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Evaluation of Analgesic and Anti-Inflammatory Properties of *Combretum racemosum* and *Combretum platypterum* Leaf (Combretaceae) on Rodents

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ABSTRACT

Members of the Combretaceae family are Combretum racemosum and Combretum platypterum. Traditional uses of Combretum racemosum include the treatment of roundworm in children, hemorrhoids, haematuria, convulsive coughing, tuberculosis, and circumcision wounds, as well as genito-urinary and gastrointestinal infections that are accompanied by bleeding. In contrast, Combretum platypterum is used to treat fever, malaria, backache, eye issues, swellings, mumps, conjunctivitis, diarrhea, sexually transmitted diseases, and cough. Aqueous leaf extracts from Combretum racemosum and Combretum platypterum were tested in this investigation to see if they had analgesic and anti-inflammatory properties. A hot plate and an acetic acid-induced writhing animal model were used to assess the analgesic property. To test the anti-inflammatory property, carrageenan paw edema in rats was utilized. At doses of 100, 200, and 400 mg/kg, the administration of Combretum racemosum aqueous leaf extract significantly ($p<0.01$) inhibits pain brought on by acetic acid, as well as significantly ($p<0.001$) inhibits inflammation brought on by carrageenan. When compared to controls, Combretum platypterum (100, 200, and 400 mg/kg) and aspirin (100 mg/kg) significantly decreased pain in mice induced by acetic acid ($P<0.05$). It is also noteworthy ($p<0.0001$, $p<0.001$, $p<0.05$) that carrageenan-induced inflammation was inhibited by Combretum platypterum and indomethacin. At any dose level ($P > 0.05$), neither plant has any influence on the hot plate model when compared to the control. In conclusion, the aqueous leaf extract of Combretum racemosum and Combretum platypterum exhibits analgesic and anti-inflammatory properties.

1. INTRODUCTION

Diseases have long been treated with medicinal herbs. There are over 40,000 tropical flowering plant species with alleged therapeutic uses for a range of illnesses (Idu et al., 2008). Most traditional therapies rely heavily on medicinal plants, and 80–85% of the world's population uses them for their basic healthcare requirements (Prakash et al., 2013).

Compared to conventional drugs, medicinal plants have one distinctive quality: they are appropriate for chronic therapy and have a greater range of therapeutic uses. They are accessible and more affordable than synthetic medications (Calixto, 2000; Idu, 2010). According to Mukeshwar et al. (2011), the world is moving toward herbal medications that strengthen, repair, and help to eradicate irritating pathogens with few or no hazardous side effects.

Combretaceae is the family name for *Combretum racemosum*. It is commonly referred to as the Christmas rose, and in Nigeria, it is also identified as 'okoso' in Edo, 'alagame' in Igbo (Umuahia), and 'ogan pupa' in Yoruba (Burkill, 1985). Historically, young leaves have served as a helminth repellent (Dalziel, 1973). It is also used to treat childhood roundworms (Oliver-Bever, 1986). The plant is used in Congo to treat genitourinary infections and gastrointestinal haemorrhage. The maceration or decoction of the root is ingested for dysentery. Bark pulp is used for bleeding during pregnancy, and leaf sap is used for haemorrhoids. Powered leaves or roots are used to treat haematuria, convulsive coughing, and tuberculosis. For the purpose of treating circumcision wounds, bark or leaves are used (Bouquet, 1969).

Okwuasa et al. (2006) conducted a study on the anti-ulcer and antibacterial properties of *Combretum racemosum* leaf extract, discovering its possession of these properties. The liver and bone marrow are protected by *Combretum racemosum* (Okwuasa *et al.,* 2012). Alkaloids, saponins, flavonoids, terpenoids, glycosides, resins, carbohydrates, and steroids are among the phytochemicals found (Okwuasa *et al*., 2012). Schepetkin et al. (2013) have demonstrated the immunomodulatory and hemagglutinating properties of an aqueous extract of *Combretum racemosum*. Antioxidant activity exists in the methanol extract (Francine *et al*., 2012). According to Okwuosa *et al*. (2012), the LD50 is greater than 5500 mg/kg.

The Combretaceae family includes *Combretum platypterum*, which is found from the east coast of Guinea to the Democratic Republic of Congo, extending through southern Sudan and as far south as northern Angola (Dalziel, 1973). Rainforests, secondary forests, scrub savannas, and occasionally marshy areas between sea level and 450 m are home to *C. platypetrum*. In Ghana, it is known as Akan Asante; in Sierra Leone, as Kissi Yekpomdeo-Chuaboa; in Liberia, as Mano Kpadah; and in Nigeria, as mmanya nza, achicha nza, Ogan dudu, and Ogan ibule (Bredenkamp, 2000). C. platypetrum is used to treat lower back pain, fever, ocular disorders, malaria, oedema, mumps, conjunctivitis, coughs, sexually transmitted infections, helminthiasis, and diarrhoea. Additionally, it is used as a febrifuge and tonic and to reduce postpartum bleeding (Bongers et al., 2005). Despite its historical applications, *Combretum platypterum* has not undergone pharmacological analysis. This investigation looked at the analgesic and anti-inflammatory properties of *Combretum racemosum* and *Combretum platypterum* aqueous leaf extracts.

2. MATERIALS AND METHODS

2.1. Collection of Plant Material

Fresh leaves of *Combretum racemosum* and *Combretum platypterum* were collected from Sakponba Forest, Edo State, Nigeria. The plant was identified and authenticated by Dr H. Akinnibosun of the Department of Plant and Biotechnology Faculty of Life Sciences, University of Benin City, Edo State, Nigeria.

2.2. Preparation of Plant Material

The leaves were washed and air-dried in the Department of Pharmacognosy, University of Benin, Benin City, for one week. Leaves of the two plants were further subjected to another 24 h of drying in an oven maintained at 40 ºC separately. Leaves were ground into powder using an impact mill. Pulverized material of each plant (200 g) was mixed with distilled water (5 L) and left for 72 h. At the end of the 72 h, the extracts were filtered separately and the filtrate was concentrated over a water bath. The

concentrated extracts of both plants were stored in Universal bottles labeled separately and refrigerated at 4 °C before use.

2.3. Experimental Animals

Albino rats weighing 100-220 g and mice weighing 20-35 g were purchased from a commercial farm in Benin City and housed in the animal facility of the Department of Biochemistry, University of Benin, Benin City. The animals were acclimatized for 2 weeks and kept under standard laboratory conditions with a 12-h light/dark cycle. They were fed with standard rodent pellets and water *ad libitum*. The litter in the cages was renewed three times a week to ensure hygiene and maximum comfort for animals. The animals were handed according to standard protocols for the use of Laboratory animals (National Institute of Health, USA: Public Health Service policy on humane care and use of Laboratory Animals, 2002).

2.4. Experimental Protocol

The analgesic effects of *Combretum racemosum and Combretum platypterum* leaf extracts were studied in mice using the hot plate method of Eddy *et al* (1953). Eight groups (n = 4) of Swiss albino mice (20-35g) were placed on a plate maintained at (55 ± 1^0C) before plant administration for 30 min. Groups 1 to 3 were administered aqueous extract of *Combretum racemosum* at doses of 100, 200, and 400 mg/kg while groups 4 to 6were administered aqueous extract of *Combretum platypterum* at doses of 100, 200, and 400 mg/kg, p. o. Group 7 received 10ml/kg of distilled water, p. o., and group 8 received morphine (5mg/kg), i.p. Each animal was placed on the hot plate maintained at 55±1 ºC 30 min after administration of plant extracts. The time the animals lick their paws is the cut-off time.

2.4.1. Writing test in mice

The analgesic effect of the extract against acetic acid-induced writhing in mice was studied using a method by Seigmund *et al*. (1957). This method was used to test possible peripheral effects of an analgesic substance. Mice were randomly allotted to an experimental group as follows:

Group 1: Received aspirin (100 mg/kg, p. o.)
Group 2: Received distilled water (10 ml/kg p. o.)
Group 3: Received 100 mg/kg of *Combretum racemosum leaf* extract
Group 4: Received 200 mg/kg of *Combretum racemosum leaf* extract
Group 5: Received 400 mg/kg of Combretum racemosum leaf extract,
Group 6: Received 100mg/kg of *Combretum platypterum leaf* extract
Group7: Received 200mg/kg of *Combretum platypterum leaf* extract
Group 8: Received 400mg/kg of *Combretum platypterum leaf* extract

One hour after treatment, each mouse was injected with 0.1ml of 1% acetic acid solution to induce the characteristic writhing. The numbers of writhing in a period of 30 minutes were counted for each mouse in each group.

2.4.2. Anti-inflammatory activity

Carrageenan-induced paw edema in rats by Winter *et al* (1962) was used. The rats were randomly allotted to groups as follows:

Group 1: Received distilled water (2ml/kg. p. o.).
Group 2: Received indomethacin (10mg/kg i. p.)
Group 3: Received 100 mg/kg of *Combretum racemosum leaf* extract
Group 4: Received 200 mg/kg of *Combretum racemosum leaf* extract
Group 5: Received 400 mg/kg of *Combretum racemosum* leaf extract,
Group 6: Received 100mg/kg of *Combretum platypterum leaf* extract
Group 7: Received 200mg/kg of *Combretum platypterum leaf* extract

Group 8: Received 400mg/kg of *Combretum platypterum leaf* extract

The diameter of right paw was taken for each group before carrageenan administration. A dose of 0.1ml of 1% carrageenan was administered to the right paw of each rat. The diameters of the paws were taken at time points 0, 15, 30, 60 and 120 min.

2.5. Statistical Analysis Data

Data are expressed as mean ± standard error of mean (SEM) and "n" represents the number of rats or mice per experimental group. One-way analysis of Variance (ANOVA) was performed with Newman Keuls' post hoc test. All data were analyzed using GraphPad Prism (UK) software version 6. $P<0.05$ shows a significant difference between compared data.

3. RESULTS AND DISCUSSION

The analgesic activity of aqueous leaf extracts of *Combretum racemosum* and *Combretum platypterum* shows no effect on the hot plate model when compared to the control (Tables 1 and 2). The morphine at 5 mg/kg shows a significant ($P < 0.05$) difference when compared to the control (Tables 1 and 2). In an acetic acid-induced writhing test in mice, the leaf extract of *Combretum racemosum* shows significant ($P < 0.01$) inhibition of pain induced by acetic acid at all dose levels (100, 200, and 400 mg/kg) and aspirin at 100 mg/kg when compared to the control (Table 3). The aqueous leaf extract of *Combretum platypterum* in acetic acid induces a writhing test in mice and shows a significant ($P < 0.05$) reduction of pain at all doses (100, 200, and 400 mg/kg) and aspirin at 100 mg/kg when compared to the control (Table 4). The acetic acid writhing-induced pain in mice was inhibited by aqueous leaf extract of *Combretum racemosum* and *Combretum platypterum*, but the pain induced by hot-plate thermal stimulation was unaffected. Animal models of pain are used to evaluate analgesic drugs, including hot-plate thermal stimulation and mice writhing in acetic acid. Testing for peripheral analgesics is done on mice using acetic acid writhing, which causes pain by increasing capillary permeability (Amico-Roxas *et al*., 1984; Sasikala *et al*., 2011). *C. platypterum's* ability to inhibit pain is dose-dependent. To assess narcotic involvement as a central-acting analgesic and to generate pain, hot-plate thermal stimulation animal models are used (Besra *et al*., 1996). The findings imply that both plants may have a nociceptive effect. The leaf extract of *Combretum racemosum* and indomethacin exhibit a significant inhibitory effect on inflammation induced by carrageenan in rat paw oedema ($P < 0.001$) (Table 5). Additionally, the leaf extract of *Combretum platypterum* significantly mitigates inflammation in carrageenan-induced paw oedema in rats compared to the control group (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$) (Table 6). Aqueous leaf extracts of *Combretum racemosum* and *Combretum platypterum* diminished carrageenan-induced paw oedema in rats.

Table 1: The analgesic effect of aqueous leaf extract of *Combretum racemosum* using a hot plate model

Dose (mg/kg)	Reacting time (s)
Control distilled H_2O (10 ml/kg)	11.03±1.49
100	8.35±0.85
200	11.33±3.30
400	12.20±193
Morphine (5 mg/kg)	22.05±4.63*

* $P < 0.05$ Compared to the value of control, Values are presented as mean ± S.E. M, n = 4

Table 2: The analgesic effect of aqueous leaf extract of *Combretum platypterum* using a hot plate

Dose (mg/kg)	Reacting time (s)
Control Distilled H2O (10ml/kg)	11.03 ± 1.49
100	15.38± 1.57
200	13.20± 1.78
400	18.57± 0.39
Morphine (5 mg/kg)	22.05± 4.63*

* $P < 0.05$ Compared to control; the values are presented as mean ± S.E.M. n = 4

Table 3: The analgesic effect of aqueous leaf extract of *Combretum racemosum* using acetic acid induces writhing in mice

Dose (mg/kg)	No writhing	% Pan inhibition
Distilled H_2O (10ml/kg)	65.00±1.47	-
100	29.75±3.88**	54.23
200	31.00±5.43**	52.31
400	25.75±3.47**	60.38
Aspirin (100 mg/kg)	37.00±12.65**	43.08

** P < 0.01 Compared to control; values are present as mean ± S.E. M, n= 4

Table 4: The analgesic effect of aqueous leaf extract of *Combretum platypterum* using acetic acid-induced writhing in mice

Dose (mg/kg)	No of writhing	% Pan inhibition
Control Distilled H2O (10ml/kg)	65.00±1.47	-
100	34.25±9.61*	47.31
200	33.75±8.25*	48.08
400	30.25±6.41*	53.46
Aspirin (100 mg/kg)	26.75±10.05*	58.85

* P <0.05 Compared to control; Values are present as mean ± S.E. M, n=4.

Paw oedema induced by carrageenan serves as a model for acute inflammation, involving various chemical mediators such as histamine, serotonin, bradykinin, and prostaglandins (Vinegar *et al*., 1987). At a dosage of 400 mg/kg, indomethacin exhibits an anti-inflammatory effect comparable to that of *Combretum racemosum* (84.21%). At a dosage of 400 mg/kg, the leaf extract of *Combretum platypterum* achieves a 90% inhibition, comparable to the 84.21% inhibition produced by the conventional medication indomethacin. Aspirin and indomethacin alleviate pain and inflammation by inhibiting cyclooxygenase activity. The analogous outcomes of *Combretum racemosum*, *Combretum platypterum*, indomethacin, and aspirin suggest a possible mechanism of action for both plants. Both *Combretum racemosum* and *Combretum platypterum* possess the capacity to inhibit cyclo-oxygenation, yet their specific mechanisms of action are not elucidated. The current investigation reveals that the leaves of *Combretum racemosum* and *Combretum platypterum* possess analgesic and anti-inflammatory properties.

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Table 5: Effect of the aqueous leaf extract of *Combretum racemosum* on Carrageenan- induced paw edema of rat

Treatment mg/kg	Mean paw diameter (mm)						
	0 min	15 min	30 min	60 min	120 min	180 min	Percent inhibition
Control	0.16 ± 0.04	0.19 ±0.04	0.22 ±0. 05	0.22 ±0.02	0.21 ±0.02	0.19 ±0.03	-
100	0.14 ±0.01	0.09 ±0.01	0.08 ±0.02***	0.09 ±0.02***	0.07 ±0.02**	0.05±0.02***	93.68
200	0.09±0.01	0.09±0.01	0.05±0.02***	0.07±0.03***	0.06±0.01***	0.05±0.01***	73.63
400	0.04±0.01	0.06±0.02***	0.07±0.02***	0.06±0.01***	0.04±0.00***	0.03±0.07***	84.21
Indomethacin	0.11±0.03	0.10±0.04	0.08±0.02***	0.04±0.01***	0.04±0.01***	0.03±0.01***	84.21

*** P < 0.001, **P<0.001, * P < 0.05 Compared to control; Values are presented as mean±SEM, n = 4.

Table 6: Effect of the aqueous leaf extract of Combretum *platypterum* on Carrageenan–induced paw edema of rat

Treatment mg/kg	Mean paw volume in ml						
	0 min	15 min	30 min	60 min	120 min	180 min	Percent inhibition
Control	0.158±0.36	0.188±0.039	0.223±0.023	0.220±0.025	0.213±0.024	0.190±0.031	-
100	0.190±0.044	0.178±0.037	0.160±0.049	0.143±0.054	0.105±0.941***	0.083±0.036***	56.32
200	0.178±0.031	0.198±0.041	0.173±0.039***	0.120±0.034	0.110±0.030***	0.095±0.030*	50.00
400	0.123±0.005	0.103±0.041*	0.050±0.008*	0.020±0.004**	0.018±0.003***	0.018±0.003***	90.00
Indomethacin	0.11±0.03	0.10±0.04	0.08±0.02***	0.04±0.01***	0.04±0.01***	0.03±0.01***	84.21

***P < 0.001, ** P < 0.01, *P < 0.05 Compared to control; Values are presented as mean±SEM n = 4.

4. CONCLUSION

This research has shown the analgesic and anti-inflammatory properties of the leaves of *Combretum racemosum* and *Combretum platypterum*. The study provides evidence that the leaves of both plants may be used to alleviate back pain, inflammation, haematuria, and haemorrhoids.

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6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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