



## **Anti-Inflammatory and Analgesic Evaluation of *Parinari curatellifolia* Methanol Leaf Extract on Albino Rats**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aim:** This research was designed to evaluate the anti-inflammatory and analgesic activities of *Parinari curatellifolia* methanol leaf extract in albino rats.

**Methodology:** Phytochemical screening was carried out using standard methods. Anti-inflammatory activity of the extract was done using egg albumin and formalin induced hind paw edema model. Analgesic effect was evaluated using hot plate induced pain and acetic acid induced writhing test. For each model twenty (20) rats were used, divided into five (5) groups of four (4) rats each.

**Results:** *Parinari curatellifolia* revealed the presence of alkaloids, flavonoids, tannins and phenols while steroids, anthraquinone, terpenoids and glycoside were not detected. For the egg albumin induced inflammation, the group treated with the standard drug (indomethacin) and the group that received the highest dose of the extract were significantly lower ( $P < 0.05$ ) than all the other groups with percentage inhibitions at 25.56% and 24.44% respectively there was no significant difference ( $P > 0.05$ ). For the formalin induced anti-inflammatory activity, at the 1st hour, the normal control group had its paw volume significantly different ( $P < 0.05$ ) from the treated groups. This trend was observed at the 2nd, 3rd and 4th hour. The hot plate method results revealed significant increased ( $P < 0.05$ ) in the analgesic activity of PCMLE at 400mg/kg body weight and the drug treated group

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when the control was compared with the treated groups with percentage inhibition of 34.32% and 52.94% respectively. The acetic acid induced writhing test revealed that the extract at the three doses of 100, 200 and 400 mg/kg body weight, showed a significant ( $P < 0.05$ ) percentage inhibition of 32.31%, 36.92% and 47.69%, respectively compared to negative control.

**Conclusion:** This justifies the use of *Parinari curatellifolia* locally in the management of pain and inflammation.

**Keywords:** *Parinari curatellifolia*; analgesic; inflammatory; phytochemicals.

## 1. INTRODUCTION

Plants have long been used by man to maintain health and well-being. The healing power of plants date back to many years. In Nigeria, application of medicinal plants especially in traditional medicine is currently well-acknowledged and established as a viable profession [1]. The use of traditional medicine in developed as well as developing countries as basis for the treatment of many ailments has been in existence for thousands of years and there is no doubt that their importance has been widely acknowledged [2].

*Parinari curatellifolia* is a large ever green, spreading tree up to 20 metres tall with a single bare stem and a dense, roundish to mushroom shaped crown. Bark of the plant is dark grey and rough [3]. The plant is used in ethnomedicine for treatment of pneumonia, fever, dressing of wounds, fractures and dislocation, and oral infections [4]. A leaf decoction is either drunk or used in bath as a fever remedy. A hot infusion of the bark is used in the treatment of pneumonia. Crushed or pulped leaves are used in dressing fractures or dislocations, and for wounds, sores and cuts. After being stripped, the twigs can be used as tooth brush. *Parinari curatellifolia* is used in treatment of various diseases but there is no previous pharmacognostic report in literature on the plant [5].

Medicinal plants are considered as the main source of new drugs due to the cost and numerous side effects associated with the use of synthetic drugs [6]. *Parinari curatellifolia* is used in folk medicine for treatment of inflammatory diseases and management of pain. However, there is no scientific backings behind its therapeutic claims. Hence, the need to validate the anti-inflammatory and analgesic activities of *Parinari curatellifolia*. The investigation could help in the search for newer, cheaper and safer alternative drug for the treatment and management of ailments, especially inflammatory disorders.

## 2. METHODOLOGY

### 2.1 Collection and Identification of the Plant

The leaves of *Parinari curatellifolia* were collected from Tungan Lalle Village, Koko/Besse Local Government Area of Kebbi State, Nigeria and were authenticated by a botanist in the Herbarium Unit, Plant Science and Biotechnology Department, Kebbi State University of Science and Technology, Aliero. A Voucher specimen (A114) was deposited at the Herbarium for future reference.

### 2.2 Extraction of *Parinari curatellifolia*

The leaves of *Parinari curatellifolia* were dried at room temperature for two weeks and were pounded into smaller pieces with mortar and pestle. Two hundred and fifty grams (250) of coarse plant material was macerated with 2000ml of methanol and kept in an air-tight container for seventy two hours. The mixture was then filtered using sterile muslin cloth. The filtrate was evaporated using a rotary evaporator at 45°C and subsequently dried in a drying cabinet at 45°C and labelled methanol extract and stored in an air-tight bottle in a refrigerator until required for analysis.

### 2.3 Phytochemical Screening

#### 2.3.1 Test for alkaloids

Five millimetres (5ml) of 1% HCl was added to 1ml of the extract and stirred on a steam bath and filtered. Three portions (1ml each) of the filtrate were treated with 3 drops of Drangendorffs, Mayers and Wagners reagents respectively. Formation of turbidity confirmed the presence of alkaloids [7].

#### 2.3.2 Test for tannins

Three (3) drops of 5% FeCl<sub>3</sub> solution was added to 3mls of the extract. Presence of brownish

green or a blue-black precipitate (when viewed on white paper) indicated the presence of tannins (condensed and hydrolysable) [7].

### 2.3.3 Test for saponins

Five millimetres (5mls) of the extract was shaken with 15mls of distilled water in a test tube. Persistent frothing on warming confirmed the presence of saponins [7].

### 2.3.4 Test for glycosides

Exactly 2.5ml of 50% sulphuric acid was added to 5ml of the extract in a test tube. The mixture was heated in boiling water for 15 minutes, cooled and neutralized with 10% NaOH. Then 5ml of Fehling's solution was added and mixture was boiled. A brick-red precipitate indicated the presence of glycosides [8].

### 2.3.5 Test for anthraquinone

Five grams (5g) of sample extract was boiled with 10ml aqueous H<sub>2</sub>SO<sub>4</sub> and filtered while hot. The filtrate was shaken with 5ml of benzene. The benzene layer separated and half of its own volume of 10% ammonia solution was added. A pink, red or violet colour indicated the presence of anthraquinone glycosides [9].

### 2.3.6 Test for flavonoids

Two millimetres (2mls) of the extract was treated with 1mls of 5% lead acetate solution in a test tube. A yellow colour indicates the presence of flavonoids [10].

### 2.3.7 Test for steroids

Two millimetres (2mls) of acetic anhydride was added to 2mls of the extract followed by the addition of 2mls of dilute H<sub>2</sub>SO<sub>4</sub>. Violet colour which changes to blue indicates the presence of steroids [9].

### 2.3.8 Test for phenols

The 2 mL of the extract was mixed with 2 mL of 1% ferric chloride. The formation of deep blue or blue-black coloration is an indication of a positive result [9].

### 2.3.9 Test for terpenoids

An amount of 0.8 g of selected plant sample was taken in a test tube, then poured 10 ml of

methanol in it, shaken well and filtered to take 5 ml extract of plant sample. Then 2 ml of chloroform were mixed in extract of selected plant sample and 3 ml of sulphuric acid were added in selected sample extract. Formation of reddish brown colour indicates the presence of terpenoids in the selected plants (Harborne, 1973).

## 2.4 Anti-inflammatory Activity

### 2.4.1 Egg albumin-induced

Wistar rats of both sexes (100 - 150 g) were used for this assay. The rats were divided into five groups of four (4) animals each. Rats were orally pre-treated with the vehicle (distilled water, 5 ml/kg), indomethacin (10 mg/kg) and the extract (100, 200 and 400 mg/kg) 1 h before inducing paw oedema. Acute inflammation was induced by injecting 0.1 ml of 0.1% freshly prepared solution of egg albumin into the subplantar region of the right hind paw of rats (Ojewole, 2006). The linear paw circumference was measured using the cotton thread method [11] for 4 hours at 1 hour intervals after the administration of the phlogistic agent.

### 2.4.2 Formalin induced inflammation

The formalin (2.5%) induced inflammation was used in this study, as described by Winter *et al.* [12]. The increase in paw diameter (edema) was measured using vernier calliper. The difference in weight of the right hind paw and the left hind paw indicates inflammation. The hind paw volume (edema) was measured immediately before 0 h and after 1, 2, 3, and 4 hours following formalin injection using vernier caliper. The percentage inhibition was calculated from the expression:

$$\text{Inhibition (\%)} = \frac{\text{Mean oedema vol. (control)} - \text{Mean oedema vol. (treated)}}{\text{Mean oedema volume (control)}}$$

## 2.5 Analgesic Activity

### 2.5.1 Hot plate methods

Albino rats of either sex weighing 150–200g were allowed to acclimatize to laboratory conditions one hour before the start of experiment with food and water available [13]. 25 rats were divided into five groups each of five rats. Group I which is the negative control group was treated with distilled water (2ml/kg), group II the positive

control group was treated with ibuprofen (50mg/kg i.p). Group III, IV and V were given 100, 200 and 400mg/kg body weight of crude extract of *P. curatellifolia* respectively. After 30min of administration, the animals were placed on hot plate and the latency time (time for which rat remains on the hot plate ( $55 \pm 0.5^\circ\text{C}$ ) without licking or flicking of hind limb or jumping) was measured in seconds. In order to prevent the tissue damage a cut-off time of 50 s were imposed for all animals. The latency time for all groups was recorded at 60mins. Percentage analgesia was calculated using the following formula:

$$\text{Analgesia (\%)} = \frac{\text{Test latency} - \text{Control latency}}{\text{Cut off time} - \text{Control latency}} \times 100$$

### 2.5.2 Acetic acid induced writhing test

The acetic acid induced writhing test in rats was used to test for analgesic effect [14]. The rats were divided into five groups of four (4) rats each. The groups were treated thus: Group 1 received 10 mL/kg b/wt. distilled water (control) intra-peritoneal, Group 2 received 10 mg/kg b/wt. of Piroxicam intra-peritoneal, Groups 3, 4 and 5 received 100, 200 and 400 mg/kg b/wt. of crude methanolic extract of *P. curatellifolia*, respectively. About 30 minutes later, the rats in the five groups were treated with 1% acetic acid intra-peritoneal. About 5 minutes later, the rats were placed in individual cages and the number of abdominal contractions counted for each rat in a 10 minutes period. Inhibition of writhing (%) was calculated using the expression:

$$\text{Inhibition (\%)} = \frac{\text{Mean no. of writhing (control)} - \text{Mean writhing (treated)}}{\text{Mean number of writhing (control)}}$$

## 3. RESULTS AND DISCUSSION

### 3.1 Percentage Yield

Methanol extraction of 500g of *Parinari curatellifolia* leaf yielded 9.44% and the extract was soluble in water, dark in colour, with a bitter taste, slimy texture with a characteristic pleasant smell.

### 3.2 Qualitative Phytochemical Screening

The presence of some phytochemicals were detected in the methanol leaf extract of *Parinari*

*curatellifolia* which revealed the presence of tannins, alkaloids, flavonoids, and phenols while steroids, anthraquinones, saponins, glycoside and terpenoids were not detected (Table 1).

**Table 1. Qualitative Phytochemical Constituents of *Parinari curatellifolia* leaves extract**

Phytochemicals	Results
Tannins	+
Steroids	-
Anthraquinones	-
Alkaloids	+
Flavonoids	+
Saponins	-
Glycosides	-
Terpenoids	-
Phenols	+
Tannins	+

KEY: + = Present, - = Not detected

### 3.3 Anti-inflammatory Activity of *P. curatellifolia* Methanol Leaves Extract

#### 3.3.1 Egg Albumin-induced anti-inflammatory activity of PCMLE

Before induction, that is at 0 hour, there was no significant difference ( $P > 0.05$ ) in the paw size of the treated groups when compared with the control group. This trend was observed at 1 hour, and 2 hour. By 3 hour and 4 hour, the group treated with the standard drug (indomethacin) and the group that received the highest dose of the extract were significantly lower ( $P < 0.05$ ) than all the other groups with percentage inhibitions of 25.56% and 24.44% respectively (Table 2; Table 3).

#### 3.3.2 Formalin induced anti-inflammatory activity of PCMLE

Before induction, that is at 0 hour, there was no significant difference ( $P > 0.05$ ) in the paw volume of the treated groups when compared with the control group. At the 1<sup>st</sup> hour, the normal control group had its paw volume significantly different ( $P < 0.05$ ) from the treated groups. This trend was observed from at the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> hour (Table 4; Table 5).

**Table 2. Egg Albumin-induced anti-inflammatory activity of PCMLE**

Treatments (mg/kg)	Paw Size (oedema)				
	0	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>
	Time (Hours)				
D. water (10ml/kg)	2.50±0.04 <sup>a</sup>	2.78±0.09 <sup>a</sup>	2.80±0.09 <sup>b</sup>	2.77±0.06 <sup>c</sup>	2.70±0.02 <sup>c</sup>
Indomethacin (40mg/kg)	2.41±0.04 <sup>a</sup>	2.38±0.09 <sup>a</sup>	2.30±0.02 <sup>a</sup>	2.21±0.04 <sup>a</sup>	2.01±0.08 <sup>a</sup>
PCMLE (100mg/kg)	2.46±0.04 <sup>a</sup>	2.40±0.02 <sup>a</sup>	2.39±0.04 <sup>a</sup>	2.33±0.01 <sup>ab</sup>	2.26±0.07 <sup>ab</sup>
PCMLE (200mg/kg)	2.43±0.13 <sup>a</sup>	2.39±0.04 <sup>a</sup>	2.35±0.04 <sup>a</sup>	2.30±0.06 <sup>ab</sup>	2.22±0.06 <sup>ab</sup>
PCMLE (400mg/kg)	2.49±0.22 <sup>a</sup>	2.37±0.15 <sup>a</sup>	2.32±0.08 <sup>a</sup>	2.24±0.08 <sup>a</sup>	2.04±0.06 <sup>a</sup>

Values are expressed as mean ± standard error of mean, n = 4. Values with the same superscript along the column are not significantly different (P<.05)

**Table 3. Egg Albumin-induced anti-inflammatory (inhibition %) activity of PCMLE**

Treatments (mg/kg)	Paw Size (oedema)				
	0	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>
	Time (Hours)				
D. water (10ml/kg)	0.00	0.00	0.00	0.00	0.00
Indomethacin (40mg/kg)	3.60	14.38	17.86	20.22	25.56
PCMLE (100mg/kg)	1.60	13.67	14.64	15.88	16.30
PCMLE (200mg/kg)	2.80	14.03	16.07	16.97	17.78
PCMLE (400mg/kg)	0.40	14.75	17.14	19.13	24.44

**Table 4. Formalin induced anti-inflammatory activity of PCMLE**

Treatments (mg/kg)	Volume (oedema)				
	0	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>
	Time (Hours)				
D. water (10ml/kg)	1.98±0.13 <sup>a</sup>	2.78±0.05 <sup>b</sup>	2.80±0.11 <sup>b</sup>	2.83±0.11 <sup>b</sup>	2.73±0.14 <sup>b</sup>
Indomethacin (40mg/kg)	2.15±0.16 <sup>a</sup>	2.05±0.06 <sup>a</sup>	1.70±0.16 <sup>a</sup>	1.53±0.09 <sup>a</sup>	1.35±0.09 <sup>a</sup>
PCMLE (100mg/kg)	2.00±0.11 <sup>a</sup>	2.20±0.11 <sup>a</sup>	1.98±0.05 <sup>a</sup>	1.73±0.09 <sup>a</sup>	1.45±0.06 <sup>a</sup>
PCMLE (200mg/kg)	2.08±0.08 <sup>a</sup>	2.15±0.12 <sup>a</sup>	1.95±0.20 <sup>a</sup>	1.65±0.06 <sup>a</sup>	1.43±0.10 <sup>a</sup>
PCMLE (400mg/kg)	1.98±0.09 <sup>a</sup>	1.98±0.11 <sup>a</sup>	1.73±0.10 <sup>a</sup>	1.60±0.11 <sup>a</sup>	1.40±0.13 <sup>a</sup>

Values are expressed as mean ± standard error of mean, n = 4. Values with the same superscript along the column are not significantly different (P<.05)

**Table 5. Formalin induced anti-inflammatory (inhibition %) activity of PCMLE**

Treatments (mg/kg)	Volume (oedema)				
	0	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>
	Time (Hours)				
D. water (10ml/kg)	0.00	0.00	0.00	0.00	0.00
Indomethacin (40mg/kg)	8.58	26.25	39.29	45.94	50.55
PCMLE (100mg/kg)	1.01	20.86	29.29	38.87	46.89
PCMLE (200mg/kg)	5.05	22.66	30.36	41.70	47.62
PCMLE (400mg/kg)	0.00	28.78	38.21	43.46	48.72

### 3.4 Analgesic Activity

#### 3.4.1 Analgesic activity of *Parinari curatellifolia* methanol leaves extract using hot plate model

The results revealed significant increased ( $P < 0.05$ ) in the analgesic activity of PCMLE at 400mg/kg body weight and the drug treated group when compared with the control group with percentage inhibition of 34.32% and 52.94% respectively. Similarly, the results revealed significant increased ( $P < 0.05$ ) analgesic activity when compared between group treated with 400mg/kg body weight of the extract with both 100mg/kg and 200mg/kg body weight treated groups. In contrast, there was significant decreased ( $P < 0.05$ ) in analgesic activity in both 100mg/kg and 200mg/kg body weight treated groups when compared with the positive control group. However, there was no significant difference ( $P > 0.05$ ) between the negative control group when compared with both 100mg/kg and 200mg/kg body weight treated groups (Table 6).

#### 3.4.2 Analgesic activity of *Parinari curatellifolia* methanol leaves extract using acetic acid induced model

The results showed a dose-dependent analgesic activity across the groups. There was significant difference ( $P < 0.05$ ) between the normal control group and the treated groups. The percentage inhibitions for 100mg/kg, 200 mg/kg and 400 mg/kg were 32.31%, 36.92% and 47.69% respectively. The percentage inhibition for the group treated with the standard drug was 56.92% (Table 7).

## 4. DISCUSSION

The medicinal properties of several medicinal plants has been attributed to the presence of chemical constituents like flavonoids, alkaloids,

phenols, tannins, carotenoids and glycosides [15]. Flavonoids are polyphenolic compounds that are ubiquitous in nature (Balasundram, 2006). Studies have shown that flavonoids and other polyphenolic compounds play a role in scavenging free radicals and in the inhibition of lipid peroxidation [16]. Plant phenolics are one of the major groups of compounds acting as primary antioxidant free radical terminators (Miliauskaa *et al.*, 2004). Alkaloids consist of most abundant naturally occurring group of secondary metabolite and have a wide range of pharmacological activities. Lin *et al.* [17] reported that alkaloids from *Rubus alceifolius* Poir may act to protect the liver through decreasing CYP2E1 enzymatic activity via decreasing its mRNA. Saponins are glucosides that consist of polycyclic aglycones attached to one or more sugar side chains. The non-sugar part of saponins have a direct antioxidant activity which may results in benefits such as reduced risk of cancer and liver damage [18]. Tannins are water-soluble phenolic compounds that have the ability to chelate metal ions such as Fe(II) and interfere with one of the reaction steps in Fenton reaction and thereby retard oxidation [19]. The inhibition of lipid peroxidation by tannin constituent can act via the inhibition of cyclooxygenase [20].

Alkaloids have been shown to possess significant anti-inflammatory potential. In fact, isolated alkaloids are known to possess anti-inflammatory activity with some of the alkaloids having greater anti-inflammatory potency than aspirin. Alkaloids have also been shown to inhibit writhing response in model animals and increase tail flick latency in the radiant heat tail-flick method; an indication that this class of compound have significant analgesic potential [21]. Tannins are bitter-tasting and polyphenolic biomolecules and represents one of the bioactive secondary metabolites contained in *Parinari curatellifolia* with strong anti-inflammatory activity [22]. Its anti-inflammatory activity has been

**Table 6. Analgesic activity of *Parinari curatellifolia* methanol leaves extract on hot plat induced heat response**

Treatments	Latency Time (s)	Inhibition (%)
Distilled water (10ml/kg)	18.04±0.73 <sup>a</sup>	0.00
Indomethacin	34.96±1.41 <sup>d</sup>	52.94
PCMLE (100mg/kg)	23.84±0.95 <sup>b</sup>	18.02
PCMLE (200mg/kg)	22.87±0.99 <sup>b</sup>	15.11
PCMLE (400mg/kg)	29.01±1.20 <sup>c</sup>	34.32

Values are expressed as mean ± standard error of mean, n = 4. Values with the same superscript along the column are not significantly different ( $P < .05$ )

**Table 7. Analgesic activity of *Parinari curatellifolia* methanol leaves extract on acetic acid induced writhing response**

Treatments	No. of Abdominal Writhing	Inhibition (%)
Distilled water (10ml/kg)	16.50±0.65 <sup>d</sup>	0.00
Indomethacin	7.25±0.63 <sup>a</sup>	56.92
PCMLE (100mg/kg)	11.25±0.85 <sup>c</sup>	32.31
PCMLE (200mg/kg)	10.50±0.85 <sup>bc</sup>	36.92
PCMLE (400mg/kg)	8.75±0.85 <sup>ab</sup>	47.69

Values are expressed as mean ± standard error of mean, n = 4. Values with the same superscript along the column are not significantly different (P<.05)

shown by its ability to prevented rat paw edema induced by both carrageenan and dextran [23]. Phenolic compounds are potent anti-inflammatory agents due to its ability to inhibit either the production or action of pro-inflammatory mediators and inhibit the leukocyte chemotaxis. The anti-inflammatory and analgesic activities of a number of plants have been attributed to the presence of saponins [24]. It exhibits the anti-inflammatory activity through suppression of NF- $\kappa$ B, phosphoinositide 3-kinase, and mitogen-activated protein kinase signaling pathways [23]. Furthermore, an in vivo study showed that saponins can significantly inhibit paw edema, and nitrite production without affecting cell viability; an indication of its potent analgesic activity. Therefore, the presence of all these bioactive compounds in *Parinari curatellifolia* somewhat justifies the use of this plant in traditional medicine for the treatment and management of inflammation and related disease conditions including body pain

The oedema developed by carrageenan and egg albumin may be classified into three phases: with the release of histamine and serotonin, the early phase (the first 90 min) is initiated; bradykinin release marks up the second phase (90–150 min); and the third phase (after 180 min) is mediated by prostaglandin [25]. The extract could possibly act by inhibiting the action of kinin or prostaglandins more significantly in the late stage rather in the early stages of inflammation, whereas the extract responded moderately in egg albumin induced model with less differences in the paw. The leaf extract of *Parinari curatellifolia* effectively suppressed the oedema produced egg albumin in a dose-dependent manner. The egg albumin induced rat paw oedema test has significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation. In the time course of oedematous inflammation induced by egg albumin, it has been shown that three main mediators are responsible for acute and chronic

inflammatory reactions (Chiurchiù *et al.*, 2018). The first phase of oedema is attributed to the release of histamine, serotonin or bradykinin by local cells. After a couple of hours, there is liberation of prostaglandins [26-27]. This indicates that the *Parinari curatellifolia* extract possibly exhibits its anti-inflammatory action by inhibiting the synthesis, release or action of inflammatory mediators including histamine, serotonin and prostaglandin known to mediate acute inflammation.

## 5. CONCLUSION

The presence of some bioactive compounds and the observed anti-inflammatory and analgesic activity in *Parinari curatellifolia* leaves extract justifies the use of this plant in traditional medicine for the treatment and management of inflammation and related disease conditions including body pain.

## NOTE

The study highlights the efficacy of "traditional medicine" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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