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 marketed 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, anthelmingtic, useful in leukoderma and bronchitis. The juice is used in gleet, gonorrhea, pain in the muscles, cough and given to children as an astringent [8]. Leaf powder stimulates articulatory system, increases secretion of urine and activates uterus [9]. The fruit juice is used in gonorrhea 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 55-85°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**

Ethanolic 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 using a GC-MS (Model: Thermo Trace GC Ultra Ver. 5.0, Therermo DSQ II), equipped with a DB-35MS capillary standard non-polar column (30 m length × 0.25 mm × internal diameter 0.25 µm) and gas chromatograph interfaced to a Mass Selective Detector (MSD) with Xcalibur software. For GC-MS detection, an electron ionization system with ionization energy of −70 eV was used. Helium was used as a carrier gas at a constant flow rate of 1 ml/minutes and the 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 libraries. NIST (Mc Lafferly, 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, tannins, terpenoids, and coumarin (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 antitumor, hepatoprotective effect, antioxidant, 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].

**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].

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

| S. No. | Phyto constituents | Aerial parts of Sarcostemma brevistigma |
|-------|-------------------|---------------------------------------|
| 1.    | Alkaloids         | In traces (++ + + +)                   |
| 2.    | Flavonoids        | ++ ++ ++ ++ ++                        |
| 3.    | Quinones          | In traces ++ + + + ++                  |
| 4.    | Phenols           | ++ ++ ++ ++ +                         |
| 5.    | Tannins           | ++ ++ ++ ++ +                         |
| 6.    | Saponins          | ++ ++ ++ ++ +                         |
| 7.    | Steroids          | ++ ++ ++ ++ +                         |
| 8.    | Anthraquinones    | ++ ++ ++ ++ +                         |
| 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*
| S. No. | RT | Compound name                                                                 | Molecular formula | Molecular weight | Area % | Functional groups | Biological activities                                                                 |
|-------|----|--------------------------------------------------------------------------------|--------------------|------------------|--------|-------------------|---------------------------------------------------------------------------------------|
| 1.    | 9.95 | 3,4-[methyleneoxy] phenethylamine                                             | C<sub>9</sub>H<sub>11</sub>N<sub>2</sub>O<sub>2</sub> | 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<sub>14</sub>H<sub>11</sub>C<sub>10</sub>O<sub>2</sub> | 230             | 1.53   | Methyl ester      | Antiinflammation and antimicrobial activity, Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antia|drgenic, haemolytic, 5-alpha reductase inhibitor |
| 3.    | 20.89 | Butyl glycol acetate                                                          | C<sub>8</sub>H<sub>16</sub>O<sub>3</sub> | 160             | 2.58   | Ester             | Antiinflammation and antimicrobial activity, Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antia|drgenic, haemolytic, 5-alpha reductase inhibitor |
| 4.    | 25.78 | 5,10-dimethyl-6,8-bisdehydropropentadecafulalene                               | C<sub>20</sub>H<sub>16</sub> | 256             | 2.98   | Alkene            | Anti-HIV activity and anticancerous activity, Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antia|drgenic, haemolytic, 5-alpha reductase inhibitor |
| 5.    | 26.37 | Methyl 2-diazo-3-oxo-4-propylhept-6-enoate                                     | C<sub>11</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub> | 224             | 1.08   | Ester             | Anti-HIV activity and anticancerous activity, Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antia|drgenic, haemolytic, 5-alpha reductase inhibitor |
| 6.    | 27.02 | 1-[dimethylamino]-4,5-dihydro-3-methyl-1H-benz[\text{g}] indole                | C<sub>2</sub>H<sub>16</sub>N<sub>2</sub> | 226             | 1.12   | Amine             | Anti-HIV activity and anticancerous activity, Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antia|drgenic, haemolytic, 5-alpha reductase inhibitor |
| 7.    | 29.24 | Cyclohexane, 1,3,5-trimethyl-2-octadecyl-\{CAS\}                                | C<sub>2</sub>H<sub>16</sub> | 378             | 4.50   | Alkane            | Anti-HIV activity and anticancerous activity, Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antia|drgenic, haemolytic, 5-alpha reductase inhibitor |
| 8.    | 30.04 | 6-Methyl-14H-benzo[6,7]cyclohepta[1,2-b]naphtha [1,2-d] indole-14-one           | C<sub>2</sub>H<sub>10</sub>NO | 309             | 1.22   | Ketone            | Anti-HIV activity and anticancerous activity, Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antia|drgenic, haemolytic, 5-alpha reductase inhibitor |
| 9.    | 30.53 | 7-Hydroxycurcuma pochromenol                                                   | C<sub>2</sub>H<sub>15</sub>BrO<sub>3</sub> | 340             | 1.89   | Hydroxyl alcohol   | Anti-HIV activity and anticancerous activity, Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antia|drgenic, haemolytic, 5-alpha reductase inhibitor |
| 10.   | 31.26 | Cis-2,3-epoxy-1-cyclohexanol                                                   | C<sub>2</sub>H<sub>10</sub>O<sub>2</sub> | 114             | 2.28   | Alcoholic compound | Anti-HIV activity and anticancerous activity, Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antia|drgenic, haemolytic, 5-alpha reductase inhibitor |
| 11.   | 31.85 | 7-hydroxymethyl-1-bromo-4-isopropyloxy-5-methoxy naphthalene                   | C<sub>2</sub>H<sub>14</sub>BrO<sub>3</sub> | 324             | 4.64   | Aromatic bicyclic compound | Anti-HIV activity and anticancerous activity, Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antia|drgenic, haemolytic, 5-alpha reductase inhibitor |
| 12.   | 32.32 | O, O-dipropyl isopropylphosphate                                               | C<sub>2</sub>H<sub>11</sub>O<sub>3</sub>P | 208             | 1.73   | Phenol            | Anti-HIV activity and anticancerous activity, Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antia|drgenic, haemolytic, 5-alpha reductase inhibitor |
| 13.   | 32.87 | Tricarbonyl [\{4\}-diethyl-2,5-dihydro-2,3-dimethyl-ex o-2-phenyl-1H-1,2,5 azasilaborol] iron | C<sub>2</sub>H<sub>15</sub>FeNO<sub>3</sub>Si | 383             | 2.87   | Phenol            | Anti-HIV activity and anticancerous activity, Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antia|drgenic, haemolytic, 5-alpha reductase inhibitor |
| 14.   | 33.32 | Methyl-2,3,4-Tris-O-[9 borabicyclo[3.3.1]Non-9-Y]-Á-D-Xylopyranoside             | C<sub>2</sub>H<sub>9</sub>B<sub>3</sub>O<sub>5</sub> | 524             | 2.47   | Heterocyclic compound | Anti-HIV activity and anticancerous activity, Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antia|drgenic, haemolytic, 5-alpha reductase inhibitor |
| 15.   | 33.68 | 2'3',5,6,6',7-hexamethylepiper [benzofuran-3-[2H]-9'-[9 H]-xanthen-2-one-3,4 bis [3,4,5-trimethoxyphenyl]-1-[2-[4-methoxyphenyl] ethyl] pyrimidine-2,5-dicarboxylic acid | C<sub>2</sub>H<sub>15</sub>O<sub>3</sub> | 384             | 5.49   | Ketone            | Anti-HIV activity and anticancerous activity, Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antia|drgenic, haemolytic, 5-alpha reductase inhibitor |
| 16.   | 33.97 | 3,4 bis [3,4,5-trimethoxyphenyl] ethyl] pyrimidine-2,5-dicarboxylic acid        | C<sub>2</sub>H<sub>11</sub>NO<sub>11</sub> | 621             | 2.90   | Carboxylic compound | Anti-HIV activity and anticancerous activity, Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antia|drgenic, haemolytic, 5-alpha reductase inhibitor |
| 17.   | 34.66 | 14,17 bis [dimethylenosamine][3.3] paracyclophan-5,8-diacetonitrile             | C<sub>2</sub>H<sub>2</sub>N<sub>4</sub> | 400             | 14.86  | Cyanide           | Anti-HIV activity and anticancerous activity, Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antia|drgenic, haemolytic, 5-alpha reductase inhibitor |

(Contd...)
Table 2: (Continued)

| S. No. | RT  | Compound name                                                                 | Molecular formula | Molecular weight | Area % | Functional groups         | Biological activities                                      |
|-------|-----|--------------------------------------------------------------------------------|--------------------|------------------|-------|---------------------------|-----------------------------------------------------------|
| 18.   | 35.11 | 6α-Acetamido-5α-hydroxyandrostane-3α,7α-diacetate                               | C_{36}H_{45}NO_{6} | 449              | 1.31  | Ester                     | Antioxidant, flavour, hypcholesterolemic, nematicide,      |
|       |      |                                                                                 |                    |                  |       |                           | pesticide, lubricant, antiandrogenic, haemolytic, 5-alpha  |
| 19.   | 35.60 | 1-[3-chloro-phenyl]-5-[(2-mercapto-phenylamino)-methylene]-2-thioxo-dihydro-     | C_{21}H_{20}ClNO_{5}S | 389              | 7.66  | Diketone                  | Antitumor, and gastroprotective drugs, antiasthmatics       |
|       |      | pyrro-pyrimidine-4,6-dione                                                      |                    |                  |       |                           | and lung diseases, carcinogenic agents, antidiabetic agents |
| 20.   | 36.23 | Butanesulfonic acid, 4-(diphenylphosphinyl)-, methyl ester                     | C_{12}H_{18}O_{5}PS | 352              | 3.15  | Methyl ester              | Antiiinflammation and antimicrobial activity                |
| 21.   | 37.49 | 12-Hydroxy-9-methyl-5H,11H-pyran-3,2',6,7'[1]benzopyrano[3,4-c] pyridine-5,11-dione | C_{15}H_{11}NO_{5} | 295              | 5.01  | Diketone                  | Antitumor, 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_{12}H_{11}ClO_{4} | 250              | 2.51  | Ketone                    | Dry asthma, colds, flu and dry cough                       |
| 23.   | 38.70 | N-fluoresceinylpropanamide                                                      | C_{14}H_{14}NO_{5} | 401              | 1.74  | Amide                     | Anesthetic agents                                          |
| 24.   | 39.39 | 2,9-bis[(diethoxyphosphinyl)methyl]-1,10-phenanthroline                         | C_{14}H_{16}N_{2}O_{6}P_{3} | 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, anti spasmodic, 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, hypcholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, hemolytic, 5-alpha 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 phytocannabinoids 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.

**ACKNOWLEDGMENT**

The authors are highly thankful to the Ranger and Guard who have provided the valuable information and accompanied us during field collection of the study plant in Pillur Beat. We are also thankful to South India Textile Research Association (SITRA) for helping us with our experiments.

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