

Phytochemical profile of leaf samples of subspecies of *Senna italica* Mill.

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Abstract

Senna italica Mill is one of the medicinally important plant belonging to the family Caesalpiniaceae commonly known as 'Nilavagai'. *Senna italica* has three subspecies *italica*, *micrantha* and *arachoides*. The subspecies *italica* and *micrantha* have been taken for GC-MS analysis to identify the bioactive compounds. Twenty eight compounds were identified in the methanol leaf extract of *Senna italica* ssp *italica*. The major phytochemicals screened was 3-O-Methyl-d-glucose, 1-Butanol,3-methyl formate, Vitamin E, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, and τ -Sitosterol. Ten phytochemicals were in the methanolic leaf extract of *Senna italica* ssp *micrantha* and Hydroperoxide 1,4-diexan-2-yl, Diazoprogerone, Squalene, Stigmasterol, was the principal compounds. The two subspecies are well distinguished by their phytochemical profile. The results provide evidence to support taxonomic separation of *Senna italica* into subspecies. Most of the phytochemicals are found to be bioactive and medicinally important.

INTRODUCTION

Cassia is large genus with about 550 species belongs to the family Caesalpiniaceae. It was split into 3 genera (*Cassia*, *Senna* and *Chamaecrista*). Leaves and seeds of *Cassia* species are used as laxative and in diabetics and leprosy (Rajesham *et al.*, 2013). *Cassia* leaf extracts were found to be effective against seed borne pathogenic fungi and also used as a ethnovetinary medicine (Mogle and Maske, 2012). *Senna* species have been of medicinal interest due to their good therapeutic values in folk medicine (Mitra *et al.*, 2008). *Senna italica* is otherwise called as *Cassia italica*, *Senegal senna*, *Italian senna*, *Cassia obovata* or Port Royal Senna. It is recognized by many other common

names based on the regions it is grown. In India, it is used to produce dye known as 'neutral henna'. In many region these plants are cultivated commercially and medicinally. The decoction and maceration of leaves and pods of *S.italica* are used for skin problems such as burns and ulcers. The roots are used for liver complains, gallbladder disorders, nausea and dysmenorrhoea (Debela *et al.*, 2006). *Senna italica* is easily distinguishable through its many distinctive features. There are 3 subspecies in *S.italica* based on the size of the inflorescence and the length of the petiole. The subspecies are *italica*, *micrantha* and *arachoides*. *S.italica* ssp *italica* and *micrantha* are distributed in India.

Plants contain a variety of phytochemicals which have found very important applications on the fields of agriculture, human and veterinary medicine (Sajal *et al.*, 2014). Medicinal plants are of great importance in the field of biotechnology and most of the pharmaceutical industries depend on phytoconstituents for the production of drug to cure many diseases. So knowledge about the chemical constituents of plants is helpful in the discovery of therapeutic agents. Analysis of the phytoconstituents is important in the determination of active ingredients of medicinal plants. GC-MS is normally used for the direct analysis of the components existing in traditional medicine or medicinal plants. Perusal of previous literature reveals that there is a fragmentary report on the phytochemistry of *S.italica* ssp *italica* and *S.italica* ssp *micrantha* was evaluated for the first time for its phytoconstituents using GC-MS analysis.

MATERIALS AND METHODS

The plant material was collected from areas of Thoothukudi, Tamil Nadu. The plant was identified and authenticated by Botanical Survey of India, Southern Circle, Coimbatore as *Senna italica* ssp *italica*, *Senna italica* ssp *micrantha* (Caesalpinaceae). Voucher specimens (SMCH-33526, SMCH-33527) were preserved in Department of Botany, St. Mary's college (Thoothukudi). The leaves were shade dried and pulverized to powder in a mechanical grinder. The powder was then extracted with methanol in soxhlet apparatus (Handa *et al.*, 2008). Then the filtrate was evaporated to dryness using a rotary evaporator. The final residue obtained was then subjected to GC-MS analysis.

GC-MS analysis of leaf extracts

GC-MS analysis of the extracts were carried out with GC-MS analysis Clarus 500 Perkin Elmer system and gas chromatograph interfaced to a mass spectrometer (GC-MS) employing the following conditions: Column Elite -1 fused silica capillary column (30mm × 0.25 mm ID × 1 μm df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70eV; Helium (99.999%) was used as a carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 μl is employed (split ratio of 10.1); injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C then 5°C/min to 280°C with a 9 minute, isothermal at 280°C. Mass spectra are taken

at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da. Total GC running time was 36 min (Hema *et al.*, 2010).

Characterization of compounds

Interpretation on mass spectra of GC-MS was conducted using the data base of National Institute of Standard and Technology (NIST). The mass spectrum of the unknown compound was compared with that of the known components stored in the NIST-library. The name, molecular weight and structure of the components of the test materials were ascertained (Table 1 and 2; Fig 1 and 2).

RESULTS AND DISCUSSION

The present study identifies that presence of twenty eight phytoconstituents in the *Senna italica* ssp *italica* with the retention time ranging from 2.20 to 33.41. 3-O-Methyl-d-glucose (49.00%) and 1-Butanol, 3-methyl-, formate (11.02%) were the phytoconstituents with high peak areas. The compounds identified were Ammonium oxalate, 1-Butanol, 3-methyl-, formate, Oxirane, 2'-[oxybis(methylene)]bis-, 3-O-Methyl-d-glucose, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 1,2-Benzenedicarboxylic acid, diheptyl ester, Phytol, Pentanal, 2-methyl-, 2-Hexen-1-ol, 2-ethyl-, 1,14-Tetradecanediol, n-Decanoic acid, 1-Iodo-2-methylundecane, 1, 14- Tetradecanediol, Undecanal, 2-methyl-, 1,2-Benzenedicarboxylic acid, diisooctyl ester, Heptacosane, Octadecane, 2-methyl-, Nonadecane, 2-methyl-, Squalene, - Tocopherol, d-Mannitol, 1-decylsulfonyl-, 2-Piperidinone, N-[4-bromo-n-butyl]-, Vitamin E, 2,4,6,-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-, 9-Octadecenoic acid(Z)-, phenylmethyl ester, τ -Sitosterol, 2,4a-Epideoxy-4a,5,6,7,8,8a-hexahydro-2-[2-benzoyloxypropyl]-5,5,8a trimethyl benzopyrane, Cedran-diol, 8S,14-. The compound that occurred with highest peak area is 3-O-Methyl-d-glucose and the compound with lowest peak area is 2-Hexen-1-ol-2-methyl.

There were ten phytoconstituents present in the *Senna italica micrantha*. The retention time was ranging from 10.36 to 31.68 mins. Hydroperoxide, 1,4-dioxan-2-yl (25%) and Diazoprogestone (23.26%) were the phytoconstituents with high peak areas. The other compounds identified were Hydroperoxide, 1,4-dioxan-2-yl, Nonamethylene glycol, Cyclohexanol, 3,5-dimethyl-, Decane 1-iodo-, 1,2-Benzenedicarboxylic acid, diisooctylester, Tridecane, 1-iodo-, Tetradecane, 1-iodo-, Squalene,

Stigmasterol, and Diazoprogerone.

The identified compounds possess many biological activities ,for instance 3-Methyl formate (3.38R/T) Alcoholic compound ,1-Iodo-2-Methylundecane (18.51R/T) Iodo compound possess antimicrobial activity. Phytol (14.62R/T),a diterpene with antimicrobial, anticancerous, cancer preventive and anti-inflammatory properties(Praveen *et al.*, 2010). Stigmasterol (30.52R/T), a steroid with antimicrobial , anticancerous, anti-inflammatory, anti-asthma, and anti-arthritic properties (Jegajeevanram *et al.* 2014). 1, 2 –Benzenedicarboxylic acid, diisooctylester

(20.45R/T) plasticiser compound with antifouling and antimicrobial properties (Sermakkani and Thangapandian 2012). Squalene is a triterpene involved in Vitamin- D synthesis and it is able to product human against cancer (Sheela and Uthayakumari ,2013). Most of the phytocomponents identified from the leaf samples have anticancerous, antioxidant, anti-inflammatory, antidiabetic and hepatoprotective activity (Table 1 & 2).Further studies are needed to isolate actual phytoconstituents involved in the bioactivity and the exact mechanism of action at the molecular level.

Table 1: Phytocomponents identified from the leaf methanol extracts *Senna italica* ssp *italica* by GC-MS analysis

No	RT	Name of the compound	Molecular formula	MW	Peak Area %	Compound Nature	**Activity
1	2.20	Ammonium oxalate	C ₂ H ₈ N ₂ O ₄	124	1.27	Oxalate compound	No activity reported
2	3.38	1-Butanol, 3-methyl-, formate	C ₆ H ₁₂ O ₂	116	11.02	Alcoholic compound	Antimicrobial
3	7.93	Oxirane, 2,2'-[oxybis(methylene)]bis-	C ₆ H ₁₀ O ₃	130	1.47	Oxirane compound	No activity reported
4	10.69	3-O-Methyl-d-glucose	C ₇ H ₁₄ O ₆	194	49.00	Sugar moiety	Preservative
5	11.35	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	4.23	Terpene alcohol	Antimicrobial, Anti-inflammatory
6	12.83	1,2-Benzenedicarboxylic acid, diheptyl ester	C ₂₂ H ₃₄ O ₄	362	0.13	Plasticizer compound	Antimicrobial, Anti-fouling
7	14.62	Phytol	C ₂₀ H ₄₀ O	296	2.49	Diterpene	Antimicrobial, Anti-inflammatory Anticancer, Diuretic
8	16.96	Pentanal, 2-methyl-	C ₆ H ₁₂ O	100	2.14	Aldehyde compound	Antimicrobial, Anti-inflammatory
9	17.10	2-Hexen-1-ol, 2-ethyl-	C ₈ H ₁₆ O	128	0.08	Unsaturated compound	No activity reported
10	17.57	1,14-Tetradecanediol	C ₁₄ H ₃₀ O ₂	230	0.10	Alcoholic compound	Antimicrobial
11	18.42	n-Decanoic acid	C ₁₀ H ₂₀ O ₂	172	0.12	Fatty acid	Insecticide
12	18.51	1-Iodo-2-methylundecane	C ₁₂ H ₂₅ I	296	0.42	Iodine compound	Antimicrobial
13	18.99	1,14-Tetradecanediol	C ₁₄ H ₃₀ O ₂	230	0.13	Alcoholic compound	Antimicrobial
14	19.44	Undecanal, 2-methyl-	C ₁₂ H ₂₄ O	184	0.30	Aldehyde compound	Antimicrobial, Anti-inflammatory

15	20.45	1,2-Benzenedicarboxylic acid, diisooctyl ester	C ₂₄ H ₃₈ O ₄	390	0.75	Plasticizer compound	Antimicrobial, Anti-fouling
16	21.33	Heptacosane	C ₂₇ H ₅₆	380	2.04	Alkane compound	No activity reported
17	22.73	Octadecane, 2-methyl-	C ₁₉ H ₄₀	268	2.67	Alkane compound	No activity reported
18	24.11	Nonadecane, 2-methyl-	C ₂₀ H ₄₂	282	1.89	Alkane compound	No activity reported
19	24.27	Squalene	C ₃₀ H ₅₀	410	3.98	Triterpene	Antibacterial, Sunscreen, Antitumor, Cancer-preventive, Perfumery Chemo preventive, Immunostimulant Pesticide, Lipoxygenase inhibitor, Antioxidant,
20	27.46	-Tocopherol	C ₂₈ H ₄₈ O ₂	416	0.57	Vitamin E Compound	Analgesic, Anticataract Anticoronary, Antidiabetic Antiaging, Antioxidant Hepatoprotective, Vasodilator, Protein-kinase-c-inhibitor, Anticancer Lipoxygenase inhibitor Antitumor, Antibronchitic
21	28.16	d-Mannitol, 1-decylsulfonyl-	C ₁₆ H ₃₄ O ₇ S	370	0.34	Sulfur compound	Antimicrobial
22	28.31	2-Piperidinone, N-[4-bromo-n-butyl]-	C ₉ H ₁₆ BrNO	233	1.45	Alkaloid	Anti-inflammatory Antimicrobial Anticancer
23	28.55	Vitamin E	C ₂₉ H ₅₀ O ₂	430	6.27	Vitamin E	Analgesic, Anticataract Anticoronary, Antidiabetic Antioxidant, Hepatoprotective, Vasodilator, Protein-kinase-c-inhibitor Anticancer, Lipoxygenase inhibitor Antitumor
24	30.01	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	C ₁₃ H ₂₂ OSi ₂	250	0.35	Ketone compound	No activity reported
25	30.49	9-Octadecenoic acid (Z)-, phenylmethyl ester	C ₂₅ H ₄₀ O ₂	372	1.03	Oleic acid ester	Insectifuge, Perfumery Cancer-Preventive, Dermatitogenic Anti-inflammatory, Antiandrogenic, Hypocholesterolemic Flavor, Anemiagenic, Allergenic
26	31.66	ς-Sitosterol	C ₂₉ H ₅₀ O	414	3.82	Steroid	Antimicrobial, Anti-inflammatory Hepatoprotective, Diuretic, Anticancer
27	32.33	2,4a-Epidioxy-4a,5,6,7,8,8a-hexahydro-2-[2-benzoyloxypropyl]-5,5,8a-	C ₂₂ H ₂₈ O ₅	372	0.90	Aromatic compound	No activity reported

		trimethylbenzopyr ane					
28	33.41	Cedran-diol, 8S,14-	$C_{15}H_{26}O_2$	238	1.06	Sesquiterpen alcohol	Antimicrobial, Anti- inflammatory Anti-cancer, Fragrance compound

**Dr. Duke's Phytochemical and Ethnobotanical Database

Table 2 : Phytocomponents identified from the leaf methanol extracts *Senna italica* ssp *micrantha* by GC-MS analysis

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %	Compound Nature	**Activity
1	10.36	Hydroperoxide, 1,4-dioxan-2-yl	$C_4H_8O_4$	120	25.00	Peroxide compound	No activity reported
2	11.34	Nonamethylene glycol	$C_{11}H_{24}O_3$	204	1.74	Alcoholic compound	Antimicrobial
3	14.63	Cyclohexanol, 3,5-dimethyl-	$C_8H_{16}O$	128	1.16	Phenolic compound	Antimicrobial, Antioxidant Analgesic, Anti-inflammatory
4	19.93	Decane, 1-iodo-	$C_{10}H_{21}I$	268	1.74	Alkane	No activity reported
5	20.46	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	8.14	Plasticizer compound	Antimicrobial, Antifouling
6	21.34	Tridecane, 1-iodo-	$C_{13}H_{27}I$	310	2.91	Iodine compound	Antimicrobial
7	22.75	Tetradecane, 1-iodo-	$C_{14}H_{29}I$	324	3.49	Iodine compound	Antimicrobial
8	24.28	Squalene	$C_{30}H_{50}$	410	18.02	Triterpene	Antibacterial, Sunscreen Antitumor, Cancer-preventive, Perfumery Chemo preventive Immunostimulant Pesticide, Lipoxygenase inhibitor, Antioxidant
9	30.52	Stigmasterol	$C_{29}H_{48}O$	412	14.53	Steroid	Antimicrobial Anti-inflammatory Anti-asthma, Hepatoprotective Diuretic, Anticancer
10	31.68	Diazoprogesterone	$C_{21}H_{30}N_4$	338	23.26	Steroid	Antimicrobial Anti-inflammatory Hepatoprotective Diuretic, Anticancer

**Dr. Duke's Phytochemical and Ethnobotanical Databases

Fig 1: GC-MS Chromatogram of leaf methanolic extract of *Senna italica* ssp *italica*

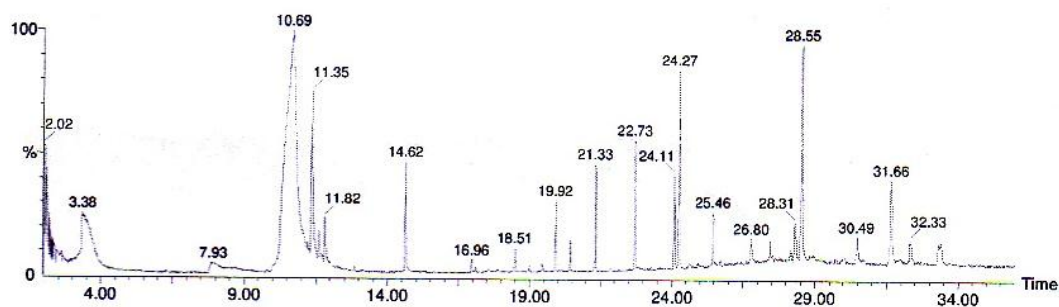
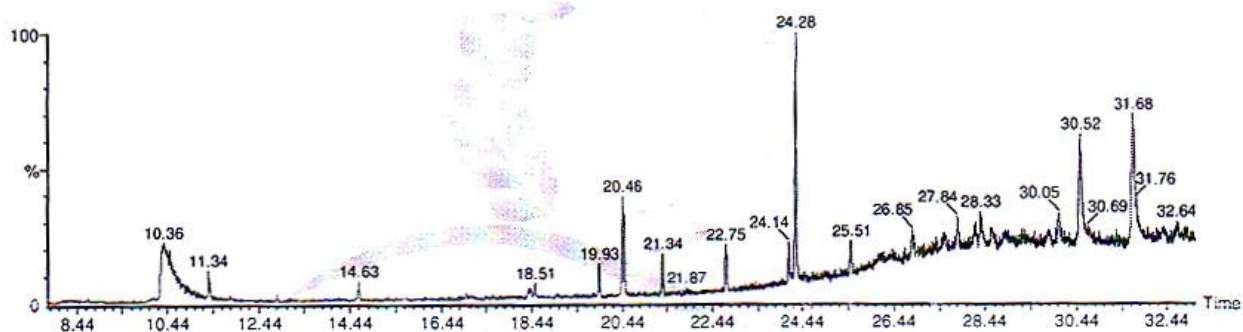


Fig 2:GC-MS Chromatogram of leaf methanolic extract of *Senna italica ssp micrantha***ACKNOWLEDGEMENT**

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