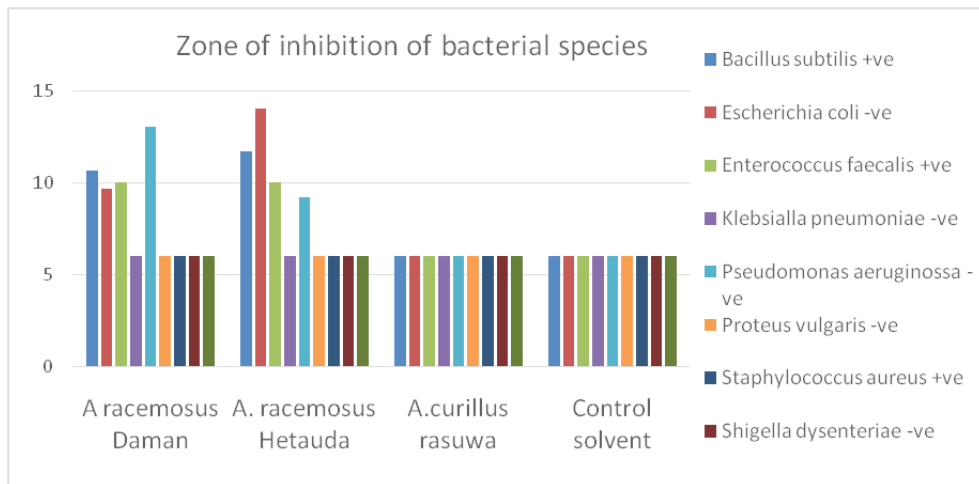
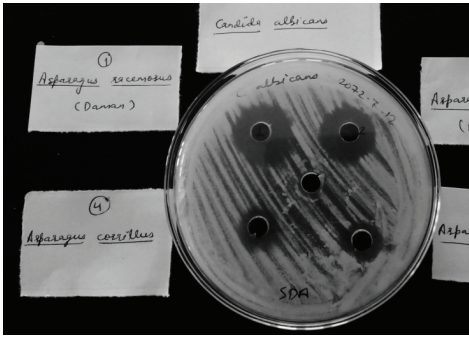
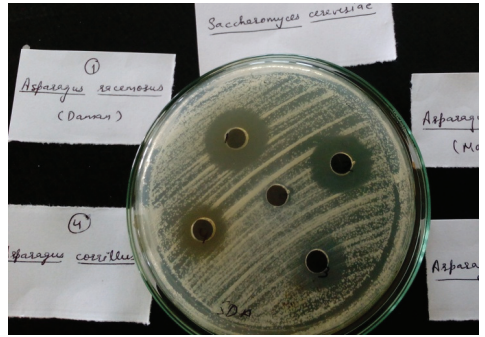


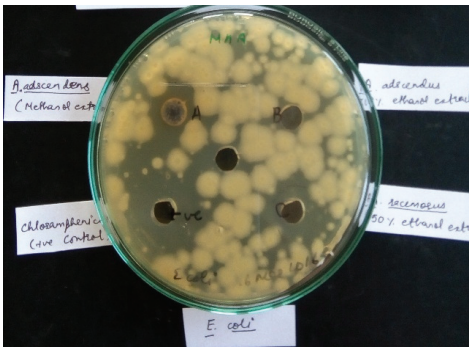
FIG. 3. Antimicrobial activities of root extracts of *Asparagus racemosus* and *Asparagus curillus*.



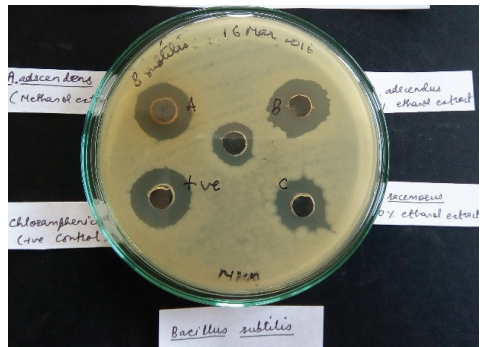
(a)



(b)

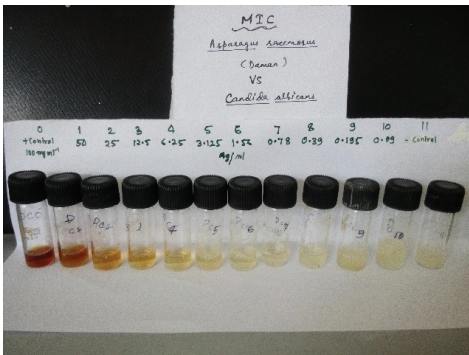


(c)



(d)

PLATE 1. Zone of inhibition by plant extract on *Candida albicans* (a), *Saccharomyces cerevisiae* (b), *Escherichia coli* (c) and *Bacillus subtilis* (d).

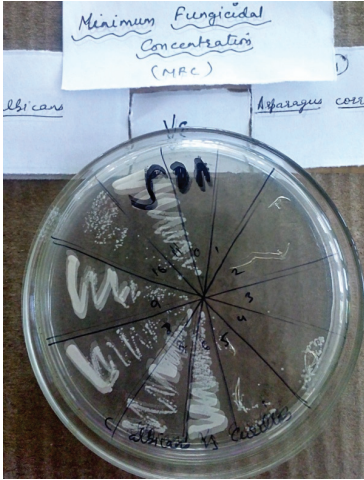


(a)

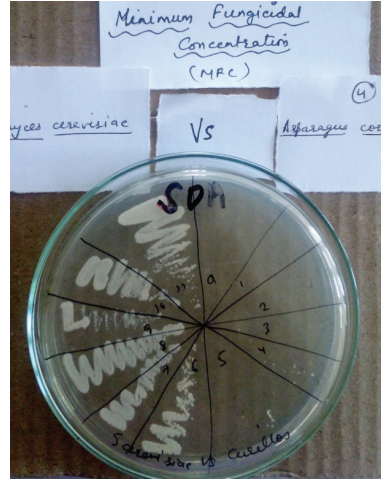


(b)

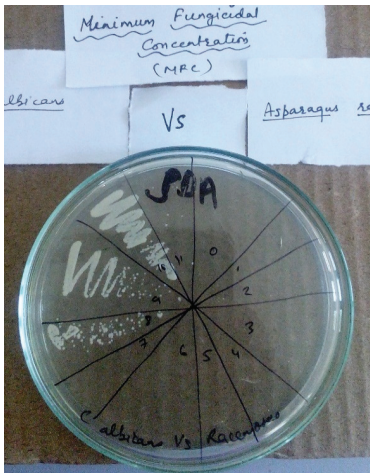
PLATE 2. Minimum inhibition Concentration (MIC) evaluation test for *Aspergillus racemosus* vs. *Candida albicans* (a) and *Saccharomyces cerevisiae* (b).



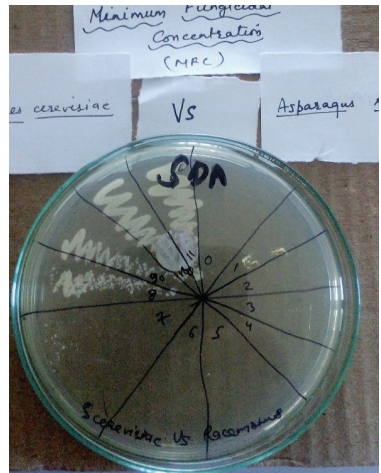
MFC values of *Asparagus curillus* vs *Candida albicans*.



MFC values of *Asparagus curillus* vs *Saccharomyces cerevisiae*.



MFC values of *Asparagus racemosus* vs *Candida albicans*.



MFC values of *Asparagus racemosus* vs *Saccharomyces cerevisiae*.

Steroidal triterpenoid of *A. racemosus* showed considerable antibacterial activities against *E. coli* and *S. aureus* while no significant activity was observed against *S. typhi*. (Shah, *et. al.* ,2014). Similarly, Devkota & Dutta (2001) studied and found antibacterial activity of *A. racemosus*, against six bacteria (*E. coli*, *Shigella dysenteriae*, *Staphylococcus aureus*, *P. aeruginosa*, *Salmonella typhi*, and *Vibrio cholerae*). The inhibition of both Gram positive and Gram negative bacteria by the solvent extracts indicated the presence of broad spectrum antibacterial substances in the plant root. The result was significant and supported the traditional use of *Asparagus racemosus* in several ailments. (Sinha & Biswas, 2011)

Phytochemical screening revealed the presence of coumarin, flavonoid, catecholic tannin, reducing compound in alcoholic extract of *A. racemosus* while its aqueous extract revealed polyuronoid, reducing compound, polyoses, saponin, gallic tannin, catecholic tannin, etc. Similarly, alcoholic extract of *A. curillus* revealed catecholic tannin, reducing compound and aqueous extract revealed polyuronoid polyoses, saponin, gallic tannin as main phytochemical compounds. Physico-chemical parameters and extractive values were in the range of standard value coated by the Indian pharmacopeia.

Antimicrobial activity was better in *A. racemosus* showing its activity with *B. subtilis*, *E. coli*, *E. faecalis*, *S. cerevisiae* and *C. albicans* than *A. curillus* which showed activity only with *S. cerevisiae* and *C. albicans*. *A. curillus* has though somehow similar in chemical constituent is found ineffective in bactericidal activity but is positive in antifungal activities

As the main objective of the study is to assess and compare phytochemical characteristics and antimicrobial properties of these two *Asparagus* species, it is concluded that *A. curillus* is lower in quality than *A. racemosus* and can be considered adulterant not substitute. This study highlighted the potential of *A. racemosus* to be further explored as a source of bioactive natural products.

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