Research

Phytochemical studies and in vitro activity of *Asparagus racemosus*

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Abstract

Phytochemical and biological screening of root of *Asparagus racemosus* was studied. The brine shrimp bioassay of its various extracts showed that the methanol (MeOH) extract was biologically active. The column chromatography of hexane, acetone and methanol extracts resulted in the isolation of 9-hexadecanoic acid-9-octadecenylester (Z,Z), *β*-Sitosterol, stigmasterol-3-O-*β*-D-glucopyranoside and diosgenin. The estimation of saponin in the root of *Asparagus racemosus* has also been carried out.

Keywords: *Asparagus racemosus*, brine shrimp bioassay, diosgenin, saponin

Introduction

Asparagus racemosus is a member of family Asparagaceae.[1] It is commonly known as *shatavarin* and *Kurilo* in Nepali. This plant is cosmopolitan in nature and is found more abundant in the jungle of Central Terai region and Himalayan at the height of 165 m to 2200 m.[2] It has been specially recommended in cases of threatened abortion and as a galactogogue.[1] Root of asparagus has been referred as bitter-sweet, emollient, cooling, nerve tonic, aphrodisiac, diuretic, rejuvenating, carminative, and stomachic antiseptic and tonic. It is reported to be useful against diarrhoea, dysentery and in general debility.[3] Saponins and an alkaloid asparagamine A exhibit antioxytocin activity which supported the traditional claim of *A. racemosus* as remedy for preventing miscarriage.[4] Root of this plant was selected for chemical and biological analysis. In the present investigation, phytochemical and biological screening, isolation and characterization of biologically active chemical constituents from the roots was carried out and the percentage of saponin in *A. racemosus* have been estimated.

Materials and methods

Collection of Plant Material

The roots of the *Asparagus racemosus* were collected from a farm of Chitwan district and identified by Prof. Dr. R. P. Chaudhary of Central Department of Botany, Tribhuvan University.

Extraction

About 850 g of dried plant material was extracted in soxhlet extractor using solvent of increasing polarity. Successive extraction of dried plant material by hexane (500 ml × 3), acetone (500 ml × 3) and methanol (400 ml × 3) yield 2.05 g, 9.4 g and 29 g extract respectively.

Phytochemical Screening

The method employed for phytochemical screening was based on the procedure put forward by Prof. I. Ciulei.[5] By using different specific reagents, the presences of main groups of natural products were analyzed in hexane, acetone and methanol extracts.

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Biological Screening of *Asparagus racemosus*

Biological screening involves the study of the effect of the crude plant extracts fractions and isolated compounds at arbitrarily fixed dose levels in a species of organism and prediction of its effect over the entire dosage range. The present research involves the screening of each fraction of the extract for their antibacterial and brine-shrimp toxicity activity.

Brine Shrimp Bioassay

The Hexane, Acetone and Methanol extracts were tested for their pharmacological potential by brine shrimp bioassay. The procedure followed for the brine-shrimp toxicity assay was carried out according to Mayer *et* al.[6] Procedure which involve determination of LC_{50}, the lethal concentration dose required to kill 50 % of the shrimps.

Anti microbial Screening

The ability to kill or inhibit the growth of pathogenic microorganisms was evaluated by anti microbial screening of the plant extracts. The three different fractions obtained were screened for its antibacterial activity by determining the Zone of Inhibition (ZOI), with the help of scale against tested organisms by agar well diffusion method given by Dingle *et al.*[7] The diameter of zone of inhibition (ZOI) measures for the estimation of potency of the antimicrobial substances. In this work *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi. Salmonella paratyphi, Proteus mirabilis, Shigella dysentriae, Pseudomonas aeruginosa, Klebsiella oxytoca* pathogenic bacteria were selected.

Separation of Compounds

Compounds were separated from Hexane, Acetone and Methanol extracts by column chromatography. In this process (1.16 g) hexane, and (8.0 g) acetone extract were loaded in a silica gel (85 g, E-merck, 60-120 mesh) packed column having internal diameter 4 cm with the adsorbent height 45 cm and were eluted with gradients of hexane, ethyl acetate and methanol to obtain number of fractions containing compounds AM_{100}, AM_{101}, AM_{102} and AM_{103}.

Determination of saponin content

Saponin content on root of *A. racemosus* was estimated by Roth Rock *et. al* Method.[8] In this method air dried powered material (50 g) was extracted with petroleum ether in soxhlet extractor for 20 hours. The defatted powder was then hydrolyzed with 5% HCl by refluxing in steam bath for 20 hours. The residue obtained by filtration under suction was washed thoroughly with water to remove last traces of HCl. The product after proper drying was extracted in a soxhlet with petroleum ether for 10 hours. The petroleum ether extract was washed thoroughly with 5% NaOH followed by water. The residue obtained on removal of the solvent was dried till to have constant weight.

Result and discussion

Phytochemical and biological Screening of *Asparagus racemosus*

Phytochemical screening of roots of *A. racemosus* was carried out on the basis of procedure developed by Prof. I. Ciulei. Fatty acids, sterols and triterpenes were identified in the Hexane extract and glycosides, quinines, flavone glycosides, reducing compounds, polyoses and saponin were revealed in methanol and aqueous extract.

Two sets of biological screening brine shrimp bioassay and antimicrobial bioassay were carried out. Brine shrimp bioassay is the simple bioassay useful for screening large number of extracts in the drug discovery from the medicinal plant. The biological activity was evaluated on the basis of their toxicity towards brine shrimp nauplli.[9] The procedure followed was of Mayer et al, [10] was adopted to determine the lethality of plant extract. In this method, the LC_{50}-values (µg/ml) for different fractions were determined and those having LC_{50} values less than 10^3 are considered to be pharmacologically active. LC_{50} of hexane, acetone and methanol extracts were found to be 2318.3, 1388.2, and 734.3 µg/ml respectively. The data suggested that methanol fraction could be biologically active for further works.

Antimicrobial inhibition studies of different fractions of root of *Asparagus racemosus* was carried out at Central Department of Microbiology, Tribhuvan University. The study showed the antimicrobial activity of methanol fraction. Hexane and acetone fractions showed moderate activity.

SN	Plant Fraction	Organisms	Zone of Inhibition (mm)	
			Control	Fractions
1.	**Hexane**	*Staphylococcus aureus*	6	**12**
		Klebsiella pneumonia	6	**12**
2.	**Acetone**	*Staphylococcus aureus*	6	**13**
3.	**Methanol**	*Staphylococcus aureus*	6	**15**
		Salmonella typhii,	6	**12**
		Salmonella paratyphi	6	**13**
		Shigella dysentriae	6	**12**

Table 1: Antimicrobial Study of Different Fractions of *A. racemosus*

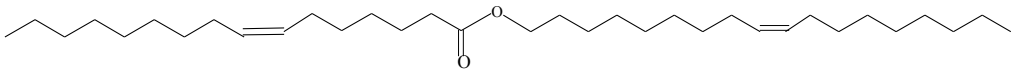
Well Diameter = 6 mm (0.6 cm), Concentration of loaded extract = 50 mg/ml

Analysis and Identification of Isolated Compounds

Chemical constituents were isolated from different extracts by column chromatography.

Compound AM_{100}

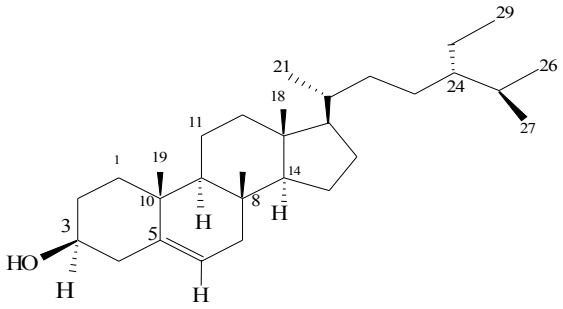
It is a white sticky solid (9 mg), isolated from hexane extract which melts at (77- 80°C) and is insoluble in methanol. One major tailing spot was observed in TLC with R_f value 0.85 (toluene: EtOAc = 99:1 + CH_3COOH vapor). The compound is UV active. It was found to be 9-hexadecanoic acid-9-octadecanoyl ester, (Z, Z) having molecular formula $C_{34}H_{64}O_2$, on the basis of match factor from EI mass analysis.



9-Hexadecanoic acid- 9-octadecenyl ester (Z, Z)

Compound AM$_{101}$

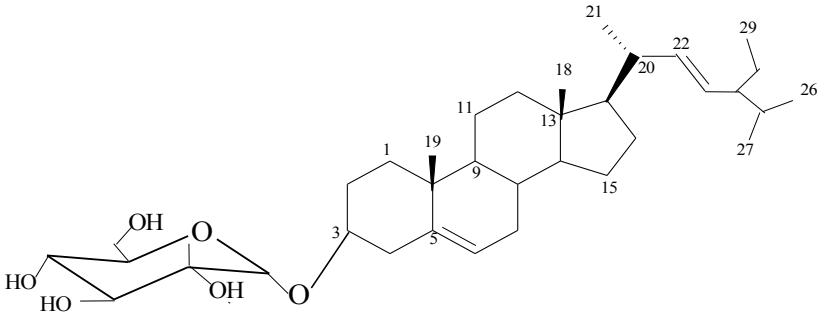
This compound was extracted from hexane extract. It was dissolved in ethyl acetate and added a few drops of hexane to the solution drop wise till turbid. The solution was kept undisturbed for overnight. White crystalline substance was crystallized. The compound was found to be single spotted on TLC with R_f value 0.45 (20% EtoAc in hexane). It was soluble in chloroform and ethyl acetate. The melting point of compound was found to be 132^{0}C. It gave positive Libermann-Burchard test with greenish red color indicating the compound to be sterol. Same compound was isolated from acetone extract by using 10% EtOAc in hexane eluting solvent. It was finally identified as to be *β*-sitosterol (14 mg) by co-TLC with authentic sample.



***β*-sitosterol**

Compound AM$_{102}$

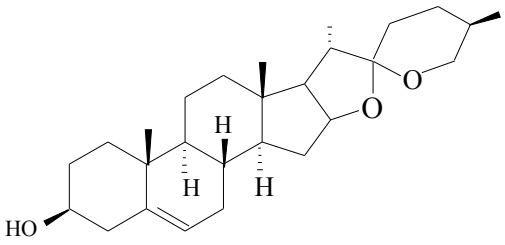
Fractions (91-100) obtained by eluting acetone extract by (40%EtOAc in hexane) resulted a white amorphous compound. TLC of the compound showed a single spot with R_f value 0.6 (15% MeOH in $CHCl_3$). The compound was found to melt at 294^{o}C. It was soluble in pyridine and mixture of methanol and chloroform (1:1). Direct comparison with authentic sample, the compound was identified as Stigmasterol-3-O-*β*-glucopyranoside (8mg).



Stigmasterol 3-O-β-D-glucopyranoside

Compound AM_{103}

Compound AM_{103} was isolated during the isolation of saponin by Roth Rock *et. al* Method.[8] The compound was soluble in chloroform and methanol solution. A single spot was observed on TLC developed in 10% acetone in pet. ether (R_f = 0.5). It was found to melt at 200°C. The compound was identified as diosgenin by Co-TLC with authentic sample.



Diosgenin

Estimation of saponin

Estimation of saponin was carried out on root of *Asparagus racemosus* by Roth Rock *et. al* Method.**[8]** The percentage of saponin was found to be 0.58, which seems quite small amount.

Conclusion

On the basis of above study, methanol extract of *A. racemosus* was identified to have antimicrobial activity which supports its therapeutic value as describe in various literature. This study results into the isolation of compounds which were identified to be 9-hexadecanoic acid-9-octadecenylester (Z,Z), *β*-sitosterol, stigmasterol-3-O-*β*-D-glucopyranoside and diosgenin. In addition to this, saponin percentage on the root was estimated, which was found to be 0.58.

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