



Anti-Inflammatory and Analgesic Effects of Methanol Extracts of *Chrysophyllum albidum* Stem Bark on Formalin Induced Paw Oedema in Albino Rats

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Abstract

Chrysophyllum albidum has reputation in Nigeria as remedy for different ailments. This study evaluated the methanol extract of *C. albidum* stem bark for its phyto-constituents, anti-inflammatory and analgesic effects in Albino rats. Phytochemical analysis was conducted using standard procedures while the anti-inflammatory and analgesic effects were determined using formalin-induced acute paw edema and nociception in rat respectively. The results of the phytochemical screening indicated the presence of anthraquinones, steroids, tannins, alkaloids, glycosides, flavonoids, saponins and phlobatannins. The percentage inhibition of the paw oedema was calculated to be 39.09%, 37.27%, 53.63% and 62.72% for groups treated with 100 mg/kg, 200 mg/kg, 400 mg/kg body weight of methanol extract of *C. albidum* stem bark and diclofenac respectively, with the group treated with 400 mg/kg body weight of the extract having the highest percentage inhibition amongst the extract-treated groups. The analgesic effect was evaluated by calculating the paw-licking rate within 60 minutes after induction of oedema. The values were calculated to be 239.75 ± 13.91 , 159.65 ± 11.62 , 75.72 ± 5.31 , 65.08 ± 9.71 and 292.04 ± 11.88 for groups treated with 100 mg/kg, 200 mg/kg, 400 mg/kg body weight of the extract, 5 mg/kg body weight of the standard drug (diclofenac) and the negative control group (induced, but not treated) respectively. It can be concluded from the result obtained in this study that methanol extract of *C. albidum* stem bark contain bioactive constituents with analgesic and anti-inflammatory potentials, which goes to support its acclaimed traditional medical use of other parts of the plant in the management of pain and inflammatory conditions.

Keywords: *Chrysophyllum albidum*; Stem bark; Anti-inflammatory; Analgesic; Paw oedema

Introduction

Inflammation is an immunological defence mechanism that is triggered in response to bacterial, viral or other parasitic infections, mechanical injuries, burns allergens and other noxious stimulus [1]. Inflammation may be acute or chronic depending on the disease course. Acute inflammation is characterized by heat, erythema, pain, swelling and loss of function [2]. Pain is a common and distressing feature of many diseases and analgesics relieve pain by acting in the central nervous system or on peripheral pain mechanisms, without significantly altering consciousness. Chronic inflammation on the other hand results in a progressive shift in inflammatory cells characterized by simultaneous destruction and healing of the injured tissue [3]. The process of inflammation is necessary in healing wounds. However, uncontrolled and persistent inflammation contributes to the progression of many chronic pathological conditions, such as rheumatoid arthritis, atherosclerosis, inflammatory bowel disease, retinitis, multiple sclerosis etc. [4]. It may also be associated with increased risk of cancer [5].

Research on analgesic and anti-inflammatory drugs has gained great attention for the past ten years. However, the number of new drugs remains low. Most analgesic and anti-inflammatory compounds available in market have adverse effects, including life-threatening, bleeding or perforation of gastro duodenal tract [6]. Consequently there results the need to search for more active compounds with less adverse effects. Many plants have long been recognized as important sources of therapeutically effective medicines. *Chrysophyllum albidum* is a small to medium buttressed tree species, up to 25-37 m in height with a mature girth varying from 1.5-2 m. The Bole is usually fluted, frequently free of branches for 21 m thin bark, pale brownish-green, slash exuding white and gummy latex [7]. *Chrysophyllum albidum* belongs to the family Sapotaceae [8]. It is a dominant canopy tree of lowland mixed rain

forest, sometimes riverine, which naturally occurs in diverse ecozones in Nigeria, Sudan Uganda, Kenya, and Niger Republic [9]. *C. albidum* is known by several local names such as white star apple (English); nkalate, mululu (Uganda), in Nigeria, it is locally known as "agbalumo" and "udara" in South Western and Eastern Nigeria respectively [10]. The fruit of *C. albidum* has been found to be rich in Vitamins B and D, iron and have a very high content of ascorbic acid with 1000 to 3,300 mg of ascorbic acid per 100g of edible fruit or about 100 times that of oranges and 10 times that of guava or cashew [10]. Several other components of the tree including the roots and leaves are used for medicinal purposes [11]. The bark is used as a remedy for yellow fever and malaria while the leaves are used as emollients and for the treatment of skin eruption, diarrhea and stomach ache [12]. Eleagnine, an alkaloid isolated from *C. albidum* seed cotyledon has been reported to have anti-nociceptive, anti-inflammatory and antioxidant activities [13]. The cotyledons are also useful in the preparation of medicine for the treatment of infertility problems in both male and female [14]. However there is paucity of information on the anti-inflammatory effect of the bark. It is therefore necessary to evaluate the phytoconstituents and anti-inflammatory activities of *Chrysophyllum albidum* stem bark extract in order to fully

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ascertain the ability of *Chrysophyllum albidum* as a potential source of new, cheap and safe anti-inflammatory drugs.

Materials and Methods

Plant material

The fresh stem bark of *Chrysophyllum albidum* was obtained from its natural habitat at a cash crop farm in Rore, Irepodun Local Government Area of Kwara State Nigeria. It was collected between the month of March and April 2014. Taxonomic identification of the plant was done at the Department of Biological Sciences, Federal University of Technology, Minna, Niger state, Nigeria. The fresh stem bark of *C. albidum* were rinsed in clean water cut into pieces, and dried at room temperature. The dried bark of *C. albidum* were grounded using mortar and pestle and then blended to powder using electronic blending machine.

Experimental animals

Twenty Swiss adult albino rats weighing between 130-200g were bought from Dare animal breeding house in Ilorin, Kwara State. They were housed in plastic cages and allowed access to constant feed and water ad-libitum. They were then allowed to acclimatize to the laboratory conditions before the commencement of the experiments.

Extraction of plant material

Methanol extraction of *C. albidum* stem bark was performed using the method described by Adewoye et al. in 2010 by weighing 1500 g of the powdered stem bark of *C. albidum* into 4 liters of 70% methanol and covered with aluminum foil [15]. The mixture was stirred every 3 hours for proper mixing and allowed to stand for 72 hours. The resulting decoction was filtered and the filtrate was concentrated in water bath at 65°C. The extract was packed in an air-tight container, labelled and stored in the refrigerator, until required.

a) Percentage yield of extracted plant material

$$\text{Percentage yield (\%)} = \frac{\text{Weight (g) of the concentrated extract}}{\text{Weight (g) of the } C. \text{ albidum stem bark}}$$

Phytochemical screening

Standard screening tests for the extract were carried out for various constituents like alkaloids, saponins, tannins, glycosides and volatile oils using standard procedures [16,17].

Anti-inflammatory test

The anti-inflammatory activity of methanol extract of *Chrysophyllum albidum* stem bark was tested using formalin induced paw oedema in rats [18]. Twenty male and female rats were divided into four groups of four rats each:

a) Group I: Rats were administered with 2 ml/kg body weight of distilled water + 0.1 ml of Formalin (negative control).

b) Group II: Rats were administered with 50 mg/kg b.w of diclofenac + 0.1 ml of Formalin (reference drug).

c) Group III: Rats were administered with 100 mg/kg b.w of methanol extract of *C. albidum* stem bark + 0.1 ml of Formalin.

d) Group IV: Rats were administered with 200 mg/kg b.w of methanol extract of *C. albidum* stem bark + 0.1 ml of Formalin.

e) Group V: Rats were administered with 400 mg/kg b.w of methanol extract of *C. albidum* stem bark + 0.1 ml of Formalin.

Inflammation was induced by injecting 0.1 ml of 2.5% Formalin into the sub-planter surface of the right hind paw one hour after extract/drug administration. The increase in volume (mm) of the hind paw was measured with a veneer calliper at 20 min intervals after the induction with formalin for a period of 1 hour. The percentage inhibition of oedema was calculated for each dose using the formula:

$$\% \text{ inhibition of oedema} = \frac{Ec - Et \times 100}{Ec}$$

Where Ec is the % oedema of the control group and Et is the % oedema of the test group.

Analgesic test

The anti-nociceptive effects of methanol extract of *Chrysophyllum albidum* stem bark was tested as described by Akuodor et al. [19]; twenty male and female rats were divided into four groups of four rats each:

a) Group I: Rats were administered 2 ml/kg body weight of distilled water + 0.1 ml of Formalin (negative control).

b) Group II: Rats were administered 50 mg/kg b.w of diclofenac + 0.1 ml of Formalin (reference drug).

c) Group III: Rats were administered 100 mg/kg b.w of methanol extract of *C. albidum* stem bark + 0.1 ml of Formalin.

d) Group IV: Rats were administered 200 mg/kg b.w of methanol extract of *C. albidum* stem bark + 0.1 ml of Formalin.

e) Group V: Rats were administered 400 mg/kg b.w of methanol extract of *C. albidum* stem bark + 0.1 ml of Formalin.

One hour after drug administration, each rat was subcutaneously injected with 0.1 ml of 2.5% formalin solution into the subplantar area of the left hind paw. They were placed in an observation chamber and monitored for one hour. The severity of pain response was recorded for each rat based on the following scale:

(0) Rat walked or stood firmly on the injected paw

(1) The injected paw was favored or partially elevated

(2) The injected paw was clearly lifted off the floor

(3) The rat licked, chewed or shook the injected paw. Anti-nociceptive effect was determined in two phases. The early phase (1-10 min) and late phase (10-60 min) after formalin injection.

Statistical analysis

The data was represented as mean \pm standard error of mean (SEM) or as percentages. The statistical significance was determined by one-way analysis of variance followed by Dunnet's test, with the level of significance set at $P < 0.05$.

Results

Percentage yield of the methanol extract of *C. albidum* stem bark

The extraction process yielded 6.06% of the methanol extract of *C. albidum* stem bark.

Phytochemical screening

Phytochemical screening of the methanol extract of *C. albidum*

stem bark revealed the presence of alkaloids, steroids, anthraquinones, saponin, flavonoids, phlobatanins, glycosides and tannins (Table 1).

Anti-inflammatory effect of methanol extract of *C. albidum* stems bark

Table 2 shows the anti-inflammatory effect of the methanol extract of *Chrysophyllum albidum* stem bark on formalin-induced paw oedema in experimental rats. The anti-inflammatory effect was observed to be dose dependent.

Analgesic effects of methanol extract of *C. albidum* stem bark on Formalin-induced paw oedema in rats

The analgesic effect of methanol extract of *C. albidum* stem bark on formalin-induced paw oedema in rats is shown in Table 2. There was no significant difference ($p < 0.05$) in nociceptiveness in animals administered 400 mg/kg bod weight of the extract and those administered with the standard drug (diclofenac) in both phases of the experiment.

Discussion

The percentage yield of methanol extract of *C. albidum* stem bark was 6.06%. There is considerable interest by phytochemist to identify the therapeutic agents contained in plants in order to establish the basis for their uses in traditional medical practice [20]. The phytochemical screening results of methanol extract of *Chrysophyllum albidum* stem bark showed that anthraquinones, steroids, tannins, saponins, alkaloids, flavonoids and glycosides were present, while reducing sugars were

absent (Table 1). The results from the anti-inflammatory studies on methanol extract of *C. albidum* stem bark showed that the three doses tested (100 mg/kg, 200 mg/kg and 400 mg/kg b.w) produced significant ($p < 0.05$) anti-inflammatory activity and reduced the paw volume by 39.09%, 37.27% and 53.63% respectively, whereas diclofenac which was used as a reference drug caused 62.72% reduction of paw volume. In the group administered with 2 ml/kg b.w distilled water (negative control), an increase in the paw oedema was observed throughout the time of experiment (Table 2). Though, the % inhibition of the paw oedema for the group treated with 100 mg/kg b.w of methanol extract of *C. albidum* stem bark was slightly higher than that treated with 200 mg/kg b.w, there was no significant difference ($p < 0.05$) between the two groups. However, the group treated with 400 mg/kg b.w. methanol extract of *C. albidum* stem bark had the highest inhibition of the paw oedema when compared to the groups administered with 100 and 200 mg/kg b.w.

The results of the analgesic activity of the methanol extract of *C. albidum* stem bark showed that there was significant ($p < 0.05$) inhibition of formalin paw nociception and paw licking in both early and late phases. In the groups administered with 100 mg and 200 mg/kg b.w of *C. albidum*, there was no significant difference ($p > 0.05$) in the inhibition of paw nociception and paw licking in the early phase while a significant difference ($p < 0.05$) was observed in the late phase of the inhibition. The highest inhibition of the paw nociception and paw licking as regards to groups treated with the methanol extract of *C. albidum* stem bark was obtained in the group administered with 400 mg/kg b.w., however, the inhibition was significantly ($p < 0.05$) lower when compared with the group administered with the standard drug (diclofenac) (Table 3).

The formalin test is believed to represent a significant model of clinical pain and formalin produced a distinct biphasic response to pain stimulus and different analgesic compounds may act differently in the early and late phases of this test [21]. The early phase is the result of direct chemical activation of nociceptive primary afferent fibers, while the factors that contribute the late phase are not well defined. Therefore, this test can be used to clarify the possible mechanisms of anti-nociceptive effect of a test compound. Centrally acting drugs such as opioids inhibit both phases equally but, peripherally acting drugs, such as cyclooxygenase inhibitors (aspirin and indomethacin) and corticosteroids only inhibit the late phase [21].

The ability of methanol extract of *C. albidum* stem bark to inhibit both first and second phases of formalin-induced pain suggests that it may possess central analgesic activity (Table 3). Similarly, the analgesic and anti-inflammatory effect of the methanol extract of *C. albidum* stem bark in this study may be attributed to the presence of appreciable amounts of secondary metabolites.

Phytochemicals	Inference
Alkaloids	±
Glycosides	±
Anthraquinones	±
Steroids	±
Tannins	±
Saponins	±
Flavonoids	±
Reducing sugar	—
Phlobatannins	±

Key: (-) Absent, (±) Present

Table 1: Phytochemical constituents of methanol extract of *C. albidum* stem bark.

Treatment	Dose (mg/kg.bwt)	Time (Min) paw oedema (mm)				Mean ± SEM	%Inhibition
		20	40	60			
D.W	2 ml	0.92	0.95	1.45	1.10 ± 0.04 ^a	-	
C.a	100	0.72	0.60	0.70	0.67 ± 0.02 ^b	39.09	
C.a	200	0.65	0.77	0.66	0.69 ± 0.03 ^b	37.27	
C.a	400	0.65	0.57	0.55	0.59 ± 0.39 ^c	53.63	
DIC	50	0.56	0.32	0.36	0.41 ± 0.02 ^d	62.72	

Key: DW- Distilled Water, C.a- *Chrysophyllum albidum*, DIC- Diclofenac
Values are in Mean ± Standard error of mean and those with the same superscript along the column are not significantly different at $P < 0.05$. Values with different superscript are significantly different at $p < 0.05$.

Table 2: Anti-inflammatory effects of methanol extract of *C. albidum* stem bark on formalin induced paw oedema in rat.

Sample	Dose (mg/kgbw)	Paw licking time (Min)	
		Phase (0-10)	Phase (10-60)
D.W	2ml	67.93 ± 6.14 ^c	292.04 ± 11.88 ^d
C.a	100	55.87 ± 7.16 ^b	239.75 ± 13.91 ^c
C.a	200	49.98 ± 5.71 ^b	159.65 ± 11.62 ^b
C.a	400	32.65 ± 6.23 ^a	75.72 ± 5.31 ^a
DIC	50	36.32 ± 6.21 ^a	65.08 ± 9.71 ^a

Key: DW- distilled water, C.a- *Chrysophyllum albidum*, DIC- diclofenac
Values are in Mean ± Standard error of mean and those with the same superscript along the column are not significantly different at $P < 0.05$. Values with different superscript are significantly different at $p < 0.05$.

Table 3: Analgesic effects of methanol extract of *C. albidum* stem bark on Formalin-induced paw oedema in rats.

Conclusion

In conclusion, methanol extract of *Chrysophyllum albidum* stem bark contain bioactive constituents with analgesic and anti-inflammatory activities, and further support the ethno medical claim of the use of the plant in the management of pain and inflammatory conditions.

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