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ISOLATION, PURIFICATION AND SPECTRAL ANALYSIS OF PURE COMPOUND OBTAINED FROM LEAVES EXTRACT OF *ASPARAGUS RACEMOSUS*

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ABSTRACT

The leaves extract of *Asparagus racemosus* was studied for isolation of pure compound by column chromatography and purified by thin layer chromatography (TLC) and identified with help of preliminary phytochemical analysis, physical properties, and structure elucidation by spectroscopic techniques such as UV-visible spectroscopy, Infrared spectroscopy, ^{1}H NMR spectroscopy and Mass spectroscopy. The compound isolated by leaves extract of *Asparagus racemosus.*

Key words: *Asparagus racemosus,* Column chromatography,TLC & UV,IR,NMR & Mass Spectroscopy.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of the plants lies in some chemical substances that produce a definite physiological action on the human body. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. The drugs are derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc. The world Health Organization is actively encouraging National Governments of member countries to utilize their traditional systems of medicines with regulations suitable to their national health care systems[1]**.** Plant based natural constituents can be derived from any part of

the plant like bark, leaves, roots, fruits, seeds.[2].*Asparagus racemosus* Wild.belonging to the family"Asparagaceae ;Liliaceae[3].Its medicinal usage has been reported in the Indian and British Pharmacopoeias as well as in the traditional Indian systems of medicine such as Ayurveda,Unani and Siddha.

Asparagus racemosus is a much branched, spinous under shrub found growing wild in tropical and sub-tropical region of India[4]. It is a woody climber growing to 1-2m in height. The leaves are linear with a stout conical spiny spur, straight or slightly curved and pine-needles, small and uniform. The inflorescence has tiny white flowers,in small spikes and the roots are finger-like and clustered[5,6,7]. The roots of the plant are white, long, fleshy, tuberous and tapering at both ends. Small white and fragrant flowers appear on this plant in the beginning of the rainy season. Fruits in the shape of small berries appear in the autumn. It grows wild in forest and can also be planted in gardens in most of the areas.

Scientific Classification of *Asparagus racemosus*

Kingdom	Plantae
Phylum	Tracheophyta
Division	Angiosperms
Class	Monocots
Order	Asparagales
Family	Asparagaceae
Genus:	*Asparagus*
Species:	***Asparagus racemosus***

It was botanically described in 1799, due to its multiple uses. The demand for *A. racemosus* is constantly on the rise.

MATERIAL AND METHODS

Collection of the plant material

The fresh plant *Asparagus racemosus* Wild.was collected from the "Sanjivani Government Nursery" at Bhopal city of M.P. India, during the month of August- September 2012. The plant material was washed thoroughly with running tap water and then leaves were separated and cut into small pieces. The leaves were shade dried at room temperature and dried leaves were crushed and powdered by mortar-pestle. And the powdered material was collected in air-tight plastic jars.

Extraction

The dried powder of plant leaves of *Asparagus racemosus* was weighed accurately. And weighed powdered material was successively extracted with Petroleum ether, Ethyl acetate and Methanol at a temperature range of 60-80^{0}C by Soxhlet apparatus. 500 gm powdered material maceratted with petroleum ether (900 ml) for 48 hrs. And then the defatted powder material was extracted with ethyl acetate and finally methanol solvent by Soxhlet apparatus at low pressure and 40^{0}C tempreture.

Phytochemistry of the Plant

The phytochemical analysis done by standard method and the phenolic compounds, anthraquinines and flavonoid present in the crude extract of Asparagus racemosus leaves extract. The main active constituents of *A.racemosus* are steroidal saponins (Shatavarins I-VI). Shatavari I is the major glycoside with 3 molecules of glucose and rhamnose moieties attached to sarsasapogenin[8,9,10]**.** Sarsasapogenin and shatavarin I-IV are present in roots, leaves,and fruits of *Asparagus* species..*A.racemosus* also contain flavonoids,glycosides of quercetin, rutin and hyperoside and tannin are present in flowers and fruits[11]**,** while diosgenin and quercetin are present in the leaves[12]**..**

Isolation & Purification of the extract

Column chromatography was used to isolation of pure the compounds. The different solvent systems petroleum ether: chloroform: ethyl acetate (90:10:10, 70:30:10, 50:50:10, 30:70:10, 10:90:10), chloroform: ethyl acetate: Methanol (90:10:10, 70:30:10, 50:50:10, 30:60:10, 10:80:20, 10:70:30,10:50:50, 10:30:70, 10:20:80, 10:10:90) were used for the isolation of active components according to the procedure given by **Harborne (1984).** The various fractions thus obtained were collected in glass vials using CHOH:EtOAc:MeOH (10:20:30) and CHOH:EtOAc:MeOH(10:80:20). Same fractions were mixed and concentrated and their purity was determined by using thin-layer chromatography and found the pure compounds as a single spot with small impurities and was washed with methanol to give pure compound**.**

Purification of the compound

Column purified fractions were subjected to TLC in order to identify the bioactive compounds. The most suitable TLC system for analysis was shown to be choloroform: methanol (5:1) with the largest discriminating power. Three major bands were found with Rf values of 0.4, 0.45 and 0.48. This values indicate the presence of flavonoids.

Confirmatory test of Flavonoids

Shinoda Test

Asparagus racemosus leaves extract in a test tube in a small quantity and few magnesium turnings and concentrated hydrochloric acid is added drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes, shows the presence of flavonoid.

Alkaline reagent test

In the small quantity of test material add few drops of sodium hydroxide solution intense yellow color is formed; which turns to colorless on addition of few drops of dilute acid indicate presence of flavonoids.

RESULT AND DISCUSSION

The results of phytochemical analysis shown the presence of flavonoids and the spectral results showed the yellowish crystalline solid ,melting point 293^0C and UV-Visible spectra showed in the 369 nm wave-lentgth (table no.1 & figure no.1),the FT-IR (KBr) spectrum of the isolated compound showed in (table no.2 & figure no.2,)demonstrated 3454cm-1(broad O-H Stretch),1636cm^{-1}(C=C stretch), 1402cm^{-1} (CH_2 deformation vibrations), 677 cm^{-1} (out of plane bending may be of O-H.) ,the appearance of a single broad peak at (3454 cm^{-1}) related to the vibration stretching for (-OH) bond indicated the presence of phenol group. The IR spectrum appear the isolated compound is aromatic compound and contains of phenol, hydroxyl group, the mass spectra showed (table no.3 & figure no.3) peaks those supports the structure of quercetin glycosides.Peaks of mass spectra m/e ions 314,256,198 Fragments from quercetin glycoside, 548, 490, 430 shows loss of sugar fragments, 134 $C_7H_2O_4$, 96 $C_5H_4O_2$ the fragments originated from flavonoid nucleus. And ^{1}H NMR spectra shows (table no.4 & figure no.3) δ (ppm) δ 1.3 (CH_2 of sugars), δ 3.5 (O-H protons substituted on benzene), δ 4.0 (O-H protons of sugars, δ 4.9 (O-H protons of sugars) , δ 6.9(aromatic protons).

Since the flavonoids in leaves of *Asparagus racemosus* are proved & the phytochemical test are positive for flavonoids. It is concluded that the isolated compound Quercetin 3 glocoronide which is one of the chemical component of *Asparagus racemosus.* The spectral data is sufficient just to identify probable compound.Number of peaks in the isolated compound possess the major functional hydroxyl groups. *Asparagus racemosus* plant also contains sterols and flavonoids as one of its phytochemical constituents (**M.K.,1991 and S.Velavan,2007;Subramanian SS,1968).**

TABLE NO. 1. UV-Visible spectra of Isolated compound

S.No.	Sample	Observation
1	Isolated compound	Maximum found at 369 nm

TABLE NO.2. Interpretation of IR Spectrum of Isolated compound (γ cm^{-1})

Serial no.	Wave number (cm^{-1})	Functional groups
1.	3454	Broad O-H Strech
2.	1636	C=C stretch
3.	1402	CH_2 deformation vibrations
4.	677	out of plane bending may be of O-H

TABLE NO. 3. Mass Spectrum of Isolated compound

S. No.	Peaks (m/e)	Fragmentation
1.	314,256,198	Fragments from quercetin glycoside
2.	548, 490, 430	loss of sugar fragments
3.	134	$C_7H_2O_4$
4.	96	$C_5H_4O_2$ the fragments originated from flavonoid nucleus

TABLE NO. 4. ^{1}HNMR Spectra of Isolated compound

S.No.	Peaks (δ ppm)	Interpretation
1.	1.3	CH_2 of sugars
2.	3.5	O-H protons substituted on benzene
3.	4.0	O-H protons of sugars
4.	4.9	O-H protons of sugars
5.	6.9	Aromatic protons

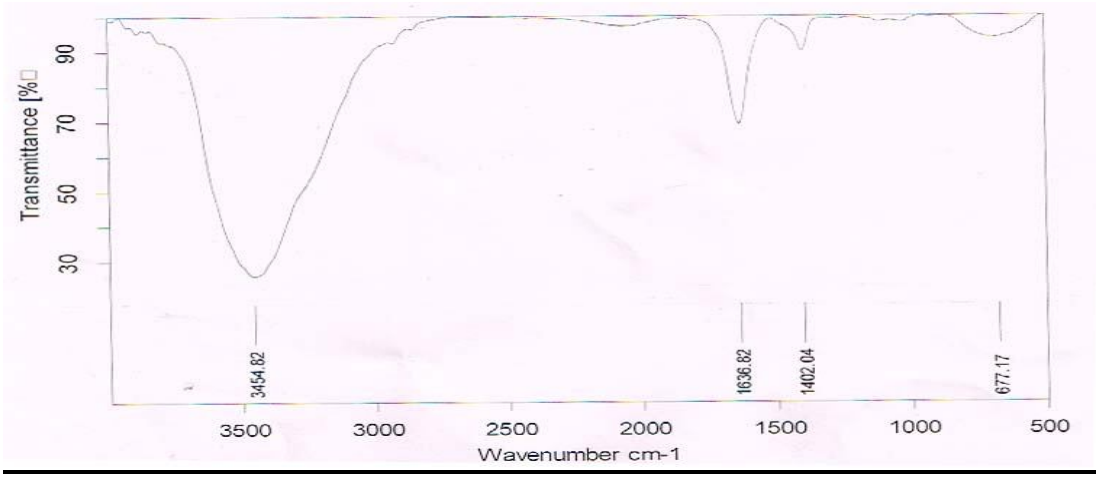
Physical Characters of isolated compounds

Colour:	Yellow Crystalline shiny powder
State:	Solid
Solubility:	Water & Methanol
Melting point:	193-195^{0}C
Rf Values**:**	0.48

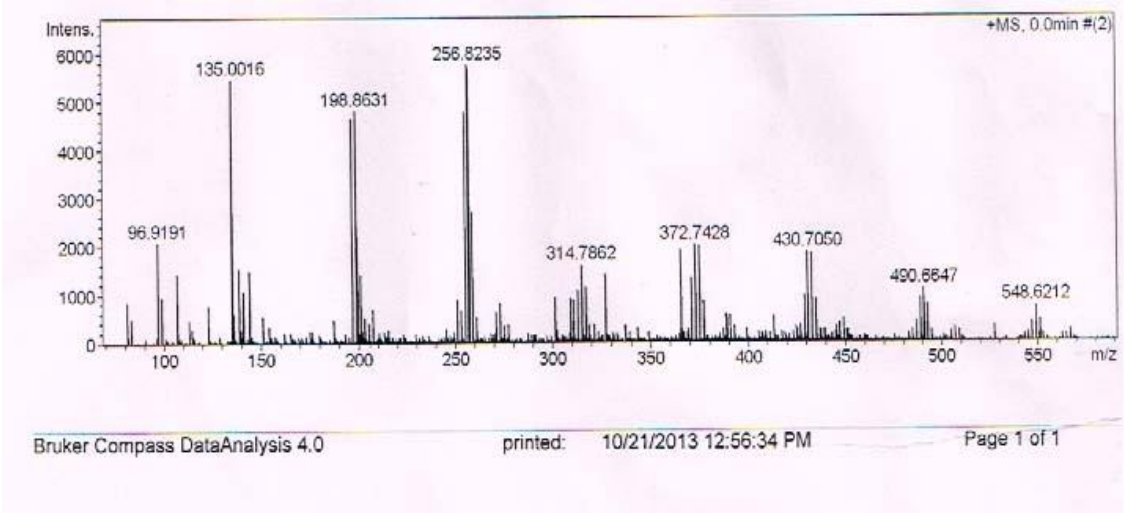
Elucidation of Pure compound

Molecular formula	$C_{21}H_{18}O_{13}$
Molecular weight	478.36
Synomous	Quercetin 3-O-β-D-glucuronide
IUPAC name	3,3',4',5,7-Pentahydroxyflavone 3-glucuronide

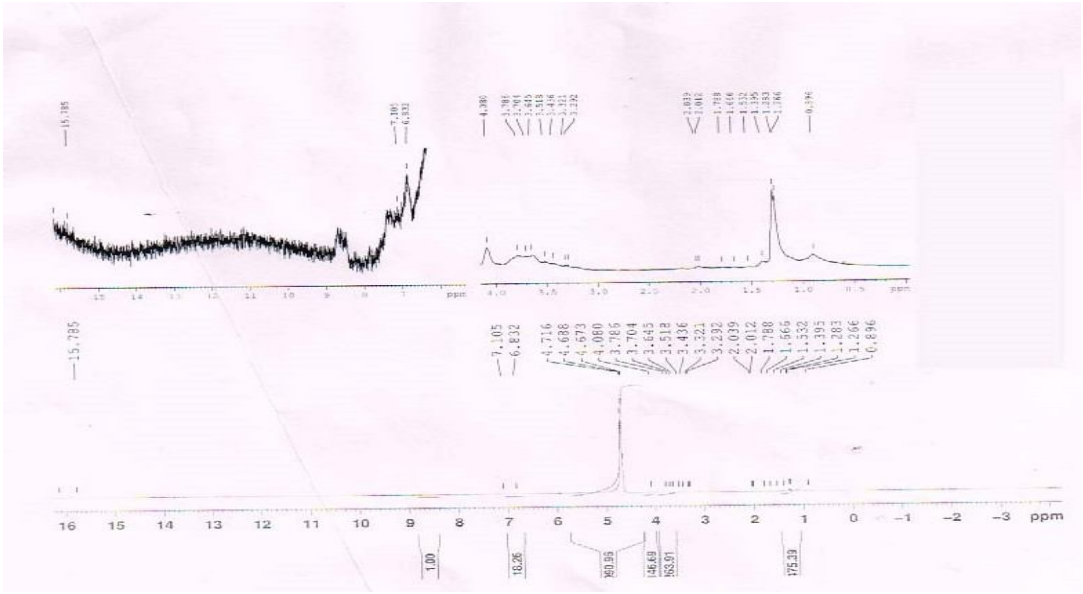
Spectroscopy Graphs



IR Graph of Isolated compound



Mass Spectra of Isolated compound



NMR Spectrascopy of Isolated compound

CONCLUSION

There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity .Phytochemical screening of active compounds from plants has lead to the discovery of new medicinal drugs, which have protection and treatment against various diseases. It is revealed that the herbal drugs are relatively safe and exhibit a remarkable efficacy in the treatment of various diseases. Medicinal plants are considered as clinically effective and safer alternatives to the synthetic antibiotics.

The therapeutic properties of the medicinal plants are due to the presence of active principles, which has to be extracted and screened for medicinal properties. Extensive research in the area of isolation and characterization of the active principles of these plants are required so that better, safer and cost effective drugs for treating bacterial infections can be developed. In recent years pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants to produce more cost effective remedies that are affordable to the population.

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