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Preliminary Phytochemical, Analgesic and Anti-inflammatory Studies of Methanol Root Extract of *Combretum hypopilinum* (Diels) Okafor, *(Combretaceace)*

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ABSTRACT

Combretum hypopilinum is an important economic ethnomedicinal plants among the locals of Northern Nigeria. In this study, the preliminary phytochemical, acute toxicity, analgesic and anti-inflammatory activities of methanol root extract of *Combretum hypopilinum* were investigated in mice and rats. Phytochemical screening was conducted using standard methods and the acute toxicity was conducted according to Lorke's method of toxicity. Acetic acid-induced abdominal constrictions and hot plate modelswere used for the analgesic study while carrageenan-induced paw oedema model was used for the anti-inflammatory study. The results revealed that the extractwas non-toxic and it exhibited significant ($P< 0.05$) analgesic and anti-inflammatory activities in the models used for this study. The analgesic and anti-inflammatory activities of the extract could be attributed to the presence of phytochemicals such as flavonoids, steroids and triterpenoid which had been found to be analgesic and anti-inflammatory agents.

Keywords: *Combretum hypopillinum,* Inflammatory diseases, Pain, Phytochemicals.

INTRODUCTION

Pain is a major global health problem associated with disease conditions and injuries.Major pathological symptom of disease is pain which interferes with general functions of the immune system [1,2]. Pain which can be severe, moderate or mild is commonly the first complaints for patients seeking medical consultation in various health care deliveries worldwide.

Inflammatory diseases are major causes of mortality and morbidity [3]. In spite of the introduction of analgesics and anti-inflammatory, it is still difficult to manage, causing constrains in the efficiency and quality of life [4, 5]. Pains and inflammatory diseases remain among the world's major health concerns and the use of commonly available analgesic and anti-

inflammatory drugs are associated with serious limitation due to side effects such as dependency, tolerance, gastrointestinal irritation and liver disorder [6]. In addition, most of these drugs are relatively expensive and not readily available. Therefore, natural occurring substances which can be used as a replacement therapy especially among the local populace should be studied [7]. Earlier studies have reported the analgesics and anti-inflammatory properties of phytochemicals such as flavonoids and steroids [8,9]

Combretum hypopilinum, also known as bush willows, belongs to the family *Combretaceae*. It is mostly harvested from the wild for local use because of its timber, gum and medical uses. It is used in ethnomedicine for the treatment of gastrointestinal disorders, including diarrhea, dysentery, and stomach aches. A twig bark and root decoction are given to relief headache, fresh roots are also chewed to treat lung problems such as cough, bronchitis, and tuberculosis and jaundice [10-13].

This study aimed to assess the preliminary phytochemicals, analgesic and anti-inflammatory activities of methanol root extract of *Combretum hypopillinum* using acid-induced abdominal constrictions and hot plate models, and carrageenan-induced paw oedema in animal models.

MATERIALS AND METHODS

Sample collection and preparation

The plant sample, root of *Combretum hypopilinum* was collected from a local market (DajinBiye) in Sabon Gari Local Government Area, Kaduna State, Nigeria. It was identified and authenticated in the Herbarium unit, Department of Botany, Ahmadu Bello University, Zaria, with voucher specimen number 900743. The root was air dried under shade and pulverized manually to a coarse powder using mortar and pestle, labeled and stored at room temperature for use. The coarse powdered plant material (200 g) was extracted with 70% methanol using maceration method for three (3) days with intermittent shaking. The extract was concentrated using rotary vacuum evaporator to yield a brownish residue, weighed and used for this study.

Experimental animals

Twenty-five (25) Swiss albino mice (17-27g) were used for the analgesic study and 25 adult Wistar rats (110 -190g) used for the anti-inflammatory study were obtained from Animal House facility of the Department of Pharmacology and Therapeutic, Ahmadu Bello University, Zaria, Nigeria. The animals were kept in a well-ventilated room in propylene cages at room temperature and were fed with laboratory diet and water *ad libitum*. They were maintained under normal humidity, temperature and light for 7 days prior to the experiment.

Preliminary phytochemical screening

Test for carbohydrates, saponins, alkaloids, anthraquinones and cardiac glycosides were carried out according to the method of Trease and Evans [14]. Also test for flavonoids, steroids were carried out according to the methods of Silva *et al*. [15].

Experimental design

Acute toxicity study (LD_{50})

The method described by Lorke [16], was used for the study. This method was carried out in two phases. In the first phase, 3 groups of 3 animals each (mice/rats) were administered the methanol extract of varying doses (10, 100 and 1000 mg/kg body weight) intraperitoneally and observed for the first 4 hours and intermittently for 24 hours for any sign of toxicity and mortality. In the second phase three groups with one mouse/rat each were treated with doses of 1,600, 2,900 and 5000 mg/kg of the extract and observed for another 24 hours for signs of toxicity and death. The median lethal dose was calculated as a geometric mean of the highest non- lethal dose (with no death) and the lowest lethal dose (where death occurred).

$$LD_{50} = \sqrt{minimum\ lethal\ dose \times maximum\ tolerated\ dose}$$

Evaluation of analgesic activities in mice

(I) Acetic acid-induced abdominal constrictions in mice

The method described by Koster *et al*. [17], was used for this study. Twenty-five (25) albino mice were divided into 5 groups of 5 mice each. Groups 1, 2 and 3 were orally administered with 250, 500 and 1000 mg/kg of the methanol root extract respectively. While Group 4 was administered with piroxicam 10 mg/kg (positive control) and Group 5 was orally administered

with 10 ml/kg of normal saline (negative control). Sixty (60) minutes after oral administration each mouse was injected intraperitoneally with 10 ml/kg of aqueous solution of acetic acid (0.6%). The number of abdominal constrictions for each mouse was counted 5 minutes after injection of acetic acid for a period of 10 minutes. A reduction in the number of writhes as compared to the negative control was considered as evidence for the presence of analgesia and expressed as percent inhibition of writhes.

The percentage inhibition of abdominal constrictions was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{mean number of writhing (control)} - \text{mean number of writhing (test)}}{\text{mean number of writhing (control)}} X100$$

(II) Thermally-Induced pain test in mice (Hot plate method)

The method described by Turner [18], was employed for this study. Twenty-five (25) albino mice were divided into five groups of five mice each. The first group served as negative control and received distilled water 10 ml/kg, the second, third and fourth groups were pre-treated with 250, 500 and 1000 mg/kgof the methanol root extract respectively while the fifth group served as positive control and was treated with 5 mg/kg of pentazocine. Thirty (30) minutes after treatment, each mouse was gently placed on Eddy's hot plate maintained at 55 ± 1 ºC and a cut off period of 15 sec [19], was observed to avoid damage to the paw. Reaction time was recorded when animals licked their hind paw or jumped at 0, 30, 60, 90 and 120 minutes after i.p administration of the test drug [20], referred toas the latency to pain response. The prolongation of the latency times was taken as an analgesic response (percent maximum possible effect {%MPE}.

$$\%\text{MPE} = \frac{Test - Basaline}{Cutoff - Baseline} \text{ x100}$$

Where: Test = latency to respond after treatment; Baseline = latency to respond prior to treatment and Cut – off (15 sec) = preset time at which the test was ended in the absence of a response.

Evaluation of anti-inflammatory activities in rats

(I) Carrageenan-induced paw oedema test in rats

The acute anti–inflammatory study was carried out using the carrageenan induced paw oedema in rats as previously described by Winter *et al.* [21]. Twenty-five (25) rats weighing between 110 to 180 g were used for this experiment. The rats were divided into five groups each containing 5 rats (n =5). Acute inflammation was induced by injecting 0.1 ml of 1% carrageenan into sub plantar surface of rat hind paw. The methanol root extract (250, 500 and 1000 mg/kg), normal saline (10 ml/kg) and piroxicam (10 mg/kg) as positive control were administrated orally sixty minutes before carrageenan injection. The paw diameter was measured at 0, 1, 2, 3, 4 and 5 hours, using vennier caliper to determine the diameter of oedema. The increase in paw diameter (oedema index) for each rat was calculated as the difference in paw diameter before carrageenan injection and after carrageenan injection at each time interval, while the percent inhibition of oedema was calculated for each group with respect to negative control group using the following relationship:

$$\frac{mean\ increase\ in\ paw\ volume\ of control - mean\ increase\ in\ paw\ volume of treated}{mean\ increase\ in\ paw\ volume\ of control} \quad X\ 100$$

Statistical analysis

The results were expressed as Mean$\pm$SEM and the mean values of the control groups were compared with the mean values of the treated groups using one-way ANOVA and repeated measure ANOVA followed by Bonferroni test for multiple comparison. The results obtained were considered statistically significant at ($P < 0.05$). Data were analyzed using the statistical software, Statistical Package for Social Science (SPSS version 21.0).

RESULTS AND DISCUSSION

Table 1: Phytochemical constituents of methanol extract

Constituents	Test	Observation
Alkaloids	Meyer	+
	Wagner	-
	Dragendoff	+
Carbohydrates	Molisch	+
Flavonoids	Shinoda	+
	Sodium hydroxide	+
	ferric chloride	+
Saponins	Frothing	+
Triterpenes	Lieberman-Bucchard	+
Steroids	Salkowski	+
Tannins	Lead acetate	+
Cardiac glycosides	Keller-Kiliani	+

+ = presence, - = absence

Table 2: Effect of methanol root extract of *Combretum hypopilinum* on acetic acid induced writhing in mice.

Treatment	**Dose (mg/kg)**	**Mean number of writhing (± SEM)**	**Inhibition (%)**
Normal Saline	10 ml/kg	32.20 ± 2.22	-
MRE	250	24.20 ± 3.15	24.84
MRE	500	19.20 ± 2.08*	40.49
MRE	1000	10.80 ± 1.80*	66.58
Piroxicam	10	11.80 ± 2.89*	63.45

Values are expressed as Mean ± SEM, n=5, NS= Normal Saline, MRE = Methanol RootExtract of *Combretum hypopilinum*, SEM= Standard Error of Mean, * = $p<0.05$ statistically significant compared with Normal Saline.

Table 3: Effect of Methanol Root Extract of *Combretum hypopilimum* on Hot Plate Test in Mice

		Mean reaction time (sec) ± SEM				
Treatment	Dose (mg/kg)	0 min	30 min	60 min	90 min	120 min
NS	10 ml/kg	5.00 ± 0.44	3.40 ± 0.68	5.40 ± 1.03	4.00 ± 0.63	4.60 ± 0.24
MRE	1000	4.800 ± 0.37	4.00 ±0.55	4.40 ± 0.81	5.20 ± 0.58	4.80 ± 0.58
MRE	500	4.80 ± 0.37	4.80 ± 0.66	5.60 ± 0.75	8.00 ± 0.89a	5.20 ± 0.73
M	250	5.60 ± 0.93	2.20 ± 0.37	5.80 ± 0.97	5.20 ± 0.37	4.80 ± 0.58
Pentazocine	5.0	6.20 ± 1.07	6.20 ± 0.58a	8.80 ± 1.07	7.40 ± 0.81a	4.20 ± 0.37

Values are expressed as Mean ± SEM, n=5, NS= Normal Saline, MRE = Methanol Root Extract of *Combretum hypopilinum*, SEM= Standard Error of Mean, a = $p<0.05$ statistically significant compared with Normal Saline.

Table 4: Effect of methanol root extract of *Combretum hypopilinum* on carrageenan induced paw edema in rats**.**

		Mean paw diameter (cm) ± SEM					
Treatment	Dose (mg/kg)	0 hour	1 hour	2 hours	3 hours	4 hours	5 hours
NS	10 ml/kg	1.97±0.08	3.47±0.11	4.09±0.03	3.65±0.08	3.60±0.09	2.77±0.24
MRE	1000	2.04±0.09	3.20±0.15	3.79±0.06	3.41±0.08	3.21±0.01*	2.44±0.04*
MRE	500	1.99±0.05	3.60±0.14	3.45±0.18*	3.79±0.08	2.94±0.24*	2.41±0.04*
MRE	250	1.79±0.03	2.80±0.06*	3.57±0.03*	3.46±0.06	2.40±0.06*	2.38±0.06*
IBU	10	1.89±0.01	2.64±0.10*	3.56±0.02*	2.32±0.04*	2.17±0.04*	2.13±0.03*

Values are expressed as Mean ± SEM, n=5, NS= Normal Saline, MRE = Methanol Root Extract of *Combretum hypopilinum*, SEM= Standard Error of Mean, * = $p<0.05$ compared with Normal Saline.

Phytochemicals are naturally occurring biochemical compounds found in plants which protect the plants from diseases and contribute to the plants' colour and aroma, and also provide health benefits for humans [22]. Preliminary phytochemical screening of methanol extract of *Combretum hypopillinum* revealed the presence of carbohydrates, flavonoids, tannins, saponins, steroids/triterpenes, cardiac glycosides and alkaloids (Table 1). These metabolites have many biological and therapeutic properties [23]. Research has shown that flavonoids, phenolic acids, and triterpenoid possessed antinociceptive and anti-inflammatory effects. Flavonoids such as rutin, quercetin, luteolin produced significant analgesic and anti-inflammatory activities [24, 25].

In this study, the acute toxicity (LD_{50}), analgesic and anti-inflammatory activities of methanol root extract of *Combretum hypopillinum* in animal models were assessed using acetic acid induced writhing in mice, thermally-induced pain test and carrageenan induced paw edema method in rats. The oral route median lethal dose (LD_{50}) of the methanol extract was found to be above 5000 mg/kg in both the mice and rats suggesting the extract to be non-toxic. Acetic acid-induced writhing reflex is a model of visceral pain [26], injection of 0.7% glacial acetic acid intraperitoneal produced writhing in this experiment. Acetic acid produces writhing reflex in animals by activating the chemo sensitive nociceptors, so the indication for level of analgesia in acetic acid-induced writhing models is reduction in the number of abdominal constrictions [27].

The methanol root extract significantly at ($P<0.05$) decreased the number of writhing in the animals at the doses tested in dose dependent manner except at dose 250 mg/kg as shown in Table 2. Percentage inhibition of pain was calculated to be 24.84% at dose of 250 mg/kg, 40.49% at dose of 500 mg/kg and 66.58% at a dose of 1000 mg/kg. The percentage inhibition was found to be higher at dose of 1000 mg/kg than the standard drug used (piroxicam) which showed a percentage inhibition of 63.45%.

The mechanism of acetic-induced model is the production of pain sensation by triggering inflammatory response leading to release of arachidonic acid from the tissue [28]. The analgesic effect of this extract could be through suppression of prostaglandin pathway because it has been reported that any agent that decreases the number of writhing will demonstrate analgesia preferably by inhibition of prostaglandin synthesis, peripheral mechanisms [28].

The hot plate is commonly used test for evaluation of the centrally acting analgesic drugs. The result of the hot plate test experiment showed a statistically significant ($P<0.05$) increase in

mean reaction time by the animals at a dose of 500 mg/kg and pentazocine at 90 min and a significant effect with the standard drug at 30 min when compared to the normal saline as shown in Table 3. The result shows that the extract has a weak central analgesic activity. It could be deduced from this result that there is involvement of centrally mediated mechanism of analgesic action of the extract, as well as peripheral mechanism as collaborated by the results obtained from the acetic acid-induced pain model.

The carrageenan-induced paw edema is a well-defined model of acute inflammation that a variety of inflammatory mediators involves in its development and has widely been used to evaluate the anti-inflammatory effects of natural products. In the carrageenan induced paw edema test, the extract showed slight decrease in paw edema after 1 hour of carrageenan injection only at dose of 250 mg/kg and dose dependent decrease in paw edema after 2 hours of carrageenan injection when compared to the control. There was however a statistically significant ($P<0.05$) decrease in paw edema at 4 -5 h of carrageenan injection at all doses tested when compared to the control as shown in the Table 4. It has been reported that neutrophil infiltration plays major role in the inflammation induced by carrageenan in hind paw [29].

Therefore, it can be inferred that the inhibitory effect of the extract on carrageenan induced inflammation could be due to the inhibition of the enzyme cyclooxygenase leading to the inhibition of prostaglandin synthesis.

CONCLUSION

The methanol root extract of *Combretum hypopillinum* was non-toxic and, possessed potential analgesic and anti-inflammatory properties on animal models and this could be attributed to the presence of phytochemicals such as flavonoids, steroids and triterpenoid. Further studies are recommended to ascertain the efficacy and potentiality of *Combretum hypopillinum* and other solvent extracts as a treatment for ailments resulting from pains and inflammation.

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