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COMPARATIVE MORPHOLOGICAL AND PHYTOCHEMICAL ANALYSES OF THREE VARIANTS OF *CISSUS QUADRANGULARIS* IN TAMIL NADU

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ABSTRACT

To study Morpho-parameters and the Phyto-constituents of Cissus quadrangularis (L.). variant I, II and III to provide its chemical constituents and find out the morphological variations between the three variants available in Tamil Nadu. The dried and powered stem materials (20 g) were extracted successively with 200 mL of methanol. Phytochemical analyses were carried out by using GC-MS and FT-IR. GC-MS identify the chemical compounds and FT-IR used to identify the functional group of Compounds. Based on GC-MS methanolic stem extract of C. quadrangularis three variants showed similar major compounds were identified. Such as 3- o- methyl- d- glucose, D-Allose, Phytol and 9, 12, 15 – octadecatrienoic acid. The FT-IR analysis showed similar functional group of compounds. The morphological character of C. quadrangularis showed various type of stems variation. Based on the morphological characters of C. quadrangularis stem shape differs in the three variants studied. Based on the GC-MS and FT-IR analyses similar phyto- constituent related to functional groups were present in all the three variants studied. The all the three variants of C. quadrangularis morphologically different in nature but the phytochemical study showed no major differents due to environmental adaptation of these plants were different from morphological character.

KEY WORDS

C. quadrangularis, FT-IR, GC-MS, Morphology, Phyto-constituents

INTRODUCTION

The world is rich with natural and unique medicinal and aromatic plants. Medicinal plants are now getting more devotion than ever because they have potential of numerous benefits to society or indeed to all mankind, especially in the line of medicine and pharmacological. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body [1]. The medicinal plants are valuable for curing of human diseases because of the presence of phytochemical constituents [2]. Phytochemicals are constituents commonly existing in plants. They give to the dye (colour), flavour and smell of plants. In addition, they form part of a plant's natural protection mechanism against diseases. Their medicinal values to human health and disease prevention have been reported [3]. Intraspecific variation of chemical compounds is common in many plant species and often shows clear geographical patterns that may reflect environmental differences within the range of a species. Hence, chemical races are often defined and provide taxonomists with a powerful tool [4]. The genus *Cissus* with 350 species is distributed in the world. In India, the genus is signified by approximately 13 species [5] and in Tamil Nadu state by approximately 11 species were occurred [6].*Cissus quadrangularis* Linn. belongs to the family Vitaceae is a climber with stout fleshy quadrangular (four angled) stem found throughout the hotter parts of India [7]. Phytochemical studies of *C. quadrangularis* variant found numerous phytochemical

constituents such as ascorbic acid, carotene, anabolic steroidal substances, calcium, beta-sitosterol, alpha-amyrin, alpha- amyrone, flavonoids, triterpenoids [8] and various secondary metabolites. Based on the stem Morphology the plant *C. quadrangularis* divided into three variants such as square-stemmed, round-stemmed and flat-stemmed are available. They are differentiated as variant I, II and III respectively [9]. *C. quadrangularis* variant I so called wild plant compare with other two variants. Pharmacological activities of *C. quadrangularis* have been reported in earlier [10-11]. In the present study to investigate the morphology and phytochemical variations of *C. quadrangularis* of three variants.

MATERIALS AND METHODS

Collection and preparation of plant material

The fresh stem plant material of *C. quadrangularis* variant I, II and III were collected from Chidambaram and Cuddalore. The herbarium specimen of the same were prepared and deposited in the Department of Botany, Annamalai University. The plants were washed completely in running tap water to remove soil particles and the plant parts were separated and shade dried. The shade dried plant parts were stored in air tight container for further analysis.

Morphological Characters of *C. quadrangularis*

Climbing herb, tendrils simple, opposite to the leaves, leaves simple or lobbed, sometimes 3 - foliate, dentate. Flowers bisexual, tetramerous, in umbellate cymes, opposite to the leaves, Calyx cup- shaped, obscurely 4-lobed. Fruit globose or obovoid fleshy berries, one seeded dark purple to black, seeds ellipsoid or pyriform. Were Observed morphological variations of *C. quadrangularis* Variant I, II and III.

Plant sample extraction

Phytochemical analyses were carried out by using Maceration methods [12]. Shade dried and powdered plant materials were successively extracted with methanol with gentle stirring for 72 hours. The extraction were passed through Whatmann No. 1 paper and collected.

Gas Chromatography- Mass Spectrum Analysis (GC-MS)

25 grams of the stem and leaves powder of *C. quadrangularis* variant I, II and III were soaked in 95 % methanol for 12 hours. The extracts were then filtered through Whatmann filter No. 41 along with 2 gm Sodium sulpate to remove the sediments and traces of water in the filtrate. Then the filtrate was concentrated by introducing bubbling nitrogen gas into the solution. The plant extract contains both polar and non-polar phytochemicals. 2 µl of the plant methanolic extract filtrate was used for GC-MS analysis. GC-MS analysis of the methanolic extract of the plant samples taken for this study was performed by using a Perkin- Elmer GC clarus- 500 system comprising an AOC 20°C auto sampler and a Gas chromatograph intergraded to a mass spectrometer equipped with a Elite – 5MS fused capillary column. Helium gas was used as a carrier gas at a constant flow rate of 1 ml/min, and an injector where of 2 µl was employed. The inject temperature was maintained at 250°C, the ion source temperature was 200°C and oven temperature was programmed from 110°C, with an increase of 10°C/min to 200°C, then 5°C/min 280°C ending with a 9 min isothermal at 280°C. The solvent delay was 0 to 2 min, and the total GC/MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The Mass detector used in this analysis was turbo- Mass gold Perkin- Elmer and the software adopted to handle Mass spectra and chromatogram was a Turbo – Mass Ver.5.2.

FT-IR analysis

The FT-IR analysis was done by utilizing Perkin Elmer Spectrum Version 10.03.09 framework, which was utilized to identify the practical gatherings of the compound. A little measure of concentrate was set specifically on the zinc solenoid piece and consistent weight. Information of infrared retentive, gathered over the wave number extended from 4000 cm^{-1} to 400 cm^{-1} utilizing spectra programming. Tests were keep running in triplicate and every one of them were embraced inside a day time span.

RESULTS AND DISCUSSION

Morphological variations of *C. quadrangularis*

Table 1. showed morphological characters of all the three variants of *C. quadrangularis* showed similar character of Root, Tendril, Flower, Fruit and Seed. The major difference in all three variants were observed in stem, *C. quadrangularis* Variant I having 4 angled stem, *C. quadrangularis* Variant II – cylinder shaped stem and *C. quadrangularis* Variant III were observed flat shaped stem. Minor different were observed in leaf character. *C. quadrangularis* Variant I and II having simple leaves

and variant III were observed palmate leaves. (Figure 1, 2 and 3)

Table 1. Morphological Character of *C. quadrangularis* variants

S.No.	Character	*C. quadrangularis* Variant I	*C. quadrangularis* Variant II	*C. quadrangularis* Variant III
1	Habit	Climber	Climber	Climber
2	Stem	Quadrangular Stems from 1 - 12 meters (Avg,) long from a tuberous rootstock	Cylinder Stems from 1-13 meter (Avg,) long from a tuberous rootstock	Flat Stems from 12 meter (Avg,) long from a tuberous rootstock
3	Root	Tap-root 10-20 Cm (Avg,)	Tap-root 8-15 Cm (Avg,)	Tap-root 5-13 Cm (Avg,)
4	Leaf	Simple, alternate, 2-6 Cm (Avg,)	Simple, alternate 2-5 Cm (Avg,)	Alternate, palmate 2-6 Cm (Avg,)
5	Tendril	These simple tendrils are long, stout, slender and leaf-opposed. 8-20 Cm (Avg,)	These simple tendrils are long, stout, slender and leaf-opposed. 8-15 Cm (Avg,)	These simple tendrils are long, stout, slender and leaf-opposed. 8-20 Cm (Avg,)
6	Flower	Umbellate cymes yellowish-Green 0.8 Cm (Avg,)	Umbellate cymes yellowish-Green 0.8 Cm (Avg,)	Umbellate cymes yellowish-Green 0.8 Cm (Avg,)
7	Fruit	Globose, succulent berry, 0.6 to 1 Cm (Avg,) These fruits are green turning red when ripe.	Globose, succulent berry, 0.6 to 1 Cm (Avg,), These fruits are green turning red when ripe.	Globose, succulent berry, 0.6 to 1 cm (Avg,), These fruits are green turning red when ripe.
8	Seed	Small with thick Testa	Small with thick Testa	Small with thick Testa

Figure 1: Morphology of *C. quadrangularis* variant I

Figure 2: Morphology of *C. quadrangularis* variant II

Figure 3: Morphology of *C. quadrangularis* variant III

GC-MS

The phytochemical constituents present in the stem methanolic extract of *C. quadrangularis* variant I stem showed fifty-four phytochemical constituents. Out of these fifty-four constituents five constituents were majorly present such as 3-O-Methyl D-glucose, D-Allose, 9, 12, 15-Octadecatrienoic acid, Phytol and Pentadecanoic Acid. Based on GC-MS spectrum confirmed the compounds with retention time 23.574, 19.237, 28.706, 25.037and 26.250 respectively (Figure 4). Apart from the above-mentioned compounds, the 3-O-Methyl-D-glucose was containing the percentage of 51.85 and it was identified as active compound of the species *C. quadrangularis* variant I. The molecular formula of the compound was $C_7H_{14}O_6$ (Table 2). GC-MS analysis of Methanolic extract of *C. quadrangularis* variant II stem demonstrates nearness of fifty-six constituents. Out of these fifty-six constituents five constituents were majorly present such as Hexadeconic acid, Ergost – 5-en- 3- ol, D-Allose, Phytol and 3-O-Methyl D-glucose with their respective retention time such as 27.425, 39.178, 39.453, 40.368 and 41.122

respectively (Figure 5). Based on the spectrum percentage area, Phytol (18.23) was identified as major active compound and the molecular formula was $C_{20}H_{40}O$ (Table 3). Methanolic stem extract of *C. quadrangularis* variant III indicates thirty-four constituents. There are five major constituents were present likely Hexadeconic acid, D-Allose, 9, 12, 15-Octadecatrienoic acid, Phytol and 3-O-Methyl D-glucose. Based on GC-MS spectrum confirmed the compounds with retention time 45.866, 46.005, 46.760, 47.285 and 47.853 respectively (Figure 6). The D- Allose (23.24) was identified as active compound of the *C. quadrangularis*. The molecular formula of the compound was $C_6H_{12}O_6$ (Table 4).

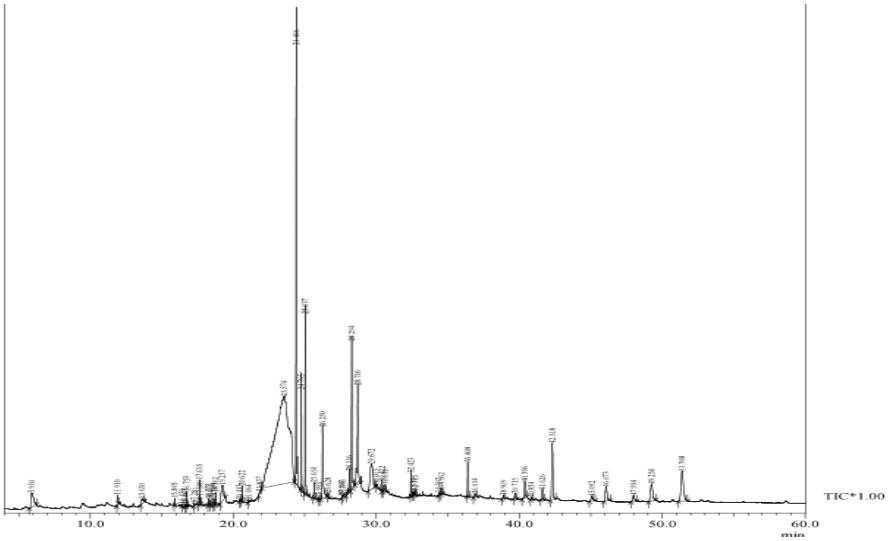

Figure 4: GC-MS Chromatogram of methanolic extract of *C. quadrangularis* stem variant I

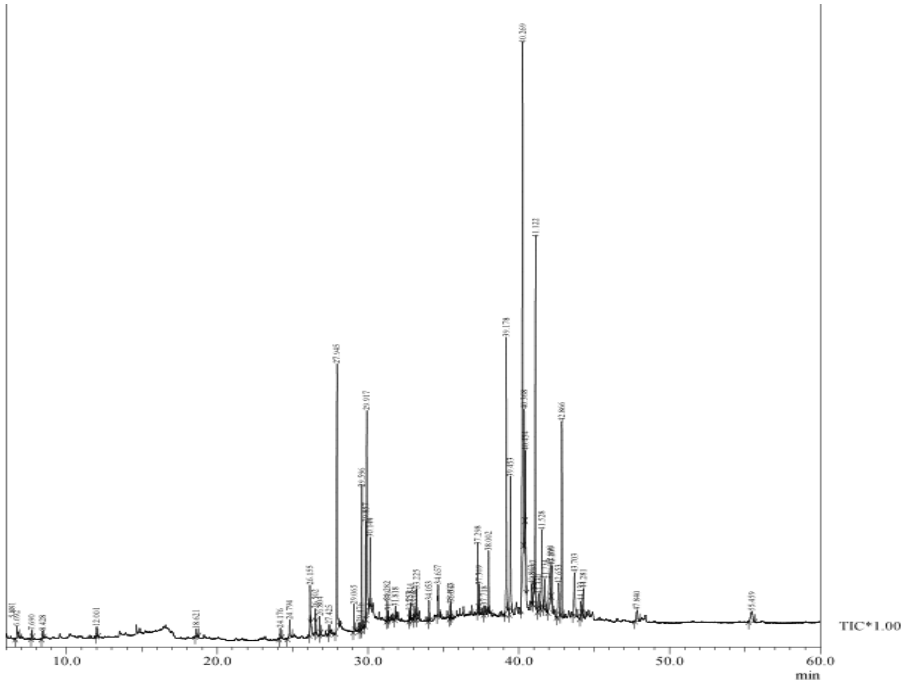

Figure 5: GC-MS Chromatogram of methanolic extract of *C. quadrangularis* Stem Variant II

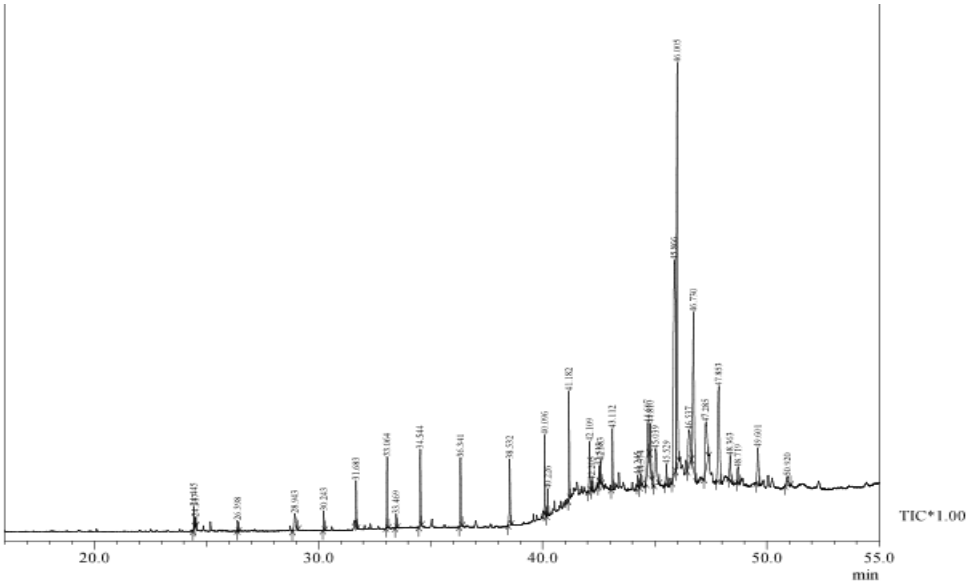

Figure 6: GC-MS Chromatogram of methanolic extract of *C. quadrangularis* Stem variant III

Table 2: GC-MS report of methanolic extract of *C. quadrangularis* stem Variant I

Peak	R. Time	Area%	Name of the compounds	Molecular Formula	Molecular weight
1	5.910	1.48	1H-Pyrrole	C_4H_5N	67
2	11.910	0.32	2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4h-Pyran-One	$C_6H_8O_4$	144
3	13.630	0.31	2h-Pyran-2-One, 5,6-Dihydro-6-Pentyl-	$C_{10}H_{16}O_2$	168
4	15.895	0.12	2.Alpha.,7,8-trimethylacenaphthylene	$C_{15}H_{24}$	204
5	16.439	0.04	2-(3-Isopropyl-4-Methyl-3-Penten-1-Ynyl)-2-Methycyclobutanone	$C_{14}H_{20}O$	204
6	16.663	0.04	3,6,6,7-Tetramethyl-3-Vinyl-2,3,3a,4,5,6-Hexahydro-1h-Indene	$C_{15}H_{24}$	204
7	16.753	0.25	Tricyclo[4.4.0.0(2,7)]Dec-3-Ene, 1,3-Di Methyl -8-(1-Methylethyl)	$C_{15}H_{24}$	204
8	17.281	0.04	Aromadenrene	$C_{15}H_{24}$	204
9	17.635	0.36	Trans (.beta.)-caryophyllene	$C_{15}H_{24}$	204
10	17.689	0.07	Isoledene	$C_{15}H_{24}$	204
11	18.27	0.11	1,4,8-Cycloundecatriene ,2,6,6,9-Tetra Methyl	$C_{15}H_{24}$	204
12	18.412	0.09	1H-Cycloprop [E]Azulene,	$C_{15}H_{24}$	204
13	18.634	0.10	6.Alpha. -Cadina-4,9-Diene, (-)-	$C_{15}H_{24}$	204
14	18.762	0.20	Beta. -ylangene	$C_{15}H_{24}$	204
15	19.237	9.28	D-Allose	$C_6H_{12}O_6$	180
16	20.443	0.03	1-Tridecanol	$C_{13}H_{28}O$	200
17	20.622	0.31	(-)-5-Oxatricyclo [8.2.0.0(4,6)] Dodecane,	$C_{15}H_{24}O$	220
18	21.064	0.05	Humulene Oxide	$C_{15}H_{24}O$	220
19	21.837	0.07	(1ar-(1aalpha, 5abeta, 9ar(*)))-5a,9,9-Trimethyloctahydrobenzo(D)Cycloprop(C)Oxepin-2,4	$C_{14}H_{20}O_3$	236
20	23.574	51.85	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194
21	24.406	1.55	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne	$C_{20}H_{38}$	278
22	24.765	2.15	Neophytadiene	$C_{20}H_{38}$	278

23	25.037	3.41	Phytol	$C_{20}H_{40}O$	296
24	25.654	0.37	Hexadecanoic Acid,	$C_{17}H_{34}O_2$	270
25	25.993	0.05	Isophytol	$C_{20}H_{40}O$	296
26	26.250	3.39	Pentadecanoic Acid	$C_{15}H_{30}O_2$	242
27	26.628	0.08	14B-Pregnane	$C_{21}H_{36}$	288
28	27.597	0.0	Methyl 4-o-benzyl-.alpha.-l-rhamnopyra noside	$C_{14}H_{20}O_5$	268
29	27.690	0.04	1H-Indene, 3-Methyl-	$C_{10}H_{10}$	130
30	28.116	0.63	9,12,15-Octadecatrie noic Acid, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292
31	28.294	2.72	3,7,11,15-Tetramethyl-, [R-[R*,R*-(E)]]	$C_{20}H_{40}O$	296
32	28.706	3.83	9,12,15-Octadecatrienoic Acid,	$C_{18}H_{30}O_2$	278
33	29.672	2.93	Cyclohexanol, 4-[(Tri Methylsilyl) Oxy] -, Cis-	$C_9H_{20}O_2$	188
34	30.015	0.25	Decanoic Acid	$C_{10}H_{20}O_2$	172
35	30.422	0.20	Octanoic acid, 2-di methylaminoethyl ester	$C_{12}H_{25}NO_2$	215
36	30.630	0.33	5-Phenyl-Pentanoic Acid, Ethyl Ester	$C_{17}H_{24}O_5$	308
37	32.42	0.42	Fumaric acid, 2-di methyl aminoethylnonyl ester	$C_{17}H_{31}NO_4$	313
38	32.610	0.06	3-Cyclopentylpropionic Acid,	$C_{12}H_{23}NO_2$	213
39	32.715	0.13	2-Ethylbutyric Acid, Eicosyl Ester	$C_{26}H_{52}O_2$	396
40	34.397	0.10	1-Penten-3-One, 4-Methyl-1-Phenyl-	$C_{12}H_{14}O$	174
41	34.562	0.20	Ethyl linolate	$C_{19}H_{32}O_2$	292
42	36.408	0.79	Squalene	$C_{30}H_{50}$	410
43	36.914	0.18	TriacontylPentafluoropropionate	$C_{33}H_{61}F_5O_2$	584
44	38.909	0.15	Chol-5-ene-3,24-diol	$C_{24}H_{40}O_2$	360
45	39.715	0.30	Ergost-5-En-3-Ol, (3. Beta.)-	$C_{28}H_{48}O$	400
46	40.396	2.98	Beta-Sitosterol	$C_{29}H_{50}O$	414
47	40.854	0.10	5-chlorostigmastan-3-yl acetate	$C_{31}H_{53}C_1O_2$	492
48	41.626	0.42	Stigmast-5-En-3-Ol, (3. Beta.)-	$C_{29}H_{50}O$	414
49	42.318	2.70	Vitamin E	$C_{29}H_{50}O_2$	430
50	4S5.092	0.40	24- Epicampesterol	$C_{28}H_{48}O$	400
51	46.073	1.05	Stigmasta-5,22-Dien-3-ol	$C_{29}H_{48}O$	412
52	47.994	0.52	Beta-di hydrofucosterol	$C_{29}H_{50}O$	414
53	49.254	1.50	,14,14a,14b-Octadbctadecahyro-2h-Picen-3-One	$C_{30}H_{48}O$	424
54	51.398	0.89	D:B-Friedo-B':A'-Neogammacer-5-En-3-one	$C_{30}H_{48}O$	424
		100.00			

Table 3: GC-MS report of Methanolic extract of *C. quadrangularis* stem Variant II

Peak	R. Time	Area%	Name of the compounds	Molecular formula	Molecular weight
1	5.237	0.56	1-Butanamine, 2-methyl-N-(2 methylbutylidene)-	$C_{10}H_{21}N$	155
2	5.435	0.69	2,3-lupetidine	$C_{10}H_{21}N$	155
3	5.881	0.52	2-Pyrrolidinone	C_4H_7NO	85
4	6.692	0.51	Benzeneethanamine	$C_8H_{11}N$	121
5	7.690	0.14	2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4- one	$C_6H_8O_4$	144
6	8.428	0.16	5-Methoxypyrrolidin-2-one	$C_5H_9NO_2$	115
7	12.001	0.26	2-Piperidineacetic acid, Alpha-phenyl-,Methylester,Threo-	$C_{14}H_{19}NO_2$	233
8	18.621	0.17	Dodecanoic acid	$C_{12}H_{24}O_2$	200
9	24.176	0.23	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180
10	24.794	0.60	Pentadeconic acid	$C_{15}H_{30}O_2$	242

11	26.155	0.79	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278
12	26.502	0.23	Tetradecanoic acid	$C_{14}H_{28}O_2$	228
13	26.804	0.34	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
14	27.425	0.12	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
15	27.945	8.00	Hexadecanoic acid	$C_{16}H_{32}O_2$	256
16	29.065	0.47	Heptadecanoic acid	$C_{16}H_{34}O_2$	270
17	29.476	0.15	9,12,15-octadecatrienoic acid, Methyl ester	$C_{19}H_{32}O_2$	292
18	29.596	2.10	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}O$	296
19	29.857	2.73	Cyclodecene	$C_{10}H_{18}$	138
20	29.917	1.11	7-Tetradecenal, (Z)-	$C_{14}H_{26}O$	210
21	30.144	1.13	Octadecanoic acid	$C_{18}H_{36}O_2$	284
22	31.282	0.26	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	$C_{12}H_{23}NO_2$	213
23	31.339	0.12	N- Nonadecanol-1	$C_{19}H_{40}O$	284
24	31.818	0.21	Cyclobutanecarboxylic acid, undec-2-enyl ester	$C_{16}H_{28}O_2$	252
25	32.757	0.10	Fumaric acid, 2-dimethylaminoethyl nonyl ester	$C_{17}H_{31}NO_4$	313
26	32.814	0.22	Fumaric acid, 2-di methyl amino ethyl heptyl ester	$C_{15}H_{27}NO_4$	285
27	33.082	0.22	n-Tetracosanol-1	$C_{24}H_{50}O$	354
28	33.225	0.40	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330
29	34.053	0.33	Cyclohexane, 1,1'-tetradecylidenebis-	$C_{26}H_{50}$	362
30	34.657	1.22	cis-9-Hexadecenal	$C_{16}H_{30}O$	238
31	35.462	0.17	Heptacosyl acetate	$C_{29}H_{58}O_2$	438
32	35.513	0.18	Squalene	$C_{30}H_{50}$	410
33	37.298	1.08	.gamma.-Tocopherol	$C_{28}H_{48}O_2$	416
34	37.369	0.39	Cholest-24-en-16-one, (5. alpha.,20.xi.)-	$C_{27}H_{44}O$	384
35	37.718	0.36	1-heneicosyl formate	$C_{22}H_{44}O_2$	340
36	38.002	3.73	Vitamin E	$C_{29}H_{50}O2$	430
37	39.178	7.54	Ergost-5-en-3-ol, (3. beta.,24R)-	$C_{28}H_{48}O$	400
38	39.453	5.24	D-Allose	$C_6H_{12}O_6$	180
39	40.269	18.23	Phytol	$C_{20}H_{40}O$	296
40	40.368	3.10	2,4a,8,8-Tetramethyl decahydro cyclopropa[d]naphthalene	$C_{15}H_{26}$	206
41	40.454	2.11	Olean-12-en-3-one	$C_{30}H_{48}O$	424
42	40.863	0.51	4,4,6a,6b,8a,11,11,14b-Octamethyl1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,	$C_{30}H_{48}O$	424
43	40.997	0.32	Stigmast-7-en-3-ol, (3. beta.,5. alpha.,24S)-	$C_{29}H_{50}O$	414
44	41.122	10.63	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194
45	41.330	0.64	9,19-Cyclolanost-24-en-3-ol, (3. beta.)-	$C_{30}H_{50}O$	426

46	41.528	3.15	Pentadecanoic Acid	$C_{15}H_{30}O_2$	242
47	41.734	1.14	. Alpha. -Tocopheryl acetate	$C_{31}H_{52}O_3$	472
48	42.090	2.04	. Beta. -Amyrin	$C_{30}H_{50}O$	426
49	42.200	1.08	9,19-Cyclolanostan-3-ol, 24-methylene-, (3. beta.)-	$C_{31}H_{52}O$	440
50	42.653	1.68	D: B-friedo-B': A'-Neogammacer-5-en-3-one	$C_{30}H_{48}O$	424
51	42.866	7.50	9,12,15-Octadecatrienoic Acid,	$C_{18}H_{30}O_2$	278
52	43.703	2.16	03027205002 Flavone 4'-OH,5-oh,7-di-O-Glucoside	$C_{27}H_{30}O_{15}$	594
53	44.133	0.46	9,19-Cycloergost-24(28)-EN-3-ol, 4,14-dimethyl-,(3.beta.,4.alpha.,	$C_{30}H_{50}O$	426
54	44.281	0.99	1-Isopropenyl-4,5-dimethylbicyclo [4.3.0] nonan-5-ylmethylphenyl sulfoxide	$C_{21}H_{30}OS$	330
55	47.840	0.58	1-Eicosanol	$C_{20}H_{42}O$	298
56	55.459	0.90	Phytol, acetate	$C_{22}H_{42}O_2$	338
		100.00			

Table 4: GC-MS report of methanolic extract of *C. quadrangularis* stem Variant III

Peak	R. Time	Area%	Name of the compounds	Molecular formula	Molecular weight
1	24.445	0.62	n-Tetracosanol-1	$C_{24}H_{50}O$	354
2	24.517	0.21	Stigmasterol	$C_{29}H_{48}O$	412
3	26.398	0.27	Cyclohexane, 1,1'-tetradecylidenebis-	$C_{26}H_{50}$	362
4	28.943	1.08	cis-9-Hexadecenal	$C_{16}H_{30}O$	238
5	30.243	0.50	Heptacosyl acetate	$C_{29}H_{58}O_2$	438
6	31.683	1.11	Squalene	$C_{30}H_{50}$	410
7	33.064	2.00	Beta-Sitosterol	$C_{29}H_{50}O$	414
8	33.469	0.40	Eicosanal-	$C_{20}H_{42}O$	298
9	34.544	2.76	Octanoic acid, 2-di methylaminoethyl ester	$C_{12}H_{25}NO_2$	215
10	36.341	2.83	Celidoniol, deoxy-	$C_{29}H_{60}$	408
11	38.532	2.78	5-Phenyl-Pentanoic Acid, Ethyl Ester	$C_{17}H_{24}O_5$	308
12	40.096	2.13	Tetracontane	$C_{40}H_{82}$	562
13	40.226	0.83	9,19-Cycloergost-24(28)-EN-3-ol, 4,14-dimethyl-,(3.beta.,4.alpha.,	$C_{30}H_{50}O$	426
14	41.182	2.51	Hexatriacontane	$C_{36}H_{74}$	506
15	42.109	1.58	Fumaric acid, 2-di methyl aminoethylnonyl ester	$C_{17}H_{31}NO_4$	313
16	42.205	0.27	1-Docosanol, acetate	$C_{24}H_{48}O_2$	368
17	42.513	0.41	Phytol, acetate	$C_{22}H_{42}O_2$	338
18	42.583	0.72	gamma. -Tocopherol	$C_{28}H_{48}O_2$	416
19	43.112	1.81	Tetratetracontane	$C_{44}H_{90}$	618

20	44.245	0.37	Alpha-d-galactopyranose, 6-O-(2,3,5-TRI-O-AC	$C_{23}H_{34}O_{13}$	518
21	44.374	0.41	Octacosyl acetate	$C_{30}H_{60}O_2$	452
22	44.697	3.14	Ergost-5-en-3-ol, (3. Beta.,24R)-	$C_{28}H_{48}O$	400
23	44.810	1.32	Henicosanal	$C_{21}H_{42}O$	310
24	45.039	3.09	. Beta. -Amyrin	$C_{30}H_{50}O$	426
25	45.529	0.84	n-Tetratetracontane	$C_{44}H_{90}$	618
26	45.866	16.27	Hexadecanoic acid	$C_{16}H_{32}O_2$	256
27	46.005	23.24	D-Allose	$C_6H_{12}O_6$	180
28	46.537	3.11	4,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6,6A,6	$C_{30}H_{48}O$	424
29	46.730	8.10	9,12,15-Octadecatrienoic Acid,	$C_{18}H_{30}O_2$	278
30	47.285	3.71	Phytol	$C_{20}H_{40}O$	296
31	47.853	6.82	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194
32	48.363	1.28	9,19-Cyclo-9. beta. -lanostane-3. beta.,25-diol	$C_{30}H_{52}O_2$	444
33	48.719	0.76	24-Norursa-3,12-diene	$C_{29}H_{46}$	394
34	49.601	2.17	03027205002 Flavone 4'-OH,5-OH,7-Di-O-Glucosid	$C_{27}H_{30}O_{15}$	594
35	50.920	0.56	Stigmastane-3,6-dione, (5. alpha.)-	$C_{29}H_{48}O_2$	428
		100.00			

FT-IR

FT-IR range was utilized to distinguish the utilitarian gathering of the dynamic segments in view of the peak an incentive in the point of infrared radiation. The FT-IR analysis results showed that the methanolic stem extract of *C. quadrangularis* variant I having the presence of Alcohol, Aldehyde, Iso cyanides, Alkane, Primary alcohol, Chloro constituent, which shows major peaks at 3420.91 cm^{-1}, 2924.27 cm^{-1}, 2846.85 cm^{-1}, 2076.74 cm^{-1}, 1647.04 cm^{-1}, 1454.75 cm^{-1}, 1409.96 cm^{-1}, 1053.44 cm^{-1}, 1032.56 cm^{-1}, 1017.56 cm^{-1}, 718.75 cm^{-1} and 666.26 cm^{-1} respectively (Figure 7 & Table 5). The FT-IR analysis of methanolic stem extract of *C. quadrangularis* variant II, the presence of Alcohol, Aldehyde, Iso cynides, Nirite, Alkane, Carboxylic acid, Tertiary alcohol, Primary alcohol and Chloro compound. Constituent which shows major peaks at 3434.81 cm^{-1}, 2867.19 cm^{-1}, 2077.67 cm^{-1}, 1640.48 cm^{-1}, 1454.51 cm^{-1}, 1400.70 cm^{-1}, 1108.01 cm^{-1}, 1054.30 cm^{-1} and 678.41 cm^{-1} respectively (Figure 8 & Table 6). The FT-IR analysis methanolic stem extract of *C. quadrangularis* variant III showed Alcohol, Aldehyde, Iso cyanides, Alkyl compound, Alkane, Primary alcohol, Chloro compound. Constituent which shows high peaks at 3434.32 cm^{-1}, 2842.29 cm^{-1}, 2519.69 cm^{-1}, 1646.67 cm^{-1},1456.31 cm^{-1}, 1017.38 cm^{-1} and 574.13 cm^{-1} (Figure 9 & Table 7).

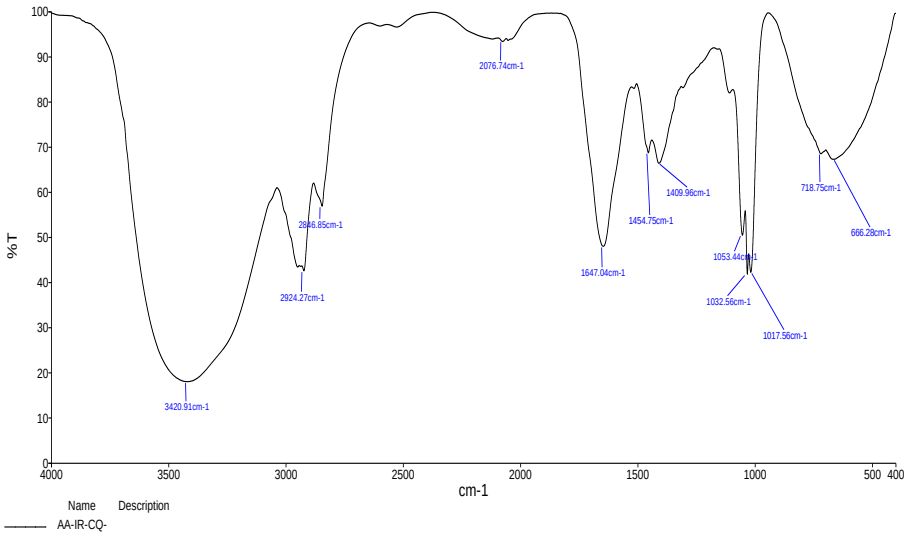

Figure 7: FT-IR Spectrum of methanolic extract of *C. quadrangularis* stem variant I

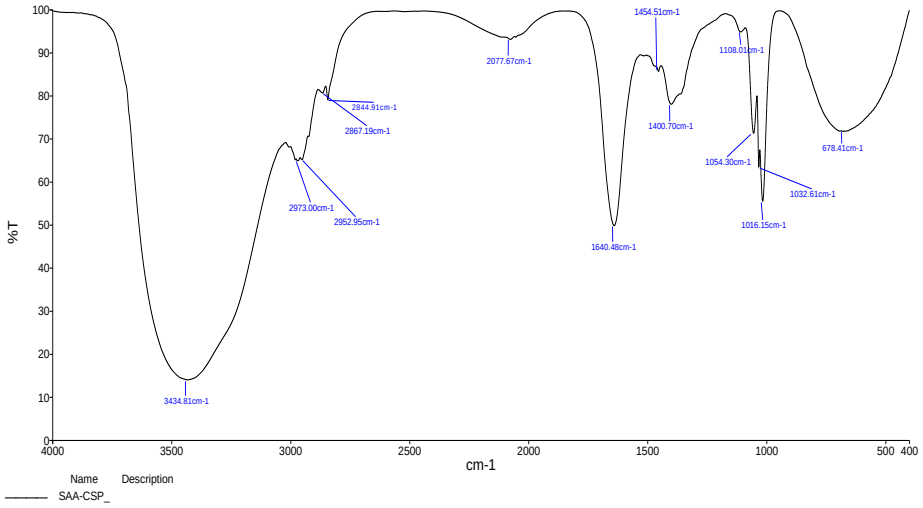

Figure 8: FT-IR Spectrum of methanolic extract of *C. quadrangularis* Stem variant II

Table 5: FT-IR absorption and functional groups of stem extract of *C. quadrangularis* variant I

S. No	Wave No.	Molecular Motion	Functional group	Absorption intensity
1	3420.91cm^{-1}	O-H	Alcohol	Strong
2	2924.27 cm^{-1}	O-H	Alcohol	Medium
3	2846.85 cm^{-1}	C=O	Aldehyde	Weak
4	2076.74 cm^{-1}	C=N	Iso cyanides	Medium
5	1647.04 cm^{-1}	O-NO	Nirite	Strong
6	1454.75 cm^{-1}	C-H	Alkane	Medium
7	1409.96 cm^{-1}	C-H	Alkane	Medium
8	1053.44 cm^{-1}	C-O	Primary alcohol	Strong
9	1032.56 cm^{-1}	C-O	Primary alcohol	Strong
10	1017.56 cm^{-1}	C-O	Primary alcohol	Strong
11	718.75 cm^{-1}	C-Cl	Chloro compound	Strong
12	666.26 cm^{-1}	C-Cl	Chloro compound	Strong

Table 6: FT-IR absorption and functional groups of stem extract of *C. quadrangularis* variant II

S. No	Wave No.	Molecular Motion	Functional group	Absorption intensity
1	3434.81 cm^{-1}	O-H	Alcohol	Medium
2	2973.00 cm^{-1}	O-H	Alcohol	Medium
3	2952.95 cm^{-1}	O-H	Alcohol	Medium
4	2867.19 cm^{-1}	C=O	Aldehyde	Weak
5	2844.91 cm^{-1}	C=O	Aldehyde	Weak
6	2077.67 cm^{-1}	C=N	Iso cynides	Medium
7	1640.48 cm^{-1}	O-NO	Nirite	Strong
8	1454.51 cm^{-1}	C-H	Alkane	Medium
9	1400.70 cm^{-1}	C-O	Carboxylic acid	Weak
10	1108.01 cm^{-1}	C-O	Tertiary alcohol	Strong
11	1054.30 cm^{-1}	C-O	Primary alcohol	Strong
12	1032.61 cm^{-1}	C-O	Primary alcohol	Strong
13	1016.15 cm^{-1}	C-O	Primary alcohol	Strong
14	678.41 cm^{-1}	C-Cl	Chloro compound	Strong

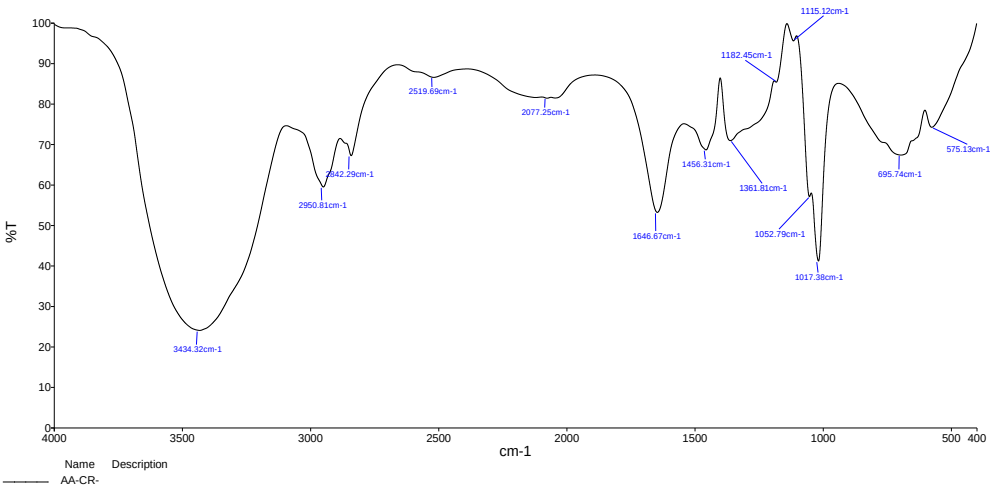

Figure 9: FT-IR Spectrum of methanolic Stem extract of *C. quadrangularis* variant III

Table 7: FT-IR absorption and functional groups of stem extract of *C. quadrangularis* variant III

S. No	Wave No.	Molecular Motion	Functional group	Absorption intensity
1	3434.32 cm^{-1}	O-H	Alcohol	Strong
2	2950.81 cm^{-1}	O-H	Alcohol	Medium
3	2842.29 cm^{-1}	C=O	Aldehyde	Weak
4	2519.69 cm^{-1}	C=N	Iso cyanides	Medium
5	2077.25 cm^{-1}	C=N	Iso cyanides	Medium
6	1646.67 cm^{-1}	C=N	Alkyl compound	Strong
7	1456.31 cm^{-1}	C-H	Alkane	Medium
8	1361.81 cm^{-1}	C-H	Alkane	Medium
9	1182.45 cm^{-1}	C-O	Primary alcohol	Strong
10	1115.12 cm^{-1}	C-O	Primary alcohol	Strong
11	1052.79 cm^{-1}	C-O	Primary alcohol	Strong
12	1017.38 cm^{-1}	C-O	Primary alcohol	Strong
13	695.74 cm^{-1}	C-Cl	Chloro compound	Strong
14	574.13 cm^{-1}	C-Cl	Chloro compound	Strong

Comparison of phytochemical compounds from *C. quadrangularis* all three-variant based on GC-MS and their pharmacological activities

Based on the GC-MS and FT-IR analyses were identified phytochemical compounds and functional groups which present in the methanolic Stem extract of *C. quadrangularis* Variant I, II and III. All the three variant of *C. quadrangularis* contain 3- o- methyl- d- glucose, D- Allose, Phytol, 9, 12, 15 – octadecatrienoic acid. These four compounds are having Anti-tubercular activity, allergic disorders and anti-microbial activities, joint disorders, wounds healing activities. Pentadeconic acid and vitamin E were present in *C. quadrangularis* variant I and II. Pharmacology activities of this compounds such as Anti-cancer drug, anti-asthmatics and anti-abortive and sexual disorders. *C. quadrangularis* variant II and III contains similar phyto-components such as hexadeconic acid, ergost – 5-en- 3- ol and Beta- amyrin. Hexadeconic acid was major active compound of the variant II and III. These three compounds having bone healing activity and wound healing activity. Beta – sitosterol only one compound found in *C. quadrangularis* variant I and III. This compound has been used for anti-cancer, bone and wound healing activities. Based on the phytochemical analysis similar phyto-components present in the all three variants and having bone and wound healing activities. Pharmacological activities of *C. quadrangularis* Variant I and II has been report from earlier [11]. Presence of 3- o- methyl- d- glucose, D- Allose, Phytol, 9, 12, 15 – octadecatrienoic acid, hexadeconic acid, ergost – 5-en- 3- ol and Beta- amyrin. Hexadeconic acid and Beta- sitosterol of Methanolic Stem extract of *C. quadrangularis* Variant III also used as pharmacology activities. Based on the FT-IR analysis similar functional group were fund in all the three variant of *C. quadrangularis.* Such as alcohol, aldehyde, iso cyanide, alkane and Cholo compound. Nirite functional groups of compounds present in *C. quadrangularis* variant I and II. Carboxylic acid fund in *C. quadrangularis* variant II. (Figure 10).

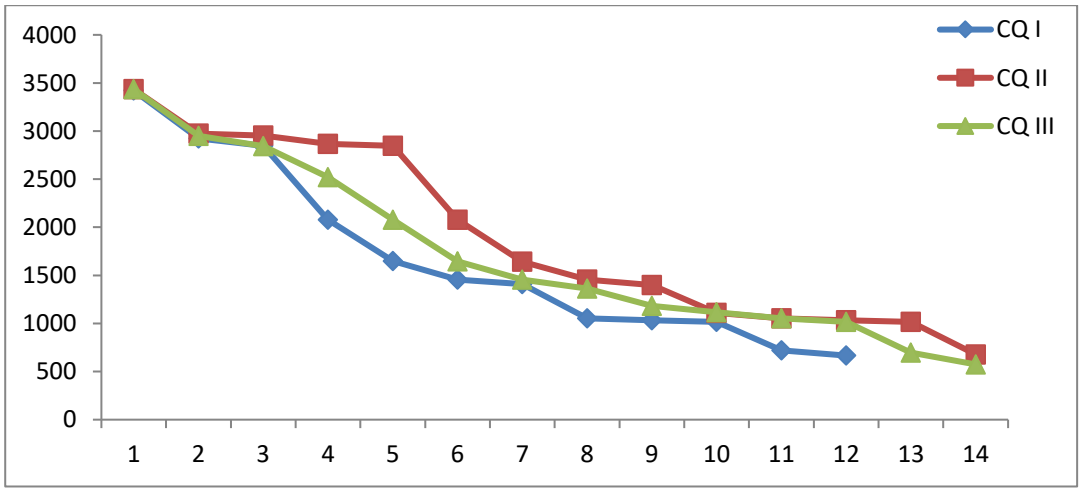

Figure 10: Similarity functional groups of *C. quadrangularis* variant I, II and III

CONCLUSION

Based on the morphology of the stem character of *C. quadrangularis* all the three variants showed distinct shaped stems. Based on the GC-MS analyses of *C. quadrangularis* variants showed similar major compounds like viz., 3- o- methyl- d- glucose, D- Allose, Phytol, 9, 12, 15 – octadecatrienoic acid. These compounds were used for anti-cancer, joint disorders, wounds healing activities and some other pharmacological activities. FT-IR analyses also showed similar functional group of compounds related with same medicinal properties.

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