

# Antioxidant and Anti-inflammatory Activity Screening of *Lasia spinosa* Rhizome and its Validation using a Computational Simulation Approach

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## ABSTRACT

**Background:** The present work assesses the antioxidant and anti-inflammatory effect of *Lasia spinosa* rhizome extracts on *in-vitro* and *in-vivo* models compiled through molecular docking study of plant-steamed phytocompounds with specific targets. **Materials and Methods:** In this study, *Lasia spinosa* rhizome was subjected to extraction using petroleum ether, ethyl acetate and methanol and the extracts were analyzed by GC-MS. Antioxidant was assessed using *in-vitro* methods such as DPPH scavenging activity and H<sub>2</sub>O<sub>2</sub> scavenging activity; anti-inflammatory activity was assessed using both *in-vitro* and *in-vivo* and molecular docking utilizing Auto dock 4.0 was done. **Results:** Tests showed that methanolic extract (MELS) has the most important dose-dependent antioxidant and anti-inflammatory efficacy at various levels. Of all compounds, Morin reported the most successful docking ranking of -8.2 to -9.8, maintaining a good binding fondness between protein and ligand. **Conclusion:** Antioxidant and anti-inflammatory of *Lasia spinosa* may be inferred from the examinations. The *in-vitro*, *in-vivo* and *in silico* assays of *L. spinosa*. Morin is confirmed by the information as a beneficial antioxidant and anti-inflammatory agent that can aid future clinical assessments.

**Key words:** *Lasia spinosa*, Antioxidant, Anti-inflammatory, *In silico*, Auto dock.

## INTRODUCTION

The natural reaction to external threats such as bacteria, viruses and parasites is inflammation.<sup>1</sup> Inflammation study is one of the major fields of public health science<sup>2</sup> owing to the side-effects of widely used NSAIDs such as renal suppression and gastrointestinal disorders like ulcers. Arachidonic acid conversion of prostaglandin (PGs) involves cyclooxygenase-1 (COX-1 and cyclooxygenase-2 (COX-2, respectively). COX-2 is not usually present and is elevated by carcinogenic or pro-inflammatory cytokines in human tissues.<sup>3</sup> Latest studies indicate that natural compounds suppress inflammation through selective COX-2 inhibition.<sup>4</sup>

Plants are known as a biologically active chemicals source and for antioxidant and anti-inflammatory function plants have been studied. *Lasia spinosa* have been used in folk medicine since ancient times, owing to anti-inflammatory, anthelmintic, emenagogue, antioxidant, anti-diabetic, antimalarial activities.<sup>5,6</sup> The present research aims to predict bioactive compounds in methanol extract from *L. spinosa* by GC-MS study. Methanolic crude *Lasia spinosa* extract's (MELS) anti-inflammatory behavior and antioxidant function was studied and tested using a numerical simulation methodology. The ADMET property and bioactivity score of the compound was checked to evaluate drug-like characteristics of chosen substances. Docking aims to integrate active

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phyto-compounds with oxidation and inflammation-enzymes such as cytochrome P450, lipoxygenase, myeloperoxidase, xanthine oxidase and active COX-2 sites. This methodology indicates a systemic framework for potential drug design utilizing active plant metabolites.

## MATERIALS AND METHODS

### Plant Material Collection and Storage

The rhizome of *Lasia spinosa* were collected from Kommulamamidi, near Kottapalli village, Paderu in Visakhapatnam (Andhra Pradesh) in November-December 2018. Prof S.B. Padal authenticated the plant materials, Department of Botany (Voucher No: 23302), Andhra University, Visakhapatnam. The collected plants were separated and its rhizome parts are processed. The collected rhizome was sliced and dried under shade for 10 days, coarsely powdered and passed through sieve no. 40 and stored in an airtight container for further study.

### Extraction of Plant material and GC-MS analysis

A 4000 mL solvent was packed with 1000 g of dried plant material. A serial non-polar to polar phytochemicals extraction was subsequently conducted utilizing petroleum ether, ethyl acetate and methanol by maceration. By the use of a 60°C rotary evaporator methanol extract was concentrated under vacuum.

### Phytochemical Analysis

The different extracts of *L. spinosa* underwent initial phytochemical testing for the recognition of chemical components according to the standard operating procedures.<sup>7,8</sup>

### Antioxidant activity

#### DPPH Assay

A slightly modified 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay<sup>9</sup> calculated the free radical scavenging activity of each extract. New methanolic DPPH solution (0.2 mM) in the dark 2 hr before examination has been packed and incubated. In their respective extraction solvent, crude extract and even normal ascorbic acid have been dissolved independently. The 0.05 ml of each solution from each sample was then moved to the 96-well plates at varying concentrations. The methanol solution of DPPH (0.195ml) was added to any well through the use of a multichannel pipette. After 1 hr of dark incubation, the absorption of the resulting mixture was measured with a micro plate reading of 540 nm. DPPH Radical scavenging activity was calculated using the following equation as as the

ascorbic acid equivalent by gram dry weight (mg TE / g dry weight):

$$\text{DPPH radical scavenging activity} = \frac{\text{Absorbance}_{\text{blank}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{blank}}} \times 100$$

### Hydrogen peroxide Assay

Dehpour's updated approach calculated the extract behavior for scavenging in relation to hydrogen peroxide radicals. The solution for hydrogen peroxide (40Mm) was made using the pH 7.4 phosphate buffer and its amount was measured using a UV spectrophotometer to determine absorbance at 560 nm. Add 0.1mg / ml of the extract and absorption, determined by using a UV spectrophotometer at 560 nm, to a blank solution with a hydrogen peroxide free phosphate tampon. The percentage of hydrogen peroxide scavenging extract and normal compound was determined with the formula:

$$\text{Hydrogen peroxide scavenging activity} = \frac{\text{Absorbance}_{\text{blank}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{blank}}} \times 100$$

### Identification of the phyto-compound through GC-MS analysis

The GC-MS analysis was conducted for the purpose of profiling the pharmacologically active portion present in methanol extract. Initial temperatures of 70 °C were sustained for 2 min, elevated higher at 6°C / min, to 260°C and held for 9 min. As a carrier gas, helium was used and a flow rate of 1 mL / min was attained. The volume of samples extracted contained 1 µL of extract.<sup>7</sup> A reference for the calculation of important peaks in mass spectrometry was screened in the Library of NIST.

### Animals

The Animal House of Andhra University, Visakhapatnam, were the source of the Wistar rats (100-150 g). It is kept in polypropylene containers, feeding the rodent with a daily diet of about 24 ± 2 centimeters in water, 12 hr in light / night, 35-60% moisture and adds *libitum* content. No food but water deprived animals 4 hr before the experiment.

### Acute toxicity studies

#### Anti-inflammatory activity screening of MELS

#### In vitro Model

#### Membrane Stabilization Assay

The anti-inflammatory function of various *Lasia spinosa* extracts. The HRBC membrane stabilization process for *in vitro L. spinosa* has been evaluated.

Blood was collected from rats and has been blended and centrifuged to isosaline in the same amount of Alsever. The same concentration of the research drug at three separate amounts of 100, 200 and 300 µg / mL has been applied to 1 mL of HRBC suspension. Both research blends were incubated and centrifuged at 37°C for 30 min. By using the spectrophotometer at 560 nm, the hemoglobin content of the solution was estimated. The percentage of hemolysis was measured using a spectrophotometer of 560 nm<sup>10</sup> as seen below hemoglobin content of the supernatant solution. The hemolysis percentage was then determined according to the following formula:

$$\% \text{ of protection} = 100 - \left( \frac{\text{OD of test}}{\text{OD of control}} \right) \times 100$$

Here “OD of test” is optical density or the test sample’s absorbance and “OD of control” is optical density or absorbance of the negative control.

Therefore, Alsever’s blood solution and no aspirin or methanol extract of the plant substance are used as a negative control in this event.

#### **In vivo Model**

##### **Carrageenan Induced Paw edema**

The mechanism for the *in vivo*, anti-inflammatory behavior of methanol extract has been used for Carrageenan-caused rat paw edema.<sup>11</sup> The rats were divided into 5 classes ( $n = 6$ ), each receiving distilled water (control), 50, 100, 200 mg/ kg of MELS and diclofenac of 20mg/kg of p.o as reference standard, respectively. In the right hind-paw subplantary tissue of each rodent, carrageenan (0.1 mL of 1 percent) had been injected. The injection volume of carrageenan into the foot was measured with a plethysmometer for 0, 30, 60, 120 and 180 min. The inhibition percentage (PI) was calculated at each interval.<sup>12</sup>

$$PI = \frac{(V_t - T_0)\text{control} - (V_t - V_0)\text{treated}}{(V_t - V_0)\text{control}} \times 100$$

$V_0$  = Mean paw volume at 0 hrs

$V_t$  = Mean paw volume at a particular time interval

#### **Statistical Analysis**

All information was revealed as the mean  $\pm$  S.D; information went through one-way ANOVA adhered to by Turkey examination. The analytical evaluation executed with Graphpad Prism (Version 3, U.S.A.) software program.  $P < 0.05$  was taken into consideration statistically considerable.

#### **Molecular Docking**

The docking studies of compounds 3-Carene, 4-hydroxy benzoic acid, Alpha pinene, Apigenin, Camphor, Cinnamic acid, Genistic acid., Morin and Syringic acid were carried out using Autodock 4.0 and Discovery studio Biovia 2019 software to find out the interaction between ligand and the target protein. The crystal structure of enzymes (receptors) that react during metabolism to the development of reactive oxygen species (ROS) are cytochrome P450 (PDB ID: 1OG5), lipoxygenase (PDB ID: 1N8Q), myeloperoxidase (PDB ID: 1DNU) and xanthine oxidase (PDB ID: 3NRZ) was derived from the protein data bank, along with pro inflammatory gene i.e., COX-2 (PDB ID: 5IKR).

#### **ADMET Screening**

ADMET of the ligands is pharmacokinetic properties calculation that is required to be examined to establish their function inside the body. The ADMET inheritance of the ligands was studied, making use of admetSAR.<sup>13</sup>

## **RESULTS**

#### **Phytochemical Screening of the extract**

Initial phytochemical testing of *L. spinosa* exposed different phytoconstituents detailed in Table 1.

#### **Antioxidant activity**

Extracts were tested with DPPH free radical scavenging and  $H_2O_2$  radical scavenging tests for their potential antioxidant function.

#### **DPPH free radical scavenging assay**

DPPH is a stable free radical which shows maximum ultraviolet and visible absorbance at 517 nm. It gets reduced in presence of antioxidant present in the sample which is considered as a measure of their antioxidant activity. The ability of samples to scavenge DPPH radical was measured on the bases of their concentrations providing 50% inhibition ( $IC_{50}$ ). The results of one way ANOVA test and post hoc test indicates significant difference of mean percentage scavenging between different concentrations of tested extracts. The scavenging activity of extract was well pronounced at higher concentrations of 80 mg/ml and 100 mg/ml with a mean percentage of  $50.89 \pm 0.58$  and  $56.22 \pm 1.41$  for MELS respectively, which was lower, compared with standard antioxidant, ascorbic acid but exhibits similar pattern of concentration dependent free radical

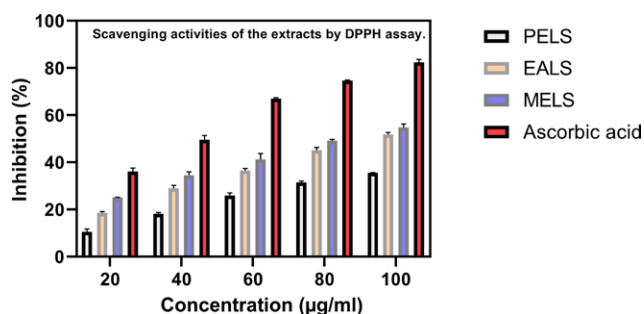
**Table 1: Phytochemical analysis of various extracts of *L. spinosa*.**

Phytoconstituents	Method	Methanol Extract	Ethyl acetate Extract	Pet. Ether Extract
Flavonoids	Shinoda Test	+	+	-
	Zn. Hydrochloride test	+	+	-
	Lead acetate Test	+	+	-
Volatile oil	Stain test	+	-	+
Alkaloids	Wagner Test	+	-	-
	Hager's Test	+	-	-
Tannins and Phenols	FeCl <sub>3</sub> Test	+	+	-
	Potassium dichromate test	+	+	-
Saponins	Foaming Test	+	-	-
Steroids	Salkowski test	+	-	+
Carbohydrates	Molish test	+	-	-
Acid compounds	Litmus test	-	-	-
Glycoside	Keller-Killani Test	+	-	-
Amino acids	Ninhydrin test	+	-	-
Proteins	Biuret test	+	-	-

"++" – Present; "-" – Absent

**Table 2: Dose-dependent DPPH free radical scavenging and H<sub>2</sub>O<sub>2</sub> radical scavenging activity of different extracts of *L. spinosa*.**

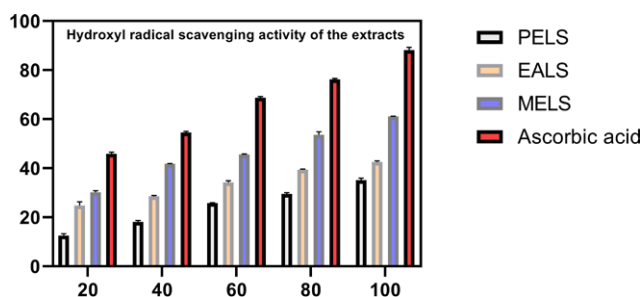
Method	Treatment	Absorbance wavelength (nm)					IC <sub>50</sub>
		20	40	60	80	100	
DPPH free radical scavenging (% Inhibition)	PELS	10.52±1.16	18.15±0.55	25.85±1.21	31.42±0.64	35.54±0.12	141.17
	EALS	18.55±0.58	28.95±1.22	36.52±0.89	45.12±1.15	51.78±0.84	93.42
	MELS	24.55±0.11	35.82±1.52	43.82±2.52	50.89±0.58	56.22±1.41	79.73
	Ascorbic acid	31.21±1.44	44.32±1.82	61.17±0.44	69.24±0.25	81.12±1.25	16.06
H <sub>2</sub> O <sub>2</sub> scavenging activity (% Inhibition)	PELS	12.52±0.75	18.12±0.52	25.78±0.15	29.55±0.52	35.11±0.88	141.78
	EALS	24.75±1.52	28.52±0.38	34.22±0.69	39.41±0.21	42.58±0.41	93.42
	MELS	30.22±0.58	41.82±0.05	45.63±0.15	53.66±1.18	61.12±0.08	79.73
	Ascorbic acid	48.89±0.68	54.58±0.47	68.67±0.52	76.22±0.41	88.12±1.14	28.52

**Figure 1: Scavenging activities of the extracts by DPPH assay. The results are expressed as mean ± SD (n=3).**

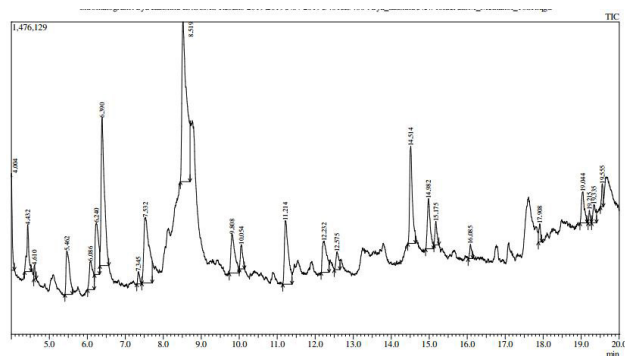
scavenging effect. The IC<sub>50</sub> values of PEELS (151.06), EALS (129.16), MELS (69.53) and ascorbic acid (28.52) were obtained using the linear regression equation. The radical scavenging ability, IC<sub>50</sub> of extracts and ascorbic acid were presented in Table 2 and Figure 1.

#### **H<sub>2</sub>O<sub>2</sub> radical scavenging assay**

Table 2 demonstrates the extract's scavenging potential on H<sub>2</sub>O<sub>2</sub>. The extract showed the dose-dependent behavior of hydrogen peroxide radical scavenging. In contrast with other samples, hydrogen peroxide radical



**Figure 2: Scavenging activities of the extracts by  $H_2O_2$  assay. The results are expressed as mean  $\pm$ SD ( $n=3$ ).**

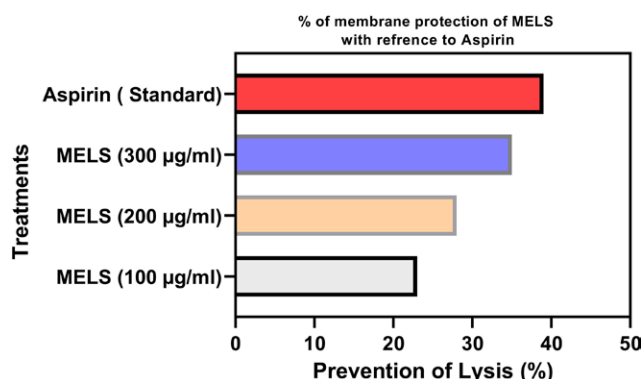


**Figure 3: GC Spectra of *Lasia spinosa* methanol crude extract.**

scavenging behaviors of MELS were significant at all concentrations. Similar dose dependent scavenging was observed for standard, ascorbic acid. The  $IC_{50}$  value of the extract was 69.53 mg/ml whereas for ascorbic acid it was 28.52 mg/ml using linear regression equation (Figure 2).

#### GC-MS analysis

The amount of methanol extract yield was 8.3%. Figure 3 reveals the main peak found in the methanol sample and the NIST Library was referred to classify each compound based on the peak area. Table 3 demonstrates bioactive compounds in *Lasia spinosa* methanol extract. Based on the GC-MS spectra, the



**Figure 4: The bar chart above represents the percentage of membrane protection of the MELS with a reference to Aspirin 100 µg/mL as standard.**

active compounds in the extract were classified as 3-Carene, 4-Hydroxy Benzoic Acid,  $\alpha$ -Pinene, Apigenin, Camphor, Cinnamic Acid, Genistic Acid, Morin and Syringic acid (CAS). Table 3 also shows molecular formulae, molecular weight and area percent of major compounds.

#### Membrane stabilization assay

MELS at a range of 100  $\mu$ g / mL to 300  $\mu$ g / mL prohibits hypotonic lysis of the human erythrocyte membranes. The RBC hemolysis was blocked with the extract at 100  $\mu$ g / mL at 23.45% compared with 39.12% for 100  $\mu$ g / mL of aspirin. Because the lysosomal membrane components of red human blood cells are similar to each other, the test of anti-inflammatory drug action was the avoidance of HRBC-induced hypotonicity membrane lysis. Results show that MELS can substantially inhibit and change the dose to HRBC hemolysis (Figure 4).

#### Carrageenan-induced paw edema model

The MELS (50, 100, 200 mg/kg, p.o.) demonstrated substantial dose-dependent reduction of carrageenan-induced rat paw edema from 0.5 hr to 3 hr after

**Table 3: Biological active compounds derived from *L. spinosa*.**

S. No	Retention Time	Compound name	Canonical Smiles	Area / Height (%)	Molecular formula	Molecular weight
	11.214	Morin	<chem>C1=CC(=C(C=C1O)O)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O</chem>	2.38	$C_{15}H_{10}O_7$	302.23
	12.575	Syringic acid	<chem>COc1cc(C(O)=O)cc(OC)c1O</chem>	0.49	$C_9H_{10}O_5$	198.173
	19.044	Apigenin	<chem>Oc(cc1)ccc1C(Oc1c2c(O)cc(O)c1)=CC2=O</chem>	0.86	$C_{15}H_{10}O_5$	270.239
	19.335	Genistic acid.	<chem>CC(OC(cc1)cc(C(O)=O)c1OC(C)=O)=O</chem>	0.48	$C_{11}H_{10}O_6$	238.194
	23.170	4-Hydroxy benzoic acid	<chem>OC(c1cc1)ccc1O=O</chem>	0.17	$C_7H_6O_3$	138.122
	23.407	3-Carene	<chem>CC1(C)[C@@H]2[C@H]1CC=C(C)C2</chem>	0.43	$C_{10}H_{16}$	136.237
	24.211	Camphor	<chem>CC(C)([C@@H](CC1)C2)[C@H]1(C)C2=O</chem>	0.03	$C_{10}H_{16}O$	152.236
	26.918	Cinnamic acid	<chem>OC/C=C/c1ccccc1=O</chem>	0.43	$C_9H_8O_2$	148.161

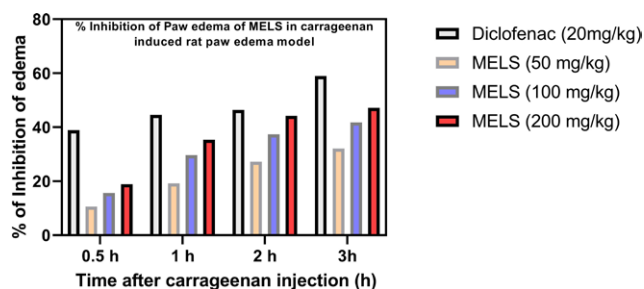


**Table 4: Effect of MELS with carrageenan-induced paw edema in rats.**

Groups	Before	Paw Volume (mL)				
		0 h	0.5 h	1 h	2 h	3 h
Distilled water	0.96±0.02	1.02±0.02	1.62±0.02	1.89±0.02	2.01±0.02	1.95±0.02
Diclofenac 20 mg/kg	0.98±0.03	1.05±0.04	1.42±0.03* (38.93)	1.54±0.01* (44.54)	1.59±0.03* (46.44)	1.44±0.01* (58.99)
MELS 50 mg/kg	0.96±0.02	1.04±0.05	1.58±0.04 (10.6)	1.75±0.02 (19.26)	1.77±0.02 (27.25)	1.68±0.03 (32.11)
MELS 100 mg/kg	0.94±0.03	1.01±0.03	1.52±0.03 (15.6)	1.63±0.01 (29.60)	1.64±0.01 (37.35)	1.56±0.02 (41.79)
MELS 200 mg/kg	0.92±0.04	0.97±0.04	1.46±0.03* (18.93)	1.54±0.03* (35.35)	1.53±0.02* (44.24)	1.47±0.01* (47.16)

Values are presented as the mean ± SEM,  $n = 6$  in each group; values given in parentheses represent PI. One-way ANOVA followed by multiple Tukey's comparison test.

\* $p < 0.05$ , as compared to the control group.



**Figure 5: Inhibition of carrageenan-induced rat paw edema by Diclofenac and MELS measured at 0.5, 1, 2 and 3 h after carrageenan injection.**

drug administration relative to control group. MELS recorded average PI of paw edema at concentrations of 50, 100, 200 mg / kg p.o. as 18.93, 35.35, 44.24 and 47.16 ( $P < 0.05$ ) (Table 4, Figure 5).

### Molecular Docking Studies

In order to identify a prospective candidate for managing antioxidant and anti-inflammatory activity, molecular docking was executed over nine phytoconstituents acquired from *L. spinosa* on the binding pocket of targets. Regarding *in-silico* antioxidant activity, Morin, apigenin and Genistic acid. Displayed best docking score, among the phytochemicals. Rest of the compounds i.e., 3-Carene, 4-hydroxy benzoic acid, Alpha pinene, Camphor, Cinnamic acid and Syringic acid also interacted well with targets showing moderate binding energies (Tables 5-10 and Figures 6-10).

Total of 3 compounds was selected based on the binding interactions with 5IKR. Out of the three compounds, Morin exhibited the best-docked score (-9.3 Kcal/mol) with COX-2(5IKR), followed by apigenin and Genistic acid. With binding affinity of -9.1 and -6.8 kcal/mol.

### Drug likeliness

The physicochemical properties of the chosen nine active compounds were studied on DruLiTo software.

**Table 5: Molecular docking of selected compounds with selected targets related to antioxidant activity.**

Sr. No	Drug	Dock Score (Kcal/mol)			
		1OG5	1N8Q	1DNU	3NRZ
1	3-Carene	-5.6	-5.6	-5.2	-5.7
2	4-hydroxy benzoic acid	-5.9	-6.2	-6.1	-7
3	Alpha pinene	-5.5	-5.8	-4.9	-5.4
4	Apigenin	-8.5	-8.1	-9.8	-7.8
5	Camphor	-5.8	-5.8	-5	-6.1
6	Cinnamic acid	-6	-5.8	-7.4	-6.1
7	Gensticacid	-6.8	-6.3	-7.2	-6.2
8	Morin	-8.5	-8.2	-9.8	-8.2
9	Syringic acid	-6	-5.8	-6.8	-5.6

**Table 6: Interactions of Cytochrome P450 (PDB ID: 1OG5) amino acid residues with ligands at receptor sites.**

Ligands	Binding Affinity, $\Delta G$ (Kcal/mol)	Amino acids involved and Distance (Å)	
		Hydrogen Binding Interactions	Hydrophobic Interactions
Apigenin	-8.5	VAL A:113 (4.67), ASN A:217 (5.25, 5.43), GLNA A:214 (4.19), LEU A:208 (4.10)	ALA A:103 (5.08, 5.11), PHE A:114 (6.14), LEU A:366 (5.25), PRO A:367 (5.30)
Gensticacid	-6.8	SER A:365 (3.54), PHE A:428 (6.31), ARG A: 433 (4.20, 6.50), ARG A:97 (5.27)	LEU A:366 (5.32), SER A: 429 (4.14)
Morin	-8.5	LEU A:208 (4.15), GLN A:214 (4.87), ASN A:217 (5.19)	PRO A:367 (5.33), PHE A:114 (6.08), LEU A:366 (5.38), ALA A:103 (4.90, 4.97), LEU A: 208 (6.32)

**Table 7: Interactions of lipoxygenase (PDB ID: 1N8Q) amino acid residues with ligands at receptor sites**

Ligands	Binding Affinity, $\Delta G$ (Kcal/mol)	Amino acids involved and Distance (Å°)		
		Hydrogen Binding Interactions	Hydrophobic Interactions	Electrostatic Interactions
Apigenin	-8.1	ARG A:200 (3.47, 4.01), ASP A:158 (4.79), GLY A:189 (4.22)	ILE A:201 (5.45)	-
Gensticacid	-6.3	ARG A:200 (4.75, 4.09, 3.44), ASN A:186 (4.90)	-	-
Morin	-8.2	VAL A:256 (5.02), THR A:274 (3.79), TYR A:275 (5.00)	LEU A:258 (6.54), ALA A:263 (4.87), PHE A:272 (6.45)	ARG A:260 (5.39, 7.42)
Syringic acid	-5.8	ARG A:200 (4.80), SER A:157 (4.11)	LYS A:156 (4.88)	-

**Table 8: Interactions of myeloperoxidase (PDB ID: 3NRZ) amino acid residues with ligands at receptor sites**

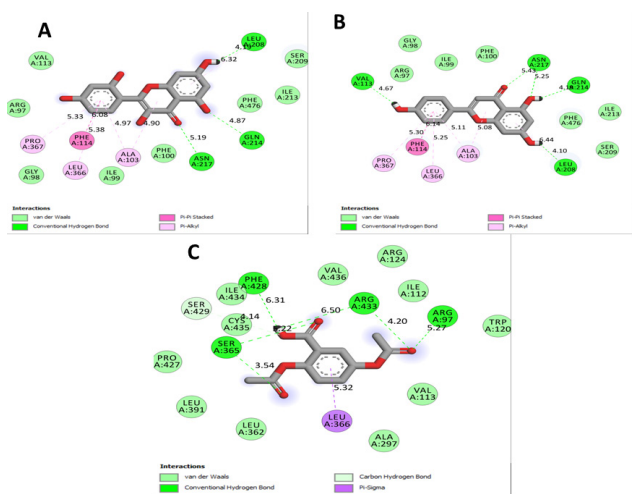
Ligands	Binding Affinity, $\Delta G$ (Kcal/mol)	Amino acids involved and Distance (Å°)		
		Hydrogen Binding Interactions	Hydrophobic Interactions	Electrostatic Interactions
Apigenin	-7.8	ILE C:1229 (3.50)GLU A:45 (4.89)	LEU A:147 (6.38), ALA C:1231 (6.39), GLY A:46 (5.65), ALA B:338 (4.84)	LYS C:1228 (5.22, 6.56)
Gensticacid	-6.2	SER A:69 (3.44), LYS B:340 (3.84), LYS B:343 (4.52)	LYS B:340 (4.11)	-
Morin	-8.2	ILE C:1229 (3.61)	ALA C:1231 (4.91, 4.99, 6.67), LEU A:147 (6.02)	LYS C:1228 (5.44, 6.08)

**Table 9: Interactions of xanthine oxidase (PDB ID: 1DNU) amino acid residues with ligands at receptor**

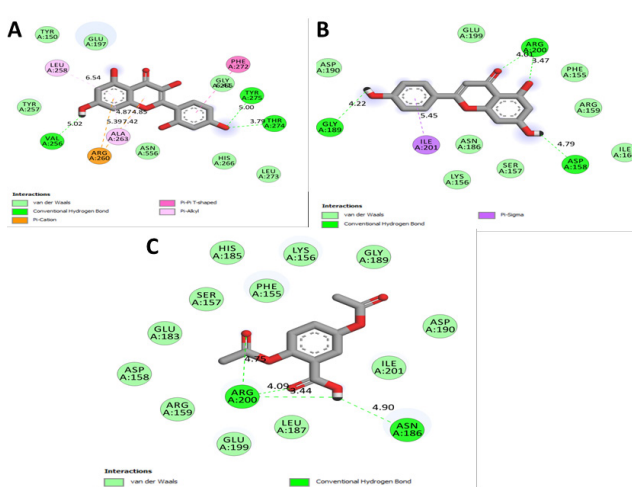
Ligands	Binding Affinity, $\Delta G$ (Kcal/mol)	Amino acids involved and Distance (Å°)		
		Hydrogen Binding Interactions	Hydrophobic Interactions	Electrostatic Interactions
Apigenin	-9.8	GLY A:335 (3.51), ARG C:333 (4.08), ASP A:98 (4.93), GLN A:91 (5.18)	GLY A:90 (6.00), ARG A:333 (5.13)	HIS A:95 (6.40), HIS C:336 (5.54), MET A:87 (7.45), ASP A:94 (5.28, 5.72)
Gensticacid	-7.2	HIS A:95 (5.52), THR C:329 (4.36), ARG C:333 (3.85), ASP A:94 (5.06)	ARG C:239 (4.05), ARG C:333 (4.97)	ASP A:94 (5.32), HIS C:336 (5.03)
Morin	-9.8	THR C:329 (3.59), ARG A:333 (4.23), PHE C:332 (3.66), MET A:87 (3.44)	GLY A:90 (5.98)	ARG C:239 (6.55), ASP A:94 (6.11, 5.41), HIS C:336 (5.36), HIS A:95 (6.60)

**Table 10: Interactions of xanthine COX-2 (PDB ID: 5IKR) amino acid residues with ligands at receptor sites**

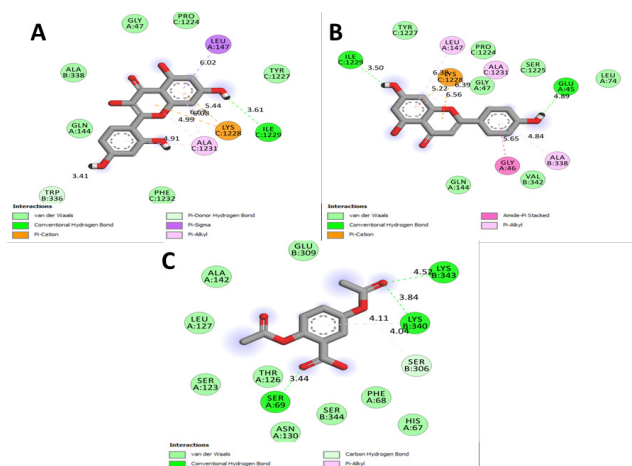
Ligands	Binding Affinity, $\Delta G$ (Kcal/mol)	Amino acids involved and Distance (Å°)	
		Hydrogen Binding Interactions	Hydrophobic Interactions
Apigenin	-9.1	CYS A:47 (3.39, 4.29), CYS A:36 (2.88), GLN A:461 (5.28)	LEU A:152 (5.27), PRO A:153 (4.91, 6.52), CYS A:36 (6.46), ARG A:469 (6.84), VAL A:46 (3.66)
Genistic acid	-6.8	ASN A:43 (4.58), GLN A:461 (5.88)	ARG A:44 (3.54), LEU A:152 (5.51), PRO A:153 (4.90)
Morin	-9.3	CYS A:41 (2.99), GLY A:135 (4.99)	CYS A:36 (6.41), ARG A:44 (4.16), LEU A:152 (5.79), PRO A:153 (4.78, 5.05)



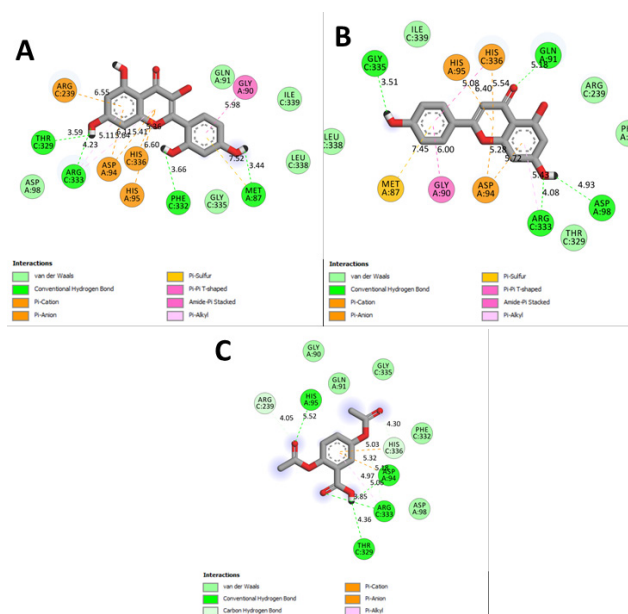
**Figure 6: Various Two-dimensional Interactions of ligands with cytochrome P450 (1PG5). A: Morin; B: Apigenin; C: Genistic acid.**



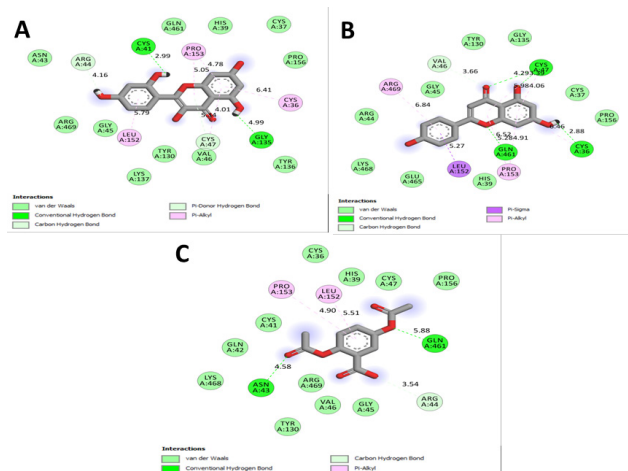
**Figure 7: Various Two-dimensional Interactions of ligands with lipoxygenase (1NBQ). A: Morin; B: Apigenin; C: Genistic acid.**



**Figure 8: Various Two-dimensional Interactions of ligands with myeloperoxidase (3NRZ). A: Morin; B: Apigenin; C: Genistic acid.**



**Figure 9: Various Two-dimensional Interactions of ligands with xanthine oxidase (1DNU). A: Morin; B: Apigenin; C: Genistic acid.**



**Figure 10: Various Two-dimensional Interactions of ligands with Cyclo oxygenase-2 (5IKR). A: Morin; B: Apigenin; C: Genistic acid.**

As all the compounds are of natural source, none of them obeyed Lipinski's rule (Table 11).

### ADME/T evaluation by using admetSAR

The ADMET properties of the ligands were assessed, making use of admetSAR. ADMET properties for the substances in the research study were evaluated, making use of admetSAR. All the substances revealed excellent human intestinal absorption (HIA), blood-brain barrier (B.B.B.) infiltration. None of the compound was found carcinogenic. All the compounds were AMES negative.



**Table 11: Physicochemical properties of the active compounds and accordance with the rules of drug-likeness**

Sr. No.	Compound	MW	logp	Alogp	HBA	HBD	TPSA	AMR	nRB	No. of Violations
1	Morin	291.96	1.405	-1.244	7	0	26.3	83.44	1	0
2	Genstic acid	227.97	0.569	-0.088	6	0	69.67	59.04	5	0
3	4-hydroxy benzoic acid	131.98	0.268	0.405	3	0	17.07	38.56	1	0
4	Cinnamic acid	139.99	1.887	1.329	2	0	17.07	47.2	2	0
5	Syringic acid	187.97	0.748	-0.592	5	0	35.53	51.85	3	0
6	Apigenin	259.97	1.138	-0.224	5	0	26.3	80.15	1	0
7	Alpha-pinene	120	4.177	1.516	0	0	0	44.54	0	0
8	3-Carene	120	4.181	2.143	0	0	0	44.99	0	0
9	Camphor	135.99	2.127	0.969	1	0	17.07	43.76	0	0

**Table 12: ADME/T Properties of different compounds from *L. spinus*.**

Ligands	HIA	BBB	AMES Toxicity	Carcinogenicity	LD <sub>50</sub> in rat (mol/kg)
Morin	0.9855	0.6286	Non AMES Toxic	Non-carcinogen	3.0825
Genstic acid	0.9541	0.9335	Non AMES Toxic	Non-carcinogen	2.7396
4-hydroxy benzoic acid	0.9872	0.532	Non AMES Toxic	Non-carcinogen	1.3983
Cinnamic acid	0.9945	0.9526	Non AMES Toxic	Non-carcinogen	1.7416
Syringic acid	0.9165	0.5861	Non AMES Toxic	Non-carcinogen	2.5353
Apigenin	0.9887	0.6364	Non AMES Toxic	Non-carcinogen	2.6983
Alpha-pinene	0.9964	0.8959	Non AMES Toxic	Non-carcinogen	1.5348
3-Carene	0.9943	0.9473	Non AMES Toxic	Non-carcinogen	1.4843
Camphor	0.9971	0.9836	Non AMES Toxic	Non-carcinogen	1.6328

The results of HIA, B.B.B., LD<sub>50</sub> values for the compounds are listed in Table 12.

## DISCUSSION

Oxygen is essential for survival but under certain abnormal physiological conditions converts some of oxygen to ROS by univalent reactions that makes them highly reactive and damages nucleic acids, proteins, lipids and carbohydrates components of cell.<sup>14</sup> Such freely available radicals control essential aspects of cell biology such as signals, gene transcriptions and controls on soluble guanylate cyclase activities.<sup>15</sup> The excess production of free radicals results in abnormal physiological conditions like oxidative stress that impedes various cellular and metabolic functions leading to neuro-degenerative diseases, gastro-duodenal disorders, cancer, cataracts, premature ageing, inflammation, cardiovascular diseases,<sup>16</sup> Chronic kidney disease<sup>17</sup> and metabolic dysfunction.<sup>18</sup> Naturally and synthetically antioxidant activity has been linked

to different processes such as chain initiation reduction, binding metal ion transition catalysts, peroxide decomposition, ongoing abstract hydrogen avoidance and radical scavenging<sup>16,19</sup> that hamper consequences of oxidative damage. Phytochemicals like flavonoids, phenols, tannins and related polyphenols<sup>20</sup> are potential natural antioxidants with strong antioxidant capacity that offers protection against oxidative deterioration by the radicals.<sup>14</sup>

H<sub>2</sub>O<sub>2</sub> has a substantial physiologic significance in which the intracellular levels of Ca<sup>2+</sup> increase, transcription factors are triggered, certain genes are repressed, cell proliferation facilitated or blocked, certain pathways for signal transduction triggered or disrupted and apoptosis facilitated or undermined.<sup>14</sup> During certain adverse physicochemical, environmental or pathological conditions the level of H<sub>2</sub>O<sub>2</sub> increases that yields potent species OH, highly reactive free radical in biological system.<sup>20,21</sup> They negatively influence lipid, proteins and DNA, in particular thiamine and guanosine and are implicated in other diseases such as cardiovascular

disorders, cytotoxicity and ageing.<sup>17,22</sup> The scavenging of  $H_2O_2$  by the extract may be attributed to active secondary metabolites, phenolics which neutralize  $H_2O_2$  by donating electrons thereby neutralizing it to water.<sup>23</sup> MELS 'scavenging capability was attributed to phytochemicals' structural specificity, which dictates their ability to donate electron. MELS demonstrated high dose-dependent  $H_2O_2$  scavenging activity. This split may be triggered by direct reactions to  $H_2O_2$ , reaction to  $H_2O_2$  intermediates and inhibiting horseradish peroxidase from  $H_2O_2$  binding.<sup>24</sup>

Numbers of epidemiological studies have highlighted the inverse correlation between antioxidants and occurrence of disease and mortality due to these diseases.<sup>20</sup> Phenolic compounds are secondary synthesis products with high redox effects, which may play an significant role in intermediate metals, lipoxygenase inhibition and non-scavenging radicals.<sup>25</sup> Preliminary phytochemical investigation revealed that root part of *L. spinosa* is rich in secondary metabolites.<sup>26</sup> The presence of phytochemicals, phenolic and polyphenolic constituents might be responsible for the free radical scavenging activities in the present experiment. Polyphenolic compounds, rich in antioxidants, can inhibit human mutagenesis and carcinogenesis<sup>27</sup> which also involves stabilizing lipid peroxidation.<sup>20</sup>

Since an animal model for extreme swelling, carrageen-induced edema remains largely to be used and is actually considered biphasic. The initial (1-2 h) is largely solved in cell-ruined environments by histamine, serotonin and increased prostaglandin formation. The latter stage (3h) is liable to release prostaglandin and regulated by tissue macrophages, bradykinin, leukotriens.<sup>28,29</sup> MELS's late-stage, substantial suppressive activity ( $P<0.05$ ) indicates its powerful anti-inflammatory effect. It is comparable to diclofenac, which avoided edema at 10 mg / kg by 61.44%. Statistically important finding ( $P<0.05$ ). Ueno et al. (2000) reported that rodent paw carragein therapy results in bradykinine production that eventually leads to prostaglandin biosynthesis, as well as many other autacoids that accumulate inflammatory exudates.<sup>30,31</sup> PGE2 is a dominant vasodilator with many endogenous vasodilators, notably histamine and bradykinine, in severe inflammatory environments. Extract action mode is firmly recommended to suppress prostaglandin synthesis. Tests revealed that MELS has essential anti-inflammatory properties at various stages. Carrageenin-induced inflammation is an essential pathway to determine anti-inflammatory function. Oedema formation in rat paw following carrageen injection stems from histamine, serotonin and prostaglandin release and associated substances. MELR's good anti-inflammatory

behavior.<sup>12,32,33</sup> Due to the anabolic steroids, flavonoids and glycosides present in the extract, this significant anti-inflammatory and even analgesic impact that result from the obstacle of any kind of inflamed negotiators. Latest results indicate *L. spinosa* efficacy in treating acute inflammation. Additionally, the end result authenticates the mythology-relevant anti-inflammatory information as well as the *L. spinosa* extracts.

Out of 9 candidates, three compounds displayed a higher binding affinity and least binding energy with the all the targets responsible for antioxidant and anti-inflammatory activity. Morin has exhibited highest dock score with least binding energy of -8.5, -8.2, -9.8, -8.2 and -9.3 Kcal/mol with 1OG5, 1N8Q, 1DNU, 3NRZ and 5IKR.

*In silico* measurement of intestinal absorption, both phytochemicals were theoretically strongly soluble in the gastrointestinal tract (Table 5). Despite effective distribution of medications in the human body, significant toxic effects such as carcinogenicity became important concerns. Accordingly, in the early phases of drug growth, identification and evaluation of novel product candidates is proposed to be important. No compounds demonstrated *in silico* toxicity in this analysis.

## CONCLUSION

Thus the findings of present investigation support the traditional ethanomedicinal claims of *L. spinosa* for the treatment of diverse infections. The antioxidant activities of *L. spinosa* can be used as a natural antioxidant or nutraceuticals and might be effective to diminish oxidative stress associated with different pathophysiological conditions. Within this analysis, the bioactive phytocompounds for drug production and testing have been identified. This combinatorial analytic procedure can be beneficial to develop phyto compounds as novel candidates for the treatment of inflammatory disorders by antioxidant and anti-inflammatory action in *invitro* and *invivo*. Such a plant screening may also provide a source of new bioactive compounds with functional characteristics that help restore health.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

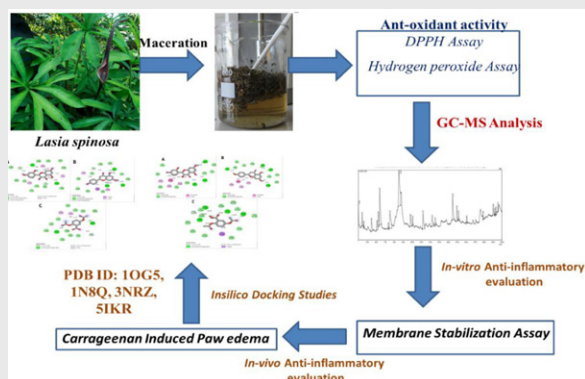
## ABBREVIATIONS

**HIA:** Human Intestinal absorption; **B.B.B:** Blood Brain Barrier; **LD<sub>50</sub>:** Lethal dose 50%; **MELR:** Methanolic extract of *Lasia spinosa*; **H<sub>2</sub>O<sub>2</sub>:** Hydrogen peroxide; **DPPH:** 2, 2-diphenyl-1-picrylhydrazyl; **MW:** Molecular Weight; **HBA:** Hydrogen bond acceptors; **HBD:** Hydrogen bond Donors; **TPSA:** Total Protein Surface area; **AMR:** Molar refractivity; **nRB:** No. of rotatable bonds.

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## PICTORIAL ABSTRACT



## SUMMARY

- The methanolic extract of *Lasia spinosa* has significant antioxidant (*invitro*) and anti-inflammatory activity (*invitro* and *invivo*) in comparison with other extract.
- The methanolic extract was subjected to GC-MS analysis, showed the presence of Morin,
- Syringic acid; Apigenin, Genistic acid, 4-Hydroxy benzoic acid, 3-Carene, Camphor, Cinnamic acid.
- Amongst the isolated compounds, Morin has potent efficacy as an antioxidant and anti-inflammatory activity, with a best docking score in comparison with other constituents.
- All the isolated compounds have no toxicity and are non-carcinogenic in nature.

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