Anti-inflammatory activity of the fruit extracts of *Carpolobia lutea* G. DON (Polygalaceae)

Oghenekevwe M. Rhuada[1], Ogacheko D. Okoko[1], Gloria A. Ayoola[1], Ismaila O. Ishola[2]
[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, PMB 12003, Lagos State, Nigeria. [2]Department of Pharmacology, Therapeutics and Toxicology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, PMB 12003, Lagos State, Nigeria.

Corresponding author: Gloria A. Ayoola
Email: gayoola@unilag.edu.ng; Phone: +2348063075179

ABSTRACT

Background: *Carpolobia lutea* G. Don (Polygalaceae) fruits are used traditionally in treating fever, infections and inflammatory conditions.

Objectives: The study sought to evaluate the phytochemical constituents and anti-inflammatory potentials of the fruit extracts of *C. lutea.*

Methods: Successive extraction was done with methanol (CLFME), n-hexane (CLFn-HE), chloroform (CLFCHE), and ethylacetate (CLFETE). Anti-inflammatory activity was evaluated *in vivo* using carrageenan-induced paw oedema and xylene-induced ear oedema models. The phytochemical constituents were identified using gas-chromatography (GC) and liquid chromatography - mass spectrometry (LC-MS).

Results: CLFME (200 mg/kg) significantly ($P< 0.05$) inhibited carrageenan-induced paw oedema (83.66%) and xylene induced ear oedema (89.85%). CLFn-HE, CLFCHE, and CLFETE (200 mg/kg) significantly reduced carrageenan-induced paw oedema with percentage inhibitions of 60.00, 70.00 and 65.00%, respectively. Moreover, CLFn-HE, CLFCHE, and CLFETE (200 mg/kg), reduced xylene-induced ear oedema by 69.14, 71.60, and 69.14%, respectively. Gas chromatography coupled to mass spectroscopic (GC–MS) analysis of CLFn-HE afforded oleic acid, squalene, cis-vaccenic acid, n-hexadecanoic acid, 9-octadecenoic acid (Z) methyl ester, cis-13-octadecenoic acid and nonacosane. CLFCHE was subjected to column chromatography, fraction-31 showed a single spot using Thin layer chromatography (TLC) (n-hexane:ethylacetate, 5:5) with an R_f value of 0.70. Liquid chromatography coupled to mass spectroscopic (LC-MS) analysis of fraction-31 afforded four peaks with retention times of 0.058, 1.161, 1.572 and 3.080 min, with similar abundant ions (Base peak) of 81.1 and molecular ion (M^+) of 208.2 indicating the presence of isomeric compounds.

Conclusion: The fruit extract of *C. lutea* possess anti-inflammatory effects which may be mediated through inhibition or release of inflammatory mediators. Thus, *C. lutea* fruit extract could be a potential phytotherapeutic agent in the management of inflammatory disorders.

Keywords: carrageenan; xylene; paw oedema; flavonoids; mass spectroscopy; chromatography.

Activité anti-inflammatoire des extraits de fruits de *Carpolobia lutea* G. DON (Polygalaceae)

Oghenekevwe M. Rhuada[1], Ogacheko D. Okoko[1], Gloria A. Ayoola[1], Ismaila O. Ishola[2]

[1]Département de chimie pharmaceutique, Faculté de pharmacie, Université de Lagos, PMB 12003, État de Lagos, Nigéria. [2]Département de pharmacologie, de thérapeutique et de toxicologie, faculté des sciences médicales de base, faculté de médecine, université de Lagos, PMB 12003, État de Lagos, Nigéria.

Auteur de correspondance : Gloria A. Ayoola
Email: gayoola@unilag.edu.ng; Téléphone: +2348063075179

RESUME

Contexte : Les fruits de la famille *Carpolobia lutea* G. Don (Polygalaceae) sont traditionnellement utilisés pour le traitement de la fièvre, des infections et des états inflammatoires.

Objectifs : L'étude visait à évaluer les constituants phytochimiques et les potentiels anti-inflammatoires des extraits de fruits de *C. lutea*.

Méthodes : L'extraction successive a été effectuée avec du méthanol (CLFME), du n-hexane (CLFn-HE), du chloroforme (CLFCHE) et de l'acétate d'éthyle (CLFETE). L'activité anti-inflammatoire a été évaluée *in vivo* à l'aide des modèles d'œdème des pattes induit par le carraghénane et de l'œdème des oreilles induit par le xylène. Les constituants phytochimiques ont été identifiés par chromatographie en phase gazeuse (GC) et par chromatographie en phase liquide - spectrométrie de masse (LC-MS).

Résultats : CLFME (200 mg/kg) inhibait de manière significative ($p<0,05$) l'œdème des pattes induit par le carraghénane (83,66%) et l'œdème des oreilles induit par le xylène (89,85%). CLFn-HE, CLFCHE et CLFETE (200 mg/kg) ont significativement réduit l'œdème de la patte induit par le carraghénane avec un pourcentage d'inhibition de 60,00, 70,00 et 65,00%, respectivement. De plus, CLFn-HE, CLFCHE et CLFETE (200 mg/kg) ont réduit l'œdème des oreilles induit par le xylène de 69,14, 71,60 et 69,14%, respectivement. La chromatographie en phase gazeuse couplée à une analyse spectroscopique de masse (CG-SM) de CLFn-HE a fourni l'ester méthylique d'acide oléique, de squalène, d'acide cis-vaccénique, d'acide n-hexadécanoïque, d'acide 9-octadécénoïque (Z), d'acide cis-13-octadécénoïque et de nonacosane. Le CLFCHE a été soumis à une chromatographie sur colonne, la fraction 31 a montré une seule tache en utilisant une Chromatographie en couche mince (CCM) (n-hexane: acétate d'éthyle, 5: 5) avec une valeur de Rf de 0,70. La chromatographie en phase liquide couplée à une analyse spectroscopique de masse (LC-MS) de la fraction 31 a donné quatre pics avec des temps de rétention de 0,058, 1,161, 1,572 et 3,080 min, avec des ions abondants similaires (Base) de 81,1 et un ion moléculaire (M+) de 208,2. indiquant la présence de composés isomères.

Conclusion : L'extrait de fruit de *C. lutea* possède des effets anti-inflammatoires qui peuvent être médiés par l'inhibition ou la libération de médiateurs inflammatoires. Ainsi, l'extrait de fruit de *C. lutea* pourrait être un agent phyto-thérapeutique potentiel dans la gestion des troubles inflammatoires.

Mots-clés : carraghénane ; le xylène ; œdème de la patte ; les flavonoïdes ; spectroscopie de masse ; chromatographie.

INTRODUCTION

Inflammation is a complex biological process in which vascularized tissues respond to invasion of pathogens, microbes and irritants by bringing cells and molecules of host defense from circulation to the sites of invasion (injured site). Inflammation is actually a protective response that is essential for survival. However, when these defense mechanisms are defective or are suppressed by drugs, they result to a wide range of inflammatory diseases. Hence, the need to resort to drug therapy that has the potential to reduce or terminate the inflammatory response. Non-steroidal anti-inflammatory drugs (NSAIDs) are used chronically to reduce pain and inflammation in patients with chronic inflammatory conditions, and also acutely as analgesics by many patients. However, regardless of their COX selectivity, reports are still appearing on the GI side effect of NSAIDs particularly on the lower gastrointestinal (GI) tract and the harmful role of their controlled release formulations. [1, 2] Hence, the need for a safer and more effective drugs from natural product.

Carpolobia lutea G. DON (Polygalaceae) is a small tree distributed in West and Central tropical Africa. It grows in rainforests and the Guinea Savannah area from Sierra Leone to Cameroon between April and September. The morphological features have been described[3]. The stem is used as chewing stick,[4] the root is also used as chewing stick and aphrodisiac effect.[5] The decoction of the root is used as an aphrodisiac,[5] also in the treatment of genitourinary infections, gingivitis and waist pains.[6] In addition, the root decoction is useful in the treatment of internal heat. The hot water extract of the root was reported to have antimicrobial activity.[7] *C. lutea* root and stem bark extracts have been reported to possess anti-inflammatory and anti-arthritic properties,[8] gastro-protective effects[9]; antinociceptive effects,[10] antimicrobial activities,[11,12] antidiarrhea and anti-ulcerogenic properties,[13] antimalarial activity and moderate toxicity.[14] The antimicrobial effects of the root have been established.[6] The root is used to facilitate childbirth, treat sterility, headache, worm infestation and as aphrodisiac and stimulant properties.[15] The leaf is reported to be used in the management of fever accompanying diarrhea, headache, leprosy, snakebite, venereal disease and wounds.[16,17] The people of Southern Nigeria consume its fruits juicy for painful and inflammatory conditions.[18] Thus, this study sought to evaluate the anti-inflammatory activity of *Carpolobia lutea* fruits in rats.

METHODS

Plant collection and extraction procedure

Fresh fruits of *Carpolobia lutea* were collected in May 2017 from a village in Ibereko, Badagry LGA, Lagos State, Nigeria. Identification was carried out by Mr. S. O. Adeleke at the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos with voucher specimen number PCGH-35. The fruits were rinsed with distilled water to remove all sand, debris and impurities. The fruits were air-dried under shade for 28 days and pulverized to dry powder with aid of grinding machine. The powdered fruit (500 g) was extracted exhaustively with 2500 ml of methanol. The procedure was repeated with 2500 ml each of n-hexane, chloroform, and ethyl acetate but with 300 g of the powdered fruit. The extracts were concentrated *in vacuo* on a rotary evaporator and lyophilized till dryness.

Gas Chromatography-Mass Spectrometry analysis

The n-Hexane fruit extract of *Carpolobia lutea* was subjected to GC-MS (Agilent technologies 7890A GC system) with helium gas as the mobile phase and HP5MS column of 30 m length and 0.25 mm diameter as stationary phase. The instrument was set to an initial temperature of 60°C and maintained for 0.5 min, the run time was 30 min. At the end of this period, the oven temperature rose to 30°C at an increment rate of 10°C / min and maintained for 6 min. Sample was injected in spitless mode of 1 µl, helium gas was used at flow rate of 1 ml/min. Mass spectra scan was set at 15-508 (m/z).

Liquid chromatography-Mass Spectrometry analysis

The chloroform extract (3 g) was subjected to column chromatography (normal phase) using silica gel as stationary phase (wet packing). A glass column of length 30 cm, diameter 3 cm and sinter pore size 0.2 mm was used and the column was eluted with solvents in order of increasing polarity. The solvent system consisted of n–hexane: ethylacetate in ratios 3:7, 4:6, 5:5, 6:4, and 7:3 using 200ml of each system and a total of 150 fractions were collected. The various fractions were spotted on a TLC plate and separation viewed under a UV lamp. Exposure of the plates to iodine using an iodine tank was also employed. A fraction - 31 was found to show a single spot using Thin Layer Chromatography (n–hexane: ethylacetate, 5:5) and was further subjected to analysis using LC-MS (liquid chromatography–mass spectrometry) equipped with poroshell 120 EC-C18 4.6 * 50mm, 2.7um, M.P: Acetonitrile: water 50:50 with a flow rate of 0.33ml/min. Spectra was obtained in a positive ion mode and the retention time and respective m/z values were recorded.

Drugs and Chemicals

Methanol, n-hexane, chloroform, ethylacetate, diclofenac, dexamethasone, carrageenan and xylene were all procured from Sigma-Aldrich chemical (St. Louis MO, USA).

Laboratory animal

This study was carried out in male albino rats (220-250 g) and mice (20-25g) obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria. The animals were housed in plastic cages with wood shavings as beddings, at room temperature under standard environmental conditions, received standard pelleted rodent diet (Livestock feed Plc, Lagos, Nigeria) and drinking water *ad libitum*. The experimental procedures adopted were in accordance with the College of Medicine, University of Lagos, Health Research Ethics Committee (CMUL/HREC/07/17/209) and United States National Institutes of Health Guidelines for Care and use of laboratory Animals in Biomedical Research (1985).

Acute toxicity test

Adult Swiss albino mice were fasted for 12 h prior to the study but had access to water. Four groups of mice (n = 5) received; distilled water (10 ml/kg), *C. lutea* (1250, 2500 and 5000 mg/kg, p.o.). They were observed for toxic symptoms and behavioral changes for 2 h post-administration and 14 days for any signs of delayed toxicity.

Assessment of *In-vivo* anti-inflammatory activity

Carrageenan-induced paw oedema

Carrageenan-induced rat paw oedema was done according to the method of Winter *et al.*[19] Rats were fasted overnight and were randomly divided into 5 groups (n=6): Group 1: vehicle (10 ml/kg, *p.o.*; 3% Tween 80). Group 2: diclofenac (10 mg/kg, p.o., reference standard), Groups 3-5:- *C. lutea* (50, 100 and 200 mg/kg, *p.o.*), respectively. The initial paw circumference was recorded and 1 h after treatment, carrageenan (0.1ml, 1%, w/v in normal saline) was subcutaneously injected intraplantar into the right hind paw[20]. Thereafter, paw circumference was recorded hourly between 1 and 6 h using a digital vernier caliper. The procedure was repeated with 200 mg/kg of n-hexane, chloroform and ethyl acetate extract and vehicle (3% Tween 80; 10 ml/kg, *p.o.*).

$$\text{Percentage inhibition} = \frac{(C_t - C_o)_{control} - (C_t - C_o)_{test}}{(C_t - C_o)_{control}} \times 100$$

Where;

$[(C_t - C_o)_{control}]$ = mean change in paw circumference of control rats

$[(C_t - C_o)_{test}]$ = mean change in paw circumference of treated rats

Xylene-induced mouse ear oedema

The effect of the methanol extract of *C. lutea* on acute topical inflammation was evaluated by a modification of the methods of Ishola *et al*.[21] Adult Swiss albino mice (20-25 g) of either sex were divided into 5 groups containing six mice per group (n=6) and were treated as follows: Group 1: 3% Tween 80, vehicle (10 ml/kg, *p.o.*), Group 2: diclofenac (10 mg/kg, p.o., reference standard), Groups 3-5:- *C. lutea* (50, 100 and 200 mg/kg, *p.o.*), respectively. One hour after treatment, xylene (20 µl) was topically applied into the right pinna ear and observed for 1 hour for acute inflammation. After which they were euthanized with di-ethyl-ether and both ears removed. Circular section of both the right (treated) and left (untreated) ears were punched out using a cork borer and weighed. The procedure was repeated with 200 mg/kg of n-hexane, chloroform and ethylacetate extract and vehicle (3% Tween 80; 10 ml/kg, *p.o.*).

$$\text{Percentage inhibition} = \frac{(R_c - L_c)_{control} - (R_t - L_t)_{test}}{(R_c - L_c)_{control}} \times 100$$

Where;

$[(R_c - L_c)_{control}]$ = mean change in weight of ear of control animals

$[(R_t - L_t)_{test}]$ = mean change in weight of ear of treated animals

(R_c); weight of right ear of control animal

(L_c); weight of left ear of control animal

(R_t); weight of right ear of treated animal

(L_t); weight of left ear of treated animal

Statistical analysis

Results are expressed as mean ± SEM (n =6). The difference between experimental groups was compared by one or two-Way Analysis of Variance (ANOVA) followed by Tukey post hoc multiple comparison test using the software Graph Pad Prism 6. A probability level of less than 5% was considered significant ($P < 0.05$).

RESULTS

Acute toxicity test

The extract up to 5000 mg/kg, p.o. produced no behavioural signs of toxicity within the first 24 h after administration and did not induce mortality up to the 14th day of observation.

Phytochemical screening

The preliminary phytochemical screening of the methanol extract of *C. lutea* fruit showed the presence of tannins, reducing sugars, saponins, flavonoids, cardiac glycosides, terpenoids and steroids.

Anti-Inflammatory activity

Effects of *C. lutea* on carrageenan-induced paw oedema

The intraplantar injection of carrageenan produced time course increase in paw size. However, the pretreatment of rats with CLFME dose-dependently and significantly decreased the carrageenan-induced increase in paw size when compared with the control rats. The highest doses tested (200 mg/kg), reduced inflammation by 83.66% at the first hour after which there was a slight reduction in effect over 6 hours. The fruit extract produced statistically significant ($P < 0.05$) inhibition throughout the duration of the experiment (1-6 h) when compared to control as shown in Table 1. Similarly, pretreatment of rats with CLFn-HE, CLFCHE, or CLFETE (200 mg/kg), significantly reduced carrageenan-induced paw oedema (Table 2).

Table 1: Effect of *C. lutea* fruit extract on carrageenan-induced paw oedema.

Treatment	Dose (mg/kg)	1 h	2 h	3 h	4 h	5 h	6 h
Vehicle	10 ml/kg	5.49 ± 0.28	5.31 ± 0.26	5.43 ± 0.19	5.29 ± 0.18	4.99 ± 0.18	4.49 ± 0.14
CLFME	50	3.82 ± 0.07* (68.09)	3.81 ± 0.09* (66.11)	3.79± 0.14* (68.58)	3.89 ± 0.10* (62.87)	3.76± 0.05* (63.29)	3.77± 1.00* (50.90)
CLFME	100	3.83 ± 0.04* (77.43)	3.93 ± 0.04* (71.55)	4.38 ± 0.19* (54.98)	4.06 ± 0.19* (65.82)	4.17± 0.21* (56.04)	3.77± 0.09* (66.88)
CLFME	200	3.79 ± 0.18* (83.66)	4.00 ± 0.09* (73.64)	4.03 ± 0.13* (73.74)	3.98 ± 0.10* (74.26)	3.86± 0.05* (76.33)	3.81± 0.10* (71.97)
Diclofenac	10	3.72 ± 0.11* (85.99)	3.84 ± 0.07* (79.92)	3.80 ± 0.13* (82.47)	3.79± 0.10* (81.86)	3.70± 0.16* (83.57)	3.77± 0.13* (73.89)

Values are expressed as mean ± SEM (mm) (n = 6). Significance relative to control: - *p < 0.05. (Two-way ANOVA followed by Tukey's multiple comparison tests). Percentages of inhibition against carrageenan induced paw oedema are in parentheses. Vehicle-3% Tween 80; CLFME: *Carpolobia Lutea* Fruit Methanol Extract.

Table 2: Effect of *Carpolobia lutea* fractions on carrageenan-induced paw oedema

Treatment	Dose (mg/kg)	1 h	2 h	3 h	4 h	5 h	6 h
Vehicle	10 ml/kg	5.90 ± 0.21	6.48 ± 0.09	6.33 ± 0.11	6.57 ± 0.29	6.47 ± 0.30	6.17± 0.23
CLFn-HE	200	4.44 ± 0.09* (53.15)	4.52 ± 0.14* (60.00)	4.81±0.18* (46.80)	5.05 ± 0.24* (42.91)	5.04± 0.23* (41.22)	5.17± 0.17* (28.92)
CLFCHE	200	4.57 ± 0.20* (53.60)	4.38 ± 0.10* (70.00)	4.59 ± 0.10* (60.38)	4.73± 0.14* (58.82)	4.70 ± 0.24* (58.42)	4.73 ± 0.15* (52.21)
CLFETE	200	4.65± 0.12* (45.95)	4.43 ± 0.09* (65.00)	4.44 ± 0.16* (62.60)	4.52± 0.10* (62.98)	4.54± 0.17* (60.93)	5.25 ± 0.17* (27.70)
Diclofenac	10	3.72 ± 0.11* (85.99)	3.84 ± 0.07* (79.92)	3.80 ± 0.13* (82.47)	3.79 ± 0.10* (81.86)	3.70 ± 0.16* (83.57)	3.77 ± 0.13* (73.89)

Value expressed as mean ± SEM (mm) (n = 6). Level of significance analysis by two way ANOVA followed by Tukey's *post hoc* multiple comparison tests * $P < 0.05$ versus vehicle treated control. Percentages of inhibition in parentheses.

CLFnHE: *Carpolobia Lutea* Fruit n-hexane Extract; CLFCHE: *Carpolobia Lutea* Fruit chloroform Extract; CLFETE: *Carpolobia Lutea* Fruit ethylacetate Extract; vehicle-3% Tween 80

Effect of *C. Lutea* extract on xylene-induced ear oedema in mice

Instillation of xylene into the pinna of the ear induced oedema (0.26 ± 0.13). However, pretreatment of mice with CLFME (200 mg/kg) significantly reduced the ear oedema by 89.85%, which was similar to the effect of dexamethasone (90.73%) (Table 3). In another set of experiments, the pretreatment of rats with CLFn-HE, CLFCHE, or CLFETE (200 mg/kg, *p.o.,* respectively) produced significant ($p < 0.05$) inhibition of ear oedema by 69.14%, 71.60% and 69.14%, respectively, relative to vehicle control treated (Table 4).

Table 3: Effects of *C. lutea* extract on xylene-induced on ear oedema in mice.

Treatment	Dose (mg/kg)	Mean difference	Percentage inhibition
Vehicle	10 ml/kg	0.26 ± 0.13	
CLFME	50	0.19 ± 0.002	48.08
CLFME	100	0.25 ± 0.11	77.61
CLFME	200	0.06 ± 0.006	89.85
Dexamethasone	10	0.06 ± 0.006 **	90.73

Values represented as mean ± S.E.M; (n=6). Level of significance relative to control: $^{*}P$ <0.05 (One way ANOVA followed by Tukey's *post hoc* multiple comparison tests). Vehicle-3% Tween 80; CLFME: *Carpolobia Lutea* Fruit Methanol Extract.

Table 4: Effects of *C. lutea* fractions on xylene induced ear oedema in mice

Treatment	Dose (mg/kg)	Mean difference	Percentage inhibition
Vehicle	10	0.13 ± 0.01	------
CLFn-HE	200	0.07 ± 0.001*	69.14
CLFCHE	200	0.07 ± 0.01*	71.60
CLFETE	200	0.07 ± 0.003*	69.14
Dexamethasone	10	0.06 ± 0.006*	90.73

Results are represented as mean ± S.E.M. (n=6), $^{*}P < 0.05$ compared with control. (One way ANOVA followed by Tukey's *post hoc* multiple comparison tests). Vehicle-3% Tween 80; CLFnHE: *Carpolobia Lutea* Fruit n-hexane Extract; CLFCHE: *Carpolobia Lutea* Fruit chloroform Extract; CLFETE: *Carpolobia Lutea* Fruit ethylacetate Extract

Results of Gas-chromatography

The results obtained from the GC-MS of n-Hexane fraction of C. lutea revealed the presence of N-hexadecanoic acid (RT 16.8min, Area 25.9%), 9-octadecenoic acid (Z) methyl ester (RT 18.2min, Area 1.8%), cis-vaccenic acid (RT 19.2min, Area 29.9%), oleic acid (RT 19.3min, Area 18.9%), cis-13-octadecenoic acid (RT 19.3min, Area 6.0%), squalene (RT 27.8min, Area 8.7%), and nonacosane (RT 28.4min, Area 1.4%) (Fig. 1).

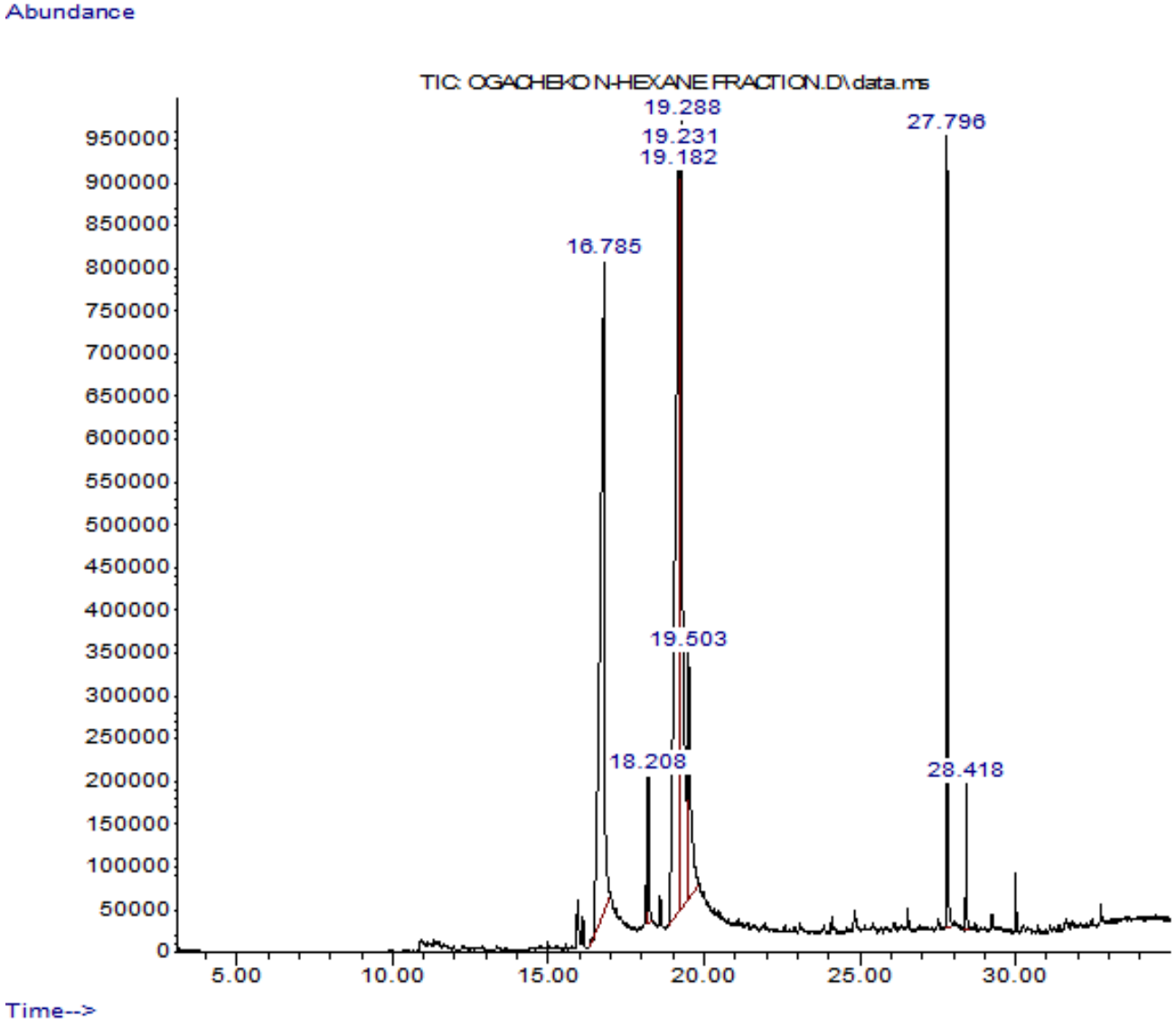

FIG 1: the spectra of n-hexane fraction GC-MS

Column chromatography and LC-MS

The chloroform extract was subjected to column chromatography and fraction 31 was found to have a single spot using thin layer chromatography (n-Hexane:Ethylacetate, 5:5) with an R_f value of 0.70. The LC-MS analysis (Poroshell 120 EC-C18 stationary phase and Acetonitrile: Water (50:50) mobile phase) of fraction 31 from column chromatography afforded four major peaks with retention time and *m/z* values (Table 5).

Table 5: Retention time and respective m/z values

S/N	RETENTION TIME (Mins)	M/Z
1	0.058	76.2, 81.2, 88.2, 106.2, 139.2, 165.2, 196.3, 208.2
2	1.161	76.2, 81.2, 88.2, 106.2, 107.2, 136.1, 139.1, 140.1, 196.2
3	1.572	76.2, 81.2, 88.2, 120.2, 139.2, 140.2, 196.2, 208.2
4	3.080	76.2, 81.2, 88.2, 97.1, 106.2, 107.2, 116.2 139.2, 157.2, 196.2, 208.2

DISCUSSION

Findings from this study showed that the fruit extracts of *Carpolobia lutea* possess anti-inflammatory properties evidenced in time course reduction of paw oedema in carrageenan model and reduction of ear oedema in xylene model. Moreover, peak anti-inflammatory actions were observed with the methanol and chloroform extracts. Interestingly, acute toxicity test showed that the fruit extract had wide margin of safety. The methanol extract up to 5000 mg/kg following oral administration is devoid of behavioural toxic effect and mortality.

Phytochemicals are biomolecules that occur in herbal drugs or phytopharmaceuticals, and possess the ability to modulate one or more metabolic processes or pathways in humans resulting in health benefits and promotion of well-being.[22,23] In this study, the preliminary phytochemical screening of the methanol extract revealed the presence of tannins, reducing sugars, saponins, flavonoids, cardiac glycosides, terpenoids and steroids. Flavonoids such as quercetin have been reported to possess anti-inflammatory effects.[18] Analysis of the n-hexane extract of *Carpolobia lutea* by GC–MS revealed the presence of the following compounds; oleic acid, squalene, cis-vaccenic acid, n-hexadecanoic acid, 9-octadecenoic acid (Z) methyl ester, cis-13-octadecenoic acid and nonacosane. Cis-vaccenic acid, the most abundant compound (29.9%), is the precursor of rumenic acid an isomer of conjugated linoleic acid. Its role in cancer and immune function remains unclear and short-term human trials have shown that vaccenic acid does not appear to affect markers of inflammation, phagocytic function or circulating immune cell phenotypes.[24] Ester bond hydrolysis of membrane phospholipids by phospholipase A_2 and consequent release of fatty acids are the initiating steps of inflammation. Inhibition of phospholipase A_2 is one of the ways to control inflammation. Previous investigation had shown that n-hexadecanoic acid, the second most abundant compound (25.9%) found in this study, inhibits phospholipase A_2.[25] Oleic acid has been widely described as an anti-inflammatory fatty acid that plays a role in the activation of different pathways of immune competent cells.[26] Diets rich in oleic acid have beneficial effects in inflammatory related diseases.[26] The flavonoids, terpenoids and fatty acids (cis-vaccenic acid, n-hexadecanoic acid and others) present in this fruit may account for the anti-inflammatory property of *Carpolobia lutea*.

The LC-MS analysis of fraction (31) from column chromatography using acetonitrile:water (50:50) afforded four major peaks with retention times of 0.058, 1.161, 1.572 and 3.080 minutes, respectively, with similar abundant ions (Base peak) of 81.1 and molecular ions (M^+) of 208.2. This indicates the

presence of isomeric compounds. Ethyl beta-D-fructofuranoside and ethyl glucoside, are saccharides with molecular formula $C_8H_{16}0_6$ and molecular weight of 208.2. Also, Sinapaldehyde extracted from the heartwood of *Populous tomentosa*, using chloroform, has the same molecular weight and is a phenylpropranoid.

The development of carrageenan induced oedema is biphasic; the first phase involving the release of histamine and serotonin occurs within one hour of carrageenan injection and the second phase (>1.0 h) results from the overproduction of prostaglandin in tissues and is also associated with the release of bradykinin, protease and lysosomal enzymes which peak at 3 h.[27] Carrageenan induces extravasation of macrophages and polymorphonuclear leucocytes (PMN leucocytes) into injected tissue and stimulates release of some pro-inflammatory mediators, such as PMN leucocytes derived reactive oxygen species and free radicals (hydrogen peroxide, superoxide and hydroxyl radical).[28] In this study, the pretreatment of rats with *C. lutea* extract reduced oedema formation in both phases indicating its ability to inhibit activity of histamine, serotonin and kinins in the early phase.[27] In addition, its ability to prevent oedematogenic effect of carrageenan in second phase suggest its possible inhibition of prostaglandin production in the tissues.[28] Xylene, a common inflammatory agent, provokes acute inflammatory response in the mouse ear, which leads to serious edematous changes of skin when applied to the surfaces of the ear.[29] The ear edema model induced by xylene has certain advantages in the evaluation of anti-inflammatory steroids as well as non-steroidal anti-inflammatory agents and has good predictive values in the screening of antiphlogistic new drugs.[30] In this study, the methanol fruit extract reduced the increase in ear weight induced by xylene possibly through inhibition of phospholipase A_2 enzyme activity.[30] Moreover, the chloroform and n-hexane extract reduced xylene-induced ear oedema possibly due to the presence of steroidal constistuents which was comparatively similar to the effect of dexamethasone.[21] The extracts also produced time course inhibition of carrageenan-induced paw oedema possibly due to the presence of flavonoids, terpenoids and fatty acids (cis-vaccenic acid, n-hexadecanoic acid.[24-26] The results of this study showed that all of the fruit extracts of *Carpolobia lutea* have the potential to be used as an anti-inflammatory agent. However, further study is ongoing to isolate and elucidate the phytochemical substance responsible for the observed effect.

CONCLUSION

The study showed that the fruit extracts of *Carpolobia lutea* possess anti-inflammatory effect possibly due to possibly due to the presence of flavonoids, terpenoids and fatty acids (cis-vaccenic acid, n-hexadecanoic acid. Thus, fruit extracts of *Carpolobia lutea* are potential natural products for the management of inflammatory conditions.

ACKNOWLEDGEMENT

The authors are thankful to the Management and Technical staff of the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, for their help and support

REFERENCES

1. Osadebe PO and Okoye FBC (2003). Anti-inflammatory effect of crude methanolic extract and fractions of A.cordifolia. Journal of Ethnopharmacology 89(1):19-24.
2. Jain KN, Kulkarni KS, Singh A (2002). Modulation of NSAID -induced antinociceptive and anti-inflammatory effects by adrenoceptor agonists with gastroprotective effects. Life sciences 70(24): 2857-2869.
3. Nwidu L, Nwafor PA, Vilegas W (2015). The aphrodisiac herb Carpolobia: A biopharmacological and phytochemical review. Pharmacognosy Reviews 9:132-139.
4. Kayode J, Omotoyinbo MA (2008). "Cultural erosion and biodiversity : conserving chewing stick knowledge in ekiti state, Nigeria" African scientist; 9(1): 41-51.
5. Etukudo A (2003). Ethnobotany: conventional and traditional use of plants. Verdict Press Limited, Uyo. Archives of Applied Science Research 2(5):184-198.
6. Ettebong E. and Nwafor P. (2009). In vitro antimicrobial activities of extracts of Carpolobia lutea root. Pakistan Journal of Pharmaceutical Sciences, 22 (3): 335-338.
7. Malcolm SA, and Sofowora EA. (1969). Antimicrobial selected folk remedies and their constituent plants. Lloydia 32(4):512-517.
8. Iwu MM, Ayanwu BN. (1982). Phytotherapeutic profile of Nigerian Herbs: Antiinflammatory and Antiarthritic agents. Journal of

Ethnopharmacology 6, (3):263-274.
9. Nwidu LL, Nwafor PA. (2009). Gastroprotective effects of leaf extracts of Carpolobia lutea (Polygalaceae) G. Don. in rats. African Journal of Biotechnology, 8(12), 15-19.
10. Nwidu LL., Nwafor PA., da Silva VC., Rodrigues CM., dos Santos LC, and Vilegas, W. (2011). Anti-nociceptive effects of Carpolobia lutea G. Don (Polygalaceae) leaf fractions in animal models. Inflammopharmacology (DOI10.1007/s10787-010-0076-y).
11. Ajibesin KK. (2005). Chemical and antimicrobial studies of the constituents of selected medicinal plants of Akwa Ibom State. A Ph.D Thesis of the Faculty of Pharmacy, University of Uyo, pp.79-83.
12. Philip AI, Jones OM, and Herbert AO. (2005). Phytochemical and antimicrobial screening of three Nigerian medicinal plants used to treat infectious diseases traditionally. Journal Pharmacy and Bioresources 2(2): 116-119.
13. Nwafor PA, Bassey A1. (2007). Evaluation of the antidiarheal and antiulcerogenic potential of ethanol extract of carpolobialutea leaves in rodent. Journals of Ethnophamacology 111(3): 619-624.
14. Bero J, Ganfon H, Jonville M.C., Frédérich M., Gbaguidi F., DeMol P., Moudachirou M., Quetin-Leclercq J. (2009). In vitro antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria. Journal of Ethnopharmacology 122: 439-444.
15. Mitaine-Offer AC, Miyamoto T, Khan IA, Delaude C and Lacaille-Dubois MA. (2002). Three new triterpene saponins from two species of Carpolobia. Journal of Natural product. 65: 553-557.
16. Ajibesin KK, Ekpo AB, Bala DN, Essien EE, Adesanya SA. (2007). Ethnobotanical survey of Awa Ibom State of Nigeria. Journal of Ethnopharmacology 115, 387-40.
17. Lewis WH, Elvin-Lewis MP. (1977). Medical Botany. New York: John Wiley and Sons, pp. 1-51.
18. Stephanie TG, Joel YG, Alambert TT (2015). Phenolic content and DPPH scavenging activity of Carpolobia lutea and Anchomanesdifformis. International Journals of Pharma Sciences and Research 6(5): 909-912.
19. Winter CA, Risley EA, Nuss GW (1962). Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proceedings Of Society Of Experimental Biology. 111:544-547
20. Ayoola GA, Akpanika GA, Awobajo FO, Sofidiya MO, Osunkalu VO, Coker HAB, Odugbemi T (2012). Anti-inflammatory properties of the fruits of Allanblanckia floribunda Oliv. (Gutifera). Botany Research; 2(1): 21-26.
21. Ishola IO, Akindele AJ, Adeyemi OO (2011). Analgesic and anti-inflammatory activities of Cnestis ferruginea Vahl ex DC (Connaraceae) methanolic root extract. Journal of Ethnopharmacology; 135(1): 55-62.
22. Abuajah CI, Ogbonna AC, Osuji CM (2015). Functional components and medicinal properties of food: a review. Journal of Food Science and Technology. 52(5): 2522-2529.
23. Toiu A, Mocan A, Vlase L, Pârvu AE, Vodnar DC, Gheldiu AM, Moldovan C, Oniga I (2018). Phytochemical Composition, Antioxidant, Antimicrobial and in Vivo Anti-inflammatory Activity of Traditionally Used Romanian Ajuga laxmannii (Murray) Benth. ("Nobleman's Beard" - Barba Împ?ratului). Frontiers in Pharmacology. 9:7.
24. Field CJ. (2009). Human health benefits of vaccenic acid. Applied Physiology, Nutrition and Metabolism 34 (5): 979-91.
25. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M (2012). Anti-inflammatory property of n-hexadecenoic acid: Structural evidence and kinetic assessment. Chemical Biology and Drug Design 80: 434-439.
26. Carrillo C, Cavia M and Alonso-Torre S. Role of Oleic acid in immune System; mechanism of action; a Review. Nutritional Hospital 2012; 27: 978-990.
27. Phelan KM, Mosholder AD. (2003). Lithium interaction with the cyclooxygenase 2 inhibitors rofecoxib and celecoxib and other nonsteroidal anti-inflammatory drugs. Journal of Clinical Psychiatry 64 (11): 1328-1334.
28. Cuzzocrea S, Zingarelli B, Gilad E, Hake P, Salzman AL, Szabó C (1997). Protective effect of melatonin in carrageenan-induced models of local inflammation: relationship to its inhibitory effect on nitric oxide production and its peroxynitrite scavenging activity. Journal of Pineal Research. 23 (2):106-116.
29. Ishola IO, Agbaje EO, Narender T, Adeyemi, OO. Shukla R. (2012). Bioactivity guided isolation of analgesic and anti-inflammatory constituents of Cnestis ferruginea Vahl ex DC (Connaraceae) root. Journal of Ethnopharmacology 142 (2): 383 - 9.
30. Ishola IO, Awodele O, Oreagba IA, Murtala AA, Chijioke MC. (2013). Antinociceptive, anti-inflammatory and antiulcerogenic activities of ethanol root extract of Strophanthus hispidus DC (Apocynaceae). Journal of Basic and Clinical Physiology and Pharmacology. 24(4): 277-286.