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Research Article

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A study on phytochemical analysis, antioxidant and larvicidal activity of dried flowers of *Tabebuia rosea*

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ABSTRACT

To study the larvicidal, antioxidant and phytochemical screening test of Methanol extract of dried Tabebuia rosea (T. rosea) flowers. The methanol extract of flowers of T. rosea were studied for their larvicidal and antioxidant activity by total antioxidant, reducing power method and lipid peroxide method. The phytochemical analysis has been done using petroleum ether, ethyl acetate and methanol extract of dried flowers of T. rosea. The flower extracts of T.rosea showed larvicidal activity against Culexquinque fasciatus and Anopheles subpictus. The regression value of culexquinque fasciatus was 0.974 and the LC_{50} value showed 586.68, and for Anopheles subpictus regression valuewas 0.981 and LC_{50} value showed 241.72.As a result of this study, it was found that the fractions T.rosea demonstrated significant larvicidal and antioxidant activity. Due to extensive use and sale of products derived from T.rosea may tend to lead pressure on the population of this species and exert use of the fractions in the treatment of other diseases in pharmaceutical field.

Keywords*: Tabebuia rosea***,** phytochemical analysis, GC-MS**,** Antioxidant activity, larvicidal activity

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INTRODUCTION

*Tabebuia rosea*plant belongs to Bignoniaceae and commonly known as Pink trumpet tree grown as an ornamental tree for its pink or purple flowers with different shades of colours.In the traditional days, aerial parts of the tree were used for the treatment of malaria and uterine cancer. A decoction of the cortex of the tree utilized for anaemia and constipation. The flowers, leaves and roots also were used to reduce fever, pain, cause sweating, tonsil inflammation and many other disorders. A lapachol is a botanical product that has been isolated from *T. rosea* considered to be an anticancer drug and also recommended for anti-malarial and anti-panasomal effects [1, 2].More recently this plant gained interest to discover new anticancer drugs and increasing the understanding of their biological importance[3].More than 3000 plants officially used for medicinal application and nearly 6000 plants used n folk, herbal and traditional medicinal system in India [4]. Although for the study of antioxidant, there are many reports showed good antioxidant activity especially in the northern district of the Tamilnadu district, India[5] and Around 104 plant species belonging to 45 families were studied for phytochemical analysis and antioxidant activity through DPPH scavenging activity by [6].Hence, there is an increasing interest in finding out the phytochemical analysis and antioxidant activity we have checked out phytochemical analysis, antioxidant activity, larvicidal activity and GCMS analysis of flower extract of *T. rosea*.

EXPERIMENTAL SECTION

2.1. Plant source

T. rosea flowers were collected from in and around VIT University, Vellore District, Tamil Nadu, India and brought to the laboratory in polythene bags. The taxonomic identification was made by Prof. Angeline, Vijayakumari, Head of Department (botany), Voorhees College, Vellore, India.

2.2. Preparation of plant extracts

The dried flowers were powdered mechanically using a commercial electrical stainless steel blender and extracted with petroleum ether (250 mL); methanol (250 mL), and ethanol (250 mL) in a soxhlet apparatus (1,700 mL) separately until exhaustion. The extract was concentrated under reduced pressure 22–26 mg Hg at 45°C,and the residue obtained was stored at 4°C.

2.3. Preliminary phytochemical analysis

The extracts were used for preliminary screening test of phytochemicals such as alkaloids (Wagner and Dragendorff's tests),flavonoids (Shinda and Lead acetate tests), phenols (lead acetate and $FeCl_3$ tests), tannins (gelatin tests), saponins (foamtests), sterols (Liberman–Burchard and Salkowski tests) andglycosides (Molish's test, Benedict's test) [7].

2.4. GC-MS analysis

The chemical composition of methanol extract of *T. rosea* flower was analyzed by Usinga clarus 680 Perkin Elmer gas chromatography equipped with an Elite-5 capillary column (5% Diphenyl 95% dimethyl poly siloxane) (30.0m X 0.25mmID X 250µm). For GC–MS detection, an electron ionization system (quadruples analyzer; mass range, 10–425 amu) with ionization energy of 70 eV was used. One µL of crude methanol extract of the flower subjected to analysis of phytochemicals and the temperature were increased from 50º to 220º C.

2.5. LARVICIDAL ACTIVITY

2.5.1. Parasites collection

A. subpictus and *C. quinquefasciatus* larvae were collected from rice field and stagnant water area of Melvisharam (12°56′23″ N, 79°14′23″ E) and identified in Zonal Entomological research Centre, Vellore (12°55′48″ N, 79°7′48″ E), Tamil Nadu, to start the colony, and larvae were kept in plastic and enamel trays containing tap water.

2.5.2. Larvicidal bioassay

One gram of crude extract was first dissolved in 100 mL of methanol (stock solution). From the stock solution, different concentrations ranging from 62.5 to 1000 ppm were prepared with dechlorinated tap water. Polysorbate 80(Qualigens) was used as an emulsifier at the concentration of 0.05%. Experiments were conducted for 24 h at room temperature (28±2°C). The larvicidal activity was assessed by the procedure of the procedure of Madhumitha et al., with some modification [8]. For bioassay test, larvae were taken in 5 batches of 20 in 249 mL of water and 1.0 mL of the desired plant extracts concentration. The control was set up with polysorbate 80. The numbers of dead larvae were counted after 24 h of exposure and the percentage mortality was reported from the average of five replicates.

2.5.3. Dose response bioassay

From the stock solution, different concentrations ranging from 62.5 to 1000 ppm for mosquito were prepared. Based on the preliminary screening results, different crude solvent extracts prepared from the flower of *T.rosea* were subjected to dose response bioassay against *A. subpictus* and *C. quinquefasciatus* respectively. The numbers of dead mosquito larvae were counted after 24 h of exposure and the percentage mortality was reported from the average of five replicates. However at the end of 24 h the selected test samples turned out to be equal in their toxic potential[8].

2.6. Statistical analysis

The average larval mortality data were subjected to calculating LC50 and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square values were calculated. Results with $p<0.05$ were considered to be statistically significant [8].

2.7 Determination of *in-vitro* antioxidant activity

2.7.1 Total antioxidant activity by phosphomolybdenum method

Different concentrations of the extracts like 250,500,750 and 1000 µl were added on the test tubes to that 1mL of reagent which contains 0.6mM of sulphuric acid, 28mM of sodium phosphate and 4mM of ammonium molybdenum was added and incubated for 95 °C for 90 min. The absorbance was measured at 635nm in UV spectrometer[9]. The total antioxidant activity was calculated by the formula

Total antioxidant $=A_o - A_1 / A_o \times 100$
A_o = Absorbance of control.
A_1 =Absorbance of standard.

2.7.2. Reducing potential activity of *T. rosea* flower
The extracts were taken in four different concentrations of 250,500,750 and 1000 µL were added and to that 0.75mL of phosphate buffer and 0.7mL of potassium ferric cyanide were added. The mixture was then incubated at 50 ºC for 20 min after the incubation 0.75mL of Tri chloroacetic acid were added. Then it was centrifuged at 3000rpm for 10 min and 1.5mL of distilled water and 0.1 mL of $FeCl_3$ were added and incubated for 10 min. Absorbance was measured at UV in 700nm [9].

2.7.3. Lipid per oxidation by Thiobarbuturic acid (TBA) method
Different concentration of the extracts was done with 250,500,750 and 1000µl. To the different concentration of the extract was added with 2 mL of 20%Trichloroacetic acid and 2 mL of 0.67% Thiobarbuturic acid and placed in water bath for 15 min then it is kept for cooling and it was centrifuged to 3000rpm for 20 min and absorbance was measured at 552 nm in UV spectrophotometer [9]. Gallic acid was used as a standard. Calculation of lipid per oxidation was done by the formula

% of lipid per oxidation $= A_o - A_1 / A_o \times 100$
A_o = Absorbance of control.
A_1 = Absorbance of standard.

RESULTS AND DISCUSSION

3.1. Phytochemical analysis
The present study on the presence of phytoconstituents showed that Protein and Diterpenes are present in the petroleum ether, ethyl acetate and methanol extracts whereas glycosides, Phenol, phytosterol, flavonoids, tannin and amino acid are commonly present in both ethyl acetate and methanol extract of *T. rosea* dried flowers. The highly polar alkaloids compounds showed positive result in methanol extract are present in Table 1.

Table 1: Phytochemical screening for petroleum ether, methanol and ethanol extracts of *T. rosea*

S.No	Test	Petroleum ether	Ethyl acetate	Methanol
1	Glycoside	_	+	+
2	Phytosterol	_	+	+
3	Phenols	_	+	+
4	Tannins	_	+	+
5	Flavonoid	_	+	+
6	Aminoacid	_	+	+
7	Protein	+	+	+
8	Diterpene	+	+	+
9	alkaloids	-	-	+

3.2. GC-MS analysis of methanol of *T.rosea*
From the GC-MS data the four major peak were obtained for Dispiro[1,3-dioxolane-2,2'bicyclo[2.2.1]heptane-3',2"(1",3"dioxolane)], 4',7',7'-trimethyl, Hentriacontane, 2-propenal, 3-phenyl and Dotriacontylpentafluoro propionate shown in Table 2.

3.3. Larvicidal activity
The potential larvicidal activity for methanol extract of *T.rosea* was carried out. The methanol extract showed moderate toxic effect on *Culexquinque fasciatus* and significant toxic effect against *An. Subpictus*at1000 mg/l concentration after 24 h of exposure. The regression value of *culexquinque fasciatus* was 0.974 and the LC_{50} value

showed 586.68, and for *Anopheles subpictus* regression value was 0.981 and LC_{50} value showed 241.72. The activity of plant crude extract depends on the presence of complex secondary metabolites present in it shown in Table 3.

Table 2: GC-MS analysis of *T.rosea*

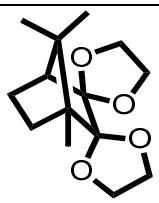
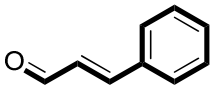
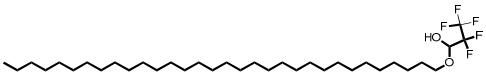
Compound name	Structure	Retention time	% of Area
Dispiro[1,3-dioxolane-2,2'bicyclo[2.2.1]heptane-3',2"(1",3"dioxolane)], 4',7',7'-trimethyl		25.92	27.21
Hentriacontane		27.43	24.46
2-propenal, 3-phenyl		10.24	1.08
Dotriacontylpentafluoropropionate		29.51	3.33

Table 3: Larvicidal activity of methanol extract of *Tabebuia rosea*

Larvae name	Concentrations (ppm)	Percent mortality[a] (ppm)±SE	LC_{50} (UCL–LCL) (ppm)	Slope	r^2
Culexquinque fasciatus	1000	69±0.78			
	500	31±0.45	586.68	24	0.974
	250	24±.1.02	(475.43-723.96)		
	125	14±0.84			
	62.5	06±0.32			
	1000	90±1.32			
Anopheles subpictus	500	74±0.79	241.72	51	0.981
	250	51±1.01	(206.60-282.80)		
	125	27±0.83			
	62.5	14±2.87			

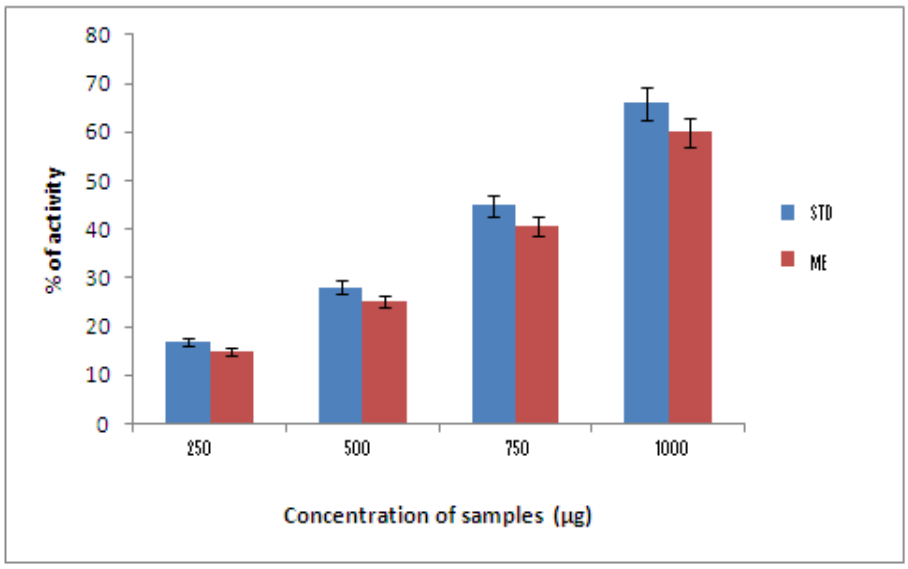


Fig 1: Total antioxidant activity

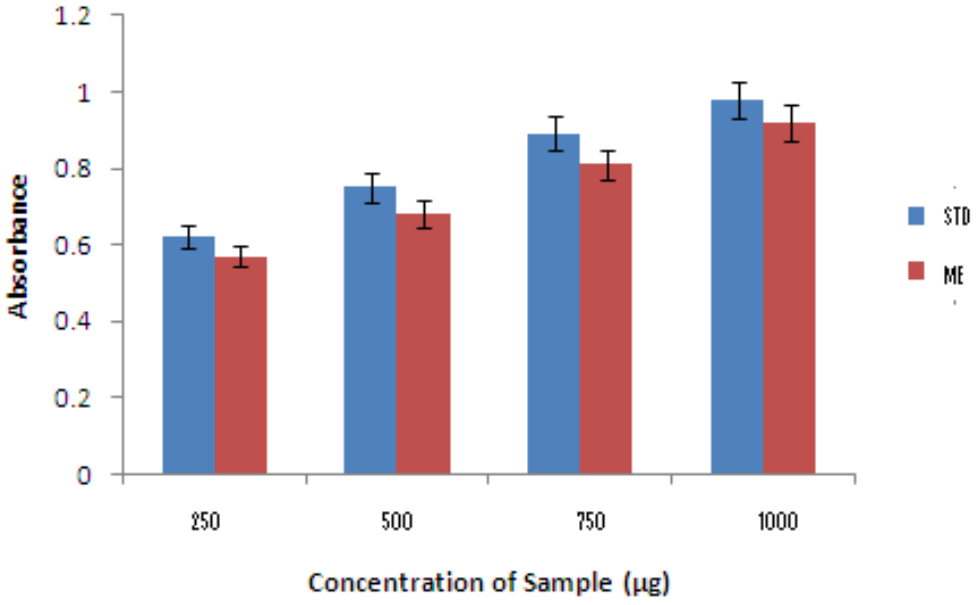


Fig 2: Reducing power assay of *T. rosea*

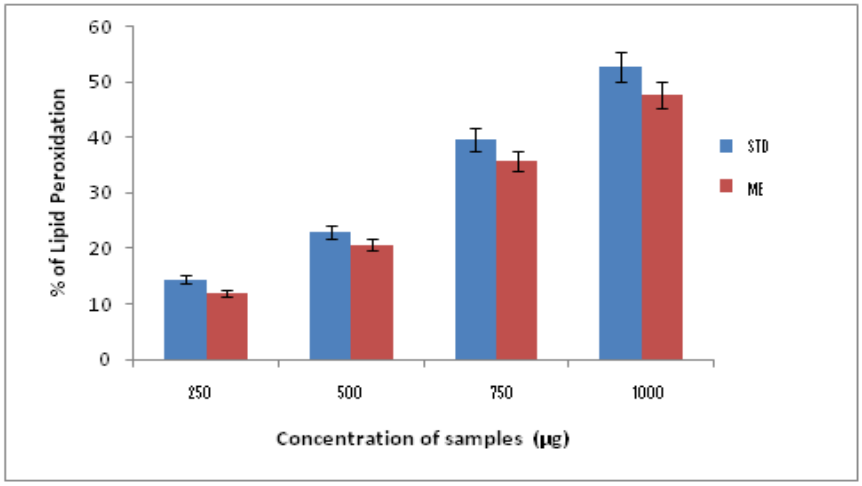


Fig 3: Lipid peroxidation by TBA method

3.4. Antioxidant activity

3.4.1. Total antioxidant activity method

The total antioxidant capacity of methanol extract of *T. rosea* was evaluated by the phosphomolybdenum method and the result was shown in **Fig 1.**It is based on the reduction of Mo (VI) to Mo (V) by the methanol extract. The result showed that concentration depends on the antioxidant capacity**Fig1.**

3.4.2. Reducing power assay

Fig 2 shows the significant reducing power of the methanol extract of *T. rosea* flower as compared to standard drug. Here the color of the solution changes from light yellow to various shades of green to blue, depending on the reducing activity of the extract. The methanol extract constituent which has reduction potential reacts with potassium ferricyanide (Fe^{3+}) and reduces it to potassium ferrocyanide (Fe^{2+}). This reduced potassium ferrocyanide reacts with ferric chloride and forms ferric ferrous complex that has an absorption maximum at 700 nm.

3.4.3. Lipid peroxidation method

Lipid per oxidation by using TBA method has been done for methanol extract of *T. rosea*. As shown in **Fig 3,** the methanol extract of *T. rosea* shows lower absorbance value corresponding to high antioxidant activity. Here gallic acid is used as standard and methanol extract shows comparatively good antioxidant activity. Control (distilled water) - nil mortality**;** LC_{50} lethal concentration that kills 50% of the exposed larvae, UCL upper confidence limit, LCL lower confidence limit, r^2 regression coefficient, Mean value of five replicates, $P<0.05$, significant level.

In summary it was identified that the flower extract of *T.rosea* showed larvicidal activity against *Culexquinque fasciatus* and *Anopheles subpictus*. The regression value of *culexquinque fasciatus* was 0.974 and the LC_{50} value showed 586.68, and for *Anopheles subpictus* regression value was 0.981 and LC_{50} value showed 241.72. GC-MS analysis was done on methanol extract. In methanol extract the antioxidant activity showed high reducing potential activity.

CONCLUSION

The study on antioxidant and larvicidal activity of *T.rosea* has demonstrated significant ability by total antioxidant, reducing power assay and lipid peroxide assay and larvicidal activity on *Culexquinque fasciatus* and *Anopheles subpictus*. Further the GC-MS analysis has been showed four major peaks that might be responsible for the activity of methanol extract. Hence, the *T. rosea* flowers are recommended for usage in pharmaceutical and nutraceutical industries.

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