Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2011-2012; 3(4); 102-106

ISSN: 0975-4873

Research Article

GC-MS Analysis of Phytochemicals in *Cyamopsis tetragonoloba* Fruit and *Cyperus rotundus* Rhizome

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ABSTRACT

Cyamopsis tetragonoloba is widely consumed by people and it is found to have varied medicinal values. *Cyperus rotundus* is also found to have different medicinal properties. *Cyamopsis tetragonoloba* fruit and *Cyperus rotundus* rhizome were therefore analyzed by GC-MS analysis to assess their phytochemical constituents. The ethanolic extract of *C. tetragonoloba* fruit showed the presence of thirty four phytochemical constituents. The major phytochemical compounds were mome inositol, ethyl alpha-d-glucopyranosidend stigmasterol. The ethanolic extract of *C. rotundus* showed the presence of twenty two phytochemical constituents. The major phytochemical compounds were 7-isopropenyl-1,4a-5,6,7,8-hexahydro-3H-naphthalen-2-one, zierone and (+)-cis-longipinan.

INTRODUCTION

Cyamopsis tetragonoloba L., (Leguminosae) is an annual legume and has been grown for centuries in India and Pakistan. This plant seems to possess antihyperglycemic and hypolipidemic activity.[1,2] It is an important leguminous herb, highly adapted to arid and semi-arid parts of the world requiring low inputs and care. The seeds are highly valued for industrial gum. It is cultivated mainly in rainy season as a rain fed crop in arid zones of India and various other parts of the world.[3] The endosperm of the legume *C. tetragonoloba* (guar) consists of an aleurone layer and a reserve polysaccharide layer (galactomannan). This reserve material is degraded during seed germination through the action of enzymes, which are released in the endosperm.[4] Guar also contains 13% dry matter (DM) crude saponin.[5] The endosperm of guar seed is a rich source of mucilage or gum, which forms a viscous gel in cold water, and is used as an emulsifier, thickener and stabilizer in a wide range of foods and industrial applications.[6] Guar is also used as a home remedy for the treatment of gastric disturbances and abdominal discomfort.[7] In recent years, guar gum–a galactomannan, prepared from *C. tetragonoloba* has been shown to be a potent hypocholesterolemic agent in both normal and hypercholesterolemic animals.[8] *Cyperus rotundus* L., (Cyperaceae) is a common perennial weed with slender and scaly creeping rhizomes.[9] It is also called as coco-grass or purple nut sedge or red nut sedge is a species of sedge native to Africa, southern and central Europe and southern Asia. It is a persistent and prolific weed occurring in the agricultural areas of tropics and subtropics.[10] The roots and rhizomes of this plant are used in different diseases like chronic diarrhea, inflammation, skin rashes and excess bleeding. It has also antiestrogenic, antimicrobial, anthelmintic, antihistaminic, antiemetic, antipyretic and antidiabetic activities.[11, 12, 13, 14] The tuber part of *C. rotundus* is one of the oldest known medicinal plants used for the treatment of dysmenorrheal and menstrual irregularities.[15] Furthermore, the 95% ethanol extract from the rhizome has been recently shown to have an inhibitory effect on human immuno-deficiency virus (HIV) and possess antifungal, antibacterial and immunomodulating properties.[16] The main active substances which have been identified in *C. rotundus* includes: alpha-cyperone, beta-selinene, cyperene, cyperotundone, patchoulenone, sugeonol, kobusone, and isokobusone that may scientifically explain its folk- and alternative-medicine uses.[17] Experimental reports have shown the potent antioxidant and free radical scavenging activity of the plant.[18] The phytochemical investigations of *C. rotundus* have revealed the presence of polyphenol, flavonol glycoside, saponin, vitamin C, sesquiterpenoids, essential oil and cardiac glycosides.[19,20] The objective of the present study is to identify the phytochemical constituents of ethanolic extract of *Cyamopsis tetragonoloba* fruit and *Cyperus rotundus* rhizome using GC/MS analysis[21].

MATERIALS AND METHODS

Collection of plant material:The rhizome of *Cyperus rotundus* and fruits *of Cyamopsis tetragonoloba* were collected from medicinal vendor, Chennai. The selected plant materials were identified and authenticated as PRAC/2010/495 and PRAC/2010/494 respectively by Prof. P. Jayaraman, Plant Anatomy Research Center, West Tambaram, Chennai, Tamil Nadu, India. Preparation of powder and extract: C. rotundus rhizome and C. tetragonoloba fruit were shade-dried and

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Table 1:Phytochemicals identified in ethanolic extracts of Cyamopsis tetragonoloba fruit by GC-MS

Peak #	R. Time	Name of Compound	Molecular Formula	Molecular Weight	Peak Area %
1	3.317	1,2-Cyclopentanedione	$C_5H_6O_2$	98	0.33
2	6.433	Isopentyl acetate	$C_7H_{14}O_2$	130	0.86
3	7.167	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	$C_6H_8O_4$	144	0.65
4	8.419	2,3-Dihydro-benzofuran	C_8H_8O	120	0.46
5	8.79	Acetyl monoglyceride	$C_5H_{10}O_4$	134	0.25
6	9.402	1-(p-methoxyphenyl)propene	$C_{10}H_{12}O$	148	0.35
7	14.001	Ethyl alpha-d-glycopyranoside	$C_8H_{16}O_6$	208	8.88
8	15.515	Mome inositol	$C_7H_{14}O_6$	194	69.54
9	16.092	N-(2-heptynyl)-n-hexylamine	$C_{13}H_{25}N$	195	0.23
10	17.343	Palmitic acid	$C_{16}H_{32}O_2$	256	1.5
11	17.603	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	284	1.56
12	18.128	Hexopyranosyl hexopyranoside	$C_{12}H_{22}O_{11}$	342	0.52
13	18.712	Phytol	$C_{20}H_{40}O$	296	0.52
14	19.176	Ethyl (9Z,12Z)-9,12-octadecadienoate	$C_{20}H_{36}O_2$	308	0.33
15	19.233	Ethyl (9Z)-9-octadecenoate	$C_{20}H_{38}O_2$	310	0.67
16	19.457	Ethyl n-octadecanoate	$C_{20}H_{40}O_2$	312	0.49
17	22.189	2-Hexadecanoyl glycerol	$C_{19}H_{38}O_4$	330	0.74
18	22.317	Mono(2-ethylhexyl) phthalate	$C_{16}H_{22}O_4$	278	0.21
19	22.742	Ethyl nonadecanoate	$C_{21}H_{42}O_2$	326	0.18
20	22.923	Aletamine	$C_{11}H_{15}N$	161	0.17
21	23.57	Propyleneglycol monoleate	$C_{21}H_{40}O_3$	340	1.31
22	23.752	alpha-Monostearin	$C_{21}H_{42}O_4$	358	0.21
23	24.205	Ethyl docosanoate	$C_{24}H_{48}O_2$	368	0.12
24	24.595	3-(2-Hydroxy-3,4-dimethoxyphenyl)-7-chromanol	$C_{17}H_{18}O_5$	302	1.53
25	24.898	Nonacosane	$C_{29}H_{60}$	408	0.16
26	24.959	4,5-Bromoacetylbenzocyclobutene	$C_{10}H_9BrO$	224	0.12
27	26.02	beta-Tocopherol	$C_{28}H_{48}O_2$	416	0.11
28	26.22	Tetracontane	$C_{44}H_{90}$	618	0.17
29	26.56	dl-alpha-Tocopherol	$C_{29}H_{50}O_2$	430	0.34
30	27.473	Ergost-5-en-3-ol	$C_{28}H_{48}O$	400	0.24
31	27.69	Stigmasterol	$C_{29}H_{48}O$	412	4.47
32	28.264	gamma-Sitosterol	$C_{29}H_{50}O$	414	0.82
33	28.788	Alpha-amyrin	$C_{30}H_{50}O$	426	0.97
34	29.305	Lupeol	$C_{30}H_{50}O$	426	0.98

pulverized to fine powder in a mechanical grinder. The powder (100g) was extracted with 1 liter of ethanol as solvent. The extracts were concentrated under reduced pressure in a rotary evaporator. The ethanolic extracts were used for GC-MS analysis.

Gas Chromatography-Mass Spectrometry (GC–MS) Analysis: The GC-MS analysis of selected samples was performed with Shimadzu GC-MS - QP2010. The inert gas helium (99.9995%) was used as carrier gas, at flow rate of 1.5 ml/min, Split ratio 10:1; sample size, 1µL injected using the split less injection technique; fused capillary silica column HP-5 (30m × 0.25mm × 0.25µm). Temperatures: injector: 260˚C, detector: 300˚C, column: 70˚C, 10˚C min−1, 260˚C (10 min). The total GC running time was 35 min. The MS was taken at 70 eV. The MS scan parameters included a mass range of m/z 40-1000, a scan interval of 0.5 s, a scan speed of 2000 amu s−1, and a detector voltage of 1.0 kV. Identification of compounds was conducted using the database of NIST08, WILEY8 and FAME Libraries. Mass spectrum of individual unknown compound was compared with the known compounds stored in the software database Libraries. The

Table 2:*Phytochemicals identified in ethanolic extracts of Cyperus rotundus rhizome by GC-MS*

Peak #	R. Time	Name of Compound	Molecular Formula	Molecular Weight	Area %
1	11.034	(-)-Alpha-gurjunen	$C_{15}H_{24}$	204	1.92
2	11.818	Campholaldehyde	$C_{10}H_{16}O$	152	1.2
3	12.167	beta-Eudesmene	$C_{15}H_{24}$	204	2.76
4	13.858	Zierone	$C_{15}H_{22}O$	218	2.33
5	13.926	1-Chlor-2,2,6-trimethyl-bicyclo(4.3.0)nonan-7-one	$C_{12}H_{19}ClO$	214	0.87
6	14.225	Longifolenaldehyde	$C_{15}H_{24}O$	220	3.95
7	14.4	5-Amino-2,2-dimethyl-1-[(1-methylethylidene)amino]-1,2-dihydro-3H-pyrrole-3,3,4-tricarbonitrile	$C_{12}H_{14}N_6$	242	1.53
8	14.451	Longiverbenone	$C_{15}H_{22}O$	218	3.14
9	14.562	Longifolene-(V4)	$C_{15}H_{24}$	204	4.38
10	14.753	Zierone	$C_{15}H_{22}O$	218	15.4
11	14.918	(E)-Carveol	$C_{10}H_{16}O$	152	1.01
12	15.042	2,4a,5,8a-Tetramethyl-1,2,3,4,4a,7,8,8a-octahydro-1-naphthalenyl acetate	$C_{16}H_{26}O_2$	250	5.59
13	15.26	7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one	$C_{15}H_{22}O$	218	23.71
14	15.415	Beta-neoclovene	$C_{15}H_{24}$	204	1.36
15	15.475	(3,8,8-Trimethyl-1,2,3,4,5,6,7,8-octahydro-2-naphthalenyl)methyl acetate	$C_{16}H_{26}O_2$	250	3.51
16	15.524	2,2,6-Trimethyl-1-[(1E)-3-methyl-1,3-butadienyl]-5-methylene-7-oxabicyclo[4.1.0]heptane	$C_{15}H_{22}O$	218	2.08
17	15.856	Acetic acid, 3-(2,2-dimethyl-6-methylene-cyclohexylidene)-1-methyl-butyl ester	$C_{16}H_{26}O_2$	250	1.54
18	16.035	1,1,4,7-tetramethyldecahydro-1H-cyclopropa[E]azulen-4-ol	$C_{15}H_{26}O$	222	3.29
19	16.18	Menthol, 1'-(butyn-3-one-1-yl)-, (1S,2S,5R)-	$C_{14}H_{22}O_2$	222	4.33
20	16.839	(+)-Cis-longipinan	$C_{15}H_{26}$	206	11.64
21	19.833	2-Isopropenyl-4,4,6b-trimethyl-4,5,5a,6,6a,6b-hexahydro-2H-cyclopropa[g][1]benzofuran	$C_{15}H_{22}O$	218	2.21
22	19.878	Digitoxigenin-4-en	$C_{23}H_{32}O_4$	372	2.27

name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

GC-MS chromatogram of the ethanolic extract of *C. tetragonoloba* fruit (Figure 1) showed 34 peaks indicating presence of thirty four phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST08, WILEY8 and FAME libraries the thirty four phytoconstituents were characterized and identified (Table 1). The major phytochemical constituent's mass spectra are mome inositol, ethyl alpha-d-glucopyranoside and stigmasterol. The ethanolic extract of *C. rotundus* rhizome (Figure 2) showed 22 peaks indicating the presence of twenty two phytochemical constituents. On comparison of the mass spectra, twenty two phytoconstituents were characterized and identified (Table 2). The major phytochemical constituent's mass spectra are 7-isopropenyl-1,4a-5,6,7,8-hexahydro-3H-naphthalen-2-one, zierone and (+)-cis-longipinan.

CONCLUSION

Some of the compounds present in both the plant extracts would contribute to its antioxidant potential. Lupeol present in *C. tetragonoloba* seem to possess anti-prophylactic effect. Further studies are undertaken to

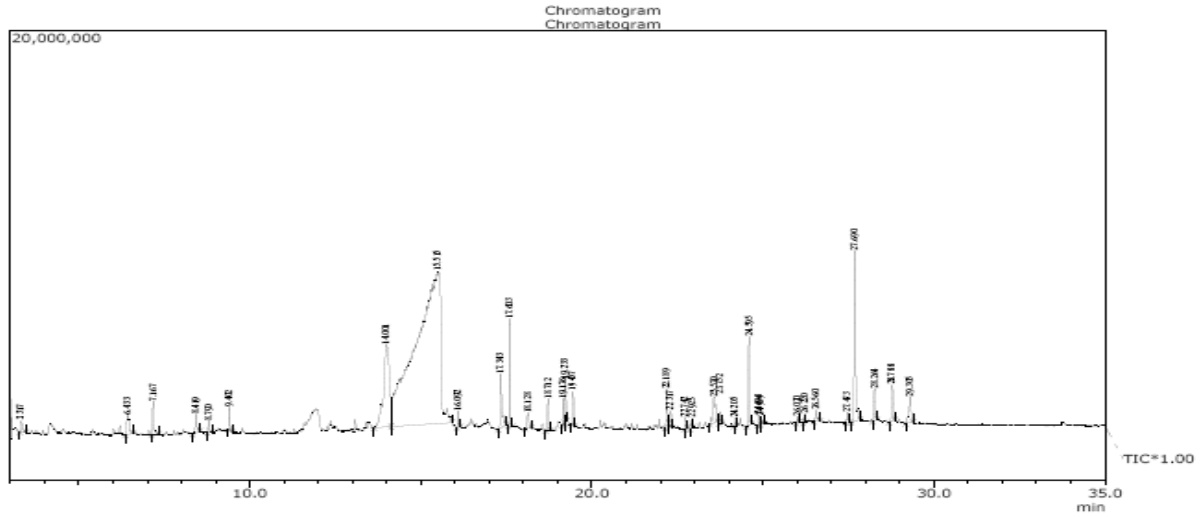

Figure 1:*GC-MS Chromatogram of ethanolic extract of Cyamopsis tetragonoloba fruit*

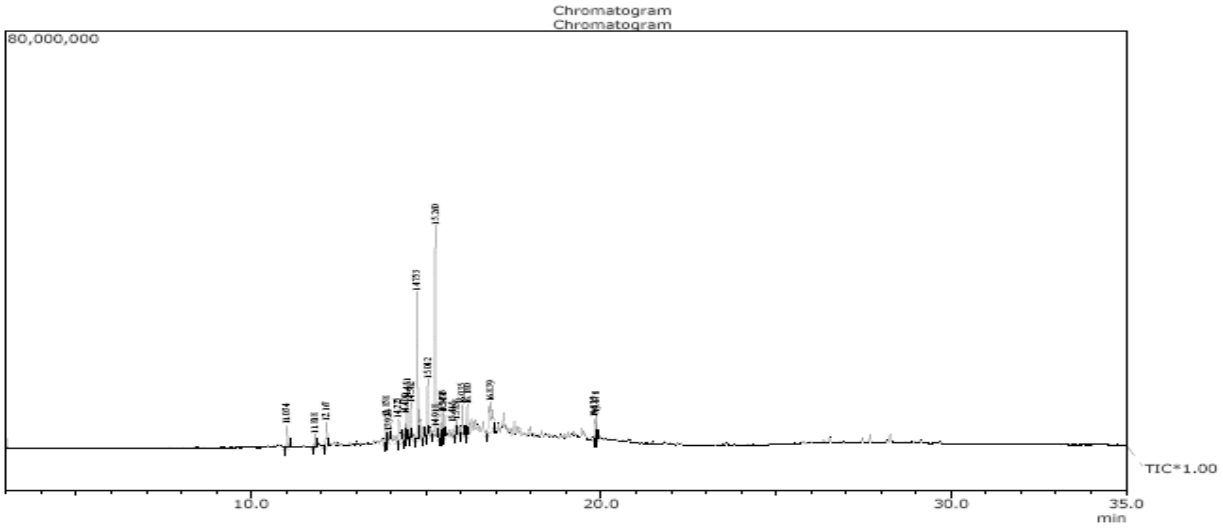

Figure 2:*GC-MS Chromatogram of ethanolic extract of Cyperus rotundus rhizome*

establish the antidiabetic effect of both extracts using animal model.

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