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Exploring the Analgesic and Anti-inflammatory Effects of Ethyl Acetate Extract of *Tapinanthus Globiferus* Growing on *Acacia Nilotica*

***[1,2]Buhari Muhammad Koko, [2]Dangoggo Sani Muhammad, [2]Mohammed Misbahu Ladan, [3]Salihu Mustapha, [3]Muhammad Abubakar Alhaji and [4]Abdullahi Salisu**

[1]Department of Chemistry, Federal University Gusau, Zamfara State, Nigeria.
[2]Department of Energy and Applied Chemistry, Usmanu Danfodiyo University, Sokoto State, Nigeria.
[3]Department of Pharmaceutical and Medicinal Chemistry, Usmanu Danfodiyo University, Sokoto State, Nigeria.
[4]Department of General Studies, College of Administration, Management and Technology Potiskum, Yobe State, Nigeria.

*Corresponding authors' email: buharee88@gmail.com Phone: +2347032832556

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ABSTRACT

Tapinanthus globiferus is a Nigerian medicinal plant used traditionally for the management of pain and inflammation. The aim of the work is to conduct phytochemical screening, acute toxicity as well as analgesic and anti-inflammatory evaluation ethylacetate extract (EET) of *T. globiferus* growing on *Acacia nilotica*. The preliminary phytochemical screening was performed using standard methods, acute toxicity (*i.p*) was assessed using Lorke's method while analgesic and anti-inflammatory effect were assessed using formalin-induced pain test and paw oedema in rats, respectively. The results of preliminary phytochemical screening revealed the presence of carbohydrates, alkaloids, glycosides, tannins, flavonoids, saponins, steroids and triterpenes while the LD_{50} value of the EET was estimated to be 3808 mg/kg indicating the extract is relatively toxic. EET exhibited significant ($p \leq 0.05$) analgesic activity in dose-dependent manner, with inhibition ranging from 34.07 & 41.15 % to 51.28 & 53.85 % at 50 to 200 mg/kg while the control had 75.46 & 81.92 %. Additionally, EET significantly inhibited the paw oedema during the 1st to 4th hrs at 200 mg/kg with inhibition ranging from 27.50, 29.20, 34.19 and 35.40 % and no significant inhibition was observed at doses of 50 and 100 mg/kg, the standard drug-piroxicam used had 46.90 to 55.75%. The findings from the study unveiled the potential of EET to significantly exhibit the analgesic and anti-inflammatory activities, thus suggest for isolation and characterization of bioactive compounds for use in the management of pain and inflammation.

Keywords: Phytochemicals, Acute Toxicity, Tapinanthus Globiferus, Analgesic, Anti-Inflammatory

INTRODUCTION

Traditional medicine remains the primary source of healthcare for approximately 80% of the population in developing countries, particularly for the management of pain, inflammation, and other common ailments. Its widespread utilization is largely attributed to its accessibility, affordability, cultural acceptability, and relatively lower incidence of adverse effects compared with many conventional therapies (Parekh, 2005). Recognizing its significant contribution to primary healthcare, the World Health Organization (WHO) advocates the integration of traditional medicine into national healthcare systems, especially in developing countries, owing to its considerable potential in disease prevention and management (Amos *et al.,* 2001).

Tapinanthus globiferus (A. Rich.) Tiegh., commonly referred to as African mistletoe and locally known as *Kauchin bagaruwa* in the Hausa language, is a medicinal plant widely employed in Northern Nigerian traditional medicine for the treatment of pain-related disorders. The plant is a woody, spreading shrub characterized by blackish, smooth stems with conspicuous lenticels and opposite, occasionally alternate, leaves measuring approximately 7–15 cm in length and 3–10 cm in width (Pithayanukal et al., 2007). Ethnomedicinally, *T. globiferus* has been used for the management of several disease conditions, including diarrhoea, dysentery, cancer, pain, and inflammatory disorders (Personal Communication). In addition, the leaves are traditionally chewed or applied topically to alleviate pain (Burkill, 2000). Phytochemical investigations of *T. globiferus* parasitizing *Acacia nilotica* have identified several bioactive constituents, including epicatechin and isoquercetin, which are believed to contribute to its pharmacological properties (Muhammad *et al.,* 2022).

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Bauman, 2002). It remains one of the leading causes of healthcare utilization worldwide and significantly impairs quality of life. Analgesics are therapeutic agents used to relieve pain without inducing loss of consciousness (Romulo et al., 2013). Among these, opioid (narcotic) analgesics are highly effective for moderate to severe pain but are associated with significant adverse effects, including tolerance, physical dependence, and respiratory depression, which limit their long-term clinical use (Howland and Mycek, 2006). Non-steroidal anti-inflammatory drugs (NSAIDs), another major class of analgesics, exert their effects primarily through inhibition of prostaglandin synthesis, thereby reducing pain, inflammation, and fever (Insel, 1996). Despite their effectiveness, prolonged NSAID therapy is frequently associated with gastrointestinal adverse effects such as gastric irritation, hyperacidity, and exacerbation of peptic ulcer disease (Insel, 1996). These limitations underscore the urgent need to identify safer, more effective, and readily accessible

analgesic and anti-inflammatory agents from natural sources (Yusuf et al., 2019).

Although several pharmacological activities of *T. globiferus* have been reported using different plant parts and extracts, there is limited scientific evidence regarding the acute toxicity, analgesic, and anti-inflammatory properties of its ethyl acetate leaf extract. Establishing the safety profile alongside its pharmacological efficacy is essential for validating its traditional use and supporting its potential therapeutic development. Therefore, the present study was designed to evaluate the acute toxicity, analgesic, and anti-inflammatory activities of the ethyl acetate leaf extract of *T. globiferus* in experimental animal models.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Tapinanthus globiferus growing on *Acacia nilotica* was collected in Dundaye village of Wamakko Local Government Area of Sokoto State, Nigeria in July, 2023. It was identified and authenticated at the Herbarium unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto where a specimen was prepared and deposited with a voucher (UDUH/ANS/0327).

Preparation and Extraction

The leaves were air shaded dried at room temperature for 7 - 10 days and then pulverized to powder with a pestle and mortal. For the extraction, 1330 g of the pulverized leaves were sequentially extracted by maceration with solvents of increasing polarity starting with n- hexane, ethyl acetate and methanol 24 hrs each with occasional shaking. The resultant mixture were filtered twice, first using muslin cloth, followed by Whatman filter paper (No.1). The filtrate was concentrated *in vacuo* with a rotary evaporator at 40°C to afford n-hexane, ethyl acetate and methanol extracts coded as HET, EET and MET, respectively. However, in this work only EET was subjected to pharmacological studies.

Drugs and Chemicals Used

Piroxicam was purchased from Life Science LLP (India) and used as positive control, while all the solvents and reagents used in the study were of analytical grades.

Experimental Animals

Swiss albino mice and adult Wister rats of either sex weighing (23-33 g) and (150-220 g) respectively were obtained from Animal House Facility, Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Kaduna state Nigeria. They were housed in animal house facility, Department of Pharmacology and Toxicology, Usmanu Danfodiyo University, Sokoto, fed with laboratory diets and water ad libitum and maintained under standard conditions (12hr light and 12hr dark cycle) in a propylene cage at room temperature. All experimental procedures were approved by the Animal Right and Ethics Community of the University.

Preliminary Phytochemical Screening

The preliminary screening of phytochemical on EET was performed using methods described by Trease and Evans (1996).

Acute Toxicity Study

The median lethal dose (LD_{50}) of EET was determined using method described by Lorke (1983). The test was carried out in two phases. In the first phase, three groups of mice were administered (*i.p*) with 10, 100 and 1000 mg/kg of EET respectively. The animals were monitored for 24 h for signs of toxicity and mortality. Based on the result of phase one, three groups of one animal each received 1600, 2900 and 5000 mg/kg of EET, respectively. The animals were monitored for signs of toxicity and mortality for 24 h. The LD_{50} was calculated using the following formula:

$$LD_{50} = \sqrt{\text{minimal lethal dose} \times \text{maximal survival dose}} \quad (1)$$

Formalin Test in Rats

The test was conducted in accordance with the method described by Dubuisson and Dennis (1977). In this method, twenty-five rats were divided into 5 groups each containing 5 rats. Groups 1 was given 10 mL/kg of distilled water *i.p.* (negative control). Groups 2, 3 and 4 were injected with 50 mg/kg, 100 mg/kg and 200 mg/kg *i.p.* of EET respectively. While group 5 was injected with piroxicam 10mg/kg *i.p.* (positive control). Thirty minutes after this treatment; 50 µl of freshly prepared 2.5 % solution of formalin was injected subcutaneously under the planter surface of the left hind paw of each rat. The rats were placed individually in an observation chamber and monitored for 1hr.

The severity of pain response was recorded for each rat based on the following scale:

(0) rat walked or stood firmly on the injected paw.
(1) the injected paw was partially elevated.
(2) the injected paw was clearly lifted off the floor.
(3) the rat licked, chewed or shook the injected paw.

Anti-nociceptive effect was determined in two phases. The early phase (phase 1) was recorded during the first 5 minutes, while the late phase (phase 2) was recorded during the last 45 minutes.

Anti-Inflammatory Study Formalin-Induced Inflammation in Rats

Formaldehyde 2.5 % v/v was used as inflammogen (Yusuf *et al.,* 2019). Rats were divided into five groups of three rats each for the extract (EET) used. Thirty minutes before injection of 2.5 % v/v formalin (50 µL volumes in the sub plantar region of the left hind paw of the rat), the groups were treated i.p. as follows:

Group I: received (10 mL distilled water per kg, as negative control).
Groups II, III and IV: received (extract, at 50, 100 and 200 mg/kg, respectively).
Group V: received (10 mg piroxicam per kg, as positive control).

The paw diameter (cm) was measured using vernier caliper at an interval of 1 – 4 hours.

Statistical Analysis

The results of the experiments were expressed as Mean ± S.E.M. The mean values of control groups were compared with the mean value of treated groups using one-way ANOVA followed by post hoc test (Dunnett t – 2-sided) using the SPSS software (version 21) and Microsoft Excel 2021 version. Results were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Phytochemical Screening of EET

The preliminary phytochemical screening conducted on EET revealed the presence of carbohydrates, proteins, lipids, flavonoids, tannins, alkaloids, saponins, steroids/triterpenes, cardial glycosides, anthraquinones and diterpenes/diterpenoid as shown in Table 1.

Table1: Phytochemical Composition of EET

Constituents	Test	Inference
Carbohydrate	i. Molisch	+
	ii. Fehling	++
Proteins	Xanthoproteic	+
Lipids	Sudan-iii	+
Phenols	Ferric chloride	++
Flavonoids	i. NaOH	+
	ii. Shinoda's	+
	iii. Ferric chloride	++
Tannins	i. Lead sub-acetate	+
	ii. Ferric chloride	++
Alkaloids	i. Dragendorff's	+
	ii. Mayer's	+
Saponins	Frothing	+
Steroids/Triterpenes	i. Salkowski's	++
	ii. Liberman-Burchard's	++
Cardial glycosides	Killer-killiani's	+
Anthraquinones	Bontrager's	-
Diterpenes/Diterpenoids	Cupper acetate	+

Key= ELE = ethylacetate leaf extract, ++ = moderately present, + = trace amount, - = Not detected

Acute Toxicity (LD_{50} Determination) on EET

The *intraperitoneal* LD_{50} (Table 2) of the EET in mice was estimated to be 3808 mg/kg indicating that the extract is relatively toxic.

Table 2: Median Lethal Dose (LD_{50}) of the EET

First Phase Dose (mg/kg)	Number of mice used	Mortality
10	3	0/3
100	3	0/3
1000	3	0/3
Second Phase Dose (mg/kg)	**Number of mice used**	**Mortality**
1600	1	0/1
2900	1	0/1
5000	1	1/1

Effect of EET 0n Formalin-Induced Pain in Rats

The analgesic activity of EET is present in Table 3 showed that, the extract exhibited significant ($p \leq 0.05$) analgesic activity in dose-dependent manner, with highest dose (200 mg/kg) had 51.28 and 53.85 % inhibitions while the least dose of 50 mg/kg having 34.07 and 41.15 % inhibitions at early and late phase respectively. However, the standard drug (piroxicam 10 mg/kg) exhibit inhibition of 75.46 and 81.92 % which is relatively higher than the inhibition observed from EET (200 mg/kg).

Table 3: Effect of EET on Formalin-Induced Pain in Rats

	Mean Pain Scores (% inhibition)	
Treatment (mg/kg)	**Early Phase**	**Late Phase**
Distilled water (10 mg/mL)	2.73 ± 0.07^{b}	2.60 ± 0.00^{b}
EET (50)	$1.80 \pm 0.20^{a, b}$ (34.07 %)	$1.53 \pm 0.07^{a, b}$ (41.15 %)
EET (100)	$1.40 \pm 0.00^{a, b}$ (48.72 %)	$1.47 \pm 0.07^{a, b}$ (43.46 %)
EET (200)	$1.33 \pm 0.07^{a, b}$ (51.28 %)	$1.20 \pm 0.00^{a, b}$ (53.85 %)
Piroxicam (10)	0.67 ± 0.07^{a} (75.46 %)	0.47 ± 0.07^{a} (81.92 %)

Each value represents mean ± SEM. $^{a}p \leq 0.05$ compared with distilled water control and $^{b}p \leq 0.05$ compared with piroxicam control.

Anti-inflammatory Effect of EET in Rats

The anti-inflammatory activity of EET at different doses (50, 100 and 200 mg/kg) was assessed using formalin-induced inflammation as presented in Table 4. The result indicates that, the EET significantly inhibited ($p \leq 0.05$) of paw oedema during 1st to 4th hour at 200 mg/kg with inhibition ranging from 27.50, 29.20, 34.19 and 35.40 % of paw oedema while at doses (50 and 100 mg/kg) no significant percentage inhibition ($p \leq 0.05$) of paw oedema by the extract was observed during 1st to 4th hour. However, the standard drug (piroxicam 10 mg/kg) used in this study was found exhibiting high activity compared to EET with paw oedema inhibition ranging from 46.90 to 55.75 during 1st to 4th hour.

Table 4: Effect of Formaldehyde-Induced Inflammation in Rats On EET

Treatment	Dose (mg/kg)	Mean paw diameter (cm) ± SEM (% inhibition)				
		0h	1h	2h	3h	4h
Piroxicam	10	0.57± 0.03	0.64± 0.03[a] (46.90 %)	0.54± 0.06[a] (47.50 %)	0.57± 0.03[a] (51.28 %)	0.50 ± 0.00[a] (55.75 %)
Distilled Water	10	0.60± 0.06	1.20± 0.12[b]	1.13± 0.12[b]	1.17± 0.12[b]	1.13 ± 0.09[b]
EET	50	0.97± 0.03	1.20± 0.06[b] (0.00 %)	1.10± 0.00[b] (2.65 %)	1.07± 0.03[b] (8.55 %)	1.00 ± 0.00[b] (11.50 %)
EET	100	0.90± 0.03	1.06± 0.03[b] (11.50 %)	0.97± 0.06[b] (14.17 %)	0.93± 0.03[b] (20.35 %)	0.90 ± 0.06[b] (20.51 %)
EET	200	0.67± 0.03	0.87± 0.03[a] (27.50 %)	0.80± 0.06[a] (29.20 %)	0.77± 0.03[a] (34.19 %)	0.73 ± 0.03[a] (35.40 %)

Each value represents mean ± SEM. $^{a}p \leq 0.05$ compared with distilled water control and $^{b}p \leq 0.05$ compared with piroxicam control.

Discussion

Preliminary phytochemical analysis of the ethyl acetate extract of *Tapinanthus globiferus* (EET) demonstrated the presence of saponins, tannins, alkaloids, cardiac glycosides, carbohydrates, steroids/triterpenes, and flavonoids (Table 1). These findings are consistent with previous reports on *T. globiferus* growing on different host plants (Abubakar *et al.,* 2016; Halilu *et al.,* 2017). The occurrence of these secondary metabolites is noteworthy, as they have been widely recognized as major contributors to the diverse pharmacological and biological activities exhibited by medicinal plants (Cragg and Newman, 2005).

Toxicological evaluation is an essential component in the development of medicinal plant products because it provides important information regarding their safety profile before therapeutic application (De Melo *et al.,* 2011). Although several species of the genus *Tapinanthus* have demonstrated promising medicinal properties in both experimental and clinical settings, their safety remains an important consideration. In the present study, the ethyl acetate extract exhibited a relatively high *intraperitoneal* median lethal dose (LD_{50}), with only one mortality recorded at the highest administered dose of 5000 mg/kg. According to Lorke's toxicity classification, substances with an LD_{50} around 1000 mg/kg are considered slightly toxic, whereas those with LD_{50} values exceeding 5000 mg/kg are regarded as practically non-toxic. Therefore, the observed toxicity profile suggests that the extract possesses low acute toxicity, which may partly explain its widespread use in traditional medicine (Nasir *et al.,* 2017).

To comprehensively investigate the antinociceptive properties of the extract, experimental models capable of assessing both peripheral and central mechanisms of analgesia were employed. Such an approach provides insight into the possible sites of action of the bioactive constituents present in the extract. Among these models, the formalin-induced nociception test is particularly valuable because it simultaneously evaluates centrally and peripherally mediated analgesic activities (Vongtau *et al.,* 2004a).

The formalin test is a well-established model of persistent pain that effectively distinguishes centrally acting analgesics, such as opioid drugs, from peripherally acting agents including acetylsalicylic acid (Vogel and Vogel, 1997; Abdullahi *et al.,* 2016). Furthermore, it differentiates between the neurogenic (early) and inflammatory (late) phases of pain based on their underlying mechanisms (Chan *et al.,* 2000). The initial phase results from direct activation of nociceptors immediately following formalin administration and predominantly reflects central pain processing, whereas the later phase is characterized by the release of inflammatory mediators that promote peripheral sensitization and sustained nociceptive transmission (Abdullahi *et al.,* 2016). The ability of the extract to significantly suppress nociceptive responses during both phases suggests that its analgesic activity involves both central and peripheral mechanisms of action, indicating a broad spectrum of antinociceptive effects (Abdullahi *et al.,* 2016).

Tapinanthus globiferus growing on *Acacia nilotica* has long been utilized in traditional medicine for the management of pain and inflammatory disorders. In the present investigation, the ethyl acetate extract produced a significant ($p \leq 0.05$) and dose-dependent reduction in formalin-induced nociceptive behaviour, with the highest inhibition observed at 200 mg/kg (Table 3). These findings indicate that the analgesic efficacy of the extract increases with dose. Although the ethyl acetate fraction exhibited lower analgesic activity than the corresponding n-hexane and methanol extracts, the observed effect nevertheless supports the ethnomedicinal application of the plant. The analgesic activity may be attributed to the individual or synergistic actions of the phytochemicals identified in the extract, particularly flavonoids, tannins, saponins, and steroidal compounds, which have previously been associated with antinociceptive properties.

The anti-inflammatory activity of the extract was further demonstrated using the formalin-induced paw oedema model. Formalin-induced inflammation occurs in two phases: an initial phase mediated primarily by histamine, serotonin, and enhanced prostaglandin synthesis, followed by a later phase involving prostaglandins, bradykinin, leukotrienes, and other inflammatory mediators (Yusuf *et al.,* 2019). In the present study, the extract significantly reduced paw oedema compared with the negative control, indicating appreciable anti-inflammatory activity. This effect may be associated with inhibition of the cyclooxygenase (COX)-dependent arachidonic acid pathway, resulting in reduced prostaglandin production, a major contributor to vascular permeability, nociceptor sensitization, and inflammatory cell recruitment (Bertram, 2001). The extract may also suppress the synthesis or release of other inflammatory mediators, including prostaglandins and polypeptide kinins.

The anti-inflammatory response was dose dependent, with the 200 mg/kg dose producing the greatest inhibition of paw oedema throughout the 1st to 4th hour of observation compared with the distilled water-treated group. Similar dose-dependent trends were observed across all treatment groups, suggesting that increasing extract concentrations enhanced its pharmacological efficacy. The observed analgesic and anti-inflammatory activities are likely attributable to the phytochemical constituents detected during preliminary screening. In particular, flavonoids, tannins,

steroids, saponins, and other phenolic compounds have been extensively reported to possess anti-inflammatory and analgesic activities through multiple mechanisms, including inhibition of inflammatory mediator synthesis, free radical scavenging, and modulation of pro-inflammatory signalling pathways (Harborne and Williams, 2000; Yusuf *et al.,* 2015).

Conclusion

The findings of this study demonstrate that the ethyl acetate leaf extract of *Tapinanthus globiferus* growing on *Acacia nilotica* possesses significant analgesic and anti-inflammatory activities, while exhibiting low acute toxicity in experimental animals. These results provide scientific evidence supporting the traditional use of the plant in the management of pain and inflammatory disorders and highlight its potential as a promising source of bioactive compounds for the development of safer analgesic and anti-inflammatory agents.

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