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Research Article

Effect of Solvent on the Phytochemical Extraction and GC-MS Analysis of *Gymnema sylvestre*

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ABSTRACT

The medicinal plant *Gymnema sylvestre* found in the Indian subcontinent and Srilanka is known for its anti-diabetic, diuretic, anti-obesity, anti-cancer, antimicrobial, anti-inflammatory properties. The current study is focused on the phyto compound extraction efficiency of different solvents like ethanol, methanol, ethyl acetate, hexane, benzene and chloroform by gas chromatography–mass spectrometry analysis of *Gymenma sylvestre*. From the results, it is concluded that *G. sylvestre* leaves extracts contains more than 38 phyto compounds with natural antioxidants potential. Further analysis of the extract will help in identifying the effective compounds which can be of potent use in the pharmacological field.

Key words: *Gymnema sylvestre*, Medicinal plants, chromatography, plant constituents, Cold maceration.

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INTRODUCTION

Since time immemorial, various parts of the plants such as leaves, roots, stem etc. are being used to treat number of diseases and infections. India is blessed with and is a source for variety of herbal plants with medicinal properties. *Gymnema sylvestre* is a woody climber shrub from the family of Apocynaceae. Gymnema genus has 50 species in the genus. It is native to India, Australia, Africa and China. It is found to be grown well in the tropical regions. *Gmnema sylvestre* is an important plant with medicinal properties. In local languages of India, it is called as Sakkarai kolli which literally means "destroyer of sugar"[1]. Because of the medicinal importance, this plant is used in the preparation of formulated medicine for treating various health ailments. In Indian Ayurvedic medicine system, *G.sylvestre* is used for treating diabetes. Therapeutically, crude extract of this plant is used as a diuretic, to cure stomachic, eye complaints, asthma, chronic cough, cardiopathy, constipation, piles etc. Apart from these, various pharmacological and biological activities such as antibacterial, antiviral, antifungal, anti-inflammatory, anticancer has been reported[2-5].

Due to the side effects associated with allopathic medicines, in recent years, research interests have turned towards plant-based phytochemicals in treating various diseases[6]. Phytocompounds from medicinal plants are used in formulations of various healthcare nutraceuticals and cosmetics products. The phytocompounds of *G. sylvestre* has been effective in controlling diabetes[7]. The phytochemical compounds like phenols, flavonoids, terpenoids, saponins, tannins of the plants are the base for modern day allopathic medicines. The active components of allopathic drugs constitute about 25 – 40% of plant-based origins[8]. The literature survey revealed that no work has been done to compare the effect of solvents on biochemical constituents of *G.sylvestre* plant extracts. To the best of our knowledge, no study has been conducted to study the effect of agroclimatic location on the antioxidant activity of *G. sylvestre* leaves. In this study, the plant samples collected from different regions of Tamil Nadu were evaluated for their antioxidant activities and the sample showing high level of antioxidant activity was extracted with six different solvents like ethyl acetate, ethanol, methanol, chloroform, hexane and benzene. The phytochemical constituents of the crude extracts of *G. sylvestre* of the different solvents were characterized by GCMS analysis. This study will reveal the relationship between the effect of sampling locations to the quantity and quality of the phytocompounds and its antioxidant activities of the plant sample and also the effect of solvents on the phytochemical availability in the crude extract. This will help to select the suitable solvent based on the actual application of the extracts.

MATERIAL AND METHODS

Chemicals

All the chemical used in the study were of analytical grade and are purchased from Sisco Research Laboratories, India. The DPPH was purchased from Sigma, India.

Plant sample

G. sylvestre leaves collected during the month of November 2016. The collected plant samples were identified and authenticated by Dr. G.V.S. Murthy, Botanical Survey of India (Ref No: BSI/SRC/5/23/2016/Tech/215). The leaves were washed with running tap water, distilled water and shade dried at room temperature. The dried leaves were ground by using a laboratory blender. The pulverized samples were stored in cold storage for further usage.

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For the present study, during the same month, the *G. sylvestre* plants were collected from seven different locations of Tamil Nadu (Figures 1 & 2) such as

1. Shenbagadevi falls at Coutrallam on the Western Ghats in Tirunelveli District
2. Thirunel, Kottamalai at Padavedu, Thiruvannamalai District
3. Irulars Tribal Women's Welfare Society at Chengalpattu District
4. Muniyankudisai Village at Arni, Tiruvanamalai District
5. Velliangiri Hills at the Western Ghats border of Coimbatore District
6. Anthyodhaya sangham at Trichy
7. Gandhi gram Trust at Dindigul district

Effect of sampling location on antioxidant activity

The effect of locations on antioxidant activity of *G.sylvestre* plants was estimated by DPPH method following the method[9] of Blois,1958. The

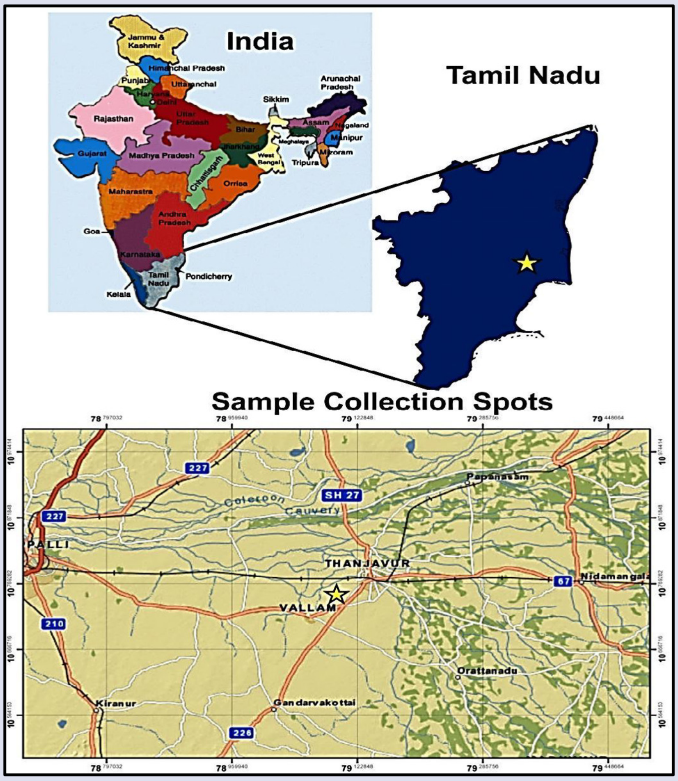


Figure 1: Shows the *G.sylvestre* collection spot (at column width).

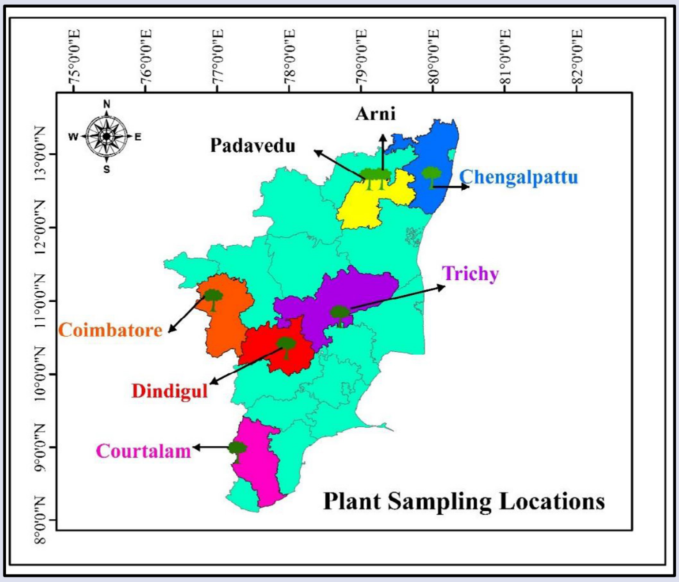


Figure 2: **Gymnema sylvestre** collection spots around Tamil Nadu (at column width).

reaction mixture (0.1mM DPPH and extract) was vortexed, incubated and its absorbance was measured at 517 nm. The scavenging ability of the plant extracts was calculated using the following equation (1)

$$\textit{DPPH scavenging Activity}(\%) = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100 \quad (1)$$

Where, $Abs_{control}$ is the absorbance of DPPH without sample; Abs_{sample} is the absorbance of DPPH with sample[10] Cieśla *et al.*

Extraction procedure

The plant sample obtained from Coutrallam is used for extraction. *G.sylvestre* leaf powder 50g was extracted with 500ml ethanol by cold maceration method for 72h. After extraction, it was filtered using Whatman N0. 41 filter paper to obtain solid particle free extract and the solvent was evaporated to dryness under vacuum using a rotary evaporator. The crude extract obtained was stored at 4 °C for further usage. The same procedure was followed for all other solvents like methanol, benzene, hexane, ethyl acetate and chloroform.

Estimation of chemical constituents by GC-MS

To determine the various volatile bioactive compounds present in each solvent extract, GC-MS analysis was conducted using SHIMADZU, QP2010 PLUS following the injecting temperature at 250°C, column temperature at 50 °C, pressure at 29.7 kPa and column flow rate at 0.72 ml/min. The total running time for the sample was 50 minutes. Based on the retention time the phytochemical compounds in various solvent extracts were identified by matching MS with available standards using NIST and Willey library.

RESULTS

DPPH radical scavenging activity

The influence of source of plant collection on the antioxidant activity was studied by DPPH method. The antioxidant activity % is presented in the Table 1. Significant influence of the location on antioxidant activity was found. Variation in the activity was witnessed with highest activity observed in the plants collected from Shenbagadevi falls at Coutrallam on the Western Ghats in Tirunelveli District with 73.40% followed by Anthyodhaya sangham at Trichy 66.10%. The plants collected from Gandhigram Trust at Dindigul district showed the least activity of 36.70%.

Chemical constituents of various extracts

The leaves obtained from coutrallam is used for the extraction and identification of phytochemicals using different solvents such as hexane, benzene, chloroform, ethyl acetate, methanol and ethanol. The results pertaining to GC-MS analysis (Figure 3) of the hexane crude extract of *G.sylvestre* leaves was analysed using GC-MS which lead to the identification of 36 different organic compounds is listed in the Table 2 with Phytol (10.294 %), Squalene (10.282 %), Tetratriacontane (>14 %) at various time intervals, n-Hexadecanoic acid (5.186 %), Eicosane (>10 %) at various time intervals, Stigmasterol (2.484 %), Phthalic acid, di(2-propylpentyl) ester (2.417 %), 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione (1.804 %), Benzoic acid, 3,5-dicyclohexyl-4-hydroxy-, methyl ester (1.787 %) comprising major area.

Table 1: Antioxidant Activity of *G. sylvestre* from different locations.

S.No	Sampling locations	Antioxidant Activity (%)
1	Shenbagadevi falls (Courtallam)	73.40
2	Anthyodhaya sangham (Trichy)	66.10
3	Vellingiri Hills (Coimbatore)	65.90
4	Muniyankudisai Village (Arni)	60.60
5	Kotta malai (Padavedu)	54.80
6	Irulars Tribal Women's Welfare Society (Chengalpattu)	49.30
7	Gandhi gram Trust (Dindigul)	36.70

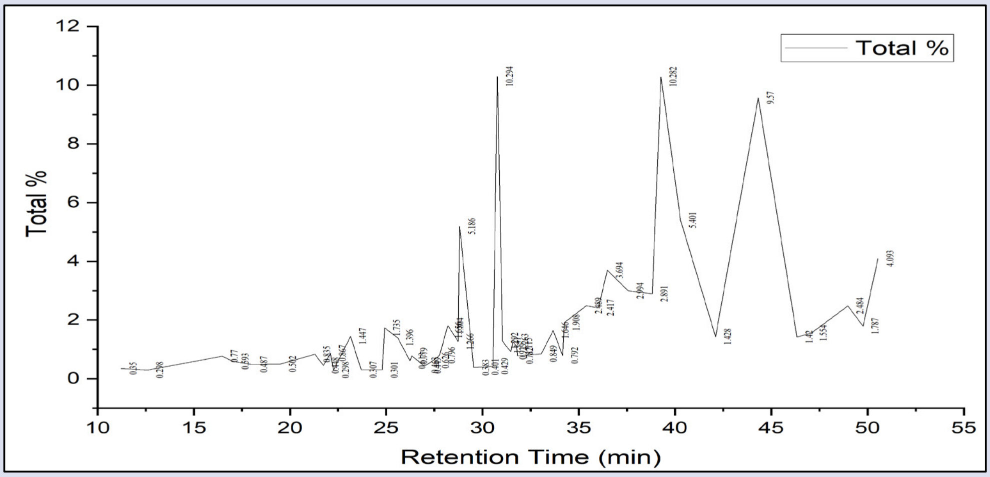


Figure 3: Abundance of the chemical constituents present in hexane extract from the *G.sylvestre* leaves.

Table 2: Chemical composition of Hexane extract of *Gymnema sylvestre* from GCMS analysis.

S. No	RT min	Name of compounds	Molecular formula	Molecular weight	% of total
1	11.219	Undecane	C11H24	156	0.350
2	16.480	Benzene, 1,3-bis(1,1-dimethylethyl)-	C14H22	190	0.770
3	17.040	Dodecane, 2,6,11-trimethyl-	C15H32	212	0.593
4	18.014	Pentadecane	C15H32	212	0.487
5	19.499	Tetradecane	C14H30	198	0.502
6	21.740	Phenol, 2,4-bis(1,1-dimethylethyl)-	C14H22O	206	0.448
7	22.263	Tetradecane, 2,6,10-trimethyl-	C17H36	240	0.298
8	23.140	Hexadecane	C16H34	226	1.447
9	23.700	Octadecane, 1-chloro-	C18H37Cl	288	0.307
10	24.784	Heptadecane	C17H36	240	0.301
11	24.918	Tridecane, 2-methyl-	C14H30	198	1.735
12	25.600	Hexadecane, 2,6,11,15-tetramethyl-	C20H42	282	1.396
13	26.233	1-Decanol, 2-hexyl-	C16H34O	242	0.613
14	26.915	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	0.463
15	27.037	Heptadecane, 2,6,10,15-tetramethyl-	C21H44	296	0.449
16	27.451	Dibutyl phthalate	C16H22O4	278	0.626
17	27.767	Ethanone, 2,2-dimethoxy-1,2-diphenyl-	C16H16O3	256	0.796
18	28.133	Nonadecane, 2-methyl-	C20H42	282	1.656
19	28.206	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	C17H24O3	276	1.804
20	28.814	n-Hexadecanoic acid	C16H32O2	256	5.186
21	29.533	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	C20H40O2	312	0.383
22	30.531	Heneicosane	C21H44	296	0.429
23	30.763	Phytol	C20H40O	296	10.294
24	31.043	Heptadecane, 2-methyl-	C18H38	254	1.292
25	31.189	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	C16H28O3	268	1.141
26	31.469	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C26H54	366	0.929
27	31.578	Eicosane, 2-methyl-	C21H44	296	1.263
28	31.761	n-Tetracosanol-1	C24H50O	354	1.115
29	33.673	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene	C16H26OSi	262	1.646
30	36.011	Phthalic acid, di(2-propylpentyl) ester	C24H38O4	390	2.417
31	39.274	Squalene	C30H50	410	10.282
32	44.315	Tetratriacontane	C34H70	478	9.570
33	46.336	Lup-20(29)-en-3-one	C30H48O	424	1.420
34	47.079	Tetracosane, 11-decyl-	C34H70	478	1.554
35	48.979	Stigmasterol	C29H48O	412	2.484
36	49.758	Benzoic acid, 3,5-dicyclohexyl-4-hydroxy-, methyl ester	C20H28O3	316	1.787

34 compounds were identified by GC-MS analysis (Figure 4) in the benzene extract of *G.sylvestre*. The compounds which occupied the major percentage in the extract are Eicosane (>20 %), Tetratriacontane (>19 %), Hexadecane, 2,6,11,15-tetramethyl- (>5 %), Benzoic acid, 3,5-dicyclohexyl-4-hydroxy-, methyl ester (5.515 %),Tetracosane (4.803 %), Squalene (4.797 %), Ethylbenzene (3.052 %), Hexadecane (2.862 %), Phytol (2.788 %), n-Hexadecanoic acid (2.622 %) (Table 3).

The GC-MS analysis (Figure 5) results of the chloroform extract of *G.sylvestre* showed the presence of 32 compounds in it. The compounds present in the chloroform extract is given in the Table 4. Among the compounds identified Eicosane (>14 %), Phytol (8.667 %), Heptadecane, 9-hexyl- (>7 %), Squalene (5.441 %), 7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione (5.074 %), n-Hexadecanoic acid (5.005 %), Tetracosane (4.469 %), Hexadecane (>4 %), Stigmasterol (3.879 %), Tetratriacontane (3.717 %), Phthalic acid, hex-3-yl isobutyl ester (3.439 %), Octadecane, 3-ethyl-5-(2-ethylbutyl)- (2.720 %) represented more than 67% of the total compounds.

The ethyl acetate *G.sylvestre* crude extract on GCMS analysis (Figure 6) revealed the presence of 43 different organic compounds listed in the Table 5 with major compounds as follows: Eicosane, 2-methyl- (>9 %), n-Tetracosanol-1 (6.579 %), Heneicosane (>6 %), Fumaric acid, 2-chloroethyl hexadecyl ester (6.100 %), Tetracosane (>5 %), Eicosane (>3 %), E-15-Heptadecenal (3.801 %), Hexadecane, 2,6,11,15-tetramethyl- (3.582 %), Triacontane, 1-bromo (3.258 %), Ethanone, 2,2-dimethoxy-1,2-diphenyl- (2.964 %), Phenol, 2,4-bis(1,1-dimethylethyl)- (2.770 %), Tetracosane (2.266 %), Cetene (1.883 %).

The GC-MS analysis (Figure 7) of the ethanolic *G.sylvestre* leaves extract based on the retention time on capillary column fused with silica is listed in the Table 6 with major compounds as 2-Pentanone, 3,3,4,4-tetramethyl (15.885%), Squalene (15.075%), n-Hexadecanoic acid (7.086%), Phytol (6.351%), Cholesterol (5.966%), Octadecane, 1,1'-[(1-methyl-1,2-ethanediyl) bis(oxy)] bis- (5.218%), Stigmasterol (4.314%), (E)-9-Octadecenoic acid ethyl ester (4.290%), trans-13-Octadecenoic acid (4.158%), 1,2,3,4-Cyclohexanetetrol (3.699%), Hexadecanoic acid, ethyl ester (3.084%), Eicosanoic acid (2.859%), Tetraethyl silicate (2.804%). From the MS chromatogram, a total of 29 compounds were identified. These compounds are members of different types of organic groups such as alcohols, amines, fatty acids, terpenes.

The methanol plant extract was analysed using GC-MS (Figure 8). A total of 17 different compounds which is listed in the Table 7 where compounds comprising major percentage are 2-Pentanone, 3,3,4,4-tetramethyl- (61.21%), Inositol, 1-deoxy- (21.218 %),

Table 3: Chemical composition of Benzene extract of *Gymnema sylvestre* from GCMS analysis.

S. No	RT min	Name of compounds	Molecular formula	Molecular weight	% of total
1	4.400	Ethylbenzene	C8H10	106	3.052
2	4.583	Benzene, 1,3-dimethyl-	C8H10	106	1.406
3	15.213	Dodecane	C12H26	170	0.478
4	16.504	Benzene, 1,3-bis(1,1-dimethylethyl)-	C14H22	190	1.636
5	16.686	2,4-Dimethyldodecane	C14H30	198	0.186
6	17.052	Dodecane, 2,7,10-trimethyl-	C15H32	212	0.648
7	18.026	Dodecane, 2,6,11-trimethyl-	C15H32	212	0.225
8	19.511	Tetradecane	C14H30	198	1.701
9	21.301	Heptadecane	C17H36	240	1.329
10	21.800	Phenol, 2,4-bis(1,1-dimethylethyl)-	C14H22O	206	0.492
11	23.128	Hexadecane	C16H34	226	2.862
12	26.221	E-15-Heptadecenal	C17H32O	252	0.879
13	26.915	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	0.416
14	27.463	Dibutyl phthalate	C16H2204	278	0.430
15	27.767	Ethanone, 2,2-dimethoxy-1,2-diphenyl-	C16H16O3	256	1.355
16	27.974	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C26H54	366	0.429
17	28.132	Hexadecane, 2,6,11,15-tetramethyl-	C20H42	282	2.466
18	28.839	n-Hexadecanoic acid	C16H32O2	256	2.622
19	29.119	10-Heneicosene (c,t)	C21H42	294	0.502
20	30.020	Nonadecane, 2-methyl-	C20H42	282	0.555
21	30.543	Heneicosane	C21H44	296	0.956
22	30.762	Phytol	C20H40O	296	2.788
23	31.042	Eicosane, 2-methyl-	C21H44	296	1.902
24	31.469	Eicosane, 7-hexyl-	C26H54	366	0.500
25	31.761	1-Heneicosyl formate	C22H44O	340	1.491
26	33.673	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene	C16H26OSi	262	2.367
27	34.245	Tetracosane	C24H50	338	4.803
28	36.023	Heneicosane, 11-(1-ethylpropyl)-	C26H54	366	0.793
29	36.485	Eicosane	C20H42	282	7.272
30	37.569	Tetratriacontane	C34H70	478	5.102
31	39.274	Squalene	C30H50	410	4.797
32	47.607	Tetracosane, 11-decyl-	C34H70	478	0.674
33	48.966	Stigmasterol	C29H48O	412	1.101
34	49.782	Benzoic acid, 3,5-dicyclohexyl-4-hydroxy-, methyl ester	C20H28O3	316	5.515

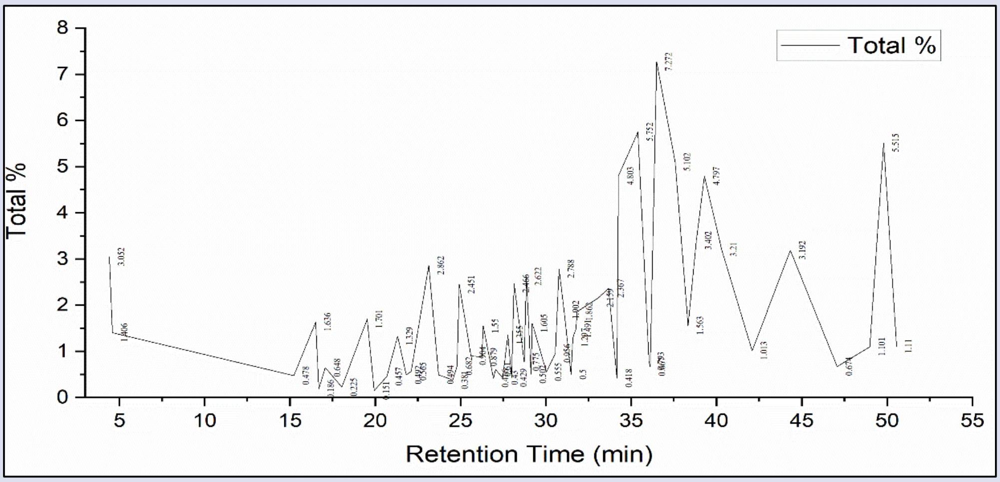


Figure 4: Abundance of the chemical constituents present in benzene extract from the *G.sylvestre* leaves.

Table 4: Chemical composition of Chloroform extract of *Gymnema sylvestre* from GCMS analysis.

S. No	RT Min	Name of the compounds	Molecular formula	Molecular weight	% of total
1	16.504	Benzene, 1,3-bis(1,1-dimethylethyl)-	C14H22	190	1.221
2	19.511	Tetradecane	C14H30	198	0.728
3	21.301	Heptadecane, 2,6,10,15-tetramethyl-	C21H44	296	0.934
4	21.788	Phenol, 2,4-bis(1,1-dimethylethyl)-	C14H22O	206	0.955
5	22.098	Dodecane, 2,6,11-trimethyl-	C15H32	212	1.095
6	23.110	Hexadecane	C16H34	226	3.781
7	23.700	Benzeneacetic acid, 4-tetradecyl ester	C22H36O2	332	0.555
8	23.931	Benzene, (1-propyloctyl)-	C17H28	232	0.808
9	24.930	Nonadecane, 2-methyl-	C20H42	282	2.383
10	25.271	Benzene, (1-pentylheptyl)-	C18H30	246	0.534
11	25.612	Heptadecane, 2,6,10,15-tetramethyl-	C21H44	296	2.091
12	27.049	1-Chloroeicosane	C20H41Cl	316	0.866
13	27.450	Phthalic acid, hex-3-yl isobutyl ester	C18H26O4	306	3.439
14	27.767	Ethanone, 2,2-dimethoxy-1,2-diphenyl-	C16H16O3	256	1.942
15	28.145	2-methyloctacosane	C29H60	408	1.502
16	28.205	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	C17H24O3	276	5.074
17	28.826	n-Hexadecanoic acid	C16H32O2	256	5.005
18	29.204	Hexadecanoic acid, ethyl ester	C18H36O2	284	2.189
19	30.775	Phytol	C20H40O	296	8.667
20	31.043	Eicosane, 2-methyl-	C21H44	296	1.283
21	31.481	Octadecanoic acid	C18H36O2	284	0.751
22	31.773	1-Decanol, 2-hexyl-	C16H32O	242	1.016
23	32.808	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene	C16H26OSi	262	0.982
24	33.673	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene	C16H26OSi	262	2.526
25	34.257	Tetracosane	C24H50	338	4.469
26	37.581	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C26H54	366	2.720
27	39.298	Squalene	C30H50	410	5.441
28	40.309	Heptadecane, 9-hexyl-	C23H48	324	3.933
29	42.009	Octadecanoic acid, 2-(hexadecyloxy)ethyl ester	C36H72O3	552	1.736
30	44.339	Eicosane	C20H42	282	7.575
31	49.015	Stigmasterol	C29H48O	412	3.879
32	50.561	Tetratriacontane	C34H70	478	3.717

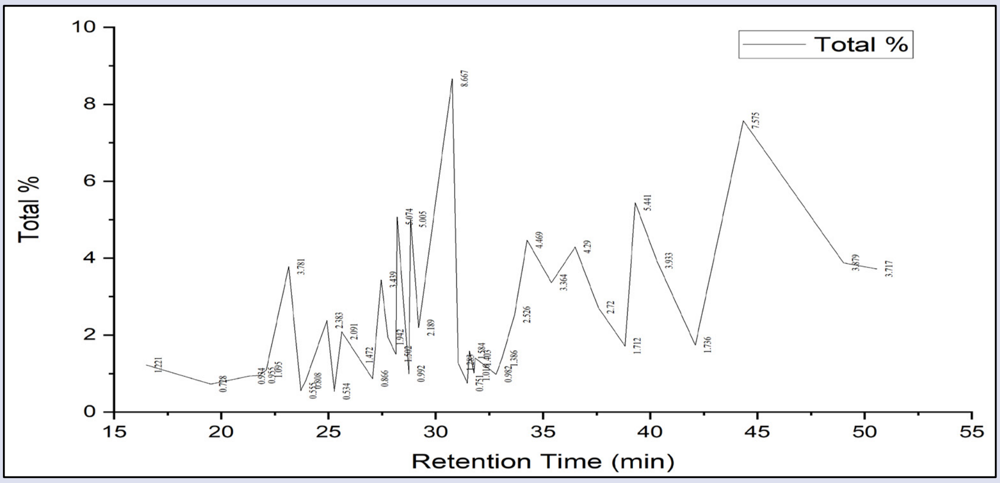


Figure 5: Abundance of the chemical constituents present in chloroform extract from the *G.sylvestre* leaves.

Table 5: Chemical composition of Ethyl acetate extract of *Gymnema sylvestre* from GCMS analysis.

S. No	RT min	Name of compounds	Molecular formula	Molecular weight	% of total
1	32.808	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene	C16H26OSi	262	0.707
2	26.915	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	0.419
3	13.094	4-Piperidinone, 2,2,6,6-tetramethyl-	C9H17NO	155	0.447
4	29.119	5-Eicosene, (E)-	C20H40	280	1.708
5	16.492	Benzene, 1,3-bis(1,1-dimethylethyl)-	C14H22	190	0.617
6	49.770	Benzoic acid, 3,5-dicyclohexyl-4-hydroxy-, methyl ester	C20H28O3	316	2.792
7	23.128	Cetene	C16H32	224	1.883
8	23.018	Dodecane, 2,6,11-trimethyl-	C15H32	212	0.729
9	26.221	E-15-Heptadecenal	C17H32O	252	3.801
10	35.390	Eicosane	C20H42	282	1.909
11	28.132	Eicosane, 2-methyl-	C21H44	296	4.922
12	34.172	Eicosane, 7-hexyl-	C26H54	366	1.174
13	27.767	Ethanone, 2,2-dimethoxy-1,2-diphenyl-	C16H16O3	256	2.964
14	33.673	Fumaric acid, 2-chloroethyl hexadecyl ester	C22H39ClO4	402	6.100
15	30.531	Heneicosane	C21H44	296	1.908
16	41.697	Heneicosane, 11-(1-ethylpropyl)-	C26H54	306	0.743
17	24.772	Heptadecane	C17H36	240	0.529
18	21.289	Heptadecane, 2,6,10,15-tetramethyl-	C21H44	296	0.998
19	46.738	Heptadecane, 9-hexyl-	C23H48	324	0.733
20	30.665	Heptadecane, 9-octyl-	C25H52	353	0.658
21	25.052	Hexadecane	C16H34	226	0.465
22	24.918	Hexadecane, 2,6,11,15-tetramethyl-	C20H42	282	3.582
23	34.635	i-Propyl 5,9,19-octacosatrienoate	C31H56O2	460	0.749
24	28.839	n-Hexadecanoic acid	C16H32O2	256	0.662
25	30.020	Nonadecane, 2-methyl-	C20H42	282	1.369
26	31.761	n-Tetracosanol-1	C24H50O	354	6.579
27	29.545	Octadecanal	C18H36O	268	0.857
28	26.306	Octadecane	C18H38	252	1.611
29	27.962	Octadecane, 2-methyl-	C19H40	268	0.606
30	50.525	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C26H54	366	0.791
31	21.740	Phenol, 2,4-bis(1,1-dimethylethyl)-	C14H22O	206	2.770
32	30.775	Phytol	C20H40O	296	1.883
33	39.274	Squalene	C30H50	410	1.698
34	48.966	Stigmasterol	C29H48O	412	0.784
35	36.486	Tetracosane	C24H50	338	2.266
36	36.023	Tetracosane, 11-decyl-	C34H70	478	2.550
37	19.511	Tetradecane	C14H30	198	0.742
38	27.536	Tetradecane, 2,6,10-trimethyl-	C17H36	240	0.788
39	28.729	Tetradecane, 2-methyl-	C15H32	212	1.499
40	40.285	Tetratriacontane	C34H70	478	0.236
41	36.108	Triacontane, 1-bromo-	C30H61Br	500	3.258
42	33.064	Tricosane	C23H48	324	0.782
43	24.540	Tridecane, 2-methyl-	C14H30	198	0.596

Table 6: Chemical composition of Ethanol extract of *Gymnema sylvestre* from GCMS analysis.

S. No	RT min	Name of the compound	Molecular formula	Molecular weight	% of total
1	7.907	Disiloxane, 1,3-diethoxy-1,1,3,3-tetramethyl-	$C_8H_{22}O_3Si_2$	222	0.174
2	9.113	Tetraethyl silicate	$C_8H_{20}O_4Si$	208	2.804
3	12.571	d-Mannitol, 1-decylsulfonyl-	$C_{16}H_{34}O_7S$	370	0.173
4	15.006	3-Dodecene, (E)-	$C_{12}H_{24}$	168	0.554
5	16.492	Benzene, 1,3-bis(1,1-dimethylethyl)-	$C_{14}H_{22}$	190	0.256
6	19.353	4-Trifluoroacetoxytetradecane	$C_{16}H_{29}F_3O_2$	310	0.389
7	19.499	2-Hexyl-1-octanol	$C_{14}H_{30}O$	214	0.830
8	21.289	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	240	0.219
9	21.764	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	0.909
10	22.312	1,2,3,4-Cyclohexanetetrol	$C_6H_{12}O_4$	148	3.699
11	22.811	2-Pentanone, 3,3,4,4-tetramethyl-	$C_9H_{18}O$	142	15.885
12	26.221	2-Dodecanol	$C_{12}H_{26}O$	186	0.531
13	26.902	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.139
14	28.802	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.086
15	29.143	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	3.084
16	30.762	Phytol	$C_{20}H_{40}O$	296	6.351
17	31.298	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	4.158
18	31.456	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310	4.290
19	31.749	Eicosanoic acid	$C_{20}H_{40}O_2$	312	2.859
20	33.052	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	0.564
21	33.746	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl) oxy propyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$	496	0.802
22	34.245	Heptadecane, 9-hexyl-	$C_{23}H_{48}$	324	1.503
23	34.975	N1-Benzyl-N2(bezylidenyl-benzylamino)-benzamidin	$C_{28}H_{25}N_3$	403	0.971
24	35.389	Docosane, 11-butyl-	$C_{26}H_{54}$	366	1.003
25	36.485	Tetracosane, 11-decyl-	$C_{34}H_{70}$	478	1.178
26	36.814	Cholesterol	$C_{27}H_{46}O$	386	5.966
27	39.274	Squalene	$C_{30}H_{50}$	410	15.075
28	44.303	Octadecane, 1,1'-[(1-methyl-1,2-ethanediyl) bis(oxy)] bis-	$C_{39}H_{80}O_2$	580	5.218
29	48.954	Stigmasterol	$C_{29}H_{48}O$	412	4.314

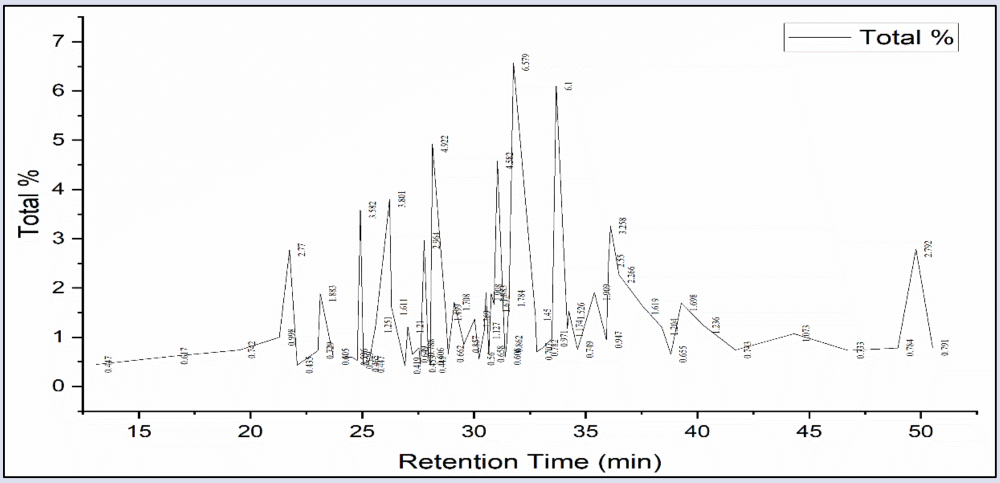


Figure 6: Abundance of the chemical constituents present in ethyl acetate extract from the *G.sylvestre* leaves.

Table 7: Chemical composition of Methanol extract of *Gymnema sylvestre* from GCMS analysis.

S. No	RT min	Name of compounds	Molecular formula	Molecular weight	% of total
1	9.088	Decane	C10H22	142	0.294
2	12.547	Undecane	C11H24	156	0.237
3	21.533	1,2,3,4-Cyclohexanetetrol	C6H12O4	148	3.768
4	23.883	2-Pentanone, 3,3,4,4-tetramethyl-	C9H18O	142	61.218
5	26.209	Inositol, 1-deoxy-	C6H12O5	164	21.218
6	26.903	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	0.912
7	28.181	Hexadecanoic acid, methyl ester	C17H34O2	270	0.195
8	28.766	n-Hexadecanoic acid	C16H32O2	256	3.616
9	30.592	10-Octadecenoic acid, methyl ester	C19H36O2	296	0.187
10	30.763	Phytol	C20H40O	296	1.693
11	31.201	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C18H30O2	278	2.521
12	31.444	Octadecanoic acid	C18H36O2	284	0.148
13	33.356	2-Pyrrolidinone, 1-(9-octadecenyl)-	C22H41NO	335	0.214
14	35.645	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	C35H68O5	568	0.140
15	36.011	Phthalic acid, di(2-propylpentyl) ester	C24H38O4	390	0.128
16	39.274	Squalene	C30H50	410	1.782
17	48.954	Stigmasterol	C29H48O	412	0.860

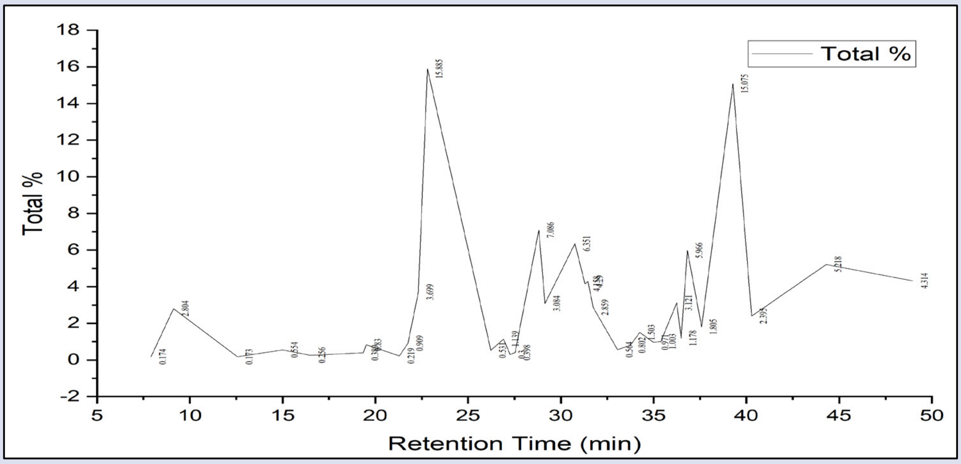


Figure 7: Abundance of the chemical constituents present in ethanol extract from the *G.sylvestre* leaves.

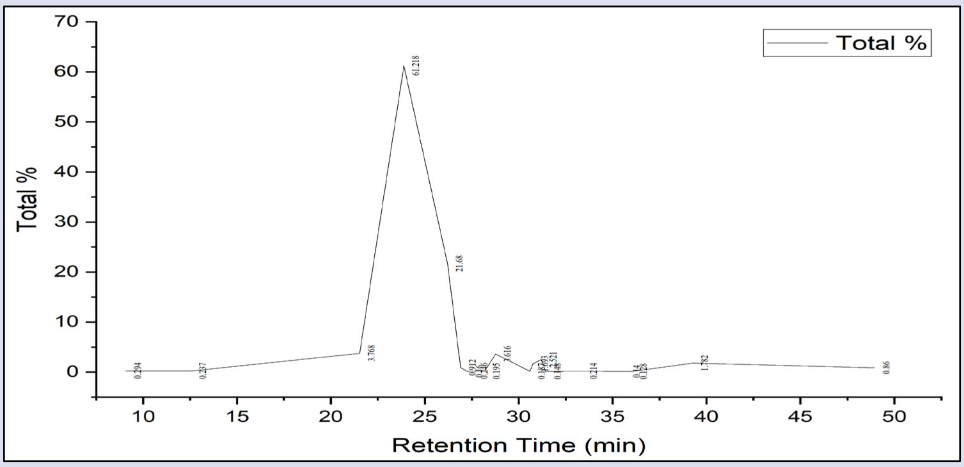


Figure 8: Abundance of the chemical constituents present in methanol extract from the *G.sylvestre* leaves.

1,2,3,4-Cyclohexanetetrol (3.768 %), n-Hexadecanoic acid (3.616), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (2.521 %), Squalene (1.782 %), Phytol (1.693 %)

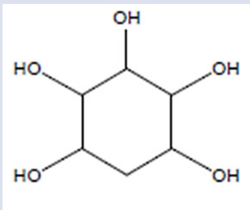
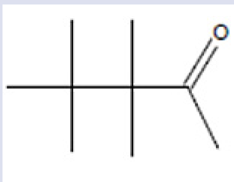
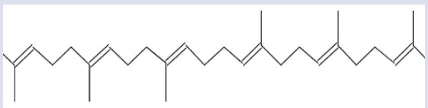
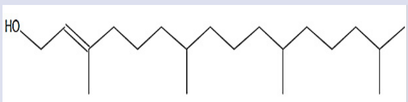
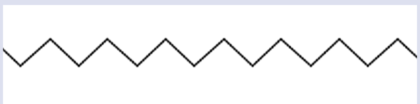
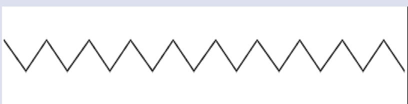
DISCUSSION

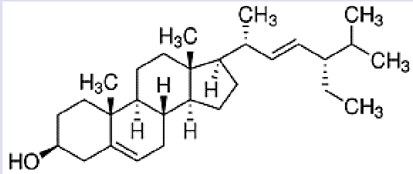
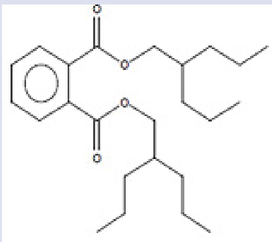
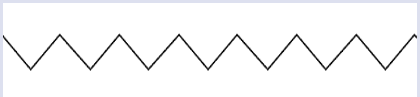
In the current study, out of various locations (Districts of Tamilnadu), the plant samples collected from Shenbagadevi falls, Courtallam showed maximum antioxidant activity and it was found to be in the order of Courtallam > Thirunelveli > Trichy > Coimbatore > Arni (Thirvannamalai) > Padavedu (Thiruvannamalai) > Chengalpattu > Dindigul. Significant variations in the antioxidant activities with respect to the sampling locations were observed. The dynamics of variation in the antioxidant content is possibly associated with the expression of variety of genes during various developmental stages of the plant or because of the environmental factors arising from seasonal variations[11-13]. In general, environmental factors like variations in the altitude, temperature, precipitation, etc varies rapidly. The sampling locations of this study falls between 36 to 1778m elevations representing diverse climatic conditions, which is associated with antioxidant activity[14]. Literature review also suggests that antioxidant activity is influenced by various species of compounds. This implies that the intake of antioxidant compounds by the *G.sylvestre* plants will have vital impact on the antioxidant activity of the plant samples collected from diverse locations. The variation of intake depends on the texture of the soil and the seasonal conditions[15]. It is noteworthy that the observed antioxidant activity is much superior to that of the total leaf extract reported recently[16].

The compounds identified in the crude extracts of *G.sylvestre* are mostly belongs to terpenes, alcohols, hydrocarbons, alkaloids and its derivatives. From the literature search, these compounds are found to be known for their therapeutic properties and are previously reported in many different medicinal plants. Some of these compounds are separately isolated in extracts and are used as antimicrobial and radical scavenging agents in medicine formulations. This study shows that the chemical compounds isolated in different crude extracts of *G.sylvestre* could be used as a vital source of antioxidant for food and pharmaceutical industry.

The crude extracts from the *G.sylvestre* leaves were subjected for GCMS analysis for identifying compounds. Various studies using GCMS has revealed the influence of different solvents in isolating the phytochemical constituents with medicinal values from crude extracts of medicinal plants[17]. The major chemical compounds identified in the *G.sylvestre* crude extracts such as Inositol, 1-deoxy- found in methanol extract, 2-Pentanone, 3,3,4,4-tetramethyl found in methanol and ethanolic extracts, Tetratriacontane and Hexadecane found in benzene, chloroform, ethyl acetate and hexane extracts, Eicosane form benzene, chloroform and ethyl acetate extracts, Heneicosane found in benzene, ethyl acetate and hexane extracts, Phthalic acid, di(2-propylpentyl) ester found in hexane and methanol extracts, Squalene, Phytol, n-Hexadecanoic acid and Stigmasterol found in all the extracts are chemically or biologically active compounds (Table 8).

Table 8: Therapeutic activity of compounds identified in the leaves of *G.sylvestre*.

S. No.	Compound Name	Structure	Nature and Therapeutic activity
1	Inositol, 1-deoxy-		Improves sensitivity to insulin and some anxiety disorders
2	2-Pentanone, 3,3,4,4-tetramethyl-		Non-central analgesic, antipyretic or antiinflammatory agents, antirheumatic agents
3	Squalene		LDP which protects skin and is an adjunctive to cancer therapy
4	Phytol		Acyclic diterpene used in making synthetic forms of vitamin E and vitamin K1
5	Tetratriacontane		Antiasthmatics, Drugs for disorders of the urinary system
6	Eicosane		Bronchodilators, Drug for throat disorders

7	n-Hexadecanoic acid		prostaglandin-E2 9-reductase) inhibitor
8	Stigmasterol		It has a role as a vitamin and a plant metabolite, Bronchodilator
9	Hexadecane		Plant metabolite
10	Phthalic acid, di(2-propylpentyl) ester		Anticancer activity
11	Heneicosane		Antineoplastic, oviposition-attractant pheromone (for trapping mosquitoes)

CONCLUSION

The present study revealed a number of compounds isolated in different solvents and its efficiency. Also, the effect of locations on the antioxidant activity exhibited by the plant was revealed. The whole plant can be used as a good source of antioxidant. Future research will be taken up for the isolation and characterization of individual compounds from the crude extracts of *Gymnema sylvestre* and tested for *in-vivo* studies for further understanding the activities of plant compounds.

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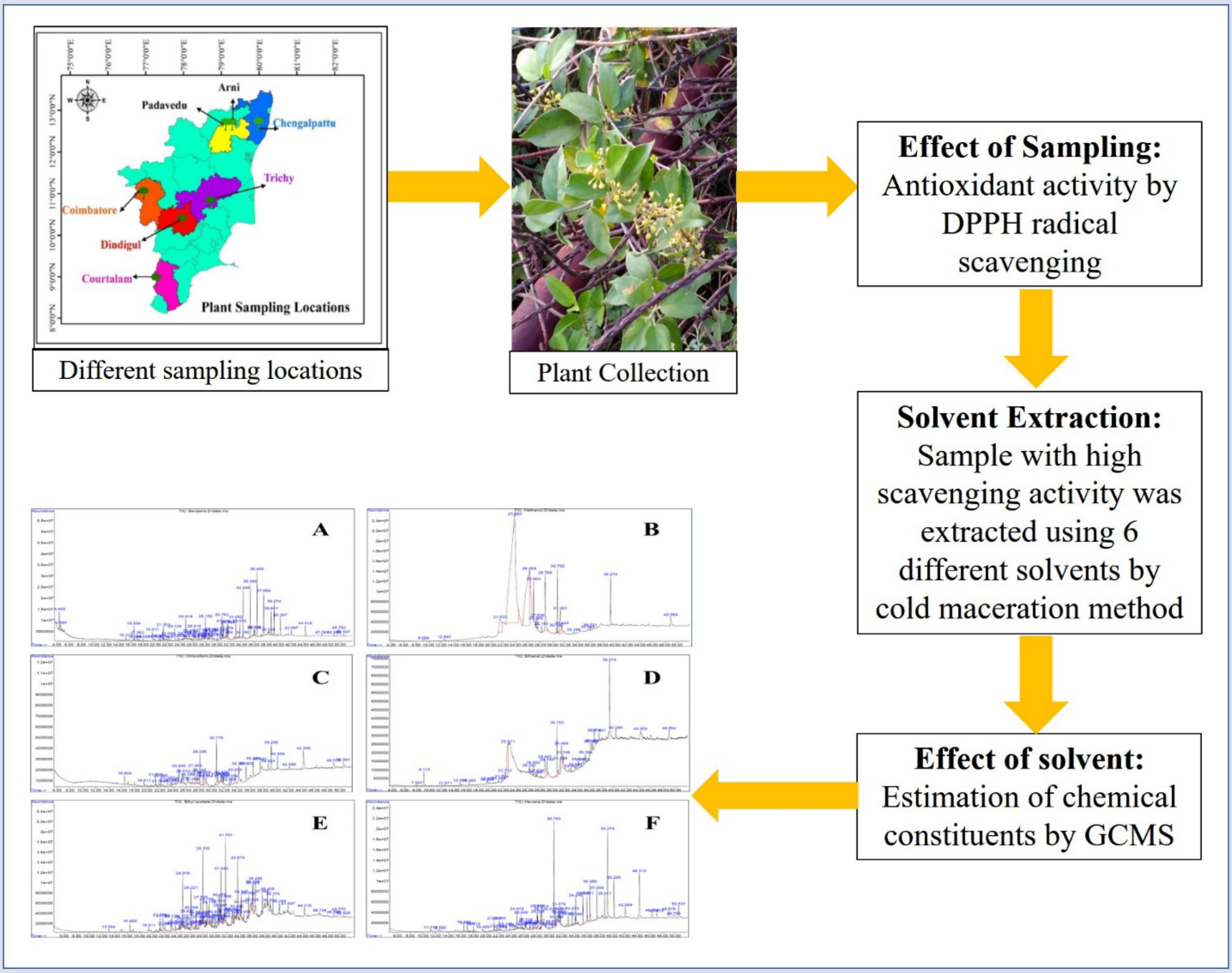
GRAPHICAL ABSTRACT



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