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Screening of phytochmical, antioxidant and larvicidal activities of *Litsea quinqueflora*

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ABSTRACT:
The present study is investigate the chemical composition, antioxidant, antimicrobial and larvicidal activities of the essential oils from the leaves of *Litsea quinqueflora* (Dennst.) Suresh in Mehamalai Hills. The essential oils were extracted by hydrodistillation and analyzed by GC and GC-MS. Sesquiterepenes were the major class of compounds present in the *L.quinquiflora* leaf oil. The antimicrobial activity of essential oils was evaluated by agar well method. Essential oils exhibited remarkable inhibitory activity against all tested Gram-positive bacteria. The maximum zone of inhibition noted in methanol extract against *S. hominis* (13.66 mm). Furthermore, the larvicidal activity of essential oils was tested using fourth-instar larvae of *C. quinquefasciatus* and *A. Aegyptii.* At 1000 ppm concentration the methanol extract showed 61 % mortality against *A. aegyptii* and 76 % against *C. quinquefasciatus* at 24 h. Our findings demonstrate that the essential oil extracted from *Litsea quinqueflora* are potential sources of natural antimicrobials and can act as inexpensive mosquito larvicidal agents.

Keywords: *Litsea quinqueflora,* Essential oil, Antioxidant activity, Antilarvicidal activity

INTRODUCTION:

Medicinal plants are majorly involved in the improvement of human health activity. Ethnobotany is the study of the relationship between plants and people and their culture. Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. The medicinal effects of plants are due to metabolites especially secondary compounds produced by plant species. *Litsea* is a genus of evergreen or deciduous trees or shrubs belonging to the Lauraceae family. The genus includes 200 to 400 species in tropical and subtropical areas of both hemispheres. The leaves can be either deciduous or evergreen depending on species and aromatic. The flowers are from greenish to white, greenish-yellow, yellowish. The genus includes species of trees and shrubs with evergreen or deciduous foliage and inconspicuous flowers. They have leaves alternate or opposite or in whorls. The inflorescences are pseudo-umbels, flat-topped or rounded flower clusters, each pseudo-umbel with an involucre of four or six decussate bracts. An antioxidant is a molecule that inhibits the oxidation of other molecules. Free radicals and reactive oxygen species (ROS) are highly reactive molecules that are generated by normal cellular processes, environmental stresses, and UV irradiation [1].

According to world health organization medicinal plants would be the best source to obtain a variety of drugs in developed countries about 80 of plants are used in traditional medicine [2]. Mostly the pharmacological activity of medicinal plants resides in its secondary metabolites which are comparatively smaller molecules in contrast to the primary molecules such as proteins, carbohydrates and lipids. These natural products provide clues to synthesize new structural types of antimicrobial and antifungal chemicals that are relatively safe to man [3]. Mosquitoes transmit many serious human diseases such as malaria, filariasis, yellow fever, dengue and other viral diseases. There are about 3500 species of mosquitoes, grouped into three subfamilies *Anopheles stephensi* is urban and rural mosquito in Iran [4]. There are several native reports about crude solvent extracts of different parts of plants, essential oils or their chromatographic fractions. They showed various levels of bioactivity against different developmental stages of malaria vectors [5].

MATERIALS AND METHODS

Classification

Kingdom	Plantae
Class	Dicot
Division	Angiosperm
Order	Laurales
Family	Lauraceae.
Genus	*Litsea*
Species	*Litsea quinqueflora* (Dennst.) Suresh
Synonym	*Litsea ligustrina* (Nees) J.Hk.

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Collection of plant material
Fresh leaves of *L. quinquiflora* (Dennst.) Suresh were collected from Varushanad hills of Tamil Nadu, India. The herbarium specimen (PLS2013001) was prepared, identified and deposited in the Department of Plant Science, Madurai Kamaraj University.

Preparation of leaves extracts
The leaves extracts were prepared by sequential extraction method using three organic solvents in the basis of polarity of solvents (Petroleum ether, Chloroform and Methanol). 40g of the dried powdered leaves sample was taken in a conical flask and 200 ml of petroleum ether was added. The conical flask was kept on mechanical shaker for 24 hours, after that the extract was filtered through what man filter paper 1 and the pellet was allowed for drying and this pellet was used for the next solvent extraction (Chloroform and Methanol). The dried extract was recovered and stored in Refrigerator -4ºCfor further analysis.

Extract recovery percentage
After drying the respective extracts under oven temperature at 40ºC, the percentage of extracts yield was calculated using the following equation.

$$\%\text{Yield} = \frac{\text{Extract + container (g)} - \text{Empty container (g)}}{\text{Sample weight (g)}} \times 100$$

Isolation of *L. quinquiflora* leaves essential oil
The fresh leaves were first washed with tap water and then dried under fan for 10 minutes. It was then weighed (200g) and chopped in to small pieces. The chopped leaves were subjected to hydrodistillation using Clevenger-type glass apparatus for 4 hours. The oil was collected and dried them over anhydrous sodium sulphate (Na_2SO_4). The oil was stored at 4^0C in air-tight container for further analysis.

GC/MS ANALYSIS OF ESSENTIAL OIL
A Shimadzu QP-2010 plus with thermal desorption system TD 20 was used to obtain the chromatograms. The name and specification of the column used is AB-Innowax (60 m X 0.25 mm X film thickness-0.25 m). The temperature was programmed from 50ºC with 5 minute initial hold to 280ºC at 4ºC min -1 and a final hold for 5 min at 280ºC. The injector and detector temperature were set at 220 and 240ºC respectively and the split ratio was 1/60. Helium was used as the carrier gas and the ionizing voltage used was 70 eV. The components were identified based on the library search carried out using NIST and WILEY library.

PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *L. QUINQUIFLORA* LEAVES
The sequentially extracted dried leaves samples of petroleum ether, chloroform and methanol extracts were used for the qualitative analysis for the identification of plant phytoconstituents [6,7].

Flavonoids
Dissolve 1ml of extract in methanol. Add a sodium hydroxide. Add concentrated hydrochloric acid drop by drop. Pink colour shows the presence of flavonoids [6,7].

Steroids
To 1ml of the extract, chloroform was added and dissolved. Add acetic anhydride. Then add concentrated sulphuric acid. Keep it undisturbed for a few minutes. Green colour shows the presence of steroids and pink colour shows the presence of terpenoids [6,7].

1. Liebermann test for steroids
Extract solvent 0.2 g of each sample was dissolved in 2 mL of acetic acid separately. The solutions were cooled well in ice followed by the addition of concentrated H2SO4 carefully. Colour development from violet to blue or bluish-green indicated the presence of a steroidal ring.

Saponins
Shake 1ml of leaf extract with 5ml of distilled water and heat it in boiling water bath. Frothing indicates the presence of saponins (Edeoga *et al*., 2005; Harbone, 1973).

Alkaloids
1 ml plant extract solvent is warmed with 10 ml of 2% hydrochloric acid for 2 minutes and filtered. 1ml of the aliquot was treated with a few drops of Dragendroff's reagent. Orange-brown precipitate shows the presence of alkaloids. Two different tests were performed for Alkaloids [6,7].

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Mayers' test:
To 1 ml of leaf extract, 6 drops of Mayer's reagent was added leading to the formation of a yellowish creamish precipitate indicating the presence of alkaloids.
Tannins
Take 1ml of the leaves extract and add 2 drops of 5% ferric chloride solution. Dirty green precipitate indicates the presence of tannins [6,7].

QUANTITATIVE PHYTOCHEMICAL ANALYSIS
After the confirmation of presence of phenol, flavonoids and tannin by preliminary qualitative phytochemical tests, the extracts were used for further quantitative estimation.
1. Estimation of total phenol content
The amount of total phenol was determined with the Folin-Ciocalteu reagent using the method [8]. This method was employed to evaluate the phenol content of the samples. A standard curve was prepared by using gallic acid as a standard. Different concentrations of gallic acid were prepared in 80% of methanol, and their absorbance was recorded at 760 nm. 100 µl of sample was dissolved in 500 µl (1/10 dilution) of the Folin–Ciocalteu reagent and 1000 µl of distilled water. The solutions were mixed and incubated at room temperature for 1 min. After 1 min, 1500 µl of 20% sodium carbonate (Na_2CO_3) solution was added. The final mixture was shaken and then incubated for 2 h in the dark at room temperature. The absorbance of all samples was measured at 760 nm using a UV–Vis spectrophotometer (Model. U.2800, Hitachi) and the results are expressed in mg of gallic acid equivalents (GAE) per mg of dry weight of the plant. The amount of phenol in plant extracts in gallic acid equivalents (GAE) was calculated by the following formula:
X = (A. mo) / (Ao.m)
where X is the phenol content, mg/mg plant extract in GAE, A is the absorption of plant extract solution, Ao is the absorption of standard gallic acid solution, m is the weight of plant extract, mg and mo is the weight of gallic acid in the solution, mg.
2. Estimation of total flavonoid content
The flavonoid content in extracts were determined spectrophotometrically using a method based on the formation of a complex flavonoid–aluminium, having the absorbtivity maximum at 430 nm [9]. Rutin was used to make the calibration curve. 1 ml of diluted sample was separately mixed with 1 ml of 2% aluminum chloride methanolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm with a UV–Vis spectrophotometer (Model. U.2800, Hitachi) and the flavonoid content was expressed in mg per mg of rutin equivalent (RE). The amount of flavonoid in plant extracts in RE was calculated by the same formula as that of phenol:
3. Estimation of Tannin content
The amount of tannin content was determined by modified Prussian blue method [10]. This method is based on the mechanism that the phenols reduce potassium ferricyanide to produce ferrous ions; these ferrous ions in turn react with ferric chloride in the presence of dilute HCL to form a Prussian blue coloured complex, which can be measured at 700 nm wavelength using a UV–Vis spectrophotometer (Model. U.2800, Hitachi). Tannic acid was used to make a calibration curve. The amount of tannin in plant extracts were expressed in mg/mg of tannic acid equivalent (TAE). The amount of tannin in plant extracts in TAE was calculated by the same formula as that of phenol:

ANTI-OXIDANT ACTIVITY
1. Free-Radical Scavenging Ability (DPPH-assay)
The scavenging ability of methanol extract on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free- radicals was estimated according to the method [11]. This method depends on the reduction of purple DPPH to a yellow coloured diphenyl picrylhydrazine and the colour was measured at 517 nm. About 2 ml of various concentrations of test sample was mixed with 0.5 mL of 1 mM DPPH in methanol. An equal amount of methanol and DPPH served as control. The mixture was shaken vigorously and then steadily stayed for 30 min at room temperature in dark. The absorbance of the resulting solution was measured at 517 nm against the blank using a UV–Vis spectrophotometer (Model. U.2800, Hitachi). The experiment was performed in triplicates. The DPPH radical scavenging activity was calculated according to the following equation:
% DPPH radical scavenging activity = (Ao – A1)/ Ao × 100%,
where Ao is the absorbance of the control reaction and A1 is the absorbance in the presence of the sample of the tested extracts. Percentage radical activity was plotted against the corresponding antioxidant substance concentration to obtain the IC50 value, which is defined as the amount of antioxidant substance required to scavenge the 50% of free radicals present in the assay system. IC50 values are inversely proportional to the antioxidant potential.
2. Total antioxidant activity by Phosphomolybdenum method
Total antioxidant capacity was expressed as ascorbic acid equivalent and was calculated using the Phosphomolybdenum method [12]. Antioxidant present in the sample reduce the Mo(VI) to Mo(V) which then react with the phosphate group sodium phosphate to form a green coloured Mo(V) – Phosphate complex (Phosphomolybdenum complex) in an acetic medium. This complex is then spectophotometrically measured at 695 nm (Model. U.2800, Hitachi). The tubes

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containing 0.2 ml of extract (100-1000µg/ml) is mixed with 1.8 ml of distilled water, 2ml of Phosphomolybdenum reagent solution. Incubate it at 95ºC for 90 minutes. The mixture is closed to room temperature and the absorbance is measured at 695 nm against reagent blank. The antioxidant capacity is expressed as Ascorbic Acid Equivalent (AAE).

3. Reducing power ability

The reducing power ability of methanol extract was determined by the method [13]. Reaction mixtures were prepared by adding 2.5 ml of phosphate buffer (0.2 M, pH 6.6), 2.5 ml Potassium Ferricyanide (0.1%) and varying concentrations of extracts (10-250 µg/mL). Then the reaction mixtures were incubated at 50°C in water bath for 30 min and allowed to cool at room temperature. Then 2.5 ml of 10% TCA (Trichloroacetic acid) were added to each reaction mixture and centrifuged at 2000 rpm for 10 min. The supernatant (2.5 ml) was separated in the test tube and added with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (1.0%). After 10 min incubation at room temperature, the absorbance was measured at 700 nm (Model. U.2800, Hitachi). Ascorbic acid solution was used as standard.

Antibacterial activity of *L. quinquiflora* leaves extracts

Antibacterial activity of leaf oil was investigated by the disc diffusion method [14]. Mueller-Hinton agar medium (MHA) was used for antibacterial susceptibility tests. The MHA medium was prepared by pouring 15 ml of molten media into sterile Petri plates. The plates were allowed to solidify and inoculums suspension was swabbed uniformly on the medium and allowed to dry for 5 min. 30 µl of leaves extracts (Petroleum ether, Chloroform and Methanol) containing 1.5mg/ml concentration was loaded on 6 mm sterile disc. The standard antibiotic disc Gentamycin (120mcg/disc) was placed on the surface of the plates. The plates were kept for incubation for 24 hrs at 37°C. The zone of inhibition was measured around the discs containing samples and standard.

Antilarvicidal activity of *L. quinquiflora* leaves extracts

The larvicidal activity of the leaf extracts of *L. quinquiflora* were tested against two mosquito vectors namely *A. aegyptii* and *C. quinquefasciatus* according to the WHO procedure [15]. The larvae were obtained from Centre for Research in Medical Entomology (CRME), Madurai. The methanol leaves extract was dissolved in methanol and to make the stock solution of 10000 ppm (10µg/ml). This stock solution was further diluted in water to make different concentrations (100, 200, 400, 600, 800 and 1000 ppm). The fourth instars larvae were collected and introduced into the test solutions as well as to the controls. A minimum of twenty larvae/ concentration were used for all the experiments, which were replicated three times. The larval mortality was recorded after 24 hrs, 48 hrs and 72 hrs. The larvae were considered dead if they do not respond to gentle prodding with a fine needle. Mortality was reported as lethal dose concentrations (LD_{50} and LD_{90}) representing the concentrations in ppm with 50% and 90% larval mortality rate in 24 hrs, 48 hrs and 72 hrs respectively. The percentage mortality was calculated using the formula,

% of mortality = Number of dead larvae/ Number of larvae introduced×100

Statistical analysis

All the experiments were repeated three times and the results were reported as mean ± SD/SE (standard deviation or standard error of estimate). All the statistical analysis was performed using Microsoft Excel. LD_{50} and LD_{90} values were calculated using probit analysis (Bio stat 2009 software).

RESULTS AND DISCUSSION

Percentage yield

The course powder of leaves material was extracted by mechanical method using petroleum ether, chloroform and methanol successively. The extract values were found to be 1.05% w/w of petroleum ether, 0.14% w/w of chloroform and 6.64% w/w of methanol extract separately.

GC-MS analysis of *L. quinqueflora* leaves essential oil

A total of 56 compounds were identified in the *L. quinqueflora* leaf essential oil (Table: 1). The GC-MS analysis chromatogram peak levels indicated the percentage of chemical compounds present in the leaf essential oil (Fig: 1). Sesquiterepenes were the major class of compounds present in the *L.quinquiflora* leaf oil. The major compounds identified were β-Elemene (16.09 %), β-Caryophyllene (13.38 %), Naphthol (12.30%), Decanal (6.5 %), α–Caryophyllene (3.63 %), Acetyl 2-methyl propanoate (3.52%), dodecane (3.5%), Ent-spathulenol (2.97%), 1,1 diethaxy ethene (2.53%), Decanal (2.29%), 1,1 diethaxy ethane (2.03%), bicyclogermacrene (1.91%), dodecanal (1.79%), D-nerolidol (1.65%), 2-menthene (1.54%) and spathulenol (1.50%) were the minor compounds were identified. The other compounds present were in trace amounts. Sequiterepenes were the predominant class of compounds present in the essential oil constituted 46.08% followed by alcohol 25.85%, aldehydes 23.65%, ester 5.25%, monoterepenes 2.33%, amine 0.77%, alkenes 0.32% and ketones 0.30% (fig: 2). From the results presented above, leaf essential oil constituents of *L. quinqueflora* were primarily sesquiterpenoides. According to the previous reports, most of the *Litsea* species essential oils such as *L. resinosa*, *L. rasilipes* and *L. Paludosa* [16] , *L. linii* [17], *L nakaii* [18] and *L. coreana* all have predominately sesquiterpenoids as their main constituents. Some recent reports demonstrated that the biological

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activities of the related *Litsea* species *L. cubeba* (Lour.) Pers. possess antibacterial [19], antifungal [20, 21], insecticidal [22,23], antioxidant [24] and anticancer properties [18] (Ho *et al*., 2009). In addition to this, the Caryophyllene was a second major constituent of *L. quinqueflora* leaf essential oil; the similar results have been reported in the previous study in the species *L. Glutinous* [25]. Caryophyllene posses several biological activities such as anti-inflammatory, antibiotic, antioxidant, anticarcinogenic and local anaesthetic activities [26].

Table 1: Chemical composition of the essential oil of *L. quinqueflora* leaves

S.No	R.Time	Area percentage	Chemical formula	Compound name	Class compounds
1	5.24	0.72	$C_6H_{13}C_lO$	2-Ethoxy-3-chlorobutane	Aldehyde
2	6.09	0.09	$C_6H_{13}C_lO$	2,4-Dimethyl-1,3-Dioxolane	Aldehyde
3	6.27	0.22	$C_7H_{14}O_3$	Ethoxypropane	Aldehyde
4	6.40	2.03	$C_6H_{14}O_2$	1,1-Diethoxyethane	Aldehyde
5	6.55	0.25	$C_2H_6O_2S$	Methyl sulfonyl methane	Aldehyde
6	6.71	0.18	$C_6H_{14}O_3$	Glycolaldehyde	Aldehydes
7	7.23	2.53	$C_6H_{14}O_2$	1,1-Diethoxyethane	Aldehyde
8	10.18	0.32	$C_{10}H_{16}$	Cyclohexene	Alkenes
9	10.27	1.54	$C_{10}H_{16}$	2-Menthene	Monoterpene
10	16.70	1.05	$C_{12}H_{24}O$	Dodecanal	Aldehyde
11	18.34	6.5	$C_{10}H_{20}O$	Decanal	Aldehyde
12	21.20	0.22	$C_{23}H_{46}O_3S$	Cyclohexylmethyl hexadecyl ester	Aldehyde
13	23.45	0.77	$C_{11}H_{19}N$	Naphthalenamine	Amine
14	23.89	1.91	$C_{15}H_{24}$	Bicyclogermacrene	Sesquiterpenes
15	24.45	0.89	$C_{14}H_{28}$	Cyclohexane	Aldehyde
16	25.89	0.15	$C_{14}H_{28}O$	Cyclohexanol	Alcohol
17	26.34	16.09	$C_{15}H_{24}$	β- Elemene	Sesquiterpenes
18	26.56	0.98	$C_{11}H_{20}O$	4- Undecenal	Aldehyde
19	26.82	0.58	$C_{15}H_{24}$	Isocaryophyllene	Sesquiterpenes
20	27.18	1.79	$C_{12}H_{24}O$	Dodecanal	Aldehyde
21	27.39	13.38	$C_{15}H_{24}$	β-Caryophyllene	Sesquiterpenes
22	27.75	0.25	$C_{16}H_{34}S_2$	2,2'-Thiodi(4-tert-octylphenol)	Alcohol
23	28.00	1.15	$C_{15}H_{24}$	γ- Elemene	Sesquiterpenes
24	28.16	1.11	$C_{13}H_{26}O_2$	Octyl 2-methylbutanoate	Ester
25	28.38	3.52	$C_{13}H_{26}O_2$	Acetyl 2-methyl propanoate	Ester
26	28.74	3.63	$C_{15}H_{24}$	α-Caryophyllene	Sesquiterpenes
27	29.03	1.50	$C_{15}H_{24}O$	Spathulenol	Alcohol
28	30.06	0.44	$C_{15}H_{24}$	Aromadendren	Sesquiterpenes
29	30.50	2.29	$C_{10}H_{20}O$	Decanal	Aldehyde
30	30.76	0.41	$C_{12}H_{26}$	n-Dodecane	Aldehyde
31	31.09	1.30	$C_{15}H_{24}$	β-Bisabolene	Sesquiterpenes
32	31.23	0.56	$C_{15}H_{24}$	Naphthalene	Sesquiterpenes
33	31.63	1.37	$C_{15}H_{24}$	δ-Cadinene	Sesquiterpenes
34	33.34	1.65	$C_{15}H_{26}O$	D-Nerolidol	Alcohol
35	33.81	2.97	$C_{15}H_{24}O$	Ent-Spathulenol	Alcohol
36	33.91	3.5	$C_{12}H_{26}$	Dodecane	Aldehyde
37	34.29	0.90	$C_{15}H_{26}O$	Ledol	Alcohol
38	34.68	0.55	$C_{15}H_{26}O$	Viridiflorol	Alcohol
39	34.89	0.76	$C_{15}H_{24}O$	Humulene epoxyde	Sesquiterpenes
40	35.02	0.79	$C_{15}H_{26}O_2$	Geranyl isopentanoate	Monoterpene
41	35.14	0.87	$C_{15}H_{26}O$	Cubenol	Alcohol
42	35.65	0.29	$C_{23}H_{42}O$	Cyclohexanol	Alcohol
43	35.99	0.65	$C_{15}H_{24}O$	10,10-Dimethyl-2,6-bis (methylene) bicyclo(7.2.0)undecan-5-ol	Alcohol
44	36.25	12.30	$C_{15}H_{26}O$	Naphthol	Alcohol
45	36.37	0.03	$C_{15}H_{26}O$	α-Muurolol	Alcohol
46	36.69	0.99	$C_{15}H_{26}O$	α-Cadinol	Alcohol
47	37.34	0.83	$C_{12}H_{20}O$	Indacenol	Alcohol

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48	38.63	0.44	$C_{38}H_{64}Si_2$	Disilacyclobutanol	Alcohol
49	39.02	0.24	$C_{15}H_{24}O$	Isospathulenol	Alcohol
50	39.56	0.34	$C_{14}H_{24}O_2$	Butenol	Alcohol
51	39.85	0.25	$C_{17}H_{28}O_4$	Nerolidol-epoxyacetate	Alcohol
52	43.10	0.24	$C_{20}H_{38}$	Neophytadiene	Sesquiterpenes
53	43.36	0.30	$C_{18}H_{36}O$	Phytone	Ketone
54	47.63	0.36	$C_{16}H_{32}O_2$	Hexadecanoic acid	Ester
55	48.40	0.26	$C_{11}H_{22}O_3S$	Cyclohexylmethyl isobutyl ester	Ester
56	51.82	0.65	$C_{20}H_{40}O$	Phytol	Alcohol

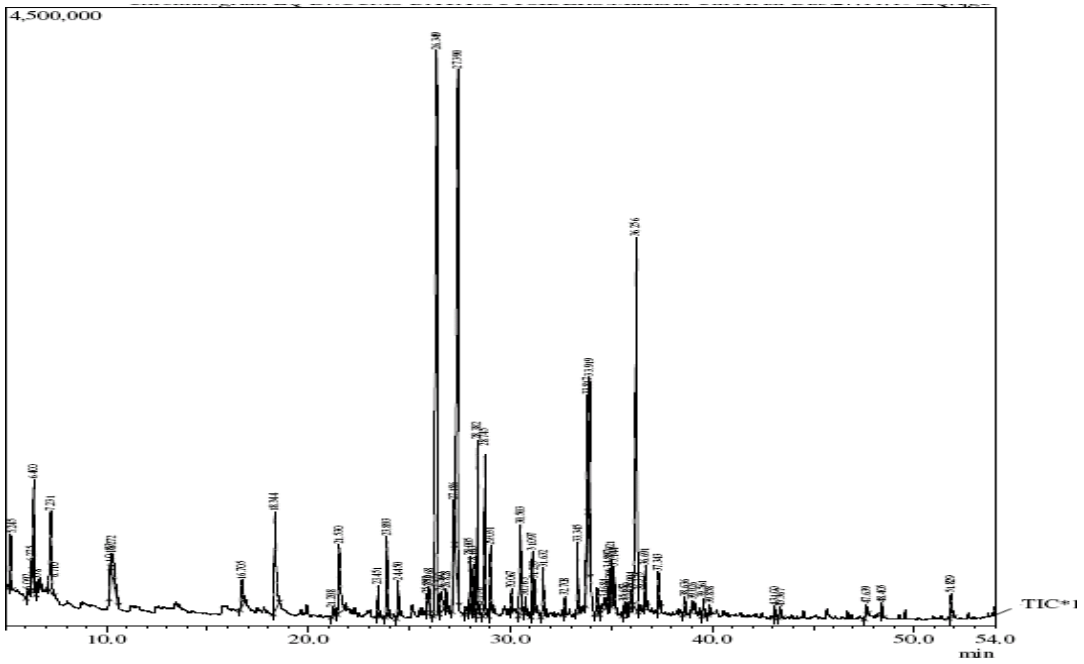

Fig 1: GC-MS Chromatogram of *L.quinquiflora* leaves essential oil

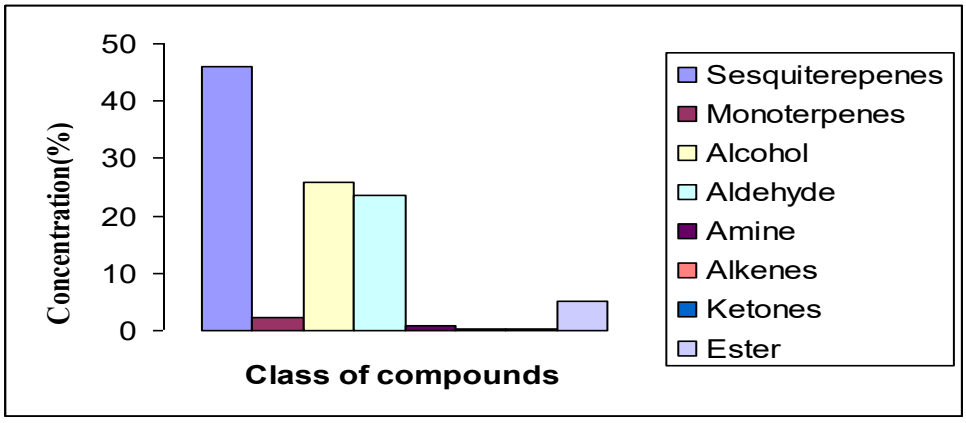

Fig 2: Composition of various classes of compounds present in the *L.quinquiflora*

PHYTOCHEMICAL ANALYSIS OF LEAVES EXTRACT

Preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, terpenoids, steroids, tannins, saponins and lipids in *L.quinquiflora* leaves extracts (Table: 2). The leaf methanolic extract showed the presence of Tannin, Flavonoid, Steroid, Alkaloid, Saponin and Lipid. The leaf chloroform extracts showed the presence of Steroid and Alkaloid while in the petroleum ether extract Tannin, Steroid and Lipid. Based on the preliminary phytochemical screening of *L. quinquiflora* leaves extracts showed that, the leaves were rich in steroids, terpenoids, tannins and lipids. On the basis of preliminary phytochemical investigation, methanol extract was chosen for the further quantitative analysis. The total phenol content of the methanol leaf extract was quantified in terms of Gallic acid equivalent (GAE) (the standard curve equation: $y=0.3107x$, $r^2=0.9681$). The obtained phenol content in methanol leaf extract was 0.222

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mg GAE/mg of dry weight of sample (fig: 3). The total flavonoids contents in the leaves extracts was 0.097 mg Rutin equivalent /mg of dry weight of sample (the standard curve equation y = 0.2526x, r^2= 0.9197) (fig: 4).While total tannin content showed higher amount compared to total phenol and total flavonoids contents. It was obtained around 1.515 mg tannic acid equivalent /mg of dry weight of sample (the standard curve equation y=0.0738x, r^2 =0.9716 (fig: 5).
Phytochemical studies on the selected plant revealed the presence of flavonoids, phenol and tannins. Phytochemical compounds are known to be biologically active, aiding these activities through different mechanisms; tannins for example, act by iron deprivation, hydrogen bonding or specific interactions with vital proteins [27]. In a previous report, phytochemical constituents of bark of *L. glutinosa* showed the effective results of antibacterial and antifungal activities [28]. Phenolic compounds are widely investigated and are naturally occurring antioxidant components present in most of the plant species. These phenolic compounds are found in medicinal plants as well as fruits and vegetables and play important roles in preventing degenerative diseases, including inflammation, cancer, and arteriosclerosis [29]. Muhammad *et al* (2008) worked on *L. monopetala* bark and found four different phenolic compounds in the methanolic extract [30]. Several studies reported that plant flavonoids showed antioxidant activity and also act as antioxidants in *in vivo* [31]. In earlier studies, many medicinal plants contained high amounts of phenolic compounds and found a positive linear correlation between the total phenolic content and antioxidant activity of the plants [32,33]. This suggested that the genus *Litsea*, possessed high levels of polyphenols and also found to have high antioxidant properties.

Table 2: Qualitative phytochemical analysis of *L.quinquiflora* leaf extracts

Phytoconstituents	Petroleum Ether	Chloroform	Methanol
Tannin	+	_	+
Flavonoid	_	_	+
Steroid	+	+	+
Alkaloid	_	+	+
Terpenoid	_	_	_
Saponin	_	_	+
Lipids	+	_	+

+ = indicates presence of phytochemicals
- = indicates absence of phytochemicals.

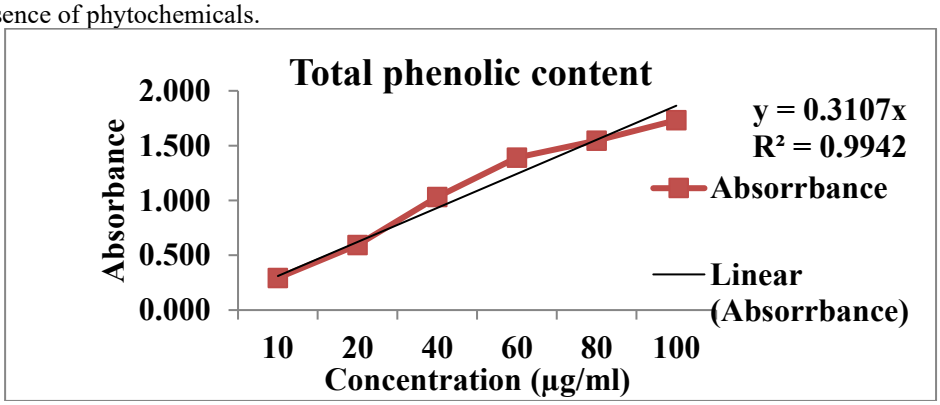

Fig 3: Standard curve of Gallic acid for total phenol estimation

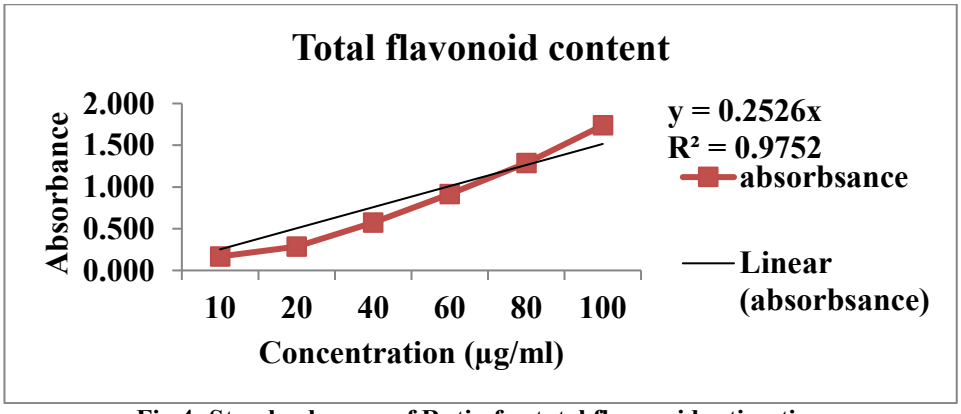

Fig 4: Standard curve of Rutin for total flavonoid estimation

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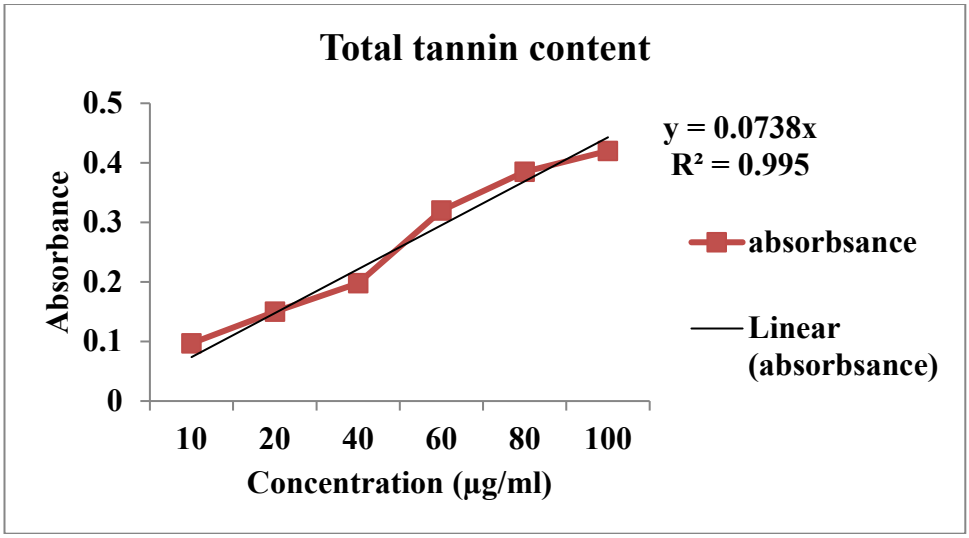
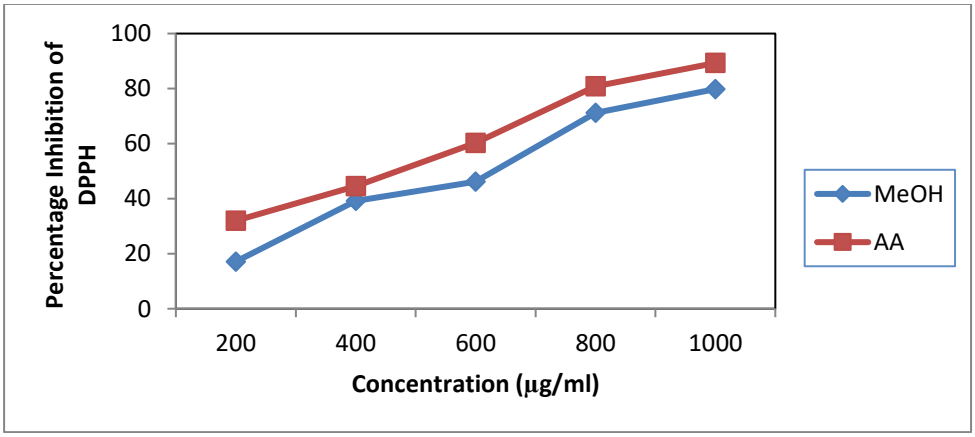


Fig 5: Standard curve of Tannic acid for total tannin estimation

ANTIOXIDANT ACTIVITY

As shown in figure 6, the DPPH radical scavenging activities of the methanol extract and the reference chemical (ascorbic acid) were exhibiting ability to reduce the stable radical diphenylpicrylhydrazyl (DPPH) to yellow-coloured diphenylpicrylhydrazine with IC50 values of 462.85 µg/ml for leaf methanol extract and 373.35 µg/ml for standard ascorbic acid respectively. Total antioxidant capacity was expressed as ascorbic acid equivalent and was calculated using the phosphomolybdenum method. The methanol extract of *L.quinquiflora* exhibited significant antioxidant activity of 3.788 mg AA equivalent /mg of dry weight of sample respectively (fig: 7). The reducing ability of a compound may serve as a significant indicator of its potential antioxidant activity. Total antioxidant capacity expressed as gallic acid equivalent and calculated using the frap method. The methanol extract of *L.quinquiflora* exhibited significant antioxidant activity of 0.277mg GAE equivalent /mg of dry weight of sample respectively (fig: 8). The leaves of *L.quinquiflora* showed the potential antioxidant activity.

In the free radical scavenging activity, superiority of the methanol extract could be attributed to the presence of phenolics in the leaf extract. Particularly, synergistic effects of phenolic acids e.g., rosmarinic acid and polyphenols as well as other chemicals such as flavonoids could also be taken into account for the radical scavenging activity observed in the methanol extracts [34]. In 2009, Kshirsagar and Upadhyay [35] found that the stem of *L. glutinosa* had high DPPH scavenging capacity than the twig of this plant. Total antioxidant activity of phosphomolybdenum assay is simple and inadequate of other antioxidant measurement employed. Also, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalent of ascorbic acid (mg/g plant extract). This method was depending on the reduction of Mo (VI) to Mo (V) by the antioxidant agents and the subsequent formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm [36]. Several scientific reports suggested that the genus *Litsea* is the rich source of natural antioxidants [37]. The results show that there was an increase in reducing power of the plant extract as the extract concentration increases. Free radical is known as a major contributor to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defence mechanism [38].



(Where MeOH-Methanol leaves extract; AA- Standard ascorbic acid)

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Fig 6: DPPH free radical scavenging activity of leaf methanol extract and standard ascorbic acid

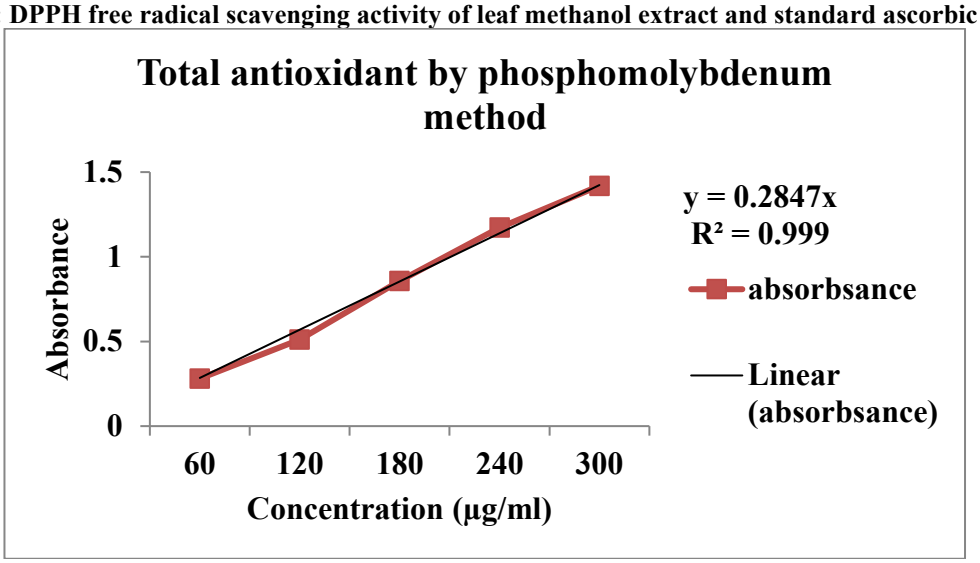

Fig 7: Phosphomolybdenum activity of Ascorbic acid

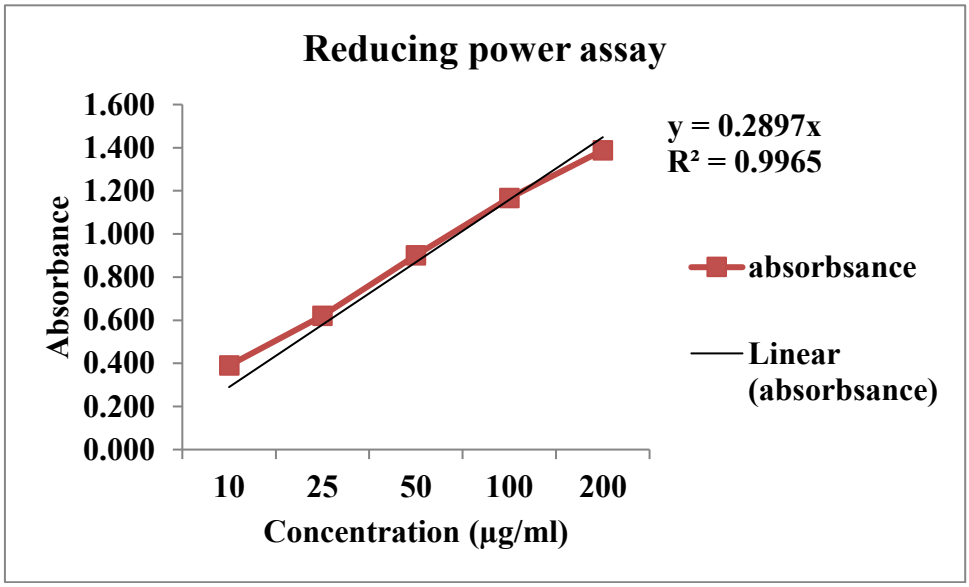

Fig 8: Reducing power capacity of ascorbic acid

Antibacterial sensitivity test

The antibacterial activity of crude extracts of leaf was determined by disc diffusion method against seven pathogenic bacterial species. Antibacterial activity of different leaves extracts (petroleum ether, chloroform and methanol) of *L.quinquiflora* against both gram positive and gram negative bacteria viz. *B. cereus, B. subtlis, B. licheniformis, S. aureus, S. hominis, A. Viridian* and *E. coli* given in the Table: 3. *L.quinquiflora* leaves methanol extract exhibited the pronounced antibacterial activity against all the bacterial strains tested. The zones of inhibition ranged from 7.33-13.66 mm in diameter. The maximum zone of inhibition noted in methanol extract against *S. hominis* (13.66 mm) and minimum zone of inhibition obtained in *A. Viridian* and *E. coli* (7.33 mm each in both the strains). The leaf methanol and chloroform extracts exerted potential effects of antibacterial activity against tested bacterial strains. However, petroleum ether extract of leaf exhibited moderate effect of antibacterial activity against some of the tested bacteria strains. The blind control (DMSO) did not inhibit any of the bacteria tested. Among the three extracts of leaf used in the antibacterial susceptibility test, the methanol and chloroform extracts showed significant antibacterial activity than that of petroleum ether extract (Table: 3).

Antimicrobial activities exhibited by the plant extracts could be due to the presence of alkaloids and flavonoids. Our results were similar to those reported earlier in other *Litsea* species *L.monopetala* [39]. According to previous report the bark petroleum ether, ethanol and aqueous extracts of *L. glutinosa* showed the effective antibacterial activity against

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both gram positive and gram negative bacterial strains and also petroleum ether extract inhibited the growth of fungal species of *Aspergillus fumigates* and *Candida albicans* [28].

Table: 3. **Antibacterial activity of different solvent extracts against bacterial species tested by disc diffusion assay**

Bacterial strain	**Solvent Extracts (Zone of inhibition in mm)**			
	Petroleum ether	**Chloroform**	**methanol**	**Gentamycin**
Staphylococcus aureus	10±0	9±2	10.66±1.52	29.33±0.57
S. hominis	10.33±0.57	12±1	13.66±0.57	31.66±1.15
Bacillus subtillis	8.33±0.57	10.66±0.57	8.66±0.57	25.33±0.57
B. lichaniformis	8.66±1.15	10±2	8.66±0.57	33.66±0.57
B. cereus	7.33±0.57	7.33±0.57	11.33±1.52	25±1
Escherichia coli	7.33±0.57	9±1.52	9±1	29.33±1.52
Aerococcus viridians	7.66±1.15	7.33±0.57	10.33±1.52	27±0

*Each value represents Mean ± Standard deviation of three replicates

Larvicidal activity

Larvicidal activity of methanol leaf extract of *L.qinquiflora* was observed against fourth instar larvae of two selected mosquito vectors such as *C. quinquefasciatus* and *A. Aegyptii* when different concentrations were taken in three replicates. It was observed that the larvae mortality was dose dependent; as the dose concentration increases the mortality rate also increases. The percentage mortality, LD_{50} and LD_{90} values were mentioned in Table 4 & 5. At 1000 ppm concentration the methanol extract showed 61 % mortality against *A. aegyptii* and 76 % against *C. quinquefasciatus* at 24 h; after 72 h observation showed that 86 % mortality against *A. aegyptii* and 76 % against *C. quinquefasciatus* respectively. The LD_{50} values of methanol extract were 92.6 ppm (24 h), 82.1 ppm (48 h) and 52.9 ppm (72 h) against *A. Aegyptii* and 78.9 ppm (24 h), 75.7 ppm (48 h) and 64.04 ppm (72 h) against *C. quinquefasciatus.* The LD_{90} values were 1738.61 ppm (24 h), 1496.53 ppm (48 h), 1062.34 ppm (72 h) against *A. Aegyptii* and 1414.26 ppm (24 h), 1183.13 ppm (48 h) and 1048 ppm(72 h) against *C. quinquefasciatus*. The LCL values of methanol extract were 635.59 (24 h), 518.52 ppm (48 h), 347.95 ppm (72 h) against *A. Aegyptii* and 367.62 ppm (24 h), 203.77 ppm (48 h), 202.81 ppm (72 h) against *C. quinquefasciatus.* The UCL values of methanol extract were 1002.41 ppm (24 h), 843.76 ppm (48 h), 585.52 ppm (72 h) against *A. Aegyptii* and 935.74 ppm (24 h), 465.45 ppm (48 h) and 426.60 ppm (72 h) against *C. quinquefasciatus.* Based on the above result, the methanol leaf extract of *L.qinquiflora* exhibited potent larvicidal activity at all the tested concentrations against *C. quinquefasciatus* and *A. aegyptii.* The leaf methanol extract of *L.quinquiflora* showed better toxicity against *C. quinquefasciatus* than *A.aegyptii*.

Most of the plant based insecticides are rapid acting and degrade quickly in the environment. The extract of whole leaf and essential oil of some plants species have been investigated against common mosquito vectors. The activity of crude plant extracts is often attributed to the presence of complex mixture of active compounds. In the preliminary screening of *Litsea* crude extracts also showed the presence of active phytoconstituents. There are several native reports about crude solvent extracts of different parts of plants, essential oils or their chromatographic fractions showed various levels of bioactivity against different developmental stages of malaria vectors [40]. In an earlier report, *L. glutinosa* plant extracts delayed the molting of larvae into pupae resulting in the abnormal development of mosquitoes at later stage [41]. Some other previous research in the *Litsea* species *(L. salicifolia L. elliptica*) extracts showed the potential larvicidal activities against *A. aegypti* and *C. quinquefasciatus* [42].

Table: 4. **Percentage Larvicidal activity of leaf methanol extracts of *Litsea quinquiflora* against *Aedes aegyptii and Culex quinquefasciatus***

	Aedes aegyptii			***Culex quinquefasciatus***		
Dose (ppm)	24h	48h	72h	24h	48h	72h
100	15±0	18.33±0.64	21.66±0.64	20±0	26±0.64	28.88±0.64
200	25±1.11	26.66±0.64	31.66±0.64	28.33±0.64	36±0.64	36.66±0.64
400	31.66±0.64	36.66±0.64	50±1.11	38.33±0.64	50±00.64	53.33±0.64
600	40± 0	48.33±0.64	66.60±0.64	48.33±1.29	65±1.11	68.33±0.64
800	51.66±0.64	56.66±0.64	75±1.11	63.33±0.64	76.66±0.64	78.33±0.64
1000	61.66±0.64	71.66±0.64	86±0.64	76.66±0.64	83.33±0.64	90±0

*Each value represents Mean ± Standard error of three replicates

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Table: 5. Probit analysis of larvicidal efficacy of methanol leaf extract of *L.quinquiflora* against *Aedes aegyptii* and *Culex quinquefasciatus*

	Aedes aegyptii				***Culex quinquefasciatus***			
Time	**LD_{50}**	**LD_{90}**	**LCL**	**UCL**	**LD_{50}**	**LD_{90}**	**LCL**	**UCL**
24h	92.6	1738.61	635.59	1002.41	78.9	1414.26	367.62	935.74
48h	82.1	1496.53	518.52	843.76	75.7	1183.13	203.77	465.45
72h	52.9	1062.34	347.95	585.52	64.04	1048.56	202.81	426.60

*Each values represents in ppm concentration; LC_{50} - Lethal concentration that kills 50 per cent of the exposed larvae; LC_{90}, Lethal concentration that kills 90 percent of the exposed larvae; UCL, upper confidence limit; LCL, lower confidence Limit.

CONCLUSION:

From the examination of the phytochemical screening, antioxidant, and larvicidal activity of extracted essential oil from *Litsea quinquiflora* leaves evidently expose that the plant extract could be explored for its good therapeutic value by pharmaceutical industries in order to increase safe drugs for various illness.

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