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Unveiling the Therapeutic Manifold of *Lagerstroemia Speciosa* (L.) Pers.: Insights on In-Vitro Antioxidant, Anti-Inflammatory, And Anticholinesterase Activities

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KEYWORDS

Lagerstroemia speciosa, phytochemicals, anti-oxidant, anti-inflammatory, anti-cholinesterase, percentage inhibition

ABSTRACT:

Introduction: A wide range of medicinal pharmacological properties against acute and chronic diseases have been demonstrated by numerous medicinal plant bioactive extracts and their identified active constituents.

Objectives: This study assesses the antioxidant, anti-inflammatory, and anti-cholinesterase properties of successive extracts derived from *Lagerstroemia speciosa* at varying concentrations in order to determine their potential medical utility.

Methods: For extraction, the Soxhlet extraction technique was utilised. Moreover, free radical scavenging, hemolytic inhibitory, protein denaturation, and cholinesterase inhibition assays were investigated to measure the pharmacological activities.

Results: The phytochemical levels in the methanol extract were higher than those in the other extracts. All the extracts showed percentage inhibition dose-dependently. Comparatively, methanol extract possessed better anti-oxidant and anti-inflammatory properties. Subsequently, at concentrations (6.25-200 µg/mL), methanol extract dose-dependently inhibited acetylcholinesterase (AChE), and butrylcholinesterase (BuChE). The IC_{50} values were determined to be 36.02 ± 0.62, and 64.58 ± 1.48 µg/mL ($p < 0.001$), respectively.

Conclusions: Thus, methanol extract should be further evaluated in search of other phytochemicals, and the *in-vitro* studies should be validated through *in-vivo* animal models.

1. Introduction

According to estimates from the World Health Organisation (WHO), approximately 80% of people worldwide receive their primary medical care through traditional medicine, with most of these treatments involving the use of herbal extracts and their active ingredients [1]. Numerous medicinal plant bioactive extracts and their identified active constituents have demonstrated a range of medicinal pharmacological properties against various acute and chronic diseases [2, 3]. At present, the effects of oxidative stress and its

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contributing factors have emerged as a significant concern for human health [1]. Stress increases the body's ability to produce reactive oxygen species (ROS) [4]. The inability of endogenous enzymatic and nonenzymatic antioxidant substances to manage the excess of ROS results in process imbalances, cell damage, and health issues [5]. A daily diet deficient in antioxidant compounds can cause several inflammatory illnesses, cancers, cardiovascular conditions, and Alzheimer's disease [5, 6, 7]. Flavonoids and phenolic content have been shown in numerous studies to contribute to the antioxidant properties of natural compounds [8]. Antioxidants based on natural compounds are one of the more effective therapeutic agents to lessen the illnesses brought on by oxidative stress because they prevent the production of free radicals [1]. The flavonoids and phenolic compounds have anti-inflammatory properties in addition to their antioxidant properties [8]. Anti-inflammatory studies have demonstrated that bioactive extracts and their natural compounds work biologically by inhibiting two key signalling pathways, including NF-κB and mitogen-activated protein kinases (MAPKs), which are responsible for the majority of proinflammatory mediator production [1].

One of the main causes of ageing is known to be oxidative stress [9]. This is regarded as a primary pathogenesis cause of Alzheimer's disease (AD) since it is linked to Aβ plaque deposition, which results in neuronal oxidative stress in AD patients [10]. According to earlier research, reactive oxygen species (ROS) are a significant factor in neurodegenerative diseases and ultimately lead to neuronal death, which impairs memory, cognitive function, and behavior [10, 11, 12]. The role of acetylcholinesterase (AChE) in AD cognitive deficits has also been clarified by recent research [13]. Acetylcholine (ACh) is hydrolyzed and broken down in the synaptic cleft by the enzyme AChE [14]. The neurotransmitter acetylcholine (ACh) is deposited within neurofibrillary tangles and amyloid beta (Ab) plaques in the central nervous system (CNS) and is responsible for cholinergic transmission in the brain [11]. Cognitive impairments and, in the worst situations, death result from the subsequent lack of cholinergic neurotransmission brought on by decreased ACh levels. Therefore, treatments that block AChE and raise ACh levels are promising short-term solutions for AD [15].

The deciduous tropical tree known as banaba, *Lagerstroemia speciosa* (L.) Pers, contains several polyphenolic compounds [16]. The leaves of *L. speciosa* have yielded more than 40 phytoconstituents that have been isolated and identified so far. These phytoconstituents, which have a variety of biological activities, include flavones, glycosides, corosolic acid, quercetin, isoquercitin, triterpenes, tannins, ellagic acid and its derivatives, and a triterpenoid [17]. There are numerous significant biological characteristics found in practically every part of the plant of *L. speciosa* [18]. Leaf is used to make slimming tea and to treat hypertension, diabetes, hypercholesterolemia, and various renal dysfunctions. Locals use the plant's bark to treat diarrhea [18, 19]. Its use is regarded as the most practical and effective method for managing diabetes due to its anti-diabetic effects and lack of apparent side effects [20]. Corosolic acid, a phytocomponent from *L. speciosa*, has recently been shown to have hypoglycemic and free radical scavenging qualities in animal models, despite the fact that it was previously believed that an insulin-like peptide hormone contributed to the hypoglycemic activity [21]. The majority of plants selected for study have a promising history of traditional use. *L. speciosa* is a very promising topic for additional research because of its identified biological characteristics, traditional uses, and isolated phytoconstituents. The anti-inflammatory, anti-cholinesterase, and antioxidant properties of leaf extract in various solvents have not yet been studied. As a result, the current study examined the extraction of plant leaves using a successive

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extraction technique and assessed their antioxidant, anti-inflammatory, and anti-cholinesterase properties.

2. Objectives

This study assesses the antioxidant, anti-inflammatory, and anti-cholinesterase properties of successive extracts derived from *Lagerstroemia speciosa* at varying concentrations in order to determine their potential medical utility.

3. Methods

3.1. Chemicals and equipment

The reagents and chemicals employed in this investigation were purchased from Sigma-Aldrich (Bangalore, India). 2,2-diphenyl-2-picrylhydrazyl (DPPH), ferric chloride, Folin–Ciocalteu reagent, hydrogen peroxide, ascorbic acid, sodium chloride, anhydrous sodium sulfate, BSA (Bovine serum albumin), salicylic acid, Whatman no.1 filter paper, acetylcholinesterase, butyrylcholinesterase, acetylthiocholine iodide, butyrylthiocholine iodide, donepezil hydrochloride, and DTNB were all used in this study. All the other solvents and reagents were of analytical grade. The colorimetric assays were analysed in UV-visible spectrophotometer (A125359, Shimadzu corp., South Korea), and micro-plate reader (BioTech 800TS, Agilent, USA).

3.2. Collection of plant material and preparation of extracts

Fresh leaves of *Lagerstroemia speciosa* (L.) were collected in September 2024 from Boragaon, Guwahati, Assam, India. The authentication of the plant was done by the curator, Department of Botany, Gauhati University, Assam, India. The voucher specimen (Ref. No. Herb./GUBH/2024/101) was later deposited at herbarium of Girijananda Chowdhury University, Assam, India. The plant was rinsed with distilled water and air-dried, followed by final drying in a tray dryer (TC-704, Kolkata, India) at 40ºC for 72 hrs. Powdered plant material (100 g) was dissolved in solvents (1L) with increasing polarity: hexane>chloroform>ethyl acetate>methanol>water. This successive extraction procedure was done in accordance with Kumar et al. [22] with minor adjustments. The entire extraction technique was carried out in Soxhlet apparatus for 72 hrs. The extracts were filtered with Whatman no. 41 filter paper and concentrated in rotary evaporator (Rotavapor-R300, Buchi Ltd., India) at 55 ± 5ºC under reduced pressure. All the extracts were stored at 4 °C until needed for further use.

3.3. Estimation of phytochemical properties of extracted materials

3.3.1. Phytochemical screening

The solvent extracts of *L. speciosa* were screened using the techniques previously outlined by Guevara [23] to determine whether any additional phytochemical constituents were present.

3.3.2. Total phenolic content

According to Nur et al. [24], with minor modifications, the total phenolic content of each solvent extract was determined. In short, this assay involved creating different samples by dissolving 10 mg in 10 mL of ethanol. A homogenized solution was obtained by sonicating the mixture for five minutes. To 300 µL of this solution in a test tube, 1 mL of ethanol, 3.16 mL of distilled water, and 200 µL Folin-Ciocalteu reagent were added. 600 µL of a 10% sodium carbonate solution was added to the test tube after it had been incubated for 8 minutes at room temperature. It was then covered with aluminium foil and heated to 40°C for 30 minutes. The same process was used to create a blank, but instead of the extract, an equivalent volume of ethanol was used. A UV spectrophotometer was set to 765 nm to measure the sample's absorbance. The same process was used to determine the gallic acid standard curve. Gallic acid equivalents (GAE) in milligrams per 100 grams were used to represent the

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total phenolic content. The total phenolic content (TPC) was expressed as:

TPC (mg GAE/100 g) = C × (V/M)

'C' denotes the equivalent of gallic acid concentration, 'V' depicts the volume of extract solution, and 'M' represents the mass of the extract utilised.

3.3.3. Total flavonoid content

The total flavonoid content was determined by using an established method [25] with minor changes. Rutin was diluted to different concentrations (31.25–1000 µg/mL) and used as a standard by dissolving it in ethanol. After 10 mg are sonicated for 10 minutes in 10 mL of ethanol, each extract created a solution. A test tube was filled with 3.4 mL of 30% aqueous ethanol to create a clear solution of a 300 µL extract (1 mg/mL of ethanol). 150 µL of aqueous sodium nitrite (0.5 M) and 150 µL of aluminum chloride solution (0.3 M) were then added to the mixture. After 5 minutes, 1 mL of sodium hydroxide solution was added to a test tube, thoroughly mixed, and its absorption was measured against a blank using a 506 nm UV-visible spectrophotometer. This continued in the same manner, with the exception of substituting an equivalent volume of ethanol for the extract. The total flavonoid content was expressed as:

TFC (mg RE/100 g) = C′ × (V/M)

'C′' represents the equivalent of rutin concentration, V' depicts the volume of extract solution, and 'M' denotes the mass of the extract utilised.

3.3.4. Total tannin content

The total tannin content was determined according to Makkar et al. [26]. Test tubes were filled with a 0.02–0.1 mL of the extract, 0.5 ml of distilled water, 0.25 ml of the Folin–Ciocalteu reagent, and 1.25 ml of the sodium carbonate solution. After 40 minutes, tubes were vortexed and the absorbance was measured at 725 nm using UV-visible spectrophotometer. The same procedure was repeated replacing the extract with tannic acid (0.1 mg/mL) as standard. The content of tannin was expressed as:

TTC (mg TAE/100 g) = [(C′′× V × DF × 100) /M]

'C′′' denotes the equivalent of tannic acid concentration, 'DF' stands for dilution factor, V' depicts the volume of extract solution, and 'M' represents the mass of the extract utilised.

3.3.5. Determination of proanthocyanidins

0.50 mL of the extract was diluted with 70% acetone into a glass test tube. The amount of acetone should be sufficient to keep the assay's absorbance (550 nm) from rising above 0.6. It may occasionally need to be ascertained by trial and error and will rely on the amount of proanthocyanidin anticipated in the sample. Next, 3 mL of the butanol-HCl (95:5 v/v) reagent and 0.1 mL of the ferric reagent were added to the tubes. After vortex, tubes covered with aluminium foil on the mouth were placed in a boiling water bath set between 97 and 100°C for 60 min. The absorbance was measured at 550 nm using UV-visible spectrophotometer [26]. The proanthocyanidin content was expressed as:

PC (mg CE/100 g) = [(C′′′× V × DF × 100) /M]

'C′′′' denotes the equivalent of catechin concentration, 'DF' stands for dilution factor, V' depicts the volume of extract solution, and 'M' represents the mass of the extract utilised.

3.4. Assessment of *in-vitro* antioxidant activity

3.4.1. DPPH scavenging assay

With a few minor modifications, the DPPH scavenging assay, as described by Sakat et al. [27], was used to measure the antioxidant activity. The assay detects the simultaneous conversion of the stable violet DPPH radical into a yellow reduced form. Consequently, absolute ethanol was used in this assay to treat the ascorbic acid and

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experimentally extracted samples. Next, 9.5 mL of freshly prepared DPPH solution (50 µg/mL) in absolute ethanol was mixed with 500 µL of each sample. 10 mL of 50 µg/mL of DPPH processed in absolute ethanol was used as a negative control, while ascorbic acid was used as a positive control. After thoroughly mixing the contents of each preparation, they were incubated for 10 minutes at 25 °C in the dark. The absorbance was measured using a UV-visible spectrophotometer at 517 nm. The following formula was used to calculate the percentage of DPPH free radicals scavenged, which was carried out in triplicate (n = 3):

Percentage inhibition = $[(A_c-A_s)/A_c] \times 100$

'A_c' indicates the absorbance of the control, and 'A_s' represents the absorbance of the sample. This equation was utilised to determine the percentage inhibition of the extracts for the other antioxidant assays.

3.4.2. ABTS assay

The ABTS assay was accomplished according to Ahmed et al. [25] with minor modifications. Plant extracts were prepared in methanol with dilutions ranging from 25 to 200 µg/mL. A sample (10 µL) was put in a test tube and thoroughly mixed with 2.99 mL of ABTS radical working solution. The working solution of ABTS•+ radical was created by reacting ABTS (9.5 mL, 7 mM) with potassium persulfate (245 µL, 100 mM) and increasing the volume to 10 mL with distilled water. The solution was then left in the dark at room temperature for 18 hours before being diluted with potassium phosphate buffer (0.1 M, pH 7.4) to an absorbance of 0.70 (±0.02) at 734 nm.

3.4.3. Reducing power assay

The reducing power was determined according to Bhatti et al. [28] with slight modifications. Separate samples of 0.2 mL of the extracts at different concentrations (25–200 µg/mL) were combined with 0.5 mL of 1% potassium ferricyanide and 0.5 mL of phosphate buffer (0.2 M, pH 6.6). For 20 min., the mixture was incubated at 50°C in a water bath. It was allowed to cool to room temperature before being centrifuged for 10 minutes at 3000 rpm with 0.5 mL of 10% trichloroacetic acid added. After collecting 0.5 mL of the supernatant, it was combined with 0.5 mL of distilled water. After adding 0.1 mL of 0.1% ferric chloride, the mixture was allowed to sit at room temperature for 10 min. At 700 nm, the absorbance was measured with UV-visible spectrophotometer. Ascorbic acid was employed as a positive control in the study.

3.4.4. Free radical scavenging assay

The capability of the extract to scavenge hydrogen peroxide (H_2O_2) was assessed using an established method developed by Bhatti et al. [28] with minor changes. An aliquot of 0.1 mL of extract (25–200 µg/mL) was added to each Eppendorf tube, and 0.6 mL of 2 mM H_2O_2 solution was added after the volume of the tubes was increased to 0.4 mL with 50 mM phosphate buffer (pH 7.4). The absorbance of the reaction mixture was measured at 230 nm after it had been vortexed for 10 minutes. The use of ascorbic acid served as the positive control.

3.5. Assessment of *in-vitro* anti-inflammatory activity

3.5.1. Denaturation of protein

Denaturation of protein inhibition was calculated using the methodology adopted by Nur et al. [24]. For each test, the extracted materials were dissolved in deionized water to produce a concentration of 1 mg/ml. In multiple test tubes, the standard and tested extract stock solutions were diluted with 0.1 M phosphate buffer solution (pH-7.4) at different concentrations (50–400 µg/ml). 5 mL reaction mixture contained 0.02 mL of the extract, 0.2 mL of 1% bovine albumin, and 4.78 mL of phosphate-buffered saline (PBS, pH 6.4). The reaction mixture was combined, incubated for 15 minutes at 37 °C in a water bath, and then scorched for 5 minutes at 70 °C. Following that, a UV-vis spectrometer was used to measure the absorbance at 660 nm. The following

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equation was used to estimate the percentage inhibition of BSA denaturation during the triplicate assay:

Percentage inhibition = $[(A_c-A_t)/A_c] \times 100$

'A_c' indicates the absorbance of the control, and 'A_t' represents the absorbance of the test sample.

3.5.2. Heat induced hemolysis inhibition assay

The heat-induced hemolysis inhibitory assay was determined according to the method established by Gunathilake et al. [29] with minor modifications. In brief, 2.90 mL of phosphate buffer (pH 7.4), 0.05 mL of the blood cell suspension, and 0.05 mL of each of the extracts (50-400 µg/mL) were combined. In a shaking water bath, the mixture was incubated for 20 min at 54 °C. A UV-vis spectrometer was then used to measure the absorbance of the supernatant at 540 nm after the incubated mixture had been centrifuged (1431 g for 5 min). The test was performed in triplicate, and the average mean value was recorded. The following formula was used to determine the hemolysis percentage:

Percentage inhibition of hemolysis = $100 \times (1-A_t/A_c)$

'A_t' indicates the absorbance of the test sample, and 'A_c' represents the absorbance of the control.

3.6. Assessment of cholinesterase inhibitory activity

3.6.1. Cholinesterase inhibition assay

The cholinesterase inhibition assay was done in accordance with method developed by Kushari et al. [30]. A 96-well microplate labelled with the concentrations of the test samples and the reference standard was used in the assay process. Each plate received 40 µL of 50 mM tris-HCl buffer, 20 µL of 0.26 U/mL enzyme solution, and 100 µL of 3 mM DTNB. After that, 20 µL of the test samples (6.25–200 µg/mL) were added to the plate, and it was incubated for 15 minutes at 25 °C. Absorbance was measured at 412 nm after 20 µL of a 15 mM acetylthiocholine iodide solution was added to each well. The same experiment was conducted with butyrylthiocholine iodide as a substrate to measure the BuChE activity of the test sample. All the measurements were conducted in triplicate and the percentage inhibition was expressed as:

Percentage inhibition = $[(A_{BLANK} - A_{SAMPLE})/A_{BLANK}] \times 100$

3.7. Statistical analysis

The results are shown as mean ± S.D (n=3). The Bonferroni post-test was used after a one-way ANOVA was used to determine whether there were significant differences between means. P-value < 0.05 was considered significant. GraphPad Prism version 5 was used to compute the IC50 value through non-linear regression analysis with a sigmoidal dose-response (variable slope) model.

4. Results

4.1. Phytochemical analyses

The phytochemical analyses of successive extracts revealed that the major phytoconstituents were present in the methanol and ethyl acetate extracts (Table 1). Moreover, the phytochemical constituents in different solvent extracts of *L. speciosa* are presented in Table 2. In Table 2, it is observed that the methanolic and aqueous extract contains a notable amount of total phenolic content, flavonoid content, and proanthocyanidin content compared to other extracts. Whereas, chloroform extract contains the maximum amount of total tannin content, contrary to others.

Table 1. Phytochemical screening of different extracts of *L. speciosa*.

		L. speciosa				
Phytochemicals	**Qualitative test**	**Hexane**	**Chloroform**	**Ethyl acetate**	**Methanol**	**Aqueous**

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Phenol	Ferric chloride test	-	-	+	+	+
Carbohydrate	Molisch test	+	+	+	+	+
Protein	Xanthoproteic test	-	-	-	+	+
Flavonoid	Alkaline reagent test	+	+	+	+	+
Alkaloid	Wagner test	-	-	-	-	-
Tannin	Lead test	+	+	+	+	+
Terpenoid	Salkowski test	+	+	+	+	+
Glycoside	Keller-killiani test	+	+	+	+	-
Saponin	Foam test	-	-	-	-	-
Essential oil	Vanillin-sulphuric acid test	+	+	+	+	-
Fixed oil	Sudan III test	+	+	+	-	-

(-): present, (+): absent

Table 2. Estimation of total phenolic content, total flavonoid content, total tannin content, and proanthocyanidin content of *L. speciosa*.

Test sample	Total phenolic content [a]	Total flavonoid content [b]	Total tannin content [c]	Proanthocyanidin content [d]
HELS	32.56 ± 2.68	286.14 ± 2.42	8.26 ± 0.36	16.48 ± 0.76
CHLS	38.48 ± 1.84	293.20 ± 3.20	12.30 ± 0.62	12.50 ± 0.42
EALS	44.18 ± 2.40	310.06 ± 3.14	6.28 ± 0.28	14.56 ± 0.84
MELS	56.04 ± 2.26	382.24 ± 2.68	8.52 ± 0.74	22.26 ± 2.08
AQLS	58.64 ± 1.88	412.68 ± 3.46	4.64 ± 0.44	18.36 ± 0.54

HELS: hexane extract of *L. speciosa*, CHLS: chloroform extract of *L. speciosa*, EALS: ethyl acetate extract of *L. speciosa*, MELS: methanol extract of *L. speciosa*, AQLS: aqueous extract of *L. speciosa*, a: mg gallic acid equivalent/100 g of sample, b: mg rutin equivalent/100 g of sample, c: mg tannic acid equivalent/100 g of sample, d: mg catechin equivalent/100 g of sample.

4.2. Assessment of *in-vitro* antioxidant activity

In this evaluation, DPPH, ABTS, reducing power, and free radical scavenging assays were performed to assess the antioxidant activity of the extracts of the *L. speciosa* (Figure 1). In the DPPH assay, at the concentrations of 25, 50, 75, 100, and 200 µg/mL, all the extracts showed antioxidant capacity. The methanolic extract of *L. speciosa* (MELS) exhibited antioxidant capacity ranging from 62.28 ± 2.34% to 88.55 ± 2.68 %, respectively. In contrast, aqueous extract of L. speciosa (AQLS) displayed from 45.05 ± 1.76% to 85.73 ± 2.52 %, ethyl acetate extract of *L. speciosa* (EALS) showed from 50.56 ± 1.08% to 81.77 ± 2.04 %, chloroform extract of *L. speciosa* (CHLS) showed from 45.26 ± 0.64% to 70.16 ± 1.47%, and hexane extract of *L. speciosa* exhibited from 44.28 ± 0.62% to 61.33 ± 0.86 %, respectively. The positive control, ascorbic acid showed DPPH scavenging activity ranging from 46.18 ± 0.52% to 93.36 ± 1.76%, respectively (Figure 1a). In the ABTS assay, MELS exhibited percentage inhibition from 19.20 ± 0.96% to 58.01 ± 0.37%, AQLS displayed from 14.62 ± 0.87% to 35.36 ± 0.60%, EALS showed from 8.42 ± 0.91% to 26.01 ± 1.10%, CHLS from 10.76 ± 1.25% to 32.01 ± 0.67%, and HELS exhibited from 13.18 ± 1.53% to 50.86 ± 1.54%, respectively. The positive control, ascorbic acid showed ABTS scavenging activity ranging from 28.86 ± 1.28% to 77.65 ± 2.35%, respectively (Figure 1b). Similarly, in the reducing power assay, MELS displayed percentage inhibition from 29.06 ± 0.38% to 81.89 ± 1.24%, AQLS ranged from 17.54 ± 0.18% to 63.45 ± 0.44%, EALS varied from 20.35 ± 0.24% to 47.01 ± 0.36%, CHLS displayed from 8.84 ± 0.10% to 46.86 ± 0.38%, and HELS showed from 3.35 ± 0.08% to 45.69 ± 0.44%, respectively. However, ascorbic acid showed percentage inhibition from 46.98 ± 0.74% to 94.27 ± 1.14%, respectively (Figure 1c). In the free radical

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scavenging assay, MELS exhibited scavenging activity from 24.50 ± 0.57% to 44.56 ± 0.48%, AQLS ranged from 17.62 ± 0.87% to 28.36 ± 0.60%, EALS showed from 16.22 ± 0.91% to 30.10 ± 0.23%, CHLS displayed from 18.76 ± 0.25% to 35.10 ± 0.67%, and HELS showed from 11.83 ± 0.68% to 26.86 ± 0.36%, respectively. Ascorbic acid showed scavenging activity from 34.86 ± 0.88% to 58.65 ± 0.35%, respectively (Figure 1d). The IC_{50} values for the extracts in antioxidant assays are presented in Table 3.

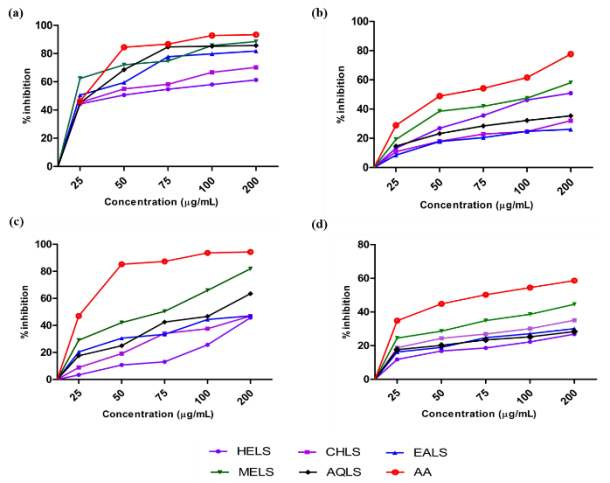

Figure 1. An illustration of the antioxidant activities of the successive extracts of *L. speciosa*. (a) and (b) represent the dose-dependent DPPH and ABTS scavenging activities of the different extracts of *L. speciosa*, (c) and (d) represent the reducing power and free radical scavenging assays of the different extracts of *L. speciosa.* Values are represented as Mean ± SD (n = 3). Ascorbic acid was used as the reference standard. HELS: hexane extract of *L. speciosa*, CHLS: chloroform extract of *L. speciosa*, EALS: ethyl acetate extract of *L. speciosa*, MELS: methanol extract of *L. speciosa*, AQLS: aqueous extract of *L. speciosa*, AA: ascorbic acid.

Table 3. IC_{50} values of different extracts for in vitro antioxidant assays.

Test sample	Solvent	IC_{50} of DPPH scavenging assay (µg/mL)	IC_{50} of ABTS scavenging assay (µg/mL)	IC_{50} of reducing power assay (µg/mL)	IC_{50} of free radical scavenging assay (µg/mL)
L. speciosa	Hexane	32.40 ± 0.86^{x}	42.16 ± 1.18^{x}	56.12 ± 1.64^{x}	26.02 ± 0.44^{x}
	Chloroform	29.08 ± 0.64^{x}	39.04 ± 0.86^{x}	44.06 ± 1.04^{x}	24.18 ± 0.76^{x}
	Ethyl acetate	26.58 ± 1.04^{x}	37.58 ± 0.66^{x}	40.50 ± 0.92^{x}	22.30 ± 0.28^{y}
	Methanol	18.26 ± 0.74^{z}	24.36 ± 0.44	21.36 ± 0.64	17.55 ± 0.14
	Aqueous	23.18 ± 0.82^{y}	28.04 ± 0.72^{z}	32.56 ± 0.38^{x}	19.68 ± 0.53^{z}
	Ascorbic acid (standard)	16.48 ± 0.58	22.64 ± 0.78	18.48 ± 0.36	15.26 ± 0.24

Results are expressed as mean ± SD (n=3); $^{x}p < 0.001$, $^{y}p < 0.01$, and $^{z}p < 0.05$ compared to standard, respectively (One-way ANOVA followed by Bonferroni's test).

4.3. Assessment of *in-vitro* anti-inflammatory activity

The protein denaturation assay and heat induced hemolytic inhibitory assay were evaluated to examine the anti-inflammatory activity of successive extracts of *L. speciosa* (Figure 2). In the protein denaturation assay, at the concentrations of 50, 100, 200, 300, and 400 µg/mL, MELS displayed percentage inhibition from 50.45 ± 0.20% to 63.68 ± 0.45%, AQLS exhibited from 39.87 ± 0.70% to 55.40 ± 0.20%, EALS varied from 34.92 ± 0.42% to 54.86 ± 0.36%, CHLS showed from 38.22 ±

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0.11% to 48.35 ± 0.14%, and HELS varied from 36.05 ± 0.11% to 45.13 ± 0.13%, respectively. Whereas, the positive control, diclofenac exhibited percentage inhibition from 60.82 ± 0.23% to 73.30 ± 0.16%, respectively (Figure 2a). Similarly, in hemolytic inhibition assay, MELS showed inhibition from 47.32 ± 0.33% to 73.86 ± 0.20%. AQLS ranged from 31.85 ± 0.32% to 56.52 ± 0.36%, EALS displayed from 31.00 ± 0.38% to 51.67 ± 0.21%, CHLS showed from 24.63 ± 0.43% to 42.51 ± 0.10%, and HELS varied from 36.75 ± 0.24% to 54.77 ± 0.07%, respectively. However, diclofenac exhibited percentage inhibition from 61.82 ± 0.41% to 81.79 ± 0.33%, respectively (Figure 2b). The IC_{50} values for the extracts in antioxidant assays are presented in Table 4.

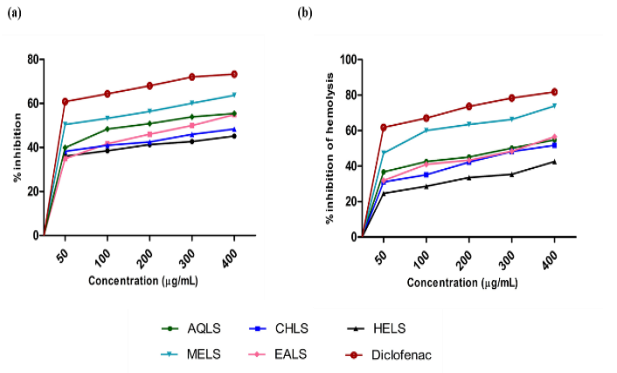

Figure 2. A schematic representation of the antioxidant activities of the successive extracts of *L. speciosa*. (a) and (b) represent the dose-dependent protein denaturation and hemolytic inhibition assays of the different extracts of *L. speciosa*. Values are expressed as Mean ± SD (n = 3). Diclofenac was used as the reference standard. HELS: hexane extract of *L. speciosa*, CHLS: chloroform extract of *L. speciosa*, EALS: ethyl acetate extract of *L. speciosa*, MELS: methanol extract of *L. speciosa*, AQLS: aqueous extract of *L. speciosa*.

Table 4. IC_{50} values of different extracts for in vitro anti-inflammatory assays.

Test sample	Solvent	IC_{50} of BSA inhibitory assay (µg/mL)	IC_{50} of hemolytic inhibitory assay (µg/mL)
L. speciosa	Hexane	42.40 ± 0.64^{x}	52.18 ± 1.48^{x}
	Chloroform	36.22 ± 0.52^{x}	44.36 ± 1.26^{x}
	Ethyl acetate	32.8 ± 1.24^{x}	42.80 ± 1.04^{x}
	Methanol	23.60 ± 0.34^{z}	34.36 ± 0.88^{y}
	Aqueous	28.48 ± 1.02^{x}	39.54 ± 1.52^{x}
	Diclofenac (standard)	19.48 ± 0.58	26.64 ± 0.78

Results are expressed as mean ± SD (n=3); $^{x}p < 0.001$, $^{y}p < 0.01$, and $^{z}p < 0.05$ compared to standard, respectively (One-way ANOVA followed by Bonferroni's test).

4.4. Assessment of *in-vitro* cholinesterase inhibitory activity

On contrary to anti-oxidant and anti-inflammatory activity, the successive extracts of *L. speciosa* were also investigated for cholinesterase inhibitory activity (Figure 3). In the acetylcholinesterase inhibitory activity, MELS at the concentrations (6.25-200 µg/mL) showed percentage inhibition from 41.53 ± 0.67% to 63.42 ± 0.65%, AQLS displayed from 28.58 ± 0.54% to 40.38 ± 0.47%, EALS showed from 35.12 ± 0.56% to 50.53 ± 0.53%, CHLS varied from 33.38 ± 0.61% to 38.36 ± 0.65%, and HELS displayed from 23.34 ± 0.43% to 34.53 ± 0.69%, respectively. Whereas, the reference standard, donepezil showed percentage inhibition from 47.46 ± 0.50% to 68.10 ± 0.60%, respectively (Figure 3a). Similarly, in butyrylcholinesterase inhibitory activity, at the same concentrations, the percentage inhibition of the extracts and donepezil are represented in Figure 3b. The IC_{50} values for the extracts in cholinesterase inhibition assays are presented in Table 5.

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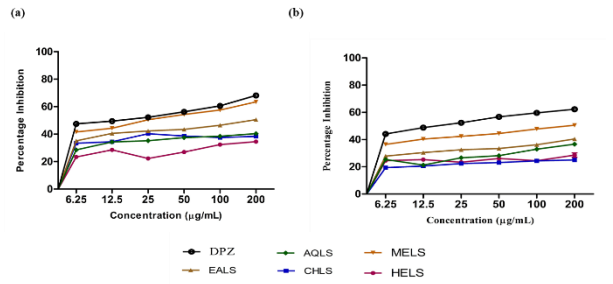

Figure 3. An illustration of the anti-cholinesterase activities of the successive extracts of *L. speciosa*. (a) and (b) represent the dose-dependent inhibition of AChE and BuChE of the different extracts of *L. speciosa*. Values are expressed as Mean ± SD (n = 3). Donepezil was used as the reference standard. HELS: hexane extract of *L. speciosa*, CHLS: chloroform extract of *L. speciosa*, EALS: ethyl acetate extract of *L. speciosa*, MELS: methanol extract of *L. speciosa*, AQLS: aqueous extract of *L. speciosa*, DPZ: donepezil.

Table 5. IC_{50} values of different extracts for in vitro cholinesterase assays.

Test sample	Solvent	IC_{50} of AChE inhibitory assay (µg/mL)	IC_{50} of BuChE inhibitory assay (µg/mL)
L. speciosa	Hexane	70.68 ± 2.04^x	152.06 ± 2.88^x
	Chloroform	86.52 ± 1.57^x	114.36 ± 2.60^x
	Ethyl acetate	68.44 ± 1.74^x	108.94 ± 2.16^x
	Methanol	36.02 ± 0.62^x	64.58 ± 1.48^x
	Aqueous	88.18 ± 2.08^x	126.37 ± 2.58^x
	Donepezil (standard)	7.48 ± 0.27	46.40 ± 1.66

Results are expressed as mean ± SD (n=3); $^x p < 0.001$ compared to standard, respectively (One-way ANOVA followed by Bonferroni's test). AChE: acetylcholinesterase, BuChE: butyrylcholinesterase.

5. Discussion

Biotechnological production and the development of novel extraction techniques to obtain phytoconstituents are favoured by the steadily increasing demand for plant-derived therapeutic molecules obtained in an environmentally responsible and sustainable manner [31]. Throughout the history of pharmaceuticals, nutraceuticals, and cosmeceuticals, phytoconstituents have a strong place. Although they are centuries old, herbal treatments in Ayurveda, homeopathy, traditional Chinese medicine, and other traditional medical systems have greatly enlightened us about plant-based cures. These herbal remedies have been validated by science and are now considered legitimate therapies. The bioactive substances derived from plants are now an essential component of drug, nutraceutical, and cosmetic research [32]. In this study, methanol and aqueous extracts contained a significant amount of total phenolic and flavonoid content compared to other extracts (Table 2). It has been observed that phenolic components and plant flavonoids have been found to influence effective anti-inflammatory actions [27]. Similarly, chloroform and methanol extract contained maximum amount of tannin and proanthocyanidin content (Table 2). According to previous reports, tannins from different plant sources are responsible for their anti-oxidant property [33, 34].

In this study, it was revealed from the anti-oxidant studies that methanol extract of the plant species exhibited the highest percentage inhibition on comparison to the other extracts and can be represented in the order: methanol>aqueous>ethyl acetate>chloroform>hexane. In addition to, IC_{50} value of methanol extract was found to be most potent on comparison to other extracts (Table 3). These findings showed that the compounds in the methanol extract can give a free radical hydrogen, which removes an odd electron and raises concerns about the radical's sensitivity. High phenolic content compounds were found to be typically associated with high radical scavenging activity

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[35]. From the study, methanol extract contained a notable phenolic content. Thus, it might be the cause of the highest percentage of antioxidant activity shown by methanol extract. Consequently, methanol extract of plant species may be a possible source of antioxidants.

One major factor contributing to protein denaturation is inflammation. Inflammatory diseases may benefit from the use of protein denaturing agents [36]. In the current investigation, anti-inflammatory properties of *L. speciosa* showed that they were dose-related. In comparison to the other extracts, this study found that the methanol extract of the plant species demonstrated greater protein denaturation inhibition at all concentrations. In contrast to the other extracts, the protein denaturation inhibition of the methanol extract was significant ($p < 0.05$) with regard to the standard, diclofenac. Additionally, the results of the hemolytic activity test for anti-inflammatory activity showed that the methanol extract of the plant species had relatively higher inhibitory hemolytic activity than other extracts at all concentrations (50–400 µg/mL). The percentage inhibition shown by different extracts for protein denaturation and hemolytic inhibition assay can be represented as: methanol>aqueous>ethyl acetate>chloroform>hexane. The IC_{50} values of methanol extract for the hemolytic inhibition activity assay and the BSA denaturation assay were found to be 34.36 ± 0.88 and 23.60 ± 0.34, respectively, in contrast to diclofenac's values of 26.64 ± 0.78 and 19.48 ± 0.58. This implies that the methanol extract of the plant species exhibits highly convincing anti-inflammatory activity with significant level of $p < 0.05$, and $p < 0.01$, respectively with regard to the standard. It has been observed that phenolic compounds and flavonoids demonstrated potent anti-inflammatory properties [37]. As shown in Table 2, methanol extract of the plant species is enriched with total phenolic and flavonoid contents, which could explain the higher inhibition of the extract. Our findings are in consistent to the study reported by Nur et al. [24].

However, previous studies have revealed the interlinkage of anti-oxidant and anti-inflammatory activities with cholinesterase inhibition activity [38, 39]. Therefore, to justify the linkage, cholinesterase inhibition assay was also investigated for the successive extracts. Interestingly, the result revealed that the methanol extract exhibited the highest percentage inhibition followed by aqueous and ethyl acetate extracts for both acetylcholinesterase and butyrylcholinesterase inhibition assays. It is well known that flavonoids have neuroprotective qualities that enhance cognitive performance and provide defense against memory loss brought on by ageing [40]. The immune resident cells of the brain, known as microglia cells, become overactivated in pathological conditions and release a range of pro-inflammatory mediators, including nitric oxide. This ultimately results in a number of CNS neurodegenerative diseases, such as Parkinson's and Alzheimer's disease [9]. The methanol extract from *L. speciosa* was examined for antioxidant activity in the current study and demonstrated strong scavenging activity of reactive oxygen species. This prompted a follow-up assay to observe its inhibitory effects on AChE and BuChE. The IC_{50} values of the extract were found to be 36.02 ± 0.62 and 64.58 ± 1.48 µg/mL, respectively (Table 5).

6. Conclusions

This study has found that the extracts of *L. speciosa* possessed anti-oxidant, anti-inflammatory, and anti-cholinesterase activities. The methanol extract, in particular, had better pharmacological properties in comparison to the other extracts. The higher amount of phytochemicals in the extract has prompted for further investigations. In conclusion, it is necessary to conduct additional research of the extract in order to determine the active ingredients and evaluate safety and bioavailability using *in vivo* animal models.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Arulselvan P., Fard M.T., Tan W.S., Gothai S., Fakurazi S., Norhaizan M.E., Kumar S.S., 2016. Role of antioxidants and natural products in inflammation. Oxidative Med Cell Longev. 2016(1), 5276130. https://doi.org/10.1155/2016/5276130
2. Wei W.C., Lin S.Y., Chen Y.J., Wen C.C., Huang C.Y., Palanisamy A., Yang N.S., Sheu, J.H., 2011. Topical application of marine briarane-type diterpenes effectively inhibits 12-O-tetradecanoylphorbol-13-acetate-induced inflammation and dermatitis in murine skin. J Biomed Sci. 18(1), 94. https://doi.org/10.1186/1423-0127-18-94
3. Arulselvan P., Ghofar H.A.A., Karthivashan G., Halim M.F.A., Ghafar M.S.A., Fakurazi, S., 2014. Antidiabetic therapeutics from natural source: A systematic review. Biomedicine & Preventive Nutrition. 4(4), 607-617. https://doi.org/10.1016/j.bionut.2014.07.009
4. Gerber M., Boutron-Ruault M.C., Hercberg S., Riboli E., Scalbert A., Siess M.H., 2002. Food and cancer: state of the art about the protective effect of fruits and vegetables. Bull du Cancer. 89(3), 293-312.
5. Bhatia S., Shukla R., Madhu S.V., Gambhir J.K., Prabhu K.M., 2003. Antioxidant status, lipid peroxidation and nitric oxide end products in patients of type 2 diabetes mellitus with nephropathy. Clin Biochem. 36(7), 557-562. https://doi.org/10.1016/S0009-9120(03)00094-8
6. Krishnaiah D., Sarbatly R., Nithyanandam R., 2011. A review of the antioxidant potential of medicinal plant species. Food and Bioproducts Processing. 89(3), 217-233. https://doi.org/10.1016/j.fbp.2010.04.008
7. Matteo V., Esposito E., 2003. Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. Current Drug Targets-CNS & Neurological Disorders. 2(2), 95-107. https://doi.org/10.2174/1568007033482959
8. Ravipati A.S., Zhang L., Koyyalamudi S.R., Jeong S.C., Reddy N., Bartlett J., Smith P.T., Shanmugam K., Münch G., Wu M.J., Satyanarayanan M., 2012. Antioxidant and anti-inflammatory activities of selected Chinese medicinal plants and their relation with antioxidant content. BMC Complement Altern Med. 12(1), 173. https://doi.org/10.1186/1472-6882-12-173
9. Lee S., Lee D., Baek J., Jung E.B., Baek J.Y., Lee I.K., Jang T.S., Kang K.S., Kim, K.H., 2017. In vitro assessment of selected Korean plants for antioxidant and antiacetylcholinesterase activities. Pharm Biol. 55(1), 2205-2210. https://doi.org/10.1080/13880209.2017.1397179
10. Zhao Y., Dou J., Wu T., Aisa H.A., 2013. Investigating the antioxidant and acetylcholinesterase inhibition activities of Gossypium herbaceam. Molecules. 18(1), 951-962. https://doi.org/10.3390/molecules18010951
11. Yousof Ali M., Jung H.A., Choi J.S., 2015. Anti-diabetic and anti-Alzheimer's disease activities of Angelica decursiva. Arch Pharmacal Res. 38(12), 2216-2227. https://doi.org/10.1007/s12272-015-0629-0
12. Wu L., Tong T., Wan S., Yan T., Ren F., Bi K., Jia, Y., 2017. Protective effects of puerarin against Aβ1–42-induced learning and memory impairments in mice. Planta Med. 83(03/04), 224-231.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(5), 2601-2614 | ISSN:2251-6727

13. Haque R., Nazir A., 2016. Identification and functional characterization of a putative IDE, C28F5. 4 (ceIDE-1), in Caenorhabditis elegans: Implications for Alzheimer's disease. Biochimica et Biophysica Acta (BBA)-General Subjects. 1860(11), 2454-2462. https://doi.org/10.1016/j.bbagen.2016.07.013
14. Dhanasekaran S., Perumal P., Palayan M., 2015. In-vitro Screening for acetylcholinesterase enzyme inhibition potential and antioxidant activity of extracts of Ipomoea aquatica Forsk: Therapeutic lead for Alzheimer's disease. J Appl Pharm Sci. 5(2), 012-016. https://dx.doi.org/10.7324/JAPS.2015.50203
15. Sallam A., Mira A., Ashour A., Shimizu, K., 2016. Acetylcholine esterase inhibitors and melanin synthesis inhibitors from Salvia officinalis. Phytomedicine. 23(10), 1005-1011. https://doi.org/10.1016/j.phymed.2016.06.014
16. Choi J., Cho J.Y., Choi S.J., Jeon H., Kim Y.D., Htwe K.M., Chin Y.W., Lee W.S., Kim J., Yoon K.D., 2015. Two new phenolic glucosides from Lagerstroemia speciosa. Molecules. 20(3), 4483-4491. https://doi.org/10.3390/molecules20034483
17. Chan E.W., Wong S.K., 2014. Phytochemistry and Pharmacology of Lagerstroemia speciosa: A Natural Remedy for Diabetes. Int J Herb Med. 2(1), 81-87.
18. Sharmin T., Rahman M.S., Mohammadi H., 2018. Investigation of biological activities of the flowers of Lagerstroemia speciosa, the Jarul flower of Bangladesh. BMC Complement Altern Med. 18(1), 231. https://doi.org/10.1186/s12906-018-2286-6
19. Park C., Lee J.S., 2011. Banaba: The natural remedy as antidiabetic drug. Biomed Res. 22(2), 125-129.
20. Deocaris C.C., Aguinaldo R.R., Ysla J.L.Y., Asencion A.S., Mojica E.E., 2005. Hypoglycemic activity of irradiated Banaba (Lagerstroemia speciosa Linn.) leaves. J Appl Sci Res. 1(1), 95-98.
21. Judy W.V., Hari S.P., Stogsdill W.W., Judy J.S., Naguib Y.M., Passwater R., 2003. Antidiabetic activity of a standardized extract (Glucosol™) from Lagerstroemia speciosa leaves in Type II diabetics: A dose-dependence study. J Ethnopharmacol. 87(1), 115-117. https://doi.org/10.1016/S0378-8741(03)00122-3
22. Kumar D, Bhat Z.A., Shah M.Y., 2012. Effect of successive extracts of Stachys tibetica Vatke (Lamiaceae) in anxiety. Orient Pharm Exp Med. 12(4), 247-253. https://doi.org/10.1007/s13596-012-0083-1
23. Guevara B.Q., 2005. A guidebook to plant screening: phytochemical and biological. University of Santo Tomas Publishing House.
24. Nur M.A., Uddin M.R., Meghla N.S., Uddin M.J., Amin M.Z., 2023. In vitro anti-oxidant, anti-inflammatory, anti-bacterial, and cytotoxic effects of extracted colorants from two species of dragon fruit (Hylocereus spp.). Food Chem Adv. 2, 100318. https://doi.org/10.1016/j.focha.2023.100318
25. Ahmed D., Khan M.M., Saeed R., 2015. Comparative analysis of phenolics, flavonoids, and antioxidant and antibacterial potential of methanolic, hexanic and aqueous extracts from Adiantum caudatum leaves. Antioxidants. 4(2), 394-409. https://doi.org/10.3390/antiox4020394
26. Makkar H.P., 2013. Quantification of tannins in tree and shrub foliage: a laboratory manual. Springer Science & Business Media.
27. Sakat S., Juvekar A.R., Gambhire M.N., 2010. In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. Int J Pharm Pharm Sci. 2(1), 146-155.
28. Bhatti M.Z., Ali A., Ahmad A., Saeed A., Malik S.A., 2015. Antioxidant and phytochemical analysis of Ranunculus arvensis L.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(5), 2601-2614 | ISSN:2251-6727

extracts. BMC research notes. 8(1), 279. https://doi.org/10.1186/s13104-015-1228-3

29. Gunathilake K.D.P.P., Ranaweera KK.D.S., Rupasinghe H.V., 2018. In vitro anti-inflammatory properties of selected green leafy vegetables. Biomedicines. 6(4), 107. https://doi.org/10.3390/biomedicines6040107
30. Kushari S., Saha D., Kalita J.M., Kalita T., Kalita P., Chakraborty J., Kumar S., Das T., Patowary L., 2025. An insight of in silico and in vitro evaluation of Catechins and L-theanine from Camellia sinensis (L.) against Alzheimer's disease targeting cholinesterases and β-secretase. In Silico Research in Biomedicine. 100020. https://doi.org/10.1016/j.insi.2025.100020
31. Fierascu R.C., Fierascu I., Ortan A., Georgiev M.I., Sieniawska E., 2020. Innovative approaches for recovery of phytoconstituents from medicinal/aromatic plants and biotechnological production. Molecules. 25(2), 309. https://doi.org/10.3390/molecules25020309
32. Shah P., Banerjee S., Singh A., Kulhari H., Anand Saharan V., 2025. From Traditional to Modern Medicine: The Role of Herbs and Phytoconstituents in Pharmaceuticals, Nutraceuticals, and Cosmetics. Formulating Pharma-, Nutra-, and Cosmeceutical Products from Herbal Substances: Dosage Forms and Delivery Systems. 3-73. https://doi.org/10.1002/9781119769484.ch1
33. Koleckar V., Kubikova K., Rehakova Z., Kuca K., Jun D., Jahodar L., Opletal L., 2008. Condensed and hydrolysable tannins as antioxidants influencing the health. Mini-Rev Med Chem. 8(5), 436-447. https://doi.org/10.2174/138955708784223486
34. Zhang L.L., Lin Y.M., 2008. Tannins from Canarium album with potent antioxidant activity. J Zhejiang Univ Sci B. 9(5), 407-415. https://doi.org/10.1631/jzus.B0820002
35. Li W., Shan F., Sun S., Corke H., Beta T., 2005. Free radical scavenging properties and phenolic content of Chinese black-grained wheat. J Agri Food Chem. 53(22), 8533-8536. https://doi.org/10.1021/jf051634y
36. Chandra S., Chatterjee P., Dey P., Bhattacharya S., 2012. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. Asian Pac J Trop Biomed. 2(1), S178-S180. https://doi.org/10.1016/S2221-1691(12)60154-3
37. Magalhães A.S., Silva B.M., Pereira J.A., Andrade P.B., Valentão P., Carvalho M., 2009. Protective effect of quince (Cydonia oblonga Miller) fruit against oxidative hemolysis of human erythrocytes. Food Chem Toxicol. 47(6), 1372-1377. https://doi.org/10.1016/j.fct.2009.03.017
38. Patil M.Y., Vadivelan R., Dhanabal S.P., Satishkumar M.N., Elango K., Antony S., 2014. Anti-oxidant, anti-inflammatory and anti-cholinergic action of Adhatoda vasica Nees contributes to amelioration of diabetic encephalopathy in rats: Behavioral and biochemical evidences. Int J Diabetes Dev Ctries. 34(1), 24-31. https://doi.org/10.1007/s13410-013-0145-z
39. Elufioye T.O., Chinaka C.G., Oyedeji A.O., 2019. Antioxidant and anticholinesterase activities of Macrosphyra longistyla (DC) Hiern relevant in the management of Alzheimer's Disease. Antioxidants. 8(9), 400. https://doi.org/10.3390/antiox8090400
40. Macready A.L., Kennedy O.B., Ellis J.A., Williams C.M., Spencer J.P., Butler L.T., 2009. Flavonoids and cognitive function: a review of human randomized controlled trial studies and recommendations for future studies. Genes Nutr. 4(4), 227-242. https://doi.org/10.1007/s12263-009-0135-4