




Research Article

Phytochemical Screening and Anti-Inflammatory Studies of *Tapinanthus globiferus* (A. Rich) Teigh. Leaves Three Extracts

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ABSTRACT

Background: *Tapinanthus globiferus* (A. Rich) Teigh. (Loranthaceae) is an excellent medicinal plant in terms of efficacy and also traditionally used for the treatment of various diseases including inflammations, cancer, diabetes and strokes. This study was designed to assess the anti-inflammatory activity of the leaf extracts of *T. globiferus* in wistar rats and identify phytochemical constituents of the extracts responsible for the observed activity.

Methods: *Tapinanthus globiferus* leaves was extracted with hexane, ethyl acetate and methanol in a soxhlet apparatus. The extracts were subjected to qualitative phytochemical analysis, toxicity and anti-inflammatory activity using carrageenan-induced paw oedema in wistar rats. Piroxicam (20 mg/kg) was used as reference standard. The data were analyzed by one-way analysis of variance with significant level set at $p \leq 0.05$.

Results: The percentage yield from the gradient extraction of *T. globiferus* leaves showed methanol to be the highest and the chromatographic analysis visualized with specific reagents confirmed the presence of steroids/triterpenes, phenolic compounds and flavonoids in the leaf of *T. globiferus*. LD₅₀ was above 2,000 mg/kg and no death was recorded. The hexane, ethyl acetate and methanol leaf extracts of *T. globiferus* at 250, 500 and 1,000 mg/kg produced a significant decrease in paw oedema ($p \leq 0.05$) with percentage inhibition at the first and third hour for hexane, ethyl acetate and methanol extract respectively. The methanol extracts recorded the highest inflammatory inhibition percentage.

Conclusion: These finding revealed that the leaf of *T. globiferus* has anti-inflammatory activity and this justified its traditional use in the treatment of inflammation.

Introduction

Drug discovery from the medicinal plant is a continuous effort in curing, preventing, managing diseases that pose a threat to human existence. Over the years, inflammation is one of the diseases that currently inflict pain and discomfort to mankind. Its characteristic signs are swollen, reddened, pain, heat, and loss of functions that could result in a hurting response in any part of the body. In more severe cases, it affects the immune cells, blood vessels and leads to other vast arrays of human diseases.^{1,2} Generally, conventional drugs from non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are synthetic drugs used in the treatment of inflammations. These drugs are often alleged as less effective and showed other toxic effects such as epigastric distress, peptic ulceration, osteoporosis, and iatrogenic cushing's syndrome.^{3,4} The need for in-depth investigation of *Tapinanthus globiferous* and its potential towards the management of inflammation could provide scientific

findings and suggest its therapeutic effect or possibly low toxicity.⁵

Tapinanthus globiferus is a species in the Loranthaceae family. It is locally called Kauchi in Hausa Language in Nigeria.⁶ It is a semi-parasite with glabrous pendulous stems up to 1.2 m long with presumably roots that mostly grows on the branches of a large number of tree species of the genera *Vitellaria* (C.F. Gaertn), *Kola* (Schott & Endl.), *Citrus* (L.), *Combretum* (Loefl.), *Acacia* (Mill.), *Aloe* (L.) and *Terminalia* (L.) as host.⁷ It is used traditionally to treat many ailments including inflammations, malaria, bacterial infections, pain relief and ulcers.⁶ It was also reported that *T. globiferus* extracts have exhibited both cytotoxic and immuno-modulatory properties which have been efficacious in the treatment of cancers.⁸

This study was designed to assess the anti-inflammatory activity of *T. globiferus* and to identify the phytochemical constituents of the extracts that might be responsible for the folkloric use of plants leaves extract.

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Materials and Methods

Experimental animals

Wistar rats of both male and female genders weighing 150 – 200 g were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were maintained under standard conditions (12 hours light /12 hours dark cycle and temperature of about $37 \pm 2^\circ\text{C}$, 35 - 60% humidity). The rats were fed with standard grower mash (Vital feed, Jos, Nigeria) and water *ad-libitum*. Animals were treated according to the standard guidelines to good practice in housing and handling laboratory animals⁹ and with approval of Animal Ethics and Care Committee, Ahmadu Bello University, Nigeria (ABUCAUC/2017/PG/018).

Collection, identification and preparation of plant material

Tapinanthus globiferus was first identified and collected from *Citrus* species in the field around Yankarfe village, Sabon gari Local Government Area, Kaduna State, Nigeria, in April, 2016. It was identified and authenticated by Mal. Namadi Sanusi of the Herbarium Unit, Department of Botany, Ahmadu Bello University, Zaria, where a specimen voucher number 1449 was deposited. The leaves were air dried under shade for two weeks, all foreign matter removed and comminuted to powder form. The powder material was then stored in a sterile airtight container for further use.

Extraction of plant material

Powdered sample (1 kg) was extracted with n-hexane, ethyl acetate and methanol (JHD, Lobal Chem, India) gradient wise in a soxhlet apparatus. The plant material was exhaustively extracted with hexane (2 L) until the solvent became clear, the same procedure was applied successively to ethyl acetate and methanol. The gradient extracts obtained with each solvent were concentrated on a rotary evaporator and finally dried to a constant weight after which it was stored in an air-tight container for subsequent use.¹⁰ The extracts were subsequently coded as TGHE (*T. globiferus* Hexane Extract), TGEE (*T. globiferus* Ethyl Acetate Extract) and TGME (*T. globiferus* Methanol Extract).

Qualitative phytochemical screening of the extracts

The Preliminary phytochemical screening of all the three extracts (TGHE, TGEE and TGME) was carried to detect the various phytochemical constituents present in the extracts using standard qualitative chemical tests.^{11,12}

Thin layer chromatographic profile of the extracts

Thin layer chromatographic analysis was performed on pre coated silica gel plates with silica gel 60 F₂₅₄ (Merck, Germany) using one way ascending technique. Developed plates were visualized using general detecting reagent (P-Anisaldehyde/H₂SO₄) and specific detecting reagents.¹³

Acute toxicity profile

The acute toxicity of the three (3) extracts of *T. globiferus* leaves were determined orally using the Organization for Economic Cooperation and Development (OECD) 425 guideline as described below.¹⁴ Nine rats were used for the study. They were divided into three groups of three rats each and the TGHE, TGEE and TGME were administered at the dose of 2000 mg/kg. The toxicity was carried out orally and observed for signs and symptoms of toxicity for 14 days.

Anti-inflammatory assessment of the extracts

The method described by Olajide and co-worker, was adopted.¹⁵ Fifty five adult albino rats were weighed, marked and randomly assorted into 11 groups (I - XI) of five rats each. Prior to the administration of the carrageenan the rats were pretreated. Group I rats were pretreated with 10 ml of normal saline orally. Group II rats were pretreated with Piroxicam 20 mg/kg body weight. Group III - XI rats were pretreated with 250, 500 and 1,000 mg/kg body weight of the TGHE, TGEE and TGME respectively. One hour after pretreatments an injection of 0.1 ml of 1% carrageenan suspension was made into the right hind paw of each rat under the sub plantar aponeurosis.¹⁶ Measurement of the paw circumference was done immediately before and 1 - 5 hours after carrageenan injection using vernier caliper. The percentage inhibitory activity was calculated using the formula.¹⁵

Percentage Inhibition =

$$\frac{(C_t - C_o)_{control} - (C_t - C_o)_{treated}}{(C_t - C_o)_{control}} \times 100$$

Where;

C_t = Paw circumference volume at time t

C_o = Paw circumference volume before administration of treatment and carrageenan

C_t - C_o = Paw edema

Statistical analysis

The results were expressed as mean \pm standard error of the mean (SEM) for all values. The data was statistically analyzed using one-way ANOVA (SPSS version 20.0 Chicago) followed by Dunnett's *post hoc*. Results were considered to be significant when P values are less than 0.05 ($p < 0.05$).

Results and Discussion

Percentage yield of the extracts

Percentage yield of the leaves of *T. globiferus* extract was presented in Table 1 below.

Table 1. Mass and percentage yield for the leaves extracts of *Tapinanthus globiferus*

S/No.	Extracts	Mass (g)	Percentage Yield (%w/w)
1.	THE	45.63	4.56
2.	TGEE	35.08	3.51
3.	TGME	248.55	24.86

TGHE = *T. globiferus* hexane extract, TGEE = *T. globiferus* ethyl acetate extract, TGME = *T. globiferus* methanol extract

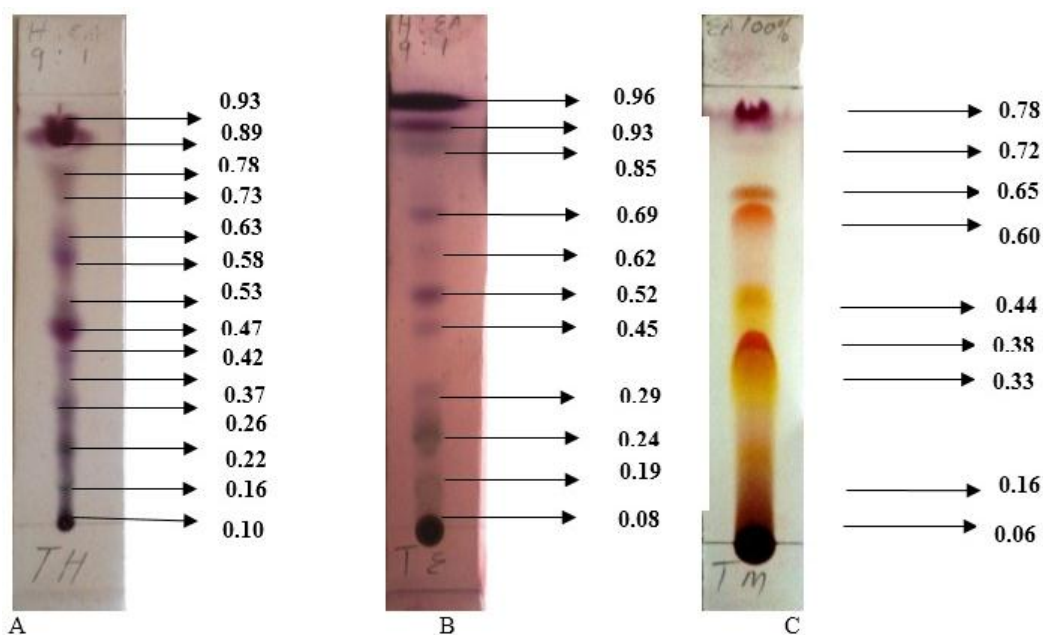


Figure 1. TLC Chromatogram of TGHE (A), TGEE (B) and TGME (C) sprayed with P-Anisaldehyde/ H_2SO_4 with R_f values TGHE = *T. globiferus* hexane extract, TGEE = *T. globiferus* ethyl acetate extract, TGME = *T. globiferus* methanol extract.

TGME had the highest yield (24.86 %) followed by TGHE (4.56 %) and TGEE has the lowest yield (3.51 %). These could be attributed to the ability of polar solvents to attract more of the phytochemical constituents present in the plant material.

Phytochemical screening

Phytochemical analysis of the leaves extracts of *T. globiferus* revealed the presence of some secondary metabolites namely alkaloids, tannins, flavonoids, cardiac glycosides, saponins, steroids and triterpenes, while anthraquinones were found to be absent (Table 2); this result is in agreement with earlier findings where the presence of phytochemicals like flavonoids, tannins, saponins, cardiac glycosides, triterpenoids and alkaloids were detected in the plant.¹⁷ Preliminary phytochemical screening gave a brief idea about the qualitative nature of active phytochemical constituents present in plant extracts, which will help the future investigators regarding the selection of the particular extract for further investigation or isolating the active principle.¹⁸

Table 2. Qualitative phytochemical screening of leaves extracts of *Tapinanthus globiferus*.

Phytoconstituents	TGHE	TGEE	TGME
Carbohydrate	+	+	+
Saponin	-	+	+
Steroids/Triterpenes	+	+	+
Flavonoids	-	+	+
Tannins	-	+	+
Alkaloids	-	+	+
Cardiac glycosides	-	+	+

Key: Present (+), Absent (-)

Thin layer chromatographic profile

Thin layer chromatographic analysis is a simple and cheap method for detection of different class of

phytochemical compounds due to its good selectivity and sensitivity of detection providing convincing results.¹⁹ It is considered a reliable technique for qualitative phytochemical screening of plant active constituents. Thin layer chromatographic analysis of TGHE, TGEE and TGME from *T. globiferus* leaves in different solvent systems produced various degree of separations.

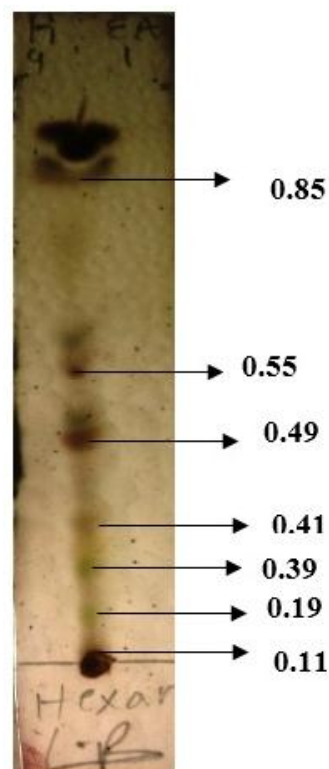


Figure 2. TLC Chromatogram of TGHE in Hexane: ethyl acetate (9:1) sprayed with Liberman Buchard (LB) Reagent and its R_f values.

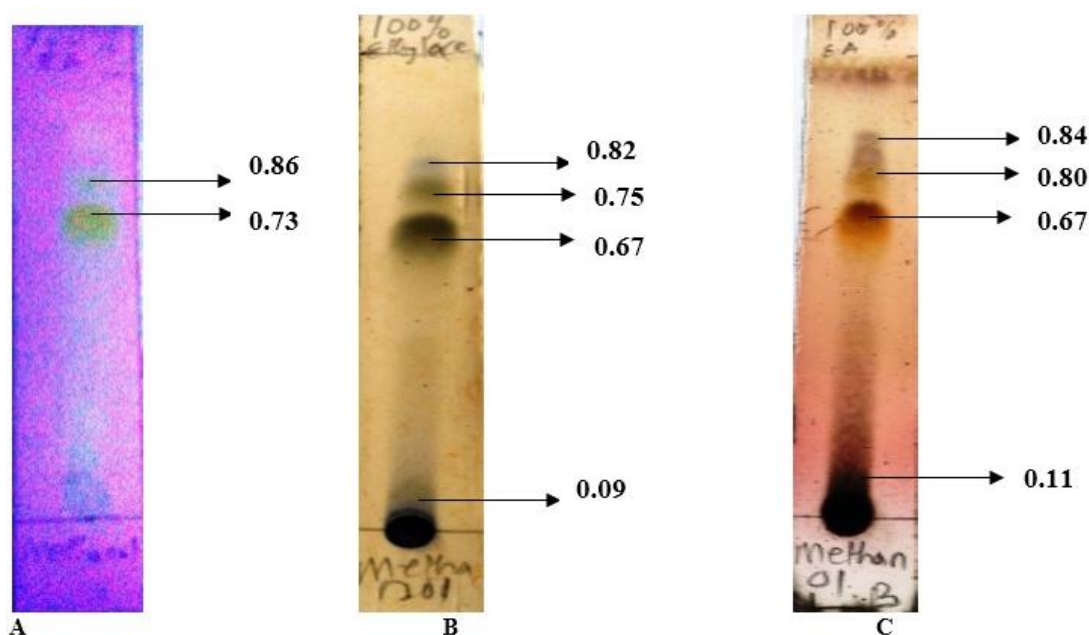


Figure 3. TLC Chromatogram of TGME Sprayed with Aluminium Chloride (AlCl_3) (A) Ferric Chloride (FeCl_3) (B) and Liebermann Buchard (C) with R_f values.

The TGHE (A) and TGEE (B) developed in Hexane : Ethyl acetate (9:1) and visualized with *p*-anisaldehyde revealed fourteen (14) clear and distinct spots for TGHE and eleven (11) spots for TGEE, while TGME (C) revealed nine (9) clear spots in 100% ethyl acetate visualized with *p*-anisaldehyde and with their R_f values (Figure 1). The chromatogram of TGHE was positive to Liebermann-Buchard reagent which revealed the presence of steroids/triterpenes (Figure 2) and it was negative to both ferric chloride and aluminum chloride reagent (absence of phenolic compounds and flavonoids respectively).

TGME was positive to Liebermann-Buchard, ferric chloride and aluminum chloride reagent which revealed the presence of steroids/triterpenes phenolic compounds and flavonoids respectively on TLC (Figure 3). The yellow fluorescence of aluminum chloride reagent (which was observed under UV light at 254 nm after spraying the plate) was faint which could be as a result of the low degree of concentration of the flavonoid compounds in the TGME. The successful separation of bio-molecules by chromatographic technique depends upon suitable solvent system which needs an ideal range of partition coefficient (k) for each target compounds.²⁰

The solvent system, hexane: ethyl acetate (9:1) gave a better separation for TGHE and TGEE in this study. For TGME 100% ethyl acetate was a good solvent for TLC of *T. globiferus* leaves. Therefore, chromatograms from the extracts confirmed the presence of steroids/triterpenes, phenolic compounds and flavonoids on TLC. The presence of these compounds support the traditional use of the plant in treatment of inflammatory diseases. It has been reported that phenolic compounds such as tannins and flavonoids possess diverse biological properties such as anti-inflammatory, antibacterial, antiulcer and anti-oxidant activities.²¹

Acute toxicity studies

In order to determine the safety margin of drugs and plant products for human use, toxicological evaluation is to be carried out in experimental animals using the Organisation for Economic Co-operation and Development (OECD 425) 2008 guideline method to predict toxicity and to provide guidelines for selecting a “safe” dose in animals and also used to estimate the therapeutic index ($\text{LD}_{50}/\text{ED}_{50}$) of drugs.^{22,23}

In this study, median lethal dose (LD_{50}) of the extracts (TGHE, TGEE and TGME) via oral route was shown to be greater than 2000 mg /kg. There was no death or any sign of toxicity recorded, showing that the leaves extracts of *T. globiferus* were practically non-toxic when administered via oral route. This is in agreement with the earlier finding where non-toxic nature of the species; *T. bangwensis* and *D. falcata* administered via oral route were reported.^{24,25}

Anti-inflammatory studies

Inflammation is a section of the complex biological response of vascular tissues to hazardous stimuli, such as pathogens, broken cells or irritants. Carrageenan-induced acute inflammation is one of the most appropriate test procedures used in screening anti-inflammatory agents. The time direction of edema improvement in carrageenan-induced paw edema model in rats is commonly represented via a biphasic curve.²⁶ The first phase of inflammation takes place within an hour of carrageenan injection and is partly due to the trauma of injection and additionally due to histamine and serotonin component.²⁷ Carrageenan induced paw edema is touchy to cyclooxygenase inhibitors and are used to evaluate the impact of non-steroidal anti-inflammatory agents, which mainly inhibit the cyclooxygenase involved in prostaglandin synthesis.²⁸

Table 3. Anti-inflammatory effects of *Tapinanthus globiferus* leaf extracts in Wister rats.

Treatments	Dose (mg/kg)	MI±SEM					
		0 hour	1 hour	2 hour	3 hour	4 hour	5 hour
Normal saline	10 ml/kg	1.63 ± 0.05	4.73 ± 0.44	4.50 ± 0.41	4.30 ± 0.34	3.52 ± 0.17	3.37 ± 0.40
TGHE	250	1.71 ± 0.03	3.71 ± 0.05	2.91 ± 0.04*	2.50 ± 0.15*	2.59 ± 0.15	2.11 ± 0.05
	500	1.74 ± 0.66	3.45 ± 0.08	3.15 ± 0.07	3.10 ± 0.06	2.34 ± 0.16*	2.24 ± 0.07
	1000	1.86 ± 0.50	2.77 ± 0.5*	2.76 ± 0.51*	2.41 ± 0.51*	2.41 ± 0.44	2.16 ± 0.50
TGEE	250	1.97 ± 0.07	3.14 ± 0.20*	3.58 ± 0.09	3.98 ± 0.19	2.97 ± 0.07	3.14 ± 0.08
	500	1.69 ± 0.10	2.83 ± 0.16*	3.97 ± 0.34	3.16 ± 0.09*	3.02 ± 0.22	2.61 ± 0.11
	1000	1.88 ± 0.08	2.79 ± 0.17*	3.84 ± 0.30	3.97 ± 0.28	2.81 ± 0.08*	3.52 ± 0.22
TGME	250	1.97 ± 0.13	2.97 ± 0.13*	2.67 ± 0.14*	2.47 ± 0.14*	2.62 ± 0.12	2.65 ± 0.10
	500	1.49 ± 0.38	3.14 ± 0.79	2.45 ± 0.61*	2.10 ± 0.53*	2.10 ± 0.51*	1.93 ± 0.49*
	1,000	1.57 ± 0.41	2.69 ± 0.68	2.22 ± 0.57*	1.97 ± 0.05*	2.05 ± 0.52*	1.85 ± 0.47*
Piroxicam	20	1.58 ± 0.15	2.55 ± 0.28*	2.57 ± 0.13*	2.62 ± 0.16*	2.32 ± 0.16*	1.99 ± 0.11*

Key: MI-mean inflammation, SEM-standard error of mean, (-) - negative, (+) – positive, *: values are statistically significant at (p<0.05) compared with negative control, (n=5). TGHE = *Tapinanthus globiferus* hexane extract, TGEE = *Tapinanthus globiferus* ethyl acetate extract, TGME= *Tapinanthus globiferus* methanol extract

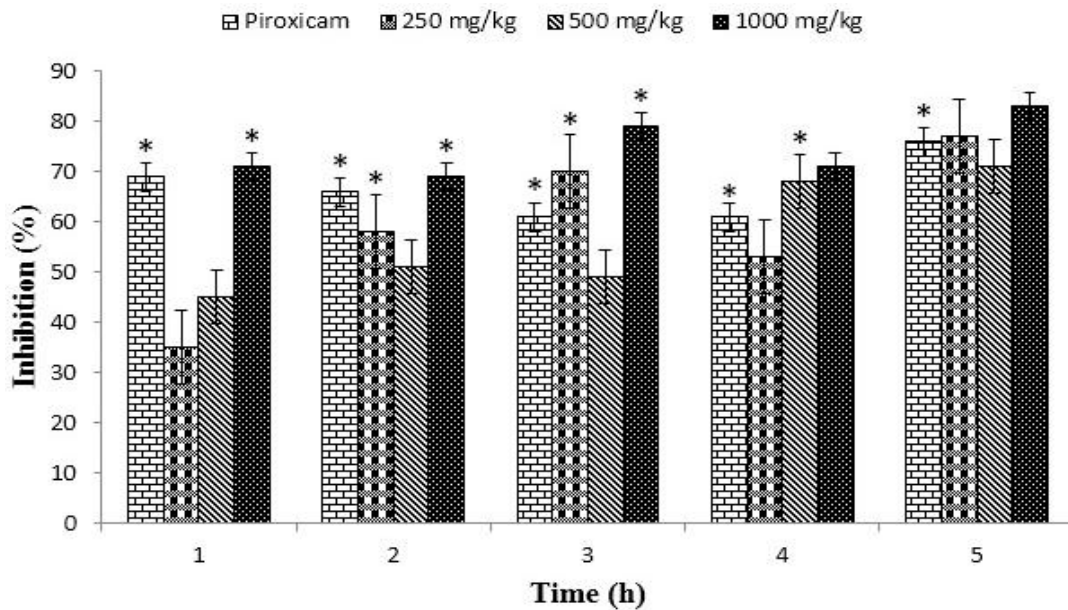


Figure 4. Effect of *Tapinanthus globiferus* hexane extract on percentage inflammatory inhibition in rats.

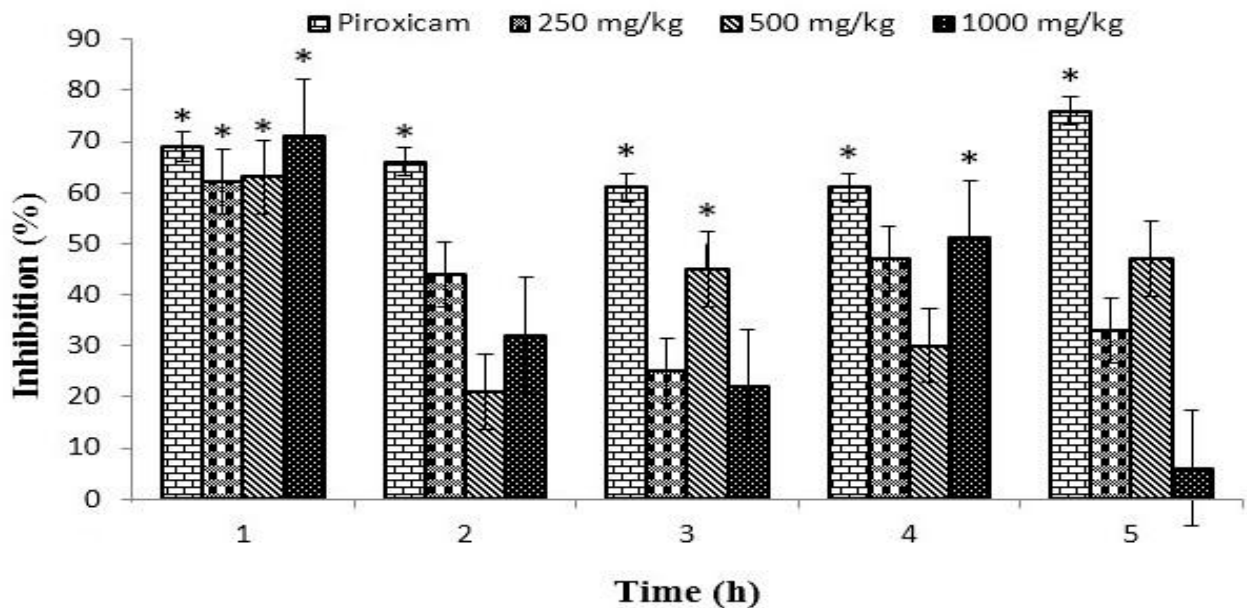


Figure 5. Effect of *Tapinanthus globiferus* ethyl acetate extract on percentage inflammatory inhibition in rats.

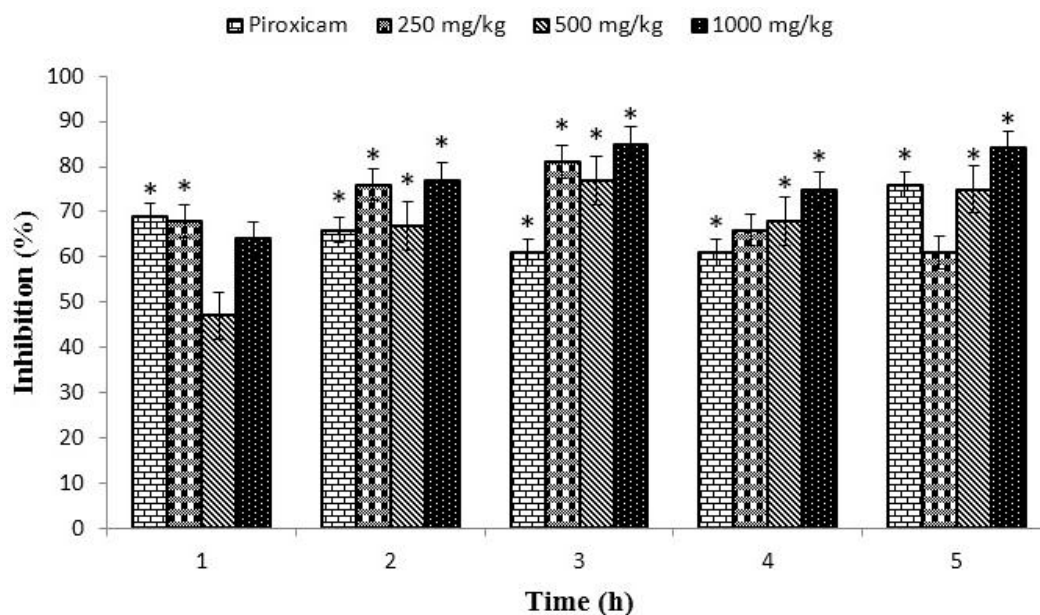


Figure 6. Effect of *Tapinanthus globiferus* methanol extract on percentage inflammatory inhibition in rats.

It performs a major role in the improvement of the second phase of inflammatory reaction, which is measured at the third hour.²⁹ The anti-inflammatory activities of the leaf extracts of *T. globiferus* (TGHE, TGEE and TGME) using carrageenan-induced inflammation have been established in this study (Table 3). There is a significant percentage inhibition ($p \leq 0.05$) of paw edema at the 1st hour for 1,000 mg/kg and at the third hour for 250 and 1,000 mg/kg doses of TGHE however there was no significant percentage inhibition ($p \geq 0.05$) for 250 and 500 mg/kg at the 1st hour (Figure 4). There was significant percentage inhibition ($p \leq 0.05$) of paw edema at the 1st hour for 250, 500, and 1,000 mg/kg doses of the TGEE (Figure 5). However at the third hour, there was no significant percentage inhibition ($p \geq 0.05$) at 500 mg/kg. TGME showed a significant percentage inhibition ($p \leq 0.05$) at the 1st hour for 250, 500 and 1,000 mg/kg doses of the extract, likewise at the third hour it also showed significant percentage inhibition ($p \leq 0.05$) for all the three doses (Figure 6). Therefore, it can be inferred that the feasible inhibitory impact of hexane, ethyl acetate and methanol extract of *T. globiferus* in carrageenan-induced inflammation might also be due to inhibition of cyclooxygenase which takes place when the drug binds the isoforms of cyclooxygenase (COX1 and COX), thereby stalling phospholipase A₂ activity and conversion of arachidonic acid into prostaglandin precursors at the rate limiting cyclooxygenase enzyme step or may also inhibit the activation of neutrophils thereby contributing to its feasible anti-inflammatory effects.²⁸ Both TGHE and TGEE at 1,000 mg/kg as well as 250, 500 and 1,000 mg/kg doses of TGME produced greater percentage inhibition when compared to 250 and 500 mg/kg for both TGHE and TGEE at the first and the third hour. TGME was observed to have the best activity compared to other extracts. These could be attributed to the presence of flavonoids, saponins, and phenolic compounds in the

extracts due to the fact flavonoids and phenolic compounds have been reported to have anti-inflammatory activity.³⁰ Flavonoids have been stated to inhibit pro-inflammatory mediators such as TNF- α and phospholipase A₂.³¹

Furthermore, some flavonoids respond by blocking both the cyclooxygenase and lipoxygenase pathways of the arachidonate cascade at relatively high concentration while at the lower level only the lipoxygenase pathway is blocked.³² Different types of saponins isolated from plants like *Phytolacca americana*, *Madhuca longifolia* and *Carissa edulis* have reportedly exhibited significant anti-inflammatory activity.³³ Steroids also attenuate inflammation by inhibiting phospholipase A₂, which hydrolyzes arachidonic acid from membrane phospholipids and subsequent formation of prostanoids and leukotrienes through the cyclooxygenase and lipoxygenase pathways.³⁴

Conclusion

It can be concluded that the leaves extracts of *T. globiferus* exhibited a significant anti-inflammatory activity on carrageenan induced paw edema in experimental rats, validating the ethno-medicine claims. These activities may be due to the strong occurrence of polyphenolic compounds such as flavonoids, tannins, steroids, and phenols in the extracts. The methanol extract of *T. globiferus* demonstrated a dose-dependent response and were comparable to piroxicam (reference drug). Based on this, further studies should be carried out to isolate, characterize and elucidate the structure of active principles responsible for the anti-inflammatory activity of the plant and investigating its mechanism of action.

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Conflict of interests

The authors claim that there is no conflict of interest.

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