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 Gymnema 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.

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. Gymnema sylvestre is an important plant with medicinal properties. In local languages of India, it is called as Sakkarai koll 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 reported²⁻⁵.

Due to the side effects associated with allopathic medicines, in recent years, research interests have turned towards plant-based phytochemicals in treating various diseases⁶. Phytochemicals from medicinal plants are used in formulations of various healthcare nutraceuticals and cosmetics products. The phytochemicals of G. sylvestre have been effective in controlling diabetes⁷. 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 origins⁸. 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 phytochemicals 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 extract.

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 Courtrallam 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, Tiruvannamalai 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 of Blois, 1958. The

![Figure 1: Shows the *G. sylvestre* collection spot (at column width).](image1)

![Figure 2: *Gymnema sylvestre* collection spots around Tamil Nadu (at column width).](image2)
reaction mixture (0.1 mM 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)

\[
\text{DPPH scavenging Activity (\%)} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \tag{1}
\]

Where, \( A_{\text{control}} \) is the absorbance of DPPH without sample; \( A_{\text{sample}} \) is the absorbance of DPPH with sample[10] Cieśla et al.

**Extraction procedure**

The plant sample obtained from Courtallam 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 Courtallam 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 courtallam 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.**
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, 3,3,4,4-tetramethyl- (2.770 %), Tetracosane (2.266 %), Cetene (1.883 %).

The GC-MS analysis (Figure 5) resulted in 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 (>20 %), Phytol (8.667 %), Heptadecane, 2-methyl- (2.788 %), n-Hexadecanoic acid (2.622 %) (Table 3).

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, 1,1'-[(1-methyl-1,2-ethanediyl) bis(oxy)] bis-(5.218 %), Stigmasterol (5.966 %), Octadecane, 3-ethyl-5- (2-ethylbutyl)- (2.622 %) (Table 3).

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- (6.211 %), Inositol, 1-deoxy- (21.218 %), 2-chloroethyl hexadecyl ester (6.100 %), Tetracosane (>5 %), Eicosane (>3 %), E-15-Heptadecenal (3.801 %), Hexadecane, 2,6,11,15-tetramethyl- (3.582 %), Triacetone, 1-bromo (3.258 %), Ethanol, 2,2-dimethoxy-1,2-diphenyl- (2.964 %), Phenol, 2,4-bis(1,1-dimethylethyl)- (2.770 %), Tetracosane (2.266 %), Cetene (1.883 %).

The Table 2: Chemical composition 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)- | C14H22O2 | 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- | C13H28O2 | 198 | 1.735 |
| 12    | 25.600 | Hexadecane, 2,6,11,15-tetramethyl- | C20H42O2 | 282 | 1.396 |
| 13    | 26.233 | 1-Decan-2-yl       | C16H34O          | 242             | 0.613      |
| 14    | 26.915 | 3,7,11,15-Tetramethyl-2-hexadec-1-enol | C20H40O2 | 296 | 0.463 |
| 15    | 27.037 | Heptadecane, 2,6,10-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- | C20H42O2 | 282 | 1.656 |
| 19    | 28.206 | 7,9-Di-tert-butyl-1-oxaspiro(4,5) dec-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-oxadecen-1-yloxy)-(Z)- | C20H40O2 | 312 | 0.383 |
| 22    | 30.531 | Hexacosane        | C21H44           | 296             | 0.429      |
| 23    | 30.763 | Phytol            | C20H40O2         | 296             | 1.292      |
| 24    | 31.043 | Heptadecane, 2-methyl- | C18H38 | 254 | 1.141 |
| 25    | 31.189 | Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate | C16H20O3 | 268 | 0.929 |
| 26    | 31.469 | Octadecane, 3-ethyl-5- (2-ethylbutyl)- | C26H54 | 366 | 0.929 |
| 27    | 31.578 | Eicosane, 2-methyl- | C20H40O2 | 296 | 1.263 |
| 28    | 31.761 | n-Tetracosanol-1  | C24H52O1         | 354             | 1.115      |
| 29    | 33.673 | 1-Cyclohexylidimethyloxy-3,5-dimethylbenzene | C16H26OSi | 262 | 1.646 |
| 30    | 36.011 | Phthalic acid, di(2-propylpentyl) ester | C24H38O4 | 390 | 2.147 |
| 31    | 39.274 | Squalene          | C30H50           | 410             | 10.282     |
| 32    | 44.315 | Tetratriacontane  | C34H70           | 478             | 9.570      |
| 33    | 46.336 | Lup-2(29)-en-3-one | C30H48O | 424 | 1.420 |
| 34    | 47.079 | Tetracosane, 11-decyl- | C34H70 | 478 | 1.554 |
| 35    | 48.979 | Stigmastanol      | C29H48O          | 412             | 2.484      |
| 36    | 49.758 | Benzoic acid, 3,5-dicyclohexyl-4-hydroxy- , methyl ester | C20H28O3 | 316 | 1.787 |

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**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)-| C₁₄H₃₂          | 190              | 1.636      |
| 5     | 16.686 | 2,4-Dimethyldecane | 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 | C₂₀H₄₀ | 296 | 0.416 |
| 14    | 27.463 | Dibutyl phthalate | C₁₆H₂₂O          | 278              | 0.430      |
| 15    | 27.767 | Ethanol, 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 | C₁₆H₃₂O          | 256              | 2.622      |
| 19    | 29.119 | 10-Heneicosene (c,t) | C₂₁H₴₂          | 294              | 0.502      |
| 20    | 30.020 | Nonadecane, 2-methyl- | C₂₀H₄₂          | 282              | 0.555      |
| 21    | 30.543 | Heneicosane       | C₂₁H₴₄          | 296              | 0.956      |
| 22    | 30.762 | Phytol            | C₂₀H₄₂O         | 296              | 2.788      |
| 23    | 31.042 | Eicosane, 2-methyl- | C₂₁H₴₂          | 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 | C₁₆H₂₆O₃Si | 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          | C₂₀H₄₂O         | 282              | 7.272      |
| 30    | 37.569 | Tetraatriacontane | 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.
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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)-               | C14H32           | 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-y1 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-                               | C16H32O2         | 242             | 1.016      |
| 23    | 32.808 | 1-Cyclohexylmethylyloxy-3,5-dimethylbenzene       | C16H26O3i        | 262             | 0.982      |
| 24    | 33.673 | 1-Cyclohexylmethylyloxy-3,5-dimethylbenzene       | C16H26O3i        | 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 | Tetraatriocantane                                 | C34H70           | 478             | 3.717      |
Table 5: Chemical composition of Ethyl acetate extract of *Gymnema sylvestre* from GC-MS analysis.

| S. No | RT min | Name of compounds | Molecular formula | Molecular weight | % of total |
|-------|--------|-------------------|-------------------|------------------|------------|
| 1     | 32.808 | 1-Cyclohexylidimethylsilyloxy-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-Piperidine, 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 | Cetone | 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 | C20H40 | 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    | 36.025 | 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 | Tetraatricontane | C34H70 | 478 | 0.236 |
| 41    | 36.108 | Triaccontane, 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  | 1,3-diethoxy-1,1,3,3-tetramethyl-disiloxane | C8H22O3Si2         | 222             | 0.174     |
| 2     | 9.113  | Tetraethyl silicate   | C8H20O4Si         | 208             | 2.804     |
| 3     | 12.571 | d-Mannitol, 1-decylsulfonyl- | C16H34O7S         | 370             | 0.173     |
| 4     | 15.096 | 3-Dodecane, (E)-      | C12H24            | 168             | 0.554     |
| 5     | 16.492 | Benzene, 1,3-bis(1,1-dimethylethyl)- | C14H22            | 190             | 0.256     |
| 6     | 19.353 | 4-Trifluoroacetoxytetradecane | C16H29F3O2        | 310             | 0.389     |
| 7     | 19.499 | 2-Hexyl-1-hexanol     | C14H30O           | 214             | 0.830     |
| 8     | 21.289 | Tetradecane, 2,6,10-trimethyl- | C17H36            | 240             | 0.219     |
| 9     | 21.764 | Phenol, 2,4-bis(1,1-dimethylethyl)- | C14H22O          | 206             | 0.909     |
| 10    | 22.312 | 1,2,3,4-Cyclohexanetetrol | C6H12O4           | 148             | 3.699     |
| 11    | 22.811 | 2-Pentanone, 3,3,4,4-tetramethyl- | C9H18O            | 142             | 15.885    |
| 12    | 26.221 | 2-Dodecanol           | C12H26O           | 186             | 0.531     |
| 13    | 26.902 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | C20H40O          | 296             | 1.139     |
| 14    | 28.802 | n-Hexadecanoic acid  | C16H32O2          | 256             | 7.086     |
| 15    | 29.143 | Hexadecanoic acid, ethyl ester | C18H36O2         | 284             | 3.084     |
| 16    | 30.762 | Phytole              | C20H40O           | 296             | 6.351     |
| 17    | 31.298 | trans-13-Octadecenoic acid | C18H34O2         | 282             | 4.158     |
| 18    | 31.456 | (E)-9-Octadecenoic acid ethyl ester | C20H38O2         | 310             | 4.290     |
| 19    | 31.749 | Eicosanoic acid      | C20H40O2          | 312             | 2.859     |
| 20    | 33.052 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C26H54           | 366             | 0.564     |
| 21    | 33.746 | 9,12,15-Octadecatrienoic acid, 2,3-bis(trimethylsilyl)oxy propyl ester, (Z,Z,Z)- | C27H52O4Si2      | 496             | 0.802     |
| 22    | 34.245 | Heptadecane, 9-hexyl- | C23H48            | 324             | 1.503     |
| 23    | 34.975 | N1-Benzyl-N2(1-ethylbenzylamino)-benzamidin | C28H25N3        | 403             | 0.971     |
| 24    | 35.389 | Docosane, 11-butyl- | C26H54            | 366             | 1.003     |
| 25    | 36.485 | Tetracosane, 11-decyl- | C34H70           | 478             | 1.178     |
| 26    | 36.814 | Cholesterol          | C27H46O           | 386             | 5.966     |
| 27    | 39.274 | Squalene             | C30H50            | 410             | 15.075    |
| 28    | 44.303 | Octadecane, 1,1’-[1-methyl-1,2-ethanediyl]bis(oxy)]bis- | C39H80O2        | 580             | 5.218     |
| 29    | 48.954 | Stigmasterol         | C29H48O           | 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)-hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester | C22H41NO   | 335              | 0.214      |
| 14    | 35.645 | Phthalic acid, di(2-propylpentyl) ester | C24H38O4     | 390              | 0.128      |
| 15    | 36.011 | Phytol            | C30H50           | 410              | 1.782      |
| 16    | 39.274 | Squalene          | 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.
Discussion

In the current study, out of various locations (Districts of Tamilnadu), the plant samples collected from Shenbaga Devi falls, Courtallam showed maximum antioxidant activity and it was found to be in the order of Courtallam > Thirunelveli > Trichy > Coimbatore > Arni (Thiruvannamalai) > 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. 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. 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. It is noteworthy that the observed antioxidant activity is much superior to that of the total leaf extract reported recently.

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. 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- | ![Inositol](image) | Improves sensitivity to insulin and some anxiety disorders |
| 2     | 2-Pentanone, 3,3,4,4-tetramethyl- | ![2-Pentanone](image) | Non-central analgesic, antipyretic or antiinflammatory agents, antirheumatic agents |
| 3     | Squalene | ![Squalene](image) | LDP which protects skin and is an adjunctive to cancer therapy |
| 4     | Phytol | ![Phytol](image) | Acyclic diterpene used in making synthetic forms of vitamin E and vitamin K1 |
| 5     | Tetraatriacontane | ![Tetraatriacontane](image) | Antiasthmatics, Drugs for disorders of the urinary system |
| 6     | Eicosane | ![Eicosane](image) | Bronchodilators, Drug for throat disorders |
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

Effect of Sampling: Antioxidant activity by DPPH radical scavenging

Solvent Extraction: Sample with high scavenging activity was extracted using 6 different solvents by cold maceration method

Effect of solvent: Estimation of chemical constituents by GCMS

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