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Anti-inflammatory Activity of the Fractions from the Methanol Extract of *Carpolobia lutea* G. Don (Polygalaceae) Fruit and Leaf

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Abstract

The use of nonsteroidal anti-inflammatory drugs (NSAIDS) is not applicable to all patients and cases, most especially in chronic inflammatory condition, because of the adverse effects which include an increased risk of ulcerative diseases, kidney and heart diseases. Therefore, this study is to evaluate the fractions from the methanol extract of the fruit and leaf of *Carpolobia lutea* for anti-inflammatory effect. The anti-inflammatory activities were evaluated using two models: inhibition of albumin denaturation and membrane stabilization test. The results showed that the different solvent fractions of *Carpolobia lutea* leaf and fruit possessed varying anti-inflammatory properties, at inhibiting the heat induced albumin denaturation and stabilizing the Red Blood Cells membrane at 200 and 1000 $\mu\text{g/ml}$ respectively. The leaf was more active than the fruit part of the plant at inhibiting inflammation for both experimental models. Maximum inhibition (64.3 %) was observed for the n-hexane fraction of the leaf at 1000 $\mu\text{g/ml}$ for the heat induced albumin denaturation while the chloroform and ethyl acetate fractions of the leaf and fruit were not active at both concentrations. The maximum inhibition was recorded for the chloroform fraction (65.54 %) of the plant leaf at the concentration of 200 $\mu\text{g/ml}$ while the n-hexane fraction was not active for the membrane stabilization test. These findings justify the ethnomedical use of this plant as an anti-inflammatory agent.

Keywords: *Carpolobia lutea*, anti-inflammatory, albumin denaturation, fractions, membrane stabilization

INTRODUCTION

Plants, which are sources of natural products, have provided promise of cure for various ailments, as they have produced the lead compounds for the synthesis/biosynthesis of drugs and which is an important source of new therapeutic agents (Andreo *et al.*, 2006). Inflammatory diseases including rheumatism and arthritis continue to be well established medical problem and a major cause of morbidity worldwide. Since the finding of aspirin from Willow's bark, more than 100

years ago, many steroidal, as well as non-steroidal anti-inflammatory drugs have been introduced such as ibuprofen, naproxen and diclofenac *e.t.c* However, the prolonged use of most of these prescription drugs reportedly causes renal diseases, gastrointestinal irritation and other adverse effects (Bertolini *et al.*, 2001). Thus, global interest has been aroused to discover potential anti-inflammatory molecules from plants which are traditionally used for aches, fever and rheumatic pain (Basu and Hazra, 2006).

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Carpolobia lutea G. Don (Polygalaceae) is known as cattle stick in English, Ikpafum (Ibibio), Agba or Angalagala (Igbo) and Egbo Oshunshun (Yoruba) in Nigeria (Ajiwhien and Bisong, 2013). The shrub, *Carpolobia lutea*, (*C. lutea*) is regarded traditionally as remedy for a lot of ailments such as sterility, dermal and venereal disease, worm infestation, diarrhoea, malaria, rheumatism, snake bite, insanity and as aphrodisiac (sexual invigorator) among other uses (Yakubu and Akanji, 2014). Ethnomedical survey of anti-inflammatory plants sold in Lagos Nigeria herb markets reveals that the stem bark, leaves and roots of the plant are used for this purpose (Sofidiya *et al.*, 2014). *C. lutea* root decoction is widely used by traditional herbal practitioners as malarial remedy (Nwafor and Basse, 2007; Ajibesin *et al.*, 2008), anti-inflammatory/anti-arthritic (Iwu and Anyawu, 1982), anti-inflammatory and antipyretic of the leaf Ndiwu and Nwafor, 2012, analgesic activity (Jackson *et al.*, 2011), anthelmintic and antisterility agent (Mitaine *et al.*, 2002)

Nonsteroidal anti-inflammatory drugs (NSAIDs) are often not suitable in all patients and cases, particularly in chronic inflammatory condition, because of their major adverse effects such as ulcerative and renal diseases. Hence, there is need for continuing search for alternative remedies. It has also been observed that not much work has been done on the anti-inflammatory efficacy of the fruit and leaf fractions of the plant used in this study. Hence, the aim of this study is to evaluate the fractions from the methanol extract of fruit and leaf of *Carpolobia lutea* for anti-inflammatory activity.

MATERIALS AND METHODS

Collection and identification of plant material

The leaves and fruits of *Carpolobia lutea* used in the study were collected from Surulere estate, Iyesi- Otta, Ado- Odo Local Government Area of Ogun State, Nigeria. The plant was identified at the Department of Pharmacognosy, University of Lagos, Nigeria with voucher specimen number PCGH-35. The test plant parts were washed, cut into small pieces and air dried under the shade for seven weeks until completely dried. The dried plant materials were ground into powder and stored in air-tight glass bottles at room temperature prior to experiments.

Extraction procedure

The powdered *Carpolobia lutea* leaf (250 g) were then subjected to cold maceration in a total of 1,500 ml of solvent system containing methanol (Analar grade) 750 ml and distilled water 750 ml over 72 hrs. The crude extract was filtered first through cotton wool, then through Whatman's filter paper of 125 mm. The filtrate was then concentrated using rotary evaporator at 35°C and further dried in the oven at 35°C. This same procedure was repeated for 330g of the powdered fruit. The dry extracts were weighed, stored in a sample bottle and preserved in the freezer before fractionation.

Extraction and Fractionation

The leaf and fruit extracts, 40g each were dissolved in 500 ml of distilled water and placed in a separating funnel to be fractionated with 3 × 500 ml of each solvent in order of polarity, starting with the least polar, n-Hexane, followed by chloroform and ethyl acetate. The fractions were dried in the oven at 35°C. The dried fractions were weighed and stored in a glass sample bottle for further experiment.

In vitro Anti-inflammatory activity

Inhibition of Albumin Denaturation:

Methods of Mizushima and Kobayashi, 1968 and Sakat *et al.*, 2010 were ensured with minor modifications. The reaction mixture consists of the test extracts (at 200 and 1000 µg/ml) and 1% aqueous solution of bovine albumin fraction. The pH of the reaction mixture was adjusted using a small amount of HCl at 37°C. The sample extracts were incubated at 37°C for 20 mins and then heated to 51°C for 20 minutes. After cooling the samples, the turbidity was measured spectrophotometrically at 660nm with a double beam UV-Visible Spectrometer (PG Instruments Ltd, T80 +, S/N 151885-01-0094). The experiment was carried out in triplicate. Aspirin was used as a standard drug. Percentage inhibition of protein denaturation was calculated using the Eq 1:

$$\% \text{ inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \text{-----}$$

-----Eq 1

Where Abs_{control} is the absorbance without sample, Abs_{sample} is the absorbance of sample extract/ standard.

Membrane Stabilization Test:**Preparation of red blood cells (RBCs) suspension**

Fresh whole human blood (10 ml) was collected from a volunteer and transferred to the laboratory centrifuge and was spin at 3000 rpm for 10 min and were washed three times with equal volume of normal saline and reconstituted as 10% v/v suspension with normal saline (Sakat *et al.*, 2010; Sadique *et al.*, 1989). The study was conducted in accordance with the nationally accepted guidelines for release of blood for non-clinical purposes and approval was given by the College of Medicine, University of Lagos, Nigeria Health Research Ethics Committee with approval number CMUL/HREC/07/17/209.

Heat Induced Haemolytic: The reaction mixture (2ml) consist of 1ml of test sample solution (200 and 1000 µg/ml) and 1ml of 10% RBCs suspension. Instead of test sample, only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 mins. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560nm. The experiment was performed in triplicates for all the test samples. Percentage membrane stabilization activity was calculated by the formula of E.q 1. (Saka *et al.*, 2010; Shinde *et al.* 1999).

Table 1: Percentage inhibition of different solvent fractions of the *C. lutea* leaf and fruit on albumin denaturation inhibition

Test fractions	Leaf		Fruit	
	% Inhibition at 200 µg/ml	% Inhibition at 1000 µg/ml	% Inhibition at 200 µg/ml	% Inhibition at 1000 µg/ml
n-Hexane	na	64.3 ± 0.01	42.9 ± 0.0005	na
Chloroform	na	na	Na	na
Ethyl acetate	na	na	Na	na
Aspirin acid	60.7± 0.0005	61.2± 0.0005	60.7± 0.0005	61.2± 0.0005

Note: na = not active

RESULTS

The weight of the *C. lutea* leaf extract was 63.55 g and fruit extract 46.17 g which translate to 19.26% and 16.13% yield respectively.

Inhibition of albumin denaturation

Maximum inhibition (64.3 ± 0.01) was observed from the n-hexane fraction of the leaf at 1000 µg/ml concentration followed by fruit (42.9 ± 0.0005) at 200 µg/ml concentration (table 1). Chloroform and ethyl acetate fractions of the *C. lutea* leaf and fruit were not active at both concentrations. Aspirin, a standard anti-inflammation drug showed the maximum inhibition 61.2 % at the concentration of 1000 µg/ml (table 1).

Membrane stabilization test

The fractions inhibited the heat induced haemolysis of RBCs to varying degree (Table 2). The maximum inhibition was recorded from chloroform fraction (65.54 %) of the plant leaf at the concentration of 200 µg/ml followed by 64.7 % at 1000 µg/ml and ethyl acetate fraction (61.73 %) of the fruit at 1000µg/ml, while the n-hexane fraction of the leaf was not active. Aspirin, a standard anti-inflammation drug showed inhibition of 86.92 % at the concentration of 1000 µg/ml (table 2).

Table 2: Percentage Inhibition of different solvent fractions of the *C. lutea* leaf and fruit on membrane stabilization inhibition

Test fractions	Leaf		Fruit	
	% Inhibition at 200 µg/ml	% Inhibition at 1000 µg/ml	% Inhibition at 200 µg/ml	% Inhibition at 1000 µg/ml
n-Hexane	na	na	14.06±0.0012	na
Chloroform	65.54±0.0007	64.7±0.0005	11.63±0.0006	na
Ethyl acetate	48.63±0.0089	na	32.77±0.0053	61.73±0.0024
Aspirin	85.96±0.002	86.92±0.002	85.96±0.002	86.92±0.002

Note: na = not active

DISCUSSION

In this study anti-inflammatory activity of fractions from *C. lutea* leaf and fruit extracts were evaluated using two different experimental models. Denaturation of proteins is a well-documented cause of inflammation (Mizushima and Kobayashi, 1968). The inflammatory drug (Aspirin) has been reported to show dose dependent ability to thermally induce protein denaturation (Mizushima and Kobayashi, 1968). Similar results were observed from this study with percentage inhibition of 60.7 % and 61.2 % at concentration of 200 and 1000 µg/ml respectively (table 1). The n-hexane fraction of *C. lutea* leaf was the most effective with a maximum inhibition of 64.3 % at concentration of 1000 µg/ml followed by the fruit (42.9 %) at concentration of 200µg/ml; this shows that the leaf was more active at a higher concentration than the fruit part of the plant at inhibiting inflammation for this type of experimental model (table 1). These fractions may likely inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophils lysosomal component include bactericidal enzymes and proteinases, which upon extracellular release cause advance tissue inflammation and injury (Chou, 1997).

The results of the stabilization of RBCs membrane revealed that the maximum inhibition of the heat induced haemolysis was recorded from chloroform fraction (65.54 %) of the leaf at the concentration of 200 µg/ml followed by (64.7 %) at 1000 µg/ml (table 2). This result is verification for membrane stabilization as a mechanism of anti-inflammatory effect. This also shows that the leaf part was further active than the fruit part of the plant at inhibiting inflammation for this type of experimental model. This effect may perhaps

inhibit the release of lysosomal content of neutrophils at the site of inflammation. However, the definite mechanism of this membrane stabilization is yet to be elucidated; it is possible that the chloroform fraction of the leaf produced this effect of surface area to volume ratio with the cells, which likely brought about an increase in size of membrane or the shrinkage of cells and an interaction with membrane proteins (Shinde *et al.*, 1999).

Furthermore, the study of Ndiwu and Nwafor *et al.*, 2012, also supported the anti-inflammatory activity of fractions from *C. lutea* leaf. The n-hexane, chloroform, ethylacetate and ethanol, fractions possessed inhibitory effects on the acute phase inflammation using the following experimental models: formalin test, egg albumin test, capsaicin test, xylene-induced ear edema test and carragenin test in rats. The ethanol fraction revealed the maximum inhibitory effect of 52.37 % followed by chloroform with 52.29 % at 770 mg/kg dose for the xylene-induced ear edema test.

CONCLUSION

In the present investigation, it was revealed that certain fractions of *Carpolobia lutea* leaf and fruit can be a potential source of anti-inflammatory agents by inhibiting the heat induced albumin denaturation and stabilizing the Red Blood Cells membrane at 200 and 1000 µg/ml respectively. The leaf part was more active than the fruit part of the plant at inhibiting inflammation for both experimental models.

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Declaration of Interest statement

The authors declare no conflict of interest concerning this research studies.

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