International Journal of Biology Research
ISSN: 2455-6548
Impact Factor: RJIF 5.22
www.biologyjournal.in
Volume 3; Issue 2; April 2018; Page No. 68-70

Isolation and identification of phytosterols from *Alhagi marorum*

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Abstract
Alhagi marorum is an important medicinal plant known for its vast potential. It is a valuable desert plant which is commonly used in traditional system of medicine for glandular tumors and antidiarrhoeal, as well as in a number of metabolic disorders such as rheumatism and hemorrhoids. In the present study, phytosterols from *Alhagi marorum* was identified and quantified in vitro. Phytosterols were identified using chromatographic and spectral studies. ß-sito sterol and stigma sterol were identified by IR and GC-MS.GC-MS profiling showed various compounds 2Propen1one, 1(4methylphenyl) 3phenyl, oxime, Benzenesulfonic acid, 3methoxy, Hydroxides 1,4Oxathiin3carboxylic acid, 2(2chloroethyl) thio methyl 5,6dihydro, methyl ester. It is the first report on phytosterols from the experimental plant (Alhagi marorum).

Keywords: *Alhagi marorum*, phytosterols, GC-MS

Introduction
Today's health care systems rely largely on plant materials. Much of the world's population depends on traditional medicine to meet daily requirements especially in developing countries. The use of plant-based remedies is also widespread in many developed countries and pharmaceuticals are based or devised from plants or plant products [1]. Plant sterols are triterpenes that are important structural components of plant membranes, and free phytosterols serve to stabilize phospholipids' bilayers in plant cell membranes just as cholesterol does in animal cell membranes [2]. Plant sterols structurally similar to cholesterol that act in the intestine to lower cholesterol absorption Because they have very low systemic absorption and are already present in healthy diets, increasing the intake of phytosterols may be a practical way to reduce coronary heart disease with minimum risk [3]. Studies now show that phytosterols consumption, of about 2 grams/day, results in an approximately 9% reduction in LDL-cholesterol, which is the "bad" cholesterol known to contribute to heart disease. Based on this tremendous benefit many food manufacturers and supplement developers are putting phytosterols into their product to benefit both consumers and to promote a healthy heart diet [4]. *Alhagi marorum* belongs to family fabacae, commonly known as Javasa. It is commonly used in traditional system of Medicine.

Material and Methods
Collection and Identification of Plant Materials
The medicinal plant *Alhagi marorum* has been collected from Jaipur-Ajmer highway in Rajasthan. The stem and leaves of the plant washed in tap water and finally were made to shade dried. Whole plant was cleaned, shade dried and pulverized to powder in a mechanical grinder. The powdered materials were stored in air tight containers till use.

Extraction
Dried and powdered plant material was defatted in petroleum ether (60-80^0 C) for 24 h on a water bath. Defatted material was air dried and hydrolyzed in 30% HCl (v/v) for 4 h. Each hydrolyzed sample was washed with distilled water till pH 7 was achieved and was dried later. The dried preparation was again extracted with benzene for 24 h. The extract was filtered and dried *in vacuo* .The crude extract was dissolved in benzene before chromatographic examination [7].

Thin layer chromatography (TLC)
Glass plates coated with silica gel G were used. Each of the extract was co-chromatographic separately with authentic sterols as marker. These plates were developed in an airtight chromatographic chamber, saturated with solvent mixture (Hexane: Acetone: 8:2), [8]. Other solvents such as benzene and ethyl acetate (85:15), [9] benzene: ethyl acetate (3:1) was also used but hexane: acetone (8:2) gave better separation. These plates were air dried and visualized under UV light and fluorescent spots corresponding to that of standards marker were marked. These developed plates were sprayed with 50% Sulphuric acid [10] and anisaldehyde reagent, separately and heated at 110^0 C for 10 min.

Preparative thin layer chromatography
PTLC was performed using silica gel G coated plates (0.4-0.5mμ) along with the reference markers. These plates were developed in hexane: acetone (8:2), air dried and examined under UV light. Each spot coinciding with that of standard marker was marked and the isolated compounds were also subjected to UV and IR spectral studies.

Result and Discussion

Identification

Melting point and IR spectra of each of the isolated compounds was taken and a comparison of the TLC color reaction was made, which was found to be in accordance with that of studied authentic compounds.

IR curves and physicochemical table

Infra-red Spectra of Isolated and Standard Stigma sterol

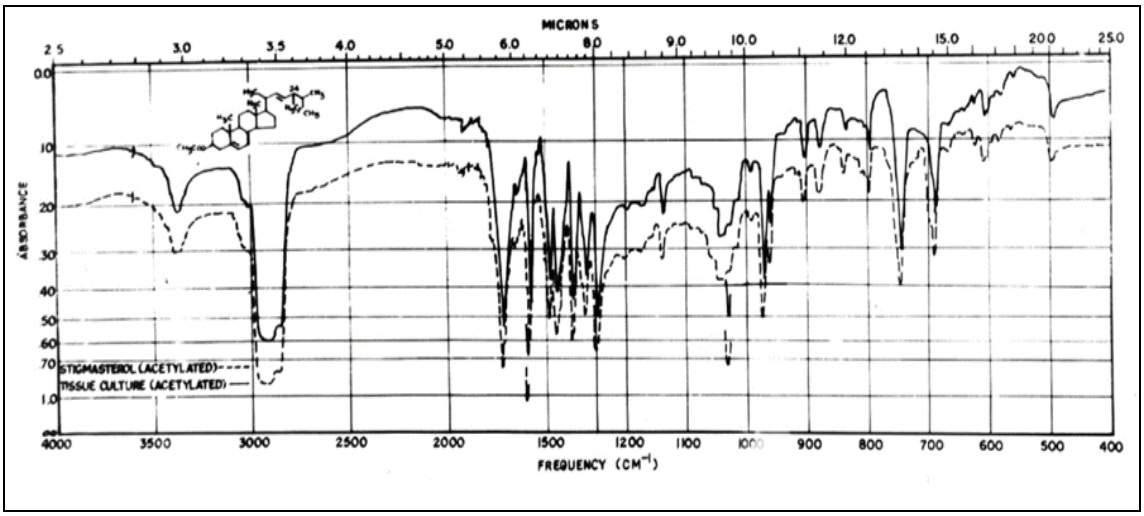

Fig 1

Infra-red Spectra of Isolated and Standard β-sitosterol

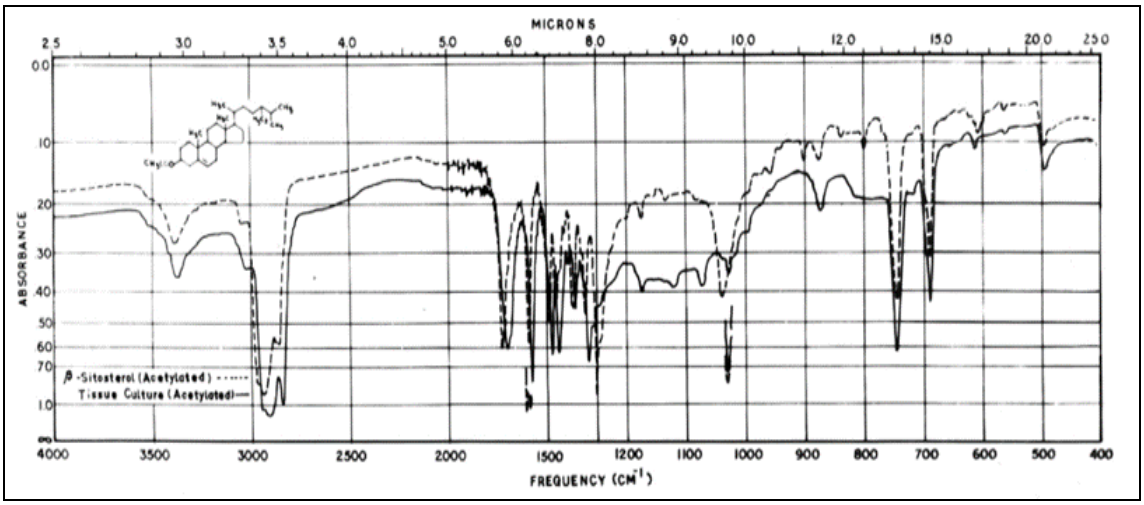

Fig 2

Chromatographic Behavior and Physico-chemical Characteristics of Isolated Phyto-sterols

Table 1

Isolated Compounds	R_f Value			Color After Spray		M.P. (°C)	IR Spectral Peaks (rept.) ν (KBr) cm^{-1}
	S_1	S_2	S_3	R_1	R_2		
β-sitosterol	0.89	0.90	0.71	PU-BN	PK	136-137	3350 (O-H), 2830, 1665 (C=C), 1470, 1300, 1052 (C-O) and 880
Stigma sterol	0.83	0.64	0.65	GY	PU	167-69	3400 (O-H). 2950, 1750, 1640 (C=O), 1035 (C-O), 991, 957, 935, 810 and 715

Abbreviations: Abbreviations: S_1 - Hexane : acetone (8 : 2), S_2 - Benzene : acetone (2 : 1), S_3 – Benzene : ethyl acetate (3 : 2),R_1 - 50% H_2SO_4, R_2 - Anisaldehyde reagent, BN - Brown, PK- Pink, PU – Purple, BL – Blue, GY – Gray

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

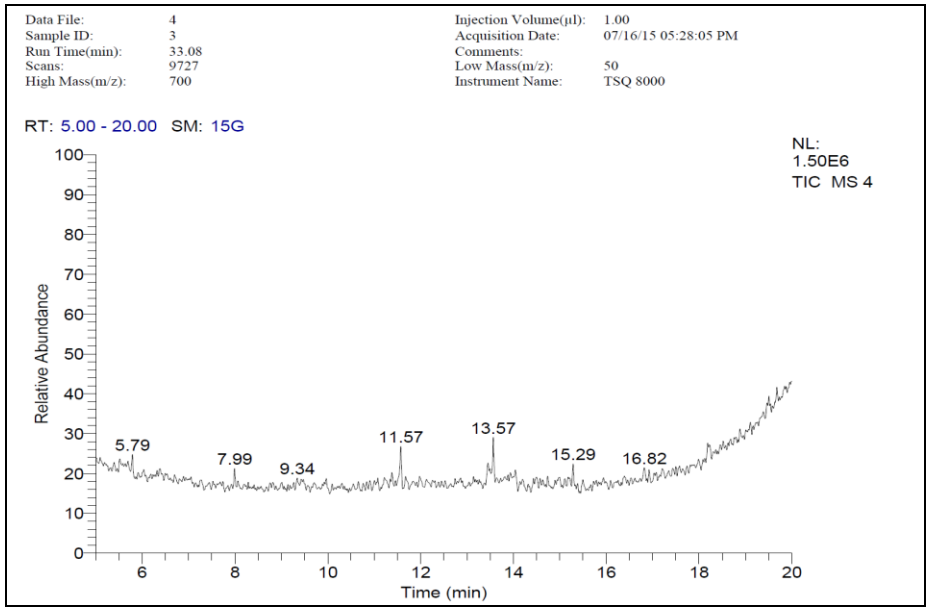

Fig 3

Table 2: GC-MS profiling of phytosterols isolated from Leave of Alhagi *marorum*

S. No.	R Time	Area	Area %	Compound Name	Molecular Formula
1.	4.09	103903	6.45	2-propane-1(,4-methylphenyl)	C16H15NO
2.	4.78	54383	3.37	Benzeneslfonic acid,3 methoxy-hydrazide	C7H10N203S

Conclusion

This investigation has given preliminary information to determine the chemical composition of sterols found in *Alhagi marorum* using IR and GC-MS. The presence of these bioactive compounds in *Alhagi marorum* lends credence to its use by the human community. It also holds for the production of novel drugs with isolation of specific compounds.

References

1. Gbile ZO. Ethno botany, taxonomy and conservation of medicinal plant, in A. Sofowora, Ed., The state of medicinal plant research in Nigeria, University of Ibadan press, Nigeria, 1986, pp.13-29.
2. RAMoreau BD, Whitaker KB. Hicks. Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses, 41, No. 6, 2002, 457-500.
3. Ostlund RE. Phytosterols in human nutrition, Annu Rev Nutr. 2002; 22:533-49.
4. Dr. Joseph Maroon. Phytosterols as both Food and as Supplements Beneficially lower cholesterol.
5. Rastogi BN. Mehrotra; Compendium of Indian Medicinal Plant, Central Drug Research Institute, Luckhnow and National Institute of science communication and information Resources , New Delhi. 2007; 1:387.
6. Shetty BV, Singh V. Flora of India (Series-II), Flora of Rajasthan, Botanical survey of India. 1991; 2:453-480.
7. Kaul B, Staba EJ. Dioscorea Tissue Cultures I. Biosynthesis and isolation of diosgenin from Dioscorea deltoid a callus and suspension cells, Lloydia. 1968; 31(2):171-179.
8. Fazli FRY, Hardman R. The spice fenugreek (Trigonella foenum-graecum); its commercial variety of seed as a source of diosgenin, Trop. Sci., 10, 66-78.
9. Heble MR, Narayanaswami S, Chadha MS. Diosgenin and β-sitosterol; Isolation from *Solanum xanthocarpum* tissue cultures, Science. 1968; 161:1145.
10. Bennet PD, Heftmann E. Thin layer chromatography of steroidal sapogenins, J. Chromatogr. 1962; 9:353.
11. Katarzyna Hąc-Wydro. Studies on β-sitosterol and ceramide-induced alterations in the properties of cholesterol/sphingomyelin/ ganglioside monolayer, BBA. 2013; 1828(11):2460-2469.
12. Herbert Scheinberg MD, Marvin E, Jaffe MD, Irmin Sternlieb MD. The Use of Trientine in Preventing the Effects of Interrupting Penicillamine Therapy in Wilson's disease, N Engl J Med. 1987; 317(4):209-213.