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ISOLATION AND IDENTIFICATION OF PHYTOSTEROLS FROM LEAVES OF *MAYTENUS EMARGINATA* PLANT

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ABSTRACT

Medicinal plants are rich source of secondary metabolites, biosynthetically derived from primary metabolites. Secondary plant products are of major interest because of their biological activities ranging from antibacterial, antibiotic, insecticidal, hormonal, pharmacological, pharmaceutical. Present study focused on isolation and identification of various phytosterols from leaves extract of Maytenus emarginata. The extraction of phytosterols done using a procedure describe by Kaul & Staba. Preliminary identification was done by TLC and PTLC chromatographic techniques, while GC-MS was used for advanced analysis. The present observation showed presence of three sterols namely β-sitosterol, Stigmasterol and Lenosterol in the leaves of this plant. Isolated phytosterols compared with standard phytosterols by using Rf value, melting point, IR and NMR spectral analysis. GC-MS analysis of leaf extract of Maytenus emarginata identified a total of 45 compounds out of many that have important pharmacological activities. From the results, it is evident that Maytenus emarginata contains various bioactive compounds and is recommended as a plant of phytopharmaceutical importance.

Keywords: *Secondary Metabolites, Phytosterols, PTLC, GC-MS, Phytopharmaceutical*.

Introduction

In today's world, health care system mainly depends on plant products or plant by-products[1]. The use of medicinal plants products for health care purpose was known as herbalism. Medicinal plants are rich source of secondary metabolites, biosynthetically derived from primary metabolites but restricted to specific taxonomic genera of plant kingdom and specific part of plant body. Secondary plant products are of major interest because of their biological activities ranging from antibacterial, antibiotic, insecticidal, hormonal, pharmacological, pharmaceutical[12]. The medicinal properties of herbs lie in plant secondary metabolites[2]. Plants synthesize two types of products through metabolic processes. Primary products or primary metabolites are which synthesize as main product during metabolic process. Additionally metabolic processes can synthesize some by-products. The latter was known as secondary products or secondary metabolites. These secondary metabolites include alkaloids (Nitrogen containing compounds), phytosterols (plant steroids), flavonoids and phenolic compounds. Primary metabolites such as proteins, carbohydrates and lipids were involved in basic cellular functions, while secondary metabolites involved in plant's accessory functions, such as defense mechanism, give characteristic color, odor and also in . Because of presence of secondary metabolites in plants parts, they can protect themselves from herbivores, insects and pathogenic micro-organisms. The property of plant secondary metabolites to prevent infections of pathogenic micro-organisms was popularly known as anti-microbial activity. This anti-microbial property of plants was extensively used by pharma-scienstists to meet requirements of world's health-care system. The current study is based on a medicinal plant "*Maytenus emarginata*", which extensively found in semi-arid climatic region of Rajasthan. The semi-arid region of Thar Desert is the major biodiversity zone of Indian sub-continent. *Maytenus emarginata* is a rare medicinal plant of semi-arid region of Rajasthan. It is a semi-evergreen deciduous tree belongs to family celestraceae. It is commonly known as kankero in local Rajasthani language and thorny staff tree in English. It is a sacred plant of north-western Rajasthan for environment friendly Bishnoi community. It is believed that lord Jambheswer (Jambhoji) had realization under the tree of *Maytenus emarginata*[3].

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(A) **(B)** **(C)** **(D)**

Maytenusemarginata (A. Plant; B. Bark; C. Leaves; D. Fruits)

In this research article, the GC-MS analysis was carried out of dried leaves for identification of phytosterols followed by qualitative and quantitave determination of compounds. Plant sterols or phytosterols are secondary metabolites found in plants in small quantities4. The most common phytosterols in plants are: β-sitosterol, stigmasterol and campesterol. β-sitosterol is the most prevalent phytosterol in higher plants. Ergosterol, the biological precursor of vitamin D2, is found in considerable quantities in fungi, algae and lichens. Phytosterols have attracted much attention since last few years due to their diverse biological activities. They reduce total cholesterol and LDL cholesterol levels in blood by inhibiting its absorption from the small intestine. Hence, they lower the atherosclerotic risk (shortening of diameter of veins) and offer protection against cardiovascular diseases[5]. Dietary phytosterols manifest a protective activity against cancer, decreasing the risks of breast, prostate and colon cancer[6]. Plant sterols also have anti-inflammatory and immunomodulatory[7] properties.

Materials and Methodology

Fresh plant material (leaves) of *Maytenus emarginata* were collected from the Jhalana forest of Jaipur district of Rajasthan. The plant materials were taxonomically identified and authenticated by herbarium, Department of Botany, University of Rajasthan, Jaipur and a specimen copy of plant material was deposited. The plant material was cleaned, dried in shade, and grind in a powdery form. The powdered plant material was stored in airtight glass-containers for further secondary metabolites analysis.

- **Extraction of phytosterols:** Dried and powdered plant material (plant leaves) was defatted in petroleum ether for 24 hours on a water bath at 60-80*C temp. Defatted material was air dried and hydrolyzed in 30% HCl (v/v) for 4 hours. This 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 hours. The benzene extract was filtered by using Whatman filter no.1. This extract was air dried in sterilized conditions. The crude extract was dissolved in benzene before chromatographic examination for phytosterol analysis[8].
- **Qualitative analysis by using thin layer chromatography (TLC):** Glass plates coated with silica gels G were used for chromatographic analysis. Plant extract was co-chromatographed separately with authentic standard sterol as marker. The plate was developed in an airtight chromatographic chamber, saturated with solvent mixture (Hexane: Acetone:: 8:2[9]. The plate was air dried and visualized under UV light and fluorescent spots corresponding to that of standard marker were marked. The developed plate was sprayed with 50% sulphuric acid[10] and anisaldehyde reagent, separately and heated at 110^0 C for 10 min. When this plate placed in a chamber saturated with I_2 vapors, it also showed dull red color of β-sitosterol. Rf value come out with the Rf value of the standard β-sitosterol (0.83-0.86)[11]. The marked spots were scrapped and collected along with the silica gel and wash with ethanol then crystallized with chloroform[12]. Finally, this purified material was subjected to its IR and NMR spectral analysis.
- **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, scraped from plates, and eluted with chloroform. The eluted reactions were subjected

to crystallization, separately and their melting point, mixed melting point were determined. The isolated compounds were also subjected to UV and IR spectral studies. Melting point and IR spectra of each of the isolated compounds was taken and a comparison of the TLC colour reaction was made, which was found to be in accordance with that of studied authentic compounds.

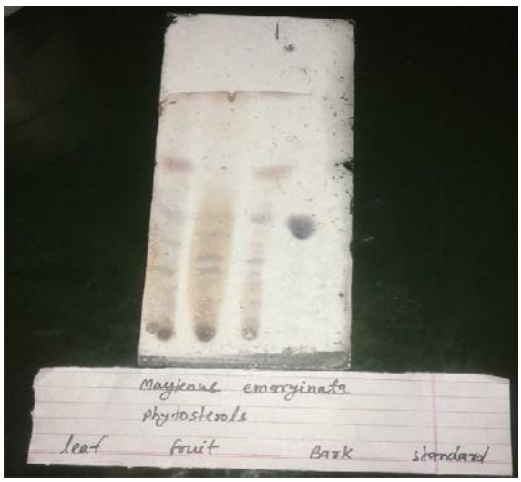
Qualitative analysis by using Gas chromatography-Mass spectroscopy (GC-MS)

- **Preparation of Sample:** Extraction of various metabolites rich fraction, such as flavonoids, steroids and alkaloids were done using standard protocols mentioned in extraction procedure section. Isolated fractions were filtered with Whatman No.1 filter paper and the residue was removed. It was again filtered through acrodisc syringe filter of having size 0.45mm in order to remove the traces of moisture. Flavonoid fractions were taken up in small volume of ethanol (2-5mL), steroid extracts were taken up in small volume of benzene for GC-MS analysis.
- **Gas Chromatography and Mass Spectroscopy (GC-MS) analysis:** Gas chromatography (GC) analysis was carried out at Advanced Instrumentation Research Facility (JNU) New Delhi. This technique is best for identification of various phytochemicals of plant. The equipment was GC-MS QP-2010 ultra. The carrier gas used in GC-MS programme was helium 1 ml/minute (split ratio=10:0). The initial oven temperature program is 80°C and final temperature is 280°C, hold time 28 min, Ion Source temperature is 220°C and interface temperature is 270°C, solvent cut time 3.50 min, Detector Gain Mode: Relative to the tuning result, Detector Gain +0.00 Kv, threshold 1000, start time 4 min, end time 49.98 min, Event time 0.20 sec, Scan speed 3333, start m/z 40.00 and end m/z 650.00. The identification of the components was based on the comparison of their mass spectra and retention time with those stored in NIST library or with mass spectra from published literature.

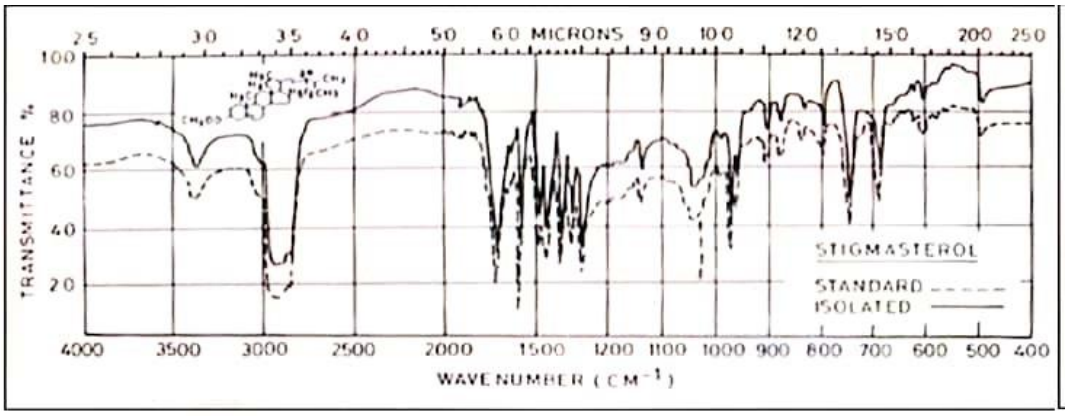
Results and Discussion

Three sterols were spotted which were common in plant parts on thin layer chromatography. The Rf values of the spots matched with authentic standards and were identified as β-sitosterol, stigmasterol, and Lenosterol. Among the various solvent systems tested best results were obtained in the solvent system Hexane: Acetone (8:2) with Rf values viz., β-sitosterol, 0.89; stigmasterol, 0.83. The characteristic colours were also developed when TLC plates were sprayed with anisaldehyde reagent (β-sitosterol - pink; stigmasterol - Purple) and with 50% sulphuric acid (β-sitosterol-Purple brown; stigmasterol- Gray) corresponding to their authentic samples. The isolated sterols were also identified and characterized with their mp, which also corresponded with those of their respective standards separately (β -sitosterol 136-137oC; stigmasterol; 167-169oC). The characteristic peaks of IR spectra of isolates (β-sitosterol, stigmasterol, and) were also found to be super imposable with the IR spectra of reference compounds.

GC-MS is the best techniques to identify the constituents of plant. Peak area, retention time and molecular formula were used for the confirmation of phytochemical compounds. The active principles compound with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented in table. From the GC-MS analysis of *Maytenus emarginata* leaves the presence of forty five compounds (phytochemical constituents) were revealed the medicinal quality of the plant.



TLC Result			
Name of Phytosterol	**Leaf**	**Bark**	**Fruit**
Stigmasterol	**++**	**++**	**++**
Sitosterol	**++**	**+**	**++**
Lenosterol	-	**+**	**+**



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

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	0.86	0.83	0.71	PU-BN	PK	136-137	3350 (O-H), 2830, 1665 (C=C), 1470, 1300, 1052 (C-O) and 880
Stigmasterol	0.84	0.63	0.65	GY	PU	167-69	3400 (O-H). 2950, 1750, 1640 (C=O), 1035 (C-O), 991, 957, 935, 810 and 715

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

Table: Compounds Identified in GC-MS analysis

S. No.	Ret. Time	Peak area (%)	Name of compound	Molecular formula	Molecular weight
1.	14.811	0.57	Neophytadiene	$C_{20}H_{38}$	278
2.	14.883	0.32	2-Pentsdecanone,6,10,14-trimethyl	C18H36O	268
3.	15.176	0.24	Pentadecanoic acid	C15H30O2	242
4.	15.259	0.19	Neophytadiene	C20H38	278
5.	16.309	5.80	n-Hexadecanoic acid	C16H32O2	256
6.	16.637	0.10	Triphenylmethane	C19H16	244
7.	16.876	0.11	Palmitic acid, TMS derivative	C19H40O2Si	328
8.	17.541	0.27	Phytol	C20H40O	296
9.	17.977	4.06	Cis-9-Hexadecenal	C16H30O	238
10.	18.127	0.50	Octadecanoic acid	C18H36O	284
11.	19.181	0.07	2-methyloctacosane	C29H60	408
12.	19.669	0.08	4,8,12,16-Tetramethylheptadecan-4-olide	C21H40O2	324
13.	20.022	0.17	Heneicosane	C21H44	296
14.	20.827	0.58	Heneicosane	C21H44	296
15.	21.116	0.08	1,2-Benzenedicarboxylic acid	C24H40O2	324
16.	21.603	1.14	Heneicosane	C21H44	296
17.	22.368	2.00	Heneicosane	C21H44	296
18.	23.221	2.90	Celidoniol, deoxy	C29H60	408
19.	23.354	1.31	Squalene	C30H50	410
20.	23.658	0.21	Alpha-Tocospiro B	C29H50O4	462
21.	23.897	0.30	Alpha-Tocospiro B	C29H50O4	462
22.	24.217	3.71	Celidoniol, deoxy	C29H60	408
23.	24.671	0.23	E,E-2,13-Octadecadien-1-ol	C18H34O	266
24.	24.894	0.13	Sulfurous acid,pentadecyl-2-propyl ester	C18H38O3S	334
25.	25.392	2.75	Celidoniol, deoxy	C29H60	408
26.	26.003	1.82	Octacosanal	C28H56O	408
27.	26.841	3.33	Tetratetracontane	C44H90	618

28.	26.983	0.52	Octacosanal	C28H58O	410
29.	27.411	0.70	Vitamin E	C29H50O2	430
30.	27.599	0.28	Hexacosanal	C26H52O	380
31.	28.603	2.12	Tetratetracontane	C44H90	618
32.	29.796	10.11	Henicosanal	C21H42O	310
33.	30.273	1.36	22-Desoxy carpesterol	C37H54O3	546
34.	31.029	1.87	Tetratetracontane	C44H90	618
35.	31.404	4.10	1-Eicosanol	C20H42O	298
36.	32.781	28.79	Beta-Amyrin	C30H50O	426
37.	32.973	1.43	Lupenone	C30H48O	424
38.	33.810	8.59	Lupeol	C30H50O	426
39.	35.195	1.08	Octadecanal	C18H36O	268
40.	35.851	0.43	Isolonifolol	C15H26O	222
41.	36.383	0.40	1,1-Propendicarbonotril,1,2-di cyclohexyl	C17H26N2	258
42.	36.934	0.64	Tetracontane	C40H82	562
43.	37.672	3.47	Friedelan-3-one	C30H50O	426
44.	39.340	0.78	25-Dehydro-neotigogenin benzoate	C41H52O5	624
45.	41.109	0.38	Tetracontane	C40H82	562

Sample Information

Analyzed by : $Admn.$
Analyzed : 1/6/2021 5:54:17 PM
Sample Type : $Organic$
Sample Name : 5
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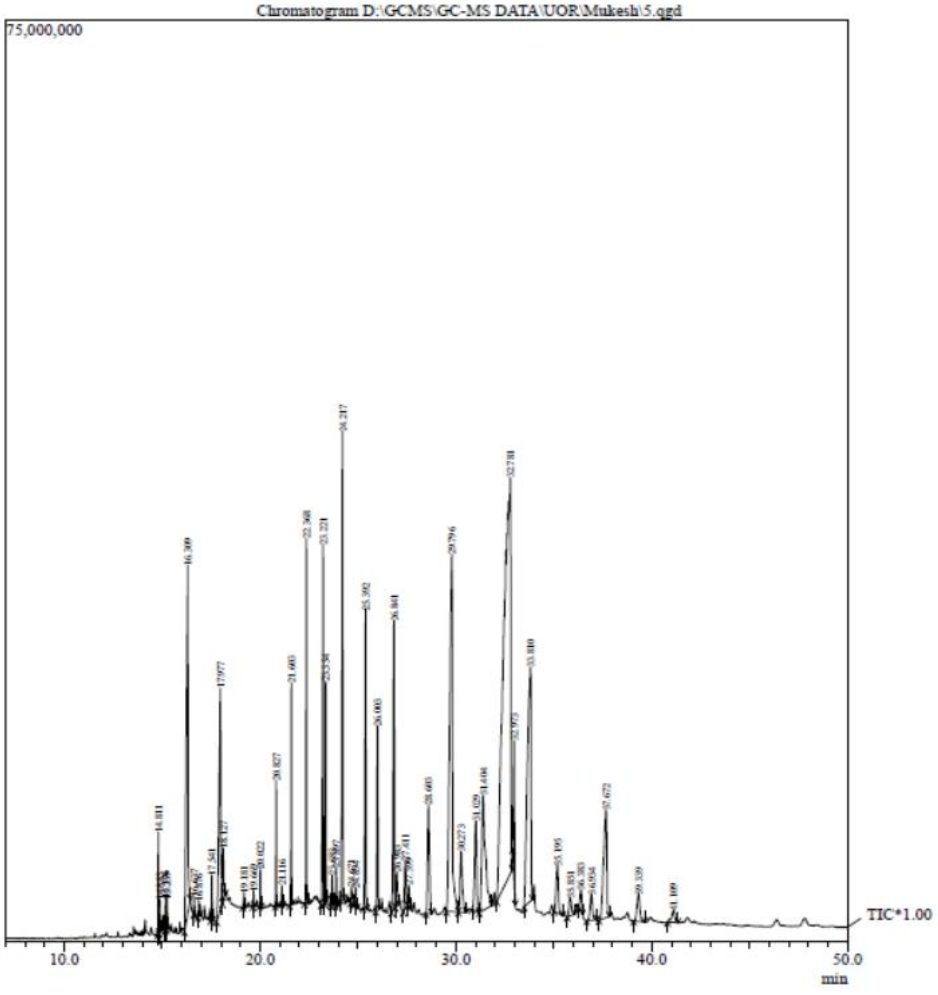

Conclusion

GCMS analysis of the extract of leaves of *Maytenus emarginata* reveals the presence of medicinally valued bioactive components like saponins, tannins, alkaloids, terpenoid, steroids and flavonoids. As the medicinal value of similar components in other plant extracts are already proved, no wonder if these components in *Maytenus emarginata* may also have equally effective. The work is in progress to ascertain its biological activity and brighten the pharmacological profile of it in the arena of traditional medicine.

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