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Qualitative and Quantitative Evaluation of Phytosterol from *Corchorus olitorius L.*

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Abstract

Corchorus olitorius is fibre yielding plant,used as vegetable for nutrition and medicine by human in rural area for health and beauty. In the present study, phytosterols from *Corchorus olitorius* was identified and quantified in vivo. Phytosterols were identified using chromatographic and spectral studies. In quantification maximum content was found in fruits (4.3mg/gdw) followed by shoots (0.35mg/gdw). Lanosterol, campesterol and stigmasterol were identified by IR and TLC.Fifty compounds were observed by GC MS analysis of sterol extracts from fruits of *Corchorus olitorius*. Maximum area was of Propanenitrile, 3[3(hexahydro2oxo1Hazepin1yl) propyl]amino] (4.71%) at retention time of 5.80 min.

Key words : *Phytosterol,TLC ,GC MS, Corchorus olitorius*

1. Introduction

Plants have played important role for well being of humans and animals. In ancient time, when modern drugs were not introduced, people used plants for the cure of many physical disorders through trial and error method. One third part of prescribed drugs used by pharmacists contains at least one active ingredient derived from plants [1].

Herbs have diverse medicinal roles that they play in the health of human and animals. The medicinal role have depends on the presence of chemical components present in the herbs [2].

C. olitorus belongs to the family, Tiliaceae and has been used in different parts of the world, as spice for food and treatment of chronic cystitis and dysuria [3]. The shoot tips and leaves are eaten and cooked by the people of Africa mostly in Nigeria, Ghana and Cameroon. In West Africa, their edible qualities are widely appreciated, where the shoots and leaves are used for making soups. The shoots and leaves contains high quantity of nutrients like Vitamin A, protein, calcium, iron, carotene and folic acid [4].

Naturally occurring compounds found in plants which resemble with cholesterol both in structure and biological function are sterols. These are structural components of the cell membrane. sterol regulate membrane fluidity and permeability as well as membrane associated metabolic processes[5].Recently beneficial effects of phytosterols related to cholesterol metabolism and atherosclerosis risk next to other metabolic processes in the human body have been reviewed [6,7]. Plant sterol played an important role as anticancer compounds have been also reported. [8]. Plant sterols are triterpenes in chemical nature, free phytosterols work to stabilize phospholipid bilayers in plant cell membranes like cholesterol does in animal cell membranes[9]. Plant sterols are already present in healthy diet, if people increasing the intake of phytosterols may be a practical way to reduce coronary heart disease with minimum risk[10]. Recent research show that intake of phytosterols of about 2 grams/day, it reduces approximately 9% LDL-cholesterol that 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 consumers and to promote a healthy heart diet[11].

2. Materials and Methods

2.1 Collection and Identification of Plant Materials

Fresh shoot and fruit of the selected plant *Corchorus olitorius L.*were collected from Jhunjhunu, India. The plant materials were taxonomically identified and authenticated by the Department of botany, University of Rajasthan (RUBL 211573) Jaipur. Whole plant were cleaned, shade dried and pulverized to powder in a mechanical grinder. The powdered materials were stored in air tight containers till use.

2.2. 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

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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 [12].

2.3 Thin layer chromatography (TLC)

Glass plates coated with silica gels G were used. Each of the extract was co-chromatographed separately with authentic sterols as marker. These plates were developed in an airtight chromatographic chamber, saturated with solvent mixture (Hexane: Acetone:: 8:2;)[13]. Other solvents such as benzene and ethyl acetate (85:15)[14] benzene: ethyl acetate (3:1)[13] 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 [15] 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, scraped from 50 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.

Identification

1. 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 those of authentic compounds studied.

3. Result and Discussion:

3.1 Qualitative estimation:

Three sterols were spotted which were common in plant parts on thin layer chromatography. The R_f values of the spots matched with authentic standards and were identified as stigmasterol, lanosterol and campesterol. Among the various solvent systems tested best results were obtained in the solvent system Hexane: Acetone (8:2) with R_f values viz., stigmasterol, 0.83; campesterol, 0.29 and lanosterol, 0.95. The characteristic colours were also developed when TLC plates were sprayed with anisaldehyde reagent (stigmasterol - Purple; campesterol - Gray; lanosterol - Gray) and with 50% sulphuric acid (stigmasterol- Gray; campesterol- Gray; lanosterol- Blue) 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 (stigmasterol-167-169^{o}C; campesterol-157-158^{o}C and lanosterol-143-144^{o}C). The characteristic peaks of IR spectra of isolates (β-sitosterol, stigmasterol, campesterol and lanosterol) were also found to be superimposable with the IR spectra of reference compounds. Sitosterol,stigmasterol, lanosterol, campesterol were reported by TLC shown in Table-1 .

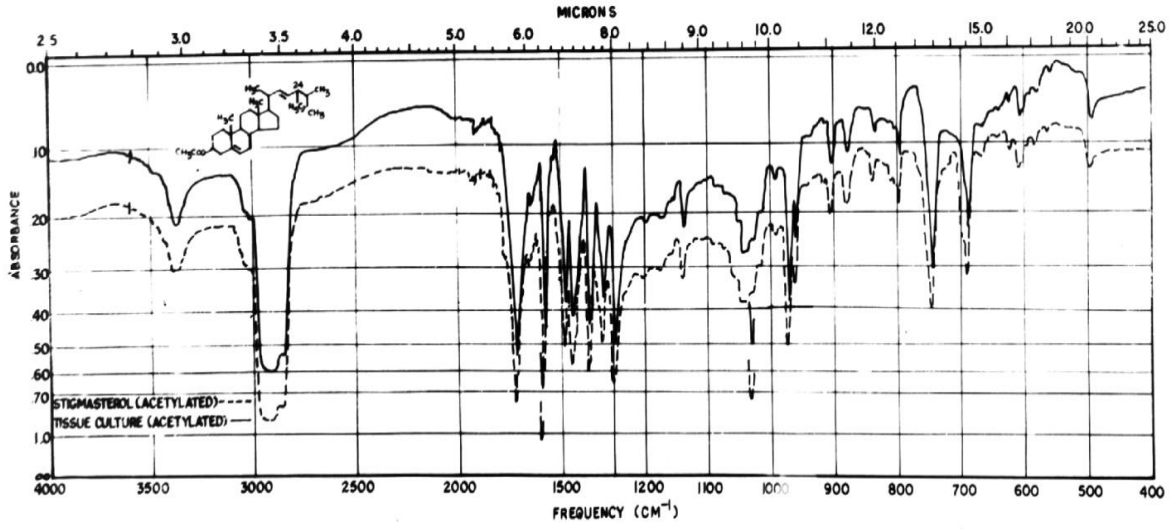

Fig 1. Infra-red Spectra of Isolated and Standard Stigmasterol

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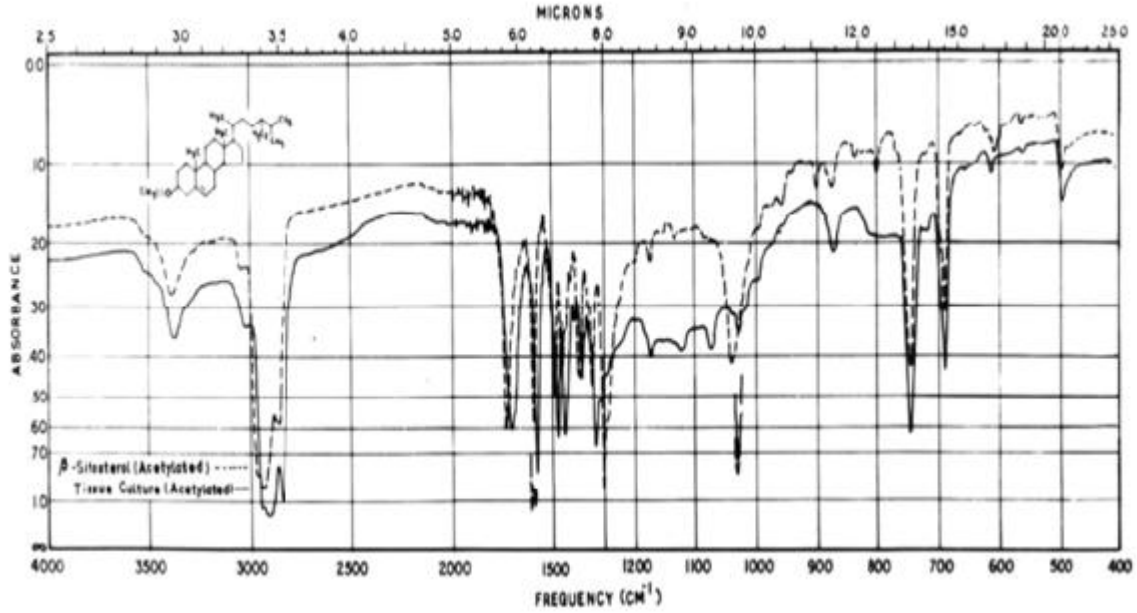

Fig-2 Infra-red Spectra of Isolated and Standard β-sitosterol

Table – 1 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	0.89	0.90	0.71	PU-BN	PK	136-137	3350 (O-H), 2830, 1665 (C=C), 1470, 1300, 1052 (C-O) and 880
Stigmasterol	0.83	0.64	0.65	GY	PU	167-169	3400 (O-H). 2950, 1750, 1640 (C=O), 1035 (C-O), 991, 957, 935, 810 and 715
Campesterol	0.29	0.23	0.21	GY	BL	157-158	3400 (O-H), 2950, 2850, 1640 (C=O), 1470, 1380, 1035, 880 and 820

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

It showed that *Corchorus* olitorius was found better source of sterols. Fifty compounds were observed by GC-MS analysis of sterol extracts from fruits of *Corchorus olitorius*. Maximum area was of Propanenitrile, 3[3(hexahydro2oxo1Hazepin1yl) propyl]amino] (4.71%) at retention time of 5.80 min. shown in Table:3 Phaytosterols are also effective when combined with cholesterol-lowering medication; adding phytosterols to statin medications can lower LDL more than doubling the statin dose . Apoptosis in various cancer cells was promoted by β-sitosterol and inhibits their growth[16].

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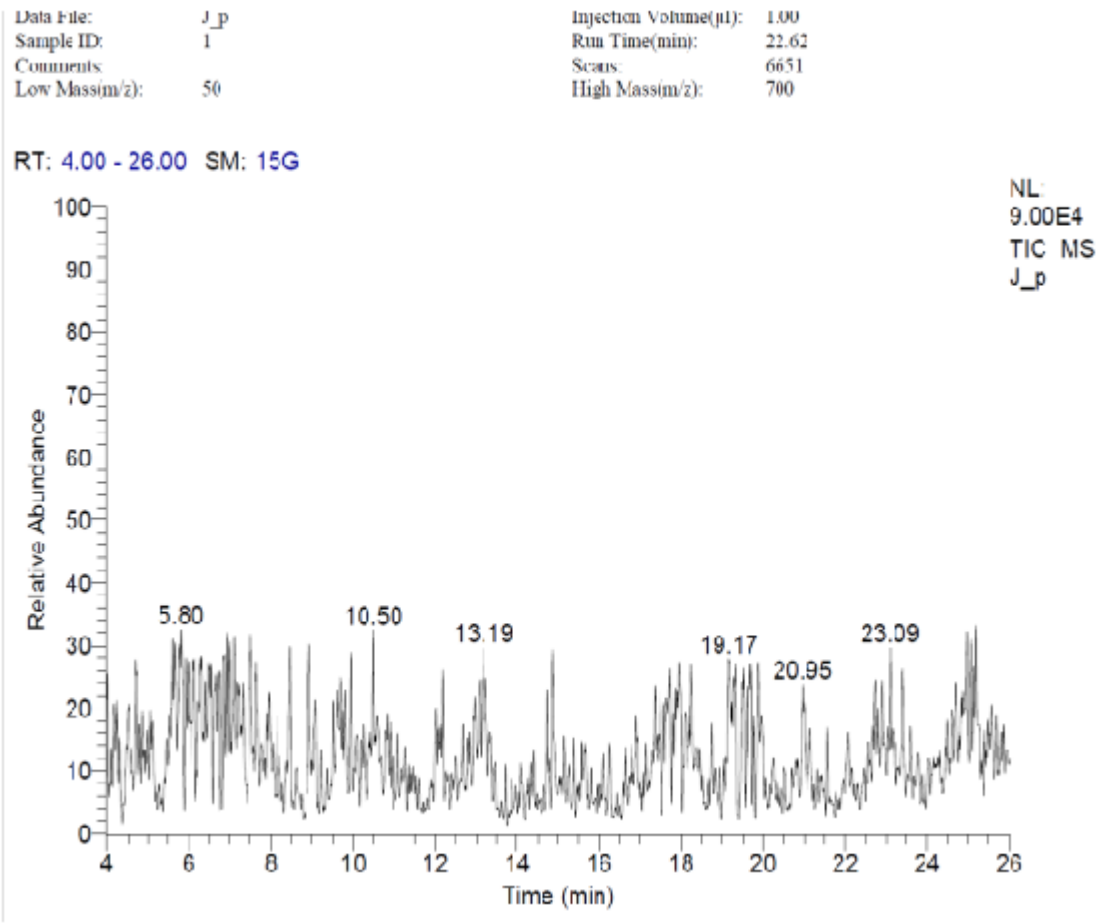

Fig 3. Gas Chromatography-Mass Spectrometry(GC-MS) Analysis

Table 2: GC MS analysis of Phytosterols from fruits of *Corchorus olitorius*

RT	Compound Name	Area	Area %
4.72	6Methyl2(4methylphenyl) 1Himidazo[2,1c][1,4]benzo xazine	43542	2.23
5.60	11bPhenyl1,2,3,5,6,11bhexahydro[1]benzothieno[3,2g]I ndolizine	15684	0.80
5.66	Methyl4,6dideoxy4(2hydroxypropionamido) 2Omethy làdmannopyranoside	33425	1.71
5.80	Propanenitrile, 3[3(hexahydro2oxo1Hazepin1yl) propyl]amino]	91899	4.71
5.92	1,2Bis(2,5diethoxyphenylthio) ethane	35036	1.80
5.96	2H1Benzopyran3(4H)one, 2phenyl, oxime	18009	0.92
6.14	Pyrrolo[3,4c] pyrrole1,4dione, 2,5dihydro3,6diphenyl	46558	2.39
6.31	Propanedioic acid, 2,2',2"(1,3,5triazine2,4,6(1H,3H,5H) trimethylidene) tris, hexaethyl ester	64505	3.31
6.40	1,4Naphthalenedione, 3,5dihydroxy2methyl	32622	1.67
6.47	Benzyl but2enoate	49685	2.55
6.53	Ethyl 2methyl1,3oxazolidine2propanoate	33320	1.71
6.60	2,4,6Trihydroxytoluene	42776	2.19
6.64	rans2,4,6Trimethylámethylánitrostyrene	36059	1.85
6.70	Cyanic acid, ethyl ester	44196	2.27

6.77	1,3,6,9bTetraazaphenalene4carbonitrile, 7,9dichloro2methyl54944	54944	2.82
6.88	Stannane, trimethyl[[(nonafluorobutyl)sulfinyl]oxy]	55684	2.86
6.94	2(5H)Thiophenone, 5methyl	31989	1.64
7.00	4Nonen3one, 1[3methoxy4[(trimethylsilyl)oxy]phenyl	28167	1.45
7.11	Iron,dicarbonyl[(4a,4b,9a,10,10aü)1,3,4,5,6,7,8,9octahydroben z[a]azulen4a(2H)yl](pentafluorophenyl)	19282	0.99
7.51	2Acetamido4phenyl5,6,7,8(4H)tetrahydro3,1benzothia zine	50078	2.57
7.64	9[5(Diethylamino)pentanamido]10,10-dimethyl9,10dihy dro10sila2azaanthracene	34793	1.79
8.47	4,2'Dihydroxy3,3' dinitrobiphenyl	50252	2.58
8.94	2Chloroquinoxaline	37984	1.95
9.09	3,4Dichlorobutane nitrile	35053	1.80
9.95	Sydnone, 3(2naphthyl	33734	1.73
10.50	3,5Nonadien7yn2ol, (E,E)	30082	1.54
12.19	1,5,7Octatrien3one, 2,6dimethyl, (E	23921	1.23
13.10	Chlorine	28789	1.48
13.19	Ketone, methyl 2methyl1cyclohexen1yl, semicarbazone	22198	1.14
14.75	Ethanimidic acid, 2cyclohexylideneNphenyl, methyl ester	22372	1.15
14.87	Rhenium, pentacarbonyliodo	42057	2.16
17.49	Methyl 3formyl1ferrocenecarboxylate	33873	1.74
17.74	Benzenepropanoic acid, à(aminooxy	56990	2.92
17.86	3,4Dihydro7methyl12hydroxymethylbenz[a] anthracenetrans3,4diol	23417	1.20
17.90	Benzo[f]cyclopenta[a]quinolizine6,7,7a,8,9,10(8H)hexaca rboxylic acid, 6,7dihydro4methyl, hexamethyl ester	27842	1.43
17.95	9(10H)Acridinone, 1hydroxy3methoxy10methyl	60790	3.12
18.26	Selenourea, N,Ndiethyl	20994	1.08
19.17	1,2Bis(diphenylamino)1,2bis(methylthio)ethylene	69468	3.56
19.32	Thieno[2,3b] pyridine, 5ethyl3nitro	85046	4.36
19.51	Methane, bromodifluoro	83037	4.26
19.60	Phosphoranamine, 1,1,1,1tetrafluoro, (TB511	25801	1.32
19.67	1,4,5,8Tetraazaphenanthrene	58896	3.02
19.72	Tricyclo[5.2.1.0(2,6)]dec3ene, 3phenyl	26615	1.37
19.78	Pentalane	21161	1.09
19.89	19Norpregna1,3,5(10),17(20)tetraene20carbonitrile, 3methoxy	52297	2.68
23.09	Ethanol, 2(phenylsulfonyl)	21334	1.09
23.41	Silane, trifluoro(2methyl2butenyl	21764	1.12
24.97	à(2,2Dicyanovinyl)(2,2)paracyclophane	24258	1.24
25.07	Benzaldehyde, 2nitroso	23713	1.22
25.18	9,9Diphenyl2methyl9sila9,10dihydro3azaantracen10 one	23124	1.19

3.2 Quantitative Estimation:

The present investigation quantify that maximum total sterol content was found in fruits (4.3mg/gdw) followed by shoots (0.35mg/gdw) showen in Table:3 It showed that *Corchorus* olitorius was found better source of sterols.

Table-3 Yield of sterols isolated (mg/gdw) from various plant parts of *Corchorus olitorius*

Plant parts	β-sitosterol	Stigmasterol	Lanosterol	Total (mg/g.dw)
Shoots	0.19	0.09	0.07	0.35
Fruits	2.02	1.36	0.92	4.3

4. Conclusion:

This investigation has given preliminary information to determine the chemical composition of sterols found in *Corchorus olitorius* using IR and GC-MS. The presence of these bioactive compounds in *Corchorus olitorius* exhibits its use by the human community. *Corchorus olitorius* is a important plant to isolate the specific compounds for the production of novel drugs.

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