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Original Research Article

Anti-inflammatory and anti-oxidant activities of *Schumanniophyton magnificum* leaves: *In vivo* and *in vitro* approaches

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Abstract

Purpose: To investigate the methanol extract of *Schumanniophyton magnificum* leaves' (MESM-L) anti-inflammatory and antioxidant properties
Methods: Acute toxicity studies were carried out for 24 hours. Albino rats were randomized into five groups for *in vivo* anti-inflammatory studies using three models namely egg albumin-induced paw edema, acetic acid-induced vascular permeability, and *in vivo* leukocyte mobilization assay. Groups include the control, standard and experimental groups (100, 200 and 400 mg/kg b.w. of MESM-L). Membrane stabilization and anti-platelet aggregatory response were carried out at the concentrations 0.1 – 0.8 mg/ml. Nitric oxide, 1, 1-diphenyl-2-picrylhydrazyl, and ferric-reducing antioxidant power assays were employed for anti-oxidant studies.
Results: : In acute toxicity investigations, MESM-L was found to be non-toxic at the maximum dose of 5000 mg/kg b.w. MESM-L (400 mg/kg b.w) inhibited edema by 73.13% and inhibited vascular permeability by 72.37%. A profound effect of MESM-L on leukocyte mobilization was detected at the lowest dose (41.17%). Inhibition of hemolysis and aggregation of platelets was exhibited with highest concentration of MESM-L on membrane stabilization and anti-platelet aggregatory. MESM-L scavenged NO and DPPH radical- with IC_{50} values of 19.52 and 12.31 µg/mL in that order. The effect of gallic acid on FRAP assay was found to be higher than MESM-L.
Conclusion: These findings proved the anti-inflammatory potential of MESM-L, although the activities exhibited by the standard drugs were significantly higher. The capacity of the plant to scavenge free radicals is responsible for the effects that have been discovered.

Keywords: *Schumanniophyton magnificum* leaves, edema, vascular permeability, leukocyte mobilization, anti-inflammatory, antioxidant

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INTRODUCTION

The term "inflammatory response" describes the immune system's main reaction to an injury or infection, and it is typified by the presence of certain cytokines and leukocyte migration to site of injury[1]. Reactive oxygen species (ROS) are also generated by mononuclear cells and polymorphonuclear leukocytes all through the inflammatory response to demonstrate a defense against the invasive pathogen.[2] Contrary to their defensive roles, excessive ROS produced deregulates cellular functions, leading to cellular and tissue damage, thereby augmenting the inflammatory state.[3] Currently available anti-inflammatory medications have adverse effects and relieve symptoms. Non-steroidal anti-inflammatory medicines (NSAIDs) cause stomach ulcers, and corticosteroids are harmful. When treatment is stopped, the problem recurs.[4] This therefore warrants the search for safer anti-inflammatory drugs, especially from medicinal plants since most populations depend on natural products derived from plants for the treatment of various ailments.[5] *Schumanniophyton magnificum* Harms (Rubiaceae) has various health benefits in African ethnomedicine. It is referred to as *mgba mmiri* in the Ibo ethnic group of Nigeria. The infusion of its bark serves as a purgative to ease stomach ache.[6] As a plant used in the treatment of snake bites, various antivenom principles have been isolated from *Schumanniophyton magnificum*.[7,8] This study therefore, explored its anti-inflammatory and anti-oxidant potentials.

MATERIAL AND METHOD

Collection of plant and preparation

The Igbo-Etiti Local Government Area in Enugu State, Nigeria, is where *Schumanniophyton magnificum* leaves were obtained in October 2018. The voucher number Intercedd/837 was submitted at the International Herbarium Unit of the Centre for Ethnomedicine and Drug Development (InterCEDD) where Mr. Alfred Ozioko confirmed the leaves' validity. Following a period of shade drying and grinding, 1.97492 kg of the leaves were macerated in methanol for 48 h. Filtering the solution, the Whatman paper was used and the resulting filtrate was then concentrated at 40 to 60°C to create the methanol extract of *Schumanniophyton magnificum* leaves (MESM-L). Following the equation 1 of the extract yield, the extract was kept at 4°C.

$$\text{Yield of extract (\%)} = \frac{\text{mass of the extract (g)}}{\text{mass of the pulverized plant material (g)}} \times 100$$

Equation 1

Experimental animals

The Zoology and Environmental Biology Department at the University of Nigeria in Nsukka provided the albino Wistar rats. In addition to having unlimited water and food, the rats were kept in well-ventilated cages. Two weeks were spent acclimating them before the experiment started.

Ethical approval

National and international standards were followed when conducting animal studies. The University of Nigeria, Nsukka's Faculty of Biological Sciences' Ethics and Biosafety Committee, provided ethical consent for animal studies (approval number: UNN/FBS/EC/1008).

Blood samples

Blood samples were drawn from volunteers who appeared to be in good health and had not taken any drug for at least fourteen days before the draw. With the consent of the volunteers, samples were collected in compliance with the Declaration of Helsinki.

Acute toxicity study

The investigation was conducted in two stages using 18 albino mice weighing between 18 - 22 g. Three groups of three mice each participated in each phase. The animals orally took MESM-L dosages of 10, 100, and 1000 mg/kg followed by 1600, 2900, and 5000 mg/kg body weight for the first and second phase, respectively. In the second phase, animals took MESM-L dosages of. Over a day, animals were watched for symptoms of noxiousness, including mortality, restlessness, anxiety, and dullness.[9]

Anti-inflammatory studies

Egg albumin-induced paw edema

Five groups of four Wistar rats each were created from twenty adult rats (200–250 g) of either sex. Before the study started, the animals were denied food and drink for eighteen hours. The volume of the rats' right hind paws was then determined using mercury displacement at time zero (t = 0). The intraperitoneal administration of the test chemicals came next. After an hour, the animals' right hind paw's sub-plantar region was treated with 0.1mL of egg albumin to cause acute inflammation.[10] In groups 1, 2, 3, and 5, rats received 3% Tween-80 (2 mL/kg b.w.), 100 mg/kg b.w. of Ibuprofen, 100, 200, 400 mg/kg b.w.,

of MESM-L, respectively. Each group received egg albumin after administration of each dose.

The development of oedema was identified by comparing the paw volume at time zero with the paw volume at 1, 2, 3, 4, and 5 h after egg albumin injection. The percentage inhibition of inflammation (IOI) was calculated using equation 2:

Average inflammation = $(V_t - V_0)$

Paw volume at time t is equal to V_t (1, 2, 3, 4, and 5 h). V_0 is the paw volume at zero time.

$$\% \text{ IOI} = \frac{(V_t - V_0)\text{control} - (V_t - V_0)\text{treated group set}}{(V_t - V_0)\text{control}} \times 100$$

Equation 2

Acetic acid-induced vascular permeability study

Five groups of four adult Wistar rats with weight between 120 and 200 g were used. After a 10 h fast, the test chemicals were given to the rats. After 3 h, 0.5 mL of 1% IV injection of Evans blue solution was administered. Induction of vascular permeability was achieved after 30 minutes, via intraperitoneal's 1 mL injection of acetic acid. The peritoneum of the rats in the respective groups was washed with 10 milliliters of regular saline after they were sacrificed twenty minutes later. After centrifuging the peritoneal fluid, the supernatant's absorbance was measured at 610 nm.[11]

Group 1 received 3% Tween-80 (2 mL/kg b.w.) + 1% Evans blue solution + 0.6% acetic acid, group 2 received Indomethacin (50 mg/kg b.w.) + 1% Evans blue solution + 0.6% acetic acid, group 3 received MESM-L (100 mg/kg b.w.) + 1% Evans blue solution + 0.6% acetic acid, group 4 was administered MESM-L (200 mg/kg b.w.) + 1% Evans blue solution + 0.6% acetic acid and group 5 received MESM-L (400 mg/kg b.w.) + 1% Evans blue solution + 0.6% acetic acid.

The percentage of vascular permeability inhibition was estimated via equation 3:

$$\% \text{ Inhibition of vascular permeability} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} x \frac{100}{1}$$

Equation 3

In vivo leukocyte mobilization assay

Leukocyte mobilization was evaluated using the method.[12] Adult Wistar rats (120 – 200 g) distributed into 5 groups of 4 rats were administered the respective treatments. Three hrs later, in normal saline, each rat was injected intraperitoneally with agar (0.5 mL of 3%) after the administration of the various doses thus: 3% Tween-80 (2 mL/kg b.w.), 50, 100, 200, and 400 mg/kg b.w. of MESM-L for group 1, 2, 3, 4 and 5, respectively.

After four hours of agar induction, five milliliter of a five percent solution of ethylenediaminetetraacetic acid in phosphate-buffered saline was used to cleanse the animals' peritoneal cavities after they were slaughtered in order to recover the peritoneal fluid. Following Wright's staining, the perfusates were subjected to a manual cell counter to determine the total and differential leukocyte counts.

Membrane Stabilization Study

Using a modified approach, the impact of MESM-L on hemolysis of human red blood cells (HRBC) were assessed.[13] For 10 minutes, the blood centrifugation was achieved at 3,000 rpm. Then, washed three times using identical amounts of regular saline. Using regular saline, the cleaned material was reconstituted as a 40% solution. Various concentrations (0.1 - 0.8 mg/mL) of 5 mL of MESM-L in were made independently in isotonic solution (normal saline) and hypotonic solution (distilled water). Three duplicates of each concentration were made. Five mL of the hypotonic solution are in the control tubes. The standard control consisted of tubes holding 5 ml of indomethacin at 0.2 and 0.4 mg/mL. To each tube was added HRBC suspension (0.1 mL) with mild agitation. At room temperature for 1 h, the tubes were incubated followed by 1300 rpm centrifugation for 3 min. Absorbance measurement was achieved at 418 nm. The percentage hemolysis were calculated using equation 4:

$$\% \text{ Inhibition of Hemolysis} = \left(1 - \frac{\text{Abs.of test in hypotonic solution} - \text{Abs.of test in isotonic solution}}{\text{Abs.of control in hypotonic solution} - \text{Abs.of test in isotonic solution}}\right) \times 100$$

Equation 4

Determination of anti-platelet aggregatory activity

For 10 minutes, centrifugation of blood samples were achieved at 3000 rpm. The supernatants were diluted twice with normal saline to get platelet-rich plasma (PRP). The cuvette was filled with 0.2 mL of PRP and various concentrations of MESM-L (0.1, 0.2, 0.4, 0.6, and 0.8 mg/mL) and indomethacin in phosphate buffer followed by adding 0.4 mL of 2 M calcium chloride to normal saline to induce aggregation. At 520 nm, absorbance were measured right away at 30-second intervals. Three duplicates of each test were conducted.[14]

In vitro antioxidant studies

Nitric oxide radical scavenging activity

In separate tubes, 5.0 mL of phosphate buffer and 2.0 mL of sodium nitroprusside (10 nM) prepared in 7.4 pH phosphate buffer were added. MESM-L (0.5 mL) was added to the corresponding tubes in various concentrations (15.63 - 1000 µg/mL) then incubated, followed by adding Griess reagent. For an additional half-hour, the incubation procedure was repeated at room temperature. At 546 nm, measurements were made of the pink chromophore's absorbance. Ascorbic acid was employed as the positive control, measurements were carried out in triplicate. Additionally, IC_{50}

(concentration needed to produce a 50% reduction in NO activity) was determined [15]. The percentage inhibition of nitric oxide was carried out using equation 5

$$\% \text{ Inhibition of nitric oxide} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} x \frac{100}{1}$$

Equation 5

Assessment of 1, 1-diphenyl-2-picrylhydrazyl's (DPPH) capacity to scavenge radicals

Different test tubes held ascorbic acid dissolved in methanol and 1 mL MESM-L varying concentrations (15.63 - 1000 µg/mL). Each tube was filled with a 0.5 mL aliquot of 0.078 mM DPPH (in methanol) and stirred then, incubated. At 517 nm, absorbance was measured. A mixture of one milliliter of methanol and 0.078 Mm DPPH solution (0.5 mL) served as the negative control [16]. Every test was conducted in triplicate. Percentage DPPH scavenging activity

$$\% \text{ DPPH scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times \frac{100}{1}$$

Assessment of ferric reducing antioxidant capacity

Different quantities (15.63 - , 31.25, 62.50, 125, 250, 500, and 1000 µg/mL) of MESM-L were combined with phosphate buffer (0.5 mL, pH 6.6) and potassium hexacyanoferrate (0.5 mL of 0.1%) followed by 20 mins incubation at 50°C. To inhibit the reaction, inclusion of trichloroacetic acid (0.5 mL of 10%) was done. The resulting solution's 1 mL was merged with equivalent amount of distilled water and $FeCl_3$ solution (0.1 mL of a 0.01 %.) Then, at 700 nm, the absorbance was measured in relative to a blank[17]. A positive control was gallic acid. Every experiment was carried out three times.

Statistical analysis

After one- and two-way Analysis of Variance (ANOVA), the acquired data were subjected to the Duncan posthoc and least significant difference (LSD) test. Variances between means were deemed significant at a 95% confidence interval. Results were displayed as Mean ± Standard Deviation (SD).

RESULT AND DISCUSSION

Yield of extraction

The yield of MESM-L was calculated to be 7.06% relative to the starting plant material.

Acute toxicity

No death or signs of noxiousness were detected even at the maximum dose of MESM-L administration within 24h.

Egg albumin-induced rat paw edema

Following the development of oedema, the rats' paw volumes considerably increased. Comparing MESM-L-treated groups to group 1, a substantial decrease in paw volumes was noted ($p < 0.05$). Group 5 showed the highest percentage inhibition of 73.13%, which was similar to that of indomethacin, and the largest drop in paw volume occurred at the fifth hour (Table 1).

Egg albumin-induced edema as an experimental method for acute inflammation using animal models involves two phases.[18] The first phase of acute inflammation (1–2 h) is marked by serotonin, histamine, and higher production of prostaglandins in tissue compromised; the belated phase is marked by mediators produced by tissue macrophages: leukotrienes, polymorphonuclear cells, bradykinin, and prostaglandins. Acute inflammation is characterized by increased passage of fluid, and to a lesser extent, inflammatory cells from dilated blood vessels into the affected region, leading to oedema.[19] At $p < 0.05$, MESM-L at all the doses observed, exhibited a significant inhibitory effect on edema relative to the control, demonstrating its inhibition of all the phases of acute inflammation. The observed anti-edematous effect could imply the phospholipase A_2 (PLA_2) – and cyclooxygenase – inhibitory potential by MESM-L. The inhibition of PLA_2 by MESM-L has been demonstrated by previous research[20] suggesting the inhibition of the synthesis of prostaglandins, which are important mediators for the edematous response.

Acetic acid-induced vascular permeability

MESM-L demonstrated dose-dependent and inhibited vascular permeability significantly ($p < 0.05$). This was revealed by the reduced values of absorbance of the treated rat's peritoneal fluids. The maximum extract dose made the highest inhibition of 57.89%. However, the inhibitory effect of indomethacin (72.37%) was significantly higher than MESM-L-treated groups at $p < 0.05$ (Table 2).

Acetic acid dilates blood vessels releasing mediators such as histamine, leukotrienes, and prostaglandins which in turn increase vascular permeability[21] leading to the exudation of plasma protein-rich fluids. Acetic acid induced vascular permeability were inhibited in a dose-dependent by MESM-L (Table 3). Peritoneal inflammation's reduction was shown via reduced absorbance of the treated rat's peritoneal fluids, as a result of reduced leakage of the Evans blue dye. Results from this study therefore, suggest that MESM-L may potentially inhibit the effusive phase of acute inflammation via inhibiting the release of inflammatory

mediators.[22] This finding is substantiated by the inhibition of rat paw edema by MESM-L.

In vivo leukocyte mobilization

Table 3 shows that giving varying doses of MESM-L led to a significant decrease in the leukocytes migration into the rat's peritoneal cavity at $p < 0.05$ compared to group 1. This effect was comparable to that of indomethacin. Percentage inhibition of leukocyte mobilization showed that MESM-L's minimum dose had the maximum inhibition (41.71%). Neutrophils and lymphocytes were solely mobilized, and the number of neutrophils mobilized were found to be > lymphocytes.

Another important facet of inflammatory response is the infiltration of cells due to the crucial function meted out by leukocytes. Leukocytes migrate and adhere to endothelial cells thereby increasing inflammatory process.

Table 1: Effect of MESM-L on egg albumin-induced rat paw edema

Groups	Paw volume (mL) and percentage (%) inhibition of edema					
	0 h	1 h	2 h	3 h	4 h	5 h
Group 1	0.37 ± 0.02^{b}	0.47 ± 0.06^{c}	0.60 ± 0.06^{c}	0.63 ± 0.05^{d}	0.65 ± 0.07^{d}	0.67 ± 0.07^{c}
Group 2	0.24 ± 0.03^{a}	0.27 ± 0.05^{a} (42.55)	0.31 ± 0.02^{a} (48.33)	0.23 ± 0.03^{a} (63.49)	0.24 ± 0.02^{ab} (63.08)	0.17 ± 0.03^{a} (74.63)
Group 3	0.37 ± 0.02^{b}	0.35 ± 0.02^{b} (25.53)	0.40 ± 0.04^{b} (33.33)	0.40 ± 0.02^{c} (36.51)	0.32 ± 0.04^{c} (50.77)	0.30 ± 0.04^{b} (55.22)
Group 4	0.36 ± 0.04^{b}	0.32 ± 0.04^{ab} (31.91)	0.39 ± 0.02^{b} (35.00)	0.38 ± 0.02^{c} (39.68)	0.30 ± 0.04^{bc} (53.85)	0.25 ± 0.05^{b} (62.69)
Group 5	0.35 ± 0.02^{b}	0.31 ± 0.04^{ab} (34.04)	0.33 ± 0.02^{a} (45.00)	0.32 ± 0.05^{b} (49.21)	0.22 ± 0.02^{a} (66.15)	0.18 ± 0.02^{a} (73.13)

Values are presented as Mean ± SD; n = 4. Values with different letters down the group are significantly ($p < 0.05$) different. Percentage (%) inhibition of edema is in parenthesis. Group 1: 3% Tween-80 (2 mL/kg b.w.) + egg albumin; Group 2: Ibuprofen (100 mg/kg b.w.) + egg albumin; Group 3: MESM-L (100 mg/kg b.w.) + egg albumin; Group 4: MESM-L (200 mg/kg b.w.) + egg albumin; Group 5: MESM-L (400 mg/kg b.w.) + egg albumin

Table 2: Effect of MESM-L on acetic acid-induced vascular permeability

Groups	Absorbance	Inhibition (%)
Group 1	0.076 ± 0.006^{d}	0.00
Group 2	0.021 ± 0.005^{a}	72.37
Group 3	0.053 ± 0.007^{c}	30.26
Group 4	0.045 ± 0.008^{c}	40.79
Group 5	0.032 ± 0.005^{b}	57.89

Values are presented as Mean ± SD, n=4. Values with different letters are significant ($p < 0.05$). Group 1: 3% Tween-80 (2 mL/kg b.w.) + 1% Evans blue solution + 0.6% acetic acid; Group 2: Indomethacin (50 mg/kg b.w.) + 1% Evans blue solution + 0.6% acetic acid; Group 3: MESM-L (100 mg/kg b.w.) + 1% Evans blue solution + 0.6% acetic acid; Group 4: MESM-L (200 mg/kg b.w.) + 1% Evans blue solution + 0.6% acetic acid; Group 5: MESM-L (400 mg/kg b.w.) + 1% Evans blue solution + 0.6% acetic acid

Table 3: Effect of MESM-L on *in vivo* leukocyte mobilization

Group	TLC (10^4/mm)	Inhibition (%)	Differential leukocyte count				
			N	L	M	E	B
1	88.20 ± 8.91^{d}	-	70.3 ± 7.96^{b}	26.6 ± 5.03^{a}	1.3 ± 0.22^{a}	1.1 ± 0.31^{a}	0.7 ± 0.09^{a}
2	41.71 ± 2.76^{a}	52.71	65.8 ± 9.66^{ab}	30.0 ± 1.40^{ab}	1.5 ± 0.10^{b}	1.4 ± 0.05^{ab}	1.3 ± 0.10^{b}
3	51.89 ± 3.38^{b}	41.17	58.5 ± 1.91^{a}	37.5 ± 4.39^{c}	1.2 ± 0.08^{a}	1.5 ± 0.17^{b}	1.3 ± 0.36^{b}
4	58.72 ± 3.01^{bc}	33.42	61.0 ± 4.56^{ab}	35.2 ± 1.91^{bc}	1.3 ± 0.17^{ab}	1.3 ± 0.13^{ab}	1.2 ± 0.17^{b}
5	64.98 ± 3.69^{c}	26.33	65.5 ± 2.44^{ab}	30.7 ± 3.95^{ab}	1.5 ± 0.08^{b}	1.0 ± 0.33^{a}	1.3 ± 0.13^{b}

Values are presented as Mean ± SD; n=4. Values with different letters down the column are significantly ($p < 0.05$ different). TLC = Total leukocyte count, N = Neutrophils, L = Lymphocytes, M = Monocytes, E = Eosinophils, B = Basophils. Group 1: 3% Tween-80 (2 mL/kg b.w.) + 3% agar; Group 2: Indomethacin (50 mg/kg b.w.) + 3% agar; Group 3: MESM-L (100 mg/kg b.w.) + 3% agar; Group 4: MESM-L (200 mg/kg b.w.) + 3% agar; Group 5: MESM-L (400 mg/kg b.w.) + 3% agar

As a defensive mechanism, these leukocytes discharge their lysosomal contents (proteases and bactericidal enzymes) during inflammation thereby causing more tissue damage and inflammation.[23] This study showed that the major cells recruited are neutrophils, which are typically the first leukocytes recruited to the site of inflammation.[24] MESM-L inhibited leukocytes mobilization into the peritoneal cavity, thereby preventing them from eliciting their effects. ME

SM-L inhibited leukocyte migration eliciting modification of the endogenous factors responsible for the relocation of leukocytes to the point of inflammation.

Effect of MESM-L on membrane stabilization

Table 4 revealed a decrease in the absorbance of the hypotonic solutions in comparison to the control. Hemolysis inhibition of MESM-L was concentration-dependent, 0.8 mg/mL had the highest inhibition (69.10%). Conversely, indomethacin's 0.4 mg/mL produced the highest inhibition of membrane stabilization (83.39%).

HRBC membrane stabilization is used to analyze the anti-inflammatory actions of MESM-L as the red blood cell and the lysosomal membrane are similar.[25] Stabilization of lysosomal membranes by the MESM-L extracts is evident in the HRBC stabilization. Stabilizing the lysosomal membrane prevents activated neutrophils from releasing their lysosomal constituents,[26] thereby limiting the inflammatory response. Red blood cell (RBC) membranes could be lysed when exposed to injurious substances such as heat and hypotonic medium, resulting in the hemolysis and oxidation of hemoglobin. As observed in this study, MESM-L inhibited lyses of HRBC exposed to hypotonic solution. It is evident in the decrease in the absorbance of MESM-L-treated groups relative to the control, as a consequence of decrease in the concentration of hemoglobin in the medium.

Table 4: Effect of MESM-L on membrane stabilization of human red blood cells

Treatment	Concentration (mg/mL)	Absorbance		Inhibition of hemolysis (%)
		Isotonic solution	Hypotonic solution	
Control	-	0.577 ± 0.008	1.381 ± 0.004	0.00
MESM-L	0.1	0.046 ± 0.002	1.098 ± 0.001*	21.20
	0.2	0.060 ± 0.002	1.038 ± 0.003*	25.74
	0.4	0.061 ± 0.002	0.846 ± 0.002*	40.53
	0.6	0.187 ± 0.002	0.746 ± 0.002*	53.18
	0.8	0.271 ± 0.001	0.614 ± 0.002*	69.10
Indomethacin	0.2	0.582 ± 0.002	0.863 ± 0.003*	64.43
	0.4	0.424 ± 0.002	0.583 ± 0.003*	83.39

Values are presented as Mean ± SD; n = 3
*$p < 0.05$ is significantly different compared with control.

Effect of MESM-L on platelet aggregation

Like the control group, the absorbance increase of the MESM-L-treated groups were concentration-dependent. A decrease in absorbance was observed across the time intervals in the respective groups. MESM-L inhibited platelet aggregation similar to indomethacin (Table 5). During tissue injury and inflammation, the synthesis of thromboxane A_2 (TXA_2) increases.[27] Platelet activation is followed by platelet aggregation in which platelets adhere to each other leading to the formation of platelet plugs as fibrin is deposited from the coagulation cascade. MESM-L inhibited the aggregation of platelets induced with $CaCl_2$. Bioactive compounds present in

MESM-L could have interacted with the calcium ions thereby inhibiting platelet aggregation as calcium ions enhance platelet aggregation in PRP and facilitate the conversion of fibrinogen to fibrin.[28] This action could also be due to PLA_2-inhibitory activity of the plant as previously demonstrated.[20] Inhibition of PLA_2 deprives cyclooxygenase (COX) of substrate for the synthesis of TXA_2 and platelet-activating factor (PAF) which promotes leukocyte migration and platelet aggregation.

Table 5: Effect of MESM-L on platelet aggregation

Treatment	Concentration (mg/mL)	Absorbance at different time intervals					
		0 s	30 s	60 s	90 s	120 s	150 s
Control	-	0.208 ± 0.11	0.198 ± 0.008	0.196 ± 0.009	0.195 ± 0.009	0.193 ± 0.009	0.192 ± 0.009
MESM-L	0.1	0.294 ± 0.043*	0.288 ± 0.044*	0.283 ± 0.044*	0.282 ± 0.044*	0.281 ± 0.044*	0.279 ± 0.044*
	0.2	0.372 ± 0.014*	0.364 ± 0.019*	0.361 ± 0.019*	0.360 ± 0.019*	0.358 ± 0.019*	0.356 ± 0.019*
	0.4	0.485 ±0.015*	0.480 ± 0.015*	0.477 ± 0.015*	0.475 ± 0.015*	0.474 ± 0.015*	0.472 ± 0.015*
	0.6	0.511 ± 0.014*	0.495 ± 0.013*	0.494 ± 0.013*	0.491 ± 0.013*	0.487 ± 0.015*	0.485 ± 0.015*
	0.8	0.526 ± 0.008*	0.507 ± 0.004*	0.506 ± 0.004*	0.503 ± 0.003*	0.503 ± 0.003*	0.502 ± 0.002*
Indomethacin	0.2	0.358 ± 0.027*	0.345 ± 0.027*	0.343 ± 0.027*	0.342 ± 0.027*	0.341 ± 0.027*	0.340 ± 0.027*
	0.4	0.467 ± 0.035*	0.457 ± 0.035*	0.454 ± 0.035*	0.451 ± 0.035*	0.450 ± 0.035*	0.448 ± 0.035*

Values are presented as Mean ± SD. *$p < 0.05$ shows significant difference compared with control

Table 6: Nitric oxide scavenging activity of MESM-L

	NO scavenging effect (%)							
	15.63	31.25	62.50	125 (µg/mL)	250	500	1000	IC_{50}
Ascorbic acid	79.03 ± 0.40^{e}	73.81 ± 0.66^{d}	70.20 ± 1.06^{c}	65.67 ± 0.80^{b}	67.36 ± 2.13^{b}	67.74 ± 1.97^{bc}	60.00 ± 1.86^{a}	16.26 ± 1.33
MESM-L	69.12 ± 0.00^{bc}	66.44 ± 3.62^{ab}	70.35 ± 2.84^{c}	73.81 ± 0.13^{d}	64.37 ± 0.27^{a}	76.04 ± 0.61^{d}	76.81 ± 0.66^{d}	19.52 ± 1.21

Values are expressed as Mean ± SD; n=3. Values with different letters across the row are significantly ($p < 0.05$) different.

Table 7: DPPH radical scavenging activity of MESM-L

	DPPH scavenging effect (%)							
	15.63	31.25	62.50	125 (µg/mL)	250	500	1000	IC_{50}
Ascorbic acid	62.61 ± 1.97^{a}	64.19 ± 1.34^{a}	63.02 ± 0.78^{a}	63.30 ± 0.68^{a}	64.13 ± 0.72^{a}	62.78 ± 0.96^{a}	64.39 ± 0.50^{a}	34.45 ± 1.27
MESM-L	69.48 ± 2.04^{a}	70.79 ± 0.71^{a}	77.42 ± 1.03^{b}	83.91 ± 0.78^{c}	90.95 ± 0.45^{e}	88.74 ± 2.94^{d}	84.92 ± 4.79^{cd}	12.31 ± 0.29

Values are expressed as Mean ± SD; n=3. Values with different letters across the row are significantly ($p < 0.05$) different.

Nitric oxide (NO) scavenging activity of MESM-L

MESM-L and ascorbic acid exhibited varying NO scavenging activity (Table 6). At 62.5 µg/mL, the scavenging activity of MESM-L was comparable to ascorbic acid. MESM-L and ascorbic acid had an IC_{50} of 19.52 and 16.26 µg/mL, respectively.

DPPH radical scavenging activity of MESM-L

As shown in Table 7, MESM-L exhibited an elevated activity up to an optimal level of 90.95% at 250 µg/mL in the DPPH assay, and similar to ascorbic acid. The IC_{50} value of MESM-L (12.31 µg/mL) was also lower in comparison with ascorbic acid (34.45 µg/mL).

Ferric reducing antioxidant power of MESM-L

MESM-L significantly ($p < 0.05$) caused a decline in absorbance at almost all the concentrations compared to gallic acid (Figure 1). The progression of many inflammatory diseases involves the generation of ROS.[29] Antioxidants exert their action either by neutralizing free radicals or preserving the antioxidant defense mechanisms.[30] This study ascertained the antioxidant potential of MESM-L using three *in vitro* approaches as a single method is insufficient to define the antioxidant activity of a sample.[31]

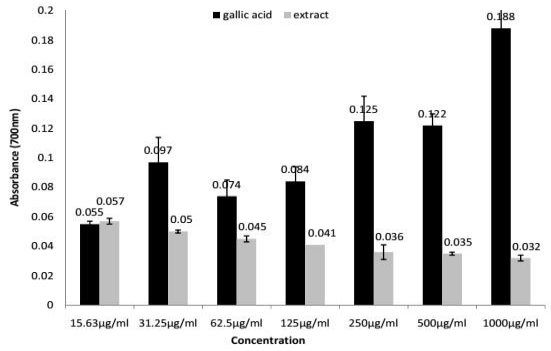

Figure 1: Ferric reducing antioxidant power of MESM-L

NO is a highly reactive nitrogen species produced during inflammation, and capable of damaging lipids, DNA, and proteins.[32] DPPH radical is a stable free radical species that loses absorption when accepting electrons, leading to a purple to yellow color change.[33] NO scavenging activity of MESM-L was comparable to that of ascorbic acid. This study also shows that MESM-L contains compounds that scavenged DPPH radicals better than ascorbic acid. Its DPPH scavenging activity is revealed by the IC_{50} value of 12.31 µg/mL when compared with the IC_{50} value of ascorbic acid - 34.45 µg/mL, suggesting that MESM-L is a very strong antioxidant like ascorbic acid since its IC_{50} is lower than 50 µg/mL.[34] In comparison with the standard gallic acid, MESM-L showed a lower absorbance of the reaction mixture, indicating a decreased ability to reduce ferric to ferrous iron**.** However, the reductive ability exhibited at 15.63 µg/mL was comparable with ascorbic acid.

CONCLUSION

This study validates MESM-L's promising anti-inflammatory activity. MESM-L's antioxidant qualities explain the observed anti-inflammatory benefits, as this study also validates its capacity to absorb free radicals. MESM-L thus contains active compounds capable of exhibiting the observed effects. Studies should be directed at isolating the anti-inflammatory and antioxidant compounds present in MESM-L, with the aim of developing potential therapeutic agents.

Conflict of interest

The authors declare no conflict of interest.

Authors Declaration

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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