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PHYTO-PHARMACOLOGICAL ACTIVITIES AND GC MS STUDIESON ETHANOLIC LEAF, FLOWER AND SEED EXTRACTS OF *LAGERSTROEMIA SPECIOSA* (L.) PERS (LYTHRACEAE)

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ABSTRACT
The medicinal activities of *Lagerstroemia speciosa* plants are generally due to their secondary metabolites. In this research, we summarize the available knowledge of phytochemical and pharmacological activities of *L. speciosa*. The phytochemical and Gas Chromatography Mass Spectroscopy (GC MS) analysis of ethanolic extracts leaf, flower and seed of *L. speciosa* revealed that it contained steroids, terpenoids, glycosides, phenolic compounds, α-amino acids, saponins, starch, alkaloids, carbohydrates, organic acids, flavonoids, reducing sugars, tannins and many other active metabolites. *L. speciosa* (Family: Lythraceae) is native to Asia-tropical and subtropical regions. It has a wide variety ofbioactive molecules used as a traditional medicine. The pharmacological properties include antimicrobial, antioxidant, anticancer, antidiabetic, hypolipidemic, antiobesity, anti- inflammatory, analgesic, gastrointestinal, diuretic, thrombolytic, cardiovascular, central nervous, inhibition of TNFα production, xanthine oxidase inhibition, hepatoprotective and nephroprotective effects. The chemical constituent, pharmacological and therapeutic effect ofthe selected parts of *L. speciosa* is discussed in this study.

KEYWORDS: *Lagerstroemia speciosa*, Phyto-constituents, Pharmacology.

INTRODUCTION
The knowledge of the active principles and medicinal compounds in plants is continuously being updated through various biochemical methods. There are a number of methods, such as Thin layer Chromatography, HPTLC, HPLC, NMR, FTIR, XRD, etc. which are used in the analysis of various biochemical and chemical parameters of plants. The present study deals with the phytochemical and GC MS analysis of the selected extracts of one such medicinal plant, *L. speciosa*. Since not much research has been conducted on this plant, the present work was undertaken. Most present-day medicines have plant origins. *L. speciosa*, locally known as Jarul, is a good timber tree with lilac flowers and winged seeds. The Western Ghats are one of the rich biodiversity regions of India, especially Coimbatore, Tamil Nadu. The active components of banaba extract are arjunolic acid[1], ellagic acid, corosolic acid, and tannic acid.[2] A wide variety of phytochemical compounds, such as secondary metabolites, are synthesised by plants. The secondary metabolites of medicinal plants have very strong antioxidant properties and act as an efficient source of natural antioxidants.[3] The present study deals with the phytochemical screening and GC MS analysis of ethanolic leaf, flower, and seed extracts of *L. speciosa*.

Common names
L. speciosa (L.) Pers to the family Lythraceae. It is distributed in the Tropical Himalaya, and Assam, Western and Eastern Ghats, up to 1000m. It is known as Pride of India, Queen's Flowers and Queen Crape Myrtle in English. It is commonly known as Poomaruthu in Tamil, Manimaruthu in Malayalam, Jarul or Banaba in many countries.[4]

MATERIAL AND METHODS
Collection and Identification
The leaves, flowers and seeds of *L. speciosa* were collected from PG Girls hostel, Government Arts College (Autonomous), Coimbatore District, Tamil Nadu, India. The *L. speciosa* were identified and authenticated at Botanical Survey of India, Coimbatore-03 (No: BSI/SRC/5/23/2020/Tech/51) and the voucher specimens were kept in Department of Zoology, Government Arts College, Coimbatore-18.

Plant extracts preparation
The selected plant parts were cleaned thoroughly before the extract preparation. The samples were then kept

under the shade at room temperature (27± 2ºC) for about 2 weeks till they dried completely. They were finely powdered using a blender. The powdered plant material (100 g) was soaked in ethanol (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 Petri dishes to dry at room temperature.[5]

Qualitative phytochemical analysis

Qualitative phytochemical analysis of the leaf, flower and seed of *L. speciosa* Ethanolic extracts were carried out according to the methodology of Horbone[6] and Trease and Evans.[7]

Test for Alkaloids (Wagner's test)

About a few ml of plant extract was 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 10% ferric chloride solution and observed for the formation of a deep blue / black colour.

Test for Reducing sugars (Fehling's Test)

To 1 ml of the extract added few drops of Fehling's reagent and the mixture was boiled in a boiling water bath for 10 minutes and observed for the appearance of 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 presenceof foam confirms the presence of saponins.

Test for Flavonoids

To about 2 ml of plant extract, a few drops of 10% ferric chloride solution was 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 added small amount of Ninhydrin reagent. A purple or violet colour formed indicates the presence of amino acids and proteins.

Test for Steroids

About 2 ml of chloroform and 0.2 ml of concentrated sulphuric acid was added to 1 ml of flower extract. The formation of a red colour precipitate indicates the presence of steroids.

Test for Tannins

To about 1 ml of plant extract, add a few drops of dilute ferric chloride solution. Thepresence 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 sulphuric acid, a formation of redcolour indicates the presence of glycoside.

Test for Quinones

To 1 ml of plant extract, 1 ml of sulphuric acid was 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 formation of yellow colour 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 non-polar column. Helium flow was 1 ml/min. The oven temperature was increased from 70ºC /min to 250ºC. Important compounds were identified in the GC-MS analysis of *L. speciosa* ethanolic extracts of leaf, flower and seed.

RESULT

Chemical constituents

***L. speciosa* Ethanolic Leaf Extract (LELE)** contains Alkaloids, flavonoids, saponins, phenols, tannins, protein and amino acids, reducing sugar, steroids, glycosides, phyto-sterols, coumarins, quinones.

***L. speciosa* Ethanolic Flower Extract (LEFE)** contains Alkaloids, flavonoids, saponins, phenols, tannins, protein and amino acids, reducing sugar, steroids, glycosides, phyto-sterols, coumarins, quinones.

***L. speciosa* Ethanolic Seed Extract (LESE)** contains Alkaloids, flavonoids, tannins, reducing sugar, steroids, glycosides, and phyto-sterols.

Pharmacological Activities

Macroscopic, microscopic and qualitative evaluations were performed according to the KMCH (KMCH Pharmacopoeia Committee, 2022) and WHO guidelines.[8] Different parts of the plant are used for the treatment of various diseases.[9]

***L. speciosa* Ethanolic Leaf Extract (LELE)**

The anti-diabetic activity of the leaf extract of *L. speciosa* [standardized to 1% corosolic acid (Glucosol)] was studied in a randomized clinical trial in Type II

diabetics. Glucosol at daily dosages of 32 mg and 48 mg for 2 w induced a significant reduction in the blood glucose levels. Glucosol in a soft gel capsule formulation showed a 30% decrease in blood glucose levels compared to a 20% drop in hard gelatin capsule formulation ($P<0.001$) suggesting that the soft gel formulation has better bioavailability than a dry-powder formulation.[10]

Quercetin, a flavonoid isolated from the leaves of *L. speciosa* showed significant in vitro cytotoxicity against MCF-7 cell lines at 500µg/ml when compared to the crude extract.[11]

Antibacterial activity of ethanol and water extracts of leaves of *L. speciosa* were tested by plate agar diffusion method against Gram positive and Gram negative bacteria. The MIC of ethanol and water extracts of leaves against *Staphylococcus aureus*: 14 and 15, *Bacillus subsilis*: 12 and 15, *Pseudomonas aeruginosa*: 14 and 17, and *Escherichia coli*: 16 and 17 mm respectively. Water extracts being the most effective.[12]

Aqueous and 50 percent ethanolic extracts of the leaves and stems of *L. speciosa* were evaluated for anti-HIV activity using in vitro reporter gene-based assays. All the extracts showed a dose- dependent inhibition of HIV-1-infection in TZM-bl and CEM-GFP cell lines, with LC50 of 1-25 µg/ml.[13]

The in vitro antioxidant activity of *L. speciosa* leaves (ethyl acetate, ethanol, methanol and water extracts) was studied by examining their superoxide, hydroxyl ion scavenging and by measuring lipid peroxidation. The ethyl acetate and ethanol extracts possessed the greater antioxidant property than the methanol and water extracts.[14]

The antioxidant activity of the 95% ethanol and water extracts (0.625, 1.25, 2.5, 5, 10, and 20 µg/ml) of *L. speciosa* leaf was studied by DPPH free radical scavenging assay method. IC50 values were found to be 2.6 and 6.2 µg/ml for ethanol extracts and 4.3 and 9.2 µg /ml for aqueous extract of leaf and fruit samples, respectively.[15]

The ethanol extract of the dried fruits of *L. speciosa* was investigated for cytotoxic activity. The extract produced a prominent cytotoxic activity against brine shrimp *Artemia salina* with (LC_{50}= 60 µg/ml and LC_{90}.[16]

The anti-inflammatory activity of *L. speciosa* leaves ethyl acetate and ethanol extracts was examined using the carrageenan-induced acute inflammation and chronic formalin- induced paw edema models. The ethyl acetate extract reduced the paw edema significantly in a dose-dependent manner in both acute and chronic inflammation models, while, ethanol extract did not show dose-dependent activity.[14]

The analgesic actions of an aqueous ethanolic extract of *L. speciosa* was investigated using formalin-induced pain, acetic acid-induced writhing and thermal (hotplate and tail immersion) tests in rats, while, the carrageenan-induced oedema of the hind paw of rats was used to study the anti-inflammatory activities. The crude plant extract significantly increased the reaction time of hot plate and immersion tests. It decreased the writhing's of acetic acid- induced abdominal contractions and lickings of formalin-induced pain. The results also showed that the aqueous ethanolic extract possessed both central and peripheral effects; this was confirmed by its effect on both phases of formalin-induced pain. The extract also significantly decreased the rat paw oedema volume at 200 mg/kg and above.[17]

L. speciosa Ethanolic Flower Extract (LEFE)

Anti- Aging activity of Flowers Among several mechanisms of skin aging, the generation of ROS has long been known to be the primary cause.[18] ROS activates cytoplasmic signal transduction pathways in skin cells that are related to differentiation, senescence, extracellular matrix degradation and pigmentation, leading to wrinkles and dark spots.[19]

L. speciosa Ethanolic Seed Extract (LESE)

The ethanol extract of the dried fruits of *L. speciosa* also produced significant ($p<0.001$) writhing inhibition in acetic acid- induced writhing in mice at the oral dose of 250 and 500 mg/kg of bw, which was comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of bw.[20]

The ethanol extract of the dried fruits of *L. speciosa* also showed antidiarrheal activity on castor oil-induced diarrhoea in mice, it increased mean latent period and decreased the frequency of defecation significantly ($P<0.001$, $P<0.01$) at the oral dose of 500 mg/kg bw comparable to the standard drug lope amide at the dose of 50 mg/kg of bw.[20]

Side effects and safety

In acute toxicity study, no mortality or toxic reaction was recorded in rats after administration of ethanolic crude extract of *L. speciosa*.[21] The control group received normal saline (2 ml/kg body weight p.o.) while other groups received 500, 1000, 2000 and 3000 mg/kg of the test extract orally. Immediately after dosing, the animals were observed continuously for the first 4 h for any behavioural changes.[22-23] They were then kept under observation for 14 days after drug administration to find out the mortality. Observations were made twice daily, once at 7 a.m. and again at 7 p.m. There were no side effects in human, with the using of the recommended dosages (8-48 mg/day). However, higher doses associated with lowered blood glucose levels, headache, dizziness, and fatigue.[24]

RESULT

The phytochemical analysis of ethanolic leaf, flower

and seed extracts of *L. speciosa* are tabulated in Table 1.

Table 1: Phytochemical analysis of Ethanolic Leaf, Flower and Seed extracts of *L.speciosa.*

Phytoconstituents	Leaf extract	Flower extract	Seed extract
Alkaloids	++	+++	+++
Flavonoids	++	+++	+
Saponins	+++	+++	_
Phenols	+++	+++	_
Tannins	+++	+++	+
Protein 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

Table 2: Bioactive phytochemicals of Ethanolic Leaf, Flower and Seed extracts of *L.speciosa.*

Classification	Phytoconstituents	Biological Functions
Anti – Neuro toxin	Alkaloids	Neuropharmacological agents, anti- oxidants, cancer chemoprevention
Anticancer	Flavonoids	Wound healing, Anti-oxidant, Anti-inflammatory, Anti-microbial.
Anti – oxidant	Saponins	strong antioxidant
Anti – oxidant	Phenols	Oxygen free radicalquenching, inhibition of lipid peroxidation, Inflammatory, Healthy Aging.
Healing Activity	Tannins	Promote rapid healing and the formation of new tissues on wounds and inflamed mucosa.
Organic compounds	Protein and Amino acids	Building blocks of life
Carbohydrate or naturalsugar	Reducing sugar	-
Anti-inflammatory	Steroids	Inflammatory
Glycone	Glycosides	Treating heart failure, anti-obesity and anti-gout activity
Steroids	Phytosterols	Control blood cholesterol levels, cardiovascular disease, heart attack or stroke.
Pigments	Quinones	Biological pigments found in arange of living organisms
Anticoagulants	Coumarins	Oral

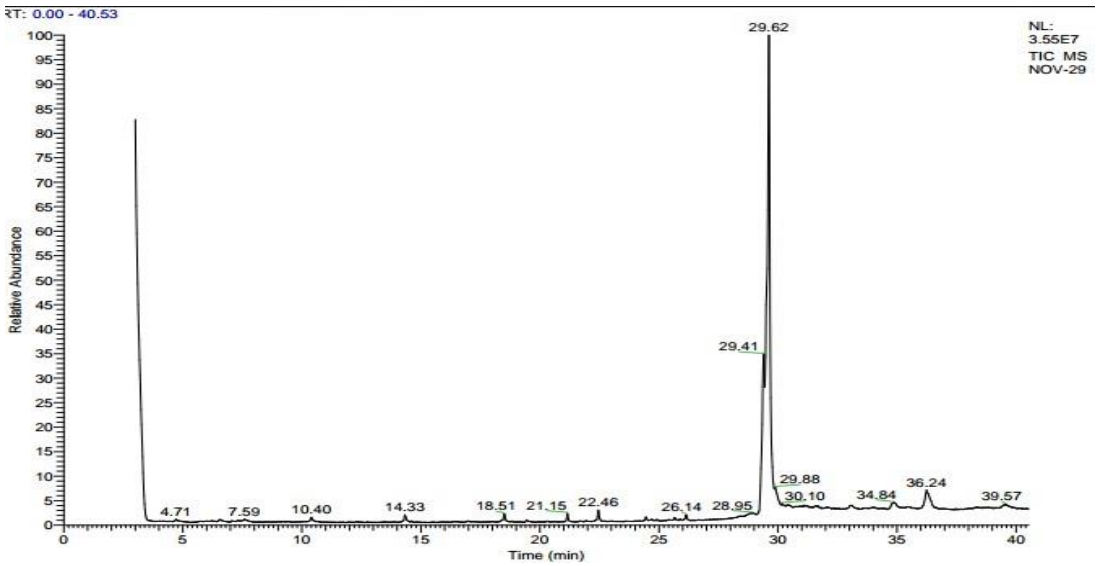

Figure 1: Shows GC-MS spectrum of Ethanolic Leaf extract of *L. speciosa.*

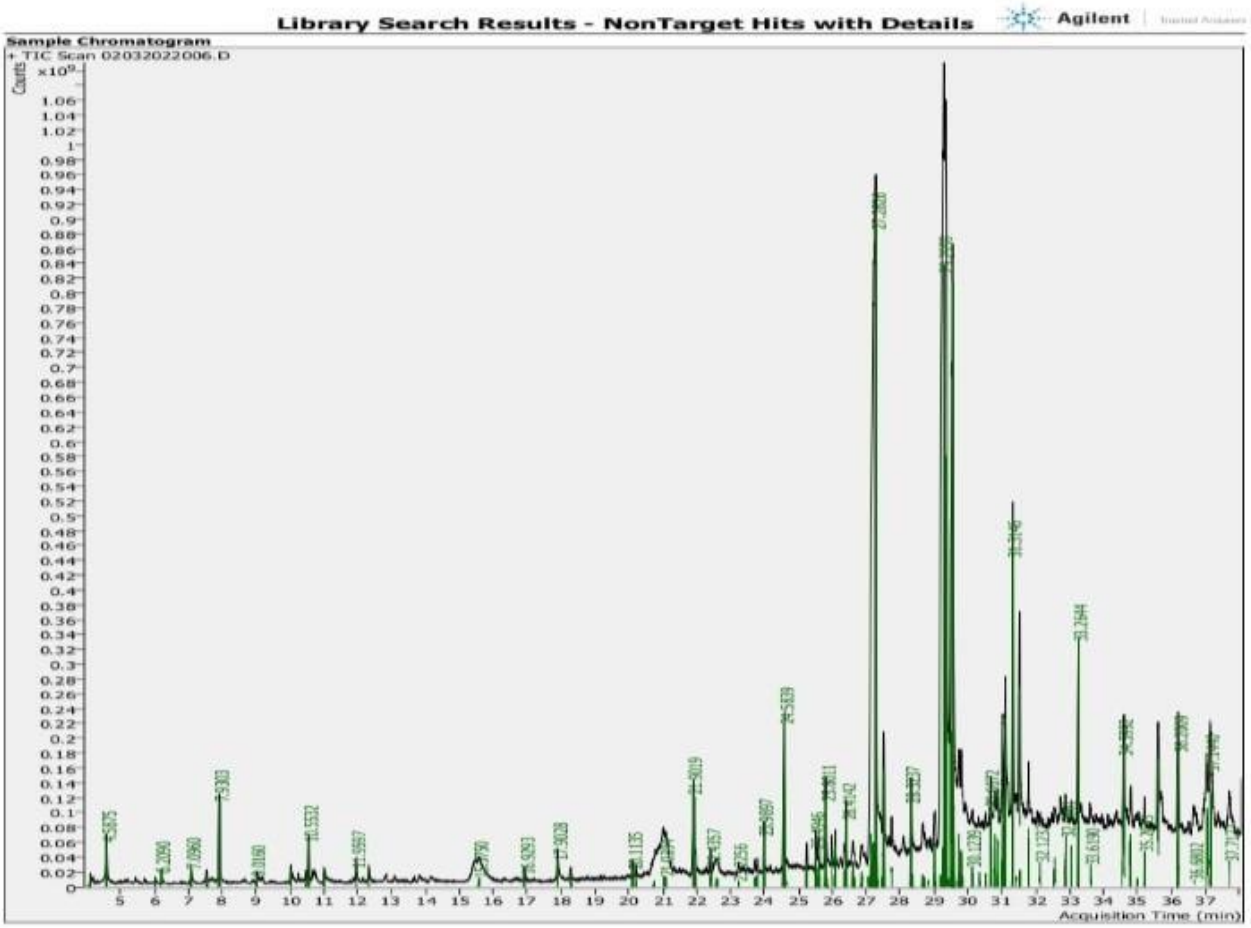

Figure 2: Shows GC-MS spectrum of Ethanolic Flower extract of *L. speciosa.*

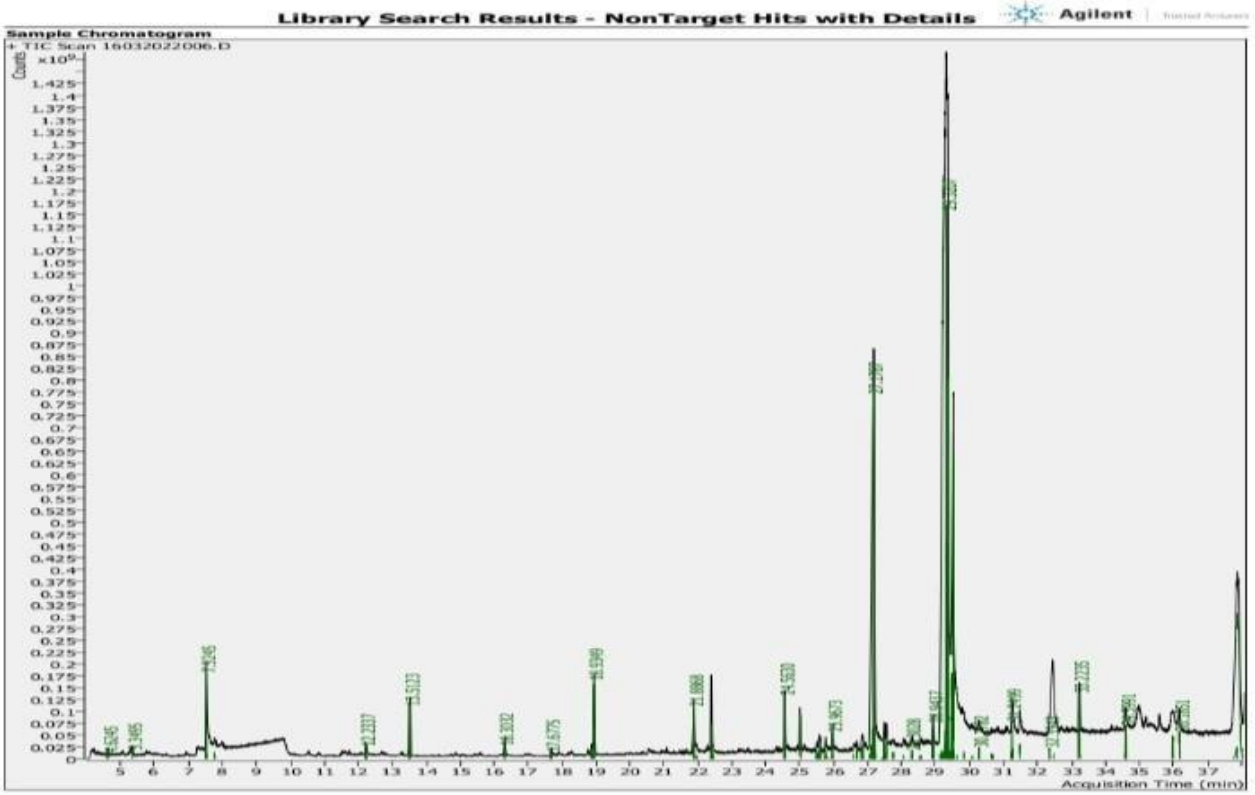

Figure 3: Shows GC-MS spectrum of Ethanolic Seed extracts of *L. speciosa.*

Table 3: Important compounds identified in GC-MS analysis of Ethanolic Leaf, Flowerand Seed extract of *L. speciosa.*

Extracts	Compounds	Molecularformula	Match factor
Ethanolic leaf extract	Cholesterol	C27H46O	84.29
	Cholesterol , 7- oxo-	C27H4420	3.42
	Lucenin- 2	C27H30O16	1.33
	Hexadecanoic acid ,ethyl ester (CAS)	C18H36O2	1.08
	Betulin	C30H48O3	0.84
Ethanolic flower extract	Furfural	C5H4O2	95.1
	Pentadecanal	C15H30O	95.0
	2-Cyclopenten-1-one, 2-hydroxy-	C5H6O2	92.7
	2,5-Furandicarboxaldehyde	C6H4O3	89.7
	3-Methylene-7,11-dimethyl-1-dodecene	C15H28	87.1
Ethanolic seed extract	Dodecanoic acid	C12H24O2	87.1
	Acetone	C3H6O	86.5
	Phytol	C20H40O	83.7
	Phytol	C20H40O	83.6
	Tetradecane	C14H30	83.4

Table 4: Major Pharmacological Activities of *L. speciosa.*

PharmacologicalActivities	Plantparts	Active Doses	References
Anti-inflammatoryactivity	Leaf	dose dependent (32 mgand 48 mg)	Nutan *et al.*, 2013
Anti-diabetic activity		1%	Judy *et al.*, 2003
Antibacterial activity		500µg/ml	Ambujakshi *et al.*, 2009
Anti-HIV activity		1-25 µg/ml	Priya *et al.*, 2008
Antioxidant activity		2.6 and 6.2 µg/ml	Myint *et al.*, 2017
Anti- Aging activity, Anti-Wrinkle activity	Flower	21.83%w/w	Masaki, 2010 & Pillai *et al.*, 2005
Antidiarrheal activity	Seed	500 mg/kg	Rahman *et al.*, 2010

DISCUSSION

Ethanol is used in medicine as a topical anti-infective agent. The choice of the solvent is very essential for being used in the extraction of various phytochemicals from plants. Most importantly, ethanol is both highly effective and perfectly safe to use for plant extraction. Ethanol retrieves a large number of biomolecules from the plant and then evaporates completely, thus minimizing the amount of product wasted. The use of this solvent in food grade and consumable goods is considered safe.

The phytochemical analysis of ethanolic leaf, flower and seed extracts of *L. speciosa* revealed that it contained steroids, terpenoids, glycosides, phenolic compounds, α-amino acids, saponins, starch, alkaloids, carbohydrates, organic acids, flavonoids, reducing sugars, tannins and many other active metabolites in Table 1. The bioactive molecules of phytochemicals show the biological functions of phytoconstituents in Table 2. Table 3, which indicate the presence of quantities of some important bio-molecules that were present in abundance in each of the selected plant parts. The Pharmacological effects of *L. speciosa* include anti-inflammatory, antimicrobial, antioxidant, anti-HIV, antidiabetic, anti-aging, anti-wrinkle, antidiarrheal activity reported by various scientists in Table 4.

CONCLUSION

Traditional medicine is mainly based on plants. Most of the crude drugs are the secondary metabolites of plants. The various parts of *L. speciosa* are the potential source of natural compounds, mainly steroids, terpenoids, glycosides, phenolic compounds, α-amino acids, saponins, starch, alkaloids, carbohydrates, organic acids, flavonoids, reducing sugars, tannins and many other active metabolites. In this review, we have summarized the phytochemical properties and pharmacological activities of *L. speciosa* for treatment of various diseases. Everyone has concluded that *L. speciosa* is an ornamental plant, but it is a very good medicinal plant that has biological activities including anti-inflammation, antimicrobial, antioxidant, anti-HIV, antidiabetic, anti- aging, anti-wrinkle, antidiarrheal activity. The present study provides enough scientific support for the pharmacological use of *L. speciosa* as a medicinal plant used for various diseases.

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