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Evaluation of Phyto - Pharmacological Properties and GC-MS Studies on Ethanolic Extracts of Red and Green Leaves of *Lagerstroemia speciosa* (L) Pers (Lythraceae)

Ganesan G[1] and Sujatha P. S*[2]

[1-2] P. G. and Research Department of Zoology, Government Arts College (Autonomous), Coimbatore - 641 018, Tamil Nadu, India

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Abstract

The majority of plants have therapeutic qualities that are frequently used by native people throughout the world. Research focuses on describing and evaluating plant components potential for fighting various illnesses; however, it is still difficult to recognize bioactive components. Asia's tropical and subtropical climates are native to *Lagerstroemia speciosa*. The sample plant we used has two different leaf types: a green leaf and a red leaf. The leaf pigments exhibit the colour variation. Focusing on a wide range of phytopharmacological characteristics and GC-MS investigations is something we're interested in. Finding the active compounds that were separated from the ethanol extracts of *L. speciosa* leaves was the objective of the study's earlier work. The pharmacological properties include antimicrobial, antioxidant, anticancer, antidiabetic, hypolipidemic, anti-obesity, anti-inflammatory, analgesic, gastrointestinal, diuretic, thrombolytic, cardiovascular, central nervous, inhibition of TNFα production, xanthine oxidase inhibition, hepatoprotective and nephroprotective effects. The chemical constituents, pharmacological effects and therapeutic effects of the selected parts of *L. speciosa* are discussed in this study.

***Key words*:** Green leaf, Red leaf, Ethanolic Extracts, Phytopharmacology, *Lagerstroemia speciosa*

Plants are widely used for medicinal properties and secondary metabolites, including medicines, agrochemicals, flavours, fragrances, colours and food additives [1]. Identifying bioactive components remains challenging. *Lagerstroemia speciosa*, a tropical and subtropical Asian plant, is a potential candidate. The Western Ghats are one of the rich biodiversity regions of India, especially Coimbatore, Tamil Nadu. The experimental plant *Lagerstroemia speciosa* commonly called Pride of India, Poomaruthu in Tamil [3]. A wide variety of phytochemical compounds, such as secondary metabolites are synthesized by plants. The secondary metabolites of medicinal plants have very strong antioxidant properties and act as an efficient source of natural antioxidants. The chemical constituents, pharmacological effects and therapeutic effects of the selected parts of *Lagerstroemia speciosa* are discussed in this study [4]. The sample plant we used has two different leaf types: a green leaf and a red leaf. The leaf pigments exhibit the colour variation [13].

The leaf of *Lagerstroemia speciosa* has dark green, oblong, leathery leaves that turn attractively red before falling in winter [5]. Carotenoids are pigments in the form of orange, red and yellow colours. Flavonoids are a type of yellow-coloured pigment that is abundantly found in lemons, grapefruit, oranges and some orange- and yellow-coloured flowers [7]. The leaves contain large amounts of corosolic acid which has previously been shown to possess antidiabetic properties [21] and significant amounts of tannins [9]. The pharmacological properties include antimicrobial, antioxidant, anticancer, antidiabetic, hypolipidemic, antiobesity, anti-inflammatory, analgesic, gastrointestinal, diuretic, thrombolytic, cardiovascular, central nervous, inhibition of TNF production, xanthine oxidase inhibition, hepatoprotective and nephroprotective effects [1]. The chemical constituents, pharmacological effects, and therapeutic effects of the selected parts of *Lagerstroemia speciosa* are discussed in this study.

MATERIALS AND METHODS

Collection, authentication and plant extracts preparation

The leaves of *Lagerstroemia speciosa* were collected from the P. G. Girls Hostel, Government Arts College (Autonomous), Coimbatore District, Tamil Nadu, India. The identification and authentication of *L. speciosa* are done by the Botanical Survey of India, Coimbatore, and the voucher specimens numbered BSI/SRC/5/23/2020/Tech/50 were placed in the Department of Zoology, Government Arts College (Autonomous), Coimbatore.

Lagerstroemia speciosa leaves were collected, washed and sun-dried for 2 weeks. The leaves were ground to powder (100g) and soaked in ethanol (1000 ml). The powder was solubilized and mixed well with intermittent stirring for 4 days. After that, the extract was filtered using Whatman No. 1 filter paper and kept in a plastic tray to dry at room temperature [15].

***Correspondence to:** Sujatha P. S, E-mail: sujatha2724@gmail.com; Tel: +91 97903 55515

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Qualitative phytochemical analysis

Qualitative phytochemical analysis of the green and red leaves of *L. speciosa* Ethanolic extracts were carried out according to the methodology of Horbone [8], Trease and Evans [28].

Test for carbohydrates

To 200 µl of plant extract, 100 µl of Molisch's reagent and a few drops of concentrated sulfuric acid were added. The presence of a purple or reddish colour indicates the presence of carbohydrates.

Test for tannins

To 100µl of plant extract, 200µl of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for saponins

To 200µl of plant extract, 200µl of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

Test for flavonoids

To 200µl of plant extract, 100µl of 2N sodium hydroxide were added. The presence of a yellow colour indicates the presence of flavonoids.

Test for alkaloids

To 200µl of plant extract, 200µl of concentrated hydrochloric acid were added. Then a few drops of Mayer's reagent were added. The presence of a green or white precipitate indicates the presence of alkaloids.

Test for quinones

To 100µl of plant extract, 100µl of concentrated sulfuric acid were added. The formation of a red colour indicates the presence of quinones.

Test for glycosides

To 200µl of plant extract, 300µl of chloroform and a 10% ammonia solution were added. The formation of a pink colour indicates the presence of glycosides.

Test for terpenoids

To 50µl of plant extract, 200µl of chloroform and concentrated sulfuric acid were carefully added. The formation of a red-brown colour at the interface indicates presence of terpenoids.

Test for triterpenoids

To 150µl of plant extract, 100µl of chloroform shaken with concentrated sulfuric acid were added. The lower layer turning yellow on standby indicates the presence of triterpenoids.

Test for phenols

To 100µl of the plant extract, 200µl of distilled water followed by a few drops of 10% ferric chloride were added. The formation of a blue or green colour indicates the presence of phenols.

Test for coumarins

To 100µl of plant extract, 100µl of 10% NaOH were added. The formation of a yellow colour indicates the presence of coumarins.

Test for steroids

To 100µl of plant extract, an equal volume of chloroform is added and subjected to a few drops of concentrated sulfuric acid. The appearance of a brown ring indicates the presence of steroids.

GC-MS analysis

The GC-MS analysis at The South Indian Textile Research Association in Coimbatore identified important compounds in *L. speciosa* ethanolic extracts of green and red leaves. The analysis used a Thermo GC-Trace Ultra ver. 5.0, Thermo MS DSQ 11 chromatography.

RESULTS AND DISCUSSION

Result 1 – (Table 1) shows the presence of the phytochemical constituents *L. speciosa* ethanolic green leaf extract. According to the phytochemical analysis, the LEGLE consists of quinone, which was absent in the LERLE.

Result 2 - The secondary metabolites of the LERLE consist of Carbohydrates, Tannins, Saponins, Flavonoids, Alkaloids, Glycosides, Terpenoids, Triterpenoids, Phenols, Coumarins, and Steroids (Table 1).

Result 3 -GC-MS Analysis of LEGLE shows the highest rate (84.29%) of Plant sterol (Table 2).

Result 4 - The (Table 3) shows that Phytol, Tetra decanoic acid, Docane, Heptacosane, 1-Butanol, 3-methyl-acetate are the highest rate of available compounds.

Result 5 -The pigments in *L. speciosa* ethanolic leaf extracts show their biological uses (Table 4).

Table 1 Phytochemical analysis of ethanolic green leaf and red leaf extracts of *L. speciosa*

Phytoconstituents	Green leaf	Red leaf
Carbohydrates	+	+
Tannins	+	+
Saponins	+	+
Flavonoids	+	+
Alkaloids	+	+
Quinones	+	-
Glycosides	+	+
Terpenoids	+	+
Triterpenoids	+	+
Phenols	+	+
Coumarins	+	+
Steroids	+	+

'+' indicates the presence of phytoconstituents
'-' indicates the absence of phytoconstituents

Evaluation of L. speciosa leaf

Macroscopic, Microscopic and qualitative evaluations were performed according to the KMCH (KMCH pharmacopeia Committee, 2022) and WHO guidelines. Different parts of the plants are used for the treatment of various diseases.

Pharmacological effects of L. speciosa LEGLE extracts
Antidiabetic activity

The antidiabetic Effect of the aqueous leaf extract of *L. speciosa* was studied in streptozotocin-induced diabetic mice. The extract effectively decreased blood glucose after the 15th day (25). Ellagitannins, Lagerstroemin, flosin B, and reginin A, isolated from the aqueous acetone extract of the leaves of *L. speciosa*, increased glucose uptake in rat adipocytes and could be responsible for lowering blood glucose levels. [9].

Table 2 Important compounds identified in GC-MS analysis of ethanolic green leaf extract of *L. speciosa*

S. No.	Compound name	Molecular formula	Match factor
1	Cholesterol	$C_{27}H_{46}O$	84.29
2	Cholesterol, 7- oxo-	$C_{27}H_{44}O_2$	3.42
3	Lucenin- 2	$C_{27}H_{30}O_{16}$	1.33
4	Hexadecanoic acid, ethyl ester (CAS)	$C_{18}H_{36}O_2$	1.08
5	Betulin	$C_{30}H_{48}O_3$	0.84
6	6-Octadecanoic acid	$C_{18}H_{34}O_2$	0.83
7	1-Hexadecanoic, acetate (CAS)	$C_{18}H_{36}O2$	0.79
8	1-Octadecanol (CAS)	$C_{18}H_{38}O$	0.73
9	Hexadecanoic acid, methyl ester (CAS)	$C_{17}H_{34}O_2$	0.72
10	Lucenin-2	$C_{27}H_{30}O_{16}$	0.58
11	Octadecanoic acid, ethyl ester (CAS)	$C_{20}H_{40}O_2$	0.56
12	9,12,15, - Octadecatrienoic acid	$C_{27}H_{52}O_4Si_2$	0.48
13	1-Hexadecanol	$C_{16}H_{34}O$	0.46
14	Cyclobutane	$C_{26}H_{40}O_4$	0.21

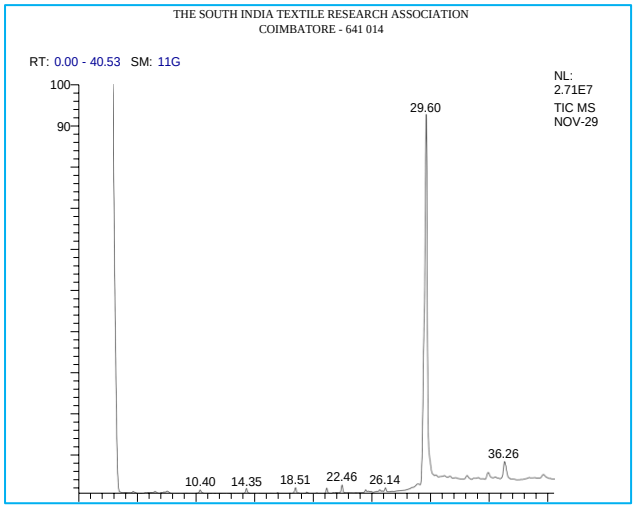

Fig 1 Shows GC-MS spectrum of ethanolic green leaf extract of *Lagerstroemia speciosa*

Antiobesity activity

The leaf extract potentially inhibited lipid peroxidation and effectively intercepted or neutralized reactive oxygen species such as superoxide, HR2ROR2R, and NO-based free radicals [25]. The effects of extracts of *L. speciosa* on glucose transport and adipocyte differentiation were studied in 3T3-L1 cells using a radioactive assay, and the ability of extracts of *L. speciosa* to induce differentiation in preadipocytes was examined by Northern and Western blot analyses [18].

Antimicrobial activity

Antibacterial activity of ethanol and water extracts of leaves of *L. speciosa* was tested by the plate agar diffusion method against gram-positive and gram-negative bacteria. The antibacterial activity of the leaves of *L. speciosa* has been reported. *L. speciosa* leaf powder extracts were tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *and Escherichia coli* with ampicillin as the standard antibiotic [2].

Table 3 Important compounds identified in GC-MS analysis of ethanolic red leaf of *L. speciosa*

S. No.	Compound name	Match factor	Molecular formula
1	4,8,12- Tetradecatrienenitrile, 5, 9, 13 - Trimethyl	59.4	$C_{17}H_{27}N$
2	Phytol	83.7	$C_{20}H_0O$
3	n- Hexadecanoic acid	73.7	$C_{16}H_{32}O_2$
4	Sulfurous acid, 2-ethyl isohexyl ester	78.8	$C_{14}H_{30}O_3S$
5	4- [(5- Hexyl- 1H- 1,2,3,4-triazol - 3-yl) amino] - 4- oxo- 2- butenoic acid	57	$C_{12}H_{18}N_4O_3$
6	Tetradecanoic acid	82.2	$C_{14}H_{28}O_2$
7	1- Hexyl- 2- nitrocyclohexane	65.6	$C_{12}H_{23}NO_2$
8	2- Undecanone	74. 9	$C_{11}H_{22}O$
9	Docane	80.2	$C_{12}H_{26}$
10	Heptacosane	82.2	$C_{27}H_{56}$
11	4(1H)- Pteridinone	55.2	$C_6H_4N_{40}$
12	Phthalic acid, di(2-propylpentyl) ester	73.5	$C_{24}H_{38}O_4$
13	Dodecanoic acid	83.9	$C_{12}H_{24}O_2$
14	4,8,12,16-Tetramethylheptadecan-4-olide	57.5	$C_{21}H_{40}O_2$
15	5,9,13-Pentadecatrien -2-one, 6,10,14-trimethyl-, (E,E)-	62.1	$C_{18}H_0O$
16	Cyclobutanecarboxylic acid, 2-dimethylaminoethylester	72	$C_9H_{17}NO_2$
17	Heptacosane	60.2	$C_{27}H_{56}$
18	1-Butanol, 3-methyl-, acetate	85.4	$C_7H_{14}O_2$

Nutraceutical effects of L. speciosa LERLE

Anthocyanins may exert beneficial effects on obesity extracts inhibit Gram-negative bacteria but not Gram-positive bacteria. Supplementation of honeysuckle anthocyanins for a 100 mg/kg diet reduced body weight gain by 17.1% compared with the control group, which was similar to the 16.9% reduction in the orlistat group [27]. The number and position of hydroxyl and methoxy substituents as electron-donating groups, along with glycosylation parameters like site and number of glycosylation and sugar type, have a great impact on the activities of anthocyanins [12]. Dyslipidaemia is a common feature of obesity. Decreasing lipogenesis and increasing lipolysis are two aspects of regulating lipid metabolism. The strategy is to stimulate triglyceride hydrolysis to reduce fat storage, which requires the oxidation of newly released fatty acids [18].

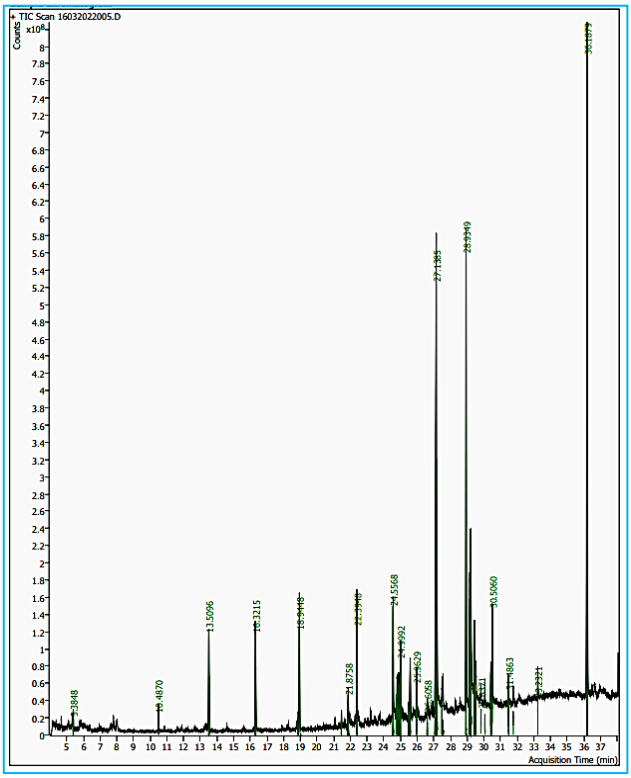

Fig 2 Shows GC-MS spectrum of ethanolic red leaf extract of *Lagerstroemia speciosa*

The leaves of *Lagerstroemia speciosa* are dark green, oblong, leathery, and turn attractively red before falling in winter [5]. Plant pigments exist in many varied forms, some with highly complex and large structures. For example, over 600 naturally occurring carotenoid structures have been identified [7]. Flavonoids provide pigments for plants, with anthocyanins and proanthocyanins being condensed tannins. The objective of the study was to reveal the presence of phytochemical compounds present in *Lagerstroemia speciosa speciosa* ethanolic green and red leaves. The corosolic acid content and distribution in *L. speciosa* finding that red leaves contain more than green leaves and other plant parts. *Lagerstroemia speciosa* leaves contain over 40 triterpenes, tannins, ellagic acids, glycosides, and flavone substances. Polyphenols, such as ellagitannins, are found in fruits, nuts, and seeds [17].

Lagerstroemia speciosa leaf extracts in Southern Western Ghats contain modest minerals, fat, fibre, protein, and carbs. Mercury, arsenic, lead, and cadmium levels are below permissible, posing potential safety risks [11]. The leaves contain six pentacyclic triterpenes, with significant variation in corosolic acid distribution among 12 natural populations. The leaves contain large amounts of corosolic acid, which has previously been shown to possess antidiabetic properties [21] and significant amounts of tannins [9]. The antidiabetic properties of leaf extracts of *Lagerstroemia speciosa* and its compounds such as ellagitannins and corosolic acids have generated much research involving in vitro, animal and human studies [14].

Ethanol efficiently retrieves biomolecules from plants, minimizing waste and ensuring safe use in food-grade and consumable goods. Phytochemical analysis reveals quinone absence in *L. speciosa* ethanolic red leaf extract, indicating the presence of quinone constituents in *L. speciosa* ethanolic green leaf extract. It contains secondary metabolites including carbohydrates, tannins, saponins, flavonoids, alkaloids, phenols, and steroids. GC-MS Analysis reveals LEGLE contains 84.29% plant sterol. (Table 3) displays the highest available compounds: Phytol, tetra decanoic acid, Docane, and heptacosane. Pigments in *Lagerstroemia speciosa* ethanolic leaf extracts reveal biological uses. Resveratrol, a polyphenol, can improve weight loss, hay fever, blood arteries, and immune system function.

Table 4 Major pigment of plants and their occurrence in other organisms (Davies 2004)

Pigments	Availability	Plant Origin	Therapeutic Values	References
Betalains	Betacyanin's Betaxanthins	Beetroot Mushroom	Anticancer Antiviral Antimicrobial Antifungal	Fu, Y.; Shi, J. 2020
Carotenoids	Carotenes Xanthophylls	Carrot	Antioxidant Arteriosclerosis Cataract Multiple sclerosis	Edge *et al*, 1997
Chlorophylls	Porphyrin	Green leafy vegetables	Chronic diseases Cardiovascular Anticancer	Sangeetha and Baskaran, 2010
Flavonoids	Flavanols Anthocyanins Proanthocyanins	Fruits and Seeds	Anti-inflammatory Neurodegenerative Metabolic diseases	Beeche, 2009

CONCLUSION

Herbal plants are excellent sources of nutrients, including pigments found in leaves, fruits, vegetables, and flowers. These pigments are used in various products, including medicines, foods, furniture, and cosmetics. The correlation between corosolic acid and cyanidin 3-O-glucoside supports traditional perceptions of Banaba tea's medicinal effects. This article describes *Lagerstroemia speciosa* phytochemical and pharmacological properties, highlighting its potential for treating various ailments. Despite being considered an ornamental plant, it has anti-inflammatory, antibacterial, antioxidant, anti-HIV, antidiabetic, anti-ageing, anti-wrinkle, and anti-diarrheal properties.

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