



Gas Chromatography - Mass Spectroscopy Analysis of *Senna uniflora* (Mill.) Irwin & Barneby Whole Plant

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ABSTRACT

To investigate the phytochemical constituents of ethanol of extract of *Senna uniflora* (Mill.) caesalpinaceae using GC-MS. The results of a GCMS analysis confirmed the presence of twenty six compounds. The most available compounds are 3-penten-2-one-4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl-E-1,2-benzene dicarboxylic acid, diisooctylester (diisooctyl phthalate). From the results, it can be concluded that the ethanolic extract of plant shows that the presence of 26 phytochemical constituents. Bioactive compounds like phytol and vitamin E and various other compounds also present, which infers that the use of whole plant for several health problems in traditional medicine.

1. Introduction

Plants are rich source of secondary metabolites with interesting biological activities [1]. Plants have been used as medicine for thousands of years and also a hallmark in the search of new medicine [2]. Many plant species have been used in traditional medicine to treat many health problems. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries. The application of modern instrument and technique for standardization and formulation is the need of the hour. A lot of physico and chemico data are available. But there are no advanced and modern methods to describe, the identification and quantification of active constituents in the plant materials.

Using gas chromatography-mass spectrometry is one of the best techniques to identify the bioactive constituents of alcohols, esters, alkaloids, flavonoids, long chain hydrocarbons, acids, steroids, phenolic glycosides, saponins, amino compounds and other nitrogen containing compounds etc., [3].

The taxon *Cassia sericea* S.W. which is currently accepted as *Senna uniflora* (Mill.) Irwin & Barneby belong to caesalpinaceae (Fig. 1) [4]. The common name is one leaf senna. Decoction of mature leaves laxative, useful in curing ring-worm and skin diseases [5]. It was reported for the first time from Eastern Karnataka and subsequently from Pune, Madhya Pradesh (Dhar and Thabua district). Andhra Pradesh, Kerala, Mangrol, Laxmipura villages, 24 km away from South East of Chittoargarh, Rajasthan [6], and Tamilnadu [7]. It is distributed in Brazil, West Indies, Central America and Mexico, introduced in many tropical countries as a weed. The poultice of leaves is applied to wounds and the extract of leaves is reported to cure eczema. The roots are used to combat dropsy. The plant has also been reported to smother the growth of *Parthenium hysterophorus* L. in Dharwar and Belgaum [8].

The medicinal importance of the plant is taken into consideration and, the ethanol extract of whole plant of *Senna uniflora* was analyzed for the first time in GC-MS. The literature survey reveals that information regarding the analysis of *Senna uniflora* is lacking. Hence the objective of the present study is to identify the phytochemical constituents using the GC-MS technique.



Fig. 1 *Senna uniflora* plant

2. Experimental Methodology

2.1 Plant Materials and Preparation Plant Extracts

The whole plant of *Senna uniflora* (Mill.) Irwin & Barneby was collected from Tiruchirappalli, Tamilnadu, South India. The plants were shade dried for about one week and pulverized to powder in an electrical grinder. The required quantity of the powdered whole plant was weighed and transferred to a stoppered flask, and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first 6 hours and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using a vacuum distillation unit. The final residue obtained was then subjected to GC-MS analysis. A voucher specimen was deposited with the Botanical Survey of India (BSI), Coimbatore, TN, South India under the accession number BSI/SRC/5/23/2012-13 Tech. 956 (11/09/2012).

2.2 GC-MS Analysis

The gas chromatography-mass spectrometry (GC-MS) is very useful to determine phytochemical compounds, multiple pesticides in grapes, musts and wines [9] and bio fluids like rat urine also can be studied by this method [10]. GC-MS analysis of this extract was performed using a GC Clarus 500 Perkin Elmer system and a gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with an Elite 5 MS capillary column (30 mm x 0.25 mm x 0.25 μ m df), composed of 5% of Diphenyl/95% of Dimethyl polysiloxane. For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as carrier gas at a constant flow rate of 1 mL/min and an injection volume

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of 2 μ L was employed (split ratio of 10:1). The injector temperature was 250 $^{\circ}$ C. Ion source temperature was kept at 280 $^{\circ}$ C. The total GC running time, 36 minutes mass spectra were taken at 70 V; a scan interval of 0.5 seconds, solvent delay 0-2 minutes fragments mass scan (m/z) from 45 to 450. The total MS running time was 36 minutes. The software adopted to handle mass spectra and chromatograms is called Turbomass 5.2. The relative percentage amount of each component was calculated by comparing its average peak area to the total area by the above software.

Interpretation on mass spectrum GC-MS was conducted using the library of National Institute Standard and technology (NIST) version year 2005 which has more than 62,000 patterns. The spectrum of unknown component was compared with the available spectrum in the NIST library. The name, molecular weight structure, retention time and peak area of the components of the test materials were ascertained.

3. Results and Discussion

The compounds present in the ethanol extract of whole plant of *Senna uniflora* were identified by GC-MS analysis (Fig. 2).

The active principle with their retention time (RT), molecular formula, molecular weight (MW) and peak area percentage of the whole plant of *Senna uniflora* are presented in Table 1.

The most abundant components in ethanol extract of whole plant of *Senna uniflora* are 3-penten-2-one,4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-(E)- or systematic name is (3E)-4-(2,2,6-Trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-3-penten-2-one (20.08%), 1,2-benzenedicarboxylic acid diisooctyl ester (18.3%), [1,1'-bicyclopropyl]-2-octanoic acid (2'-hexyl methyl ester) (12.77%) in addition to that vitamin E (1.06%) and phytol (2.46%).

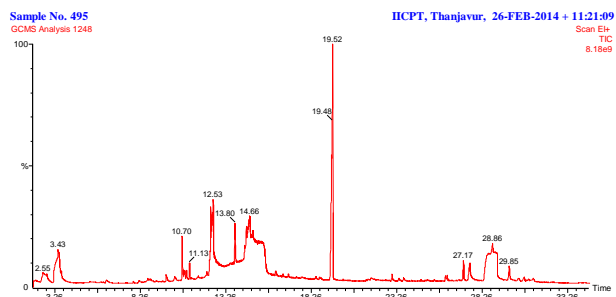


Fig. 1 GC-MS chromatogram of the whole plant ethanol extract of *Senna uniflora*

Table 1 Components detected in the whole plant ethanol extract of *Senna uniflora*

S.No.	RT	Name of the compound	Molecular Formula	MW	Peak Area%
1	2.55	DL-Arabinitol	C ₅ H ₁₂ O ₅	152	2.99
2	3.43	Acetic acid, pentyl ester	C ₇ H ₁₄ O ₂	130	8.86
3	9.77	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-	C ₁₃ H ₂₂ O ₂	210	0.53
4	10.27	á-D-Glucopyranose, 4-O-á-D-galactopyranosyl-	C ₁₂ H ₂₂ O ₁₁	342	0.56
5	10.70	10-Methyl-E-11-tridecen-1-ol propionate	C ₁₇ H ₃₂ O ₂	268	1.65
6	10.85	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	C ₁₁ H ₁₈ N ₂ O ₂	210	0.26
7	10.95	E-7-Tetradecenol	C ₁₄ H ₂₈ O	212	0.31
8	11.13	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	0.50
9	12.38	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	3.20
10	12.53	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	9.62
11	13.80	Phytol	C ₂₀ H ₄₀ O	296	2.46
12	14.66	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	C ₂₁ H ₃₈ O ₂	322	12.77
13	14.85	13,16-Octadecadiynoic acid, methyl ester	C ₁₉ H ₃₀ O ₂	290	10.54
14	19.52	1,2-Benzenedicarboxylic acid, diisooctyl ester	C ₂₄ H ₃₈ O ₄	390	18.30
15	22.99	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	C ₂₀ H ₃₄ O ₂	306	0.40
16	23.39	1-Heptatriacotanol	C ₃₇ H ₇₆ O	536	0.25
17	23.66	9,12,15-Octadecatrienoic acid, 2,3-bis(acetyloxy)propyl ester, (Z,Z,Z)-	C ₂₅ H ₄₀ O ₆	436	0.20
18	26.11	Cholestan-3-ol, 2-methylene-, (3á,5á)-	C ₂₈ H ₄₈ O	400	0.24
19	26.21	Limonen-6-ol, pivalate	C ₁₅ H ₂₄ O ₂	236	0.39
20	27.17	Vitamin E	C ₂₉ H ₅₀ O ₂	430	1.06
21	27.54	Dihydrofuran-2-one, 4-(3,4-dimethoxybenzyl)-3-(4-hydroxy-3-methoxybenzyl)-	C ₂₁ H ₂₄ O ₆	372	2.48

22	28.86	3-Penten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-(E)- or systematic name((3E)-4-(2,2,6-Trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-3-penten-2-one)	C ₁₄ H ₂₂ O ₂	222	20.08
23	29.85	2H-Pyran, 2-(7-heptadecyloxy)tetrahydro-	C ₂₂ H ₄₀ O ₂	336	1.16
24	30.40	Corymbolone	C ₁₅ H ₂₄ O ₂	236	0.34
26	30.72	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	C ₃₀ H ₅₂ O	428	0.42
26	31.26	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	C ₂₃ H ₃₂ O	324	0.43

Standardization of medicinal plants by modern techniques is useful in further research and commercial preparations. For any plant, animal (or) any organism identification is important for distinguishing itself within their group or family and among other groups and families. It has been widely accepted and observed that medicinal value of a plant lies in the bioactive compounds present in it [7].

Gas Chromatography-Mass Spectrometry (GC-MS) is one of the valuable tools to identify phytochemical compounds [3]. In the present study 26 compounds have been identified from ethanol extract of the whole plant of *Senna uniflora* by Gas Chromatography (Table 2). In Mass spectrometry analysis, among 26 identified phytochemicals some of the compounds are having many biological activities, some have few biological activities. Among them, n-Hexadecanoic acid (palmitic acid) has peak area of 9.62% and acts as antioxidant, hypocholesterolemic, nematocidal, pesticide, lubricant, antiandrogenic, flavor, hemolytic 5-alpha reductase, inhibitor, 9,12,15-octadecatrienoic acid, 2,3-bio(acetyloxy) propyl ester, (z,z,z)-has peak area 0.20% has lot of biological activity and acts as anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematocidal, insectifuge, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary. Vitamin E has 1.06% peak area and acts as antiageing, analgesic, antidiabetic, anti-inflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, anticoronary. And also Phytol has 2.46% peak area but it has very valuable activities like antimicrobial, anti-inflammatory, anticancer and diuretic properties.

Among 26 compounds some have high peak area but no activity is reported. Those compounds are 3-Penten-2-one,(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-(E)- or systematic name((3E)-4-(2,2,6-Trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-3-penten-2-one), has 20.08%, 1,2-Benzenedicarboxylic acid, diisooctyl ester has 18.30%, [1,1'-Bicyclopropyl]-2-Octanoic acid, 2'-hexyl-methyl ester has 12.77%, 13,16-octadecadiynoic acid, methyl ester has 10.54% peak area, these compounds have high percentage of peak area have reported no biological activity. Although some chemical compounds among 26 identified compounds have not being reported regarding biological activity can be investigated further to they find useful information related to biomedical applications.

Table 2 Activity of Components identified in the whole plant powder sample

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Compound Nature	**Activity
1	2.55	DL-Arabinitol	C ₅ H ₁₂ O ₅	152	2.99	Sugar alcohol	Antimicrobial
2	3.43	Acetic acid, pentyl ester	C ₇ H ₁₄ O ₂	130	8.86	Acetic acid compound	Antimicrobial
3	9.77	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-	C ₁₃ H ₂₂ O ₂	210	0.53	Ketone compound	No activity reported
4	10.27	á-D-Glucopyranose, 4-O-á-D-galactopyranosyl-	C ₁₂ H ₂₂ O ₁₁	342	0.56	Sugar moiety	Preservative
5	10.70	10-Methyl-E-11-tridecen-1-ol propionate	C ₁₇ H ₃₂ O ₂	268	1.65	Alcoholic compound	Antimicrobial;
6	10.85	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	C ₁₁ H ₁₈ N ₂ O ₂	210	0.26	Alkaloid compound	Antimicrobial Anti-inflammatory
7	10.95	E-7-Tetradecenol	C ₁₄ H ₂₈ O	212	0.31	Unsaturated alcoholic compound	No activity reported
8	11.13	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	0.50	Terpene alcohol	Antimicrobial Anti-inflammatory
9	12.38	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	3.20	Stearic acid	Antibacterial and Antifungal.

10	12.53	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	9.62	Palmitic acid	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
11	13.80	Phytol	C ₂₀ H ₄₀ O	296	2.46	Diterpene	Antimicrobial Anti-inflammatory Anticancer Diuretic No activity reported
12	14.66	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	C ₂₁ H ₃₈ O ₂	322	12.77	Ester compound	No activity reported
13	14.85	13,16-Octadecadiynoic acid, methyl ester	C ₁₉ H ₃₀ O ₂	290	10.54	Unsaturated fatty acid ester	No activity reported
14	19.52	1,2-Benzenedicarboxylic acid, diisooctyl ester	C ₂₄ H ₃₈ O ₄	390	18.30	Plasticizer compound	Antimicrobial Anti-fouling
15	22.99	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	C ₂₀ H ₃₄ O ₂	306	0.40	Unsaturated fatty acid compound	Cardio protective Hypocholesteromic
16	23.39	1-Heptatriacotanol	C ₃₇ H ₇₆ O	536	0.25	Alcoholic compound	Antimicrobial
17	23.66	9,12,15-Octadecatrienoic acid, 2,3-bis(acetyloxy)propyl ester, (Z,Z,Z)-	C ₂₅ H ₄₀ O ₆	436	0.20	Unsaturated fatty acid ester	Anti-inflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogeni, Antiarthritic, Anticoronary, Insectifuge
18	26.11	Cholestan-3-ol, 2-methylene-, (3 α ,5 α)-	C ₂₈ H ₄₈ O	400	0.24	Steroid	Antimicrobial Anti-inflammatory Anticancer Diuretic Antiarthritic Antiasthma
19	26.21	Limonen-6-ol, pivalate	C ₁₅ H ₂₄ O ₂	236	0.39	Sesquiterpene alcohol	Anti-tumor, Analgesic, Antibacterial, Antiinflammatory, Sedative, Fungicide.
20	27.17	Vitamin E	C ₂₉ H ₅₀ O ₂	430	1.06	Vitamin compound	Antiageing, Analgesic, Antidiabetic Antiinflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogeni, Vasodilator, Antispasmodic, Antibronchitic, Anticoronary
21	27.54	Dihydrofuran-2-one, 4-(3,4-dimethoxybenzyl)-3-(4-hydroxy-3-methoxybenzyl)-	C ₂₁ H ₂₄ O ₆	372	2.48	Furan compound	No activity reported

22	28.86	3-Penten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-, (E)- or systematic name((3E)-4-(2,2,6-Trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-3-penten-2-one)	C ₁₄ H ₂₂ O ₂	222	20.08	Ketone compound	No activity reported
23	29.85	2H-Pyran, 2-(7-heptadecyloxy)tetrahydro	C ₂₂ H ₄₀ O ₂	336	1.16	Pyran compound	No activity reported
24	30.40	Corymbolone	C ₁₅ H ₂₄ O ₂	236	0.34	Sesquiterpene	Anti-tumor, Analgesic, Antibacterial, Anti-inflammatory, Sedative, Fungicide. Antimicrobial
25	30.72	2,2,4-Trimethyl-3-(3,8,12,16-tetramethylheptadeca-3,7,11,15-tetraenyl)-cyclohexanol	C ₃₀ H ₅₂ O	428	0.42	Alcoholic compound	
26	31.26	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	C ₂₃ H ₃₂ O	324	0.43	Aldehyde compound	Antimicrobial Anti-inflammatory

**Source: - Dr. Duke's Phytochemical and Ethnobotanical Databases

4. Conclusion

This type of GC-MS analysis is the first step towards understanding nature of active compounds in this medicinal plant and helpful for the further detailed study. The information on the biological activities of the above compounds have taken from the Dr. Dukes phytochemical and ethno botanical databases. Further investigation and detailed Phytochemistry and pharmacological studies may add new information to the existing information in the medical field and prove the therapeutic values of *Senna uniflora*.

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