CHAPTER III

IDENTIFICATION OF PHYTOCHEMICAL CONSTITUENTS IN *MEMECYLON SISPARENSE* GAMBLE LEAVES AND ITS ANTIMICROBIAL ACTIVITY

3. INTRODUCTION

Phytochemicals are formed in plants during their normal metabolic process. These phytochemicals are used by most of the people especially in developing countries for their health care. These phytochemicals exhibit best sources of antioxidant, anti-inflammatory, antimicrobial, psoriasis, anticancer activities and used towards the cure of many diseases. Plants are the best sources of bioactive compounds, used as dietary supplements and folk medicines etc. The natural extract having potential bioactive phenolic compounds with more antioxidant capacity can be obtained by doing extraction in solvents such as ethyl acetate (63). The studies on *M.edule* reveal that ethyl acetate is the suitable solvent for extracting potential bioactive components which had shown broad spectrum antimicrobial activity (64).

The bacteria develop resistance by protecting themselves from antibiotics by various drug resistant mechanisms there by developing antibiotic or drug resistant bacteria (65). Due to the occurrence of antibiotic or drug resistant bacteria, search for new anti-microbial agents particularly from plant origin became pivotal because of having safety and also to overcome the undesired side effects of some antibiotics.

3.1 MATERIALS AND METHODS

3.1.1 Collection of *Memecylon sisparense* Gamble leaves

Memecylon sisparense Gamble (MSG) leaves were collected from the forest of Tirumala hills in Chittoor district, Andhra Pradesh, India. The authentication of leaves was done by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupathi against the voucher specimen No.984 deposited at the herbarium of Sri Venkateswara University.

3.1.2. Preparation of *Memecylon sisparense* Gamble leaves extracts

The leaves of *Memecylon sisparense* Gamble are dried in shade, powdered and sieved. The coarse fine powdered leaf materials of 150 gm were extracted successively with 600 ml of methanol, ethyl acetate, n-hexane respectively in soxhlet apparatus. These crude extracts were concentrated to dryness by using Heidolph rotary evaporator (Model: Hei vap advantage ML/HB/G3) at a temperature not exceeding 40 ^{o} C and freeze dried the crude extracts using a

Verdant Scientific lyophilizer (Model: Sub-Zero). Five mg/ml stock solutions were prepared in DMSO and diluted with autoclaved water for desired concentration.

3.1.3. Estimation of total phenolic content of *Memecylon sisparense* Gamble leaves extracts

This is a method for polyphenolic content quantification based on colorimetric measurements with Folin-Ciocalteu reaction. MSG leaves crude extracts were determined spectrophotometrically for total phenolic content by using gallic acid as a standard by *Scalbert method* with slight modification (66). 0.2 ml of FC reagent was added along with 7.5 % sodium carbonate solution (0.75 ml) to 0.1 ml of MSG leaf extracts and incubated about 30 min at room temperature. Measured the absorbance at 765 nm and the result is expressed as GAE (gallic acid equivalents) in mg/ gm of dry extract.

3.1.4 Estimation of total flavonoid content of *Memecylon sisparense* Gamble leaves extracts

MSG leaves crude extracts were determined spectrophotometrically for total flavonoid content with rutin as a standard by *Brighente method* with slight modification (67). 0.03 ml of sodium nitrite (5 %) was added to 0.1 ml of MSG leaves extracts and incubated for five minutes at 25 °C and then 0.03 ml of 10% aluminium nitrate added and incubated for five minutes at room temperature. Latter treated this reaction mixture with 1M NaOH (0.2 ml) and diluted with water (0.5 ml), absorbance measured at 510 nm. The result was expressed as RE (rutin equivalents) in milligram per gram of dry extract. In this assay, the sample solutions react with 5.0 % w/v $NaNO_2$ solution. In this reaction, a flavonoid-aluminum complex occurs in alkaline condition using aluminum nitrite.

3.1.5 DPPH free radical scavenging activity of *Memecylon sisparense* Gamble leaves extracts

The scavenge free radicals against the MSG leaf crude extracts capacity was determined spectrophotometrically by using Ascorbic acid, Curcumin, Butylated hydroxyltoluene (BHT), Trolox as standard in DPPH free radical scavenging assay (68). To 0.1 ml of MSG leaves extracts, DPPH methanolic solution of 1 ml was added and incubated the reaction mixture for 20 min at room temperature and measured the absorbance at 517 nm. The % FRSA (percentage of free radical scavenging activity) was calculated by using the formula:

$$\% \text{ FRSA} = (A_{\text{control}} - A_{\text{extracts}}/A_{\text{control}}) \times 100$$

3.1.6 Preliminary phytochemical screening of *Memecylon sisparense* Gamble leaves ethyl acetate extract

The preliminary phytochemical studies were done on *Memecylon sisparense* Gamble leaves ethyl acetate extract in order to ascertain major groups of phytochemical constituents by following the standard methods (69-72).

Ninhydrin test for aminoacids: To 2 ml of MSG leaves ethyl acetate extract solution, add two drops of ninhydrin solution (1 mg in 100 ml of acetone) and the appearance of purple colour shows the presence of amino acids.

Molish's test for carbohydrates: To 2 ml of MSG leaves ethyl acetate extract solution, add two drops of α-naphthol alcoholic solution, mix thoroughly. Then add a few drops of sulphuric acid slowly to the mixture along with the test tube sides and the violet ring appearance shows the presence of carbohydrates.

Borntrager's test for glycosides: Hydrolyse MSG leaves ethyl acetate extract with hydrochloric acid and then collect the hydrolysate. To 2 ml of hydrolysate, add equal volume of chloroform and mix thoroughly until the chloroform layer separates which will be collected into a test tube. Then, add half the volume of 10 % ammonia solution to the separated chloroform layer. The pink colour appearance shows the presence of glycosides.

Ferric chloride test for phenols: Add few drops of neutral solution of 5 % ferric chloride to 2 ml of MSG leaves ethyl acetate extract. The presence of phenolic compounds was revealed by dark green color appearance.

Alkaline reagent test for flavonoids: Add few drops of sodium hydroxide solution to 2 ml of MSG leaves ethyl acetate extract solution. The flavonoids presence is indicated by the yellow fluorescence appearance and that disappears upon addition of diluted hydrochloric acid.

Foam test for saponins: Add 20 ml of water to 50 mg of the ethyl acetate extract and shake vigorously in graduated cylinder for 15 min. The occurrence of foam layer around 2 cm indicates the presence of saponins.

Gelatin test for tannins: Add 2 ml of 1 % gelatin solution with 10 % sodium chloride to 2 ml of MSG leaves ethyl acetate extract. White precipitate appearance shows the presence of tannins.

Test for steroids: To 1mg of MSG leaves ethyl acetate extract, add 5 ml of chloroform and equal volume of concentrated sulphuric acid along with the sides. The formation of an array of deep

red color in upper layer, yellow color with green fluorescence in the lower layer of sulphuric acid shows the presence of steroids.

Test for fixed oils: Press gently the ethyl acetate extract of MSG leaves between two filter paper and appearance of oil stain shows the presence of fixed oils.

Test for gums and mucilages: Mix 2 ml of absolute alcohol with 1 ml of ethyl acetate extract under constant stirring. White precipitate appearance shows the presence of gums and mucilages.

Test for anthraquinone: Hydrolyse ethyl acetate extract with diluted sulphuric acid and extract with benzene. Add diluted ammonia solution (4-5 drops) to the extract. The rose pink colour appearance shows the presence of anthraquinone.

Test for alkaloids: Add 2 ml of hydrochloric acid and 1ml of Dragendroff's reagent to 5 ml of ethyl acetate extract solution. The appearance of red precipitate shows the presence of alkaloids.

Test for triterpenoids: Add 50 mg of ethyl acetate extract to 2 ml of chloroform and 1ml of acetic anhydride. Then, add 1ml of concentrated sulphuric acid to this mixture from the sides of the test tube. Reddish violet ring appearance indicates the presence of triterpenoids.

3.1.7 HPTLC finger printing of *Memecylon sisparense* Gamble leaves ethyl acetate extract

Chromatography was performed on HPTLC plates (5 cm X 10 cm) coated with silica gel of 0.25 mm layer. These plates had undergone methanol washing before using and activated for 5 min at 110 ^{o}C. The MSG leaves ethyl acetate extract was applied as wide bands of 4 mm with 6 mm apart. The application rate of 6 µl/s was used constantly. Toluene: Ethyl acetate: Methanol: Formic acid was used as mobile phase in 3:1:1:0.1 (v/v/v/v) ratio.

3.1.8 GC-MS analysis of *Memecylon sisparense* Gamble leaves ethyl acetate extract

The GC-MS analysis was carried out on Agilent 7890A (Agilent Technologies, USA). Gas chromatography system was equipped with a time-of-flight mass spectrometer (Pegasus HT TOF, LECO Corporation, USA). Separation of analytes were carried out by a capillary column (Agilent J and W HP-5ms, (5 %) Phenyl-methylpolysiloxane 30 m x 0.25 mm, film thickness 0.25 µm). Ultra-high pure helium (99.999%) was used as carrier gas at a constant flow rate of 1ml/min in a split less mode.

To calculate the retention indices, C_7 to C_{40} n-alkane mixture (1 µg/µl) was run prior to analysis of MSG leaves ethyl acetate extract (MSGLEAE). 1 µl of MSGLEAE was manually injected into inlet of column at 250 ^{o}C operating in a split less mode. Retention indices of each compound

were calculated according to Van den Dool and Kratz (73). The parameters, such as the Retention time, similarity and Retention Index (RI) values were matched with that of peaks and subsequently identified through a NIST/EPA/NIH Mass Spectral Library 2011 library.

3.1.9 Anti-bacterial activity of *Memecylon sisparense* Gamble leaves ethyl acetate extract

Antibacterial activity of MSG leaves EAE was done against the following bacterial strains: *Staphylococcus aureus* (MTCC 11949), *Staphylococcus epidermidis* (MTCC 3615), *Bacillus subtilis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC 424), *Bacillus cereus* (MTCC 430), and *Eschericia coli* (MTCC 118). These strains are maintained in 20% glycerol stock and sub cultured before use. The antibacterial activities were done by both qualitative technique of agar well diffusion assay (72, 74-76) and quantitative assay of Resazurin based microplate assay (77, 78) for determination of MIC, MBC.

3.1.9.1 Agar well diffusion assay:

After preparation of nutrient agar media, poured into sterile Petri plates, then inoculated with respective bacteria after solidification. By using a sterile cork borer, 8 mm diameter holes were punched aseptically and loaded with 100 µl of MSG leaves ethyl acetate extract dilutions ranging from 5000, 2000 to 62.5 µg/ml in two fold dilution against blank and Ciprofloxacin (10 µg/ml) was used as a standard drug. The agar plates are then incubated at 37^{o}C in an incubator. The anti-microbial agent present in the extract diffuses into the agar media and inhibits the growth of bacteria there by showing the zone of inhibition. The diameters of the inhibitory zones were measured in mm in three different directions and the experiments were repeated thrice by calculating mean ± standard deviation of the readings.

3.1.9.2 Micro dilution method:

After preparation of nutrient agar media, poured into sterile Petri plates, then inoculated with respective bacteria after solidification and incubated at 37^{o} C for 24 h in an incubator. After 24 h, 2-3 colonies were picked by sterile inoculating loop and transferred into saline (0.85 % w/v), vortexed and adjusted the turbidity to 0.5 McFarland scale. The sterile nutrient broth was prepared and 50 µl of media was transferred to 96 well plates. The ethyl acetate extract stock (5 mg/ml) was prepared in dimethylsulphoxide (DMSO). Then two fold dilutions ranging from 2.5 mg/ml to 0.04 mg/ml were done in the wells with media. Then, aseptically 50 µl of inoculum was transferred into all the wells. To negative control wells, only media was added, whereas 50 µl media and 50 µl inoculum wells serves as positive control, and only extract dilutions in broth

serves as a blank. Then plates incubated for 18 h at 37^{o} C in an incubator. After 18 h, 10 µl of 0.02 % (w/v) resazurin dye was added and further incubated for 2 h and read at 570 nm to determine minimum inhibitory concentration (MIC).

A loop full of broth was collected from wells of MIC and streaked upon the nutrient agar plates, incubated for 48 h at 37^{o} C for determining the minimum bactericidal concentration (MBC). After incubation of plates, the concentration of MSG leaves ethyl acetate extract at which no visible growth of bacteria was seen are recorded as MBC. All the experiments were performed in triplicates and repeated thrice.

3.2 RESULTS AND DISCUSSION

3.2.1 Results

3.2.1.1 Yield of *Memecylon sisparense* Gamble leaves extracts

The yield of MSG leaves n-hexane, methanolic and ethyl acetate extracts was found to be 4.7 %, 10.0 %, and 8.7 % respectively.

3.2.1.2 Total phenolic content of *Memecylon sisparense* Gamble leaves extracts

Total phenolic content is a laboratory index of antioxidant strength**.** The total phenolic content of *Memecylon sisparense* Gamble leaves extracts obtained from the Fig. 3.1 calibration curve is in the following order: ethyl acetate extract (238.99 ±28), methanolic extract (49.07±0.6) GAE/gm of dry extract respectively.

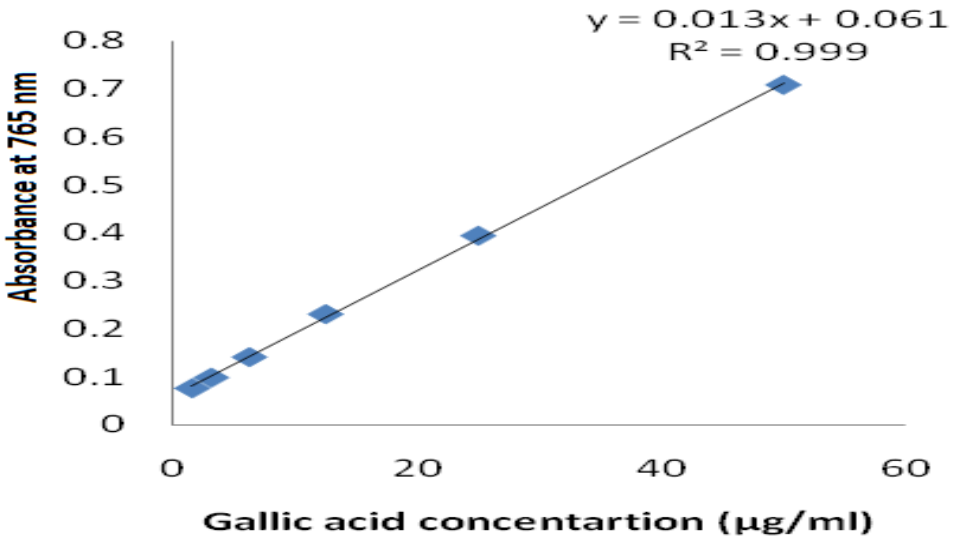

Fig. 3.1: Gallic acid calibration curve for estimating total phenolic content in *Memecylon sisparense* Gamble leaves extracts

3.2.1.3 Total flavonoid content of *Memecylon sisparense* Gamble leaves extracts

The total flavonoid contents of MSG leaves obtained from the Fig. 3.2 calibration curve is in the following order: ethyl acetate leaves extract (99.41±1), methanolic leaves extract (16.5±13) RE/gm of dry extract respectively.

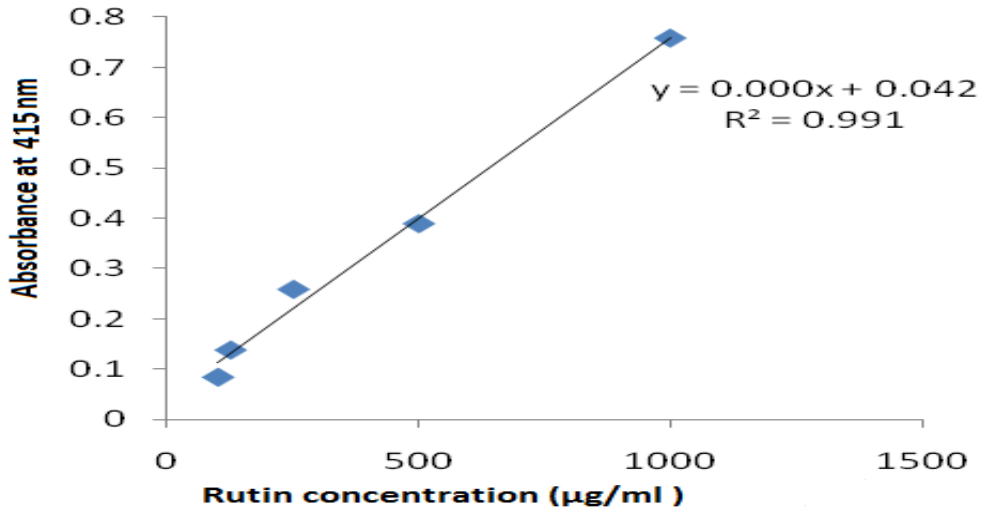

Fig. 3.2: Rutin calibration curve for estimating total flavanoid content in *Memecylon sisparense* Gamble leaves extracts

The MSG leaves hexane extract was devoid of phenolic and flavonoid compounds.

3.2.1.4 DPPH free radical scavenging activity of *Memecylon sisparense* Gamble leaves extracts

In the percentage inhibition of the DPPH radical, a steady rise was observed by the ethyl acetate extracts up to 250 µg/ml concentration, there after a levelling off with much slow rise in inhibition. At 250 µg/ml concentration of the MSG leaves extracts, the DPPH radical inhibition along with the standard references like ascorbic acid, curcumin, BHT and trolox was in the following order: trolox (96.58 %) > ethyl acetate leaves extract (93.67 %) > ascorbic acid (90.23 %) > methanolic leaves extract (78.56 %) > BHT (76.27 %) > curcumin (70.24 %) > hexane leaves extract (7.21 %), shown in Fig 3.3. The EC_{50} values (± SEM) of ascorbic acid, curcumin, BHT, trolox, hexane leaves extract, ethyl acetate leaves extract and methanolic leaves extract were 80.35±11.9, 172.91±7.28, 116.05±30.8, 97.2±9.98, 1483.02± 5.58, 64.40± 3.45 and 90.98± 6.91 µg/ml respectively. As MSG leaves ethylacetate extract is having more free radical scavenging activity compared to hexane and methanolic extracts, hence selected for further screening activities.

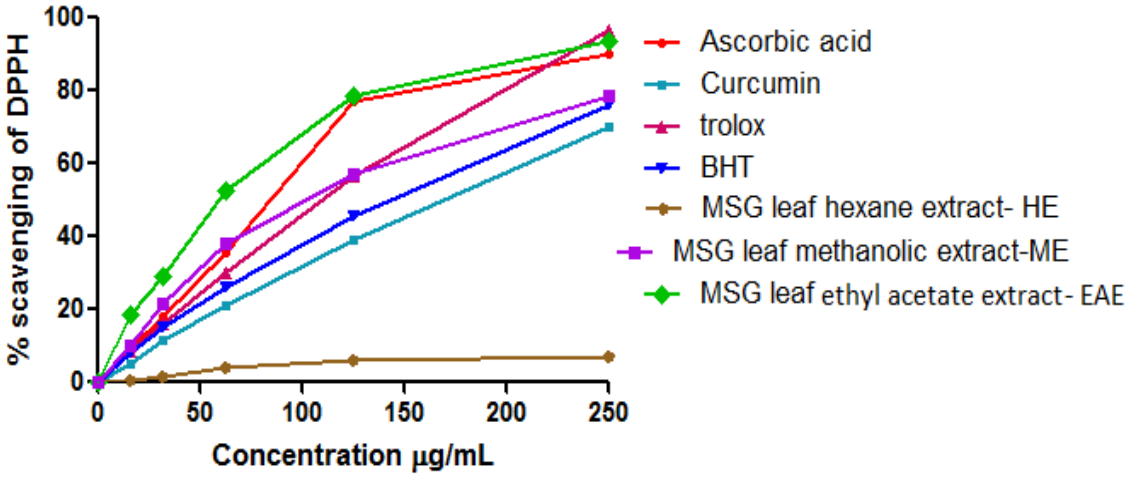

Fig. 3.3: DPPH free radical scavenging activity of *Memecylon sisparense* Gamble leaves extracts

3.2.1.5 Preliminary phytochemical screening of *Memecylon sisparense* Gamble leaves ethyl acetate extract

Screening for qualitative analysis of MSG leaves ethyl acetate extract for phytochemicals revealed the presence of phenols, flavonoids, amino acids, carbohydrates, triterpenoids, tannins, steroids, saponins, glycosides, alkaloids and fixed oils (Table 3.1).

Table 3.1 Preliminary phytochemical screening of *Memecylon sisparense* Gamble ethyl acetate extract

S.No.	Name of the test	Phytochemical constituent	Result
1	Molish's test	Carbohydrates	+
2	Borntrager's test	Glycosides	+
3	Ninhydrin test	Amino acids	+
4	Gelatin test	Tannins	+
5	Ferric chloride test	Phenols	+
6	Alkaline reagent test	Flavonoids	+
7	Triterpenoids test	Triterpenoids	+
8	Steroids test	Steroids	+
9	Foam test	Saponins	+
10	Fixed oils test	Fixed oils	+
11	Gums and mucilages test	Gum and mucilages	-
12	Anthraquinones test	Anthraquinones	-
13	Alkaloids test	Alkaloids	+

'+' indicates positive and '_' indicates negative.

3.2.1.6 HPTLC finger printing of *Memecylon sisparense* Gamble leaves ethyl acetate extract

The HPTLC of MSG leaves ethyl acetate extract gave 11 spots in the solvent system of Tolune: Ethyl acetate: Methanol: Formic acid 3:1:1:0.1 (v/v/v/v) at the retention time values (min): 0.11, 0.23, 0.35, 0.5, 0.54, 0.63, 0.69, 0.81, 0.83, 0.94 and 0.98 respectively and the chromatogram is represented below in Fig. 3.4.

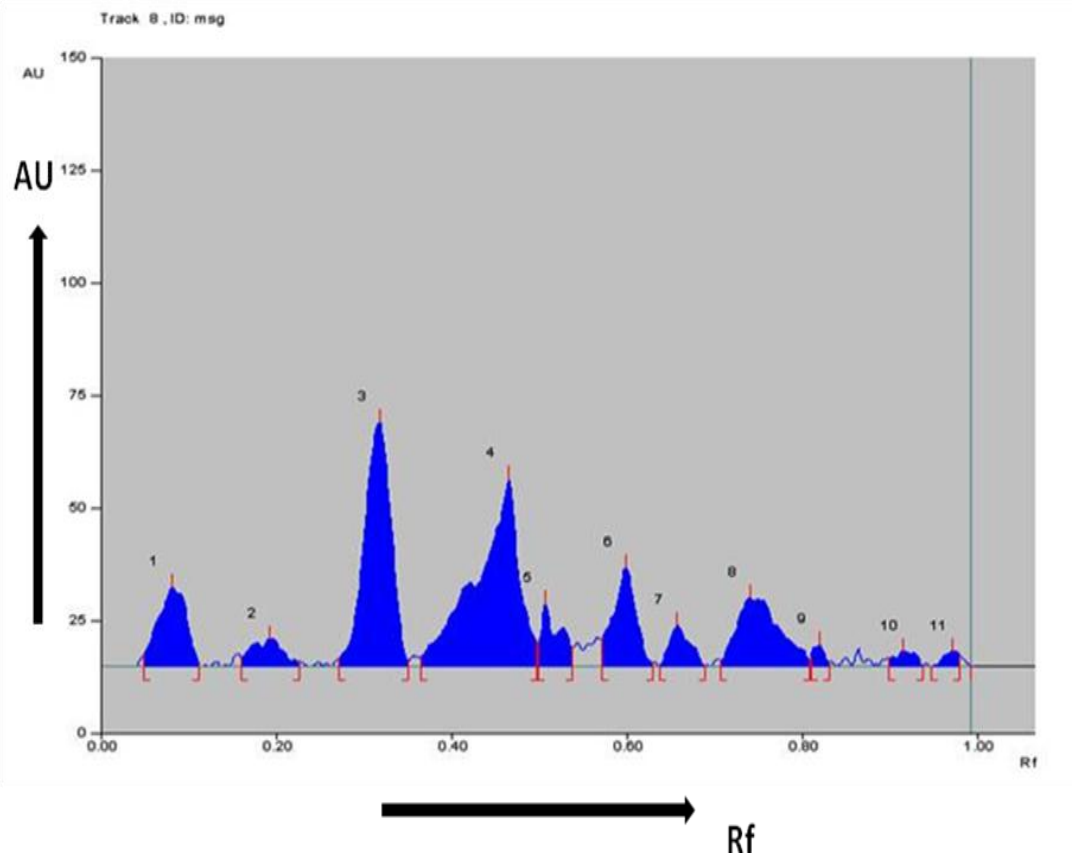

Fig. 3.4: HPTLC Chromatogram of *Memecylon sisparense* Gamble leaves ethyl acetate extract

3.2.1.7 GC-MS analysis of *Memecylon sisparense* Gamble leaves ethyl acetate extract

In GC-MS analysis of MSG leaves ethyl acetate extract, forty one compounds were identified by using NIST11 library based on retention time, retention index (RI) with difference of ± 30 of experimental values compared to library values, molecular formula and molecular weight along with similarity above 700 and shown in Table 3.2 . Some of the peaks in GC-MS corresponds to Methylglyoxal (2.59 %), Diglycolamine (4.17 %), 1H-Imidazole,1-acetyl- (3.76 %), m-Cresyl acetate (0.97 %), 2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl- (1.69 %), Heptadecane 9-Octyl (13.87 %) etc, chromatogram represented in Fig. 3.5.

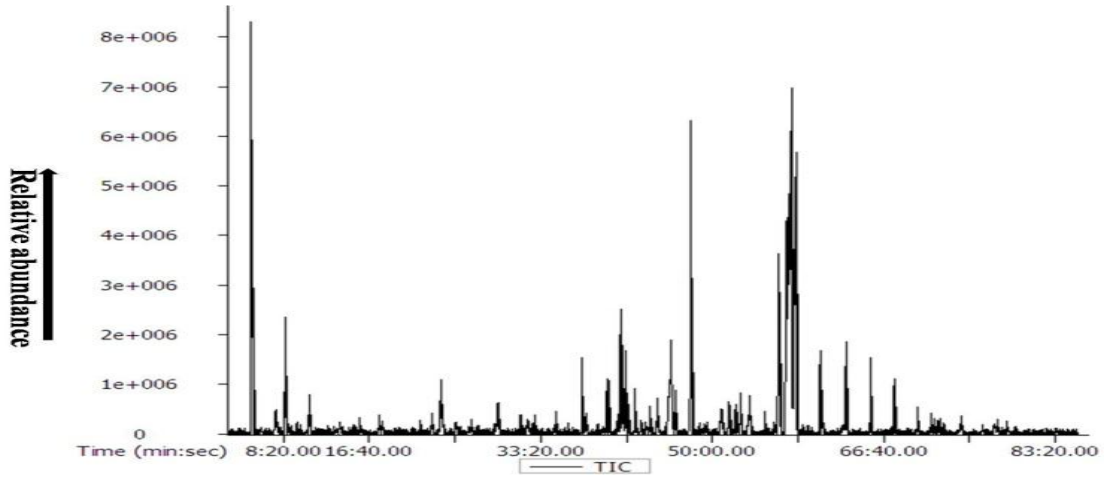

Fig. 3.5: GC-MS Chromatogram of *Memecylon sisparense* Gamble leaves ethyl acetate extract

Table 3.2 Identification of phytochemical constituents in *Memecylon sisparense* Gamble leaves ethyl acetate extract by GC-MS

S.No.	RT (min)	Name	Empirical formula	Exact Mass	RI_{Exp}	RI_{Lib}	Area %
1	05:11	3,3-Dimethyl-4-methylamino-butan-2-one	$C_7H_{15}NO$	129.1154	968	967	2.28
2	05:14	Methyl glyoxal	$C_3H_4O_2$	72.0211	972	970	2.59
3	05:17	Diglycolamine	$C_4H_{11}NO_2$	105.079	974	980	4.17
4	08:34	1H-Imidazole, 1-acetyl-	$C_5H_6N_2O$	110.048	1069	1054	3.76
5	09:32	1-Amino-2,6-dimethylpiperidine	$C_7H_{16}N_2$	128.1313	1095	1098	0.01
6	09:39	N,N,O-Triacetylhydroxylamine	$C_6H_9NO_4$	159.0532	1099	1122	0.24
7	10:50	m-Cresyl acetate	C_7H_8O	108.0575	1126	1136	0.97
8	13:48	Phenol, 2,4-dimethyl-, acetate	$C_{10}H_{12}O_2$	164.0837	1194	1217	0.11
9	17:39	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	$C_7H_9NO_2$	139.0633	1281	1265	0.36
10	17:57	Benzene, 1,3-bis(1,1-dimethylethyl)-	$C_{14}H_{22}$	190.1722	1287	1249	0.11
11	19:01	Phenol, 4-ethyl-2-methoxy-	$C_9H_{12}O_2$	152.0837	1312	1303	0.02
12	29:01	Cyclohexanecarboxylic acid, 3-fluorophenyl ester	$C_{13}H_{15}FO_2$	222.1056	1554	1596	0.13
13	29:05	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.1671	1557	1539	0.51
14	29:14	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	180.115	1560	1532	1.69
15	29:16	Naphthalene, 1,6,7-trimethyl-	$C_{13}H_{14}$	170.1096	1561	1552	0.22

16	32:44	Pentadecane, 2,6,10-trimethyl-	$C_{18}H_{38}$	254.2974	1653	1654	0.45
17	34:45	Azulene, 1,4-dimethyl-7-(1-methylethyl)-	$C_{15}H_{18}$	198.1409	1709	1734	0.49
18	35:58	Chamazulene	$C_{14}H_{16}$	184.1252	1743	1728	0.18
19	36:24	Heptadecane, 2,3-dimethyl-	$C_{19}H_{40}$	268.313	1756	1782	0.06
20	37:43	3-Octadecene, (E)-	$C_{18}H_{36}$	252.2817	1794	1795	1.43
21	38:55	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.2246	1829	1823	0.20
22	39:39	9-Nonadecene	$C_{19}H_{38}$	266.2974	1851	1880	1.53
23	41:05	7-Octadecyne, 2-methyl-	$C_{19}H_{36}$	264.2817	1895	1863	2.17
24	41:14	2-Heptadecanone	$C_{17}H_{34}O$	198.1984	1899	1878	1.31
25	41:18	9-Nonadecene	$C_{19}H_{38}$	266.2974	1901	1880	0.52
26	41:19	1,2-Dioctylcyclopropene	$C_{19}H_{36}$	264.2817	1902	1913	1.61
27	42:04.	1H-Indene, 5-decyloctahydro-	$C_{19}H_{36}$	264.2817	1925	1937	1.42
28	44:00	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284.2715	1986	1975.6	0.42
29	44:39	Butyric acid, 4-pentadecyl ester	$C_{19}H_{38}O_2$	298.2872	2007	2013	0.91
30	45:58	10-Undecenoic acid, octyl ester	$C_{19}H_{36}O_2$	296.2715	2050	2067	1.23
31	46:26	Eicosane, 10-methyl-	$C_{21}H_{44}$	296.3443	2065	2041.6	1.81
32	48:44	Oxalic acid, allyl tridecyl ester	$C_{18}H_{32}O_4$	312.2301	2143	2135	0.13
33	52:11	5-Eicosene, (E)-	$C_{20}H_{40}$	280.313	2265	2285	0.36
34	52:23	Docosane, 2,21-dimethyl-	$C_{24}H_{50}$	338.3913	2271	2279	1.48
35	55:47	5-Isobenzofurancarboxylic acid, 1,3-dihydro-3-oxo-, nonyl ester	$C_{18}H_{24}O_4$	304.1675	2397	2447	10.71
36	57:56	Heptadecane, 9-octyl-	$C_{25}H_{52}$	352.4069	2481	2442	13.87
37	58:48	3-Hexadecylaminopyridine	$C_{21}H_{38}N_2$	318.3035	2515	2477	0.24
38	59:21	N-{(4-Hydroxy-3-methoxyphenyl)methyl}-8-methyl-6-nonenamide	$C_{18}H_{27}NO_3$	305.1991	2537	2541	2.10
39	60:29	Docosane, 11-butyl-	$C_{26}H_{54}$	366.4226	2582	2542	1.33
40	72:05	Hentriacontane	$C_{31}H_{64}$	436.5008	3096	3100	0.32
41	74:06	dl-α-Tocopherol	$C_{29}H_{50}O_2$	430.3811	3194	3149	0.16

Among the 41 compounds, 20 compounds are found to have various biological activities which are reported from Dr.Dukes Phytochemical and Ethnobotanical Databases-USDA (79) and mentioned in Table 3.3.

Table 3.3 Biological activity of phytochemical constituents identified by GC-MS in *Memecylon sisparense* Gamble leaves ethyl acetate extract

Name of the compound	**Activity***
Methyl glyoxal	Increase Glyoxalate Transamination, Catechol-O-Methyl - Transferase inhibitor, Methyl donor, Methyl-Guanidine inhibitor
1H-Imidazole, 1-acetyl-	Actyl-choline antagonist, 5-HT inhibitor, Antidote, Hallucinogenic, Hepatoprotective
1-Amino-2,6-dimethylpiperidine	Increase Aromatic Amino Acid Decarboxylase Activity
N,N,O-Triacetylhydroxylamine	Inhibit production of Tumor Necrosis factor, Nitric-Oxide-Synthase-Inhibitor, Antitumor (Nasopharynx), Nephroprotective, Neuromuscular-Blocker, NF-kB inhibitor, NO-Scavenger, Nociceptive, Inhibit Production of Uric acid, Oxidant.
m-Cresyl acetate	Anticancer (mammary), Inhibit Microtubule formation, MAPK inhibitor, Mast cell stabilizer, Microphagocytogenic, Mitogen, Regulate calcium metabolism
1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	Methyl-Guanidine inhibitor, Anti-5-HT, Antidote, Hallucinogenic, Hepatoprotective, Increase T-Helper
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	Histamine Inhibitor, HIV-RT-Inhibitor, Hyperglycemic, Increase T-helper, ACE Inhibitor, Analgesic, Antiarthritic, Antacid, Antibiotic, Anticancer, Anticoagulant, Antidiuretic, Antimicrobial, Antioxidant, GABA Antagonist
Cyclohexanecarboxylic acid, 3-fluorophenyl ester	Arachidonic acid Inhibitor, Inhibit Production of Uric acid
Pentadecanoic acid	Arachidonic acid Inhibitor, Inhibit Production of uric acid
7-Octadecyne, 2-methyl-	Methyl-Guanidine-Inhibitor, Methyl donor
1H-Indene, 5-decyloctahydro-	Anti-5-HT, Hepatoprotective, HIV-RT-Inhibitor, Increase T-Helper
Hexadecanoic acid, 15-methyl-, methyl ester	Methyl-Guanidine-Inhibitor, Methyl donor, Arachidonic acid Inhibitor, Inhibit Production of Uric acid
Butyric acid, 4-pentadecyl ester	Arachidonic acid Inhibitor, Inhibit Production of Uric acid
10-Undecenoic acid, octyl ester	Arachidonic acid Inhibitor, Inhibit Production of Uric acid
Eicosane, 10-methyl-	Methyl-Guanidine-Inhibitor, Methyl donor
Oxalic acid, allyl tridecyl ester	Arachidonic acid Inhibitor, Inhibit Production of Uric acid
5-Eicosene, (E)-	Anticancer (oesophagus), Decrease Epinephrin production, Decrease Oxalate excretion, ER-Beta-Binder
N-{(4-Hydroxy-3-methoxyphenyl)methyl}-8-	Antitumor, Nephroprotective, NO Scavenger, Tumor Necrosis factor Inhibitor

methyl-6-nonenamide	
5-Isobenzofurancarboxylic acid, 1,3-dihydro-3-oxo-, nonyl ester	Arachidonic acid Inhibitor, Inhibit Production of Uric acid
dl-α-Tocopherol	Tocopherol Synergist

*reported from Dr.Dukes Phytochemical and Ethnobotanical Databases-USDA

3.2.1.8 Antibacterial activity of *Memecylon sisparense* Gamble leaves ethyl acetate extract

The potential of the antibacterial activity of MSGLEAE was evaluated according to the zone of inhibition against the bacteria, and ciprofloxacin as standard drug. The results revealed that the extract had shown activity against *B. subtilis, B. cereus, S. aureus*, *S. epidermidis, E. coli, P. aeuriginosa* at concentrations of 5000, 2000 µg/ml respectively. At 1000 µg/ml concentration, MSG leaves EAE extract showed activity against *S. epidermidis, S. aureus, B. cereus, E. coli* and *P. aeuriginosa* compared to *B. subtilis,* whereas 500 µg/ml extract showed activity against *S. epidermidis, S. aureus* and *P. aeuriginosa* as represented in Fig.3.6.

In eukaryotic and bacterial cell proliferative activity, Resazurin is used as a colorimetric indicator of cell viability which normally appears blue color in oxidized form that enters the cytosol and gets converted to reduced form, resorufin that appears red color. The MIC of MSG leaves EAE against *Staphylococcus aureus, S. epidermidis, Bacillus subtilis, B. cereus, Eschericia coli* and *Pseudomonas aeuriginosa* are 625, 1250, 1250, 1250, 1250, 625 µg/ml respectively whereas MBC was found to be 1250, 1250, 2500, 2500, 2500, 1250 µg/ml respectively, that are presented in Table 3.4.

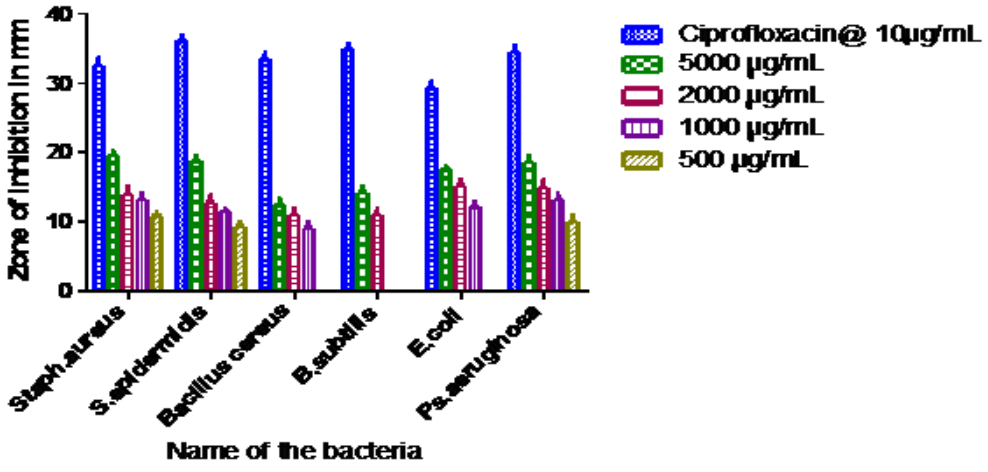

Fig. 3.6: Agar well diffusion assay for *Memecylon sisparense* Gamble leaves ethyl acetate extract

Table 3.4 Determination of MIC and MBC in *Memecylon sisparense* Gamble leaves ethyl acetate extract

Name of the microorganism	**MIC (mg/ml)**	**MBC (mg/ml)**
Staphylococcus aureus (MTCC 11949)	0.63	1.25
Staphylococcus epidermis (MTCC 3615)	1.25	2.50
Bacillus subtilis (MTCC 441)	1.25	1.25
Bacillus cereus (MTCC 430)	1.25	2.50
Eschericia coli (MTCC 118)	1.25	2.50
Pseudomonas aeuriginosa (MTCC 424)	0.63	1.25

3.2.2 Discussion

In Ayurvedic medicine, saponins, flavonoids and polyphenols are known to be the major bioactive compounds. Tocopherol is identified in *M. malabaricum, M. umbellatum, M. talbotianum, M. edule, M. wightii* (80). The methanolic extract of *M. umbellatum* showed pronounced antioxidant capacity compared to ascorbic acid (81). Ethyl acetate and methanol extract of *M. umbellatum* seed and leaf showed good antimicrobial activity compared to other extracts (82, 83). Thirty two *Memecylon* species leaf methanolic extracts were screened for antibacterial activity, broad spectral activity was observed in *M. sessile* as well as in *M. clarkeanum* (54).

The present study reveals that MSG leaves ethyl acetate extract has more phenolic and flavonoid content compared to methanolic extract where as DPPH free radical scavenging activity shown that ethyl acetate extract is having good anti-oxidant activity. So, we selected ethyl acetate extract for our present studies which possess bioactive phytoconstituents having anti-microbial, anti-cancer, nephroprotective, hepatoprotective, antioxidant compounds. The GC-MS analysis of MSG leaves EAE had shown the presence of phytochemicals like Phenol, 2,4-bis(1,1-dimethylethyl)- and Hexadecanoic acid, 15-methyl-, methyl ester which were also identified in *M. umbellatum* leaf petroleum ether extract (84), whereas 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- were found in chloroform extract of *M. umbellatum* leaves (84). In the present study, N,N,O-Triacetylhydroxylamine, N-{(4-Hydroxy-3-methoxyphenyl) methyl}-8-methyl-6-nonenamide, m-Cresyl acetate etc., are identified and the antibacterial activity was

observed on a dose dependent manner on both Gram positive and Gram negative bacterial strains.

3.3 Summary

Memecylon sisparense Gamble leaves possess bioactive phytoconstituents having anti-microbial, nephroprotective, hepatoprotective, and antioxidant compounds. The MSG leaves EAE is found to be antibacterial with highest zone of inhibition on *Staphylococcus aureus* followed by *S. epidermidis* then *Pseudomonas aeuriginosa* there by *Eschericia coli* followed by *Bacillus subtilis, B. cereus* with lowest zone of inhibition respectively.