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Research Article

ANTI-INFLAMMATORY AND ANTIPYRETIC EFFECTS OF *CARPOLOBIA LUTEA* LEAF EXTRACT IN RODENTS

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ABSTRACT
The polar and non-polar fractions from the leaf of *Carpolobia lutea* G. Don (Family Polygalaceae) were assessed for anti-inflammatory and antipyretic effects. Anti-inflammatory activities were evaluated using acute anti-inflammatory models. Antipyretic activities of the fractions were also evaluated. It was found that all the fractions possessed significant (P<0.05-0.001) inhibitory effects on the acute phase of inflammation as seen in formalin, egg albumin, capsaicin-induced oedema and xylene-induced ear edema as well as in carrageenin-induced paw edema in rats. In the antipyretic model, significant (0.05-0.001) inhibition of pyrexia was exerted in 2, 4-dinitrophenol but not in yeast induced–pyrexia. No effects of the fractions were observed on the normal body temperature of the rats. The structural elucidation of the potent fraction reveals the presence of polyphenols. These findings justify ethno-medicinal uses as anti-inflammatory and antipyretic.
KEYWORDS: *Carpolobia lutea*, polygalaceae, anti-inflammatory, antipyretic, polyphenols

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDS) are often not suitable in all patients and cases, particularly in chronic inflammatory condition on account of their limitations, e.g. numerous side effects, including ulcerative diseases. As a result, the continuing searches for alternative remedies. Medicinal plants are known to be an important source of new chemical substances with potential therapeutic effects [1-5]. The research on plants which are employed as anti-inflammatory in traditional ethno-medicine is therefore one of the productive and logical strategies in the search for new drugs with anti-inflammatory potentials.

Carpolobia lutea (*C. lutea)* is called cattle stick (English), Ikpafum (Ibibio), Agba or Angalagala (Igbo) and Egbo Oshunshun (Yoruba) in Nigeria. It is used to cure rheumatism, fever, muscular pains, insanity, dermal infection, venereal diseases, combat sterility and promote child birth, used as taeniafuge and vermifuge [6-8]. The leaf is reported to be useful as anti-inflammatory and anti-arthritis[9]; antidiabetic (Etefia, Personal communication); treatment for fever, leprosy, snakebite, venereal disease and wound healing.[10] Members of Polygalaceae are known to contain a variety of different chemical species-polyphenolic compounds such as xanthones, flavonoids, and biphenyl derivatives -many of which exhibit significant biological activities.[11] Three triterpines saponins (presenegenin 1-3) were isolated from the root of the plant[12]; alkaloids in detectable quantity and saponins and cardenolides in plant root extract[13]. Phytochemical screenings confirmed the presence of tannins, saponins and flavoniods in the leaf and root of *C. lutea.*[14,15] Tannins, flavonoids, saponins, cardiac glycosides and anthraquinone were screened from *C. lutea* chewing stick. [16] Pharmacological assay on the leaf extract confirms ethnomedicinal use as anti-diarrheal and anti-ulcer [14] and gastroprotectives[17]. Although *C. lutea* leaf has enjoyed wide patronage traditionally for the management of migraine, inflammatory and arthritic conditions, these biological activities are yet to be scientifically verified.

Two new cinnamoyl 1-deoxyglucopyranosides (**1** and **2**) and two new *p*-coumaroyl 1-deoxyglucopyranosides (**4** and **5**), besides cinnamic acid (**3**) were isolated and characterized (Fig. 1). This is a first report of detail anti-inflammatory and antipyretic investigation of the plant leaf fractions.

MATERIALS AND METHODS

Collection and Identification of Plant

C. lutea leaves were collected and supplied in October 2006 by Mr. Okon Etefia, the traditional herbalist, attached to the Pharmacognosy Department, University of Uyo. The plant was identified and authenticated by Dr. (Mrs.) Margaret Bassey of the Department of Botany of same University. A voucher specimen (UUH 998) was deposited at the University Herbarium. The leaves were air dried, powdered with pestle and mortar. The pulverized leaves were stored at room temperature.

Extraction Procedure

The pulverized air-dried leaf (0.75 kg) was extracted as reported earlier[17].

Extraction and fractionation

Procedure of gradient solvent extraction has been described in previous article[17] and fraction in [18].

HPLC analysis and isolation

HPLC analysis and isolation of pure compounds is similar to procedure adopted in earlier report.[18] This separation provided the isolation of compounds **1** (42.0 mg), **2** (327.0 mg), **3** (58.0 mg) and a mixture of **4** + **5** (8.0 mg).

Structural elucidation

The structure elucidation of the isolated compounds was based on 1D and 2D - NMR experiments (^{1}H, HOMODEC, COSY, TOCSY, HMQC and HMBC) similar to earlier report.[18]

Drugs and Chemicals

The chemicals used were all of analytical grade: absolute ethanol, ethyl acetate, chloroform, n-hexane (Reidel-de Haem, Germany), methanol (Synth, Brazil), carragenin, acetyl salicylic acids, indomethacin were all purchased from Sigma Chemical Co (St. Louis, USA).

Animals

Adult albino mice and rats were used. All the animals were housed in standard cages under laboratory conditions in the University of Uyo and Pharmacology department, UNESP, Brazil. The animals in Nigeria were fed with pellet feeds (Vital Feed and Flour Mill Limited, Edo State, Nigeria) and water *ad libitum*. All Animals have free access to tap water under standard conditions of 12 h dark–12h light, and temperature (21±1%). The protocols were approved by the University of Uyo Institutional animal Care and Use Committee (UUAEC) which follows the guidelines of CPSCEA (Committee for the purpose of control and supervision of experimental animals) (UUAEC No. 2004/013)).

Anti-inflammatory Assay

Formalin test

Adult albino mice (30-33g) of either sex were used for this study according to the method of Gomes *et al.*[19]. Mice were divided into six groups, each consisting of six mice per group and were treated as follows: Group I received 10ml / kg (i.p.) of 20% Tween 80 as vehicle-treated control group, Group 2-5 receive 770 mg/kg leaf fractions, Ethanol fraction (ETF), ethyl acetate fraction (EAF), chloroform fraction (CHF) and *n*-hexane fraction (*n*-HF) and group 6 receive indomethacine (INDO) (10 mg kg $^{-1}$ i.p.) as (positive control mice). The animals were pre-treated with samples 1h before being challenged with formalin 0.02 mL (2.5% in distilled water) which was injected into the subplantar area of the right hind paw of mouse according to the method of Gorski *et al*.[20] with modification. After 3 h of formalin injection, the animals were killed by cervical dislocation and the paws were cut at the knee-joint and the increase of the weight of the right hind paw versus the left hind paw was measured and recorded.

Carragenin test

The test of carrageenin-induced rat paw edema was done according previously reported technique.[21] Male and female Wistar rats (150-210g) were divided into six groups of 6 rats per group. Group I received 10ml/kg (i.p.) of 20% Tween 80 as vehicle-treated control group. Group 2-5 received 770 mg/kg leaf fractions (ETF, EAF, CHF and *n*-HF) and group 6 received indomethacin (INDO) (10 mg kg $^{-1}$ i.p.) as (positive control rat). Each rat received drug orally 1 h before the injection of carrageenin. Paw volume was measured immediately after carrageenin injection and at 1-5 h intervals after the administration of the edematogenic agent using a vernier calliper. Anti-inflammatory activity was assessed on the basis of inhibition of paw edema induced by the injection of 0.1 ml 0.2% carrageenin (an edematogenic agent) into the subplantar region of the right hind paw of the rat.[21]

Egg Albumin test

The test of egg-albumin-induced rat paw oedema was investigated using a previously reported technique[21]. The procedure is similar to the methods adopted with carragenin as stated above.

Capsaicin test

Increase in rat hind paw linear circumference induced by sub-plantar injection of a phlogistic agent was used as the measure of acute inflammation[22]. The phlogistic agent employed in this study was capsiacin[23, 24]. Adult wistar rats of either sex (160-180g) were used after 24 h fast and deprived of water only during the experiment. Inflammation of the hind paw was induced by injecting 0.1ml of 5 µg/kg capsaicin dissolved in 20% Tween 80 into the subplantar surface of the right hind paw. Other procedure is as evaluated for carragenin above. Average oedema was calculated[25, 26].

Xylene- induced Ear Oedema test

The xylene-induced ear oedema test was carried out according to previously described method by De Melo e*t al.,*[27]. Male and female mice (30-32g) were divided into six groups of 6 mice per group. Group I received 10 ml/kg (i.p.) of 20% Tween 80 as vehicle-treated control group. Group 2-5 received 770 mg/kg leaf fractions (ETF, EAF, CHF and *n*-HF) and group 6 receive diclofenac sodium (DCS) (10 mg kg $^{-1}$ i.p.) as (positive control mice). A total of 30µl of xylene was given on the anterior and posterior surfaces of the right ear lobe of each mouse. The test samples, diclofenac, and the distilled water were administered 1 h prior to giving xylene. 1 h later, the animals were sacrificed by cervical dislocation, and the right and left ears of each animal were removed. The left ear was considered as control. Circular sections were taken with a cork borer (diameter of 7mm) and weighed.

Antipyretic Assay

Normal Body Temperature assay

The method of Arya and Kumar[22] were adopted in this evaluation. Thirty six wistar rats (160-170g) were randomly divided into 6 groups. Male and female wistar rats were divided into six groups of 6 rats (3 male and 3 female) per group. Group I received 10 ml/kg (i.p.) of 20% Tween 80 as vehicle-treated control group. Group 2-5 received 770 mg/kg leaf fractions (ETF, EAF, CHF and *n*-HF) and group 6 received diclofenac sodium (DCS) (10 mg kg $^{-1}$ i.p.) as (positive control rat). The basal body temperature of the rat was determined by the average of two tests, and the rat with body temperature more than 38 °C were the options for afterwards observation. Fraction administration was conducted 1 h before obtaining the body temperature. Body temperature was sequentially tested at seven time points: 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 4 h and 5 h after drug administration. The differences between these seven body temperatures and the initial temperatures of rats were used as pharmacological data.

Yeast -induced Pyrexia test

Antipyretic activity of *C. lutea* fractions were measured according to the method described by Adams *et al*.[28], but with slight modification. Thirty six wistar rats (170-180g) were randomly divided into 6 groups. Group I received 10 ml/kg (i.p.) of 20% Tween 80 as vehicle-treated control group. Group 2-5 received 770 mg/kg leaf fractions (ETF, EAF, CHF and n-HF) and group 6 received diclofenac sodium (10 mg kg $^{-1}$ i.p.) as (positive control rat). The basal body temperature of the rat was determined by the average of two tests, and the rat with body temperature more than 38 °C were the options for afterwards observation. Fever was induced by hypodermically dorsal injection of yeast solution (1 ml/100g body weight). Drug administration was conducted 5 h after yeast solution injection; equivalent dosage of distil water was simultaneously applied to control group. Body temperature was sequentially tested at seven time points: 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 4 h and 5 h after drug administration. The differences between these seven body temperatures and the basic temperatures of rats were used as pharmacological data.

2-4 Dinitrophenol -induced pyrexia test

Adult albino rats (160-180 g) of both sexes fasted for 24 h but allowed water *ad libitum* were used for the experiment. They were randomized into groups of 6 rats each. DNP (10mg/kg, i.p) was administered to the rats after obtaining the basal rectal temperatures. Hyperthermia developed within 30 min of DNP administration. Group I received 10 ml/kg (i.p.) of 20% Tween 80 as vehicle-treated control group. Group 2-

5 received 770 mg/kg leaf fractions (ETF, EAF, CHF and *n*-HF) and group 6 received diclofenac sodium (DCS) (10 mg kg^{-1} i.p.) as (positive control rat). Rectal temperatures of the animals were obtained at an hour interval for 5 hrs [21, 29, 30].

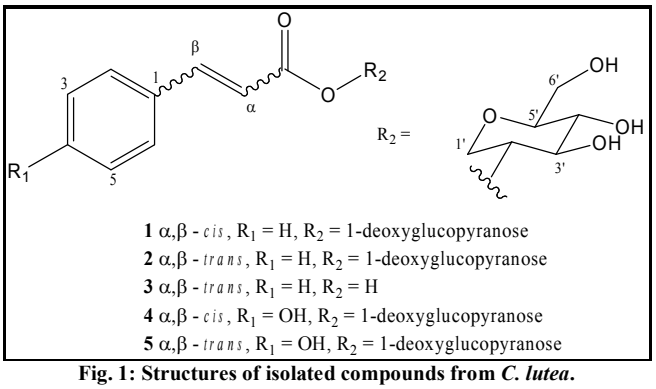
Statistical Analysis and Data Evaluation

Data obtained from this work were analysed statistically using Students' T-test and by multiple comparisons of Mean ± S.E.M by one way and two way analysis of variance (ANOVA, One- or Two –way) followed by a post test (Turkey- Kramer multiple comparison test). A probability level of less than 5% was considered significant ($P \leq 0.05$).

RESULTS

Phytochemical evaluation

Phytochemical characterization and isolation steps of *C. lutea* leaf fraction reveal that the plant contain basically polyphenols. Polyphenols such *E*-cinnamic acid-β-D-2-deoxyglucosyl ester, *Z*-cinnamic acid- β-D-2-deoxyglucosyl ester (**1 and 2**), *E*-p-coumaric acid- β-D-2-deoxyglucosyl ester, *Z*-p-coumaric acid- β-D-2-deoxyglucosyl ester (**4 and 5**) and *Z-cinnamic* acid (**3**) were isolated. This is shown in Fig. 1.



1 α,β - *cis*, R_1 = H, R_2 = 1-deoxyglucopyranose
2 α,β - *trans*, R_1 = H, R_2 = 1-deoxyglucopyranose
3 α,β - *trans*, R_1 = H, R_2 = H
4 α,β - *cis*, R_1 = OH, R_2 = 1-deoxyglucopyranose
5 α,β - *trans*, R_1 = OH, R_2 = 1-deoxyglucopyranose

Fig. 1: Structures of isolated compounds from *C. lutea*.

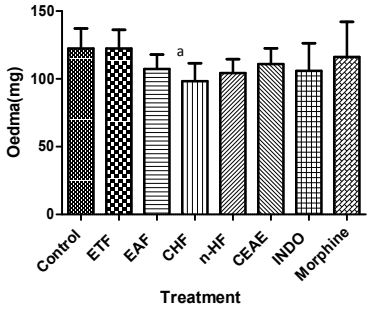
Anti-inflammatory tests

Formalin-induced Oedema Test in Mice

The anti-inflammatory effects of CLL on formalin-induced oedema in mice are as shown in Fig. 2. The CHF (770 mg/kg) significantly ($p<0.05$) inhibited the formation of formalin-induced oedema when measured by weight in mice relative to control. The inhibitions of inflammation by other extract when compared to control were not statistically significant.

Carragenin-induced Oedema Test in Rats

The anti-inflammatory effects of CLL extracts, ETF, EAF, CHF, n-HF on carragenin- induced hind paw oedema in rats is as shown in Table 1. EAF and n-HF showed a significant inhibition ($p<0.05$-0.001) throughout the duration of the experiment (1-5 h) when compared to control. However, the antagonism of ETF on carragenin-induced oedema was for only 2 h ($p < 0.05$-0.001) while CHF showed no significant effect. The result is as shown in Table 1.



Significance relative to control: [a]$p<0.05$; values represent mean ±S.E.M (n=6)

Fig. 2: Effects of *C. lutea Leaf* Fractions on Formalin-induced Oedema in Mice

Egg Albumin-induced Oedema Test in Rats

The anti-inflammatory effects of CLL extract on egg albumin -induced oedema in rats is as shown in Table 2. All the fractions inhibited the egg albumin induced -oedema in rats. This inhibition was statistically significant ($p<0.05$- 0.001) relative to control.

Capsaicin-induced Oedema Test in Rats

The antiinflammatory effect of CLL extracts on capsaicin-induced oedema in rats is as shown in Table 3. CHF and *n*-HF fractions showed the maximal inhibitory effect that lasted for 3-5 h while ETE and EAE exhibited their antagonism for only 2 h. These inhibitions were statistically significant ($p <$ 0.05-0.001) relative to control.

Xylene-induced Oedema test in Mice

Topical anti-inflammatory activity of CLL extracts on xylene-induced ear edema in mice is shown in Table 4. The application of xylene to mice ear induced cutaneous inflammation which caused significant increase in ear plug weight of the right ear when compared to the vehicle-treated left ear. Administration of diclofenac sodium (DCS), an anti-inflammatory drug (25 mg/kg, p.o.) gave rise to a significant ($p < 0.001$) inhibition of 85.71% in ear plug weight relative to control. The CLL extracts however do not demonstrate any significant inhibition of xylene-induced oedema. The inhibition percentage are 52.37, 33.34, 52.29 and 47.63%, for ETF, EAF, CHF, n-HF respectively in ear plug weight and is as shown in Table 4.

Antipyretics Activity

Effects of *C. lutea* Fractions on Normal Body Temperature in Rats

The antipyretic effects of CLL extract, *n*-HF, EAF, CHF and ETF on normal body temperature of rats are shown in Table 5. It was observed that there was no statistically significant change in temperature relative to control.

Effects of *C. lutea* Fractions on Yeast-induced Pyrexia in Rats

The antipyretic effects of CLL extracts, CLL extract, *n*-HF, EAF, CHF and ETF on yeast-induced pyrexia in rats are shown in Table 6. It was observed that there was no statistically significant change in temperature relative to control.

Effects of *C. lutea* Fractions on 2, 4-Dinitrophenol-induced Pyrexia in Rats

The antipyretic effects of different CLL extracts on 2, 4-dinitrophenol-induced pyrexia in rats is as shown in Table 7. The result reveals that administration of ethanol and n-hexane extract.

Table 1: Effect Of Leaf Extracts Of *C. lutea* on carrageenin-induced Inflammation in rats measurement of hind paw oedema (CM)

DOSE (mg/kg)	INITIAL MEASUREMENT	0.5 HR	1.0 HR	1.5 HRS	2.0 HRS	2.5 HRS	3.0 HRS	3.5 HRS	4.0 HRS	4.5 HRS	5.0 HRS
Control	0.38 ± 0.01	0.57 ± 0.03	0.65 ± 0.03	0.68^{c} ± 0.04	0.67 ± 0.03	0.67 ± 0.03	0.66 ± 0.02	0.64 ± 0.03	0.63 ± 0.04	0.63 ± 0.04	0.62 ± 0.04
ETF 770	0.38 ± 0.01	0.51^{c} ± 0.01	0.53^{c} ± 0.01	0.58 ± 0.02	0.57^{c} ± 0.03	0.55^{c} ± 0.02	0.53^{c} ± 0.03	0.52^{a} ± 0.03	0.53^{c} ± 0.02	0.53^{c} ± 0.02	0.54^{c} ± 0.02
EAF 770	0.40 ± 0.01	0.54 ± 0.91	0.60 ± 0.01	0.64 ± 0.02	0.64 ± 0.02	0.67 ± 0.01	0.69 ± 0.02	0.72 ± 0.01	0.71^{b} ± 0.02	0.69^{b} ± 0.01	0.69^{a} ± 0.02
CHF 770	0.38 ± 0.01	0.57 ± 0.02	0.65 ± 0.02	0.68 ± 0.02	0.69 ± 0.02	0.73^{b} ± 0.03	0.74^{c} ± 0.02	0.75 ± 0.02	0.73^{c} ± 0.02	0.73^{c} ± 0.02	0.70^{b} ± 0.03
n-HF 770	0.39 ± 0.01	0.55 ± 0.02	0.58^{c} ± 0.02	0.62^{b} ± 0.03	0.64 ± 0.03	0.61^{b} ± 0.03	0.59^{c} ± 0.04	0.54 ± 0.03	0.54^{c} ± 0.04	0.54^{c} ± 0.04	0.56^{a} ± 0.04
INDO 10	0.39 ± 0.01	0.58 ± 0.01	0.59^{c} ± 0.01	0.59^{c} ± 0.01	0.57^{c} ± 0.01	0.55^{c} ± 0.02	0.55^{c} ± 0.02	0.55 ± 0.02	0.55^{c} ± 0.03	0.54^{c} ± 0.01	0.53^{c} ± 0.02

Significance relative to control:- $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$, n=6

Table 2: Effect Of Leaf Extracts Of *C. lutea* On Egg Albumin- Induced Inflammation In Rats Measurement Of Hind Paw Oedema (CM)

DOSE (mg/kg)	INITIAL MEASUREMENT	0.5 HR	1.0 HR	1.5 HRS	2.0 HRS	2.5 HRS	3.0 HRS	3.5 HRS	4.0 HRS	4.5 HRS	5.0 HRS
Control	0.44 ± 0.01	0.74 ± 0.02	0.74 ±0.02	0.71 ± 0.02	0.70 ± 0.03	0.68 ± 0.02	0.74 ± 0.03	0.66 ± 0.02	0.61 ± 0.01	0.62 ± 0.01	0.60 ± 0.01
ETF 770	0.32^{c} ± 0.01	0.59^{c} ± 0.02	0.62^{c} ± 0.03	0.65 ± 0.03	0.60^{c} ± 0.02	0.57^{c} ± 0.03	0.56^{c} ± 0.02	0.56^{c} ± 0.03	0.53^{c} ± 0.02	0.51^{c} ± 0.03	0.47^{c} ± 0.02
EAF 770	0.41 ± 0.01	0.65^{c} ± 0.03	0.63^{c} ± 0.05	0.62 ± 0.04	0.59^{c} ± 0.04	0.58^{c} ± 0.04	0.57^{c} ± 0.04	0.58^{c} ± 0.02	0.56 ± 0.04	0.55^{b} ± 0.03	0.54^{b} ± 0.03
CHF 770	0.39 ± 0.02	0.52^{c} ± 0.01	0.54^{c} ± 0.01	0.50^{c} ± 0.01	0.48^{c} ± 0.02	0.52^{c} ± 0.03	0.55^{c} ± 0.03	0.54^{c} ± 0.03	0.51^{c} ± 0.04	0.50^{c} ± 0.05	0.47^{c} ± 0.04
n-HF 770	0.35^{b} ± 0.02	0.58^{c} ±0.03	0.59^{c} ± 0.02	0.59^{a} ± 0.02	0.57^{c} ± 0.05	0.61^{b} ± 0.02	0.60^{c} ± 0.02	0.57^{c} ± 0.02	0.58 ± 0.03	0.59 ± 0.03	0.54^{b} ± 0.03
INDO 10	0.38 ± 0.01	0.68^{a} ± 0.05	0.66^{c} ± 0.03	0.62 ± 0.03	0.61^{c} ± 0.03	0.58^{c} ± 0.03	0.58^{c} ± 0.04	0.56^{c} ± 0.02	0.54^{b} ± 0.02	0.54^{b} ± 0.03	0.52^{c} ± 0.03

Significance relative to control: - $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$, n=6

Table 3: Effect Of Leaf Extracts Of *C. lutea* 770mg On Capciacin- Induced Inflammation In Rats Measurement Of Hind Paw Oedema (CM)

DOSE (mg/kg)	INITIAL MEASUREMENT	0.5 HR	1.0 HR	1.5 HRS	2.0 HRS	2.5 HRS	3.0 HRS	3.5 HRS	4.0 HRS	4.5 HRS	5.0 HRS
Control	0.48 ± 0.01	0.66 ± 0.02	0.66 ± 0.01	0.61 ± 0.02	0.66 ± 0.02	0.59 ± 0.01	0.60 ±0.04	0.59 ± 0.02	0.55 ± 0.02	0.52 ± 0.02	0.51 ± 0.02
ETF 770	0.39^{b} ± 0.01	0.53^{C} ± 0.04	0.61^{C} ±0.02	0.61^{C} ± 0.02	0.61^{C} ± 0.02	0.58 ± 0.02	0.58 ± 0.02	0.58 ± 0.02	0.57 ± 0.02	0.54 ± 0.03	0.53 ± 0.02
EAF 770	0.39 ± 0.02	0.55^{C} ± 0.02	0.58^{C} ± 0.02	0.59^{C} ± 0.03	0.59^{C} ± 0.03	0.60 ± 0.02	0.60 ± 0.02	0.59 ± 0.01	0.55 ± 0.02	0.51 ± 0.02	0.51 ± 0.03
CHF 770	0.44 ± 0.02	0.65 ± 0.03	0.59^{C} ± 0.02	0.61^{C} ± 0.02	0.56^{C} ± 0.02	0.56 ± 0.02	0.53^{C} ± 0.02	0.53^{C} ±0.02	0.54 ± 0.01	0.54 ±0.01	0.56 ± 0.03
n-HF 770	0.46 ± 0.01	0.62 ± 0.02	0.62^{b} ± 0.02	0.58^{C} ± 0.02	0.55^{C} ± 0.02	0.54^{b} ± 0.02	0.53^{C} ± 0.03	0.52^{C} ± 0.02	0.55 ± 0.03	0.54 ± 0.03	0.57 ±0.02
INDO 10	0.44 ± 0.01	0.64 ± 0.02	0.61^{C} ± 0.02	0.56^{C} ± 0.02	0.56^{C} ± 0.01	0.53^{C} ±0.03	0.53^{C} ±0.02	0.51^{C} ± 0.01	0.50^{C} ±0.02	0.41^{C} ± 0.02	0.37^{C} ± 0.02

Significance relative to control:- $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$, n=6

TABLE 4: Effects of leaf extracts of *c. lutea* on xylen induced on ear oedema in mice.

Treatment	Dose (mg/kg)	Mean difference	Inhibition (%)o
Control		35.0 ± 7.87	-
ETF	770	16.67 ± 10.83	52.37
EAF	770	23.33 ± 6.73	33.34
CHF	770	16.70 ± 5.40	52.29
n-HF	770	18.33 ± 5.94	47.63
DCS	25	5.00 ± 2.45	85.71

Significance relative to control: Not significant, values represent mean ±S.E.M (n=6)

Table 5: Effects Of Leaf Extracts of *C. lutea* On Normal Body Temperature Rise In Rats Measurement Of Temperature (C)

Extracts / DOSE (mg/kg)	Pre-drug rectal temp. ^{0}c	1.0 HR	2.0 HRS	3.0 HRS	4.0 HRS	5.0 HRS
Control	36.50 ±0.37	36.68 ±0.31	36.88 ±0.31	37.73 ±0.25	37.22 ±0.07	36.95 ±0.20
ETF 770	35.12^a ±0.35	35.45^c ±0.41	35.97^c ±0.22	36.12^c ±0.20	36.40^c ±0.12	36.62 ±0.38
EAF 770	37.32 ±0.27	37.32^a ±0.23	37.13 ±0.24	37.70 ±0.15	37.07 ±0.23	37.97^a ±0.38
CHF 770	36.40 ±0.18	36.32 ±0.06	36.20^c ±0.09	36.97^c ±0.11	36.38 ±0.26c	36.57 ±0.30
n-HF 770	35.68 ±0.19	36.23 ±0.27	36.10 ±0.20	36.27^c ±0.29	36.75^a ±0.25	36.23^a ±0.38

Significance relative to control: ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001; values represent mean ±S.E.M (n=6)

Table 6: Effects Of Leaf Exctracts Of *C. lutea* On Brewer's Yeast-Induced Temperature Rise In Rats Measurement Of Temperature (°C)

Extracts / DOSE (mg/kg)	CONTROL	1.0 HR	2.0 HRS	3.0 HRS	4.0 HRS	5.0 HRS
Control	36.90±0.27	37.00±0.08	37.28±0.16	37.77±0.08	38.05±0.13	38.45±0.13
ETF 770	37.33±0.26	37.07±0.19	37.38±0.32	37.77±0.25	38.07±0.33	38.61±0.23
EAF 770	37.33±0.35	36.78±0.36	37.30±0.17	37.53±0.14	37.62±0.21^a	38.03±0.26^a
CHF 770	37.22±0.34	36.88±0.15	37.18±0.16	37.38±0.11	37.58±0.23^b	37.75±0.34^c
n-HF 770	36.87±0.26	37.50±0.21^b	37.27±0.15	37.58±0.16	38.18±0.19^c	38.22±0.08
DCS	37.17±0.28	37.55±0.11^c	37.43±0.18	37.43±0.15	37.33±0.08^c	37.32±0.18^c

Significance relative to control: ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001, n=6,

Table 7: Effects Of Leaf Exctracts of *C. lutea* on 2,4-dinitro phenol-induced temperature rise in Rats measurement of temperature (°C)

Extracts / Dose (mg/kg)	CONTROL	1.0 HR	2.0 HRS	3.0 HRS	4.0 HRS	5.0 HRS
Control	36.98±0.26	37.47±0.27	37.65±0.33	37.30±0.30	37.0±0.25	37.34±0.16
ETF 770	36.88±0.44	37.22±0.14^a	37.47±0.26^b	37.02±0.34^C	37.0±0.45	36.95±0.37
EAF 770	37.58±0.10	39.87±0.09	37.98±0.20	37.82±0.10	37.78±0.13	37.56±0.11
CHF 770	37.43±0.08	37.92±0.09	37.53±0.11	37.48±0.18	37.62±0.17	37.70±0.14
n-HF 770	36.47±0.19^c	36.72±0.24^c	36.73±0.35^c	36.22±0.34^c	36.82±0.32^c	36.97±0.36^a
ASA	37.23±0.10	37.87±0.21	36.88±0.23	37.50±0.14	37.87±0.09	38.05±0.06

Significance relative to control: ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001, n=6,

(770 mg /kg) reduced pyrogen induced temperature rise significantly ($p < 0.05$-0.001) relative to control (Table. 7). The EAF and CHF and ASA do not exert any significant reduction of pyrexia when compared to control.

DISCUSSION

In this study the anti-inflammatory and antipyretic activity of polar and non-polar extracts of CLL was evaluated using different experimental models.

Carrageenin-induced rat paw oedema model is the most commonly used primary test for the screening of new anti-inflammatory agents[21]. The oedema formation is a biphasic event. The initial phase, observed during the first hour, is attributed to the release of serotonin, histamine and bradykinin[31] and the delayed oedema is due to the release of bradykinin and prostaglandins and NO[31]. It has been reported that the second phase of oedema is sensitive to steroidal and non-steroidal anti-inflammatory agents[32]. The CHF and EAF of CLL reduced the paw volume significantly from 1 to 5 h, the highest effects were found at the second and third hour respectively. These results tend to suggest the anti-inflammatory activity of these extracts act at both phases through the suppression of mediator release. The effects of ETF and n-HF are insignificant. CLL active extracts could exert its anti-inflammatory effects by inhibiting the cyclooxygenase pathway, considering that the mechanism involved in the pathogenesis of the carrageenin induced oedema could cause the release of prostaglandin, kinins, serotonin and histamines.

Similarly, for the egg albumin-induced oedema the extracts pre-treated rats significantly reduced paw oedema. All the extracts at the tested dose (770 mg/kg), significantly inhibited rat paw oedema than the standard drug, indomethacin. The CHF gave the highest inhibition of oedema induced by egg albumin relative the effect of the standard drug, indomthacin at the tested dose. Egg albumin is known to induce two mediators of inflammation which are basically histamine and serotonin which the extract inhibited to reduce inflammation[23].

For the capsaicin model, all the extracts at the tested dose were very effective up to two hours of induction of the inflammation induced by capsaicin. Capsaicin induces neurogenic inflammation mediated by neuropeptides such as substance, P, neurokinin A, vasoactive intestinal peptides and calcitonin gene related peptides released from capsaicin stimulated neurons. The absence of prolong inhibition of capsaicin- induced oedema suggests in part that its anti-inflammatory effects may not be mediated through neurogenic mechanism[33]. Due to a primary stimulus, two mechanisms contribute to the development of edema caused by increased vascular permeability. One induced by local release or formation of various autacoids and another induced neurogenically by stimulation of primary sensory neurons and subsequent mediator (substance P) release from

peripheral endings of nerve fibers[34, 35,36]. According to Lemback and Holzer[37], the neurogenic component plays an important role in maintaining the non-neurogenic plasma extravasations since the stimulation of peripheral neurons and subsequent release of substance P from peripheral sensory endings causes further release of histamine from mast cells. It therefore means that the possible specific action of these extracts in blocking the neurogenic component of the stimulated vascular permeability can stop the series of pathogenic events locally evoked by noxious stimuli. Therefore, the anti-inflammatory properties of these fractions strongly support the evidence of a major anti-edematous component.

It is of interest to note that the fractions of *C. lutea* aerial parts did not produced any significant effect in the body temperature in normothermic rats but it does in chemical but not in biological (yeast) -induced pyrexia. It is well known that pyretic activity involves stimulation of the region in the hypothalamus that controls body temperature; via prostaglandins synthesized within the central nervous system (CNS)[38] and that the blood–brain barrier (BBB) prevents drug molecules or other chemicals from entering the CNS[39]. The ability to cross the BBB could be one of the factors contributing to antinocciceptive effects of all the fractions[18] but the antipyretic activity was more in the chemical induced but not the biological induced pyrexia.

Clinically available antipyretic drugs, such as paracetamol and the non-steroidal anti-inflammatory drugs are able to lower the body temperature[40]. In general, non-steroidal anti-inflammatory drugs produce their antipyretic action through inhibition of prostaglandin synthetase within the hypothalamus[41, 42.] Therefore, it appears that the antipyretic action of the extract may also be related to the inhibition of prostaglandin synthesis. The antipyretic effects of the extracts in chemical-induced model do corroborate its ethnomedicinal uses in feverish condition.

CONCLUSION

Our present study is a first report of the anti-inflammatory and antipyretic activities of *C. lutea* leaves. It confirms the traditional use of this plant in treating ailments associated with inflammation and fever. We suggest that the coumaroyl and cinamoyl glucosides contents in the leaves are major contributors to the anti-inflammatory and antipyretic effects of the leaves. A chemical investigation by NMR and IR of our fractions showed the presence of different polyphenols. These natural products have been implicated as active anti-inflammatory[43] and decreasing inflammatory mediator production in human whole blood cultures[44]. However, further detailed bioactivity guided studies are required to determine the active ingredients responsible for these effects and to determine the mechanism of action of these compounds in the anti-inflammatory and analgesic processes. These findings support the local use of the plant as anti-inflammatory and antipyretic.

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