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Phytochemical Screening and Anti-inflammatory Properties of Henna Leaves (*Lawsonia inermis*)

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Authors' contributions

This work was carried out in collaboration among all authors. Author LCC designed the study, served as the general supervisor. Author NCC performed the preliminary aspect of the study, carried out the statistical analysis, wrote the protocol and wrote the first and final draft of the manuscript and author DD managed the literature searches, the bench work and analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

In traditional West African setting, plants are commonly used for cosmetic and medicinal benefits such as antibacterial, antifungal, anti-inflammatory, antioxidant and antiparasitic effect of which Henna (*Lawsonia inermis*), a natural occurring plant of the lythraceae family is not an exception. A preliminary qualitative phytochemical screening and anti-inflammatory properties of *Lawsonia inermis* leaves extracted with N-butanol and ethyl acetate was investigated. Standard analytical procedures were observed, and statistical analysis using ANOVA version 20.0 was conducted to analyze for mean ± standard deviation (SD) and the least significant difference (LSD) obtained from the study. Results revealed the presence of flavonoids, tannins, terpenes, cyanogenic and cardiac glycosides; while *In vivo* anti-inflammatory activity wasobserved on a week old cockerel chicks induced carrageenan inflammation post extract administration, using aspirin tablet as control. The anti-inflammatory activity of the extract as portrayed could be attributed to the plants rich phytochemical potential.

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1. INTRODUCTION

Henna or Hina (*Lawsonia inermis*), a flowering plant and lone specie of the Lawsonia genus. It is commonly used as a dye for various uses (skin, hair, finger nails and fabrics). The plant is localized in the Sahel, Central Africa and slightly in the Middle East. Aside its external use, it serves some medicinal and therapeutic purposes ranging from hepatoprotective and immunomodulatory effects, antimicrobial, antifungal and antioxidant properties with scientific evidence [1]. In south west Nigeria, the aqueous leaves of *L. inermis* is used as antimalaria decoction according to scientific research [1]. Reports by Aguwa [2] also captured the abortifacient activity of *L. inermis* methanol root extract in mice, albino rats and guinea pig in a dose dependent ratio.

Secondary metabolites produced by plants, which confers attributes like colour, flavour and natural defence to plants are referred to as phytochemicals [3]. Human health over time has been influenced positively by the contributory protective effect of phytochemicals against a variety of degenerative diseases [4,5]. A wide range of mechanism suggests that plant phytochemicals are important components of human and animal diets. These phytochemicals commonly obtained from vegetables, fruits and seeds act as antioxidants, modulators of gene expression and signal transduction pathways [5]; (Okwu, 2005). Irritants, cell damage and pathogens which are harmful to the body tissues constitute a complex biological response referred to as inflammation [6]. Molecular mediators, blood vessels and immune cells constitute a protective response leading to inflammation. It is essentially the elimination of initial causes of cell injury, necrotic cells and tissue damage, followed by the initiation of tissue repair (Tanko *et al*., 2008). The study evaluates the therapeutic efficacy of N-butanol and ethyl acetate extracts of *L. inermis* leaves on carrageenan induced inflammation in a week old cockerel chicks and to ascertain the respectively phytochemicals responsible for the anti-inflammatory activity.

2. METHODOLOGY

2.1 Materials and Reagents

Distilled water, olive oil, ethanol,acetyl acetate, N-butanol, hydrochloric acid, ferric chloride, lead acetate, mayers reagent, wagners reagent, dragendoffs reagent, hagers reagent, alpha naphtol, sodium hydroxide, fehlings solution A and B, ethyl acetate, ammonium hydroxide, chloroform, millions reagent, sulphuric acid, phlorglucinol and iodine were of analytical grade. Aspirin (200 mg) used was obtained from LUTH pharmacy.

2.2 Plant Collection and Identification

Fresh *L. inermis* leaves were harvested from farm stead in Elele, Ikwerre L.G.A., Rivers State. Harvesting was carried out during the day and in good condition. Plant identification was done by Mr. I. Ozioko (rtd), a renowned botanist and former staff of Botany Department, University of Nigeria, Nsukka, Enugu State.

2.3 Procurement of Chicks

Twenty white cockerels (*Gallus gallus domesticus*, strain shaver 5790) weighing between 40-55 g were procured from Jocarl Breeder Farm, Umudioga road, Elele, Rivers State, Nigeria, conveyed to the animal house of pharmacognosy, Madonna University, Nigeria. They were properly housed with soft wood shavings as beddings, vaccinated and had access to clean water and commercially available starter pellet diet (Top feed, Nigeria) *ad libitum* prior to the study, with a 12/12 hours light and dark cycle maintained.

2.4 Preparation of Plant Material

Harvested and identified fresh leaves of Henna (*L. inermis*) were properly washed with distilled water to get rid of debris, and then air dried in natural environment afterwards. The dried leaves were homogenized into fine powder and 250 g each of powdered leaves was poured into two 1000 ml round bottom flasks. A 1250 ml each of N-butanol and acetyl acetate was measured and poured into the two flasks respectively. The resultant mixture was allowed to stand for 24 hours with occasional shaking, prior filtration. The filtrate was evaporated, first with a porcelain bag, and then with Whatman No. 1 filter paper before a rotary evaporator was used to dry to paste with the aid of a thermostatic water bath at 40°C. The concentrate was transferred into storage amber bottles and then stored in the refrigerator at 4°C for use.

2.5 Preliminary Qualitative Phytochemical Screening

Quantitative phytochemical screening for the detection of secondary metabolites (phenols and flavonoids) was carried out on the plant extracts based on the methods of Harbourne, [7] and Evans [8].

2.5.1 Test for carbohydrates

Molisch's test: 0.5 g of the extract was boiled with 2ml of distilled water and filtered. To the filtrate, few drops of α-naphthol solution in ethanol (Molisch reagent) was added and concentrated sulphuric acid was then gently poured down the side of the tube to form a lower layer. A purple interfacial ring formed indicates the presence of carbohydrate.

2.5.2 Test for saponins

20 ml of distilled water was added to 0.5 g of the respective extracts and boiled on a hot water bath for 2 minutes and filtered. The filtrate was allowed to cool and then used for the following tests;

1. Frothing test: 5 ml of the filtrate was diluted with 15 ml of distilled water and shaken vigorously. The formation of a stable froth indicates the presence of saponins.
2. Emulsion test: To the frothing solution was added 2 drops of olive oil and the contents shaken vigorously. The formation of emulsion indicates the presence of saponins.
3. Haemolytic test: 2 ml of the plant extracts were respectively mixed with 2 ml of anti-agglutinated blood (whole blood) and shaken. Haemolysis of the red blood cells indicates the presence of saponins.

2.5.3 Test for tannins

1. Lead acetate test: 1 ml of lead acetate solution was added to a test tube containing 1 ml of the extract and the formation of a white precipitate is indicative of the presence of tannins.
2. Brayer's test: 1 ml of ferric chloride solution was added to a test tube containing 1 ml of the extract and the formation of a dark blue colour indicates the presence of Phenolic compound.
3. Ferric chloride test: To 3 ml of the filtrate, few drops of ferric chloride was added and the formation of a greenish black precipitate indicates the presence of tannins.

2.5.4 Tests for flavonoids

10 ml of ethyl acetate was added to 0.2 g of the extract and heated on a water bath for 3 minutes. The mixture was cooled, filtered and the filtrate used for the following test;

1. Ammonium test: 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate and a yellow colour in the ammonical layer indicates the presence of flavonoids.
2. Aluminium chloride solution test: 4 ml of the filtrate was shaken with 1 ml of 1% aluminium chloride solution and the layers were allowed to separate. The formation of yellow colour in the aluminium chloride layer indicates the presence of flavonoids.

2.5.5 Test for reducing sugars

1. Fehling's solution test: 5 ml of a mixture of equal volume of fehling's solution I and II were added to 5 ml of the extract and then heated on a water bath for 5 minutes. A brick red precipitate indicates the presence of reducing sugars.
2. Benedict's solution test: 5 ml of Benedict's solution was added to 5 ml of the extract and then heated on a water bath for 5 minutes. A brick red precipitate indicates the presence of reducing sugar.

2.5.6 Test for protein

0.5 g of extract was dissolved in 20 ml of distilled water, filtered and the filtrate was used for the following test:

1. Million's test: To an aliquot of the filtrate in a test tube, and 2 drops of million's reagent was added. A white precipitate indicates the presence of protein.
2. Xanthoproteic test: 5 ml of the filtrate was heated with few drops of concentrated nitric acid. A yellow coloured precipitate changes to orange on addition of an alkali (dilute sodium hydroxide) indicates the presence of protein.

2.5.7 Test for steroids

9 ml of ethanol was added to 1 g of the extract and refluxed for a few minutes and filtered. The

filtrate was concentrated to 2.5 ml on a boiling water bath. 5 ml of hot distilled water was added to the concentrated solution, the mixture was allowed to stand for 1 hour and the waxy substance was filtered off. The filtrate was extracted with 2.5 ml of chloroform using separating funnel. 1 ml of absolute sulphuric acid was carefully added to 0.5 ml of chloroform extract to form a lower layer. A reddish brown interface indicates the presence of steroids.

2.5.8 Test for alkaloids

20 ml of 3% sulphuric acid in 50% ethanol was added to 2 g of the extract and heated on a boiling water bath for 10 minutes, cooled and filtered. 2 ml of the filtrate was tested with a few drops of Mayer's reagent (potassium mercuric iodide solution), Dragendorff's reagent (Bismuth potassium iodide solution), Wagner's reagent (iodopotassium iodide solution) and picric acid solution (1%). Alkaloids give milky precipitate with Mayer's reagent; reddish brown precipitate with Wagner's reagent; yellowish precipitate with picric acid and brick red precipitate with Dragendorff's reagent based on Anowi CF, et al. [9].

2.5.9 Test for glycosides

10 ml of 1% sulphuric acid was added to 0.5 g of the pastry extract in a test tube and boiled for 15 minutes on a water bath, then cooled and neutralized with 20% potassium hydroxide solution. 10 ml of a mixture of equal volume of fehling's solution I and II was added and boiled for 5 minutes. A brick red precipitate indicates the presence of glycoside.

2.6 Anti-inflammatory Assay

Carrageenan-induced paw edema is the most common method employed in the evaluation of anti-inflammatory therapies. The use of carrageenan stimulated inflammation and the determination of the anti-inflammatory effects of *Lawsonia inermis* leaves extract in 7 day old domestic chicks was experimented. The time course effect of foot withdrawal laterncy to a thermal stimulus and edema were examined over a 6 hour period following an intraplantar carragenan injection. The extract was suspended in sterile water by adding 2% of tween 20 to enhance the suspension of the extract. Varying concentrations (50, 100 and 200 mg/kg) of the extract suspensions were administered to the chicks (n=5) and this served as the negative control. Carrageenan (10 µl of 2% suspension in saline) was injected subplantar into the right footpad of the chicks. The foot volume was measured before injection and at hourly interval for 5 hours after injection using a digital venier calliper. The edema induced by the inflammation was quantified by measuring the difference in foot thickness before carrageenan injection and at the various time points. The values were individually normalized as a percentage change in foot edema relative to the corresponding values at time zero, and then averaged for the respective treatment groups. The percentage inhibition for each treatment was determined thus:

$$\%\ inhibition\ = \frac{(Acontrol\ -\ Asample)}{Acontrol} \times 100$$

2.7 Data Analysis

Data obtained were statistically analyzed for mean ± standard deviation (SD), comparison of mean and LSD correlation was done using analysis of variance (ANOVA) version 20.0 and significance set at $p<0.05$.

3. RESULTS

The result of qualitative phytochemical screening of N-butanol and ethylacetate extracts of *L. inermis* leaves carried out in triplicate evaluations indicated significant ($p<0.05$) presence of beneficial phytochemicals with low levels of proteins and carbohydrates (Table 1).

Carrageenan induced edema has a course of action that lasts for approximately 2 hours post injection and the carrageenan induced inflammation was seen at all concentrations tested. The activities of the extract were compared with that of a reference standard non-steroidal anti-inflammatory drug, diclofenac sodium which served as a positive control (see Tables 2 and 3).

4. DISCUSSION

4.1 Qualitative Phytochemical Screening of N-butanol and Ethylacetate Extracts of *L. inermis* Leaves

The qualitative phytochemical screening on N-butanol and ethylacetate extracts of *L. inermis* leaves carried out in triplicate indicated the presence of flavonoids, alkaloids, glycosides, saponins, tannins and steroids, with flavonoids having the most significant level. On the contrary, there were absence of carbohydrate and protein. Plants containing flavonoids and alkaloids have shown antispasmodic, anti-inflammatory and

Table 1. Qualitative phytochemical screening of *L. inermis* extracts

Phytochemicals	N-butanol leaf extract	Ethylacetate leaf extract
Carbohydrate	-	-
Saponins	+	+
Tannins	+	+
Flavonoids	+++	+++
Protein	-	-
Steroids	+	+
Alkaloids	++	++
Glycosides	++	++

Key: + = slightly present; ++ = Moderately present; +++ = Highly present; - = Absent

Table 2. Anti-inflammatory activity of N-butanol extract of *L. inermis* leaves on carrageenan-induced edema in chicks

Groups	IVP	VAI (0 Mins)	30 Mins	60 Mins	120 Mins	180 Mins	240 Mins
Group 1	2.34±0.30	3.10±0.42^{a}	2.94±0.30^{a}	2.88±0.22^{a}	2.80±0.20^{a}	2.56±0.24	2.38±0.26
Group 2	2.38±0.19	3.24±0.27	3.12±0.23^{a}	3.02±0.24^{a}	2.84±0.21^{a}	2.68±0.23	2.58±0.16
Group 3	2.32±0.15	3.22±0.30^{a}	3.18±0.26	2.98±0.19	2.82±0.15	2.62±0.19	2.44±0.11^{a}
Group 4	2.36±0.21^{b}	3.24±0.30^{b}	3.20±0.24	2.98±0.26^{a}	2.84±0.23	2.68±0.15^{ab}	2.58±0.15^{ab}
Group 5	2.38±0.22	3.34±0.30^{a}	3.26±0.26^{b}	3.10±0.21^{b}	2.96±0.18^{ab}	2.80±0.16	2.58±0.22

Data are presented as the mean ± standard deviation (SD). ^{a}P<0.05 vs. IVP (NC); ^{b}P<0.05 vs. VAI; ^{ab}P<0.05 vs. IVP & VAI. IVP, Initial volume of paw; VAI, volume after inoculation. Group 1 (Negative control on distilled water); Group 2 (Positive control on diclofenac); Group 3 (50 mg/kg bwt); Group 4 (100 mg/kg bwt); Group 5 (200 mg/kg bwt)

Table 3. Anti-inflammatory activity of ethylacetate extract of *L. inermis* leaves on carrageenan-induced edema in chicks

Groups	IVP	VAI (0 Mins)	30 Mins	60 Mins	120 Mins	180 Mins	240 Mins
Group 1	2.20±0.10	3.22±0.41^{a}	3.18±0.45^{a}	3.06±0.51^{a}	2.94±0.46^{a}	2.82±0.33^{a}	2.58±0.22
Group 2	2.22±0.13	3.58±0.28	3.48±0.19	3.22±0.30^{a}	2.96±0.52^{a}	2.74±0.23	2.54±0.15
Group 3	2.18±0.19	3.32±0.22^{a}	3.20±0.20	3.00±1.00	2.82±0.11	2.58±0.13	2.36±0.17
Group 4	2.26±0.11^{b}	3.34±0.15^{b}	3.26±0.19^{b}	3.06±0.15^{b}	2.94±0.11^{ab}	2.76±0.15	2.56±0.09
Group 5	2.24±0.21	3.30±0.20^{ab}	3.18±0.26^{a}	2.86±0.27^{b}	2.82±0.19^{ab}	2.64±0.18	2.42±0.41

Data are presented as the mean ± standard deviation (SD). ^{a}P<0.05 vs. IVP (NC); ^{b}P<0.05 vs. VAI; ^{ab}P<0.05 vs. IVP & VAI. IVP, Initial volume of paw; VAI, volume after inoculation. Group 1 (Negative control on distilled water); Group 2 (Positive control on diclofenac); Group 3 (50 mg/kg bwt); Group 4 (100 mg/kg bwt); Group 5 (200 mg/kg bwt)

antioxidative properties (Table 1). However, from the results, there was no difference in the extract type (N-butanol and actylacetate) used which was attributed to the similarity as regards polarity index.

4.2 Anti-inflammatory Activity of N-butanol Extract of *L. Inermis* Leaves on Carrageenan-induced Edema in Chicks

The anti-inflammatory activity of N- butanol extract of *L. inermis* leaves on carrageenan-induced edema in chicks showed a significant (p<0.05) reduction with increase in time (30 to 240 mins) on the level of edema within each group when compared with IVP and VAI respectively. However, group 3 (50 mg/kg bwt) demonstrated the most effective anti-inflammatory and therapeutic efficacy followed by group 4 (100 mg/kg bwt). The observed effect and reduction in edema was time dependent (see Table 2).

4.3 Anti-inflammatory Activity of Ethylacetate Extract of *L. inermis* Leaves on Carrageenan-induced Edema in Chicks

The ethylacetate extract of *L. inermis* leaves on carrageenan-induced edema in chicks was observed with significant (p<0.05) difference in anti-inflammatory activity. The leave extract had a reducing effect on carrageenan-induced edema with group 3 (50 mg/kg btw) showing the most effective time dependent activity. The anti-inflammatory activity was also observed to be consistent with increase in time (Table 3).

The N-butanol and ethylacetate extracts of *L. inermis* leaves illustrated a mild anti-inflammatory effect oncarrragenan-induced edema on chick paw which was compared statistically with diclofenac (standard drug). The observed effect was concentration and time dependent as observed across groups with a peak activity of 50mg/kg weight. Most human clinical studies on the safety and therapeutic activity of green lipped mussel extract (GMLE) was shown to useful(Ahmad et al., 2018; Gibson, 2000; Halpern, 2000, 2008; Lyprinol, 2017; Treschow et al., 2007; Whitehouse et al., 1997).

5. CONCLUSION

The anti-inflammatory and therapeutic activity of *L. inermis* extracts (N-butanol and ethyl acetate) has been reported with little or no scientific evidence. This investigation demonstrates the possibility of a promising anti-inflammatory activity which is time and concentration dependent, with an optimum therapeutic range of 50 mg/kg body weight irrespective of the mode of extraction. This assertion on the anti-inflammatory activity is affirmed by the result of the investigation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Strict compliance with National Institute of Health (NIH) Nigeria guideline for the care and use of laboratory animals was maintained and permission sought from the animal handling and ethics committee of Madonna University, Nigeria [MAU/EC/20/029].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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