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Anti-inflammatory Activity of Leaf Extract and Fractions of *Tapinanthus bangwensis* (Engl. & K.Krause) Danser Parasitic on *Citrus angustifolia*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors COO and SKN designed the study, performed the statistical analysis and wrote the protocol. Author ICE wrote the first draft of the manuscript. Author TCA managed the analyses of the study. Authors SKN and ICE managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: This study was undertaken to evaluate the anti-inflammatory activity of extract of the leaves of the plant.
Study Design: The study adopted the experimental design.
Place and Duration of Study: Department of Pharmacology & Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria, between 2014-2016.
Methodology: The methanol extract (ME), obtained by cold maceration was fractionated in a silica gel column to afford n-hexane (HF), ethylacetate (EF) and methanol (MF) fractions. The extract and fractions were subjected to phytochemical analysis using standard methods. Acute toxicity (oral,

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intraperitoneal) and median lethal dose (LD_{50}) of the extract was determined in mice. Acute anti-inflammatory activity of the extract and fractions was evaluated using topical acute edema of mouse ear induced by xylene and systemic acute edema of rat paw induced by carrageenan. Chronic anti-inflammatory activity was evaluated using formaldehyde arthritis test in rats and cotton pellet granuloma test in rats.

Results: In topical acute inflammation, ME, EF and MF caused significant (P = .05, P < 0.01) inhibition of mouse ear edema and their effects were comparable to those of indomethacin. In systemic acute inflammation, ME, EF and MF produced significant (P = .05, P < .001) and sustained inhibition of the development of paw edema in rats. HF did not produce any significant edema inhibition in these models of inflammation. Studies in chronic inflammation showed that the extract and fractions caused significant (P = .05) inhibition of the global edematous response to formaldehyde arthritis in rats. They also significantly (P < .01) inhibited the formation of granuloma on implanted cotton pellets in rats.

Conclusion: These findings show that *T. bangwensis* parasitic on *C. angustifolia* leaf extracts and fractions of increasing polarity possess anti-inflammatory properties in acute and chronic inflammation.

Keywords: Anti-inflammatory agents; Loranthaceae; Tapinanthus bangwensis; Citrus angustifolia.

1. INTRODUCTION

The folkloric use of plants in inflammatory diseases is common in traditional medicine practices around the world. In conventional settings, on-steroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of mild to moderate inflammatory and painful conditions but use of this class of drugs is associated with unwanted, sometimes life-threatening side effects such as gastrointestinal bleeding and perforation [1]. Hence, the discovery of safer, effective alternatives is important for rational management of pain and inflammation. In the continuous search for new anti-inflammatory drugs, medicinal plants play an important role as staring points. One of such, *Tapinanthus bangwensis* (Loranthaceae), is a hemi parasitic evergreen woody shoot parasite found growing on a host of evergreen and decidous economically important fruit and medicinal trees like *Citrus* species, *Cola acuminata*, *Irvingia* spp, *Erythroxylum cocoa*, *Leiba pentandra*, *Newboldia leavis* and *Theobroma cacao*. It is found in the tropical forest region from Senegal to Cameroun and extends over the Congo basin to Zaire and Nigeria [2]. Its folkloric use in diseases like diabetes, hypertension, asthma, epilepsy and inflammatory disorders is well documented [3]. The leaves are used as a worm expeller in Ghana and as a hepato-protectant in Nigeria [2,4]. Although the economic impact of mistletoe infestation was found to be negligible, its unfettered growth could become a serious threat to the survival of host trees and can lead to their death [5]. Thus, its potential application as an alternative source of new medicines would favor natural conservation of its native hosts.

The medicinal effects of *Tapinanthus bangwensis* has been evaluated and reported by different investigators. An extract of the plant exhibited hepato-protective properties against tetracloride induced hepato-toxicity in rats [6]. The aqueous and ethanol leaf extracts of *T. bangwensis* was shown to possess antioxidant and inhibitory effect on Fe^{2+}-induced lipid peroxidation in the pancreas *in vitro* [7]. Its butanol fraction was found to possess significant anti-inflammatory activity against systemic acute inflammation [8]. The antibacterial property as well as the isolation of three gallic acid derivatives; methyl syringate, 3,4,5- triomethyl gallic acid and 3, 4- dimethoxy-5- hydroxyl benzoic acid from the leaves have been reported [3,9].

In the present study, we report the evaluation of anti-inflammatory activity of extract and solvent fractions of leaves of *T. bangwensis* parasitic on *Citrus angustifolia* in topical and systemic acute and proliferative and granulomatous forms of chronic inflammation in murine models. This was to elucidate the anti-inflammatory activity profile of extract of this plant and provide justification for its use in traditional medicine practice.

2. MATERIALS AND METHODS

2.1 Animals

Adult albino rats (80 – 230 g) and mice (15– 23 g) of either sex were used. The animals were

obtained from the laboratory animal facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka and housed in steel cages under standard conditions with natural lighting within the facility. They were allowed free access to clean drinking water and standard rodent feed. Prior to commencement of experiments, the animals were acclimatized for 2 weeks to laboratory conditions. Ethical approval was obtained from the ethics committee of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. Animal experiments and handling were conducted in accordance with the National Institute of Health Guide for care and use of Laboratory Animal [10].

2.2 Plant Material

Fresh leaves of *T. bangwensis* growing on *Citrus angustifolia* were collected in Umuabo Eha-Alumona, Enugu State. The plant material was authenticated at the International Centre for Ethnomedicine and Drug Development (Inter CEDD) Nsukka, where a voucher specimen is maintained. The leaves were cleaned and dried away from direct sunlight for 5 days.

2.3 Extraction of Plant Material

The dried plant material was pulverized to coarse powder using an electrical blender. About 3 kg of the powdered leaf was extracted with absolute methanol by cold maceration for 48 h. The mixture was filtered and the marc repeatedly washed with fresh solvent and filtered. The filtrates were pooled and concentrated in a rotary evaporator under reduced pressure to obtain 320 g of the methanol extract (ME; 10.67% w/w).

2.4 Fractionation of Extract

A 200 g quantity of ME was subjected to solvent-guided fractionation over silica gel (200 mesh size) in a glass column successively eluted with n-hexane, ethyl acetate and methanol. The solvent fractions were concentrated in a rotary evaporator under reduced pressure to obtain 5 g (2.5% w/w), 85 g (42.5% w/w) and 80.5 g (40.5% w/w) of n-hexane (HF), methanol (MF) and ethyl acetate (EF) fractions respectively.

2.5 Phytochemical Analysis of Extract and Fractions

Phytochemical screening of ME for tentative identification of the presence of carbohydrates, free reducing sugar, combined reducing sugars, free anthraquinone glycosides, combined anthraquinone glycosides, saponins, terpenes, steroids, flavonoids, oleo-resins and alkaloids was carried out in accordance with standard test procedures [11].

2.6 Acute Toxicity (LD_{50}) Test

The acute toxicity and median lethal dose (LD_{50}) of ME in mice was estimated using the method described by Lorke in two phases [12]. In phase one, three groups of mice (n=3) received orally; 10, 100 or 1000 mg/kg respectively of ME. They were closely monitored for signs of intoxication or mortality within 4 h. Thereafter, they were monitored daily for 14 days for signs of delayed toxicity. In the second phase of the test, 1600, 2900 and 5000 mg/kg doses of ME were administered to three mice respectively (n = 1). The mice were monitored as described in phase one. The test was also repeated in a separate group of mice by administering the same doses of ME intraperitoneally.

2.7 Pharmacological Studies

2.7.1 Topical acute anti-inflammatory activity test

The effect of the extract and fractions on acute topical inflammation was evaluated by the method Atta and Alkohafi, [13] as modified by Okoli et al. [14]. For each experiment, mice were divided into ten groups of five mice each. The treatment groups received ME or fractions (5 mg or 10 mg dissolved in 6%v/v tween 80) applied on the anterior surface of the right ear. Control groups received topically, either vehicle or indomethacin (0.25 mg dissolved in the vehicle). Topical inflammation was immediately induced on the posterior surface of the same ear by application of xylene (0.05 ml). Two hours after induction of inflammation, mice were euthanized by chloroform inhalation and both ears removed. Circular sections (7 mm diameter) of both the right (treated) and left (untreated) ears were removed using a cork borer, and weighed. Edema was quantified as the weight difference between the two ear plugs. The anti-inflammatory activity was evaluated as percentage reduction of edema in the treated animals relative to control animals, using the relation:

$$\text{Edema inhibition (\%)} = 1 - \{(Wt/Wc) \times 100\}$$

Where:

Wt= difference between mean weight of right ear plug of treated mice and mean weight of left ear plug of treated mice.
Wc= difference between mean weight of right ear plug of vehicle-treated control mice and mean weight of left ear plug of vehicle-treated control mice.

2.7.2 Systemic acute anti-inflammatory test

The method of Winter et al. was used with slight modification [15]. Acute inflammation was measured as increase of the rat hind paw volume induced by sub-plantar injection of carrageenan. The initial volume of distilled water displaced by the right hind paw was measured. Four groups of five animals each received 50, 100, 200 or 400 mg/kg of extract or fractions administered orally. Control groups received either equivalent volume of the vehicle (distilled water in 6% Tween 80) or indomethacin (10 mg/kg). One hour after treatment, carrageenan (0.1 ml) was injected into the sub-plantar region of the right hind paw. Volume of distilled water displaced by the inflamed paw was recorded after 30 min and thereafter, at 1 h intervals up to 6 h. Inflammation was assessed as the difference between the initial volume of the treated paw and the volume at the various times after the administration of ph logistic agent.

Percentage inhibition of edema was calculated using the relation:

Inhibition of edema (%) = 1- {(Vt/Vc) × 100}

Where;

Vt= difference between mean paw volume of treated groups at different times after carrageenan injection and mean paw volume of treatment groups before carrageenan injection
Vc= mean paw volume of untreated control at different times after carrageenan injection and mean paw volume of untreated control before carrageenan injection.

2.7.3 Formaldehyde-induced arthritis test

The effect of the extract and fractions on chronic inflammation was evaluated in establishing arthritis induced by formaldehyde in rats using the method of Brownlee [16], as modified by Raval et al. [17]. On day 0, rats were divided into ten groups of five each. The initial volume of distilled water displaced by the right hind paw was measured. Treatment groups received the extract or fractions (200 - 400 mg/kg) administered orally, while control groups received either indomethacin (10 mg/kg) or equivalent volume of vehicle (6% Tween 80). After 1 h, arthritis was induced by injecting 0.1 ml of 2% formaldehyde solution into the sub-plantar region. This was repeated on day 3 and volume of distilled water displaced by the paw was measured. Treatment was continued once daily for ten days. For each treatment, a plot of the difference in paw volume against time (days) was prepared and the areas under the curve (AUC) were calculated. The level of inhibition of arthritis was calculated as:

Inhibition of arthritis = $[1 - (AUC_t)/(AUC_c)] \times 100$

Where;

AUC_c= mean AUC of untreated control group.
AUC_t= mean AUC of treatment groups.

2.7.4 Cotton pellet-induced granuloma test

This test was performed according to the method described by Winter and Porter [18]. Fifty rats were divided into ten groups of five rats each. Four sterile pre-weighed cotton pellets (20 ± 1 mg) were implanted subcutaneously in each axilla of the rats under ketamine anesthesia. Treatment was carried out orally for seven consecutive days, the first dose being given immediately after the implantation of pellets. Groups I and II served as the control groups and received Tween 80 (5 ml/kg) and indomethacin (10 mg/kg) respectively. Groups III-X were the test groups and received 200 - 400 mg/kg of ME or fractions (HF, EF and MF) respectively. On day 8, the animals were euthanized and the pellets dissected out, freed from extraneous tissues and dried overnight in a hot air oven at 60ºC. Each pellet was weighed and the difference between the initial and the final weights of the cotton pellets recorded. The percentage inhibition of granuloma tissue formation was calculated using the following:

% inhibition= (x-y/x) × 100

Where;

x= mean increase in pellet weight in untreated control group
y= mean increase in pellet weight in treatment groups

2.8 Statistical Methods

Data was analyzed using One Way ANOVA (SPSS version 16). Differences between means were accepted significant at P = .05 when compared with the control. The results are presented as Mean ± SEM after least significant difference was deduced from *post hoc* analysis.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Composition

The methanol extract (ME) and methanol fraction (MF) tested positive to alkaloids, glycoside, saponins, tannins, flavonoids terpenoids (Table 1). Tannins were undetected in the hexane (HF) and ethylacetate (EF) fractions.

Table 1. Phytochemical constituents of extract and fractions

Phyto-constituents	Relative presence			
	HF	EF	MF	ME
Alkaloids	+	++	++	+++
Carbohydrate	-	+	+	++
Flavonoids	-	+++	+	++
Glycosides	-	+	+	++
Reducing sugars	+	-	+	++
Resins	-	+	+	++
Saponins	++	-	++	++
Steroids	-	++	++	++
Tannins	-	-	+	++
Terpenoids	-	++	++	+++

MF= methanol extract; HF: hexane fraction; EF= ethyl acetate fraction; MF= methanol fraction
+= Slightly present; ++= moderately present; +++= abundantly present; - = absent

Mistletoes such as *T. bangwensis* have many diverse ethnomedicinal uses associated with them in traditional medicine practice. These multiple ethnomedicinal applications may be due to interactions of their phytoconstituents with aspects of the inflammatory response pathway usually accompanying pathological processes associated with disease states. Hemi-parasites including *T. bangwensis* synthesize carbohydrates by photosynthesis and obtain minerals and water from its host. Thus, the parasitic host (for example, *Citrus* spp.) mineral and water content may indirectly influence phytochemical composition of the hemi-parasite and therefore, its ethnomedicinal application [19].

3.2 Acute Toxicity

Acutely, oral or intraperitoneal administration of ME at doses up to 5000 mg/kg caused no death or observable signs of intoxication. Therefore, the oral and intraperitoneal LD_{50} of ME in mice was estimated to be greater than 5 g/kg. The high LD_{50} values suggest a remote risk of acute intoxication following oral or intraperitoneal administration.

3.3 Anti-inflammatory Activity

The leaf extract of this plant used in this study and its solvent fractions exhibited potent anti-inflammatory action by inhibiting topical and systemic acute inflammation as well as chronic inflammation of exudative and proliferative natures.

3.3.1 Acute anti-inflammatory effect

3.3.1.1 Effect on topical edema

The extract and fractions significantly (P = .05, P < .01) inhibited acute topical edema induced by xylene in the mouse ear, as shown in Table 2. The ethylacetate fraction (EF) caused the highest inhibition (52.75%) followed by ME (52.7%). The inhibition produced by EF was comparable to that of indomethacin (53%). Topical application of the extract and fractions is consistent with the traditional use in the treatment of acute topical inflammation.

Table 2. Effect of extract and fractions on topical acute edema

Treatment	Dose (mg/ear)	Edema (mg)	Inhibition (%)
ME	5	2.88 ± 0.87[a]	52.00
	10	2.84 ± 1.04[b]	52.70
HF	5	5.49 ± 1.04[b]	8.57
	10	5.13 ± 1.02[b]	14.45
EF	5	2.87 ± 0.67[b]	52.17
	10	2.83 ± 0.66[b]	52.75
MF	5	2.86 ± 0.87[b]	52.33
	10	2.83 ± 0.82[b]	52.28
Indomethacin	0.25	2.82 ± 0.23[a]	53.00
Control	-	6.00 ± 1.07	-

[a]P < .001, [b]P = .05 compared to control (One Way ANOVA; LSD post hoc)
ME = methanol extract; HF = hexane fraction; EF = ethylacetate fraction; MF = methanol fraction

Table 3. Effect of methanol extract on acute systemic inflammation of the rat paw

Treatment	Dose	Edema (ml)						
	mg/kg	0.5 h	1 h	2 h	3 h	4 h	5 h	6 h
ME	50	0.72±0.09	0.66±0.07	0.70±0.06	0.97±0.07	1.18± 0.05	0.82±0.02[a]	0.70±0.16[a]
	100	0.53±0.12	0.67±0.09	0.82±0.16	0.70±0.19[a]	1.00±0.16	0.90±0.10[b]	0.76±0.14[b]
	200	0.36±1.29	0.73±0.12	0.77±0.11	0.83±0.77	0.94±0 .05	0.83±0.02[b]	0.68±0.09[a]
	400	0.32±0.06	0.56±0.67	0.79±0.10	0.94±0.09	0.80±0 .11[c]	0.42±0.09[a]	0.60±0.09[a]
Indometh.	10	0.49±0.08	0.65±0.08	0.48±0.10[b]	0.75±0.15	0.62±0.06[a]	0.42±0.09[a]	0.30±0.08[a]
Control	5	0.59±0.09	0.58±0.12	0.98±0.13	1.04±0.07	1 .20±0.14	1.42±0.18	1.38±0.20

[a]P < .001, [b]P < .005, [c]P = .05 compared to control (One Way ANOVA; LSD post hoc)

ME = Methanol extract; Mean ± S.E.M = Mean values ± Standard error of means of five experiments

Table 4. Effect of ethylacetate fraction on systemic acute edema of the rat paw

Treatment	Dose	Edema (ml)						
	mg/kg	0.5 h	1 h	2 h	3 h	4 h	5 h	6 h
EF	100	0.84±0.08	1.02±0.09	0.82±0.04	0.54±0.02	0.58 ±0.04	0.54±0.0[a]	0.58±0.08
	200	0.82±0.05	1.04±0.08[c]	0.86±0.07	0.52±0.09	0.50±0.05[c]	0.62±0.07[c]	0.50±0.06
	400	0.70±0.08	0.86±0.07	0.64±0.10	0.54±0.05	0.52±0 .07	0.44±0.06[a]	0.48±0.07
Indometh.	10	0.67±0.07	0.70±0.06	0.68±0.15	0.56±0.0 7	0.50±0.08[c]	0.26±0.08[a]	0.14±0.06[a]
Control	5	0.66±0.07	0.80±0.11	0.74±0.06	0.70±0.11	0 .68±0.04	0.80±0.03	0.66±0.08

[a]P < .001, [b]P < .005, [c]P = .05 compared to control (One Way ANOVA; LSD post hoc)

EF = Ethylacetate fraction; Mean ± S.E.M = Mean values ± Standard error of means of five experiments

Table 5. Effect of methanol fraction on systemic acute edema of the rat paw

Treatment	Dose	Paw volume difference (ml)						
	mg/kg	0.5 h	1 h	2 h	3 h	4 h	5 h	6 h
MF	100	0.38±0.09	0.52±0.06	0.52±0.06	0.34±0.14[b]	0.30±0.13[c]	0.26±0.07[b]	0.24±0.10[a]
	200	0.64±0.05	0.78±0.05	0.80±0.06	0.72±0.06	0.56±0 .05	0.60±0.56	0.54±0.07[c]
	400	0.50±0.05	0.56±0.08	0.64±0.06	0.60±0.07	0.46±0 .09	0.60±0.07	0.56±0.11
Indometh.	10	0.46±0.06	0.72±0.02	0.88±0.05	0.06±0.0 4	0.48±0.06	0.44±0.10	0.10±0.08[a]
Control	5	0.48±0.08	0.44±0.05	0.72±0.14	0.77±0.07	0 .60±0.07	0.68±0.09	0.80±0.05

[a]P < .001, [b]P < .005, [c]P = .05 compared tocontrol (One Way ANOVA; LSD post hoc)
MF = Methanol fraction; Mean ± S.E.M = Mean values ± Standard error of means of five experiments

Table 6. Effect of hexane fraction on systemic acute inflammation of the rat paw

Treatment	Dose	Paw volume difference (ml)						
	mg/kg	0.5 h	1 h	2 h	3 h	4 h	5 h	6 h
HF	100	0.66±0.05	1.02±0.06	1.00±0.11	0.88±0.16	1.02 ±0.08	0.92±0.08[a]	0.86±0.04[a]
	200	0.70±0.16	0.72±0.17[c]	0.70±0.15[c]	0.80±0.11	0.78±0.17	0.86±0.17[b]	0.82±0.16
	400	0.84±0.04	0.82±0.31	0.80±0.07	0.88±0.05	0.83±0.04	0.96±0.12	1.02±0.10
Indometh.	10	0.64±0.08	0.54±0.12[b]	0.38±0.05[a]	0.28±0.09[a]	0.24±0.08[a]	0.92±0.10[a]	0.92±0.05[a]
Control	-	0.88±0.05	1.08±0.09	1.08±0.09	0.90±0.05	1 .06±0.08	0.92±0.10	0.92±0.05

[a]P < .001, [b]P < .005, [c]P = .05 compared to control (One Way ANOVA; LSD post hoc);
HF = Hexane fraction; Mean ± S.E.M = Mean values ± Standard error of means of five experiments

3.3.1.2 Acute systemic anti-inflammatory effect

In similar trend, the extract and fractions significantly (P = .05, P < .001) inhibited the development of acute systemic edema and this effect was sustained for up to 5 h (Tables 3-6). Their effect on systemic acute edema clearly indicates true anti-inflammatory action. The acute inflammatory reaction is a physiological characteristic of vascularized tissues in response to inflammatory mediators [20]. Assault or tissue injury that activates the acute inflammatory response leads to increased vascular permeability with consequent exudation of fluid containing plasma proteins such as immunoglobulins, coagulation factors and cells into the damaged tissue, causing edema at the site [21]. Thus, leaves of this plant may contain constituents which inhibit processes associated with acute inflammation such as leukocyte transport and biological actions of mediators.

3.3.2 Chronic anti-inflammatory activity

3.3.2.1 Effect on formaldehyde arthritis

The extract and fractions caused significant (P<0.05) dose-dependent inhibition of global edematous response to formaldehyde arthritis in rats (Table 7). The EF showed the highest level of inhibition (62.04%) and was comparable to indomethacin (60.50%).The least inhibition was shown by HF which reduced the area under curve by 23.99% relative to the untreated control group. Chronic inflammation is associated with the release and actions of macrophages and other leukocytes at the site of inflammation [22]. The formaldehyde arthritis rodent model closely mimics human arthritis in the pathogenesis with the associated tissue damage, joint pain and dysfunction and tissue necrosis in some cases [23]. Thus, the extract and factions may be effective in disorders of chronic inflammation such as rheumatoid arthritis.

3.3.2.2 Inhibitory effect on granuloma formation

The extract and fractions also elicited significant (P<0.05) dose-dependent inhibition of granuloma formation in rats (Table 8). ME displayed the highest inhibition (57.05%) comparable to indomethacin (55.5%) whereas HF showed the least inhibition (11.6%). Further evaluation of the effect on chronic inflammation of the granuloma type showed that the extract and fraction inhibited granuloma formation associated with cellular infiltration and accumulation. The cotton-pellet granuloma test is used to screen inhibitors of transudative and proliferative components of the chronic inflammatory process. Although classical anti-inflammatory drugs such as the non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to hinder granuloma formation by inhibiting granulocyte infiltration, collagen fiber development and mucopolysaccharides [24], the extent to which constituents of the extract and fractions interact with these mechanisms is not known. The presence of alkaloids, terpenoids and flavonoids in the extract and active polar fractions may account for the observed activity of the extract and fractions. Some compounds belonging to different chemical classes such as alkaloids, steroids, terpenoids, polyphenolics, phenylpropanoids, fatty acids and lipids have been reported to show good anti-inflammatory properties [25].

Table 7. Effects of extract and fractions on global edematous response to formaldehyde arthritis

Treatment	Dose (mg/kg)	AUC	Inhibition (%)
ME	200	0.38±0.06[b]	54.71
	400	0.36±0.04[a]	57.1
HF	200	0.75±0.08[b]	12.8
	400	0.65±0.07[b]	23.99
EF	200	0.39±0.14[a]	54.40
	400	0.33±0.03[a]	62.04
MF	200	0.39±0.05[a]	54.80
	400	0.34±0.05[a]	60.10
Indomethacin	10	0.34±0.08[a]	60.50
Control	-	0.86±0.09[b]	-

[a]P < .001; [b]P = .05 compared to control (One Way ANOVA; LSD post hoc); Values of AUC shown are mean values ± Standard error of means of five experiments, Inhibition (%) was calculated relative to the control; ME = Methanol extract; HF = Hexane fraction; EF = Ethylacetate fraction; MF = Methanol fraction

Table 8. Effect of extract and fractions on cotton pellet granuloma

Treatment	Dose (mg/kg)	Weight of granulomatous tissue (mg)	Inhibition (%)
ME	200	35.16 ± 0.54[a]	53.32
	400	32.47 ± 0.34[a]	57.05
HF	200	68.96 ± 1.03[a]	8.8
	400	66.77 ± 1.05[a]	11.69
EF	200	36.24 ± 0.62[a]	52.1
	400	34.55 ± 0.31[a]	54.3
MF	200	37.69 ± 0.79[a]	50.2
	400	36.83 ± 0.31[a]	51.3
Indomethacin	10	33.67 ± 0.52[a]	55.5
Control	-	75.61 ± 107	-

[a]P < .001 compared to control (One Way ANOVA; LSD post hoc)

Values of granuloma weight shown are mean values ± Standard error of means of five experiments, Inhibition (%) was calculated relative to the control; ME = Methanol extract; HF = Hexane fraction; EF = Ethylacetate fraction; MF = Methanol fraction

4. CONCLUSION

The findings of this study suggest that polar constituents of leaves of *T. bangwensis* parasitic on *C. angustifolia* may possess anti-inflammatory activity and be useful in the management of both acute and chronic inflammatory disorders. The mechanisms of anti-inflammatory action remain to be elucidated as well as the phytochemical constituents responsible for the pharmacological activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sostres C, Gargallo CJ, Arroyo MT, Lanas A. Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. Best Pract Res Clin Gastroenterol. 2010; 24(2):121-32.
2. Bassey P, Sowemimo A, Lasore O, Spies L, van de Venter M. Biological activities and nutritional value of *Tapinanthus bangwensis* leaves. Afr J Biotechnol. 2012; 11(73):13821-6.
3. Ekhaise F, Ofoezie V, Enobakhare D. Antibacterial properties and preliminary phytochemical analysis of methanolic extract of mistletoe (*Tapinanthus bangwensis).* Bayero J Pure App Sci. 2001;3(2):65-8.
4. Burkill HM. The useful plants of West Africa. Kew: Royal Botanical Garden. 1995;3:857.
5. Edagbo D, Ajiboye T, Borokin T, Ighere D, Alowonle A, Michael C. The influence of African misttetoe (*Tapinanthus bangwensis*) on the conversation status of *Citrus sinesis* in MOOR plantation. Int J Curr Res. 2012;4(12):484-7.
6. Patrick-Iwuanyanwu KC, Onyeike EN, Wegwu MO. Hepatoprotective effects of methanolic extract and fractions of African mistletoe *Tapinanthus bangwensis* (Engl. & K. Krause) from Nigeria. EXCLI J. 2010; 9:187-94.
7. Molehin OR, Adefegha SA. Antioxidant and inhibitory effects of aqueous and ethanolic extract of *Tapinanthus bangwensis* leaves on Fe2+-induced lipid peroxidation in pancreas (*in vitro*). Int Food Res J. 2015;22(1):269-74.
8. Patrick-Iwuanyanwu, KC, Onyeike EN, Wegwu MO. Anti-inflammatory effect of crude methanolic extract and fractions of African mistletoe *Tapinanthus bangwensis* (Engl. & K. Krause) on Wistar albino rats. Der Pharmacia Lettre. 2010;2(6):76-83.
9. Patrick-Iwuanyanwu KC, Onyeike EN, Adhikari A. Isolation, identification and characterization of gallic acid derivatives

from leaves of *Tapinanthus bangwensis*. J Nat Prod. 2014;7:14-9.
10. National Institutes of Health. Guide for the Care and Use of Laboratory Animals, 8th ed. Bethesda MD: NIH; 2011.
11. Trease GE, Evans WC, Pharmacognosy. 12th ed. London: BaillereTindall; 1983.
12. Lorke D. A new approach to practical acute toxicity. Arch Toxicol. 1982;53:275-89.
13. Atta AH, Alkohafi A. Antinociceptive and anti-inflammatory effects of some Jordanian medicinal plants extracts. J Ethnopharmacol. 1998;60:117-24.
14. Okoli C, Akah P, Onuoha N, Okoye T, Nwoye A, Nwosu S. An experimental evaluation of the antimicrobial, anti-inflammatory and immunological properties of a traditional remedy for furuncles. BMC Complement Altern Med. 2008;8:27.
15. Winter C, Risley E, Nuss G. Carrageenan induced edema in hind paw of rats as an assay for anti-inflammatory drugs. Proc Soc Exp Bio Med. 1962;111:544-7.
16. Brownlee G. Effect of deoxycortone and ascorbic acid on formaldehyde-induced arthritis in normal and adrenalectomized rats. Lancet. 1950;1:157–9.
17. Raval ND, Ravishankar B, Ashok BK. Anti-inflammatory effect of Chandrashura (*Lepidium sativum* Linn.) an experimental study. Ayu. 2013;34(3):302-4.
18. Winter A, Porter C. Effect of alteration in side chain upon anti-inflammatory and liver glycogen activities in hydrocortisone esters. J Am Pharm Assoc. 1957;46:515–9.
19. Adesina SK, Illoh HC, Imoh IJ, Imoh EJ. African mistletoes (Loranthaceae); Ethnopharmacology, chemistry and medicinal values: An update. Afr J Trad Complement Altern Med. 2013;10(4):161-70.
20. Rang, HP, Dale MM, Ritter JM. Textbook of Pharmacology. 4th ed. UK: Churchill LivingStone; 1999.
21. Cotran RS, Kumar V, Collins T. Robbin's pathological basis of disease. 6th ed. Philadelphia: W. B. Saunders Co; 1999.
22. Sallusto F, Mackay CR, Lanzavecchia A. The role of chemokine receptors in primary, effector, and memory immune responses. Ann Rev Immunol. 2000; 18:593-620.
23. Greenwald R. Animal models for evolution of arthritic drug. Method Find Exp Clin Pharmacol. 1991;13:75-83.
24. Suleyman H, Demirezer LO, Kuruuzum A, Banoglu ZN, Gocer F, Ozbakir G et al. Anti-inflammatory effect of the aqueous extract from *Remex patientia* L. roots. J. Ethnopharmacol. 1999;65:141-8.
25. Gautam R, Jachak SM. Recent developments in anti-inflammatory natural products. Med Res Rev. 2009;29(5):767-820.

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