Research Article

GCMS Analysis of Bioactive Constituents from *Cycas circinalis.L* and *Ionidium suffruticosum.Ging*

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ABSTRACT

The aim of the study was to investigate phytochemicals and GCMS analysis of ethanolic extract of *Cycas circinalis* and *Ionidium suffruticosum*. The bioactive constituents were traced out in *C.circinalis* and *I.suffruticosum* by phytochemical screening (qualitative methods) and the GCMS method. *C.circinalis* showed the presence of terpenoids, flavonoids, cardiac glycosides, Phytosterols, amino acids, and alkaloids. In GCMS analysis *C.circinalis* showed 16 bioactive components with high concentration of α-D-Glucopyranoside, α -D-glucopyranosyl (77.73%), Decanoic acid, ethyl ester (11.92%) whereas *I.suffruticosum* showed the presence of tanins, flavonoids, terpenoids, saponin, cardiac glycosides, amino acids and alkaloids. In GCMS analysis *I.suffruticosum* showed 18 bioactive components with high concentration of 3-Trifluoroacetoxydodecane (16.76%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (15.32%), Decanoic acid, ethyl ester (14.98%), E-2-Tetradecen-1-ol (10.46%), Spiro[2.2]pentane-1-carboxylic acid, 2-cyclopropyl-2-methyl (5.01%), 2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro (8.31%), Squalene (6.24%). The phytochemical constituent's common in both *C.circinalis* and *I.suffruticosum* was Decanoic acid, ethyl ester and 3-Trifluoroacetoxydodecane. The presence of some of these bioactive constituents in the plant extract may provide the scientific evidences for many medicinal effects of these plants.

Keywords: 3-Trifluoroacetoxydodecane, *Cycas circinalis*, Cardiac glycosides, Decanoic acid ethyl ester, GCMS, *Ionidium suffruticosum,* Phytosterols, Terpenoids.

INTRODUCTION

The medicinal herbs were gaining much importance in recent years due to wide applications of its bioactive molecules. Different strategies have been developed for the selection of particular herbs for the study. The herbs selected were screened for the active phytoconstituents. The specific compound present in the herbs were active subjected to isolation with different analytical techniques. The analogues of isolated molecules are characterized and structural modification has been done to enhance the desired activity and minimize the unwanted side effects.[1] Herbal plants synthesize lower molecular weight organic compounds called secondary metabolites which possess various biological activities.[2]

Cycas circinalis .L (Family –Cycadaceae), a sago palm commonly known as Madana Kaman in Tamilnadu. The genus is native of eastern and southeastern asia and is cultivtated in many tropical and subtropical areas for ornamental purpose.[3] The male sago cone has aphrodisiac activity.[4] *Ionidium suffruticosum. Ging* (Family–Violaceae) a perennial herb known as Orithazthamarai. Ionidium leaves are alternate, flowers purple, sepals 5, petals 5, anthers connate or free, style clavat and incurved, stigma oblique, capsule elastically 3 valved, seeds globose.[5] The whole plant has aphrodisiac activity and it is used as rejuvenating herb in Siddha system of Medicine.[4]

MATERIALS AND METHODS

50 grams of powder of male cone of *C.circinalis* and whole plant of *I.suffruticosum* were successively extracted with 500ml of absolute ethanol solvents by hot continuous percolation method in Soxhlet apparatus for 6 hrs separately. The extracts were concentrated by using hot water bath and subjected to drying in a hot air oven and stored at 4°C for further use.[6]

Phytochemical analysis

The ethanolic extracts of *C.circinalis* and *I.suffruticosum* were subjected to standard chemical tests to determine the presence (Qualitatively) or absence of alkaloids, steroids, glycosides, Saponins, flavonoids, carbohydrates, proteins and amino acids, fats, oils and phenols.

Gas Chromatography Mass Spectroscopy Analysis[1]

The ethanolic extract of both herbs were analyzed through GCMS for the identification of different compounds.

Instruments and Chromatographic conditions

GC Programme

Column: Elite-5MS (5% Diphenyl /

95% Dimethyl poly siloxane), 30 x 0.25mm x 0.25m df.

Equipment: GC Clarus 500 Perkin Elmer

Carrier gas: 1ml per min, Split: 10:1

Detector: Mass detector Turbo mass gold-Perkin Elmer

Software: Turbomass 5.2

Sample injected: 2l

Oven temperature Programme - 110° C -2 min hold

Up to 200° C at the rate of 10° C/min-No hold

Up to 280° C at the rate of 5° C / min-9 min hold

Injector temperature 250° C

Total GC running time 36 min

MS Programme

Library used NIST Version-Year 2005

Inlet line temperature 200° C

Source temperature 200° C

Electron energy: 70 eV

Mass scan (m/z): 45-450

Solvent Delay: 0-2 min

Total MS running time: 36 min

The extract was dissolve in ethanol and filtered with Elite-5MScolumn and analyzed in GCMS for different constituents. The phytoconstituents obtained as a result was interpreted on Mass – Spectrum GCMC using NIST (2005) having more than 62,000 patterns.[1]

RESULTS

C.circinalis showed the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, phytosterols, cardiac glycosides and amino acids. *I.suffruticosum* showed the presence of alkaloids, flavonoids, saponins, tannin, cardiac glycosides, amino acids and terpenoids (Table 1).

Table 1: Phytochemical screening of *Ionidium Suffruticosum* and *Cycas circinalis*

Parameters	*C.circinalis*	*I.suffruticosum*
Phlobatannins	-	-
Terpenoids	+	+
Flavonoid	+	+
Tannin	-	+
Saponins	-	+
Cardiac glycosides	+	+
Phytosterols	+	-
Amino acids	+	+
Alkaloids	+	+

+: Present, - : Absent

The active constituents with their retention time (RT), molecular weight (MW) Molecular formula (MF), concentration (peak area %) were presented in Table 2 & 3. GCMS analysis of *C.circinalis* showed 16 bioactive compounds with high concentration of α-D-Glucopyranoside, α -D-glucopyranosyl (Trehalose) having retention time (RT) 11.10 and peak area 77.73 %Decanoic acid, ethyl ester (RT 12.72 & 11.92 %) followed by some constituents 3-Trifluoroacetoxydodecane (RT 14.78 & 1.40%) Vitamin d3 (RT 9.51 & 1.04 %) and the rest of the compounds showing peak area ranging from 1.99% to 0.17%, (Table 2). GCMS chromatogram of *C.circinalis* was shown in Figure 1.

Table 2: Bioactive Constituents of *Cycas circinalis* Extract by GCMS Analysis

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	7.29	1-(5-Bicyclo[2.2.1]heptyl)ethylamine	$C_9H_{17}N$	139	1.90
2	7.56	1-Adamantanemethylamine, α -methyl-	$C_{12}H_{21}N$	179	1.99
3	9.01	3,4-Anhydro-d-galactosan	$C_6H_8O_4$	144	1.06
4	9.51	Vitamin d3	$C_{27}H_{44}O$	384	1.04
5	11.10	α -D-Glucopyranoside, α -D-glucopyranosyl	$C_{12}H_{22}O_{11}$	342	77.73
6	12.72	Decanoic acid, ethyl ester	$C_{12}H_{24}O_2$	200	11.92
7	14.78	3-Trifluoroacetoxydodecane	$C_{14}H_{25}F_3O_2$	282	1.40
8	15.24	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	$C_6H_7N_3O_2$	153	0.33
9	19.17	2,6,6-Trimethyl-bicyclo[3.1.1]hept-3-ylamine	$C_{10}H_{19}N$	153	0.17
10	20.58	1-Methyldecylamine	$C_{11}H_{25}N$	171	0.24
11	21.96	2-Aminononadecane	$C_{19}H_{41}N$	283	0.28
12	23.34	1,4-Dioxaspiro[4.5]decane, 8-(methylthio)-	$C_9H_{16}O_2S$	188	0.31
13	24.71	8,9,9,10,10,11-Hexafluoro-4,4-dimethyl-3,5 -dioxatetracyclo[5.4.1.0(2,6).0(8,11)]dodecane	$C_{12}H_{12}F_6O_2$	302	0.32
14	25.57	Spiro[2.2]pentane-1-carboxylic acid, 2-cyclopropyl-2- methyl-	$C_{10}H_{14}O_2$	166	0.19
15	27.62	Cyclopenta[c]furo[3',2':4,5]furo[2,3-h][1]benzopyran- 11(1H)-one, 2,3,6a,9a-tetrahydro-1,3-dihydroxy-4- methoxy-	$C_{17}H_{14}O_7$	330	0.54
16	30.78	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	$C_{13}H_{20}O_2$	208	0.58

Table 3: Bioactive Constituents of *Ionidium suffruticosum* Extract by GCMS Analysis

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	9.84	Tetrahydro-4H-pyran-4-ol	$C_5H_{10}O_2$	102	3.75
2	10.95	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	15.32
3	11.20	Z-1,9-Hexadecadiene	$C_{16}H_{30}$	222	7.31
4	11.40	E-2-Tetradecen-1-ol	$C_{14}H_{28}O$	212	10.46
5	12.71	Decanoic acid, ethyl ester	$C_{12}H_{24}O_2$	200	14.98
6	14.80	3-Trifluoroacetoxydodecane	$C_{14}H_{25}F_3O_2$	282	16.76
7	15.14	Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester	$C_{20}H_{40}O_2$	312	1.78
8	17.62	2-Cyclopentene-1-undecanoic acid, (+)-	$C_{16}H_{28}O_2$	252	1.09
9	20.57	1-Methyldecylamine	$C_{11}H_{25}N$	171	1.06
10	21.94	2-Aminononadecane	$C_{19}H_{41}N$	283	0.98
11	23.50	Squalene	$C_{30}H_{50}$	410	6.24
12	24.19	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	$C_{24}H_{45}N_2O_3$	409	1.35
13	25.32	Spiro[2.2]pentane-1-carboxylic acid, 2-cyclopropyl-2-methyl-	$C_{10}H_{14}O_2$	166	5.01
14	26.87	5 α -Androstan-16-one, cyclic ethylene mercaptole	$C_{21}H_{34}S_2$	350	1.74
15	27.07	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	$C_{22}H_{40}O_2$	336	8.13
16	27.80	Bisnorallocholanic acid	$C_{22}H_{36}O_2$	332	0.67
17	31.19	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	$C_{13}H_{20}O_2$	208	1.56
18	32.17	Benzeneethanamine, α -methyl-3-[4-methylphenyloxy]-	$C_{16}H_{19}NO$	241	1.79

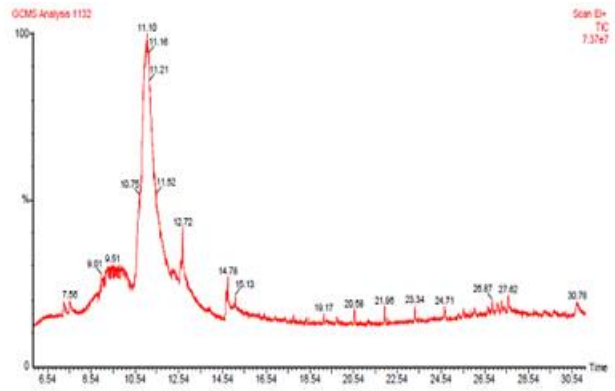

Figure 1: GCMS Chromatogram of Ethanolic Extract of *Cycas circinalis*

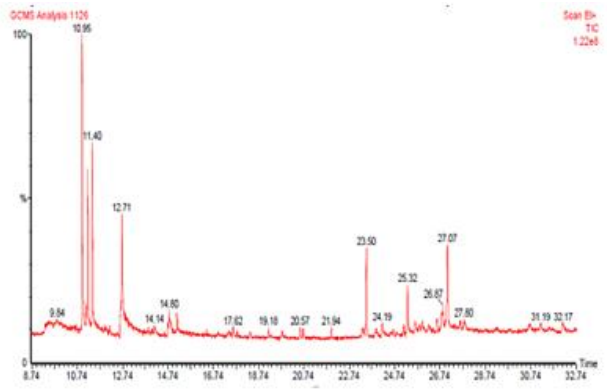

Figure 2: GCMS Chromatogram of Ethanolic extract of *Ionidium Suffruticosum*

I.suffruticosum showed 18 bioactive compounds with high concentration of 3-Trifluoroacetoxydodecane, having retention time 14.80 and peak area 16.76 % 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, (RT 10.95 & 15.32 %) Decanoic acid, ethyl ester having (RT 12.71&14.98 %) E-2-Tetradecen-1-ol (RT 11.40 and peak area 10.46 %. Followed by four constituents such as 2H-Pyran, 2-(7-heptadecynyloxy) tetrahydro (8.13%), Squalene (6.24%). Spiro [2.2] pentane-1-carboxylic acid, 2-cyclopropyl-2-methyl (5.01 %), Tetrahydro-4H-pyran-4-ol (3.75%) showed moderate concentration. Nine constituents were least ranging with a peak area from 1.79% to 0.67% (Table 3). GCMS chromatogram of *I.suffruticosum* was shown in figure 2.

DISCUSSION

Abeer moawad et al., had done phytochemical investigation on leaflets of *C.circinalis* and found out new bioflavonoid and in the present study the male cone of *C.circinalis* showed the presence of flavonoids along with terpenoids, glycosides, Phytosterols, amino acids and alkaloids.[3] Ramamoorthy et al., carried out phytochemical investigation on *I.suffruticosum* showed the presence of alkaloids, glycosides, carbohydrates, steroids, flavonoids, proteins and Coumarins, which goes in hand with the present study showing all phytochemicals and also showed presence of 3 more components which includes terpenoids, tannin and saponins.[5]

Trehalose a disaccharide (α -D-Glucopyranoside, α -D-glucopyranosyl) compound in *C.circinalis* have effects on

osteoporosis in a recent research done by Takanobu Higashiyama on the physiological function of trehalose on bone resorption in ovariectomized mice, the trabeculae was significantly decreased when compared to control. In contrast, ovariectomized mice having trehalose did not show any sign of decrease of the trabeculae.[7] These results suggest that trehalose might have a kind of suppressive effect on the development of osteoporosis. These results further imply that the daily ingestion of trehalose containing foods could be useful both for prevention of osteoporosis.[8] It has been shown that trehalose could protect corneal epithelial cells in culture from death by desiccation and suppress tissue denaturalization.[9, 10]

Vitamin d3 is an active compound found in *C. circinalis.* Various researchers have proved the effect of Vitamin D in male reproductive system. In the genital tract of male rodents, Vitamin D receptor (VDR) has been found in the smooth muscles of the epididymis, spermatogonia, and Sertoli cells, indicating a role of vitamin D in spermatogenesis and sperm maturation in rats.[11,12] VDR was detected in human testicular tissue homogenates using titrated vitamin D.[13] VDR was detected in human sperm, with binding sites in the nucleus and the midpiece of the sperm.[14] VDR and vitamin D metabolizing enzymes were expressed in spermatids, vesicles within the caput epididymis, glandular epithelium of cauda epididymis, seminal vesicle, and prostate.[15] Among the other identified phytoconstituents, hexadecanoic acid ethyl ester, have the property of antioxidant activity.[16] 3-Trifluoroacetoxydodecane has antimicrobial and anticancer activity.[16]

I.suffruticosum showed the presence of squalene. Squalene has the property of antioxidant activity. Squalene possesses chemo preventive activity against colon carcinogenesis. Antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant, chemo preventive, lipoxygenase-inhibitor, pesticide. Squalene is a hydrocarbon and triterpene.[16] It is a natural and vital part of the synthesis of cholesterol, steroid hormones, and vitamin D in the human body.[18] 3,7,11,15-Tetramethyl-2- hexadecen-1-ol is a Terpene alcohol possessing antimicrobial, anti-inflammatory activity.[16] 1-(5-Bicyclo[2.2.1]heptyl)ethylamine has antibacterial effect.[19] E-2-Tetradecen-1-ol has antimicrobial activity.[20] 2H-Pyran, 2-(7-heptadecynyloxy) tetrahydro is a phytoconstituents of Andrographis paniculata which has antimicrobial activity.[21]

CONCLUSION

GC-MS analysis is the initial step for understanding the nature of active principles in the herbs followed by the isolation of individual phytochemical constituent and finding out its biological activity. To conclude the present study, *C.circinalis and I.suffruticosum* possess various bioactive compounds with pharmaceutical importance. The present research is still under process for its bioactivity and toxicity profile. Isolation of various compounds followed by its toxicological aspects and clinical trials to come out with safer natural bioactive compounds for the treatment of diseases.

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