ANTIFUNGAL ACTIVITY OF *Asparagus racemosus* (Willd)

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Abstract

Plants represent a vast and largely unexplored resource for the discovery of novel metabolites with interesting biological activities. Plant extracts are widely used in folk medicine in tropical and subtropical areas. Most of the plants finding in nature itself new weapons with insecticidal, fungicidal, herbicidal and antiparasiticidal properties. Research projects dealing with medicinal plants often justify their relevance by stating that the plants are used in ayurveda. The research methodology itself involves phytochemical analysis of the plant with the emphasis being on interesting new structures. As bioactivity is considered to be important, fractions and compounds are screened to develop new drugs and standardize existing drugs. Theplant Asparagus racemosus(Willd)is widely distributed in the tropicaland sub-tropical regions of South Asia. Based on preliminary reports, there is a lot of interest in using the roots and leaves of this plant for treating so many disorders. Present investigation initiated with the aim of Antifungal activity of the medicinal plant Asparagus racemosus. In this study leaves of the plant were collected from natural environment,washed, shade dried pulverised and extracted with methanol. Methanol extract was subjected to column chromatography and fractionated by using various organic solvents in a different combination in order of gradually increased polarity of the solvents. The dried fractions were subjected to antifungal assay.The main objectives of the research work were to identify the maximum inhibitory fractionof the medicinal plant against the fungus and check the presence or absence of the phytochemical constituentsof the selected medicinal plant leaf methanolic extract.Antifungal activity was observed only for 13 fractions out of 41 fractions ;while the remainder did not show any antifungal activity. Among that the fraction of65%hexane:35%ethyl acetate and 50%hexane:50%ethyl acetate showed the maximum zone of inhibition of against the Aspergillus sp. And the results of the Qualitative Phytochemical screening of these medicinal plant showed that steroid was found to be present in theAsparagus racemosus leaf methanolic extract.

Key words: *Asparagus racemosus* (Willd), column chromatography, antifungal assay,*Aspergillus*sp., steroid

Introduction

Asparagus racemosus(Willd) commonly known as 'Shatavari' ,it was previously included under the family Liliaceae, but now it has been shifted to a newly created family i.e. Asparagaceae (Sharma*et a*l, 2011)It is a much-branched, spinous under shrub found growing wild in tropical and sub-tropical parts of South Asia like India, Sri Lanka, Nepal and Himalayas. Shatavari is a woody climber growing to 1-2 m length and prefers to take root in gravelly, grows well in rocky soil at 1,300 to 1400 m elevation. The leaves are like pine-needles, small and uniform. The inflorescence has tiny white flowers, in small spikes and the roots are finger-like and clustered. The plant preferslight (sandy), medium (loamy) and heavy (clay) soil. Black, well drained and fertile soil is good for cultivation. But can be cultivated in loose and medium black soil. Crop responses well to tropical and hot climate.

Medicinal properties of this plant have been described in traditional medicine, such as the Ayurveda (Himalayan), Siddha and Unani system of medicine (Verma *et al*, 2014).It is a well known Ayurvedic rasayana which prevent ageing, increase

longevity, impart immunity, improve mental function, vigor and add vitality to the body. It is also usedin nervous disorders, dyspepsia, tumors, inflammation, neuropathy, cough, bronchitis, hyperactivity, hepatopathy and certain infectious diseases (Chawla *et al*, 2011).

The rootsof *Asparagus Racemosus* are used in manyayurvedic preparations in Indian systemof medicine. In ayurveda, it has been referred as bitter-sweet, emollient, cooling, constipating, galactogogue, antiseptic, nervine tonic, aphrodiasic, diuretic, rejuvenating, carminative, stomachic, antispasmodic and tonic. Reports indicate that the pharmacological activities of root extracts include antiulcer, anti-tussive, antidiarrhoeal, antidiabetic antioxidant, antifungal, antibacterial activities, adaptogenic activity, antiprotozoal activity, immunomodulatory activity and central nervous system stimulant activity. They are also useful in hypertension and in treatment of epilepsy (Shastry *et al*, 2015).

A study of ancient classical Ayurvedic literature claimed several therapeutic attributes for the root of A. racemosus and has been specially recommended in cases of threatened abortion and as a galactogogue as tonic.[17] It is a pharmacologically acclaimed phytoestrogenic medicinal plant used for its immunomodulatory effects (Verma *et al*, 2014).

Recent chemical analysis indicate that the following active constituents are present is Shatavari plant: Steroidal saponins, known as shatavarins (I, IV), sarsasapogenin, adscendin (A, B), asparanin (A,B,C). Shatavarin I is the major glycoside with 3 glucose and rhamnose moieties attached to sarsasapogenin. Shatavarin IV is a glycoside of sarsasapogenin having 2 molecules of Asparagus rhamnose and 1 molecule of glucose. Sarsasapogenin and shatavarin I-IV are present in roots, leaves, and fruits of Asparagus species.Synthesis of sarsasapogenin in the callus culture of *Asparagus racemosus* was also reported. A new isoflavone, 8-methoxy-5, 6, 4′trihydroxyisoflavone-7-O-β-d-glucopyranoside was also reported from *Asparagus racemosus* previously. The isolation and characterization of polycyclic alkaloid called asparagamine, a new 9,10-dihydrophenanthrene derivative named racemosol and kaempferol were also isolated from the ethanolic root extract of *Asparagus racemosus*. Oligofurostanosides (curillins G and H) and spirostanosides (curilloside G and H) have been isolated from the roots and sarsasapogenin from leaves of A.curillus.

This plant also contains vitamins A, B_1, B_2, C, E, Mg, P, Ca, Fe, Polysaccharides, mucilage, and folic acid. Other primary chemical constituents of *Asparagus racemosus* are essential oils, asparagine, arginine, tyrosine, flavonoids (kaempferol, quercetin, and rutin), resin, and tannin. (Chawla *et al*, 2011)

Materials and Methods

Collection of plant leaves

The Leaves of the plant *Asparagus racemosus* were collected in the month of March 2016 from Anuradhapura district, North Central province of Sri Lanka. Plant leaves of were washed thoroughly with running tap water followed by rinsing with distilled water to remove sand particles and other debris. The leaves were shade dried at room temperature for 45 days, then pulverized into powder by using a grinder.

Preparationof methanol extract

The dried powder of plant leaves (200 g) was successively extracted with Methanol at room temperature for 24 hours by using a mechanical stirrer with 500 rpm. This process was repeated four times that afforded crude methanolic extract .The extract was filtered through a funnel contained cotton wool and a clear filtrate was obtained. The total filtrate of methanolic extract was evaporated by using a rotary evaporator under reduced pressure at (30-40) °C. The thick greenish black residue (27 g) was obtained. It was dried and stored for further use.

Separation by using column chromatographic technique

Dried crude extract (25g) was dissolved in minimum quantity of Dichloromethane and mixed with silica gel 60 (50g) and dried by using rotary evaporator under reduced pressure at (30-40) °C. Theresidue obtained was finely powdered by using mortar and pastel and stored for column Chromatographic extraction. Silica gel G (60-120) was used as stationery phase. Sample was eluted with various organic solvents hexane, ethylacetate andmethanol. Sample was eluted initially with increasing polarity of hexane in combination with ethyl acetate ranging from 5% ethyl acetate in hexane to 100% ethyl acetate. Afterwards, increasing polarity was used by combination of methanol with ethyl acetate, ranging from 5%methanol in ethyl acetate to 100% methanol. This process afforded 41 fractions.

Assay of antifungal activity against *Aspergillus* sp.

Disk diffusion method was slightly modified in order to use for filamentous fungi like *Aspergillus.* In this method a liquid culture of *Aspergillus* on CDB was prepared by inoculating 7 days old fungus were grown on PDA and incubated for 3 days at 30°C. Sterile disk papers (Whatman No-4 filter paper – Diameter 6 mm) were soaked in the test samples dissolved in MeOH in order to get 200 µgof the sample per disk. (i.e. 4 mg of the sample was dissolved in 100 µl of methanol and each disk paper was soaked in 5µl of the sample solution in order to get 200µg). Meanwhile, PDA medium was prepared, autoclaved and cooled to about 45°C and then inoculated with the liquid culture of *Aspergillus* on CDB(0.5 ml of liquid culture for 25 ml of PDA medium). Then the medium was poured into sterilized Petri dishes (20 ml per each) and left until solidify. After the solidification, dried disk papers with the sample were placed on the inoculated medium and the dishes were transferred into an incubator (30°C)and moisture conditionwas given for 3 days. Diameter of the inhibition zones were measured along the two axes at right angle to each other. Two replicates were used for each sample and MeOH was used as negative control.

Qualitative phytochemical analysis

The leaf extract of*Asparagus racemosus* was subjected to different chemical tests for the detection of phytoconstituents such as alkaloid, glycoside, terpenoid, steroid, flavonoid and tannin.

Alkaloid:*Asparagus racemosus* leaf methanolic extract was evaporated to dryness and the residue was heated on boiling water bath with 2% HCl, reaction mixture was cooled, filtered, treated with a few drops of 5% Sodium Hydroxide and observed for the presence of turbidity or yellow precipitation.

Glycoside:*Asparagus racemosus* leaf methanolic extract of 5 mg was treated with 0.5 ml glacial acetic acid and few drops of ferric chloride; to this concentratedSulphuric acid was added and observed for a reddish brown colour at the junction of two layers and the upper layer for bluish green colour.

Terpenoid and steroid:*Asparagus racemosus* leaf methanolic extract of 4 mg was treated with 0.5 ml of acetic anhydride, 0.5 ml of chloroform, concentrated solution ofsulphuric acid wasadded slowly and red violet colour was observed for terpenoid and bluish green colour for presence of steroid.
Flavonoid:*Asparagus racemosus* leaf methanolic extract of 1 ml solution was treated with 0.5 ml of lead acetate solution and white colour precipitation was observed for the presence of flavonoids.
Tannin: To 0.5 ml of *Asparagus racemosus* leaf methanolic extract solution 1 ml of water and 1-2 drops of ferric chloride solution were added and observed for green precipitate anindication for the presence of tannins.

Data Analysis
One-way ANOVA test was carried out for the zone of inhibition measured from various fractions. Mean comparison test – Turkey's pair wise comparison was carried out to compare the average length of the zone of inhibition among the various fractions

Results and Discussion

The antifungal activity of the fractions of the *Asparagus racemosus* by the disk diffusion method was observed after 3 days. Out of41 fractions from *Asparagus racemosus*,only 13 fractions were showed the antifungal activity against *Aspergillus* sp. as shown in Table1.Among them, the fraction of 65%hexane:35%ethyl acetate and 50%hexane:50%ethyl acetatewere showedmaximum zone of inhibition of 12.83 mm as shown inFigure 1 and Figure 2.

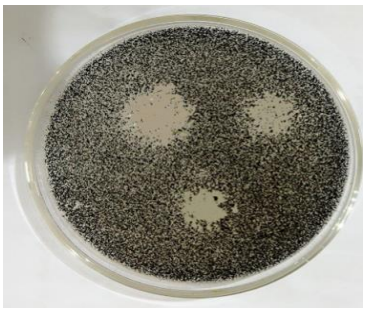

Figure 1.Fraction of 65% hexane: 35% ethyl acetate against Aspergillus sp.

Figure 2. Fraction of 50% hexane: 50% ethyl acetate against Aspergillus sp.

Figure 3.Negative control test (100% methanol) against Aspergillus sp.

Additionally, another 10 fractions of *Asparagus racemosus*were showed moderate antifungal activity against *Aspergillus*sp. and the zone of inhibition were 12.41 mm, 12.16 mm, 12.08 mm, 11.75 mm, 11.33 mm,10.16 mm, 7.25 mm ,6.75 mm, 6.66 mm and 6.41 mm. Comparative results of antifungal activity against *Aspergillus* sp. of negative control used as Methanol didn't show any zone of inhibition.

Table 1.Interaction between Aspergillus sp.and various fractions of Asparagus racemosus

Fractions of Asparagus racemosus	Average Inhibition Diameter [mm]
80% hexane:20% ethyl acetate	6.75
65% hexane:35% ethyl acetate	12.83
60% hexane:40% ethyl acetate	12.08
55% hexane:45% ethyl acetate	11.75
50% hexane:50% ethyl acetate	12.83
45% hexane:55% ethyl acetate	12.41
35% hexane:65% ethyl acetate	12.16
30% hexane:70% ethyl acetate	11.33
15% hexane:85% ethyl acetate	11.33
10% hexane:90% ethyl acetate	6.41
75% ethyl acetate:25% methanol	6.66
45% ethyl acetate:55% methanol	7.25
00% ethyl acetate:100% methanol	10.16
Methanol (control)	0.00

According to the ANOVA and Turkey's Pair wise comparisions tests ,$p = 0.000 < 0.05$ vs control; i.e. The mean difference is statistically significant at 0.05 level.

Different phytochemical analysis was done for Alkaloid, Glycoside, Terpenoid, steroid, Flavonoid and Tannin. *Asparagus racemosus* leaf methanolic extract was found to be steroid positive. Development of bluish green colour upon reaction of the extract with acetic anhydride and chloroform confirms the presence of steroid in the leaf extract.

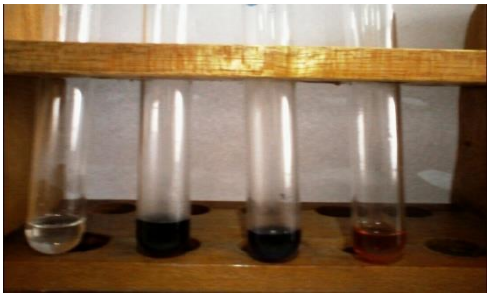

Figure 4. A: Test for Alkaloid, B: Test for Glycoside, C: Test for Flavanoid, D: Test for Tannin

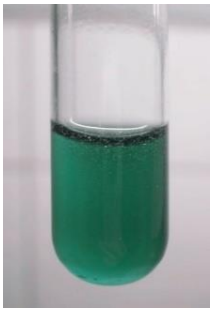

Figure 5. Test for Steroid

Conclusion

On the basis of this present study; it can be concluded that the fraction of65%hexan:35%ethyl acetate and 50%hexan:50%ethyl acetate were showed the maximum zone of inhibition against the *Aspergillus*sp.of 12.83 mm.*Asparagus racemosus* leaves are good source for steroids. The knowledge gained from this study will be helpful in the isolation, purification and characterisation of new biologically active compounds , the active compounds associated with the antifungal activity, the identification of Minimum Inhibitory Concentration (MIC), environmental friendly fungicides and new drugs in future.

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