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Analgesic and Anti-Inflammatory (Acute and Sub-Acute) Properties of the Methanol Extract of the Whole *Cassytha Foliformis* Plant

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ABSTRACT

***Background*:** *Cassytha filiformis* (family Lauraceae) is traditionally used to treat pain and inflammation, but scientific validation is limited. This study evaluated the analgesic and anti-inflammatory activities of its methanol whole plant extract.

Methods: The powdered plant was extracted by cold maceration in methanol. Acute oral toxicity was assessed using Lorke's method. The extract was tested at oral doses of 100, 200, and 400 mg/kg. Analgesic activity was evaluated using acetic acid-induced writhing, hot plate, and formalin-induced pain tests in mice. Aspirin (100 mg/kg, oral) and pentazocine (10 mg/kg, i.p.) served as positive controls; distilled water (2 ml/kg, oral) was the negative control. Anti-inflammatory activity was assessed using carrageenan-, dextran-, and formalin-induced paw edema in rats, with indomethacin (10 mg/kg, oral) as the reference drug.

Results: The extract showed no signs of toxicity. It significantly ($p<0.05$) reduced writhing in the acetic acid model and increased pain threshold in the hot plate and formalin models compared to the control. It also significantly ($p<0.05$) inhibited paw edema in all inflammatory models, indicating both acute and sub-acute anti-inflammatory effects.

Conclusion: The methanol extract of *Cassytha filiformis* whole plant possesses significant analgesic and anti-inflammatory activities, supporting its traditional use in pain and inflammation management.

Keywords: Cassytha foliformis, analgesia, anti-inflammatory, ethnomedicine.

1. INTRODUCTION

It has been suggested that in the last three decades not less than 80% of people globally rely on herbal for some part of primary care [1]. More so, the users of herbal remedies believe they are cheaper and freer from adverse effects if compared with orthodox medicines [2, 3]. Cassytha foliformis L (CF), a member of the Lauraceae family, frequently called "love-vine," is one plant of interest [4, 5]. It is a climbing parasitic vine that is found in South-East Asia, northern Australia, central and southern China, and Japan. It is propagated from Africa to Asia [5]. The plant (especially the stem) contains a series of alkaloids belonging to the aporphine type, biosynthetically derived from the amino acid phenylalanine [4]. In India, Vietnam, China and also in Central America, an infusion of the stems is taken

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as a tonic, for biliousness, piles, diarrhea and spermatorrhoea [5]. Externally, the stems are used for cleaning ulcers and an infusion as eyewash [5]. In Indonesia, the pounded stems are given as a vermifuge and for other intestinal troubles. In Brunei, a decoction of the stems is drunk or applied to the skin to relieve itch and eczema. In the Philippines, a decoction of the plant is taken to hasten parturition, reduce labour pains and to prevent haemoptysis [4]. In Fiji, a drink made with crushed stems in water is drunk to treat indigestion, difficult parturition and to reduce fever. It is also taken for hemorrhoids, to treat sinusitis and to promote menstruation. In Peninsular Malaysia and India, the stems are dried, powdered and mixed with sesame oil to make a mucilaginous hair tonic [5], but this use may be attributed to the luxuriant hair-like appearance of the stems. In several African countries including Nigeria, the whole plant is used for treating venereal discharges, urethritis, diarrhea, gonorrhea and syphilis. It also has a widespread reputation for its use against parasitic conditions of the skin and scalp. In southern Nigeria, a decoction is taken by women to suppress lactation after a stillbirth and used as an analgesic and anti-inflammatory [4, 6]. CF is reported to be a useful medicine in the treatment of gonorrhea, kidney ailments and as a diuretic. It is also used as a vasorelaxant, an adrenoreceptor antagonist, and as an antitrypanosomal agent [7]. Despite all these ethnomedicinal claims, scientific data to corroborate these claims are thin, hence this study focused on evaluating the plant's analgesic and anti-inflammatory activities using various animal models.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Drugs and Chemicals

Indomethacin (Yangzhou Norier Pharmaceutical Co. Ltd, China); Dextran (Sigma Adrich, 3050 Spruce Street, St Louis, USA); Carrageenan (Sigma Adrich, St Louis); Formaldehyde (Pharmacology Laboratory, University of Benin); Acetic acid (BDH Chemicals), Pentazocine (Elysium pharmaceuticals limited, India) Aspirin powder (Department of Pharmaceutics and Pharmaceutical Technology, University of Benin, Benin City), Distilled water, normal saline, 3% Tween 80.

2.1.2 Experimental animals

Male and female albino rats weighing 140-290 g were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Edo State, Nigeria. The animals were housed within the animal house in plastic and wire gauze cages and maintained on standard pellets (Chikun feeds) and water ad libitum. All animal experiments were in accordance with the National Institution of Health guide for care and use of laboratory animals (Pub No.85-23 revised 2011) [8]. Ethical approval for the use of animal for this study was obtained from the Animal Ethics Committee of the Faculty of Pharmacy University of Benin, Nigeria.

2.2 Methods

2.2.1 Plant collection, Identification, and extraction

Whole plant was collected in the northern part of Nigeria in 2017 and it was identified and authenticated by a botanist at the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. The voucher specimens were deposited in the herbarium for future reference. Soil particles were rinsed off the whole plant of *Cassytha foliformis*, dried and pulverized into a powdered sample. 500 g of the powdered plant material was soaked in 2.5 L methanol in a maceration jar and stirred over 72 hours. The extract was then filtered using a filter clothe and cotton wool. The filtrate was allowed to dry in an oven at a temperature of 40° C. The dried methanol extract of *Cassytha foliformis* (MECF) was stored in the refrigerator prior to use and reconstituted with distilled water prior to administration.

2.2.2 Acute toxicity study

Oral acute toxicological evaluation was determined using Lorke's method [9]. This involved the use of fifteen animals, divided into two phases, Phase I and Phase II. In the first phase, twelve animals were divided into four groups of three animals each and distilled water (0.2 ml) was administered to group 1. Oral doses of MECF, 10, 100, and 1000 mg/kg body weight were administered to groups 2, 3, and 4 respectively and they were observed for signs of toxicity and death for 24 hours. The second phase was carried out using three rats. The three animals were administered MECF at doses of 1600, 2900 and 5000 mg/kg body weight and observed for signs of toxicity and death for 24 hours.

2.2.3 Anti-inflammatory screening

2.2.3.1 Dextran-induced hind paw edema

The effect of the MECF on acute inflammation was evaluated in rats using dextran-induced paw edema. The rats were divided into five groups of four animals in each group. Group 1 served as negative control and received distilled water

(2 ml/kg) orally. Animals in group 2 served as positive control and received indomethacin (10 mg/kg) orally while groups 3, 4, and 5 received orally 100, 200, and 400 mg/kg MECF respectively. One hour after oral administration of drug/extracts, inflammation was induced by injecting 0.1 ml of 1.5% w/v dextran in normal saline into the subplantar tissue of the right hind paw of each rat [10]. Before dextran administration, the paw sizes were first measured (0 hour) and thereafter paw thickness was measured using the vernier caliper at 1, 2, 3, 4 and 5 hours after dextran administration [11]. The mean inflammation seen in the extract treated groups was compared with that of the negative and positive controls. The mean value obtained for the negative control group was considered 100%.

2.2.3.2 Carrageenan-induced hind paw edema

The effect of MECF on acute inflammation was also evaluated in rats using the carrageenan-induced paw edema model by Winter et al [12]. Rats were divided into five groups of four animals each. Group1 which served as a negative control group received distilled water (2 ml/kg) orally. Group 2 served as positive control and received indomethacin (10 mg/kg) orally while group 3, 4 and 5 received oral doses of 100, 200 and 400 mg/kg doses of MECF respectively. Paw sizes were measured before induction of carrageenan and thereafter one hour after oral administration of drug/extracts, inflammation was induced by injecting 0.1 ml of 1% w/v of carrageenan suspension in normal saline into the right hind paw of each rat. Paw volumes were measured hourly for 5hours following carrageenan administration [13]. The mean inflammation observed in the extract group was compared with the negative and positive control groups. The mean value obtained for the negative control group was considered as 100%.

2.2.3.3 Formaldehyde induced hind paw edema

The effect of the MECF on sub-acute inflammation was evaluated in rats using formaldehyde-induced hind paw edema. Rats were divided into five groups of four animals each. Group 1 received distilled water (2 ml/kg) orally and served as the negative control group. Group 2 served as the positive control group and received indomethacin (10 mg/kg) orally while group 3, 4 and 5 received 100, 200 and 400 mg/kg of MECF respectively orally. Doses of MECF, indomethacin and distilled water were administered once daily for a period of 2 days. An hour after the last dose was administered; 0.2 ml of 1% w/v formaldehyde was injected into the right hind paw of each rat. Prior to formaldehyde administration, paw volumes were measured. Then, paw volumes after formaldehyde administration were measured at 1, 3, 6, 24 hours, day 4, day 7, day 10 and day 14. The anti-inflammatory activity of MECF was assessed by comparing with means of both the positive and negative controls. The mean value obtained by the negative control was considered as 100%.

2.2.3 Analgesic screening

2.2.3.1 Acetic Acid Induced Mouse Writhing Test

This study was carried out using the method of Koster et al [14]. The mice were divided into five groups of animals. Distilled water (0.2ml) was used as control and administered to group one orally with an orogastric tube. 100, 200 and 400 mg/kg of MECF were administered to groups two, three and four respectively. 100 mg/kg of Aspirin was given to the last group orally. One hour after pretreating the animals, 0.6% of Acetic Acid was administered intraperitoneally at a dose of 10 ml/kg body weight of the animals. Immediately after the injection of acetic acid, the animals were isolated in individual cages and observed for a period of 30 minutes and the numbers of writhes were counted.

2.2.3.2 Hot Plate Test

The method used was as described by Shethy and Anika [15] and modified by Franzotti et al [16]. Following the initial screening, the mice were divided into five groups of four animals and before treatment, the animals were gently dropped on the hot plate (Ugo Basile 35100) maintained at 54.8 ± 0.5degree Celsius to obtain their reaction time (time in seconds for the mice to either try to jump or lick its hind paw). The animals were then orally administered 0.2ml of distilled water to group one, 100, 200 and 400 mg/kg of the extract to group two, three and four respectively. The reference drug Pentazocin (10 mg/kg) was administered to the fifth group intraperitoneally. Each animal was then dropped at 30, 60, 90, 120, 150 and 180 minutes respectively on the hot plate after treatment. The mean values were recorded.

2.2.3.3 Formalin-Induced pain test

This method used was as described by Shibata et al [17]. The mice were divided into five groups of four animals each. The first group was pretreated with 0.2 ml of distilled water orally, extract at doses of 100, 200 and 400 mg/kg were administered orally to the second, third, and fourth group respectively. The fifth group was given the reference drug, pentazocin, intraperitoneally at a dose of 10 mg/kg. After one hour, 0.02 ml of 1% formalin was injected subcutaneously into the right hind paw of the mice in all groups. Thereafter, the time in seconds spent in licking the injected paw was taken as an indicator of pain response. These responses were measured first for 5 minutes after

formalin was injected indicative of the first phase (neurogenic pain), and then for another 15-30 minutes as second phase (inflammatory pain)

2.3 Statistical analysis

The results obtained were expressed as mean ± standard error of mean (SEM). The statistical significance of the difference between the groups was determined by a two-way mixed and one-way analysis of variance (ANOVA) with Tukey post-hoc test conducted on GraphPad Prism® (version 9.5.1). Level of significance was set at $p< 0.05$.

3. RESULTS

3.1 Oral acute toxicity of MECF

Oral acute toxicological evaluation of MECF did not result in any sign of toxicity or death in both phases (table 1).

Table 1: Acute toxicity study of both phase I and II

Groups/Phase	Doses (mg/kg)	Signs of toxicity	Mortality (%)
Control	0.2 ml	Nil	0
1/I	10	Nil	0
2/I	100	Nil	0
3/I	1000	Nil	0
1/II	1600	Nil	0
2/II	2900	Nil	0
3/II	5000	Nil	0

Signs of toxicity includes alertness, tremor, writhing reflex, touch response, and pinna reflex.
Control animals received distilled water.

3.2 Anti-inflammatory effects of MECF

3.2.1 *Anti-inflammatory effects of MECF on Dextran-induced paw edema*

Treatment with MECF significantly ($p<0.05$) reduced the paw sizes from the 1st to the 4th hour by the 400 mg/kg dose, 1st, 3rd and 4th hour by the 200 mg/kg dose and the 1st and 4th hour by the 100 mg/kg dose in comparison with the negative control. However, at the 5th hour, it does appear to be some form of resolution of oedema in all groups including the negative control. However, the anti-inflammatory effect of MECF at 400 mg/kg was observed to produce the most significant reduction in the paw sizes even better than that of indomethacin (table 2).

Table 2: Anti-inflammatory effects of MECF on dextran-induced paw edema

Time (hrs)	Mean paw size (mm)				
	Control	MECF 100	MECF 200	MECF 400	Indo 10
0	0.52 ± 0.05	0.51 ± 0.005	0.54 ± 0.03	0.50 ± 0.04	0.46 ± 0.03
1	0.62 ± 0.08	0.48 ± 0.07a	0.55 ± 0.04a	0.52 ± 0.05a	0.64 ± 0.03
2	0.62 ± 0.04	0.62 ± 0.05	0.58 ± 0.04	0.57 ± 0.03a	0.62 ± 0.05
3	0.67 ± 0.05	0.65 ± 0.06	0.52 ± 0.07a	0.42 ± 0.03a	0.57 ± 0.03a
4	0.65 ± 0.07	0.57 ± 0.03a	0.55 ± 0.04a	0.52 ± 0.04a	0.57 ± 0.03a
5	0.42 ± 0.07	0.41 ± 0.04	0.38 ± 0.08	0.35 ± 0.04	0.34 ± 0.05

Values are mean ± SEM, n=4 per group, [a]P<0.05 significantly different from the control.
Indo: Indomethacin, MECF: Methanol extract of *Cassytha filiformis*. Control animals received distilled water and doses are in mg/kg.

3.2.2 *Anti-inflammatory effect of MECF on carrageenan-induced paw edema*

MECF appears to have a dose dependent effect on inflammation as treatment with MECF at 400 mg/kg significantly reduced paw sizes from the 2nd, 3rd and 5th hour in comparison with the negative control. The effect of the extract at the highest dose is comparable to that of indomethacin. The effect of the 200 mg/kg dose was only significant at the 3rd and 5th in comparison to the negative control. This can be seen in table 3.

Table 3: Anti-inflammatory effect of MECF on carrageenan-induced paw edema.

Time (hrs)	Mean paw size (mm)				
	Control	MECF 100	MECF 200	MECF 400	Indo 10
0	0.42 ± 0.01	0.39 ± 0.03	0.42 ± 0.02	0.39 ± 0.03	0.46 ± 0.11
1	0.59 ± 0.07	0.59 ± 0.06	0.59 ± 0.03	0.57 ± 0.03	0.55 ± 0.03
2	0.65 ± 0.09	0.69 ± 0.08	0.66 ± 0.04	0.57± 0.03[a]	0.52± 0.05[a]
3	0.67 ± 0.03	0.72 ± 0.05	0.62 ± 0.02[a]	0.54± 0.06[a]	0.55± 0.03[a]
4	0.69 ± 0.03	0.75 ± 0.06	0.76 ± 0.04	0.67 ± 0.03	0.62± 0.05[a]
5	0.57 ± 0.03	0.54 ± 0.08	0.45 ± 0.04[a]	0.44± 0.07[a]	0.39± 0.03[a]

Values are mean ± SEM, n=4 per group, [a]P<0.05 significantly different from the control.
Indo: Indomethacin, MECF: Methanol extract of *Cassytha filiformis.* Control animals received 0.2 ml distilled water and doses are in mg/kg.

3.2.3 *Anti-inflammatory effect of MECF on formaldehyde-induced paw edema*

The effect of MECF on formaldehyde induced inflammation is presented in table 4. Here, the effect of the extract at the 100 mg/kg dose is significant, however at the 200 and 400 mg/kg doses of the extract, a dose dependent on significant reduction in the mean paw size was observed from the 2nd hour up to the 4th day in comparison to the negative control ($p<0.05$). A significant reduction was also observed at the first hour for just the 200 mg/kg dose and indomethacin in comparison with the control ($p<0.05$). From the 7th to the 14th day all mean paw sizes appeared similar. The effect of the extract at the 200 and 400 mg/kg dose compares well with the standard. This is seen in table 4.

Table 4: Anti-inflammatory effects of MECF on formaldehyde-induced paw edema

Time	Mean paw size (mm)				
	Negative Control	MECF 100	MECF 200	MECF 400	Positive Control
0 hr	0.39 ±0.03	0.42 ± 0.12	0.32 ± 0.03	0.37 ± 0.03	0.29 ± 0.03
1 hr	0.57 ± 0.06	0.55 ± 0.07	0.49 ± 0.03[a]	0.57 ± 0.03	0.47± 0.06[a]
2 hr	0.62 ± 0.05	0.55 ± 0.11	0.47 ± 0.05[a]	0.51± 0.06[a]	0.49± 0.06[a]
3 hr	0.62 ± 0.05	0.62 ± 0.08	0.49 ± 0.03[a]	0.57± 0.03[a]	0.54± 0.08[a]
4 hr	0.59 ± 0.06	0.65 ± 0.05	0.49 ± 0.05[a]	0.59± 0.07	0.52± 0.05[a]
5 hr	0.42 ± 0.02	0.37 ± 0.05[a]	0.34 ± 0.03[a]	0.32± 0.05[a]	0.38± 0.04[a]
Day 4	0.35 ± 0.04	0.39 ± 0.08	0.34 ± 0.03	0.34 ± 0.03	0.32±0.004
Day 7	0.28 ± 0.04	0.39 ± 0.03	0.34 ± 0.06	0.32 ± 0.05	0.35 ± 0.04
Day 14	0.28 ± 0.08	0.34 ± 0.03	0.32 ± 0.04	0.27 ± 0.03	0.28 ± 0.05

Values are mean ± SEM, n=4 per group, [a]P<0.05 significantly different from the control.
Indo: Indomethacin, MECF: Methanol extract of *Cassytha filiformis.* Control animals received distilled water and doses are in mg/kg.

3.3 Analgesic effects of MECF

3.3.1 *Acetic acid induced writhing*

As shown in table 5, 200 and 400 mg/kg doses of the extract significantly inhibited acetic acid induced writhing in mice ($P < 0.05$) as there was a significant reduction in the number of writhes counted when compared to the control group. When compared to the reference drug, aspirin, the number of writhes counted for the extract was far less with the 400 mg/kg dose and this effect was most significant ($P < 0.05$). The 400 mg/kg dose produced the highest percentage inhibition of 84.48% far better than aspirin with a percentage inhibition of 56.62%.

Table 5: The effect of MECF on acetic acid induced writhing test in mice.

Groups mg/kg	Number of writhes	Percentage inhibition (%)
Control (0.2 ml)	58.0 ± 14.0	-
MECF 100	24.0 ± 7.3[a]	56.62
MECF 200	62.0 ± 7.1	-6.9
MECF 400	51.0 ± 1.89[a]	12.06
Aspirin 100	9.0 ± 2.2[a]	84.48

Values are mean number of writhes ± standard deviation (SD), (n=4, per group) [a]p< 0.05 significantly different from the control group. MECF: Methanol extract of *Cassytha filiformis*

3.3.2 *Hot plate test*

The results as shown in table 6 shows that the different doses of the extract caused significant ($p< 0.05$) increase in the mean reaction time of the mice on the hot plate from the 30th minute to 180th minute. This effect was most significant with the 100 mg/kg of the extract at the 120th minute where the mean reaction time was 11.0 ± 0.5 secs compared to the control which was 7.10 ± 0.6 secs and the reference drug which was 8.30 ± 0.8 secs. At the 180th minute, the 100 mg/kg dose had the most significant result ($p< 0.05$) followed by the 400 mg/kg dose.

Table 6: The effect of MECF on hot plate model in mice.

Treatment	Mean Reaction time in sec(s)						
(mg/kg)	0min	30mins	60mins	90mins	120mins	150mins	180mins
Control	5.05 ± 0.2	5.25 ± 1.3	6.9 ± 1.6	7.8 ± 2.0	7.1 ± 2.6	6.3 ± 0.5	7.5 ± 0.5
Pen 10	5.05 ± 0.9	7.6 ± 2.1[a]	6.3 ± 0.9	7.6 ± 0.3	8.3 ± 0.8[a]	7.8 ± 0.3[a]	11.0 ± 0.4[a]
MECF 100	5.9 ± 1.6	8.2 ± 0.3[a, b]	9.4 ± 0.3[a, b]	10 ± 0.6[a, b]	11.0 ± 0.5[a, b]	10.2 ± 0.4[a, b]	8.7 ± 1.4[a]
MECF 200	5.6 ± 0.6	8.1 ± 2.2[a, b]	8.6 ± 0.3[a, b]	8.9 ± 1.8a[, b]	7.8 ± 0.3[a]	9.5 ± 0.3[a, b]	7.9 ± 1.5[a]
MECF 400	5.7 ± 1.1	10.2 ± 0.8[a, b]	8.8 ± 0.2[a, b]	7.5 ± 1.5	10.8 ± 1.3[a, b]	10.0 ± 0.3[a, b]	9.9 ± 1.7[a]

Values are mean reaction time in secs ± standard deviation (SD), (n=4, per group) [a]$p< 0.05$ significantly different from control, [b]$p< 0.05$, significantly different from the pentazocin group. MECF: Methanol extract of *Cassytha filiformis*

3.3.3 *Formalin induced pain*

The results from the formalin induced pain test is presented in table 7. The result shows significant ($p<0.05$) inhibitory effect by all doses of the extract in both phases (neurogenic and inflammatory), when compared to the control. When compared with pentazocin, the reference drug, the inhibitory effect of the extract seemed significantly better ($p< 0.05$) at all doses tested in the second phase with the 400 mg/kg dose of the extract producing the most significant effect for both phases in comparison with the control group. There was prolonged mean reaction time at all doses when compared with the control ($p< 0.05$).

Table 7: The effect of MECF on Formalin Induced pain test in mice.

Treatment/mg/kg	1st Phase 0-5 minutes (secs)	2nd phase 15-30 minutes(secs)
Control (0.2 ml)	57.8 ± 1.4	107.5 ± 2.6
Pentazocin10	6.0 ± 0.7[a]	29.0 ± 3.9[a]
MECF 100	13.8 ± 0.5[a]	20.2 ± 1.9[a, b]
MECF 200	13.0 ± 3.6[a]	20.0 ± 1.9[a, b]
MECF 400	13.0 ± 4.0[a]	19.0 ± 0.5[a, b]

Values are mean reaction time in seconds ± standard deviation (SD), (n=4, per group), [a]$P<0.05$ and [b]$P<0.05$, significantly different from control and reference drug respectively. MECF: Methanol extract of *Cassytha filiformis.*

4. DISCUSSION

Assessment of the acute toxic potential of any substance is required to determine the adverse effects that might occur due to accidental or short-term exposure [18]. The oral acute toxicity test of the methanol extract of the whole plant of *CF* evaluated using the Lorke's method showed that the animals in both phases had no signs of toxicity nor was their death during the period of observation. Hence, the plant can be said to be relatively safe. From our findings, the methanol extract of *CF* inhibited paw edema induced by dextran at all doses, but the 400 mg/kg dose was observed to reduce the paw sizes better compared to the lower doses of the extract. Dextran induces anaphylactic reaction that is characterized by extravasation and edema formation, because of release of histamine and serotonin from mast cells [19]. This therefore suggests that the extract may likely have an inhibitory effect on the release of histamine and serotonin. It is possible that the extract also inhibits the cyclooxygenase enzyme thus functioning like indomethacin which inhibits the synthesis of prostaglandins produced primarily by the cyclooxygenase enzymes [20]. Furthermore, our results showed that the methanol extract of *CF* significantly inhibited carrageenan paw-induced edema at doses of 200 and 400 mg/kg when compared to the negative control group especially between the 3rd and the 5th hour. Carrageenan is a sulphated polysaccharide obtained from seaweed and is commonly used to induce acute inflammation [21]. Its mode of action is believed to be biphasic. The first phase is due to the release of histamine and serotonin

while the second phase is caused by the release of bradykinin, proteases, prostaglandins, and lysosomes [21]. Prostaglandins play a major role in the second phase of edema which sets in by the third hour of the test. The suppression of the first and second phases of carrageenan may be due to inhibition of the release of the early mediators such as histamine, serotonin, and kinins. Based on the results, it can be inferred that the inhibitory effect of the methanol extract of *CF* on carrageenan-induced inflammation in rats might be due to the inhibition of the release of the mediators responsible for the second phase of inflammation, thus the extract is most likely exerting its effect via the inhibition of the release of bradykinin, protease, prostaglandins and lysosomes, this is in addition to the extract's inhibitory effect on histamine and serotonin release as seen from its effect on dextran induced inflammation [13]. The effect of the extract was observed to be similar to the anti-inflammatory drug indomethacin whose mechanism of action is via the inhibition of cyclooxygenase enzyme, a prostaglandin derivative [7]. Additionally, 200 and 400 mg/kg doses of the methanol extract *of CF* caused significant reduction of formaldehyde-induced inflammation. The formaldehyde induced paw edema in rats is one of the most suitable test procedures to screen anti-arthritis and anti-inflammatory agents as it closes resembles human arthritis [22]. Formaldehyde induced paw edema is a sub-acute inflammation that results from cell damage, which promotes the production of endogenous mediators such as histamine, serotonin, prostaglandin and bradykinin [19]. Interestingly, the methanol extract of *CF* was observed to produce significant inhibitory effect at mainly the 200 and 400 mg/kg from the2nd hour to the 4thday. Therefore, the methanol extract of *CF* showed significant reduction in paw sizes as its effect extended to the 4th day. From the 7th to the 14th day the differences in paw size between the extract treated groups were no longer significant in comparison with the control. Thus, it can be said that the extract may have a role to play in some form of sub-acute inflammatory processes. Results from the analgesic screening show that the extract could inhibit pain. In the acetic acid induced mouse writhing model, doses (200 and 400 mg/kg) of the extract produced inhibitory effects that seemed higher than that of the positive control (aspirin). On the other hand, 100 mg/kg dose of the extract had a slightly increased number of writhes compared to the control suggesting less analgesic effect. Writhes are twisting of the body or a bodily part in response to pain. The abdominal constriction response induced by acetic acid is a sensitive procedure used to determine peripherally acting analgesics [23]. Hence the extract activity might be peripherally mediated as seen in the results presented where the inhibitory effects of the doses were as significant ($P<0.05$) as that of aspirin the positive control used. In the hot plate test, all the doses of the extract used significantly increased the mean reaction time of the mouse on the hot plate compared to the control animals ($P<0.05$). Centrally acting analgesic function can be evaluated via time spent on the hotplate and as indicated in our result, there was an increase in reaction time following administration of the extract, thus pointing to a central analgesic effect. The central analgesic effect was further confirmed from the result where the effect of the extract was more significant than the effect of pentazocine at 100 mg/kg dose. The increase in response time shows that the extract also has a central analgesic effect. The hot plate test involves higher brain function and is a supraspinally organized response [24]. From the formalin induced pain in mice, all doses of the extract significantly increased the reaction time of the mice in the formalin induced pain test for both phases (neurogenic and inflammatory phase). From the results obtained, it can be inferred that the extract possesses some centrally acting analgesic activity which was previously seen in the hot plate test as well as some peripherally mediated analgesic activity seen in the acetic acid induced writhing test. Formalin produces pain by two phases: neurogenic pain and inflammatory pain. The effect of the extract at all doses used was comparable to pentazocine in both phases of pain ($P<0.05$) which was the standard centrally acting analgesic used. It was also significantly different from the control. Neurogenic pain is caused by production of substance P, while inflammatory pain is caused by the release of histamine, serotonin as well as bradykinin [17]. Methanol extracts significantly reduced the mean reaction time in both phases of pain caused by formalin which indicates that it may function both as a narcotic analgesic as well as a non-steroidal anti-inflammatory drug (NSAID) acting via inhibitory effect on the release of substance P, histamine, serotonin, and bradykinin [17]. The initial acute phase (phase 1), which is recorded for 5mins just after formalin administration, reflects acute peripheral pain, due to direct activation of nociceptors through TRPA1 channels, while phase 2 recorded after the periods of 5-15mins commonly recorded for 15-30mins is due to inflammatory input and central nociceptive sensitization [25]. The effect of the extract was significant in both phases as seen in the result. This observation therefore suggests that the analgesic effect of the extract may be mediated through the inhibition of the release of substance P and the fact that the methanol extract of *CF* may also possess some anti-inflammatory effect which is also mediated by the inhibition of the release of prostaglandins, bradykinins, histamine, and other inflammatory mediators [15]. Hence from the results obtained from this study, methanol extract of *Cassytha filiformis* can be further investigated as a potential agent for the management of neurogenic pain as well as inflammatory pain such as rheumatoid arthritis.

5. CONCLUSION

Our study has demonstrated that methanol extract of *CF* possesses an acute and sub-acute anti-inflammatory activity. This anti-inflammatory effect appears to be mediated via inhibition of prostaglandins synthesis and blockade of the release of bradykinins, histamine, and serotonin. Also, our study suggests that the plant also possesses significant analgesic effect which may be mediated via both central and peripheral inhibitory mechanisms. Our findings have therefore provided scientific insights into the ethnomedicinal use of the plant in the treatment of inflammation and pain. Future work is recommended and could focus on isolation and characterization of the pharmacologically active constituents of the plant.

DECLARATIONS

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Authors Contribution

Conceptualization, O.O.J., A.A.M., and B.I.O.; Methodology, O.O.J., A.A.M., I.S.O., O.E.W., O.E.S., and B.I.O.; Formal Analysis, O.O.J., A.A.M., I.S.O., and B.I.O.; Investigation, I.S.O., O.E.W., and O.E.S.; Writing—Original Draft, O.O.J., A.A.M., and I.S.O..; Writing—Review and Editing, O.O.J., A.A.M., and B.I.O.; Supervision, O.O.J., A.A.M., and B.I.O.; Project Administration, O.O.J., A.A.M., and B.I.O. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Data Availability Statement

The data presented in this study are available on request form the corresponding author.

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