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Evaluation of the Anti-Inflammatory Effects of *Blumea Aurita*

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Abstract - *Background :* There are repeated evidences suggesting potential therapeutic effects of *Blumea aurita*; however, the literature lacks scientific proofs for these benefits.

Aims : 1) to determine phytochemical constituents of *Blumea aurita*, 2) to evaluate anti-inflammatory, anti-pyretic and analgesic effects of *Blumea aurita* 3) to assess the membrane stabilizing activity of *Blumea aurita* as a possible mechanism for its therapeutic effects.

Material and Methods : Phytochemical constituents were determined according to the standard methods. A series of experiments were conducted in animal models using Wister albino rats to evaluate the possible effects of *Blumea aurita*. Edema-inhibition percent (EI %) and granuloma tissue-formation inhibition (GTI %) were used to evaluate anti-inflammatory effects, the hot plate method to assess analgesic effects and inhibition percent of heat-induced and hypotonic solution-induced RBCs haemolysis to determine membrane stabilizing activity.

Results : The phytochemical screening of *Blumea aurita* revealed presence of triterpenes, flavonoids, saponin, cumarins, tannins and traces of alkaloids. The herb is devoted from unsaturated sterols and anthraquinon. Experimental evaluation of the anti-inflammatory effects of *Blumea aurita* revealed highest EI % after 4 hours of oral administration of *Blumea aurita* extract at a dose of 400 mg/kg (EI% = 53%), and 6 hours at 800mg/kg (EI% = 67%).

Conclusion : The current results strongly suggest anti-inflammatory, anti-pyretic, analgesic and membrane stabilizing effects of *Blumea aurita*. The relevance of the potential therapeutic effects of *Blumea aurita* to its phytoconstituents was discussed.

Keywords : *analgesic, anti-inflammatory, antipyretic, blumea aurita, membrane stabilizing activity.*

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Abodola MA [α], Lutfi MF [σ], Bakhiet AO [ρ] & Mohamed AH [ω]

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Conclusion : The current results strongly suggest anti-inflammatory, anti-pyretic, analgesic and membrane stabilizing effects of *Blumea aurita*. The relevance of the potential therapeutic effects of *Blumea aurita* to its phytoconstituents was discussed.

Keywords : analgesic, anti-inflammatory, antipyretic, blumea aurita, membrane stabilizing activity.

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I. Introduction

Blumea aurita (Synonyms: *Laggera aurita*; local name: Raihan Aljroof) belongs to the family Asteraceae which is one of the longest families of flowering plants. The family is of worldwide distribution and particularly well represented in semi-arid regions of the tropics and subtropics [1]. It is pubescent pale herbs up to 1m high, strongly scented herb [2]; strongly unpleasant aromatic [3] or aromatic herb [4], erect or decumbent annual herbs. Leaves alternate sessile, oblong-obovate, auriculate and interruptedly decurrent, margin dentate. It is inflorescences compound monochasial heads, 5-6, 7-8 mm; head heterogamous, outer florets filiform, inner one tubular. Its Habitat is water catchments areas. It is found in Central and Southern Sudan [4], mainly in Rahad, Nile Bank and Khartoum.

There are no previous phytochemical reports on *Blumea aurita*; however, flavones, flavonoids, essential oils and organic acids were reported from various *Blumea spp*. [5]. The boiled water extract of the leaves is used for jaundice [6]. The antibacterial activity of seven essential oils of *Laggera aurita* has been studied [4, 5, 7].

Blumea aurita is used in traditional medicinal practice by Sudanese healers to treat; pain; and rheumatism. There were no previous studies in the possible anti-inflammatory, antipyretic or analgesic effects of this plant.

There were repeated evidences that support potential therapeutic effects of *Blumea aurita*. In eastern Sudan, *Blumea aurita* was used by traditional Sudanese herbalists for the treatment of connective tissue inflammatory conditions, pain, fever and jaundice. However, the present literature lack any scientific proofs for these therapeutic benefits. The aims of this study were to screen for the possible phytoconstituents of *Blumea aurita* and to evaluate its anti-inflammatory, anti-pyretic and analgesic effects. In addition, the membrane stabilizing activity of *Blumea aurita* as a possible mechanism for its therapeutic benefits was also evaluated.

II. Experimental

a) Collection and extraction of plant materials

The whole plant was collected from Kasala in Eastern Sudan; after it had been authentificated by

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taxonomists of Medicinal and Aromatic Plants Research Institute (MAPRI) - Sudan. A sample was deposited at the herbarium in the institute. The plant material was then allowed to dry at room temperature for three days. Then the plant material was coarsely powdered.

The dried coarsely powdered plant material was extracted using the soxhlet apparatus. The extraction was first run by petroleum ether to extract the fats and fatty constituent; then by chloroform to separate the non polar compounds; and finally by 70% ethanol to separate the polar compounds. The ethanolic extract was evaporated to dryness under reduced pressure, and kept into a refrigerator to be used for the different tests.

b) Animals

Adult male and female Wister albino rats weighing 90-200 g (a total of 230 rats), were purchased, at the time of each experiment, from the animal center of MAPRI, National Center for Research, Khartoum. All animals had free access to food and water and were kept at room temperature 25±1 ^{o}C, on a 12/12 light/dark cycle. Before each study, animals were submitted to fasting for at least 12 hours.

c) Phytochemical screening

i. *Test for unsaturated sterols and triterpenes*

One ml chloroform was added to the ethanolic extract, and then 0.5 ml of acetic acid anhydride was added followed by 2 drops of concentrated sulphuric acid. The gradual appearance of green, blue, pink to purple color was taken as an evidence of the presence of sterols (green to blue) and triterpenes (pink to purple) in the sample.

ii. *Test for alkaloids*

Five ml of 2N hydrochloric acid were added to 0.5 gm of the extract and stirred while heating in a water bath for 10 minutes. The mixture was cooled, filtered and divided into two test tubes. Few drops of Mayer's reagent were added to one test tube. Few drops of Velser's reagent were added to the other tube. A slight turbidity or heavy precipitate in either tube was taken as presumptive evidence for the presence of alkaloids

iii. *Test for flavonoids*

Half gram of the ethanolic extract of the plant was dissolved in 1 ml ethanol and then 1 ml of 1% KOH was added. Dark yellow color indicates the presence of flavonoids. For conformation, 1 ml of aluminum chloride was added to the extract. Appearance of yellow color confirms presence of flavonoids.

iv. *Test for saponin*

One ml of distilled water was added to the extract in a test tube and was shacked. Formation of foam is considered positive for the presence of saponin.

v. *Test for cumarins*

Half gram of the extract was added to 20 ml of distilled water and boiled. A filter paper was attached to the test tube to be saturated with the vapor then a spot of 0.5 N KOH was put on it. The filter paper was inspected under ultraviolet light. Adsorption of ultraviolet light confirms presence of cumarins.

vi. *Test for anthraquinon*

Half gram of the extract was boiled in 10 ml of 0.5 N KOH containing 1 ml of 3% hydrogen peroxide solution. The mixture was shaken with 5 ml benzene, and allowed to separate into two layers, and then 3 ml of 10% ammonium hydroxide solution were added. The presence of anthraquinones was indicated if the alkaline layer was changed to pink or red color.

vii. *Test for tannins*

Ten ml of hot normal saline were added to 1 gm of the extract and allowed to cool, and then gelatin salt reagent was added to 5ml of the mixture. Immediate precipitation was considered positive for the presence of tannins. In addition, ferric chloride test reagent was added to the other 5 ml of the mixture, Blue, black or green colors were considered positive for the presence of tannins.

d) Evaluation of anti-inflammatory activity

i. *Rat-paw edema model*

The anti-inflammatory activity of ethanolic extract was studied using a modification of rat paw formalin edema method as described by Domenjoz *et. al* [8] and Ramadan *et. al* [9]. The anti-inflammatory effect was determined after measuring the paw's thickness before the formalin injection, and then 1, 2,3,4,6, and 24h post-treatment [9]. The inflammatory response to formalin was evaluated by:

1. Mean paw thickness (MPT) in mm: the mean of the increase in paw thickness after inducing inflammation by formalin.
2. Edema inhibition percentage (EI %) [10]: EI is calculated based on edema formation percentage as follows:

$$\text{Edema formation percentage (EF\%)} = \frac{\text{Tt_To}}{\text{To}} \times 100$$

$$\text{Edema inhibition percentage (EI\%)} = \frac{\text{EFc_EFt}}{\text{EFt}} \times 100$$

Where:

- To = the paw thickness before formalin injection (mm)
- Tt = the paw thickness after t hours of formalin injection (mm)
- EFc = edema formation rate of the control group
- EFt = edema formation rate of the treated group at t hours time

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The observations were statistically analyzed using analysis of variance followed by multiple comparisons [11, 12] via SPSS program

ii. *Cotton pellet granuloma-formation inhibition method*

The method described by Goldstain et al [13] was employed. Cotton pellet weighing 500mg were sterilized in an autoclave. The cotton pellet was implanted subcutaneously in the groin region of each rat under light ether anaesthesia. The cavity was stitched to avoid the drop out of the pellet and exudates. The groups were then orally dosed with aqueous suspension of the ethanolic extracts of Blumea aurita, indomethacin and normal saline once a day as follows:

- Group 1 (N = 5 rats): Blumea aurita 800mg/kg
- Group 2 (N = 5 rats): Blumea aurita 400mg/kg,
- Group 3 (N = 5 rats): Indomethacin 5mg/kg.
- Group 4 (N = 5 rats): normal saline 1ml/kg (control group)

The treatment continues for 5 consecutive days. On day 6 the rats were scarified under light ether anesthesia, the pellets were separately removed and the extraneous materials were removed. The pellets were allowed to dry in an oven at 60 °C overnight. The cotton pellets were weighed individually and the increase in weights were calculated, and considered as the granuloma tissue deposits. Values of granuloma tissue weight were expressed as means ± standard error of the mean (S.E.M).

The mean increase in cotton-pellet weight of the control group was considered as 100% and the rest groups were compared to it as follows:

$$\text{Granuloma tissue formation inhibition percentage(GTI\%)} = \frac{C0_C1}{C0} \times 100$$

Where;

- C0 = the mean of the differences of the control group
- Ct = the mean of the differences of the treated group

Statistical analysis was determined using ANOVA followed by Dunnett's test for multiple comparisons and was employed via SPSS program.

e) Analgesic Activity

The hot plate method as described by Jacob and Bosvski [14] was adopted. The groups were then orally dosed with aqueous suspension of the ethanolic extracts of Blumea aurita, indomethacin and normal saline once as follows:

- Group 1 (N = 5 rats): Blumea aurita 800mg/kg
- Group 2 (N = 5 rats): Blumea aurita 400mg/kg,
- Group 3 (N = 5 rats): Asprin 100mg/kg.
- Group 4 (N = 5 rats): normal saline 1ml/kg (control group)

The rats were dropped on a hot plate maintained at 55 ± 0.50C. The response time was defined as the interval from the instant the animal reached the hot plate until the moment the animal licked its feet or jumped out. The response time was recorded at 10 minutes before treatment, 5 minutes before treatment, 60, 90, and 150 minutes after treatment (using the Hot plate model 39, Wagtech International Ltd - England). Statistical analysis was determined using ANOVA followed by Dunnett's test for multiple comparisons.

f) Antipyretic activity

Hyperpyrexia was induced in rats by subcutaneous administration of 20 ml/kg of 20% aqueous suspension of Brewer's yeast [15]. The rat groups were then orally dosed with aqueous suspension of the ethanolic extracts of Blumea aurita, indomethacin and normal saline once as follows:

- Group 1 (N = 5 rats): Blumea aurita 800mg/kg
- Group 2 (N = 5 rats): Blumea aurita 400mg/kg,
- Group 3 (N = 5 rats): Asprin 100mg/kg.
- Group 4 (N = 5 rats): normal saline 1ml/kg (control group)

Temperatures were then recorded 5 min before and 1, 2 and 4 hours after treatment using Themalert model No.TH5 (Physitemp -U.S.A). Statistical analysis was determined using ANOVA followed by Dunnett's test for multiple comparisons and was employed via SPSS program.

g) Assessment of membrane stabilizing ability

The membrane stabilizing activity of *Blumea aurita* was evaluted according to Shinde *et al.* [16] and Abe *et al.* [17]. Erythrocytes were separated from untreated control rats and suspended in 10mM Na_3PO_4 as 40%. Membrane stabilizing ability was determined as follows:

i. *Heat-induced haemolysis*

5 ml of the isotonic solution (10mM sodium phosphate buffer) containing 50, 100, and 200μg/ml of ethanolic extract of *Blumea aurita* were put into two duplicate sets of centrifuge tubes. 5 ml of the isotonic buffer serve as a control. Erythrocyte suspension (30μl) was added to each tube and mixed gently. One pair of the tubes was incubated at 54 °C for 20 min in a water bath. The other was maintained at 0-5 °C in an ice bath. The reaction mixtures were centrifuged and optic

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densities of the supernatant were measured at 540nm using UV-160A spectrophotometer. Optic density of each solution was used as an indicator for the degree of hemolysis and hence cell membrane stability. Acetyl salicylic acid (aspirin) 200μg/ml was used as a reference standard.

ii. *Hypotonic solution-induced haemolysis*

Same as described above but using hypotonic solution (154mM NaCl), erythrocyte suspension (30μl) was mixed with 5 ml of the hypotonic solution containing *Blumea aurita* ethanolic extracts at concentrations of 50, 100, and 200 μg/ml. The control sample was mixed with drug free solution. The mixtures were left for 10 minutes at room temperature and centrifuged for 3 min at 1300g. Optic density of each solution was measured and used as an indicator for the degree of cell membrane stability. Acetyl salicylic acid (aspirin) 200μg/ml was used as a reference standard.

In experiment, the percentage inhibition or acceleration of haemolysis were calculated according to the equation:

$$\% \text{ Acceleration or inhibition of haemolysis} = \left(1 - \frac{OD2 - OD1}{OD3 - OD1}\right) 100$$

Where:

- OD_1=test sample unheated or in isotonic solution;
- OD_2= test sample heated or in hypotonic solution;
- OD_3=control sample heated or in hypotonic solution.

Statistical analysis was determined using ANOVA followed by Dunnett's test for multiple comparisons.

III. Results

The findings of the phytochemical screening of the of *Blumea aurita* revealed presence of triterpenes, flavonoids, saponin, cumarins, tannins and traces of alkaloids. In contrast, *Blumea aurita* is devoted from unsaturated sterols and anthraquinon (table-1). Table-2 shows the effects of ethanolic extracts of *Blumea aurita* and indomethacin on rat MPT and EI% at the studied time intervals. The highest EI% for both indomethacin and *Blumea aurita* at a dose of 400mg/kg were reported after 4 hours of oral administration of the aqueous suspension. *Blumea aurita* at a dose of 800mg/kg showed a peak EI% after 6 hours (tabl-2). The effects of ethanolic extract of *Blumea aurita* was dose-dependent reduction in MPT and EI%. As shown in table-3, granuloma tissue-formation inhibition percentage of *Blumea aurita* at a dose of 800mglkg (63.79%), and *Blumea aurita* at 400mg/kg (56.72%) were significantly more compared to indomethacin (32.25%). The peak rats' response to analgesia was recorded after 60 minute of oral administration of *Blumea aurita* in a dose-dependent manner (table-4). *Blumea aurita,* at a dose of of 800 mg/kg, significantly reduced body temperature of hyperthermic rats compared to acetylsalicylic acid; however, there was no significant difference between acetylsalicylic acid and *Blumea aurita* at a dose of 400 mg/kg (table-5). *Blumea aurita* ethanolic extracts at a concentration of 50μ/ml, 100μ/ml, 200μ/ml, showed significant inhibition of heat-induced and hypotonic solution-induced red cell hemolysis compared to acetylsalicylic acid at 200μ/ml concentration, (table-6, $P < 0.05$ using Dunnett test).

Table 1 : Phytoconstituents of the ethanolic extracts of *Blumea aurita*

Blumea aurita	Ingredients
Negative	Unsaturated sterols
Positive	Triterpenes
Traces	Alkaloids
Positive	Flavonoids
Positive	Saponin
Positive	Cumarins
Negative	Anthraquinon
Positive	Tannins

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Table 2 : Effects of ethanolic extracts of B. aurita and indomethacin on rat paws' thickness and edema inhibition percentage at studied time intervals

Extract/drug		Time interval						Mean
		1 hour	2 hours	3 hours	4 hours	6 hours	24 hours	
B. aurita	EI%	41.6	59.5	47.2	52.8	99.86*	95.6	67
(800 mg/kg)	MPT(mm)	7.41±.31	6.51±.21	6.51±.37	5.29±.22	5.88±.28	5.36±.14	6.03±.79
B.aurita (400mg/kg)	EI%	48.92	51.8	24.83	87.31*	46.21	68.87	53.15
	MPT(mm)	7.16±.39	6.84±.33	7.30±.15	5.83±.31	7.05±.12	5.71±.35	6.43±.85
Indomethacin	EI%	24.2	32.2	50.9	97*	83.4	71.4	64
(5mg/kg)	MPT(mm)	8.29±.64	7.59±.39	6.67±.52	5.63±.33	5.91±.25	6.05±.17	6.52±1.06
Normal saline	EI%	9.03±.71	8.45±.39	7.71±136	8.24±.41	7.69±.32	7.21±.20	7.68±1.15

* The highest edema-inhibition percentage.

Table 3 : Granuloma tissue formation inhibition percentage for *B. aurita* ethanolic extracts and indomethacin

Extract/drug	Granuloma weight (mg) Mean±SEM	Percent inhibition
B. aurita (800 mg/kg)	42.67±.71	63.79%
B.aurita (400mg/kg)	51±2.27	56.72%
Indomethacin (5mg/kg)	79.83±4.46	32.25%
Normal saline	117.83±.60	

Table 4 : The analgesic effect of B. aurita ethanolic extracts and acetylsalicylic acid

Extract/drug	Mean response time /time interval					Mean response time /time interval
	10 minutes before treatment	10 minutes before treatment	10 minutes before treatment	10 minutes before treatment	10 minutes before treatment	
B. aurita (800 mg/kg)	6.89	7.51	22.09	18.87	11.84	13.43±.1.15
B. aurita (400 mg/kg)	7.48	8.26	18.58	14.94	9.45	11.76±9.89
Acetylsalicylic acid (100 mg/kg)	7.91	10.91	13.42	11.68	9.24	10.63±0.57
Normal saline	6.75	7.87	16.88	14.19	10.19	5.49±0.20

Table 5 : Antipyretic activity, mean rectal temperature at intervals for rats treated with B. aurita ethanolic extracts and acetylsalicylic acid

Extract/drug	Body temperature/interval (°C)				mean±SEM (°C)
	Before treatment	1hour after treatment	2h0urs after treatment	4hours after treatment	
B. aurita (800 mg/kg)	39.06±0.46	36.28±0.07	36.24±0.16	36.37±0.14	36.83±0.14
B. aurita (400 mg/kg)	39.36±0.42	36.36±0.11	36.38±0.1	36.29±0.11	37.08±0.16
Acetylsalicylic acid (100 mg/kg)	39.44±0.3	36.58±0.27	36.36±0.06	36.39±0.11	37.32±0.17
Normal saline	38.7±0.81	38.24±0.34	38.9±0.44	37.46±20.29	38.46±0.14

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Table 6 : Membrane stabilizing ability percentage inhibition of RBCs haemolysis produced by *B. aurita* ethanolic extracts and acetylsalicylic acid

Extract/Drug	Concentrations	Heat-induced haemolysis % inhibition Mean%±SEM	Hypotonic solution-induced haemolysis % inhibition Mean%±SEM
B.aurita	50 µg/l	27.73±.19*	67.91±.82*
	100 µg/l	48.74±.80*	79.61±.84*
	200 µg/l	65.60±99*	91.86±1.88*
Aspirin	200 µg/l	25.19.28	76.41±.61

IV. Discussion

The findings of the current study give scientific confirmation for the anti-inflammatory, anti-pyretic and analgesic effects of *Blumea aurita*. These effects are probably attributed to the unique phytoconstituents of *Blumea aurita* which deserve further investigations. Interestingly, the effects of *Blumea aurita* exceed the classical non-steroidal anti-inflammatory drugs (NSAID) used in clinical practice, namely indomethacin and acetylsalicylic acid. *Blumea aurita* was used by traditional Sudanese herbalists for the treatment of many inflammatory conditions, including rheumatoid arthritis, and for pain relief. However, the plant did not receive any scientific attention, and the results of the current study represent the first report on the possible therapeutic effects of *Blumea aurita*.

The phytochemical screening of *B.aurita* revealed presence of triterpenes, Alkaloids, Flavonoids, Saponins, Cumarins and Tannins; however, the plant is devoid of unsaturated sterols and Anthraquinon. The existence of flavonoids in the *Blumea aurita*, may account for the observed anti-inflammatory activity [18, 19]. Fan *et al* [20] attributed the anti-inflammatory activity of *Terminalia catappa* to triterpenic acids, and since *Blumea aurita* contains triterpens this finding may be applied to it. In addition, the current results showed significant granuloma tissue formation inhibition, indicating that the *Blumea aurita* has the ability to interfere with one or more responses of the inflammatory processes especially those concerned with the inflammatory cells migration and proliferation. The reduction in granuloma tissue weight could be due to better maturation of collagen which invariably leads to shrinkage of granulation tissue [22].

The analgesic activity of *Blumea aurita* may in part be attributed to the saponine, triterpenes, sterols, flavonoids and glycosides [9]. . In the folklore medicine of different cultures, the plants rich in triterpenes are commonly used for the treatment of inflammation [23]. Although it is not possible to pin point the exact phytoconstituent(s) responsible for the anti-inflammatory, anti-pyretic and analgesic activities, these effects appear to be due to the flavonoids or glycosides as well. Actually, these later two phytoconstituents were present in *Caralluma tuberculata* when studied by Ahmed *et al* and can elucidate the anti-inflammatory and analgesic effects of this herb [23]. Alternatively, Ramadan *et al* [9] studied the anti-inflammatory, analgesic of *Adansonia digitata* and reported that the anti-inflammatory effect may be due to the presence of sterols, saponins and triterpenes in their aqueous extract, same as *Blumea aurita*.

On the other hand, according to the current data *Blumea aurita* possess antipyretic activity more than acetylsalicylic acid at a dose rate of 100 mg/kg. The *Blumea aurita* is also rich in saponins which explain its antipyretic activity as proved by Mohsin *et al* [15] when studying therapeutic effects of *Tamarix nilotica*.

The plant significantly reduced erythrocytes heat-induced and hypotonic solution-induced haemolysis compared to acetylsalicylic acid at a concentration of 200µm/L. According to Abe *et al* [17], saponins are claimed to have a membrane stabilizing action. The possible explanation for the membrane stabilizing activity could be an increase in the surface area/volume ratio of the cells which could be brought about by an expansion of membrane or shrinkage of the cell by interacting with certain cytoskeletal proteins [17, 24]. Theoretical speaking, the membrane stabilizing activity of *Blumea aurita* interferes with the release of the mediators of inflammation, fever and pain producing substances and therefore explain the therapeutic effects of *Blumea aurita* [24].

In conclusion, the current data prove beyond doubt the potential therapeutic effects of *Blumea aurita* in treating acute inflammation as indicated by edema inhibition, chronic inflammation as indicated by inhibition of granuloma tissue formation, pain and hyperpyrexia. In addition, current results augment what was acknowledged by traditional Sudanese herbalists that *Blumea aurita* is an effective treatment of many inflammatory conditions, including rheumatoid arthritis. Detailed phytochemical and toxicological investigations are desirable to determine the active ingredients responsible to the therapeutic effects of *Blumea aurita* and the potential side effects.

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