

Neuroprotective Effect of triterpenoids rich fraction *Lagerstroemia Speciosa* Leaves and its Nano-formulation in Albino mice

Vaishali D. Shewale^{*1}, Dr. Amit Kumar², Dr. Sarika M. Kamble³

¹ Research Scholar, Department of Pharmacognosy, Maulana Azad University, Jodhpur, Rajasthan, India.0009-0003-7669-5515

² Professor, Department of Pharmaceutics, Faculty of Pharmacy, Maulana Azad University, Jodhpur, Rajasthan, India.0009-0001-9952-0203

³ Professor, Department of Pharmacology, SMBT College of Pharmacy, Dhamangaon, Igatpuri, Nashik, Maharashtra, India.0000-0002-8222-2736

***Corresponding author:** Vaishali D. Shewale,

*Research scholar, Maulana Azad University, Jodhpur-342802, Rajasthan Email id: shewale.vaishu@gmail.com.

ABSTRACT

The present study was designed to evaluate the neuroprotective potential of the triterpenoids-rich fraction of *Lagerstroemia speciosa* leaves and its nanoformulation in albino mice. Neurotoxicity was induced using standard models to assess behavioral, biochemical, and histopathological parameters. The triterpenoids-rich fraction was extracted and characterized, and a nanoformulation was developed to enhance bioavailability and brain targeting. Treatment with the triterpenoids-rich fraction and its nanoformulation significantly improved behavioral deficits, reduced oxidative stress markers (lipid peroxidation, nitrite levels), restored antioxidant enzyme levels (superoxide dismutase, catalase, reduced glutathione), and attenuated neuroinflammation. Histopathological examination revealed marked protection of neuronal architecture in treated groups compared to toxicant controls. The nanoformulation demonstrated superior neuroprotective activity compared to the plain triterpenoids-rich fraction, likely due to enhanced brain bioavailability. The findings suggest that *Lagerstroemia speciosa* triterpenoids-rich fraction and its nanoformulation possess significant neuroprotective effects, potentially through antioxidant and anti-inflammatory mechanisms, and may offer therapeutic promise for neurodegenerative disorders.

Keywords: *Lagerstroemia speciosa*, Triterpenoids, Nanoformulation, Neuroprotection, Oxidative stress, Neuroinflammation.

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1. Introduction:

As a result of their high levels of pentacyclic triterpenoids, which are associated with neuroprotective properties and antioxidant activities in rodent models, *Lagerstroemia speciosa* (Banaba) leaves have received considerable interest in recent integrated drug development studies. Recent studies have revealed that alcohol and aqueous-ethanol extract preparations of the *L. speciosa* leaves alleviate hyperalgesia induced by mechanical, thermal, and cold stimuli in streptozotocin-diabetic Wistar rats, which indicates a possible reduction in painful diabetic neuropathy. Normalizing effects on lipid peroxidation, excessive production of nitric oxide, and depletion of glutathione in the sciatic nerve and central nervous system coincide with improved neurobehavioral function, implying an antioxidant and anti-inflammatory process (Elsheshtawy et al., 2026; Hou et al., 2009; Kiran et al., 2016).

Corosolic acid, oleanolic acid, asiatic acid, and similar pentacyclic derivatives are among the triterpenoids that have been identified from *L. speciosa* leaves. These compounds are well-known modulators of oxidative stress, mitochondrial function, and pro-inflammatory signaling networks. In diabetic neuropathy rat models, corosolic acid in particular has been demonstrated to have antidiabetic and neuroprotective properties, with dose-dependent reductions in neuropathic pain indices

and concurrent decreases in oxidative stress indicators. The idea that triterpenoids generated from banaba may arrest both metabolic and neurodegenerative cascades in the peripheral nervous system is supported by the fact that systemic injection of triterpenoid-rich fractions in diabetes mice also normalizes blood-glucose-related neuronal dysfunction (Upaganlawar, 2022; Okada et al., 2003).

It was demonstrated that the triterpenoids found in *L. speciosa* are capable of modulating the cellular processes involved in chronic neuropathic pain conditions through the modulation of the intracellular signaling pathways such as NF- κ B, TNF- α , and Nrf2/HO-1 signaling pathways, which are responsible for maintaining the balance between oxidative stress and cytoprotection. The involvement of the said metabolites in neurodegeneration through neuroinflammation can also be deduced from their capability to reduce the activity of pro-inflammatory cytokines such as IL-6, IL-1 β , and TNF- α both in the spinal cord and hippocampus with other triterpenoid-enriched fractions. This makes the triterpenoid-enriched fraction of *L. speciosa* leaf an ideal candidate for neuroprotection due to the fact that oxidative stress, inflammation, and apoptosis are hallmark features of neurodegeneration, including diabetes-induced neuropathy (Obloh et al., 2021; Bankar et al., 2024; Moradi et al., 2020).

*Author for Correspondence: shewale.vaishu@gmail.com

Although all these results sound promising, inadequate water solubility, rapid degradation through metabolism, and elimination processes involving transporters such as efflux pumps tend to reduce the oral availability and penetrability of triterpenoids extracted from *L. speciosa*, hence reducing the levels at which they can achieve an effective concentration within the nervous tissues. It has been shown that delivery to the brain, the half-life, and controlled release of neuroprotective agents from plants such as triterpenoids and flavonoids can be enhanced using different types of nano-delivery systems including polymer and lipid nanoparticles. Wistar rats can thus benefit from triterpenoid-rich fractions of *L. speciosa* encapsulated in nanocarriers.

Nanofabricated triterpenoid-rich fractions are usually tested against standard neurotoxic or neurodegenerative models in experimental neuroprotection study designs, such as diabetic neuropathy induced by streptozotocin, cognitive impairment induced by scopolamine, or chronic inflammatory neuropathy paradigms in Wistar rats. In addition to biochemical indicators of oxidative stress, inflammation, and apoptosis in the sciatic nerve, hippocampus, and brain, behavioral endpoints like mechanical and thermal hyperalgesia, cold allodynia, and cognitive tests are frequently used to measure neuroprotection. By differentiating the effects of the crude extract, the enhanced triterpenoid fraction, and its nano-formulation, this comprehensive assessment makes it clear if nano-encapsulation in and of itself enhances neurobehavioral and neurochemical outcomes beyond the parent fraction (Oboh et al., 2021; Bankar et al., 2024).

The proposed study on the neuroprotective activity of a triterpenoid-enriched extract of *L. speciosa* leaves and its nanoformulation in Wistar rats would be based on earlier research findings that compounds obtained from banaba plants exhibit protective properties against oxidative stress and pain due to diabetic neuropathy. However, by designing a nanoparticle system, the approach could enhance delivery of the substance to target sites within neural cells and tissues, thereby increasing its efficacy in preventing neuroinflammation through inhibition of the NF- κ B pathway, preserving mitochondrial functions, and regulating the antioxidant response element (ARE) via Nrf2. Ultimately, demonstrating the superiority of the nanotechnology-based formulation compared to the unencapsulated triterpenoid mixture would provide compelling arguments for further preclinical testing of the new treatment modality (Kamble et al., 2017).

2. Material and Methods:

2.1: Plant material collection and Extraction:

In order to ensure there would be plenty of leaves and young twigs for research, plant material of *Lagerstroemia speciosa* (Jarul/ Banaba/Pride of India) was collected from the Nashik district of Maharashtra, India, during the post-monsoon season while the plant was in full vegetative and early flowering stage. Healthy, disease-free branches with mature leaves and terminal shoots were hand-harvested from specific trees that were

dispersed across Nashik's gardens, roadside plantations, and institutional buildings; locations with significant industrial or vehicular pollution were avoided. Following collecting, extra soil and debris were removed, the plant parts were quickly rinsed with distilled water, and a representative voucher sample—which included leaves, twigs, and flowering portions—was made. It was then pressed between blotting sheets and shade-dried for preservation.

For taxonomic verification, the dried voucher was next sent to the Botanical Survey of India (BSI), ideally the regional BSI station having jurisdiction over Maharashtra, along with all the collection details such as location, date, habitat, and information about the collector. A taxonomic certification was subsequently issued by experts at the institution following morphological, macro- and microscopic examination of the voucher against herbarium specimens available in the Central National Herbarium, where necessary using molecular tools, verifying the species as *Lagerstroemia speciosa* L., family Lythraceae. For traceability and reproducibility purposes of the plant sample in question, this authentic voucher specimen ie DDGLS-1 was archived in the herbarium at the institution.

2.2 Morphological and microscopical Characterisation:

In order to establish diagnostic features for authenticity and quality control, *Lagerstroemia speciosa* leaves were characterized both macroscopically and microscopically using established pharmacognostic approaches. In order to describe common morphological features like elliptic-lanceolate, opposite, coriaceous, and glabrous foliage with a distinct midrib and venation pattern, fresh leaves were analyzed for sensory parameters like size, shape, margin, apex, base, surface texture, color, odor, and taste. These observations were recorded alongside dried leaf specimens. To verify the botanical identity and guarantee consistency of the plant material utilized in further phytochemical and pharmacological investigations, these macroscopical and microscopical characteristics were compared with published pharmacognostic and morphological data for *L. speciosa*.

2.3 Pulverization and Soxhlet Extraction:

The leaves of *Lagerstroemia speciosa*, previously dried under shade conditions, were mechanically grounded into a fine powder and passed through a standard sieve, like 40-60 mesh, in order to obtain a standardized particle size. The powder was then stored in an airtight storage container to prevent any loss due to moisture exposure. To ensure high extraction efficiency of the polar and mildly non-polar triterpenoids and phenols, the medicinal herb powder was subjected to repeated solvent extraction with a hydroalcoholic mixture of 30/70 water-to-alcohol ratio.

The rough hydro-alcoholic extract was prepared by filtration of the combined extract through Whatman filter paper, concentration of the extract under reduced pressure via rotary evaporation and drying to constant

weight after several hours of the extraction process (6-8 rotations). (Zhang et al., 2018; Abubakar et al., 2020).

2.4 Docking for Nrf2 antioxidant by Phytoconstituents of L. Speciosa:

A structure-based virtual screening was performed on an Apple workstation with an 8-core CPU running Windows XP using the Glide and QuickPro modules of Schrodinger, LLC, New York, USA, 2008. A previously published protein structure (PDB ID: 2FLU) that was acquired from the protein data bank for the docking study was used to generate the keap1 grid. The inhibitors of Nrf2-Keap1 protein-protein interaction (PPI) are identified using the high resolution (1.5 Å) co-crystal structure of the human Keap1 Kelch domain-Nrf2 peptide complex. This structure, which is accessible in PDB as 2FLU, has been used to study gedunin, a tetracyclic triterpenoid, in connection to the Nrf2-Keap1 PPI domain (Abed et al., 2015; Kamble et al., 2017).

2.5 Herbal Nano- Emulsion Formulation:

Oil-in-water or water-in-oil systems that have enhanced solubility, stability, and bioactivity of poorly water-soluble herbal extracts or essential oils can be referred to as nanoemulsion herbal formulation systems. In order to achieve small-sized droplets below 200 nanometers by kinetic stability, a herb oil or extract can be used in the oil phase together with surfactants such as Tween 20, Tween 80, and others combined with nonionic surfactants and a co-surfactant of polyethylene glycol. Spherical nanoparticles with zeta potential values of 172 nanometers were obtained from a nanoemulsion system using Alhagi maurorum essential oil and surfactants that include chitosan and Tween 80. This again emphasizes the potential for herbal nanoemulsions to be an important vehicle for transforming traditional phytochemicals to more effective and bioavailable drug products for cosmetic, anti-infective, and anticancer applications (Rahmani et al., 2024; Iskandar et al., 2024; Wang et al., 2023). Finalize formula is as below:

Table 1: L. Speciosa Nano-emulsion formula

Component	Percentage (%)
Herbal extract	5
MCT oil	5-10
Surfactant (Tween 80)	10-15
Co-surfactant (PEG 400)	5-10
Purified water q.s. to	100

2.6 Application of Design Expert: Construction of and Formula optimization using Extracts

A 2³ full factorial design was employed using Design-Expert® software to optimize the nanoemulsion formulation containing *Extracts*. Three independent variables surfactant concentration (X1=10-15%), co-surfactant concentration (X2=5-10%), and oil concentration (X3=5-10%) were selected based on their potential impact on the formulation's characteristics. The dependent variables used to evaluate performance were

particle size (Y1) and entrapment efficiency (Y2). Eight formulations were generated to cover all combinations of the selected factor levels, and each was evaluated for the defined responses. The experimental data were analyzed statistically using ANOVA and model fitting to determine the optimal conditions for achieving minimal particle size and maximum entrapment efficiency. The formulation layout for the factorial design batches was shown in Table 2. The particle size and zeta potential was calculated with Horiba instrument.

Table 2: Factor level for 2³ Full Factorial Designs

Batch Code	X1: Surfactant (%)	X2: surfactant (%)	Co- X3: oil (%)	Y1: Particle (nm)	Y2: %EE (%)
F1	10	10	5	168	82
F2	10	5	5	190	75
F3	10	5	10	125	88
F4	15	5	10	148	85
F5	15	5	5	155	81
F6	15	10	10	112	93
F7	15	10	5	110	94
F8	10	10	10	114	91

2.7 Experimental Design:

The animals were divided into many treatment groups, each consisting of six animals. Eight-week-old males were given time to acclimate to their surroundings. With a strict 12:12 h light-dark cycle and unrestricted access to food and water, the animals were kept in a

temperature-controlled environment (25±1°C, 55.5% humidity). The IAEC committee approved the experimental protocol with protocol number IAEC/1858/2025/09A. the treatment schedule was as given below

The treatment schedule for the experiment is as follows:

Group 1: Treated with $AlCl_3$ at the dose by 100 mg/kg *po*

Group 2: Treated with 100 mg/kg *Lagerstroemia speciosa* extract intranasal + $AlCl_3$ at the dose by 100 mg/kg *po*

Group 3: Treated with 300 mg/kg *Lagerstroemia speciosa* extract intranasal + $AlCl_3$ at the dose by 100 mg/kg *po*

Group 4: Treated with 100 mg/kg nanoemulsion of *Lagerstroemia speciosa* extract intranasal + $AlCl_3$ at the dose by 100 mg/kg *po*

Group 5: Treated with 300 mg/kg nanoemulsion of *Lagerstroemia speciosa* extract intranasal + $AlCl_3$ at the dose by 100 mg/kg *po*

Group 6: Treated with 10 mg/kg Levetiracetam + $AlCl_3$ at the dose by 100 mg/kg *po* (Khalil et al., 2022)

2.8 Elevated Plus Maze Test:

The Elevated Plus Maze (EPM) test was used to evaluate the anxiolytic effect of the test chemical in adult albino mice. The apparatus featured two open arms (50×10 cm) and two closed arms ($50 \times 10 \times 40$ cm) arranged in a plus (+) shape, and it was 50 cm above the ground. Every group received the treatment shown above. Each mouse was placed individually in the center of the maze, facing one of the open arms, following a half-hour intraperitoneal drug injection. The behavior of each animal was observed for five minutes. Both the number of admissions and the amount of time spent in the open and closed arms were recorded. An animal was said to have penetrated an arm when it put all four of its paws inside. Higher time spent and number of entries into the open arms were regarded as signs of anxiolytic effects. All experiments were carried out during the light period of the cycle, and the maze was cleansed with 70% ethanol before each test session to remove olfactory cues (Abu-Elfotuh et al., 2021).

2.9 Rotarod Test:

To assess motor coordination, a rotarod treadmill was employed. The subjects were trained on the treadmill for 3 days prior to the study. Preparation of the rotarod was done prior to the study. The tests were performed during the light phase of the cycle when there were stable environmental conditions. An increase of acceleration in 10 seconds was done gradually from 10 to 30 rounds per minute, while the duration of the test was set at 300 seconds. Cleaning of the maze between tests using 70% ethanol was done to remove any olfactory cues that might influence the result. In the course of the whole study, the subject stayed on the horizontal rod; the time

taken for each individual to fall off was recorded (Alavi et al., 2024).

2.10 Marble Burying Test:

Standard polycarbonate rat cages with fitted filter-top lids that are 26 cm by 48 cm by 20 cm are used for the test. Place another cage of the same size on top of the bedding to level the surface, then fill each cage with fresh, unscented mouse bedding material down to a depth of 5 cm. This also has the advantage of leaving a parallel line template that may be used to put marble on the bedding surface. Gently arrange the normal glass toy marbles, which come in a range of colors and forms, weigh 5.2 g, and have a diameter of 15 mm, in five rows of four on the bedding.

2.11 Nestlet Shredded Test:

Employ regular polycarbonate cages for rats that are 19 cm wide, 29 cm long, and 13 cm high, complete with filter coverings. Put unscented mouse bedding in each cage with a depth of up to 0.5 cm. Follow that by pressing down on the bedding by putting an identical cage over the bedding. Weigh commercial cotton fiber nestlets ($5 \text{ cm} \times 5 \text{ cm}$, 5 mm thickness, weighing about 2.5 grams each) using an analytical scale. After that, put a single nestlet on top of each test cage's bedding. Put a mouse with a single nestlet, which has already been weighed, into one cage and put a filter top on top of the cage. Do not consume any food or water during the test period. Keep the mouse alone with the nestlet in the cage for thirty minutes. After the test period, take the mouse out of the cage and put it back into its cage. Take out any unused intact nestlet from the cage with the help of the forceps and let it dry. Calculate the percentage of shredded nestlet by dividing the weight of unshredded nestlet by the weight of intact nestlet. If required, score the subject mouse's nest's shape using a particular 5-point nest rating scale¹⁷, being in mind that this scale is usually designed to assess nest creation rather than obsessive shredding. Throw away any leftover bedding and shredded nestlet material (Pyatha et al., 2023).

3. Results and Discussion:

3.1 Plant material collection and Extraction:

plant material of *Lagerstroemia speciosa* (Jarul/Banaba/Pride of India) was collected from the Nashik district of Maharashtra, India and authenticated by BSI with voucher no DDGLS-1.

3.2 Morphological and microscopical Characterisation:

The morphology and microscopy was studied for *Lagerstroemia speciosa* and results were as follows:

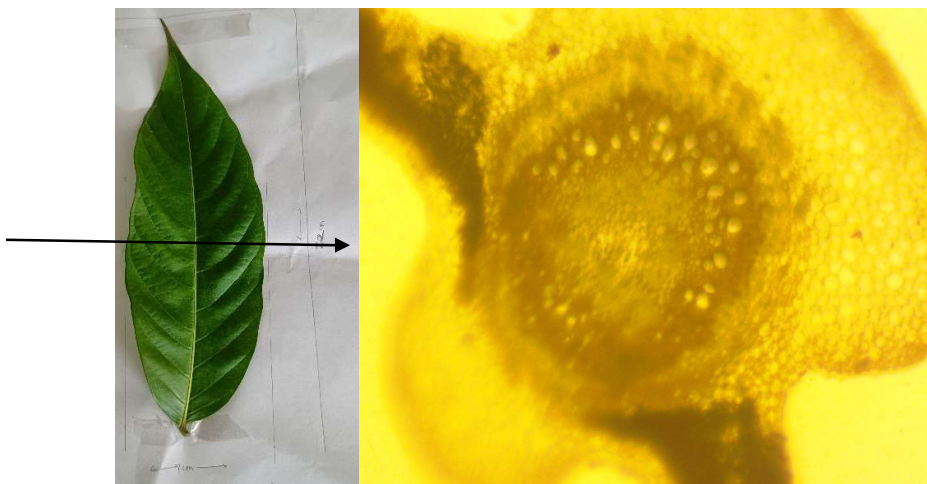


Figure 1: Morphology and Microscopical characteristics of *L. Speciosa*

Table 3: Morphology of *Lagerstroemia speciosa*:

Parameter	Typical for <i>Lagerstroemia speciosa</i>
Leaf type	Simple leaves, entire margin
Arrangement (on twig)*	Opposite
Shape	Elliptic to oblong-lanceolate
Apex	Acute to acuminate
Base	Cuneate to attenuate
Margin	Entire
Size (approx.)	Large, 8–20 × 3–9 cm
Texture	Coriaceous, glabrous
Colour	Dark-green upper, paler lower
Venation pattern	Strong midrib, closely spaced curved lateral veins
Surface characters	Entire, non-pubescent lamina

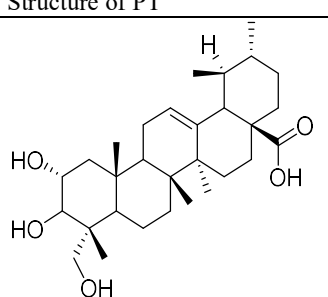
Microscopical analysis shown Epidermis is single layered, cutinized, and protective in nature. Cortex is broad, parenchymatous with intercellular spaces. Vascular bundles are conjoint, collateral, open, and arranged in a ring with distinct xylem and phloem. Central pith is large, parenchymatous, and meant for storage.

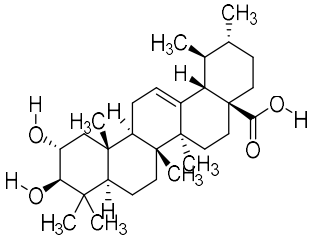
3.3 Extraction:

The percentage extraction of the hydro-alcoholic extract was determined to be 23%, implying that a large amount of the solubilizable plant compounds had been extracted from the powdered plant. The percentage yield of the extract was calculated relative to the dried plant material.

3.4 Docking for potential phytoconstituents:

Table 4: Docking of phytoconstituents on NrF2 receptor

Sr. No	Name of PT	Structure of PT	Glide score	Glide emodel	Glide energy
1	Asiatic acid		-8.13	-36.337	-29.684

2	Corosolic acid		-8.63	-54.069	-39.293
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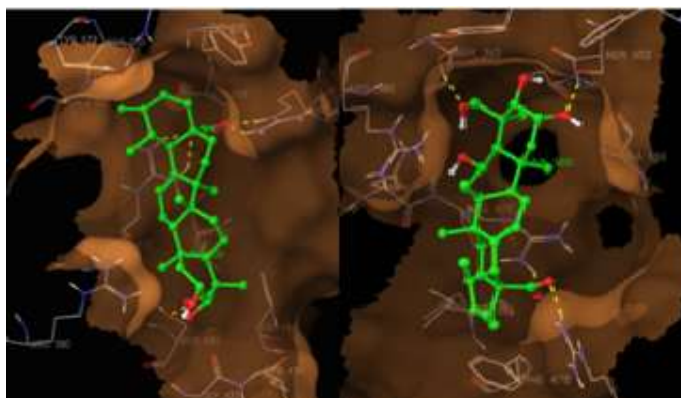


Figure 2: Surface interaction of Asiatic acid Corosolic acid with NrF2 protein

The combination of multiple H-bonds (especially involving two VAL residues and ARG) plus a hydrophobic cage suggests good complementarity between the ligand and the binding pocket. The binding mode is consistent with what would be expected for a rigid triterpenoid — the scaffold locks into the hydrophobic core while the polar functional groups (COOH, OH) point toward H-bond donors/acceptors at the pocket rim.

Herbal nanoemulsion was prepared successfully with analysis of response surface plot particle size of the Nanoemulsion is one of the crucial elements because the rate and efficiency of the Extract release and its absorption depend on it. The smaller particle size provides a higher surface area for Extracts to absorb. In order to calculate the polydispersity index, the average particle size, solvent refractive index, angle, and dispersion should be considered. Small polydispersity index values indicate a high level of homogeneity in particles, while large ones point to a broad distribution or several different populations.

3.4 Herbal Nano- Emulsion Formulation:

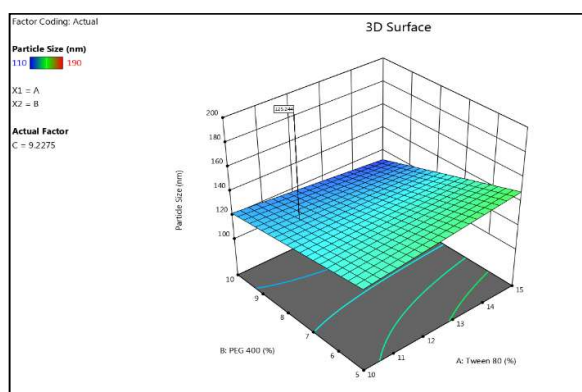


Figure 3: DOE Analysis of particle size surface response plot

3.5 Experimental

3.5.1 Elevated Plus maze:

In the elevated plus maze study, the negative control group displayed the least amount of open arm entries due to their higher level of anxiety-related behavior. The positive control, levetiracetam, elicited a significant rise in the percentage of time spent in the open arm, followed

by the nanoemulsions of *Lagerstroemia speciosa* extract at concentrations of 100 mg/kg and 300 mg/kg. The raw extract at the same dose of 100 mg/kg and 300 mg/kg demonstrated some increase in open arm activity; however, the effects were less compared to those of the nanoemulsions. There was a steady day-wise increase in all groups of animals from day 0 to day 6.

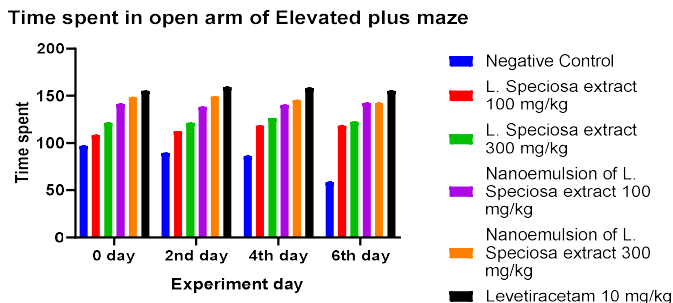


Figure 4: Effect of L. Speciosa extract and its nanoemulsion of elevated plus maze test

Elevated plus maze testing has often been employed for evaluating both exploratory behavior and anxiety-related behavior. Increased time spent in the open arms usually means low levels of anxiety and good CNS activity. From the graph above, it can be observed that there was a dose- and time-dependent response, meaning the leaves of Lagerstroemia speciosa are helpful for improving behavior in the central nervous system. However, it should be noted that the formulation method of nanoemulsion had a better result compared to the non-formulated extract. This is because of better solubility, absorption, and penetration into the brain of the phytoconstituents.

This trend also shows that the nanoemulsion in a dose of 300 mg/kg demonstrated almost equivalent efficacy as the conventional drug. This proves that the nanoemulsion is a promising drug for clinical application. Since the nanoemulsion is superior to the crude extract in terms of efficacy, it can be assumed that

improving the drug delivery method could play an important role in increasing the pharmacological properties of the drug. This is crucial information for future neuroprotection studies, since the efficiency of plant extract drugs depends on the effectiveness of the drug delivery method used.

3.5.2 Rotarod Test:

In the graph, latency time (seconds) is plotted against different time points such as Day 0, 2nd, 4th, and 5th day using six experimental groups including negative control, crude L. speciosum at 100 mg/kg and 300 mg/kg, nanoemulsion L. speciosum at 100 mg/kg and 300 mg/kg, and levofloxacin at 10 mg/kg as the standard. The graph represents that there is an initial uniformity in the case of negative control group on Day 0, followed by a dose-dependent increase in all other experimental groups along with significant superiority of nanoemulsion over crude at equal doses by Day 5.

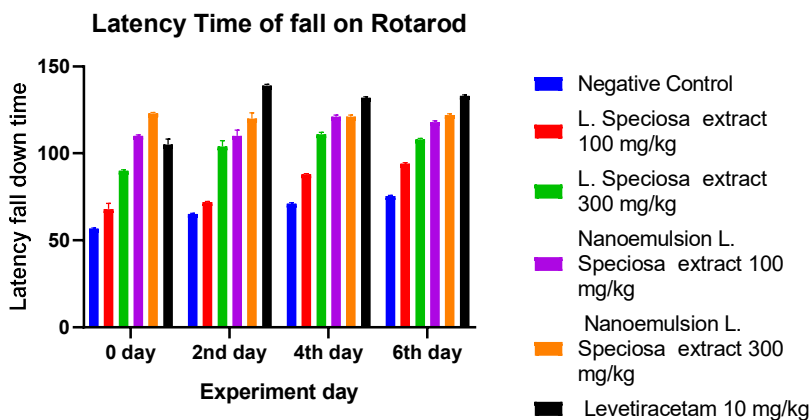


Figure 5: Effect of L. Speciosa extract and its nanoemulsion of Rotarod test

3.5.3 Marble burying test:

Results show accurate values for group means, highlights the dose-dependent reduction in marble-burying behavior induced by crude extract, the significant increase in efficacy of both nanoemulsion doses, and the close-to-normal behavior exhibited by

Nanoemulsion 300 mg/kg relative to Levetiracetam. provides context for results within the neuropharmacological basis of marble-burying, explains the increased bioavailability conferred by nanoemulsification, and makes comparisons to the mechanism of Levetiracetam.

*Author for Correspondence: shewale.vaishu@gmail.com

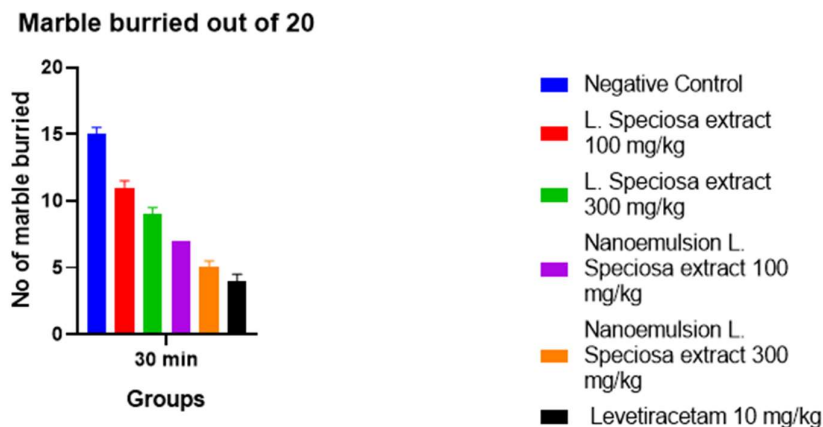


Figure 6: Effect of *L. Speciosa* extract and its nanoemulsion of marble burying test

3.5.4 Nestlet Shredding:

systematically presents the step-wise decrease in % nestlet shredded, describes the dose-response curve for the crude extract, and compares the better effectiveness of both nanoemulsion doses when compared to the respective crude extracts. describes the nestlet shredding test as an OCD-related animal model, provides the

neuropharmacological background (GABAergic system, 5-HT system, CSTC pathway), discusses the rationale behind the nanoemulsion being more effective (blood-brain barrier crossing, lymphatic uptake, avoiding the first-pass effect), and compares it to Levetiracetam with its SV2A action.

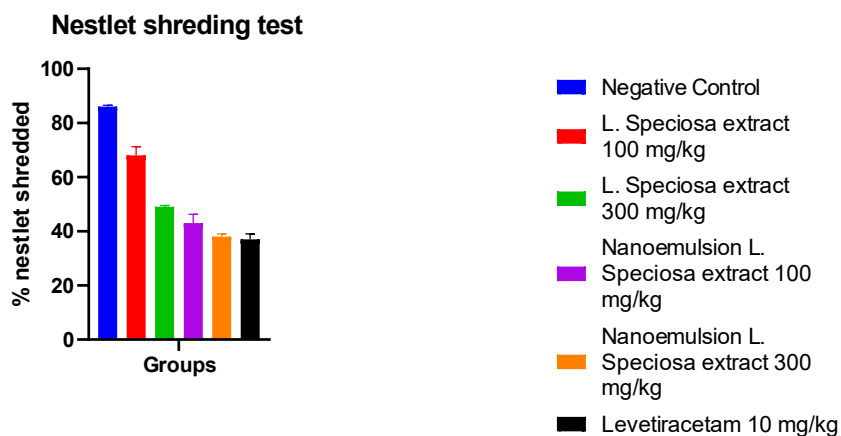


Figure 7: Effect of *L. Speciosa* extract and its nanoemulsion of Nestlet shredding test

4. Conclusion:

This Elevated plus maze test shows that the extract of *Lagerstroemia speciosa* enhances the exploratory behavior in the open arm of the elevated plus maze test, while the nanoemulsion formulation amplifies this efficacy even more of all groups tested, those treated with the combination of levetiracetam and nanoemulsion had the most promising results, thereby proving the superiority of nanoformulations compared to the mere extract. Interprets results from rotarod test shown that a phytochemical perspective (flavonoids, terpenoids and alkaloids), pharmacokinetic considerations of nanoemulsion (size of droplets, passage through BBB, enhancing bioavailability), and underlying mechanisms of rotarod performance enhancement. For example, in a marble burying test, the findings reveal that the

nanoemulsion formulation enhances anticompetitive behavior. In addition, four potential future research pathways are identified based on the key finding (the role of nanoemulsions in enhancing anti-obsessive properties). as demonstrated by the Nestlet Shredding test conducted on mice that consolidates evidence presented, emphasizes the convergence of findings in both models through the marble burying test and proposes 5 distinct avenues for further investigation in the future.

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