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Phytochemical Analysis, Identification, Isolation, and Biological Activity of Ellagitannin from Acetone Leaf Extracts of *Lagerstroemia speciosa* (L.) Pers (Lythraceae)

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Abstract

Medicinal plants have bioactive compounds known as phytochemicals that play an important role in curing and healing of various diseases in human. Ellagitannins occur naturally in some fruits, nuts, and seeds. They form a diverse group of bioactive polyphenols with anti-Inflammatory, anticancer, antioxidant and antimicrobial activity. The medicinal plant *Lagerstroemia speciosa* that grows in Tamil Nadu's Western Ghats was the subject of the current study. For assurance of quality, samples of acetone leaf extract (LALE) were used to identify the phytochemical components of the plants. The amounts of the major components are given by the GC-MS and HPLC. The present study involves the phytocompounds analysis, identification, isolation and biological activities of ellagitannin from acetone leaf extracts of *L. speciosa*. The phytochemical analysis shows the presents of phytocompounds in the acetone leaf extracts of experimental plant. The GC-MS analysis shows the major compounds of leaf of *L. speciosa*. The HPLC shows the quantitative and qualitative amount of ellagitannin in the leaf of *L. speciosa*. Bioassay-guided fractionation of the aqueous acetone extract of the leaves afforded three active ellagitannins, lagerstroemia, flosin B and reginin A.

***Key words*:** Ellagitannins, *Lagerstroemia* speciosa acetone, Leaf extract, Phytocompounds, GC-MS, HPLC

Phytochemicals, found in medicinal plants, are essential for preventing and curing various human ailments. These bioactive polyphenols, found in fruits, nuts, and seeds, possess anti-inflammatory, anticancer, antioxidant, and antimicrobial properties [1]. These bioactive polyphenols, present in fruits, nuts, and seeds, possess anti-inflammatory, anticancer, antioxidant, and antimicrobial properties. This study investigates the phytochemical components of three distinct medicinal herbs. Pharmaceutical companies prioritize phytochemical analysis for novel pharmaceuticals due to their immunological stimulation, digestive secretion, antibacterial, anthelmintic, antiviral, anti-inflammatory, inhibitory, and antioxidant activities. These active plant phytochemicals, including carotenoids, phytosterols, polyphenols, and glycosylates, can be categorized based on their chemical makeup. This study investigates three distinct medicinal herbs for their phytochemical components. Pharmaceutical companies prioritize phytochemical analysis for novel pharmaceuticals as they offer immunological stimulation, digestive secretion, anti-bacterial, anthelmintic, antiviral, anti-inflammatory, inhibitory, and antioxidant activities. These active plant phytochemicals, including carotenoids, phytosterols, polyphenols, and glycosylates, can be categorized based on their chemical makeup. Phytochemicals, produced by medicinal plants, are essential for treating various illnesses. Known as ellagitannins, these compounds possess anti-inflammatory, anticancer, antioxidant, and antimicrobial properties. Ellagitannins (ETs) are hydrolysable tannins, one of the two primary types of tannins, with high in vitro free radical scavenging activity. They consist of ellagic acid and gallic acid with a sugar core, while condensed tannins are flavonoids without a sugar core. Both groups have adverse effects on health [2]. The Western Ghats are one of the rich biodiversity regions of India, especially Coimbatore, Tamil Nadu. The experimental plant *Lagerstroemia speciosa* is commonly called Pride of India, or Poomaruthu in Tamil Nadu [3-4]. 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. This research aimed to screen the phytochemical compounds, identification, isolation, and biological activities of ellagitannin from acetone leaf extracts of *Lagerstroemia*.

MATERIALS AND METHODS

Collection and identification

The leaves of *Lagerstroemia speciosa* were collected from the PG Girls Hostel, Government Arts College (Autonomous), Coimbatore District, Tamil Nadu, India. The *L. speciosa* were identified and authenticated at the Botanical Survey of India, Coimbatore (No. BSI/ SRC/ 5/ 23/ 2020/ Tech/ 52) and the voucher specimens were kept in the Department of Zoology, Government Arts College, Coimbatore-18.

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Plant extracts preparation

The selected plant leaf was cleaned thoroughly before the extract preparation. The samples were then kept under the shade at room temperature (27± 2 °C) for about 2 weeks until they dried completely. They were finely powdered using a blender. The powdered plant material (100 g) was soaked in acetone (1000 ml) in an airtight, wide-mouthed bottle and kept for 4 days with periodic shaking. After that, the extract was filtered using Whatman No. 1 filter paper and kept in a plastic tray to dry at room temperature [5].

Qualitative phytochemical analysis

Qualitative phytochemical analysis of the acetone leaf extracts of *Lagerstroemia speciosa* was carried out according to the methodologies of Horbone [6] and Trease and Evans [7].

Test for alkaloids (Wagner's test)

About a few ml of plant extract were treated with 4-5 drops of Wagner's reagent. The formation of reddish-brown precipitates confirms the presence of alkaloids.

Test for phenols (Ferric chloride test)

About 2 ml of the extract was treated with a 10% ferric chloride solution and observed for the formation of a deep blue or black colour.

Test for reducing sugars (Fehling's Test)

To 1 ml of the extract, a few drops of Fehling's reagent were added, and the mixture was boiled in a boiling water bath for 10 minutes and observed for the appearance of a blue colour.

Test for saponins (Foam test)

To 2 ml of the plant extract, add 6 ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam for a few seconds. The presence of foam confirms the presence of saponins.

Test for flavonoids

To about 2 ml of plant extract, a few drops of a 10% ferric chloride solution were added. The formation of a green or blue colour indicates the presence of flavonoids.

Test for phytosterols (Salkowski's Test)

One ml of the plant extract was treated with 2 ml of chloroform and a few drops of acetic anhydride were added. To that mixture, an equal amount of concentrated sulphuric acid was added. The formation of a bluish green colour indicates the presence of Phytosterols.

Test for amino acids and proteins (Ninhydrin test)

To a few ml of plant extract, add a small amount of Ninhydrin reagent. A purple or violet colour indicates the presence of amino acids and proteins.

Test for steroids

About 2 ml of chloroform and 0.2 ml of concentrated sulfuric acid were added to 1 ml of leaf extract. The formation of a red precipitate indicates the presence of steroids.

Test for tannins

To about 1 ml of plant extract, add a few drops of a dilute ferric chloride solution. The presence of tannin is confirmed by the formation of a dark green or blue colour.

Test for glycosides

To 1 ml of plant extract, add a few ml of concentrated sulfuric acid; the formation of a red colour indicates the presence of glycoside.

Test for quinones

1 ml of plant extract and 1 ml of sulfuric acid were added. The formation of a red colour indicates the presence of quinones.

Test for coumarins

To 1 ml of plant extract, 1 ml of 10% NaOH was added. A yellow formation indicates the presence of Coumarins.

GC-MS analysis

The GC-MS analysis was conducted at The South Indian Textile Research Association, Coimbatore. 1µl of sample powder was injected into a Thermo GC –Trace ultra ver. 5.0, Thermo MS DSQ 11. The chromatography was performed using the DB 35-MS capillary standard nonpolar column. Helium flow was 1 ml/min. The oven temperature was increased from 70 °C /per minute to 250 °C. Important compounds were identified in the GC- MS analysis of *Lagerstroemia speciosa* acetone extracts of the leaf.

HPLC analysis

HPLC analysis was performed in liquid chromatography (Shimadzu LC-8A, Japan) pumps equipped with SPD-20 A UV/Vis detector. The HPLC profile of the acetone leaf extract of *Lagerstroemia speciosa* was recorded with a modified method by Govindarajan [8]. Detection was carried out at 264 nm. Quantification of Ellagitannin compounds achieved by absorbance recorded using chromatogram relative external standards with the following equation:

$$B(b)=(A(b)) / (A (st)) \times B (st).$$

In which B (b) is the concentration of compound in the sample, A (b) is the peak area of the compound in the sample chromatograms, B (st) is the concentration of standard in the reference solution, and A (st) is the area of peak for the standards in the reference chromatograms [9].

Table 1 Phytochemical analysis of acetone leaf extract of *Lagerstroemia speciosa*

Phytoconstituents	Acetone leaf extract
Alkaloids	++
Flavonoids	+++
Saponins	++
Phenols	++
Tannins	+++
Proteins and amino acids	++
Reducing sugar	++
Steroids	-
Glycosides	++
Phytosterols	+++
Quinones	-
Coumarins	+

'+' indicates the presence of Phytoconstituents
'-'indicates the absence of Phytoconstituents
'++' indicates the Phytoconstituents present in a moderate level
'+++' indicates the Phytoconstituents present abundantly

RESULTS AND DISCUSSION

Lagerstroemia speciosa L. Pers, finding that red leaves contain more than green leaves and other plant parts. *L. speciosa*

leaves contain over 40 triterpenes, tannins, ellagic acids, glycosides, and flavone substances. Polyphenols, such as ellagitannins, are found in fruits, nuts, and seeds. Retention times and percentage area of Ellagitannin such as Ellagic acid were given in (Fig 2). Retention times of ellagitannin namely, Ellagic acids were of 4.881 and 4.904 min, respectively in acetone leaf extract of *Lagerstroemia speciosa.*

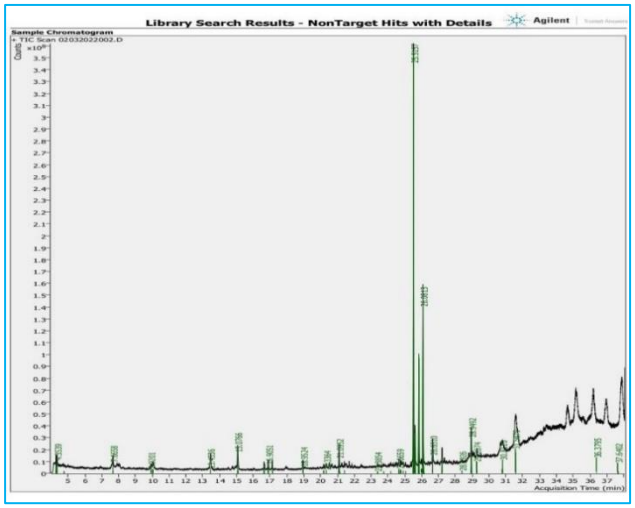

Fig 1 Shows GC-MS spectrum of acetone leaf extract of *Lagerstroemia speciosa*

Tannins, high molecular weight plant polyphenols, are divided into condensed tannins, hydrolyzable tannins (ETs), and gallotannins (GTs). Originally popular in the commercial tanninning industry, they have biological activities and potential health benefits [10]. Some plants rich in ellagitannins are commonly used for medical purposes, especially in Asia [11]. Ellagitannins (ETs) and ellagic acid (EA) are polyphenols present in some fruits, nuts, and seeds, such as pomegranates, black raspberries, strawberries, walnuts, and almonds. ETs are hydrolyzed to EA under physiological conditions [12]. The Leaf of *L. speciosa* has dark green, oblong, leathery leaves that turn attractively red before falling in winter. The leaves contain large amounts of corosolic acid, which has previously been shown to possess antidiabetic properties [13], and significant amounts of tannins [14]. *L. speciosa* leaves contain over 40 triterpenes, tannins, ellagic acids, glycosides, and flavone substances. Polyphenols, such as ellagitannins, are found in fruits, nuts, and seeds (12). 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.

The bioavailability of ellagitannins and free ellagic acid depends on the part of gastrointestinal tract in which these compounds are absorbed. In vitro studies have shown that ellagitannins are stable under the conditions of the acidic gastric environment (HCl, pH 1.8- 2.0) and in the presence of gastric enzymes (pepsin, rennin, gastric lipase) and undergo neither hydrolysis to free ellagic acid nor degradation [15]. Moreover, poly phenols extracted from blackberry fruits show stronger antimicrobial properties than those extracted from the roots.

Table 2 Important compounds identified in GC-MS analysis of Acetone leaf extract of *Lagerstroemia speciosa*

Compounds	Molecular formula	Match factors
Neophytadiene	$C_{20}H_{38}$	87.8
3,7,11,15-Tetramethyl-2-hexadecen-1ol	$C_{20}H_{40}O$	82.9
1-Hexadecyne	$C_{16}H_{30}$	82.6
Cyclopentane, 1, 1,3,4-tetramethyl-, cis-	C_9H_{18}	81.9
3,7,11,15-Tetramethyl-2-hexadecen-1ol	$C_{20}H_{40}O$	81.5
2-Pentadecanone, 6, 10, 14-trimethyl-	$C_{18}H_{36}O$	81.3
Phytol	$C_{20}H_{40}O$	81.1
Neophytadiene	$C_{20}H_{38}$	78.3
2- Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	78.2
2-Pentadecanone, 6, 10, 14-trimethyl-	$C_{18}H_{36}O$	77.5
1, 14- Tetradecanediol	$C_{14}H_{30}O_2$	77.5

Standard ellagic acid

Acetone leaf extract of *Lagerstroemia speciosa*

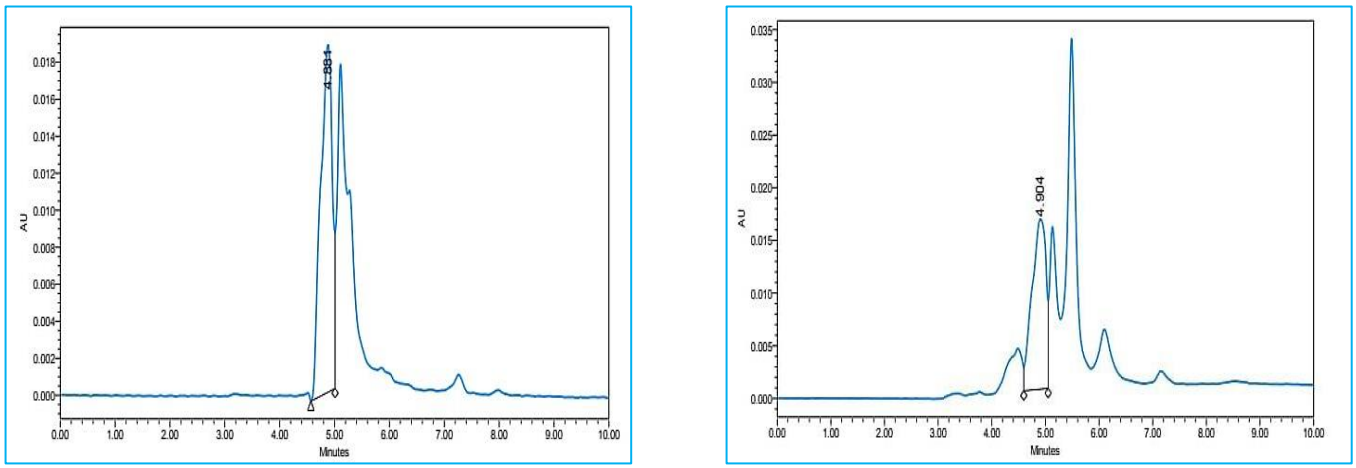

Fig 2 HPLC analysis of standard ellagic acid, acetone leaf extract of *Lagerstroemia speciosa*

CONCLUSION

Investigating ellagitannins' bioavailability and biological activity, current research is insufficient in anti-tumour and antifungal properties, as yeast and moulds are primary causes of food rotting. Ellagitannins, used as food preservatives, have limited biological activity. Consumption of food rich in ellagitannins inhibits tumorigenesis, but the mechanism is not yet understood. Further studies on their biological activity are needed.

Table 3 HPLC analysis of acetone leaf extract of *Lagerstroemia speciosa*

RT	Area	% Area	Height
4.881	278548	100.00	18761
4.904	292827	100.00	16115

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