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Anti - Obesity Effects of *Lagerstroemia speciosa* Ethanolic Green and Red Leaf Extracts against Caprylic Acid and a High Fat Diet in the Albino Rat

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Abstract

Obesity, is a global health issue affecting 300 million people, is caused by excessive fat accumulation in specialized fat cells. *Lagerstroemia speciosa*, a native of Southeast Asia's Western and Eastern Ghats, produces abundant flowers with horticultural and therapeutic value. The study investigates the therapeutic value of *L. speciosa* ethanolic green and red leaf extracts against caprylic acid and a high-fat diet in Albino rats. The phytochemical profile reveals abundant phytoconstituents, including corosolic acid, lagerstroemin, and anthocyanin at high levels. The experimental animals were divided into seven groups with n=6 animals and fed a high-fat diet for 21 days, except the control group. The groups IV, V, VI, and VII are the experimental groups, orally fed with LELE Low Dose and High Dose (250 and 500 mg/kg body weight), and the II and III are the standard groups treated with HFD and Caprylic acid. The control group receives a normal diet and is evaluated for the estimation of biochemical parameters. A histopathological evaluation was also performed. The results show that the treated groups showed a significant (***P<0.001, **P<0.01, *P<0.05) elevation compared to the control group. The blood test reveals an increase in the number of blood cells and the Hg level. The antiobesity activity of LELE in HCD has considerably changed body weight and biochemical aspects. Administration of LELE significantly reduced body weight, total lipid profile, and blood glucose in a dose-dependent manner. Finally, the acute toxicity of LELE demonstrates that it is safe and non-toxic, while the treated group demonstrated dose dependence and decreased obesity. These effects could be due to the presence of phytoconstituents such as corosolic acid and lagerstroemin in the green leaf and the red pigment anthocyanin, present in the red leaf of *L. speciosa* an ethanolic leaf plant extract.

***Key words*:** Obesity, Hyperlipidemia, *Lagerstroemia speciosa* ethanolic leaf extract, *Lagerstroemia speciosa* ethanolic leaf extract, High fat diet

Obesity is global disease-causing hypertension and type 2 diabetes mellitus. It began in the 1990s due to processed foods high in fat and sugar, particularly fructose [1]. Obesity is exacerbated by reduced physical activity and sedentary behaviour [2]. Excessive fat accumulation in specialized fat cells leads to various illnesses, including diabetes, heart disease, osteoarthritis, and cancer with a rapidly increasing number [3]. High-fat diet lard, or saturated oil, added to diet takes 3 weeks to develop obesity. Most commonly used model HFD (high fat diet) contains 32.6% Protein, 33% Fat, 30% carbohydrate, normal chow, lard, casein, cholesterol, vitamins, minerals, yeast powder, methionine, and NaCl [4].

The induction with HFD treatment causes increased free fatty acids, LDL, cholesterol, and adipocyte differentiation. Dietary fat intake often has been claimed as responsible for the increase in adiposity. Human studies have shown that high-fat diets (30% of energy from fat) can easily induce obesity [5]. Epidemiological studies conducted in countries such as China, Canada, and the USA have shown that, when the average amount of fat in the diet increases, the incidence of obesity also increases [6].

This has led to a worldwide effort to decrease the amount of fat in the human diet. Diets rich in fat not only induce obesity in humans but also make animals obese [7]. In both rats [8] and mice [9] a positive relationship has been found between the level of fat in the diet and body weight, or fat gain. In the scientific literature, it was first shown that rats consuming diets containing high proportions of fat gained weight faster than those on diets containing minimal amounts of fat [10]. Animal studies have shown that various herbs, including fenugreek, cayenne pepper, ginger, oregano, and ginseng, aid in weight loss and fat reduction [11]. These herbs reduce appetite, increase metabolism, and alter fat synthesis while also stimulating weight loss and modifying fat formation [12].

Natural antioxidants gain popularity as complementary and alternative drugs due to less side effects compared to synthetic drugs in the cosmetic, pharmaceutical, and food industries [13].

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Lagerstroemia speciosa, a native to Southeast Asia, has medicinal and horticultural properties, with bioactive phytochemicals showing hypoglycemic, antibacterial, antiinflammatory, antioxidant, and hepatoprotective properties [14]. *Lagerstroemia speciosa* is popularly called as "Jarul" in West Bengal, India, and it belongs to the family Lythraceae. It is known as Pride of India, and is also called Queen's Flowers or Queen Crape Myrtle in English. This plant is widely distributed in the Southeast Asian countries, Philippine and India. The GC-MS analysis shows the presence of Phytocompounds [15].

Gymnemic acid (from *Gymnema sylvestre*), oleanolic acid (from *Panax ginseng*), and corosolic acid from *L. speciosa* have potential action on obesity [16]. The Corosolic acid was identified as an effective component of the Banaba extract responsible for the anti-obesity activity [17]. The objective of the study is to evaluate the anti-obesity potential of *L. speciosa* ethanolic green (LEGLE) and red leaf extract (LERLE) in Albino rats induced with a high fat diet (HFD).

MATERIALS AND METHODS

Collection and authentication of plant samples

The leaves of *L. speciosa* were collected from the PG 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.

Plant extracts preparation

L. speciosa leaves were collected, washed, and shade-dried for 2 weeks. The leaves were ground to powder (100g) and soaked in ethanol (1000ml). 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 [18].

Quality control analysis

Qualitative phytochemical analysis of the green and red leaves of *Lagerstroemia speciosa* Ethanolic extracts were carried out according to the methodology of [19], Trease and Evans [20]. 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 [21].

Determination of corosolic acid

It includes of plant material preparation. Extraction of corosolic acid, concentration and evaporation fractionation, identification of corosolic acid, data analysis, and reporting [22].

Determination of lagerstroemin

A naturally occurring substance, also known as corosolic acid, is present in several plants, most notably *Lagerstroemia speciosa*. Chromatography on a thin layer (TLC): TLC is a simple and reliable technique for qualitative analysis. Its presence in the sample can be determined by comparing the migration distance of the sample with that of a standard Lagerstroemin [23].

Determination of tannin

Various techniques can be used to determine the presence of tannins in a sample, depending on the kind of tannins and the goal of the analysis. Using high-performance liquid chromatography (HPLC), it is possible to separate and quantify the various tannin components in a sample. The identification and measurement of various tannin components are made possible by its great specificity and precision [24].

In order to synthesise 4,6-O-(S)-hexahydroxydiphenoyl gluconic acid (20 mg), fraction 1, which was previously obtained by Sephadex LH-20 Chromatography from the 70% aqueous acetone extract of the dried leaves was chromatographed on Sephadex-20 ethanol and then MCI-gel CHP 20P with water containing an increasing amount of methanol. Lagerstannin C (8) (40 mg) and 7 (100 mg) were synthesised by repeated chromatography of fraction 23a on MCI-gel CHP 20P, Cosmosil 75 C18-OPN, Toyopearl HW-40F, and Sephadex LH-20 with water and methanol [25].

Determination of anthocyanin

The vanillin-HCL method is specifically used to identify condensed tannins (proanthocyanins and anthocyanins). A complex that is pink to red in colour forms after the material reacts with the vanillin-HCL reagent [26]. An established standard curve with known tannin concentrations is used to calculate the complex's absorbance at a certain wavelength, and the tannin content is then calculated. The spectrophotometric approach is one of various techniques for determining anthocyanins, and it is frequently employed [27].

Feed composition

High fat diet (HFD) cocktail combines a normal diet with cholesterol, Cholic acid, Vanaspati ghee, and coconut oil for antihyperlipidemic properties [4].

Acute oral toxicity studies

Behavioural alterations observed in experimental groups after oral administration of ethanolic leaf extract; toxicological analysis confirmed mortality, trial continued [28].

Experimental groups

Animals were divided into three major groups: the control group fed with a normal diet, and the standard laboratory chow (STD) fed with HFD, and Caprylic acid with HFD and the experimental groups fed with a high-fat diet (HFD) along with leaf extract. The animals in the experimental group were fed an HFD containing 15% carbohydrate, 20% protein, and 75% fat for 3 weeks.

Experimental design

Female Albino Wistar rats aged 10-12 weeks weighing between 100 and 200 g were used in this study. They were raised in the animal house of the Department of Pharmacy, KMCH, Coimbatore, Tamil Nadu. All the animals were placed in polypropylene cages at room temperature with a 12/12 h cycle (light/dark). They had free access to water and standard diet.

Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. This study was approved by the Institutional Animal Ethics Committee of KMCH College of Pharmacy in Coimbatore, Tamil Nadu (Approval No: KMCRET/ReRc/Ph. D/24/2021). The animal protocol was accomplished in accordance with the guidelines of Institutional Animal Ethics Committee of KMCH College of Pharmacy and Use of Laboratory Animals Manual (8th Edition).

Forty-two (42) rats were randomly divided into 6 groups (n = 6) and treated orally daily for 21 days. Group I

(normal control): Rats received normal diet and 10 mL/kg of distilled water; Group II (obesity standard): Rats received HFD (high fat diet) and 10 mL/kg of distilled water; Group III (positive control): Rats received HFD and caprylic acid (1mL/100mg/kg); Groups IV, V, VI, and VII: Rats received HFD, and leaf extracts (250 and 500 mg/kg) respectively. Body weight, Blood sample, food and water consumption of the animals were evaluated on the 1st, 21st and 42nd days of the experiment.

Biochemical analysis

The rats were sacrificed, and their blood was collected for haematological studies. Blood was drawn from the medial cantus and placed in EDTA bottles for biochemical analysis. The serum was separated by centrifugation at 2500 rpm for 15 minutes at 37°C, and the total cholesterol, total glycerides, HDL, LDL, and VLDL levels were determined [29]. Antioxidant enzymes, viz., Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidise (GPx), Reduced glutathione (GSH), and Lipid peroxidation (LPO), were determined in all the liver tissues of all the tested rats [30].

Histological slides

Histological examinations of adipose tissues and kidney specimens were performed by the method described by Lillie and Fulman, [31]. The tissue samples were embedded in paraffin wax after being fixed with 10% formalin. The paraffin-embedded tissues were sliced into sequential sections that were 5 micrometres thick. Using an Olympus BX51 light microscope, the tissues were stained with hematoxylin and eosin. Evaluations were made of the epithelization, necrosis, and ulcerations.

Statistical analysis

Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett comparison was performed. (***P<0.001) control group was compared with std group-II. (***P<0.001, **P<0.01, *P<0.05) treated groups III, IV, V, VI, and VII was compared with Group I. GraphPad Prism 8.02 (California, United States of America) was the statistical software used for the analysis of biodata obtained.

Table 1 Effect of ethanolic green and red leaf extract of *Lagerstroemia speciosa* against HFD on body weight

Group	Control	Only HFD	HFD + Caprylic acid	HFD + LGL L. D	HFD + LGL H. D	HFD + LRL L. D	HFD + LRL H. D
1st day	159.8±7.391	148±17.07ns	159±8.266ns	145±5.209ns	135.8±8.723ns	117.5±4.801*	146.3±1.116ns
21st day	178.8±11.12	231.2±7.761***	229±9.869***	215.5±7.562*	204.5±6.937ns	212.2±5.753*	228.3±5.289***
42nd day	189.2±10.65	320.8±4.52***	213.8±11.51ns	190.8±4.82ns	188.7±9.443ns	190±8.339ns	191.3±7.566ns

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett comparison was performed. (***P<0.001) control group was compared with std group-III. (***P<0.001**P<0.01, *P<0.05) treated groups IV, V, VI and VII was compared With Group I.

RESULTS AND DISCUSSION

Result 1: *Analysis of quality control*

Phytochemical analysis involves the study of plant-derived compounds to identify and quantify various bioactive components (Table 5). The qualitative and quantitative analysis shows the presences of Corosolic acid, Lagerstroemin, Tannin, Anthocyanin.

Result 2: *Acute oral toxicity*

Acute toxicity testing with LS at a dose of 2000 mg/kg on rats revealed no toxicity, mortality, or morbidity, and there were also no noticeable alterations in behaviour or gait.

Result 3: *Body weight*

The initial and final body weights of the control group 159.8±7.391 and 189.2±10.65. The negative control shows 148±17.07 and 320.8±4.52 at the end of the experiment after 42 days. The total percentage of increased body weight is 52% in HFD (high fat diet), compared to control group.

Table 2 Lipid profile analysis (TC, TG and HDL) of *Lagerstroemia speciosa* ethanolic green and red leaf extract against HFD

	Initial lipid profile			Lipid profile after 21st days HFD			Lipid profile after 42nd days		
	TC	TG	HDL	TC	TG	HDL	TC	TG	HDL
Control	75.03±0.9597	91±0.7	29±1.3	176±8.45	140.3±10.65	11.4±0.524	250±73.4	260±40.4	34±1.15
Only HFD	77.57±3.749ns	87±9 ns	29±4ns	387±34.9**	224±17.24***	30.4±4.05**	715±57.3**	582±88.1ns	82±8.33***
HFD + Caprylic acid	77.43±2.461 ns	89±2.6 ns	19±0.57*	345±48.6*	177.3±12.13 ns	12.4±3.47 ns	357±75.4 ns	288±57.3 ns	48±4.16 ns
HFD + LEGLE L. D	82.83±7.879 ns	78±1.6 ns	25±1.5ns	287±21.3 ns	165.7±6.064 ns	13.3±3.02 ns	412±105 ns	438±76.3 ns	68±4.62**
HFD + LEGLE H. D	71.57±4.572 ns	81±4.7 ns	13±0.46***	238±28.9 ns	134±6.658 ns	11.6±2.03 ns	279±20.7 ns	216±23.9 ns	42.7±3.53 ns
HFD + LERLE L. D	74.7±4.661 ns	90±4.2 ns	13±0.78***	256±32.9 ns	141.3±10.09 ns	11.4±2.89 ns	473±107 ns	386±158 ns	74±8.72***
HFD + LERLE H. D	81.47±4.474 ns	83±5 ns	13±2.6***	290±45.8 ns	124±4.163 ns	8.37±1.27 ns	223±32.6 ns	212±24.6 ns	32±2.31 ns

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett comparison was performed. (***P<0.001) control group was compared with std group-III. (***P<0.001**P<0.01, *P<0.05) treated groups IV, V, VI and VII was compared With Group I

Result 4: *Biochemical analysis*

The ethanol extract of *Lagerstroemia speciosa* green and red leaves is provided, decreasing cholesterol levels in experimental animals given HFD (high fat diet). The results of these analyses of the level of LDL in the treatment giving 250 and 500 mg/1 ml/100 g BW ethanol extract of *Lagerstroemia speciosa* showed significant differences compared to the experimental animals given HFD (high fat diet).

The treatment of 250 and 500 mg/ 1ml/100 g BW ethanolic extract of *Lagerstroemia speciosa* extract showed significant differences in HDL (high-density lipoprotein), VLDL (very low-density lipoprotein), Triglycerides, and Lipid Lowering levels compared to the experimental animals given HFD (high fat diet). The results are shown in the (Table 2).

Result 5: *Antioxidant activity*

Continuous administration of leaf extracts significantly increased levels of nonantioxidant enzymes (GSH) and endogenous antioxidant enzymes (SOD, CAT, and GPx), prevented membrane damage by reducing lipid peroxidation, and prevented HFD (high fat diet) control.

Result 6: *Histological analysis*

Obese white adipose tissue (WAT) and Kidney showed increased adipocyte quantity due to hyperplasia, indicating stem cell differentiation. Young mice and rats undergo cell differentiation of tiny cells into triacylglycerol-filled cells [32]. The supplementation group shows at 87% showing comparable histology to the control group, suggesting suppression of fat cell hyperlipidaemia development.

Table 3 Important compounds identified in GC-MS analysis of ethanolic green and red leaf extract of *Lagerstroemia speciosa*

	Ethanolic green leaf extract of *L. speciosa*			Ethanolic red leaf extract of *L. speciosa*		
S. No.	Compound name	Molecular formula	Match factor	Compound name	Molecular formula	Match factor
1.	Cholesterol	$C_{27}H_{46}O$	84.29	1-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	85.4
2.	Cholesterol, 7- oxo-	$C_{27}H_{4420}$	3.42	Dodecanoic acid	$C_{12}H_{24}O_2$	83.9
3.	Lucenin- 2	$C_{27}H_{30}O_{16}$	1.33	Phytol	$C_{20}H_0O$	83.7
4.	Hexadecanoic acid, ethyl ester (CAS)	$C_{18}H_{36}O_2$	1.08	Tetradecanoic acid	$C_{14}H_{28}O_2$	82.2
5.	Betulin	$C_{30}H_{48}O_3$	0.84	Heptacosane	$C_{27}H_{56}$	82.2
6.	6-Octadecanoic acid	$C_{18}H_{34}O_2$	0.83	Docane	$C_{12}H_{26}$	80.2
7.	1-Hexadecanoic, acetate (CAS)	$C_{18}H_{36}O_2$	0.79	Sulfurous acid, 2-ethyl isohexyl ester	$C_{14}H_{30}O_3S$	78.8
8.	1-Octadecanol (CAS)	$C_{18}H_{380}$	0.73	2- Undecanone	$C_{11}H_{22}O$	74. 9
9.	Hexadecanoic acid, methyl ester (CAS)	$C_{17}H_{34}O_2$	0.72	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	73.7
10.	Lucenin-2	$C_{27}H_{30}O_{16}$	0.58	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$	73.5
11.	Octadecanoic acid, ethyl ester (CAS)	$C_{20}H_{40}O_2$	0.56	Cyclobutanecarboxylic acid, 2-dimethylaminoethylester	$C_9H_{17}NO_2$	72
12.	9,12,15, - Octadecatrienoic acid	$C_{27}H_{52}O_4Si_2$	0.48	1- Hexyl- 2- nitrocyclohexane	$C_{12}H_{23}NO_2$	65.6
13.	1-Hexadecanol	$C_{16}H_{340}$	0.46	5,9,13-Pentadecatrien -2-one, 6,10,14-trimethyl-, (E, E)-	$C_{18}H_0O$	62.1
14.	Cyclobutane	$C_{26}H_{40}O_4$	0.21	Heptacosane	$C_{27}H_{56}$	60.2

Obesity is a global issue causing health issues like diabetes, heart disease, and malignancies. Unbalances in lipogenesis and lipolysis disrupt metabolic pathways, potentially causing adipose tissue toxicity [33]. Obesity treatments include metabolic promoters, gene product inhibitors, digestion blockers, central appetite suppressants, and other medications [34]. Traditional medicinal herbs and their active phytoconstituents have shown anti-obesity benefits in rats, reducing body weight and cholesterol levels. *Lagerstroemia speciosa* leaf extracts contain sugars, tannins, saponins, flavonoids, alkaloids, quinones, glycosides, triterpenoids, coumarins, and steroids, with antilipidemic substances like corosolic acid and anthocyanin [35]. The experimental animal shows the great impact on the body weight gain during HFD (high fat diet) treatment. For Group II mice (320.8±4.52***), from the second week after providing HFD (high fat diet), the body weight increased to a significant higher level than control. Body mass index (BMI) is the most commonly used measure to evaluate the degree of obesity. Obesity is a metabolic disorder characterized by an excess accumulation of fat in the body due to energy intake exceeding energy expenditure [36]. From the day one to the end of the experiment, HFD (high fat diet) treated mice (64.8%) are heavier than control.

The anti-oxidative role of herbal plants in different kinds of human diseases, such as diabetes mellitus, obesity, and hyperlipidemia, has already been reported in the literature [35]. Phenolic chemicals play a crucial role in managing obesity, with plant-based meals containing phenolic compounds like quercetin reducing body weight. Decursin in Angelica gigas improves glucose tolerance and decreases adipocytokines released by HFD (high fat diet) [37]. Sitosterol in *Boerhaavia diffusa* may lower cholesterol by raising LDL cholesterol. *Citrus aurantium* psynephrine enhances metabolic rate, energy expenditure, and weight loss [38].

Flavonoids inhibit pancreatic lipase activity and adipocyte growth in *Nelumbo nucifera*. Anti-obesity activity of catechins resulted from the combined actions of appetite reduction, greater lipolytic activity, energy expenditure and adipocyte differentiation [39].

The Group III, no difference revealed for the body weight between control and obesity model (Table 1). Hence in our present experiment conditions, HFD treated IV, V, VI and VII groups are failed to gain much higher body weight than control. The experimental plant *L. speciosa* ethanolic leaf extract contains corosolic, lagerstroemin, tannins and anthocyanin. Corosolic acid (CRA), a constituent of Banaba leaves, has been reported to exert antihypertension, anti-hyperinsulinemia, anti-hyperglycemia, and anti-hyperlipidemia effects.

The experimental Group IV and V shows the antilipidemic activity at moderate level. The green leaves of *Lagerstroemia speciosa* constitute abundant amount of Corosolic acid [40]. The (Table 2) shows the moderate reduced amount of TC (Total count), TG (Triglycerides), HDL (High-density lipoprotein) in higher dose of LEGLE. The anthocyanin

are condensed tannins present in the red leaf of *Lagerstroemia speciosa* is responsible for higher rate of anti-obesity effect in the experimental group VI and VII. Natural sources have been actively researched to develop effective medicines for obesity treatment. Corosolic acid, Lagerstroemin, tannin, and anthocyanin are found in *Lagerstroemia speciosa*, an ornamental plant with potential for medicinal use.

CONCLUSION

Traditional medicinal plants have long been a rich source for the discovery and development of new pharmaceuticals. In particular, the phytochemical analysis of *Lagerstroemia speciosa* has revealed the presence of various bioactive compounds, including terpenoids, flavonoids, tannins, alkaloids, sterols, cardiac glycosides, and saponins. Research indicates that the phenolic compounds present in *Lagerstroemia speciosa* exhibit lipase inhibitory properties, which are significant because lipase is an enzyme involved in the digestion and absorption of fats. By inhibiting this enzyme, these compounds can potentially reduce fat absorption, making *Lagerstroemia speciosa* a promising candidate for the development of treatments for obesity. The therapeutic potential of *Lagerstroemia speciosa* for obesity is supported by its ability to interfere with the breakdown and absorption of dietary fats, thereby possibly aiding in weight management and the reduction of body fat accumulation. The various bioactive compounds found in this plant may also contribute to other health benefits, making it a valuable subject for further research in medicinal plant studies.

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