ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (100-116), Month: July - September 2018, Available at: www.researchpublish.com

INVESTIGATION ON ANTIOXIDANT, ANTIBACTERIAL, CYTOTOXIC POTENTIAL AND ESSENTIAL OILS OF FLOWER AND FLOWER STALK OF *SAMADERA INDICA* FROM SOUTH INDIA

[1]Sindhu C, [2]Beena Jose

[1]Research & Development Centre, Bharathiar University, Coimbatore-641046, India
[2]Assistant Professor, Department of Chemistry, Vimala College, Thrissur, Kerala-680009, India,

Abstract: **Objective is to investigate the total phenols, flavonoids, carotenoids, antioxidant, antimicrobial and cytotoxic activites of *Samadera indica* flower and flower stalk extracts and also to investigate components of essential oils from flower and flower stalk of *Samadera indica*.**

Method: Total phenols, flavonoids, carotenoids content, DPPH scavenging activity, reducing power activity, phosphomolybdenum activity, metal chelating activity, hydrogen peroxide and hydroxyl radical scavenging activity of crude extracts, cytotoxic activity, antibacterial screening were evaluated. The extraction of essential oils by steam distillation and GCMS data were analyzed.

Results: The total phenols, flavonoids and carotenoids of *Samadera indica* methanol extract of flower and flower stalk were determined and were found to be 96.64 ± 0.136 mg catechol equ per gram, 119.81 ± 0.236 mg quercetin equ per gram and 80.46 ± 0.135 mg β-carotene equ per gram for flower and 87.69151 ± 0.15 mg catechol equ per gram, 104.48 ± 0.136 mg quercetin equ per gram and 65.71 ± 0.581 mg β-carotene equ per gram for flower stalk. The antioxidant activity of the petroleum ether, ethyl acetate, chloroform, methanol and aqueous extracts of *Samadera indica* flower and flower stalk were investigated using reducing power assay, metal chelating assay, DPPH radical scavenging assay, Hydrogen peroxide radical scavenging assay, Hydroxyl radical scavenging assay and phosphomolybdenum assay. Methanol extracts of flower and flower stalk showed more activity on DPPH assay, reducing power assay and metal chelating activity assay than other extracts and their activity increased with increasing concentration of extracts. The antioxidant activity of the flower and flower stalk methanol extracts were found to be 96.314 and 104.18 µg/ml for hydrogen peroxide scavenging assay, 115.28 and 135.96 mg equivalent of ascorbic acid/100 g of plant extract by phosphomolybdenum method. The hydroxyl radical scavenging activity of methanol extracts are quite comparable to the standard Mannitol of the assay. The antibacterial studies on various extracts of flower and flower stalk of plant showed that methanol extracts showed good inhibitory activity against all the test pathogens compared with standard antibiotics streptomycin and penicillin.The remarkable cytotoxic activity showed by flower and flower stalk methanol extracts could be attributed to the synergic effect of the active compounds present in them.

Conclusions: The presence of major bioactive compound, phytol in flower essential oil and 1,2-Benzenedicarboxylic acid, butyl methyl ester in flower stalk essential oil revealed to use flower and flower stalk (pedicel and peduncle) of the plant in drug delivery, nutritional or pharmaceutical fields.

Keywords: **Flower and flower stalk of *Samadera indica*, GC-MS analysis, bioactive compounds.**

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (100-116), Month: July - September 2018, Available at: www.researchpublish.com

1. INTRODUCTION

Chemicals derived from plants can serve as natural antioxidants that can scavenge free radicals that are produced in the body and which have the ability to damage biomolecules that are the root cause of malevolent diseases like cancer. The curing of such diseases often comes at the price of various side effects and high expense. This makes ventures for safe, cheap cures more necessary. This necessitates the need to highlight on folk knowledge and also to identify the potential and save the depleting plants. *Samadera indica* is one such plant that is found in the coastal areas of Kerala, and has great potential as an antioxidant and antimicrobial, therefore triggering the need to save the endangered plant. There must also be more researches on the plant to isolate potential components from the plant.

Samadera indica (Simaroubaceae) is a small bitter tree up to 11 m in height with stout branches and pale yellow bark. The leaves of the plant are large, up to 25 cm long and 9 cm broad, elliptic-oblong, shortly acuminate, entire, shining and base rounded. Flowers are pinkish yellow in few or many flowered umbels, peduncles longer than the leaves, pedicels red. Fruits are large, flat, pear shaped, much compressed, smooth reticulate.[1] The leaves are used to cure puritis, leprosy, scabies, pruritus, skin diseases, constipation and bilious fever. The seed oil is astringent, acrid, thermogenic, depurative, emetic, purgative and febrifuge.[2,3,4] Indaquassins C1, samaderins C2, B3 and A4 were isolated from seed and bark of *Samadera indica* Taraxerone, stigmastanon, stigmasterol, 2-f-dimethoxy-benzoquinone, leupenone, simalikalactone D. Samaderin B and C isolated from the bark of *Samdera indica* were shown to exhibit anti-feedant activity against *Spodopteralitura*.[5] *Samadera indica* was evaluated for its physiochemical property and antiseptic activity, which was formulated using its extract.[6]*Samadera indica* exhibits significant anti-inflammatory activity.[7] Flavone-o-glycoside, luteoline-7-o-β-D-glucopyranoside were isolated from the kernels of *Samadera indica*.[8]The bitterness of *Samadera indica* is due to the presence of flavanoids like Quassinoids which is reported as an antioxidant.[9]The leaves of *Samadera indica* (Gaertn) shows remarkable termite control properties.[10,11,12]

The past studies revealed that so far there are no detailed study pertaining phytochemical components and pharmacological evaluation of the flowering part and flower stalks of the plant. So the present work is to investigate the presence of secondary metabolites in the petroleum ether, ethyl acetate, chloroform, methanol and aqueous extracts of flower and flower stalk of *Samadera indica*, to investigate the total phenols, flavonoids, carotenoids contents from their methanol extracts, antioxidant, antimicrobial and cytotoxic activites through reliable methods and also the identification of the active compounds present in the essential oils of flower and stalk of flower by GC-MS, extracted by steam distillation .Reactivity patterns and mechanisms are difficult to interpret without detailed information about the composition and structures of antioxidants being tested. Considering all factors the results of investigation showed that the methanol extract of *Samadera indica* have higher activity on these assays and are considered as good source of potential compounds and natural antioxidants[13].

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (100-116), Month: July - September 2018, Available at: www.researchpublish.com

2. MATERIALS AND METHODS

2.1 Chemicals:

2, 2-diphenyl-1-picryl-hydrazyl (DPPH), sodium phosphate, potassium ferricyanide, ammonium molybdate, quercetin, ascorbic acid, aluminium chloride, potassium hydroxide, potassium acetate, trichloroacetic acid, ferric chloride, ferrozine, EDTA, diethyl ether, and Catechol, mannitol, β-carotene, 2-thiobarbituric acid (TBA), butylated hydroxyl anisole (BHA), Follin-Ciocalteu, 2- deoxyribose and H_2O_2 (30 %, v/v) .All other chemicals and solvents used were of analytical grade.

2.2 Plant Material:

From the coastal regions of Thrissur District, Kerala (Vellankallur), India, *Samadera indica* flower and flower stalk (pedicel and peduncle) were separately collected in the month of february 2016 and authenticated by Dr. Kochuthressia M.V., HOD, Department of Botany, Vimala College, Thrissur, Kerala, India.

2.3 Preparation of Extracts:

Half kilogram powdered flower and flower stalk of the plant material were separately extracted successively with petroleum ether, ethyl acetate, chloroform, methanol and water using soxhlet apparatus.

2.4 Preliminary Phytochemical analysis:

The sample extracts were analysed for the presence of various phytoconstituents like of flavonoids, alkaloids, glycosides, steroids, phenols, saponins and tannins according to standard methods.[14,15]

2.5 Determination of total phenolics:

Folin-Ciocalteu (FC) assay was used to the determination of the total phenolics (TP) content of the flower and flower stalk extracts of *Samadera indica* . Eight ml of water was added into 1 ml of extract in a 10 ml volumetric flask. 0.5 ml of FC reagent was added and mixed for 15 min followed by addition of 1.5 ml of 20 % sodium carbonate solution. After 2 h at ambient temperature the absorbance of the colored reaction product was measured at 765 nm, where different concentrations of standard catechol solutions were used for calibration curve and results were expressed as mg of Catechol equivalent per gram of dried extract(standard plot: y =0.0966x, R^2 = 0.9878).[16,17]

2.6 Determination of total flavonoids:

The total flavonoid content of flower and flower stalk extracts of *Samadera indica* were determined by using aluminium chloride colorimetric method[18,19]. Quercetin (standard plot: y =0.0148x R^2 = 0.9878) was used as a standard to make the calibration curve. The sample solution (0.5 ml) was mixed with 1.5 ml of 95 % ethanol, 0.1 ml of 10 % aluminium chloride hexahydrate, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. After incubation at room temperature for 40 min the absorbance of the reaction mixture was measured at 415 nm. The same amount (0.1 ml) of distilled water substituted for the amount of 10 % aluminum chloride as the blank and a seven point standard curve (0-500 µg/ml) was obtained.

2.7 Determination of carotenoids:

Total carotenoids content of flower and flower stalk of *Samadera indica* were determined[20]. One gram sample was extracted with 100 ml of 80 % methanol solution and centrifuged at 4000 rpm for 30 min. The supernatant was concentrated to dryness. The residue was dissolved in 15 ml of diethyl ether and after addition of 15 ml of 10 % methanolic KOH the mixture was washed with 5 % ice-cold saline water to remove alkali. The free ether extract was dried over anhydrous sodium sulphate for 2 h. The ether extracts were filtered and its absorbance was measured at 450 nm by using ether as blank. β–carotene (standard plot: y = 0.022x-0.008 R^2 = 0.9997) has taken as standard.

2.8 Antioxidant Activity:

2.8.1 DPPH free radical scavenging assay

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating free radical-scavenging activities of antioxidants [21]. The hydrogen or electron donation abilities of the compounds were measured from the bleaching of the purple-colored ethanol solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). This spectro-

photometer assay uses the stable radical DPPH as a reagent. The sample solution of material (50 µl) at four concentrations (1.0, 0.5, 0.25 and 0.125 mg/ml) was mixed with freshly prepared methanolic solution of DPPH (634 μM) and allowed to stand for 30 min at room temperature. The absorbance was then measured at 515nm using a spectrophotometer and the inhibition of free radical DPPH in percent (%) was calculated using the formula below:

The percent of inhibition of DPPH reduction (decolourization)

$$\% \text{ of inhibition} = \frac{A_0 - A\text{sample}}{A_0} \times 100$$

The percent of inhibition of DPPH reduction (decolourization) where (A_0) is the absorbance of the control (blank) and (A (sample)) is the absorbance of the test compound. The compound concentration demonstrating 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against sample concentration. Samples and DPPH were dissolved in methanol. L-ascorbic acid was used as positive control.

All data were expressed as mean $\pm$ standard deviation (SD). The extract concentration corresponding to 50 percent inhibition (IC_{50}) was calculated from the curve of percentage inhibition against extract concentration. Each sample was assayed in triplicate for each concentration.

2.8.2 Reducing power assay

Aliquots of each extracts were taken in test tubes and dissolved in 1 ml of 0.2 M phosphate buffer in a test tube to which was added 5 ml of 0.1 % solution of potassium ferric cyanide [22]. The mixture was incubated 50^0 C for 20 min. Following this, 5 ml of trichloroacetic acid (10 %) (w/v) solution was added and the mixture was then centrifuged at 7000 rpm for 10 min. A 5 ml of aliquot of the upper layer was combined with 5 ml of distilled water and 1 ml of ferric chloride solution (0.1%) and absorbance was recorded at 700 nm against reagent blank. A higher absorbance of the reaction mixture indicates greater reducing power of the sample.[22]

2.8.3 Metal chelating activity

The chelating of ferrous ions by *Samadera indica* flower and flower stalk were estimated [23]. Briefly, the extract samples (250 µl) were added to a solution of 2 mmol/l $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5mmol/l ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min, after which the absorbance was measured spectrophotometrically at 562 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The metal chelating activity of the extract is expressed as mg EDTA equivalent/g extract.

2.8.4 Phosphomolybdenum activity

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation [24]. An aliquot of 100 µL of sample solution was combined with 1ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a 4 ml vial. The vials were canned and incubated in a water bath at 95^0 C for 90 min. After the samples have cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported (Ascorbic acid equivalent antioxidant activity) are mean values expressed as g of ascorbic acid equivalents/100g extract.

2.8.5 Hydroxyl radical scavenging activity

Hydroxyl radicals were generated by a Fenton reaction (Fe^{3+}-ascorbate-EDTA-H_2O_2 system), and the scavenging capacity towards the hydroxyl radicals was measured by using deoxyribose method [25]. The reaction mixture contained 2-deoxy-2-ribose (2.8 mM), phosphate buffer (0.1 mM, pH 7.4), ferric chloride (20 µM), EDTA (100 µM), hydrogen peroxide (500 µM), ascorbic acid (100 µM) and various concentrations (10-1000 µg/ml) of the test sample in a final volume of 1 ml. The mixture was incubated for 1 h at 37 °C. After the incubation an aliquot of the reaction mixture (0.8 ml) was added to 2.8% TCA solution (1.5 ml), followed by TBA solution (1% in 50 mM sodium hydroxide, 1 ml) and sodium dodecyl sulphate (0.2ml). The mixture was then heated (20 min at 90 °C) to develop the colour. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. All experiments were performed in triplicates. The percentage of inhibition was expressed, according to the following equation:

% Inhibition = $[A_0 - (A_1 - A_2)]/A_0 \times 100$, where, A_0 is the absorbance of the control without a sample, A_1 is the absorbance in the presence of the sample and deoxyribose and A_2 is the absorbance of the sample without deoxyribose.

2.8.6 Hydrogen peroxide radical scavenging activity

Hydrogen peroxide assay [26]was carried out for the determination of antioxidant activity of compounds for their ability to scavenge the oxidant hydrogen peroxide and compared the results with standard L ascorbic acid. The reaction mixture contained phosphate buffer (pH-7.4) and hydrogen peroxide solution prepared in phosphate buffer (40 mM). Plant extracts at the concentration of 10 mg/10µL was added to hydrogen peroxide solution (0.6 ml, 40 mM). The total volume was made up to 3 ml. The absorbance of the reaction mixture was recorded at 230 nm. The blank solution contained phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenged by the plant extract was calculated as follows:

$$\text{Percentage of scavenged } H_2O_2 = \frac{A_0 - A_{sample}}{A_0} \times 100$$

Where, A_0-Absorbance of contro

A_{sample}- Absorbance in the presence of plant extract.

2.9 Antibacterial activity assay:

2.9.1 Test microorganisms

The microorganisms used for antibacterial activity evaluation were obtained from Microbial Type Culture Collection and Gene Bank (IMTECH) Chandigarh, India). They are *Bacillus cereus* ,*Staphylococcus aureus*, *Pseudomonas aeruginosa* , *Escherichia coli* , *Klebsiella pneumonia* and *Proteus vulgaris*.

2.9.2 Culture medium and inoculum

The stock cultures of microorganisms used in this study were maintained on Plate Count Agar slants at 4^0 C. Inoculum was prepared by suspending on loop full of bacterial cultures into 10ml of nutrient broth and was incubated at 37^0 C for 24 hours. On the next day Muller- Hinton agar (MHA) (Merk) sterilized in a flask and cooled to 45-50^0 C was distributed by pipette (20ml) into each sterile Petri dish and swirled to distribute the medium homogeneously. About 0.1ml of bacterial suspension was taken and poured into Petri plates containing 20ml nutrient agar medium. Using the L- shaped sterile glass spreader bacterial suspensions were spread to get a uniform lawn culture.

2.9.3 Antibacterial activity screening

The flower and flower stalk methanol extracts at concentration 1mg/ml in various solvents were tested for antibacterial activity using agar well diffusion method . Wells of 8mm (0.8cm) diameter were dug on the inoculated nutrient agar medium with sterile cork borer and 50 µL of the methanol extracts of the flower and flower stalk of *Samadera indica* were added in each well. Wells introduced with 50 µL of pure petroleum ether, ethyl acetate, chloroform and methanol served as negative controls. The plates were incubated at 37°C over night and examined for the zone of inhibition. The diameter of the inhibition zone was measured in mm. The standard antibiotic drugs such as streptomycin (10µg/disc) and penicillin (10 µg/disc) were also screened under similar conditions for comparison. An extract was classified as active when the diameter of the inhibition was equal to or larger than 8mm. All the assays were performed in triplicate and mean values were presented.[27]

2.10. Cytotoxicity analysis:

2.10.1. Cell Lines:

Dalton's Lymphoma Ascites (DLA) cells maintained in the intraperitoneal cavity of mouse, were used for the study

2.10.2. In vitro cytotoxicity analysis

The short term *invitro* cytotoxic activity of flower and flower stalk methanol extracts of *Samadera indica* were assayed by determining the percentage viability of DLA cells using Trypan blue dye exclusion method. The cells were aspirated from the peritoneal cavity of tumour bearing mice. The cells were washed three times using phosphate buffered saline**.** The viability of the cells were checked using Trypan blue (Cell viability should be above 98%). Differenciated dilution of 10^{-1},10^{-2} and 10^{-3} were made.The number of cells in the 10^{-3} dilution was counted using a haemocytometer and the cell number was adjusted to 1×10^{-6} cells/mL. The experiment was setup by incubating different concentrations of the drug with 1×10^{-6} cells.The final volume of the assay mixture was made up to 1mL using PBS (Phosphate Buffered Saline) and was incubated at 37^0C for about 3 hours.100µL of Trypan blue was added after incubation and the number of dead cell was counted using a haemocytometer. The percentage viability was calculated.[28]

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (100-116), Month: July - September 2018, Available at: www.researchpublish.com

2.11 Extraction of Essential Oils from flower and flower stalk of *Samadera indica* by steam distillation:

214g of the flower and 262g of flower stalks are separately crushed and ground to reduce the particle size and to rupture some of the cell walls of oil bearing glands using an electric mixer grinder. The ground masses were then subjected to steam distillation for 3 hours. About 2 litres of the distillate were collected and were extracted using pure organic solvent ie diethyl ether (3×100ml) and dried using anhydrous sodium sulphate. The dry ether extract on evaporation yielded 0.45g flower oil with a pale yellow colour (0.21% of fresh weight of the sample) and 0.34g flower stalk oil with pale orange yellow colour (0.13% of fresh weight of the sample). 2 µL of the essential oils of flower and flower stalk of *Samadera indica* employed for GC/MS analysis.

2.12 Gas chromatography- Mass spectrometry:

2.12.1 Instruments and chromatographic conditions

The GC-MS analyses (fig. 4) were carried out on Agilent 6890 GC system equipped with a 5973 inert mass selective detector (Agilent Technologies, USA). A CO Sil 8 CB (Varian, Middleburg, Netherlands) column of 30m length, 0.25mm i.d, and 0.25µm film thickness was used. The oven was programmed from an initial temperature 500C (hold for 2 min) to the final temperature 2800C at the rate of 100C/min. The final temperature hold up time was 5min. Helium at the rate of 1 ml/min was used as the carrier gas in constant flow mode. The inlet and interface temperatures were kept at 2800C. The EI source was operated at 2300C and the quadrupole temperature was 1500C. The MS was scanned from 30 to 500 mass units. One micro litre of the sample was injected in split mode at a split ratio of 10:1. For compound identifications,

2.12.2 Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of CSIR- Indian Institute of chemical technology (IICT) Hyderabad. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the IICT library. The name, molecular weight and structure of the components of the test materials were ascertained.

2.13 Statistical analysis:

Results were expressed as the means of three replicates ± the standard deviation of triplicate analysis.

3. RESULTS

3.1 Preliminary Phytochemical analysis

Phytochemical screening of the petroleum ether, ethyl acetate, chloroform, methanol and aqueous extracts of flower and flower stalk of *Samadera indica* revealed that potential phytochemicals are present in large amount in the methanol extracts than other extracts.(Table 1) The many reported pharmacological activities of the plant could be attributed to the synergic effect of these active compounds present in it.[29]

Table 1: Phytochemical analysis of extracts of *Samadera indica*

Phyto constituent	Solvents used	Phenolic compounds	Flavanoids	Glycosides	Steroids	Terpenoids	Saponins	Alkaloids	Tannin
Flower	A	+	+	-	-	-	-	+	-
	B	++	++	+	++	++	+	+	-
	C	++	++	++	++	++	+	+	+++
	D	+++	++	++	+++	+++	++	+++	+++
	E	+	+	++	++	++	++	++	+
Flower stalk	A	-	+	+	+	-	+	+	+
	B	++	+++	++	++	++	++	++	+
	C	++	++	++	++	++	++	++	+
	D	+++	+++	++	++	+++	++	++	++
	E	+	++	++	+	+	+	+	+

+ present ++ moderately present +++ appreciable amount - absent

A-Petroleum ether B-Ethyl acetate C-Chloroform D-Methanol E-Water

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (100-116), Month: July - September 2018, Available at: www.researchpublish.com

3.2 Determination of total phenols, flavonoids and carotenoids

Total phenolic, flavonoid and carotenoid content of the flower and flower stalk of methanol extracts of *Samadera indica* were compared with standards , catechol for total phenolics, Quercetin for total flavonoids and β-Carotenoids for total carotenoids and datashave given in Table 2. The phenolic compounds, flavonoids and carotenoids attribute antioxidant activity and can act as free radical scavengers thereby having significant effect on human health [30]. Since the flower methanol extracts of *Samadera indica* found to have higher amount of phenolic compounds, flavanoids as well as carotenoids suggesting their usage as a good source of natural antioxidant, preventing free radical-mediated oxidative damage.

Table 2: Total phenolic, Flavanoid and Carotenoid content of *Samadera indica*

	Total phenolic in mg catechol equivalent per gram of plant extract	Total flavonoid in mg Quercetin equivalent per gram of plant extreme	Total carotenoids in mg β-carotene equivalent per gram of plants extracts
Flower	96.64 ± 0.136	119.81 ± 0.236	80.46 ± 0.135
Flower stalk	87.69151 ± 0.15	104.48 ± 0.136	65.71 ± 0.581

3.3 Antioxidant activity (DPPH free radical scavenging activity) determination

The antioxidant activity of the flower and flower stalk extracts of *Samadera indica* in solvents of varying polarity were measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH. The IC_{50} values of the free radical scavenging activity of extracts of *Samadera indica* assessed by DPPH assay was summarized in tables 3. Lower IC_{50} value indicates higher antioxidant activity. IC_{50} values of the *extracts* were comparable with the authentic antioxidant L-ascorbic acid .[31] The flower and flower stalk methanol extracts showed significant radical scavenging activity than the other solvent extracts.

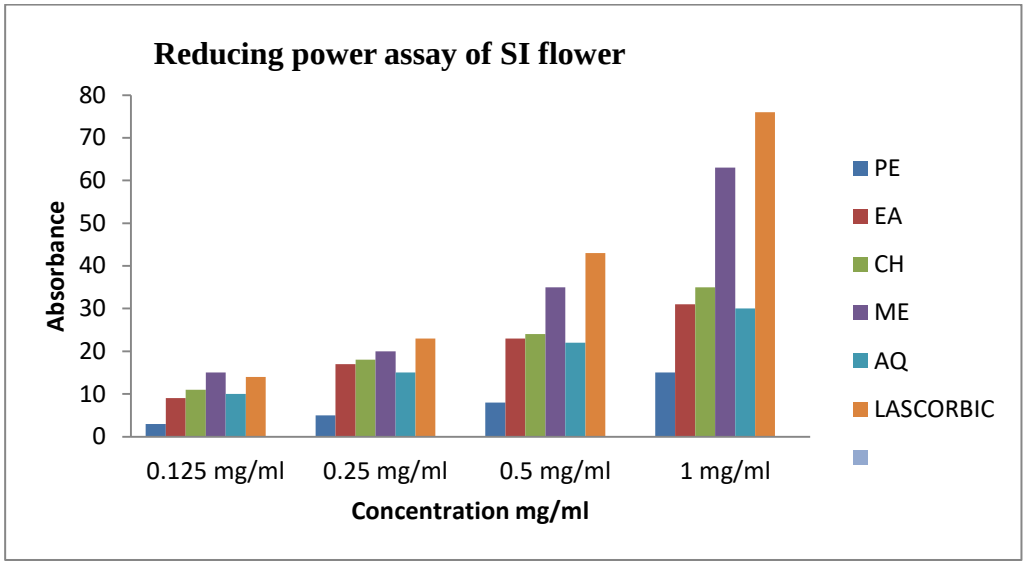
Table 3: IC_{50} values of DPPH scavenging activities of extracts of *Samadera indica*

Samples	IC_{50} values of DPPH radical scavenging activity of				
	Petroleum Extracts	Ethyl acetate Extracts	Chloroform Extracts	Methanol Extracts	Aqueous Extracts
SI flower Extracts	388± 0.93	252± 0.73	250± 0.92	95± 0.66	295± 1.53
SI Flower stalk extracts	863± 2.95	367± 2.53	355± 0.99	195± 0.59	385± 1.83
L. Ascorbic acid (standard) IC_{50} value-=53.19 ± 0.312					

3.4 Reducing power assay

Figures 1 shows the reducing power of the petroleum ether, ethyl acetate, chloroform, methanol and aqueous extracts of flower and flower Stalk of *Samadera indica* using potassium ferricyanide reduction method. The absorbance value of the extract shows higher increase with increase in concentration, when compared to standard L ascorbic acid. The yellow colour of test solution changes to various shades of green and blue due to the reduction of Fe^{3+}/ Ferric cyanide complex to ferrous form by the antioxidants present in the extract. Flower and flower stalk methanol extracts have higher reducing power than solvent extracts.Thus, the reducing power of medicinal plants and vegetables are said to be well associated with the antioxidant activity .[32]

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (100-116), Month: July - September 2018, Available at: www.researchpublish.com



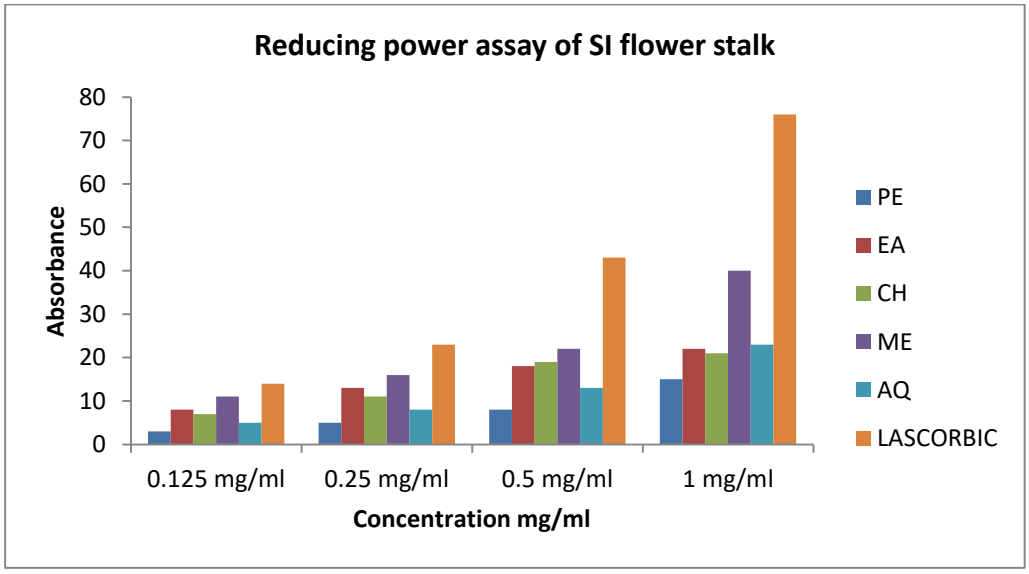


Figure 1: Reducing power assay

3.5 Metal chelating Activity

The metal chelating activity of the petroleum ether, ethyl acetate, chloroform, methanol and aqueous extracts of flower and flower stalk extracts of *Samadera indica has given in Table 4*. The IC_{50} value of plant extracts were compared to the standard EDTA 39.49 µg/ml and it was found that the IC_{50} values of the extracts were close to the IC_{50} value of EDTA. In this metal chelating activity, the presence of chelating agents in the extract of *Samadera indica* disrupts the ferrozine-Fe^{2+} complex formation, thus decreasing the red colour. It was reported that chelating agents are effective as secondary antioxidants as they stabilize the oxidized form of the metal ion by reducing the redox potential.[33]

Table 4: Metal chelating activity of *Samadera indica* extracts

Samples	IC_{50} values of metal chelating activity of				
	Petroleum Extracts	Ethyl acetate Extracts	Chloroform Extracts	Methanol Extracts	Aqueous Extracts
SI flower extracts	189+ 1.73	85+ 1.58	82+ 1.02	64+ 0.63	76+ 1.62

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (100-116), Month: July - September 2018, Available at: www.researchpublish.com

SI flower stalk extracts	364+ 1.83	162+ 1.53	128+ 0.69	**94**+ 0.92	118+ 0.69
EDTA (standard) IC_{50} value- 39.49 ± 0.22					

3.6 Phosphomolybdenum activity

The total antioxidant activity of the methanol extracts of flower and flowerstalk of *Samadera indica* was found to be 115.28 and 135.96 mg equivalent of ascorbic acid/100 g of plant extract as determined by phosphomolybdenum method. This method is based on the formation of green phosphomolybdenum complex at 95 ^{0} C measured at an intensity of absorbance at 695 nm. In this method, reduction of Mo (VI) to Mo (V) by the antioxidant compounds present in the plant extract, forming green phosphate/Mo (V) complex takes place[34].

3.7 Hydroxyl radical scavenging activity

At a concentration of 1 mg/ml, the scavenging activity of flower and flower stalk of methanol extracts of *Samadera indica* and standard mannitol were found to be 71%, 68% and 82.70% respectively.The hydroxyl radical can induce oxidative damage to DNA, lipids and proteins [35]. The hydroxyl radical scavenging ability of the extracts was determined by its ability to compete with deoxyribose for hydroxyl radical. The *Samadera indica* flower and flower stalk extract compete with deoxyribose and diminish chromogen formation in a dose dependant manner. In this assay, 2-deoxy-2-ribose was oxidized when exposed to hydroxyl radicals generated by Fenton-type reaction. The oxidative degradation can be detected by heating the products with TBA under acid conditions to develop a pink chromogen (thiobarbituric acid reactive species) with a maximum absorbance at 532 nm .[35]

3.8 Hydrogen peroxide radical scavenging activity

The hydrogen peroxide radical scavenging activity of the methanol extracts of flower and flower stalk of *Samadera indica* is significantly high. The IC_{50} values of 96.314 and 104.18 µg/ml were compared with standard ascorbic acid (IC_{50}=51.23 µg/ml). Hydrogen peroxide, though not reactive, is said to be highly important because of its ability to penetrate biological membranes, releasing toxic hydroxyl radicals in the cells[36,37]. Thus the plant extracts can be considered as natural antioxidants.

3.9 Antibacterial screening of *Samadera indica* extracts

As can be seen from Table 5, the flower and flower stalk extracts of *Samadera indica* were analysed for antibacterial activity at concentration 1mg/ml against microorganisms *Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia and Proteus vulgaris,*. Among the extracts of flower and flower stalk methanol extracts exhibited higher activity than the other extracts. The methanol extracts of the plant exhibited marked activity against all the tested organisms and are comparable with the standard antibiotic Streptomycin and Penicillin at a concentration of 10µg .So the methanol extracts of the plant can exhibit remarkable activity against bacterial infections as external antiseptic.

Table 5: Antibacterial activity of flower and flower stalk of *Samadera indica* in aqueous and organic solventsagainst gram positive and gram negative bacterial strains (mean ± SE; *n* = 3)

sample	Solvent	**Inhibition zone in diameter (mm/50µl) at concentration 1mg/ml of *Samadera indica* extracts**					
		B cereus	*S aureus*	*P aeruginosa*	*E.coli*	*K pneumonia*	*P vulgaris*
Flower	Petro- ether	-	-	-	-	10±0.68	-
	Ethyl-acetate	-	12±0.80	-	-	12±0.64	-
	Chloroform	13±0.8	12±0.6	-	13±0.8	12±0.82	-
	Methanol	18±0.8	15±0.9	15±0.86	16±1.5	17±0.48	16±1.42
	Aqueous	10±0.2	-	-	10±0.4	-	-

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (100-116), Month: July - September 2018, Available at: www.researchpublish.com

Flower Stalk	Petro- ether	-	-	-	-	-	-
	Ethyl-acetate			10±0.68			
	Chloroform	10±1.5		12±0.45	10±1.6		
	Methanol	16±0.4	10±0.3	14±1.84	13±0.4	16±1.54	14±0.53
	Aqueous			10±0.68			10±0.84
Streptomycin	10µg	18.80±0.5	15.70±0.65	13.10±0.61	14.0.82	20.10±0.31	19.±0.42
Penicilin	10µg	19.36±0.47	14.50±0.35	17.30±0.81	15.60±0.25	20.10±0.85	20.10±0.85

3.10 *IN VITRO* CYTOTOXICIY ANALYSIS

Cytotoxicity of the flower and flower stalk methanol extracts towards DLA cells at concentrations 10,20,50,100 and 200 µg/ml were studied. Dose dependant increase in cytotoxicity was found in both extracts and was found to be significantly toxic at concentration 200µg/ml to DLA cells .

Table 6: Cytotoxicity of *Samadera indica* methanol extracts towards DLA Cells

Samples- *Samadera indica*	% Cytotoxicity at concentrations				
	10µg/ml	20µg/ml	50µg/ml	100µg/ml	200µg/ml
Flower methanol extracts	11%	20%	41	**64**%	88%
Flower pedicel methanol extracts	10%	23%	45%	**70**%	91%

3.11 GC-MS analysis

On comparison of the mass spectra of the constituents with NIST library, 43 peaks for flower oil and 17 peaks for flower stalk were identified (Tables 7,9). The extracts show presence of many methyl ethyl esters, phenols, terpenoids in them. Phytol a diterpene alcohol is the major component in flower and 1,2-Benzenedicarboxylic acid, butyl methyl ester in flower stalk extracts. Both are reported as potential antioxidants and antimicrobials (Tables) hinting the use of the flower and flower stalk of *Samadera indica* for pharmaceutical advantages.[38,39]

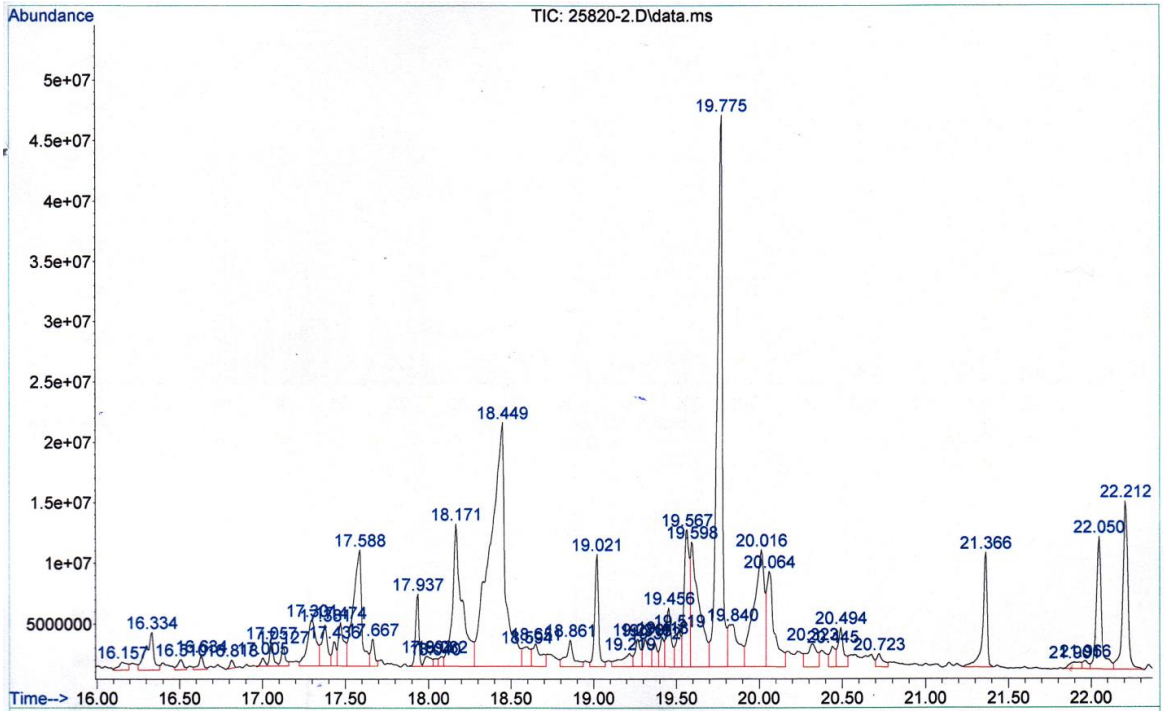


Figure 2 - Phytochemical present in flower essential oil of *Samadera indica*

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (100-116), Month: July - September 2018, Available at: www.researchpublish.com

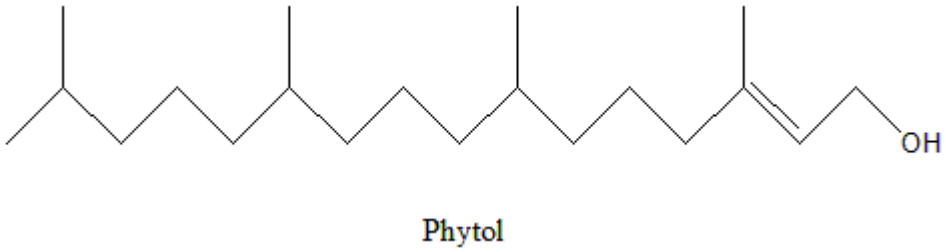
Table 7: Phytochemical present in flower essential oil of *Samadera indica*

Sl.No	RT	Name of the component	Percentage composition (%)
1	4.314	3-Hexen-1-Ol, (Z)	0.83
2	4.445	1- Hexanol	0.78
3	6.258	2- Butanoicacis, 2- methyl- (E)	0.64
4	6.876	Hexanoic acid	0.82
5	7.086	3-Hexenoic acid, (E)	1.01
6	7.388	2- Hexenoic acid	0.90
7	7.697	2-Hexenal, (E)	0.84
8	7.795	1,6- Octadien-3 Ol, 3,7-dimethyl	0.86
9	7.822	Nonanal	0.72
10	8.032	Phenyl ethyl Alcohol	1.06
11	8.124	2-Cyclohexen-1-one,3,5,5-trimethyl	0.67
12	8.465	Bicyclo[2,2,1]heptan-2-one,1,7,7trimethyl-(1S)	0.69
13	9.188	3-Cyclohexen-1-methanol,alpha.,alpha,4-trimethyl	0.89
14	9.641	Benzothiazole	1.25
15	9.694	Cyclopropane,1,1'- ethenylidenebis-	0.83
16	10.042	2,6-Octadien-1-Ol,3,7-dimethyl- (E)	0.73
17	10.449	Hydroquinone	1.62
18	10.869	2-Methoxy-4-vinylphenol-	3.96
19	11.789	Heptanoic acid, 3-hexenyl ester, (Z)-	2.98
20	11.828	3 Hexenoic acid (E)	1.90
21	11.888	1-Hexene,6-bromo	1.95
22	12.919	1-Decene	1.64
23	13.352	Alpha- Farnesene	1.07
24	13.523	Dodecanoicacid,methyl ester	0.78
25	13.589	Naphthalene,1,2,3,5,6,8a-hexahydro-4,7,dimethyl- (1-methyl ethyl)-, (1S-Cis)-	1.4
26	13.753	(E)- 9,11-Dodecadien-1-ol	2.88
27	14.134	3-Hexen-1-ol, benzoate,(Z)-	2.25
28	14.285	Phenyl tert-butyl ketone	
29	14.377	Azulene,1,2,3,3a,4,5,6,7-Octahydro-1,4-dimethyl-7- (1-methyl ethenyl)-[1R-(1.alpha.,3a.beta.,4-alpha.,7. Beta)]	0.84
30	14.916	2-Naphthalene methanol,1,2,3,4,4a,5,6,7-octahydro-alpha.,alpha.,4a,8-tetramethyl-(2R-cis)-	3.36
31	15.014	Tau-Muurolol	0.57
32	15.172	Alpha-Cadinol	2.88
33	15.310	Cyclotetradecane	0.48
34	15.717	2-Tridecenal,(E)	1.08
35	16.328	Benzyl Benzoate	1.77
36	17.937	Hexadecanoicacid,methyl ester	6.4
37	18.174	Isophytol	4.88
38	18.436	n-Hexadecanoic acid	4.96
39	19.566	9-Octadecyne	1.39
40	19.757	phytol	22.8
41	20.006	9,12-Octadecadienoic acid(Z,Z)	2.8
42	21.360	Tricosane	2.05
43	22.212	Z-12-Pentacosene	1.34

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (100-116), Month: July - September 2018, Available at: www.researchpublish.com

Table.8: Activity of Phyto-Components identified in the flower essential oil of *Samadera indica* by GC-MS Dr.Duke's Phytochemical and Ethnobotanical Databases (Online Database)**

SL.No	Name of compound	Activity
1.	2-Naphthalene methanol,1,2,3,4,4a,5,6,7-octahydro-alpha.,alpha.,4a,8-tetramethyl-(2R-cis)-	Anti inflammatory, Cytotoxic,Antimicrobial
2.	Iso-phytol	Terpenes, antioxidants and antimicrobial
3.	Hexadecanoicacid,methyl ester	Antioxidant, hypocholesterolemicnematicide, pesticide, anti-androgenic flavor, haemolytic and 5-alpha reductase inhibitor
4.	Phytol	antidepressant, antiseptic, antispasmodic, expectorant



Phytol

Figure 3: Phytochemical present in the flower essential oil of *Samadera indica*

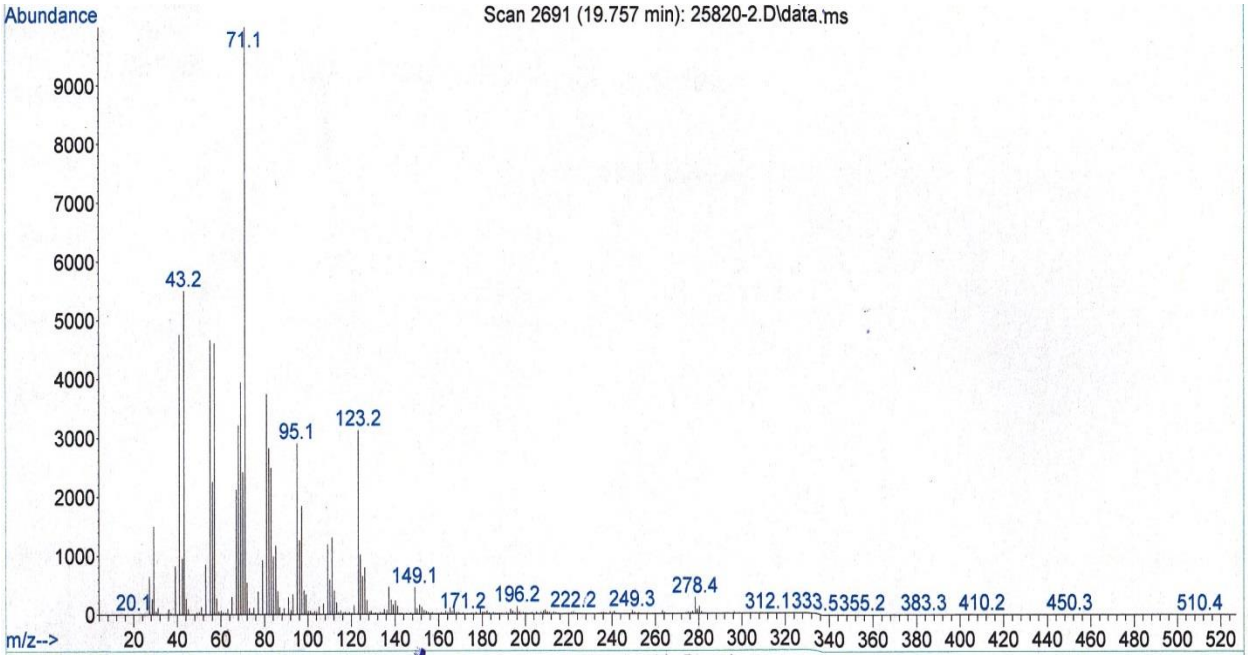


Figure 4: Phytochemical present in the flower stalk essential oil of *Samadera indica*

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (100-116), Month: July - September 2018, Available at: www.researchpublish.com

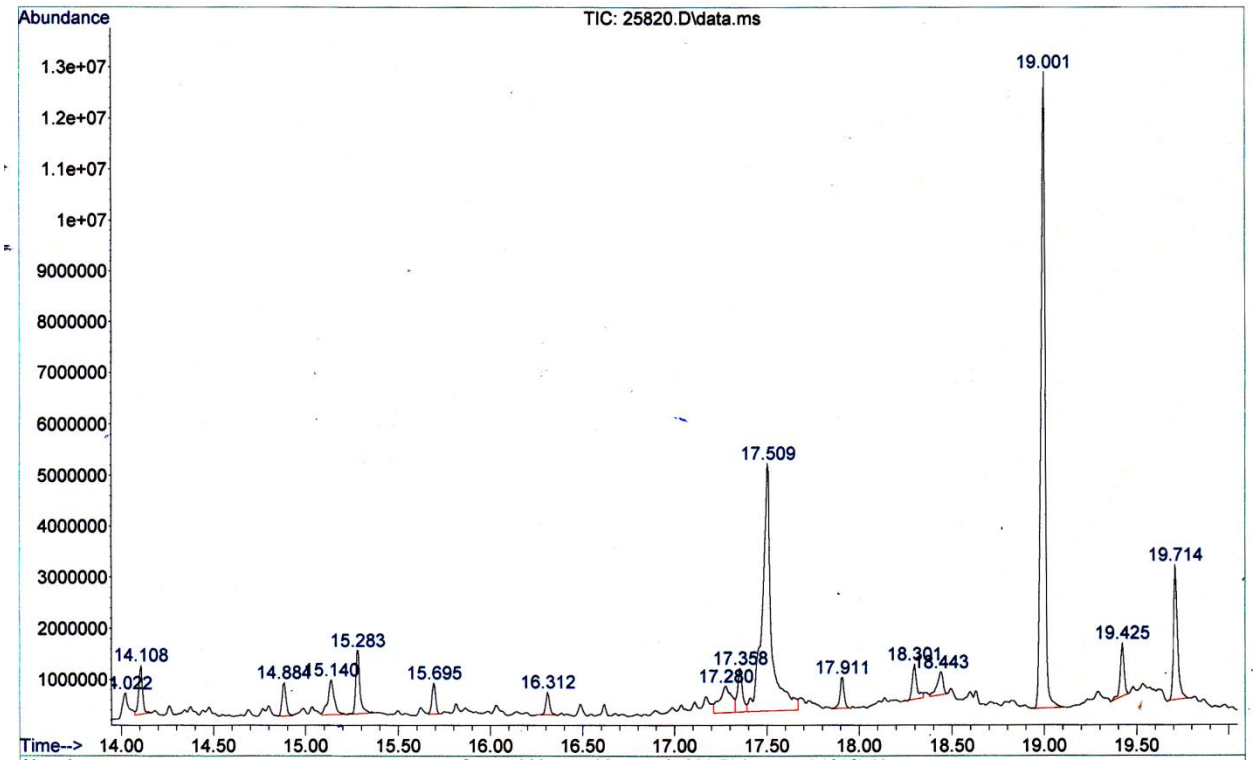


Table 9: Phytochemical present in the flower stalk essential oil of *Samadera indica*

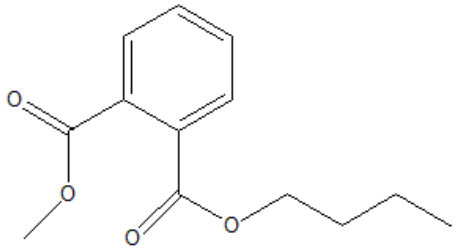
Sl.No	RT	Name of the component	Percentage composition
1	6.659	2-ethyl-1-Hexanol	4.9
2	10.292	Hydroquinone	1.12
3	12.886	nonyl- Cyclopropane	1.5
4	13.510	Dodecanoic acid,methyl ester	0.24
5	14.108	3-Hexen-1-Ol, benzoate, (Z)	0.54
6	14.883	2- Naphthalene methanol,1,2,3,4,4a,5,6,7-Octahydro-alpha.,alpha.,4a,8-tetramethyl- (2R- Cis)	0.5
7	15.139	Cyclohexene, 6-ethenyl-6-methyl-1- (1-methylethyl)-3-(1-methyl ethyllidene)-,(S)	0.45
8	15.284	Cycloteradecane	1.7
9	16.315	Benzoic acid,2-ethylhexyl ester	0.43
10	17.353	Benzyl Benzoate	0.68
11	17.504	1,2- Benzenedicarboxylic acid, bis(2-methyl propyl)ester	16.53
12	17.911	Propanil	0.46
13	18.301	Hexadecanoicacid,methyl ester	0.57
14	18.443	Dibutyl phthalate	0.54
15	18.995	1,2-Benzenedicarboxylic acid, butyl methyl ester	66.7
16	19.422	1-Octadecene	0.23
17	19.714	Phytol	2.5

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (100-116), Month: July - September 2018, Available at: www.researchpublish.com

Table.10: Activity of Phyto-Components identified in the flower stalk essential oil of *Samadera indica* by GC-MS

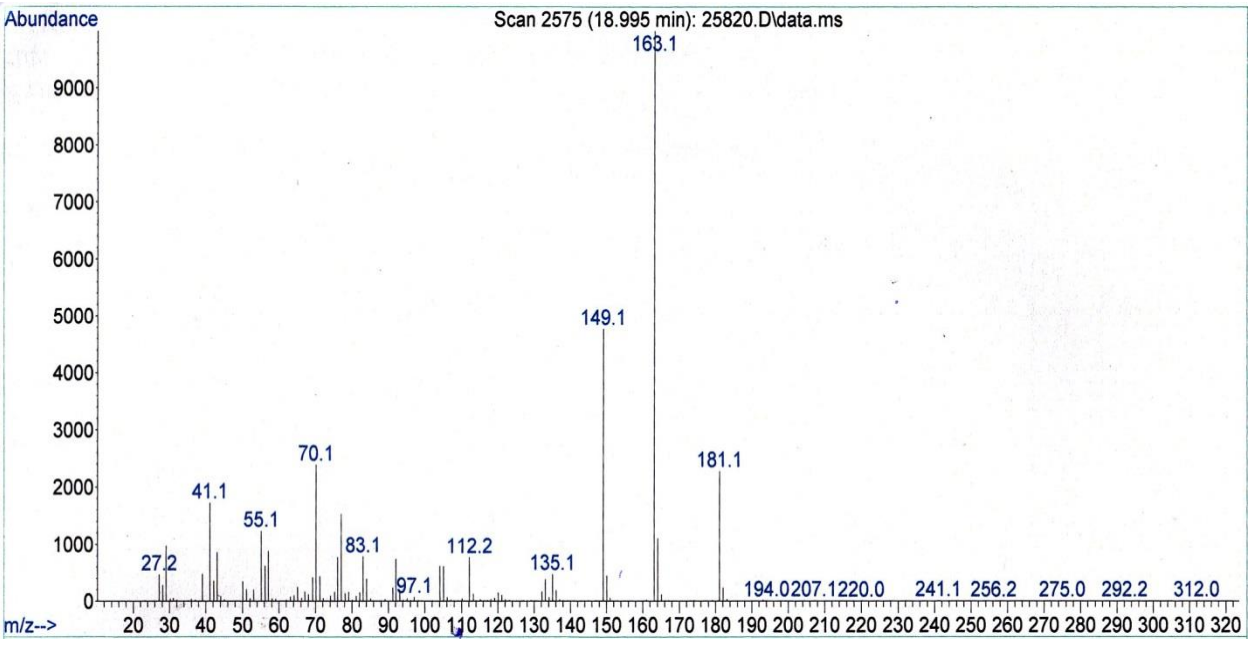
** Dr.Duke's Phytochemical and Ethnobotanical Databases (Online Database)

SL.No	Name of compound	Activity
1.	1,2-Benzenedicarboxylic acid, butyl methyl ester	Antimicrobial Antifouling
2.	1,2- Benzenedicarboxylic acid, bis(2-methyl propyl)ester	Antibacterial, Antioxidants
3.	2-ethyl-1-Hexanol	fragrant organic compounds, Antibacterial
4.	Phytol	antidepressant, antiseptic, antispasmodic, expectorant



1, 2 - Benzenedicarboxylic acid, butyl methyl ester

Figure 5: 1,2 – Benzenedicarboxylic acid, butyl methyl ester



: 1,2 – Benzenedicarboxylic acid, butyl methyl ester

4. CONCLUSION

The present study is relevant and maybe requisite in knowing more about the presence of phytoconstituents in *Samadera indica* is the main cause for the widely agreed pharmacological activity of the plant. Phytochemical studies revealed the presence of various secondary metabolites in *Samadera indica*. The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences and confer antibacterial, antioxidant, anticancer activity on the flower and flower stalk extracts of the plant. The plant carries chemicals constituents such as alkaloids, proteins, steroids, saponins, flavonoids, phenolic compounds, tannins, samaderaA,B,C, taraxerone, stigmastanon, stigmasterol,2-f-dimethoxy benzoquinone,leupenone, simalikalactoneD. These isolated components are potentialantimicrobial and

antioxidants.So this plant is identified as the source of natural antioxidants that can protect body from oxidative decay. The flower and flower stalk methanolic extracts of *Samadera indica* are high in phenolic, flavonoid and carotenoid content. Flavonoids are potent antioxidants having characteristics of scavenging free radical, chelating metal and inhibiting lipid peroxidation. Out of petroleum ether, ethyl acetate, chloroform, methanol and aqueous extracts of flower and flower stalk, the methanol extract shows strong antioxidant activity. Also the results of scavenging activities observed against DPPH, reducing power, phosphomolybdenum activities, show that flower and flower stalk of *Samadera indica* as promising natural sources of antioxidants suitable for preventing free radical-mediated reaction. The flower and flower stalk extracts exhibited high antibacterial activity against important bacterial strains[36]. Among the five solvent extracts, methanol was found to be the best solvents of choice to extract natural products to get maximum medicinal benefits. Methanol extracts of flower and inflorescence of the plant are highly cytotoxic and show high cytotoxicity at 200µg/ml concentration against DLA cells. The major components of flower essential oil are phytol, 2-Naphthalene methanol,1,2,3,4,4a,5,6,7-octahydro-alpha.,alpha.,4a,8-tetramethyl-(2R-cis)-, Hexadecanoic acid,methyl ester,Heptanoic acid, 3-hexenyl ester, (Z)-, n-Hexadecanoic acid, Isophytol, Alpha-Cadinol,Tricosane, 3-Hexen-1-ol, benzoate,(Z). Out of 43 isolated components of flower essential oils, these are the components present in high percentages. It includes esters, phenols as well as terpenoids, which are reported to be antioxidants, antimicrobials and cytotoxic .*Samadera indica* flower stalk essential oil contains 1,2-Benzenedicarboxylic acid, butyl methyl ester, 1,2- Benzenedicarboxylic acid, bis(2-methyl propyl)ester, 2-ethyl-1-Hexanol, phytol and other esters. Out of 17 components identified, these four are the components present in high percentages. The flower and flower stalks can be considered as a source of natural antioxidants, antimicrobials and cytotoxic.Further studies are needed to explore the potential phenolics and flavonoids, terpenoids compounds from flower and flower stalk of *Samadera indica* for application in drug delivery, nutritional or pharmaceutical fields.

Conflict of interest statement

We declare that we have no conflict of interest

Acknowledgment

The authors are grateful to Principal, Vimala College and St. Marys College Thrissur, Kerala, Director Amala Cancer Research Centre, Amala Nagar, Thrissur, Kerala, IICT- Hyderadad for encouragement and providing facilities for doing this research work

REFERENCES

[1] Warrier PK, Nambiar VPK.andRamankutty C: Indian Medicinal Plants A Companion of 500 species. Orient Longman Ltd, Madras 2000

[2] Kirtikar KR and Basu BD: Indian Medicinal Plants. Orient Longman Ltd, Dehra Dun 1998.

[3] Mbatchi SF, Mbatchi JT, Banzouzi T, Nesonde GF and Ouambaand JM: *In vitro* anti plasmodial activity of 18 plants used in Congo Brazzaville traditional medicine. J. Ethnopharmacol 2006; 2:168-74.

[4] Malathi R, Rajan SS, Suresh G, Krisishnakumari GN and Gopalakrishnan G: Samaderin B and C from *Samadera indica*.Acta Crystallography 2003; 59:416-18.

[5] Rajalakshmi GR and Harindran J: Anti-inflammatory activity of *Samadera indica* leaves by Membrane Stabilization. *Int J Pharm Sci Res*. 2013; 4(2); 721-723.

[6] P.H. Rajasree, VidyaVishwanad, Merin Cherian, JincyEldhose and Ranjith Singh- Formalution and evaluation of antiseptic polyherbal ointment – International Journal of Pharmacy and Life Sciences- ISSN 0976-7126. 3(10): Oct.,2012.

[7] AlokSahai, SudhanshuDwivedia And NidhiSaxena- Luteolin-7-O-B-D-Glucopyranoside From The Kernals Of *Samadera Indica*-Gaertn - Int. J. Chem. Sci.: 10(1), 2012, 104-107 .

[8] VidyaViswanad, N.A Aleykutty, B. Jaykar, Subin Mary Zachariah, Litha Thomas- Studies on Antimicrobial And Antioxident Activity of Methanolic extract of *Samadera indica*- Volume 11, Issue 2, November – December 2011; Article-012 Issn 0976 – 044x

[9] VidyaViswanad, N.A Aleykutty, Subin Mary Zachariah, Litha Thomas- Evaluation of Antioxidant and Free Radical Scavenging Activity of *Samadera indica* Using *In vitro* Models- Pharmacognosy Journal- Volume 3,Issue 23- Pages 85-90, July 2011

[10] **'**Organic Termite Control'(1994) P Forshaw. HDRA undergraduate report.

[11] 'Natural Pest and Disease Control' (undated) H Elwell and A Mass, published by the Natural Farming Network, PO Box 8515, Causeway, Harare, Zimbabwe.

[12] 'Natural Pest and Disease Control'(1996) Gaby Stoll, published by Magraf Verlag, PO Box 105 97985 Weikersheim, Germany

[13] Tharakan Dhanasekaran M and Manyam BV2005 PhototherapyResearch.**19** 669

[14] J.B. Harborne, Pytochemical methods, A guide to Modern techniques of plant analysis, Chapman and Hall, London, 1973.

[15] Talwar G P (1971) Hand book of practical immunology In: Talwar G P (Eds) National Book Trust, New Delhi PP - 336-339.

[16] Aquino R, Morelli S, Lauro M R , Abdo S, Saija A and Tomaino A (2001). Phenolic constituents and antioxidant activity of an extract of Anthuriumversicularleaves.Journal of Natural products 64, 1019-1023.

[17] Siddhuraja R, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringaloifera Lam.) leaves. J Agr Food Chem 2003; 51: 2144-2155.

[18] Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002; 10: 178-182.

[19] RamalakshmiS,Edayulla N, Ramesh P,Muthuchelian K. Investigation on cytotoxic, antioxidant, antimicrobial and volatile profile of *Wrightiatinctoria*(Roxb) R.Br. flower used in Indian medicine. Asian Pacific Journal of Tropical Disease 2012;D68-S75.

[20] Jensen A. Chlorophyll and carotenoids. In: Hallebust JA, Craigie JS. (eds). Handbook of physiochemical and biochemical methods. Cambridge: Cambridge University Press; 1978, p. 5-70.

[21] Sindhu C, Beena Jose, Evaluation of DPPH radical scavenging activity of the leaf and bark extracts of Samadera indica from South India; WJPR,Vol 4,8-2015

[22] Siddhuraju P, Mohan PS, Beaker K. Studies on the antioxidant activity of Indian Laburnum (Cassia fistula L.). A preliminary assessment of crude extracts from stem, bark, leaves and fruit pulp. Food chem 2002; 79: 61-67.

[23] Dinis TCP, Madeira VMC, Almeida LM.Action of phenolic derivatives (acetoaminophen, salicilate and 5-aminosalicilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers.Arch BiochemBiophys 1994; 315: 161-169.

[24] Kannan RRR, Arumugam R, Anantharaman P. In vitro antioxidant activities of ethanol extract from Enhalusacoroides (L.F.) Royle. Asian Pacific J Trop Med 2010; 3(11): 898-901.

[25] Aquino R, Morelli S, Lauro M R , Abdo S, Saija A and Tomaino A (2001). Phenolic constituents and antioxidant activity of an extract of Anthuriumversicularleaves.Journal of Natural products 64, 1019-1023.

[26] Jayaprakasha GK, Jaganmohan RL, Sakariah KK. Antioxidant activities of flavidin in different in vitro model systems.Bioorg Med Chem 2004; 12(19): 5141-5146.

[27] Omar S, Lemonnier B, Jones N, Ficker C, Smith ML and Neena C. Antimicrobial activity of extracts of eatern North American hardwood trees and relation to traditional medicines. 2000 JEthnopharmacol**73** 170.

[28] Moldeus P, Hogberg J and Orrenius S (1978),Isolation and use of liver cells. Methods in Enzymology 52, 60-71

[29] Dr. RaghuveerIrchhaiya, Anurag Kumar, Anumalikyadav, Nitika Gupta ,Swadesh Kumar1, Nikhil Gupta, Santosh Kumar2, Vinay Yadav2 , Anuj Prakash2 andHimanshu Gurjar3,Metabolites in plants and its classification- WJPPS- 4- 1(2015)

[30] Lu Y, Foo Y. Antioxidant and radical scavenging activities of polyphenols from apple pomace. Food Chem 2000; 68: 81-85.

[31] Yamaguchi T, Takamura H, Matoba T, Terao J. HPLC method for evaluation of the free radical scavenging activity of foods by using 2, 2-diphenyl-1-picryl hydrazyl. Bio Sci Biotechnol Bio Chem 2002; 62: 1201-1204

[32] Isabel C.F.R. Ferreira , Paula Baptista, Miguel Vilas-Boas, Lillian Barros- Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity- Food Chemistry 100 (2007) 1511–1516

[33] Gardner PT, White TC, Mcphail DB, Duthie GG.The relative contributions of Vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. Food Chem 2000; 68: 471-474.

[34] Manilal A, Sujith S, SeghalKiran G, Selvin J, Shakir C. Cytotoxic potentials of Red Alga, Laurenciabrandenii collected from the Indian Coast. Global J Pharm 2009; 3(2): 90-94.

[35] PratapChandran R., Vysakhi, M.V., Manju, S., Kannan, M., Abdul Kader, S And Sreekumaran Nair, *In Vitro* Free Radical Scavenging Activity Of Aqueous And Methanolic Leaf Extracts Of *AegleTamilnadensis*Abdul Kader (Rutaceae),Int J Pharm Pharm Sci, 5, Suppl 3, 819-823(2013)

[36] Arulmozhi S, Papiya MM, Purnima A, Sathiya N. In vitro antioxidant and free radical scavenging activity of Alstoniascholaris Linn. R.Br. Iran J Pharm Therap 2008; 6: 19

[37] Geronikaki A A and Gavalas A M (2006) Antioxidants and anti-inflammatory diseases: synthetic and natural antioxidants with anti inflammatory activity. Combinatorial Chemistry and High Throuhput Screening9, 425- 442

[38] Cai Y, Luo Q, Sun M and Corke H (2004). Antioxidant activity and Phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sciences 74, 2157- 2184.

[39] Sezik, E., Tumen, G., Kirimer, N., Ozek, T., and Baser, K.H.C. 1993. Essential Oil Composition of Four Origanumvulgare Subspecies of Anatolian Origin. J. Ess. Oil Res., 5: 425-431.