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THE ANTI-AGEING POTENTIAL OF PHYTOCONSTITUENTS IN *LAGERSTROEMIA SPECIOSA*: RESVERATROL AND COROSOLIC ACID

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ABSTRACT

Background: Ageing is a multifactorial biological process characterized by cellular senescence, oxidative stress, and loss of regenerative capacity. Natural polyphenols such as resveratrol and corosolic acid from *Lagerstroemia speciosa* have shown promising anti-ageing effects through antioxidant and senolytic mechanisms.

Objective: To evaluate the anti-ageing potential and senolytic activity of resveratrol and corosolic acid using human fibroblast cell lines.

Methods: Human fibroblast cells were cultured and induced to senescence using hydrogen peroxide (H_2O_2). Cells were treated with resveratrol (10–100 µM), corosolic acid (10–100 µM), and *L. speciosa* ethanolic leaf extract (10–500 µg/mL). Antioxidant enzyme activities (SOD, CAT), lipid peroxidation (LPO), and protein content were quantified. All experiments were performed in triplicate, and statistical analysis was conducted using one-way ANOVA with Dunnett's post hoc test (GraphPad Prism v20.0).

Results: Resveratrol and corosolic acid significantly enhanced SOD ($p < 0.001$) and CAT ($p < 0.001$) activity, while *L. speciosa* extract showed moderate but significant SOD improvement at higher doses ($p < 0.05$). LPO levels were moderately reduced across treated groups, suggesting attenuation of oxidative stress.

Conclusion: Both resveratrol and corosolic acid exerted potent anti-ageing and antioxidant effects, while *L. speciosa* extract showed moderate senolytic activity. Further mechanistic studies are warranted to explore the molecular pathways involved in SASP modulation and mitochondrial protection.

Keywords: Senolytic cells, *Lagerstroemia speciosa,* Polyphenols, Resveratrol and Corosolic acid.

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1. INTRODUCTION:

Ageing is a complex, multifaceted process marked by progressive cellular damage, a decline in regenerative capacity, and the accumulation of senescent cells, which can lead to various age-associated diseases [1]. Senescence, a state of irreversible growth arrest in response to stress or damage, has evolved as a protective mechanism against cancer; however, the persistence of senescent cells contributes significantly to chronic inflammation and tissue dysfunction. The accumulation of these cells over time results in a detrimental phenomenon often called "inflammation," which exacerbates ageing and the progression of degenerative diseases, including cardiovascular disease, diabetes, and neurodegenerative disorders. Senescent cells secrete a pro-inflammatory mix of cytokines, chemokines, and growth factors collectively referred to as the senescence-associated secretory phenotype (SASP), which disrupts the function of surrounding healthy cells and propagates age-related decline [2].

Research into natural compounds for promoting cellular health and mitigating ageing-related damage has gained momentum, particularly with polyphenols is a diverse group of naturally occurring antioxidants found in plants. Polyphenolic compounds, such as resveratrol and corosolic acid, have shown potential in counteracting cellular ageing processes due to their robust antioxidant and anti-inflammatory properties. Resveratrol, a polyphenol found in grapes and berries, has demonstrated protective effects against oxidative stress, DNA damage, and inflammation, making it one of the most studied compounds for lifespan extension [3]. Corosolic acid, derived from *Lagerstroemia speciosa* (Banaba), is another polyphenolic compound of interest due to its beneficial impact on metabolic health and glucose regulation. This compound also exhibits potent antioxidant and anti-inflammatory actions, which have been shown to contribute to reduced cellular senescence and improved cellular resilience [4].

Metformin is an oral medication primarily prescribed to manage Type 2 diabetes by improving insulin sensitivity and lowering blood sugar levels. Originating from the plant *Galega officinalis*, metformin has gained attention beyond its traditional use due to its potential anti-ageing properties [5]. Research suggests that metformin may activate pathways associated with increased lifespan, such as AMP-activated protein kinase (AMPK), which plays a key role in cellular energy homeostasis and stress response. Metformin's anti-ageing benefits are believed to stem from its ability to reduce oxidative stress, enhance autophagy, and improve metabolic functions [5].

Emerging evidence suggests that targeting senescent cells with senolytic compounds that selectively induce apoptosis in these damaged cells may alleviate age-related inflammation and enhance tissue function, promoting healthier ageing. Unlike traditional antioxidants, which broadly reduce oxidative damage, senolytics specifically target the elimination of senescent cells, thus addressing one of the root causes of ageing. Polyphenolic compounds, including resveratrol and corosolic acid, have demonstrated senolytic properties in vitro and in animal studies, suggesting their potential utility in enhancing cellular health and longevity [6].

This study investigates the senolytic properties of *Lagerstroemia speciosa* extracts, particularly focusing on polyphenols like corosolic acid, as well as other plant-based compounds with known antioxidant and anti-inflammatory effects. The research aims to assess these compounds' ability to reduce cellular senescence, enhance cell health, and suppress the senescence-associated secretory phenotype (SASP). By exploring the molecular mechanisms involved, the study seeks to contribute to the development of plant-based, anti-ageing therapeutics. Emphasizing cellular resilience, anti-inflammatory actions, and senescent cell clearance, the research aims to support safe, natural strategies for promoting healthy ageing and combating age-related diseases. The study incorporated in vitro experimental approaches to investigate the anti-ageing effects of *L. speciosa* extracts and related plant-based compounds.

1.1 *In Vitro* Studies: The in vitro experiments involved culturing human or animal cell lines known to exhibit ageing characteristics. These cells were treated with various concentrations of polyphenol-rich extracts, including corosolic acid, to assess their impact on cellular senescence markers, cell viability, and the suppression of the SASP. Techniques such as cell proliferation assays, senescence-associated β-galactosidase staining, and analysis of reactive oxygen species (ROS) levels were used.

2. MATERIALS AND METHODS:

2.1 Plant Profile: *L. speciosa*, commonly known as the Queen's Crape Myrtle or Pride of India, is a deciduous tree native to tropical and subtropical regions of Southeast Asia. It belongs to the family Lythraceae and is admired for its vibrant, showy flowers that bloom in shades of pink, purple, and white. The plant thrives in warm climates and is often cultivated for ornamental purposes due to its appealing aesthetic [7]. Beyond its decorative appeal, *L. speciosa* is valued for its significant medicinal properties. Its leaves contain bioactive compounds such as corosolic acid, flavonoids, and

ellagic acid, which exhibit potent antioxidant, anti-inflammatory, and anti-diabetic effects [8]. Traditionally used in herbal medicine, extracts from the leaves have been employed to manage conditions like diabetes, metabolic syndrome, and age-related disorders. Research has highlighted the potential anti-ageing properties of the plant, attributed to its ability to modulate oxidative stress and support cellular health, all while demonstrating a favourable safety profile.

2.2 Collection and Authentication: 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/51 were placed in the Department of Zoology, Government Arts College (Autonomous), Coimbatore.

2.3 Extraction Process: The green leaves of *L. speciosa* were collected, washed, and shade-dried for 2 weeks. The leaves were ground into a fine powder (100 g) and soaked in ethanol (1000 ml). The extract is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The crude drug was strained, pressed, clarified, and filtered before being dried at room temperature [9].

2.4 Extraction of Phytochemicals:

i. **Solvent Extraction**: Use a hydroethanolic solution (70-80% ethanol) for extraction, as both resveratrol and corosolic acid dissolve effectively in ethanol. The plant powder is soaked in ethanol (e.g., 1:10, w/v) and kept for 24-48 hours.
ii. **Sonication/Heat Reflux**: Enhance extraction by either sonication or heating. Sonication with ultrasound waves improves solvent penetration, while reflux extraction (e.g., Soxhlet) maximizes yield.
iii. **Storage**: Once isolated, resveratrol and corosolic acid are sensitive to light and oxygen, so storing them under low temperatures and in inert conditions (e.g., nitrogen) is advisable.
iv. **Quality Control Analysis:** The ethanolic leaf extracts of *L. speciosa* were subjected to a qualitative phytochemical analysis using methods described by Horborne [10] and Trease and Evans [11]. 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 Thermo GC-Trace Ultra ver. 5.0 and Thermo MS DSQ 11 chromatography [12]. TLC was performed to analyse the variation in bioactive chemical constituents.

2.5 *In-vitro* study: The experimental design included treatment groups exposed to varying concentrations of *Lagerstroemia speciosa* ethanol leaf extract to assess dose-dependent effects on cellular ageing markers. A control group received only vehicle treatment to establish baseline conditions. To enable meaningful comparisons, metformin was used as a negative standard, providing insight into baseline cellular responses to a common anti-ageing agent. Resveratrol and corosolic acid were included as positive standards due to their known senolytic and anti-ageing properties.

Cells were cultured and then divided into specific treatment groups. The low and high dose groups were treated with different concentrations of the *Lagerstroemia speciosa* ethanol leaf extract. The metformin-treated group helped establish baseline anti-ageing effects, while the resveratrol and corosolic acid groups served as benchmarks for evaluating the efficacy of the plant extract. This design allowed for the assessment of *Lagerstroemia speciosa*'s ability to mitigate cellular senescence and promote cell health, compared with established anti-ageing agents.

The in-vitro study evaluated the oxidative stress biomarkers such as total protein [13], superoxide dismutase (SOD) [14], catalase (CAT) [15], and lipid peroxidation (LPO) [16].

2.6 Application of Doses to Cell Lines:

1. Preparation of Stock Solution: Corosolic acid is first dissolved in a small amount of ethanol to create a concentrated stock solution. This stock solution should be sterile-filtered and stored at the appropriate conditions (e.g., -20°C) until use.

2. Dilution for Cell Treatment: The stock solution is diluted with culture media to obtain final working concentrations. Common effective concentrations for corosolic acid in cell line studies typically range from 1 to 100 µM, depending on the desired biological effect and the cell type [5].

3. Resveratrol has been widely studied in concentrations ranging from 1 to 100 µM in various cell lines to evaluate its anti-aging and antioxidant effects [5, 17].

4. Metformin is commonly used in vitro at doses between 0.1 mM to 10 mM in studies investigating its impact on cellular metabolism and aging [5].

5. Plant extracts, including polyphenol-rich ones like *Lagerstroemia speciosa*, are used in in vitro models at concentrations determined by dose-response studies or previous literature, often between 10–500 µg/mL, depending on the extract's potency and the cell type.

2.7 Experimental Groups:
1. Control Group: Cells treated with ethanol in culture media.
2. Negative Standard: Cells treated with metformin (0.1–10 mM).
3. Positive Standard (Resveratrol): Cells treated with resveratrol (1–100 µM).
4. Positive Standard (Corosolic Acid): Cells treated with corosolic acid (1–100 µM).
5. Experimental Group 1: Cells treated with *Lagerstroemia speciosa* extract (10–50 µg/mL).
6. Experimental Group 2: Cells treated with *Lagerstroemia speciosa* extract (100–500 µg/mL).

2.8 Cell Line : Human dermal fibroblast adult cell line (HDFa; ATCC® PCS-201-012™) was procured from the National Centre for Cell Science (NCCS), Pune, India. Cells were cultured in DMEM supplemented with 10% foetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 °C in a 5% CO_2 incubator.

2.9 Replicates and Statistical Analysis: All experiments were performed in triplicate (n = 3). Data were expressed as mean ± SEM. Statistical comparisons were made using one-way ANOVA followed by Dunnett's test (GraphPad Prism 20.0). Significance was set at $p < 0.05$.

RESULTS:

3.1 Phytochemical Analysis: *Lagerstroemia speciosa* ethanolic leaf extract (LELE) is rich in various bioactive phytochemicals, including alkaloids, flavonoids, saponins, phenols, tannins, proteins, amino acids, reducing sugars, steroids, glycosides, phytosterols, coumarins, and quinones (Table 1). Notably, LELE contains a significant presence of corosolic acid and quinones. GC-MS analysis of LELE highlights the predominance of plant sterols at a high rate (84.29%). (Figure 1 and Table 1). The plant's main contributions to antioxidant and anti-ageing properties are attributed more to corosolic acid and other polyphenolic compounds.

Table 1: Phytochemical analysis of ethanolic leaf extracts of *Lagerstroemia speciosa*

S. No	Phytoconstituents	LELE
1	Carbohydrates	+
2	Tannins	+
3	Saponins	+
4	Flavonoids	+
5	Alkaloids	+
6	Quinones	+
7	Glycosides	+
8	Terpenoids	+
9	Triterpenoids	+
10	Phenols	+
11	Coumarins	+
12	Steroids	+

'+' indicates the presence of Phytoconstituents

'-' indicates the absence of Phytoconstituents

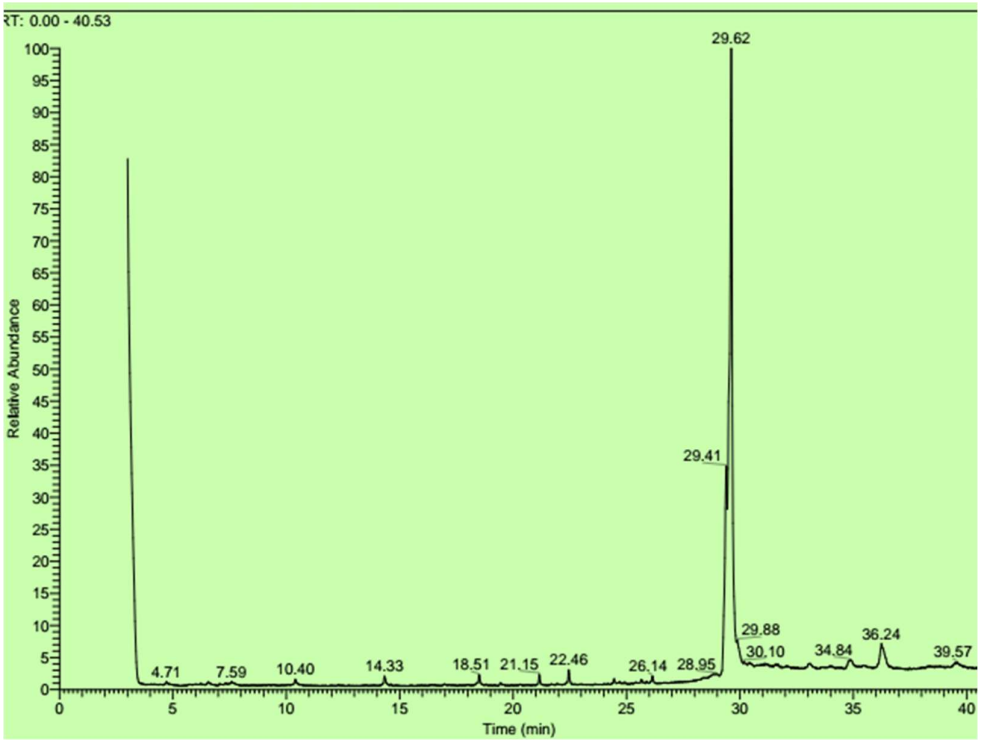

Figure 1: Shows GC-MS spectrum of ethanolic leaf extract of *Lagerstroemia speciosa* [18]

3.2 Oxidative stress and Antioxidant markers: Oxidative stress and antioxidant markers are crucial indicators of cellular ageing. Antioxidant enzyme activity, including superoxide dismutase (SOD), catalase, and glutathione peroxidase, is assessed through calorimetric or fluorometric assays to evaluate the cell's defence mechanisms. Additionally, lipid peroxidation, marked by products like malondialdehyde (MDA), is measured using the TBARS assay, as it rises with oxidative stress and ageing. Together, these markers provide insights into cellular oxidative damage and the effectiveness of antioxidant defences. Compared with control, resveratrol increased total protein levels by approximately 163%, SOD activity by 165%, and CAT activity by 187% ($p < 0.001$). Corosolic acid enhanced SOD and CAT activities by about 65% and 50%, respectively, though changes in LPO were not significant. LELE at high dose (LELE H.D) produced moderate increases in SOD (≈ 32%) but showed limited effects on CAT and total protein levels. These results confirm the ranking of antioxidant potency as RV > CA > LELE.

Table 2: Antioxidant activity of resveratrol, corosolic acid, and *Lagerstroemia speciosa* ethanolic leaf extract in *In-vitro* anti-ageing assays

Groups	Control	Std	RV	CA	LELE L.D	LELE H.D
Total Protein (mg/dl)	0.327±0.03	0.713±0.011***	0.861±0.05***	0.386±0.011^{ns}	0.322±0.05^{ns}	0.494±0.14^{ns}
SOD (Unit/min/ mg protein)	0.178±0.06	0.455±0.027***	0.471±0.01***	0.358±0.042^{ns}	0.318±0.03*	0.392±0.019*
CAT (µmol of H_2O_2 consumed/ min/ mg protein)	0.214±0.027	0.812±0.06***	0.616±0.03***	0.431±0.016^{ns}	0.512±0.05^{ns}	0.492±0.018^{ns}
LPO (nmol of MDA/ mg protein)	0.125±0.005	0.369±0.012^{ns}	0.352±0.15^{ns}	0.332±0.061^{ns}	0.286±0.12^{ns}	0.431±0.013^{ns}

All the values are expressed as mean ± SEM. ***: Highly significant ($P< 0.001$), *: Significant ($P< 0.05$), ns: Not significant

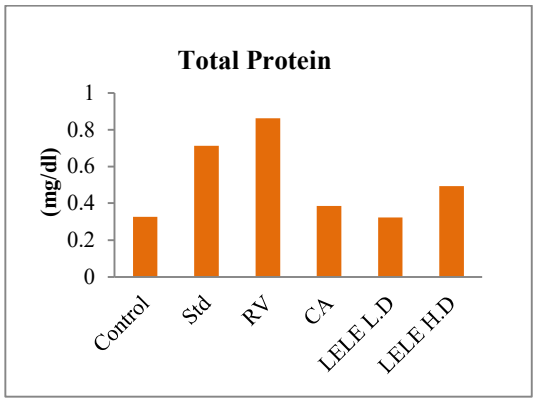

Figure 2: Total Protein content in fibroblast cells treated with standard drug, resveratrol (RV), corosolic acid (CA), and *Lagerstroemia speciosa* ethanolic leaf extract (LELE). Values represent mean ± SEM (n = 3). Statistical analysis was performed using one-way ANOVA followed by Dunnett's test. ***p < 0.001, **p < 0.01, *p < 0.05 compared with control group.*

All experiments were conducted in triplicate, and data are expressed as mean ± standard error of the mean (SEM). Statistical differences between groups were analysed using one-way ANOVA followed by Dunnett's multiple comparison test ($p < 0.05$).

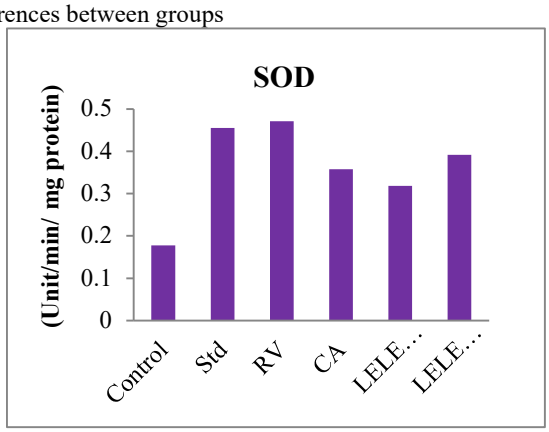

Figure 3: Superoxide dismutase (SOD) activity showing significant elevation in RV > CA > LELE groups. Values represent mean ± SEM (n = 3). ***p < 0.001, **p < 0.01, *p < 0.05 vs control.*

All experiments were conducted in triplicate, and data are expressed as mean ± standard error of the mean (SEM). Statistical differences between groups were analysed using one-way ANOVA followed by Dunnett's multiple comparison test ($p < 0.05$).

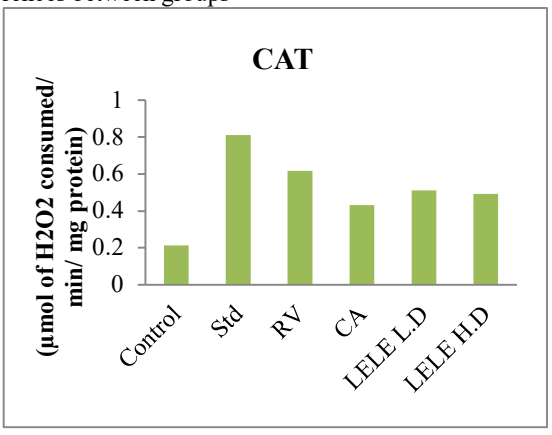

Figure 4: Catalase (CAT) Activity showing significant elevation in RV > CA > LELE groups. Values represent mean ± SEM (n = 3). ***p < 0.001, **p < 0.01, *p < 0.05 vs control.*

All experiments were conducted in triplicate, and data are expressed as mean ± standard error of the mean (SEM). Statistical differences between groups were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test ($p < 0.05$).

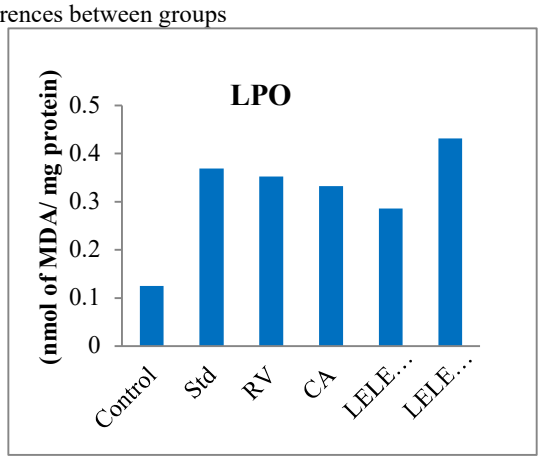

Figure 5: Lipid Peroxidation (LPO) Activity showing significant elevation in RV > CA > LELE groups. Values represent mean ± SEM (n = 3). ***p < 0.001, **p < 0.01, *p < 0.05 vs control.*

All experiments were conducted in triplicate, and data are expressed as mean ± standard error of the mean (SEM). Statistical differences between groups were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test ($p < 0.05$).

3. DISCUSSION:

The in-vitro study evaluated the antioxidant activity of resveratrol (RV), corosolic acid (CA), and *Lagerstroemia speciosa* ethanolic leaf extract (LELE) at both low (L.D) and high doses (H.D). The findings for total protein, superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation (LPO) are summarized in Table 2.

Total Protein Content (mg/dl): The standard group and the RV group demonstrated significantly higher total protein levels (0.713±0.011*** and 0.861±0.05***, respectively) compared to the control (0.327±0.03), indicating notable protein synthesis and cellular health (Figure 2 and Table 2). In contrast, CA and both LELE groups (L.D. and H.D.) did not show significant differences compared to the control, suggesting that these treatments did not substantially enhance total protein production.

SOD Activity (Unit/min/mg protein): The standard and RV groups exhibited significant improvements in SOD activity (0.455±0.027*** and 0.471±0.01***, respectively), highlighting their potent antioxidant properties (Figure 3 and Table 2). The LELE H.D. group also showed moderate activity (0.392±0.019*), indicating its potential as an antioxidant. However, CA and LELE L.D. showed no significant changes.

CAT Activity (µmol of H_2O_2 consumed/min/mg protein): Both the standard (0.812±0.06***) and RV (0.616±0.03***) groups demonstrated substantial increases in catalase activity compared to the control (0.214±0.027) (Figure 4 and Table 2). The LELE groups, however, did not exhibit significant differences, suggesting that the extracts may have a limited impact on catalase activity under these conditions.

LPO Levels (nmol of MDA/mg protein): LPO measurements indicated that none of the treatment groups showed significant changes compared to the control. This result implies that while RV and the standard group had potent antioxidant activity in other metrics, they did not significantly affect lipid peroxidation levels. The LELE H.D group's slight increase (0.431 ± 0.013^{ns}) suggests that higher doses might not effectively reduce lipid peroxidation (Figure 5 and Table 2).

4. CONCLUSION:

The in vitro study highlights that resveratrol and the standard treatment demonstrated significant antioxidant activity, reflected in enhanced total protein content, SOD, and CAT activity. LELE, particularly at high doses, showed moderate antioxidant potential, notably in SOD activity, but did not significantly affect total protein, CAT, or LPO levels. These results indicate that while *Lagerstroemia speciosa* ethanolic leaf extract possesses antioxidant and potential anti-ageing properties, its efficacy may not be as robust as resveratrol under the conditions tested. Importantly, LELE showed these effects without evident side effects, emphasizing its safety as a natural treatment. These findings suggest a potency gradient of RV > CA > LELE, reflecting differences in molecular stability and bioavailability among the tested polyphenolic compounds. Future studies could

explore optimizing extract concentrations or combining it with other antioxidants to enhance its anti-ageing activity.

Declaration of Competing Interest: The authors declare no conflict of interest with respect to the research, authorship, and/or publication of this article.

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