

PHYTOCHEMICAL STUDIES AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF *SARCOSTEMMA BREVISTIGMA*, WIGHT & ARN.

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ABSTRACT

Objective: To explore the phytochemical constituents present in *Sarcostemma brevistigma* using gas chromatography-mass spectrometry (GC-MS).

Methods: Preliminary phytochemical analysis of secondary metabolites was made by following standard procedures. GC-MS analysis of the plant extracts was performed by using GC-MS equipped with a DB-35MS capillary standard non-polar column and gas chromatograph interfaced to a Mass Selective Detector (MS-DSQ-II) with Xcalibur software.

Results: The qualitative phytochemical analysis of this species exhibited the presence of alkaloids, glycosides, flavonoids, phenols, steroids, amino acids, tannins, terpenoids, quinones, and coumarin. The GC-MS analysis revealed the presence of 24 compounds in the ethanolic extract of aerial part of *Sarcostemma brevistigma*. The most prevailing compound was 14,17 bis(dimethylaminoamino)[3.3]paracyclophan-5,8-diacetonitrile (14.86%).

Conclusion: The study concludes that the species *Sarcostemma brevistigma* is a potential source for bioactive compounds such as esters, alkanes, alcohols, alkenes, amide, amine, phenol, ketone, and so forth. This study justifies the traditional usage of this species.

Keywords: Gas chromatography-mass spectrometry, *Sarcostemma brevistigma*, Phytochemical compounds, Medicinal plants.

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INTRODUCTION

For millennia, people around the world have healed the sick with herbal derived remedies and handed down through generations. Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illness [1]. Various types of traditional medicine and other medical practices referred to as complementary or alternative medicine are increasingly used in both developing and developed countries.

Presently, there is an increasing interest worldwide in herbal medicines accompanied by increased laboratory investigations into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases. Various drugs have entered into the international market through exploration of ethnopharmacology and traditional medicine. Although scientific studies are carried out on a large number of plants, smaller number of marketable drugs or phytochemical entities has entered the evidence-based therapeutics [2]. Even today, bioactive compounds from plants continue to play a major role in health-care benefits [3]. GC for bioactive components is the more appropriate technique to identify the new phytochemicals of medicinal importance which have higher activity against many diseases [4,5].

Sarcostemma brevistigma, Wight & Arn. is a potential medicinal plant belonging to the family Asclepiadaceae. It is used in the traditional systems of medicine for various ailments. The decoction of the plant is useful to gargle for throat and mouth infection. Fresh roots are prescribed for jaundice [6,7]. The plant is hot, bitter, tonic, expectorant, pungent, dry and indigestible causes flatulence, diuretic, laxative, aphrodisiac, anthelmintic, useful in leukoderma and bronchitis. The juice is used in gleet, gonorrhoea, pain in the muscles, cough and given to children as an astringent [8]. Leaf powder stimulates articular system, increases secretion of urine and activates uterus [9]. The fruit

juice is used in gonorrhoea and to relieve pain in muscles [10]. The leaves, roots, and latex are employed in treating asthma, rheumatism, arthritis, chronic ulcer, fever, cough, snake bite, bronchitis, dysentery, purgative, leprosy, tumor, vesicant, constipation, skin diseases, and stomach distension [11] in Tamil Nadu, India. However, no much scientific validation has been made for this species for its medicinal uses.

To address the lacuna, this study was aimed to evaluate the phytochemical compounds present in the ethanolic extract of aerial part of *Sarcostemma brevistigma* using gas chromatography-mass spectrometry (GC-MS) analysis.

METHODS

Chemicals

In this study, all the chemicals were purchased from Hi Media Pvt. Ltd., Mumbai. The chemicals used were of analytical grade.

Collection and identification of plant material

Sarcostemma brevistigma was collected from Pillur Beat (Pillur slope RF and Nellithurai RF), Karamadai Range, Western Ghats, Tamil Nadu, India. The authenticity of the plant was confirmed in Botanical Survey of India, Southern Circle, Coimbatore by referring the deposited specimen. The voucher number of the specimen was BSI/SRC/5/23/2015/Tech./2334.

Preparation of extract

The aerial parts were washed under running tap water, shade dried at room temperature, and powdered. The powdered plant sample (50 g/250 ml) was extracted successively with ethanol, methanol, hexane and water using Soxhlet apparatus at 55-850°C for 8-10 hrs to extract the polar and non-polar compounds [12]. For each solvent extraction, the powdered pack material was air dried and then used. The solvents of the respective extracts were reduced under room

temperature and stored at 4°C for further use. The dried plant extracts were then redissolved in dimethyl sulfoxide to get the solution of 10 mg/10 ml for each extract which was subjected to analysis of phytochemicals and GC-MS studies.

Phytochemical screening

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in ethanol, methanol, hexane, and aqueous extract of aerial part of test plant.

GC-MS analysis

Ethanol extract of aerial part of *Sarcostemma brevistigma* was analyzed for the presence of different volatile compounds by GC-MS technique. GC-MS analysis of some of the potent volatile constituents present in the extract was performed at the South India Textile Research Association, Coimbatore, Tamil Nadu, India. GC analysis of the extracts was performed using a GC-MS (Model; Thermo Trace GC Ultra Ver. 5.0, Thermo MS DSQ II) equipped with a DB-35MS capillary standard non-polar column (30 m length × outside diameter 0.25 mm × internal diameter 0.25 μm) and gas chromatograph interfaced to a Mass Selective Detector (MS-DSQ-II) with Xcalibur software. For GC-MS detection, an electron ionization system with ionization energy of -70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1 ml/minutes and the sample injected was 1 μl; Injector temperature 260°C; Ion source temperature 200°C. The oven temperature was programmed from 75° to 260°C at the rate of 10°C/minutes, held isothermal for 1 minute and finally raised to 260°C at 6°C/minutes. Interface temperature was kept at 260°C. Total GC run time was 37.53 minutes. The relative percentage of each extract constituent was expressed as a percentage with peak area normalization.

Identification of bioactive compounds

The identification of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST (Mc Lafferty, 1989), WILEY (Stein, 1990) library sources were also used for matching the identified components from the plant material.

RESULTS

The preliminary phytochemical screening of *Sarcostemma brevistigma* revealed the presence of alkaloids, glycosides, flavonoids, phenols, saponins, steroids, amino acids, tannins, terpenoids, quinones, anthraquinones, and coumarin (Table 1).

The total ion chromatogram of ethanol extract of *Sarcostemma brevistigma* showing the GC-MS profile of the compounds identified was given in Fig. 1. The peaks in the chromatogram were integrated and were compared with the database of the spectrum of known

components stored in the GC-MS library. The detailed tabulation of GC-MS analysis was given in Table 2.

A total of 24 compounds were identified in the ethanolic extract of the plant. The identification of phytochemical compounds was based on the peak area, retention time, molecular weight, and molecular formula. The highest peak area (%) of 14.86 was obtained by 14,17 bis(dimethylamino amino)[3,3] paracyclophan-5,8-diacetonitrile (cyanide) with retention time 34.66 and the lowest peak area (%) of 1.73 was obtained by O,O-dipropyl isopropylphosphonate (phenol) with retention time 32.32.

DISCUSSION

The phytochemical analysis revealed the presence of alkaloids, glycosides, flavonoids, phenols, saponins, steroids, tannins, terpenoids, anthraquinones, quinones, starch, gum, amino acid, coumarin, and fixed oil (Table 1). These phytochemicals exhibited a wide range of biological effects as consequence of their antioxidant properties [13,14].

The bioactive compounds phenols, flavonoids, alkaloids, tannins, and many other compounds have been reported to be free radical scavengers and inhibitors of lipid peroxidation [15], and these compounds show important properties such as anticancer, hepatoprotective effect, antioxidant, antiglycemic, anti-inflammatory, wound healing, analgesic, and many more [16,17]. The antioxidant properties of phenolic acids and flavonoids were due to their redox properties, ability to chelate metals, and quenching of singlet oxygen [18].

Alkaloids have been associated with medicinal uses for centuries, and one of their common biological properties was their cytotoxicity [19],

Table 1: Qualitative phytochemical screening of the plant extracts of *Sarcostemma brevistigma*

S. No.	Phyto constituents	Aerial parts of <i>Sarcostemma brevistigma</i>			
		Hexane	Ethanol	Methanol	Aqueous
1.	Alkaloids	In traces	++	++	In traces
2.	Flavonoids	++	+++	+++	++
3.	Quinones	In traces	++	In traces	In traces
4.	Phenols	++	++	++	++
5.	Tannins	++	++	++	++
6.	Saponins	-	+	-	-
7.	Steroids	+	++	++	+
8.	Antraquinones	+	+	+	+
9.	Terpenoids	++	++	++	+
10.	Coumarin	+	+	+	++
11.	Glycosides	+++	+++	+++	+++

(+++): Highly present, (++) : Moderately present, (+): Low Present, (-): Absent

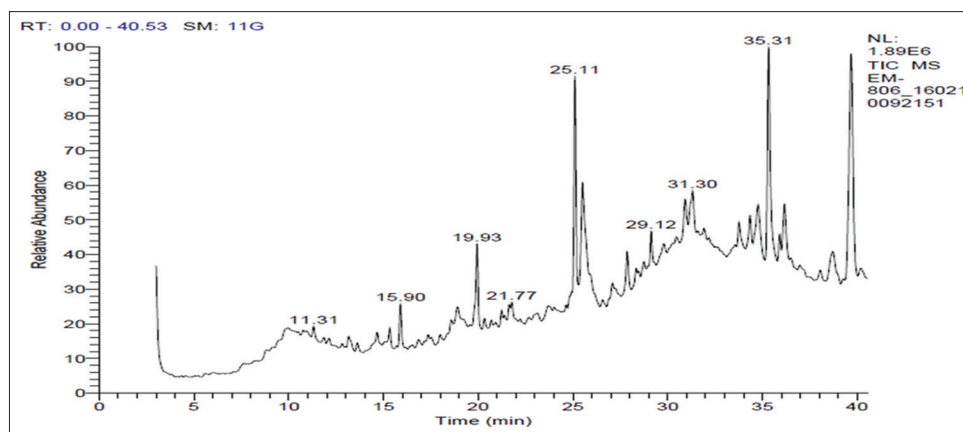


Fig. 1: Gas chromatography-mass spectrometry chromatogram of ethanolic extract of *Sarcostemma brevistigma*

Table 2: Bioactive compounds identified in the ethanolic extract of *Sarcostemma brevistigma*

S. No.	RT	Compound name	Molecular formula	Molecular weight	Area %	Functional groups	Biological activities
1.	9.95	3,4[methylenedioxy] phenethylamine	C ₉ H ₁₁ NO ₂	165	1.33	Amine	Analgesics that are pain killers, increase brain function, disinfection of drinking water
2.	17.32	9-chloro-9-methoxy-9H-fluorene	C ₁₄ H ₁₁ ClO	230	1.53	Methyl ester	Antiinflammation and antimicrobial activity
3.	20.89	Butyl glycol acetate	C ₈ H ₁₆ O ₃	160	2.58	Ester	Antioxidant, flavour, hypocholesterolemic, nematocidal, pesticide, lubricant, antiandrogenic, haemolytic, 5-alpha reductase inhibitor
4.	25.78	5,10-dimethyl-6,8-bisdehydropentadecavalene	C ₂₀ H ₁₆	256	2.98	Alkene	Anti-HIV activity and anticancerous activity
5.	26.37	Methyl 2-diazo-3-oxo-4-propylhept-6-enoate	C ₁₁ H ₁₆ N ₂ O ₃	224	1.08	Ester	Antioxidant, flavour, hypocholesterolemic, nematocidal, pesticide, lubricant, antiandrogenic, haemolytic, 5-alpha reductase inhibitor
6.	27.02	1-(dimethylamino)-4,5-dihydro-3-methyl-1H-benz[<i>g</i>] indole	C ₁₅ H ₁₈ N ₂	226	1.12	Amine	Analgesics that are pain killers, increase brain function, disinfection of drinking water
7.	29.24	Cyclohexane, 1,3,5-trimethyl-2-octadecyl- (CAS)	C ₂₇ H ₅₄	378	4.50	Alkane	Antimicrobial agents, transducer for immunosensor and its method of production, carcinogens, enzymes inhibitors
8.	30.04	6-Methyl-14H-benzo[6,7]cyclohepta[1,2- <i>b</i>] naphtha [1,2- <i>d</i>] indole-14-one	C ₂₂ H ₁₅ NO	309	1.22	Ketone	Dry asthma, colds, flu and dry cough
9.	30.53	7-Hydroxycyclopentachromenol	C ₁₆ H ₂₁ BrO ₃	340	1.89	Hydroxyl	Antimicrobial activity
10.	31.26	Cis-2,3-epoxy-1-cyclohexanol	C ₆ H ₁₀ O ₂	114	2.28	Alcoholic compound	Antimicrobial activity
11.	31.85	7-hydroxymethyl-1-bromo-4-isopropoxy-5-methoxy naphthalene	C ₁₅ H ₁₇ BrO ₃	324	4.64	Aromatic bicyclic compound	Anticancerous activity
12.	32.32	O, O-dipropyl isopropylphosphonate	C ₉ H ₂₁ O ₃ P	208	1.73	Phenol	Antioxidant, analgesics antipyretic and antiinflammatory
13.	32.87	Tricarbonyl [(4)-diethyl-2,5-dihydro-2,3-dimethyl-exo-2-phenyl-1H-1,2,5 azasilaborol] iron	C ₁₇ H ₂₂ BFeNO ₃ Si	383	2.87	Phenol	Antioxidant, analgesics, antipyretic and antiinflammatory
14.	33.32	Methyl-2,3,4-Tris-O-[9 borabicyclo[3.3.1]Non-9-Yl]-D-Xylopyranoside	C ₃₀ H ₅₁ B ₃ O ₅	524	2.47	Heterocyclic compound	Antiinflammatory, anti-HIV, anticancerous, antifungal and antiviral activity
15.	33.68	2',3',5,6,6',7'-hexamethylspiro [benzofuran-3 (2H)-9'-[9 H]-xanthen-2-one	C ₂₆ H ₂₄ O ₃	384	5.49	Ketone	Dry asthma, colds, flu and dry cough
16.	33.97	3,4 bis (3,4,5-trimethoxyphenyl)-1-[2-(4-methoxyphenyl) ethyl] pyrrole-2,5-dicarboxylic acid	C ₃₃ H ₃₅ NO ₁₁	621	2.90	Carboxylic compound	Joint pain and fever
17.	34.66	14,17 bis (dimethylaminoamino)[3.3] paracyclophan-5,8-diacetonitrile	C ₂₆ H ₃₂ N ₄	400	14.86	Cyanide	Tuberculosis and leprosy

(Contd...)

Table 2: (Continued)

S. No.	RT	Compound name	Molecular formula	Molecular weight	Area %	Functional groups	Biological activities
18.	35.11	6á-Acetylamido-5à-hydroxyandrostane-3á,7á-diacetate	C ₂₅ H ₃₉ NO ₆	449	1.31	Ester	Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, haemolytic, 5-α reductase inhibitor
19.	35.60	1-(3-chloro-phenyl)-5-[(2-mercapto-phe nylamino)-methylene]-2-thioxo-dihyd ro-pyrimidine-4,6-dione	C ₁₇ H ₁₂ ClN ₃ O ₂ S ₂	389	7.66	Diketone	Antiulcer, and gastroprotective drugs, antiasthmatics and lung diseases, carcinogenic agents, antidiabetic agents
20.	36.23	Butanesulfonic acid, 4-(diphenylphosphinyl)-, methyl ester	C ₁₇ H ₂₁ O ₄ PS	352	3.15	Methyl ester	Antiinflammation and antimicrobial activity
21.	37.49	12-Hydroxy-9-methyl-5H,11H-pyrano[3',2':6,7][1]benzopyrano[3,4-c]pyridine-5,11-dione (Isoschumanniphytine)	C ₁₆ H ₉ NO ₅	295	5.01	Diketone	Antiulcer, and gastroprotective drugs, antiasthmatics and lung diseases, carcinogenic agents, antidiabetic agents
22.	37.76	1-(ethoxycarbonyl)-5-chloro-4-methoxy-3-ethylpent an-2-one	C ₁₁ H ₁₉ ClO ₄	250	2.51	Ketone	Dry asthma, colds, flu and dry cough
23.	38.70	N-fluoresceinylpropanamide	C ₂₃ H ₁₅ NO ₆	401	1.74	Amide	Anesthetic agents
24.	39.39	2,9-bis[(diethoxyphosphinyl)methyl]-1,10-phenanthroline	C ₂₂ H ₃₀ N ₂ O ₆ P ₂	480	1.68	Amine	Analgesics that are pain killers, increase brain function, disinfection of drinking water

used as central nervous system stimulant, topical anesthetic in ophthalmology, powerful pain reliever [20], antipyretic, antispasmodic, and antibacterial [21]. Cardiac glycosides were known to lower the blood pressure according to many reports [22].

In general, the reliability of medicinal plant for its usage was evaluated by correlating the phytochemical compounds with their biological activities [23]. GC-MS analysis of plant extract showed the presence of 24 compounds (Table 2 and Fig. 1). Out of these, various compounds have got their applications in pharmaceutical industries. The methyl ester in the plant extract had anti-inflammatory, antiandrogenic, antioxidant, hypercholesterolemic and antimicrobial activities [24-26] and the esters have been reported to have antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, hemolytic, 5-α reductase inhibitor activities. Alkanes were antimicrobial agents, transducer for immunosensor and anticarcinogens [27]. Ketone could act on dry asthma, colds, flu, and dry cough. Phenol was an antioxidant and showed analgesics, antipyretic and anti-inflammatory properties. As GC-MS was the first step toward understanding the nature of active principles [28,29], further investigation in this species was suggested for the development of novel drugs.

As per our knowledge, this is the first study on the identification of important phytoconstituents in ethanolic extract of *Sarcostemma brevistigma* aerial parts. Plant extract has shown the presence of various compounds of pharmaceutical and industrial importance. Therefore, aerial parts could be used for the sourcing of these compounds from the extract prepared through the method described herein.

CONCLUSION

The results obtained in this study thus suggested that the aerial part of *Sarcostemma brevistigma* was an increasingly valuable reservoir of potential bioactive compounds with socioeconomic importance. This study also helps to predict the formula and structure of biomolecules

which can be used as drugs. Further investigation may lead to the development of drug formulation.

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