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Original Article

Spectral Analysis of Steroidal Saponin Isolated and Purified from Leaves Extract of *Asparagus racemosus* (Family – Asparagaceae)

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ABSTRACT

Asparagus racemosus is an important medicinal plant. Its medicinal usages has been reported in Indian and British pharmacopoeias. Qualitative preliminary phytochemical analysis was performed on the crude extract for the presence of bioactive compounds. The pure compound isolated to the Column chromatography and purified this compound by Thin Layer Chromatography with suitable solvent systems and visualizing reagents.
Structure elucidation of pure compound has been accomplished through the extensive use of UV-Visible, IR, ^{1}HNMR and Mass spectroscopy. Phytochemistry and spectral analysis showed the presence of steroidal saponin in *Asparagus racemosus* leaves extract.

Keywords: Saponin, *Asparagus racemosus*, IR,UV,HNMR ,Mass Spectroscopy.

INTRODUCTION

Plant steroids (or steroid glycosides) are one of the most naturally occurring plant phytoconstituents that have found therapeutic applications There are two types of saponins include: steroidal saponins and triterpene saponins. The sugar is attached at C-3 in saponins because in most sapogenins there is a hydroxyl group at C-3. The two major types of steroidal sapogenin are diosgenin and hecogenin. Saponins are regarded as high molecular weight compounds and soluble in water and insoluble in non-polar organic solvents and they are mostly amorphous in nature[1]. Saponins are also necessary for activity of cardiac glycosides. The two major types of steroidal sapogenin are diosgenin and hecogenin. Steroidal saponins are used in

the commercial production of sex harmones for clinical use.

Asparagus racemosus also called Shatavari and it is the main Ayurvedic rejuvenative tonic for the female; it is used for sexual debility and infertility in both sexes. It is also used for menopausal symptoms and to increase lactation[2]. The plant is a spinous under-shrub, with tuberous root, short rootstock bearing numerous succulent tuberous roots 30–100 cm long and 1–2 cm thick, that are silvery white or ash colored externally and white internally[3]. The plant flowers during February-March leaving a mild fragrance in its surrounding and the leaves are reduced to small scales or needle-like spines called cladodes[4].

Collection of Plant material

Fresh plant leaves were collected from Govt.Science & Commerce College, Benazir,Bhopal (M.P.) in July-September 2012. The taxonomic identification of plant was confirmed by Dr. Madhuri Modak, Professor, Department of Chemistry, M.V.M. Govt. College, Bhopal. After authentification of the plant, herbarium was kept and the plant is used for the analysis.

Preparation of crude extract

The dried plant leaves of *Asparagus racemosus* was grounded mechanically till the fine powder in a mixer grinder and weighed accurately. The powdered material was subjected to solvent extraction with ethyl acetate & methanol by Soxhlet apparatus at room temperature for 48 hours.The resulting mixture was filtered and evaporated by rotatary evaporator temperature maintained at 40-60^{0}C. The obtained dried crude extract was used for phytochemical analysis.

Phytochemical screening

A small portion of the dry crude extract was used for the phytochemical analysis. Dried methanolic extract of *Asparagus racemosus* was investigated with the various chemical tests by Harborne, 1973[5] & Kokate CK.,*et al*[6].

Isolation and purification

Chromatographic separation of substances using column is perhaps the most simple technique and can be easily performed with even a small glass tube or burette packed with solid adsorbent. This technique used to isolate and purify the constituents present in the extracts. It was chromatographed on the silica gel (230-400 mesh size) and elucidated with chloroform and methanol (6:4) to give crude steroidal saponins and the column fractions was purified by Thin Layer Chromatography plates with different systems but the best separations by $CHCl_3$:EtOH:MeOH (7:3:1) solvent system.

RESULTS AND DISCUSSION

The fresh leaves of Asparagus racemosus were extracted with ethyl acetate and methanol. Concentrated the extract under reduced pressure and temperature.In chromatographic separations of the extract used silica gel (230-400 mesh size) gave pure compound and the isolated compound was purified with Thin Layer Chromatography with solvent system and suitable visualizing reagents.

Isolated compound was gave a positive test Liebermann-Burchard test for steroidal saponin and the compound obtained as dark brownish amorphous powder as a single spot with 0.37 Rf value.UV spectra showed maximum wavelength at 425 & 417 nm and the IR showed 2922cm-1 C-H stretch of methylene, 2855cm^{-1} (C-H stretch of

methyl), 1738cm^{-1} (C=O carbonyl stretch), 1660 cm^{-1} (C=C stretch), 1451cm^{-1} (C=C stretch Aromatic peaks),1374cm^{-1} (methyl deformation vibrations),1235cm^{-1}(C-O Stretch of carbonyl), 1167cm^{-1}(C-O-C Stretch), 937, 1032cm^{-1} (CH_2 Out of plane bend), 3400 (OH) ,980, 915, 896, 850 (intensity 915>896), 255-spiroketal, 839,721cm^{-1}(Aromatic, Out of plane bend)**.**It possesses Carbonyl, Aromatic ring ,Methylene , etheral stretch, mass spectra (m/e) 685,507,470,413 & 347 elimination of sugar, 301 formation of fragment $C_{15}H_{10}O_7$ & complete removal of sugar, 242 elemination of C_3H_7O ,214 elemination on C_2H_4 ethene elemination confirm unsaturation, 98 fragmentation of sugar. And the HNMR spectra of isolated compound displayed the characteristic signals **δ** 1.2 methylene CH_2 protons , **δ** 1.5 CH_2 protons down field shift due to oxygen , 2.0 CH_2 protons down field shift due to oxygen , **δ** 3.5 ,4.0 ,4.5 O-H protons different shift positions due to electro negativity of Oxygen , 5.0 & 5.5 alkene protons. **δ** 4.82 (d,J = 6.0 H_Z,H-1 of Glc), 4.98 (br.S,H-1 of Rha), 5.23 (br S, H-1 of Rha). All the above data identified the this compound is Adscendin,a steroida saponin isolated from *Asparagus racemosus* leaves extrat.

The UV-Visible spectra shown in (Table no.1 & figure no.1), IR spectra in (Table no. 2 & figure no. 2), Mass spectra shown in (Table no. 3 & figure no. 3) and HNMR spectra also shown in (Table no. 4 & figure no.4)

Confirmatory test for isolated compound

Leibermann –Burchard

2 ml of acetic anhydride was added to 0.5g of the plant extract with 2 ml of sulphuric acid. The color change from violet to blue green in the sample indicates the presence of steroids.

Structure elucidation of the Isolated compound

The structure of isolated compound was elucidated by employing spectroscopic analysis UV, IR, MASS, & NMR spectroscopy.

Molecular Formula : $C_{39}H_{62}O_{12}$
Molecular Weight : 722
Melting point : 219 – 221^{0}C
IUPAC Name : Spirostan-5- en -3β – ol3 -O-[α – L-rhamnopyranosy -(1 -->6)– β –D-glucopyranoside

Physical characters of Isolated compound

Color	:	Dark brown
State	:	Dry amorphous powder
Solubility	:	Chloroform & Methanol
Melting point	:	219^{0}C
Rf Values	:	0.37

CONCLUSION

The herbal drugs are proved to be safe, clinically effective and less expensive than the allopathic drugs. It is strongly believed that the phytochemical and various biological properties of the extracts might provide detailed evidence for the use of this plant in different medicines. *Asparagus racemosus* leaves contain Saponins, steroids make this plant a natural chemical source which play a major role in treatment of various diseases. Phytochemistry of plants defining the chemical profiles of medicinal herbs and an understanding the analytical tests for identification of the herbs and for the quantitative assessment of any known bioactive ingredients in plants[7]**.**The present study carried out on the plant samples

revealed the presence of medicinally active constituents.

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Table 1. UV-Visible spectra of Isolated compound

S.No.	Sample	Observation
1	Isolated compound	Maximum found at 417 &425 nm

Table 2. IR Spectrum of isolated compound

S. No.	Wave number (cm^{-})	Functional group
1.	2922	C-H stretch of methylene
2.	2855	C-H stretch of methyl
3.	1738	C=O carbonyl stretch
4.	1660	C=C stretch
5.	1451	C=C stretch Aromatic peaks
6.	1374	methyl deformation vibrations
7.	1235	C-O Stretch of carbonyl
8.	1167	C-O-C Stretch
9.	937, 1032	CH_2 Out of plane bend
10.	839,721	Aromatic, Out of plane bend

Table 3. Mass Spectrum of Isolated compound

Peaks No.	Molecular Weight	Fragmentation of Molecule
1	685,507,470,413 & 347	Elimination of sugar
2	301	Formation of fragment $C_{15}H_{10}O_7$ & complete removal of sugar
3	242	Elemination of C_3H_7O
4	214	Elemination on C_2H_4 ethene, elemination confirm unsaturation,
5	98	Fragmentation of sugar

Table 4. ^{1}HNMR Spectra of Isolated compound

S.No.	Peaks (δ ppm)	Interpretation
1.	1.2	Methylene CH_2 protons
2.	1.5	CH_2 protons down field shift due to oxygen
3.	3.5,4.0,4.5	O-H protons different shift positions due to electro negativity of Oxygen
4.	5.0 & 5.5	Alkene protons
5.	2.0	CH_2 protons down field shift due to Oxygen

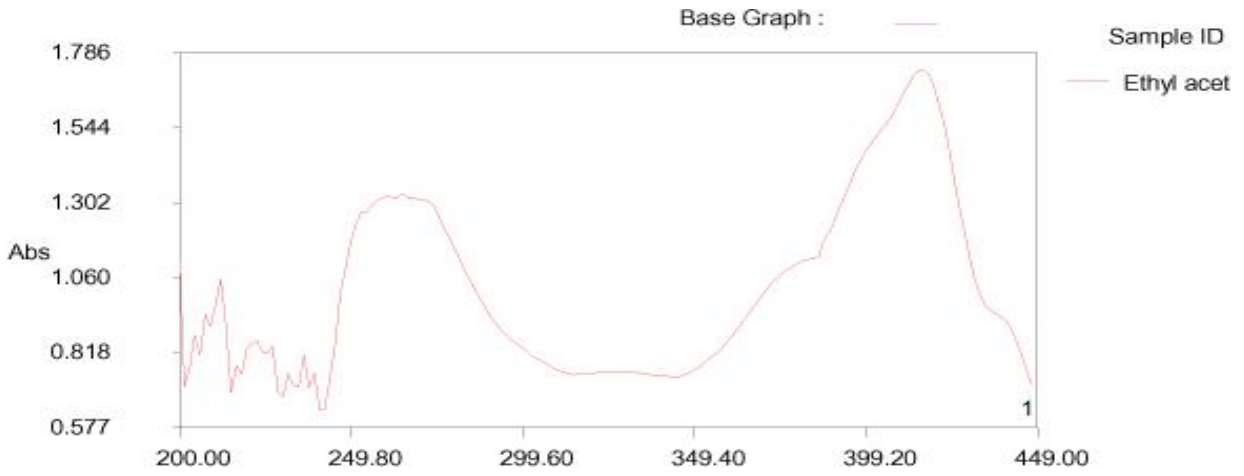

Figure 1. UV-Visible spectra of Isolated compound

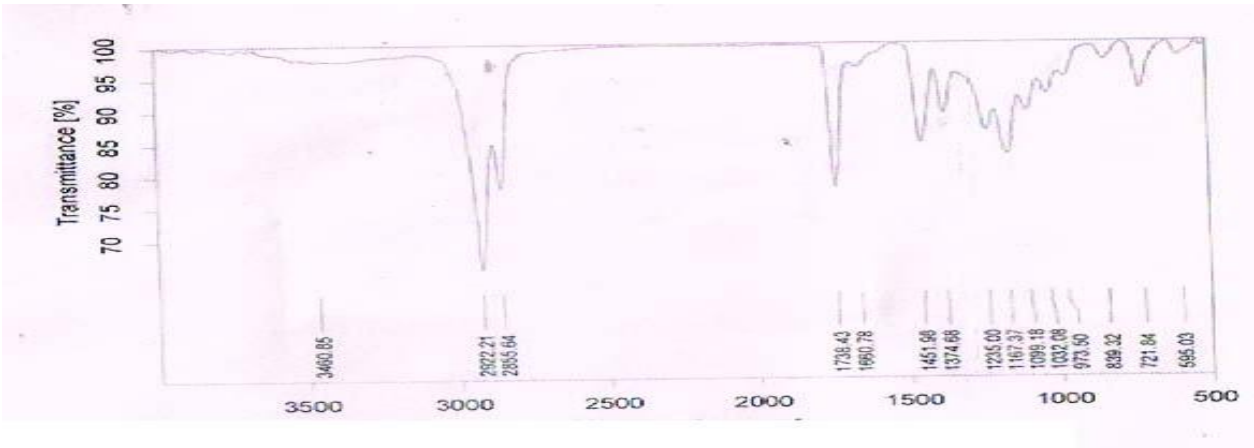

Figure 2. IR spectra of Isolated compound

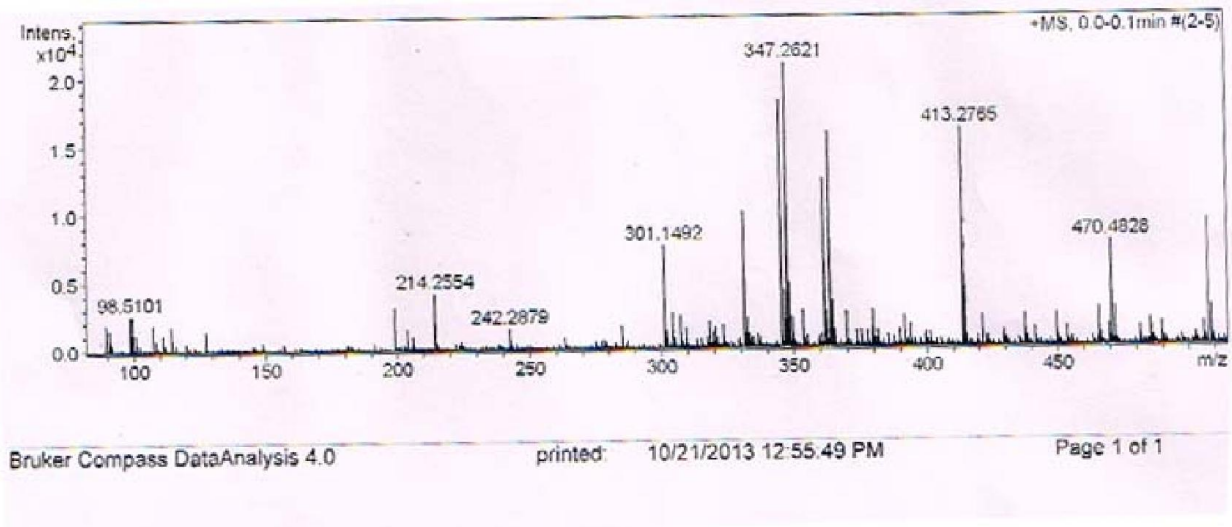

Figure 3. Mass spectra of Isolated compound

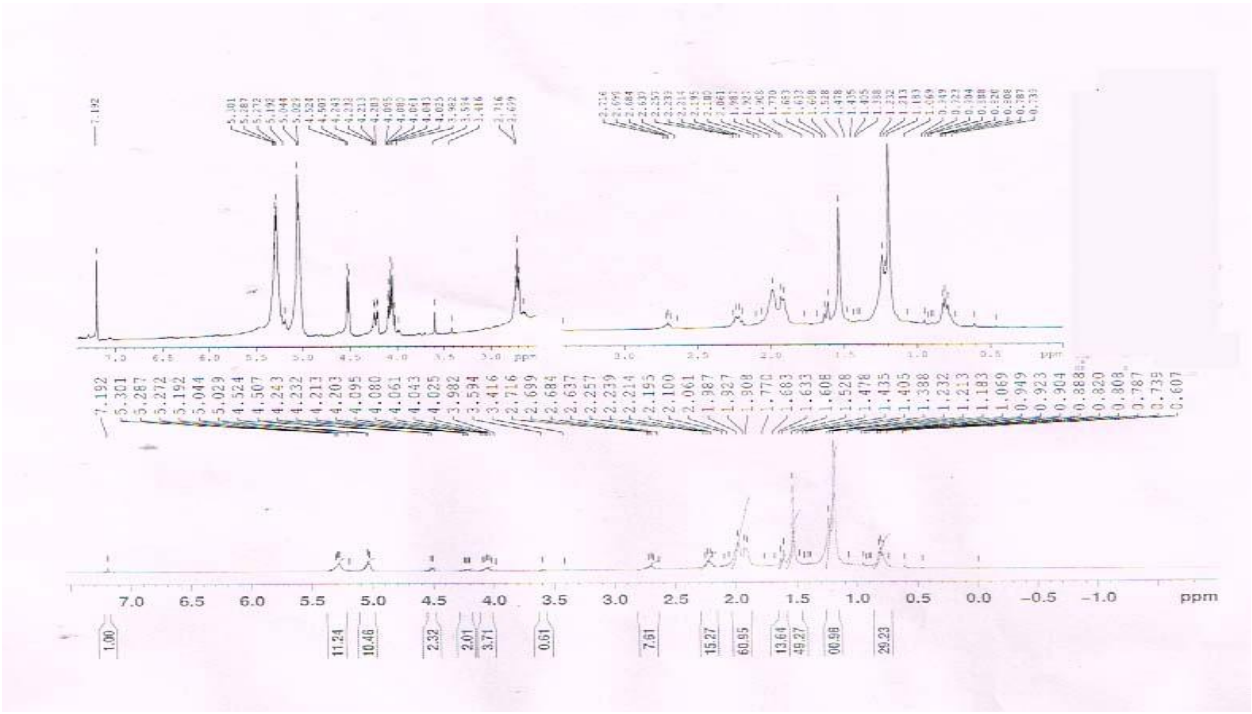

Figure 4. [1]HNMR spectra of Isolated compound