International Journal of Research in Pharmacology & Pharmacotherapeutics (IJRPP)

IJRPP |Vol.13 | Issue 2 | Apr - June -2024

www.ijrpp.com

DOI : https://doi.org/10.61096/ijrpp.v13.iss2.2024.126-133

ISSN: 2278-2648

Research

Evaluation of analgesic and anti-inflammatory activity of ethanolic extract from talinum triangulare on experimental animals

Mumtaj Begum. A[*1], Pavithra. K P S[2], Tamilselvan. G[2], Senthil Kumar. K L[3]

*[*1] Scholar, Department of pharmacology, Sri Vijay Vidyalaya College of Pharmacy, Nallampalli, Dharmapuri Affiliated to the TN Dr.MGR Medical University, Guindy, Chennai, Tamil Nadu, India.*
[2]Associate Professor, Department of pharmacology, Sri Vijay Vidyalaya College of Pharmacy, Nallampalli, Dharmapuri Affiliated to the TN Dr.MGR Medical University, Guindy, Chennai, Tamil Nadu, India.
[3]Principal, Sri Vijay Vidyalaya College of Pharmacy, Nallampalli, Dharmapuri Affiliated to the TN Dr.MGR Medical University, Guindy, Chennai, Tamil Nadu, India.

*Author for Correspondence: A. Mumtaj Begum
Email: mumtajabdulla96@gmail.com

Published on: 18 May 2024

Published by:
DrSriram Publications

Abstract

Aim: The study aimed to evaluate the analgesic and anti- inflammatory activity of ethanolic extract from *Talinum Triangulare* on experimental study.
Methods:*Talinum triangulare* plant was extracted with ethanol and the leaves were dried, grind, and extracted with ethanol.The Phytochemical analysis was conducted using various test.Albino Wistar rats of either sex weighing (180-200g) and adult male albino mice (25-35g)were used in this study.*Talinum Triangulare* leaves extract was used to evaluate analgesic activity by acetic acid induced writhing method and anti-inflammatory activity by carrageenan-induced hind pawedema method.The study was conducted with prior approval of Institutional Animal Ethical Committee.
Result: In acetic acid induced writhing method,*talinum triangulare* shows analgesic activity which was statistically significant as compared to control($P<0.05$) but less than aceclofenac sodium. In the model of acute inflammation i.e.,carrageenin -induced paw edema in rats , *talinum triangulare* shows anti -inflammatory activity which was statistically significant as compared to control($P<0.05$) but less than indomethacin.
Conclusion: The result of this study suggests that the ethanolic extract of *talinum triangulare* leaves has a potential analgesic and anti-inflammatory activity.

Keywords: Anti-Inflammatory, analgesic, talinum triangulare.

INTRODUCTION

Inflammation acts as a protective mechanism aimed at eliminating the initial cause of cell injury, as well as necrotic cells and tissues resulting from the insult. Various factors such as pathogens, abrasions, chemical irritants, cellular distortions, and extreme temperatures can trigger inflammation.[1] It represents the body's concerted effort to eradicate microbes, toxins, or foreign substances at the site of injury, preventing their spread to other tissues, and initiating the process of tissue repair to restore tissue homeostasis.[2] *Talinum triangulare*, also known as Water leaf, is a leafy vegetable belonging to the family Portulaceae. It is a herbaceous perennial plant

typically characterized by its above-ground stem.[3] While the genus Talinum is generally self-pollinated, there is a tendency for population heterogeneity due to the plant's inclination towards insect pollination.This erect, glabrous perennial herb features succulent stems and leaves, with swollen, fleshy rootsand purple flowers.[4] Widely consumed across tropical regions, particularly in West and Central African countries like Nigeria and Cameroon, *Talinum triangulare* is commonly cultivated, primarily through seed propagation and cuttings.[5] It is valued not only for its taste but also for its nutritional andpotential medicinal properties. While there may not be an exhaustive list of phytochemical constituents specific to *Talinum triangulare* readily available, several studies have investigated its chemical composition and potential health benefits. Here are some of the phytochemical constituents that have been identified in *Talinum triangulare*.[6]They include flavonoids, Glycosides, saponins, steroids, phenolic acids, and tannins, alkaloids, carbohydrates, fats, resins, terpenoids. *Talinum triangulare* is rich in various vitamins and minerals, including vitamin C, vitamin A, iron, calcium, and potassium. These nutrients are essentialfor human health and contribute to the nutritional value of the plant.[7]

METHODS

Collection and Authentication of plant

The plant was collected from Bargur, Krishnagiri district, Tamil Nadu. It was identified and authenticated by Assistant Professor Dr.S.Jagatheshkumar, Department of Botany, Sri Vijay Vidyalaya College of Arts and Science, Nallampalli, Dharmapuri.[8]

Cleaning and Drying

Thoroughly clean the collected plant material to remove any dirt ordebris. Allow it to air dry in a shaded area to prevent degradation of active compounds Alternatively, you can use a food dehydrator or an oven set to a low temperature for drying.[9]

Grinding or Crushing

Once dried, grind the plant material into a coarse powder using amortar and pestle or an electric grinder. Ensure that the powder is uniform in texture to facilitate extraction.

Ethanol Extraction

Place the powdered plant material in a clean, dry container and cover it with ethanol (e.g., 70% ethanol). Seal the container and let it soak fora specified period, with occasional shaking or stirring. After extraction, filter the mixture to remove solid particles, and then evaporate the ethanol under reduced pressure using a rotary evaporator or by air-drying to obtain the crude extract.[10]

Phytochemical Screening

They include flavonoids, Glycosides, saponins, steroids, phenolic acids, and tannins, alkaloids, carbohydrates, fats, resins, terpenoids.[11]

Table 1: Preliminary Phytochemical Studies of various test

Sl. No	Phytoconstituents	Name of the test	Procedure	Inference
1.	Alkaloids	Dregendroffs test	1ml extract + 2drops of dregendroffs reagent	Formation of orange red precipitate
2.	Tannins	Ferric chloride test	2ml of extract+ few drops of 1%ferric chloride	Formation of blue green precipitate
3.	Glycoside	Fehling's test	1ml of extract+ 1ml of Fehling's A and B solution, water bath for 2-4 mins	tion of redcolour
4.	Saponins	Froth formation test	1ml of extract+ 1ml or 2ml of distill water, shake well	Formation of 1cm foam layer
5.	Flavonoids	Shinoda test	1ml of extract + 3ml of ethanoate solution	Appearance of pale yellow brown colour

Experimental Animals

Young adult Wistar albino rats (150-250g) of either sex and adult male albino mice(25-35g) wereobtained from the small animals breeding station. They were housed in polypropylene cages understandard environmental conditions, including a 12-hour light/dark cycle, a temperature of 25±2°C,and humidity ranging from 35% to 60%. Adequate air ventilation was provided, and the rats weregiven standard pellet diet (from M/s. Hindustan Lever Ltd., Mumbai, India) and fresh water ad libitum. Prior to the commencement of the experiments, the animals were

allowed to acclimatize to the laboratory environment for two weeks.[12]

Pharmacological Screening
Anti-inflammatory activity by carrageenan-induced paw oedema method.

Procedure

Carrageenan-induced rat paw oedema is used widely as a working model of inflammation in the search for new anti-inflammatory drug. The anti-inflammatory activity of the ethanolic extract of*Talinum triangulare* was evaluated by carrageenan-induced rat paw oedema method.[13] Adult wistar albino rats (150-250g) were used. Anti-inflammatory activity was measured using carrageenan induced rat paw oedema method. The rats were divided into 5 groups of 5 animals each. Group I. were given normal saline and treated as negative control. Rats of Group II was treated with carrageenan (1%w/v) in saline in the sub-planter region of the right hind paw. Rats inGroup III were administered Indomethacin (10 mg/kg, b.w) and considered as standard. Rats fromGroup IV and V were given two doses of ethanolic extract of *Talinum triangulare* (200 and 400 mg/kg b.w). Acute paw edema was induced by injecting 0.1 ml of 1% (w/v) carrageenan solution,prepared in normal saline. After 1 h, 0.1 ml, 1% carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. The linear paw circumference will be measured at hourly interval for 4 h. The perimeter of paw was measured by using digital pleythsmometer. Measurements were taken at 0–4 h after the administration of the carrageenan. The anti-inflammatory activity was calculated by using the relation:

$$\% \text{ inhibition of edema} = \frac{T - T0}{V} \times 100$$

T: Thickness of paw in control group
T0: Thickness of paw edema in the test compound treated group

Anti- Inflammatory Carrageenan Induced Pleurisy in Rats

The animals were divided into five groups of five rats each as described in the carrageenan inducedpaw edema model [14][15] and each were pretreated with ethanolic extract of *Talinum triangulare* (200 and 400 mg/kg, p.o.), Indomethacin (10 mg/kg, p.o.) or normal saline (0.1 ml). One hour laterall the animals were received 0.25 ml of an intrapleural injection of 1 % carrageenan on the right side of the thorax. The animals were sacrificed 3 h after carrageenan injection by ether inhalation.One ml of heparinized Hank's solution was injected into the pleural cavity and gently massaged to mix its contents. The fluid was aspirated out of the cavity and the exudates were collected. Thenumber of migrating leukocytes in the exudates was determined with Neubauer chamber.
The values of each experimental group were expressed as mean ± SEM and compared with the control group.

Statistical analysis

Results of anti-inflammatory activity were expressed as Mean increase in paw diameter ± SD. Results were analyzed using one way ANOVA. Differences were considered as statisticallysignificant at $P < 0.05$ are compared to control

Analgesic activity was assessed by acetic acid induced writhing test
Procedure

The acetic-acid writhing test was performed using the Aoki *et al* procedure. Groups of rats (n=6), were administered with ethanolic extract of *Talinum triangulare* at a dose of200 and 400mg/kg and 10 mg/Kg aceclofenac as positive control group and 5 ml distilled water as negative control group. After 30 minutes the animals were administered with i.p.injection of 0.1 ml acetic acid (0.6%). Then the count of abdominal contractions of animals during 30 minutesafter acetic acid injection was reported and the Percentage Analgesic Activity (PAA) wascalculated by using the following formula:

$$\mathbf{PAA = ((C- CD)/CD) \times 100}$$

C = Mean of contractions' count in animals treated with different doses of ethanolic extract of *Talinum triangulare* and aceclofenac sodium.
CD = Mean of contractions' count in animals served as negative control

Statistical analysis

The results are reported as mean ± S.E.M. The statistical analyses were performed using one wayanalysis of variance (ANOVA). Group differences were calculated by post hoc analysis using newmann keuls multiple range tests. For all tests, differences with values of $P<0.05$ were considered significant.

RESULT

Acetic acid-induced writhing response

The study showed that the application of different doses of ethanolic extract of *Talinum triangulare* had significant analgesic effects in the animals under investigation. The results of doses 200 and 400 mg/kg were significant and comparable with the effect of aceclofenac sodium in analgesic activity (Table 1)

Table 2: Effects of ethanolic extract of *Talinum triangulare* on acetic acid induced writhingresponse (N=6 in each group).

Groups	Treatment	(number of writhing movements) (Mean ± S.E)	Percentage %
Control	Distilled water	38.5±3.0	-
Standard	Aceclofenac sodium 10mg/kg	9.6±0.85	75.06%*b
Treatment	ethanolic extract of *Talinum triangulare* 200mg/kg	13.4±1.20	65.19%*b
Treatment	ethanolic extract of *Talinumtriangulare* 400mg/kg	12.2±1.12	68.31%*b

- Values are expressed as mean ± SEM.
- Values are significantly different from Toxic control G1 at P<0.01.

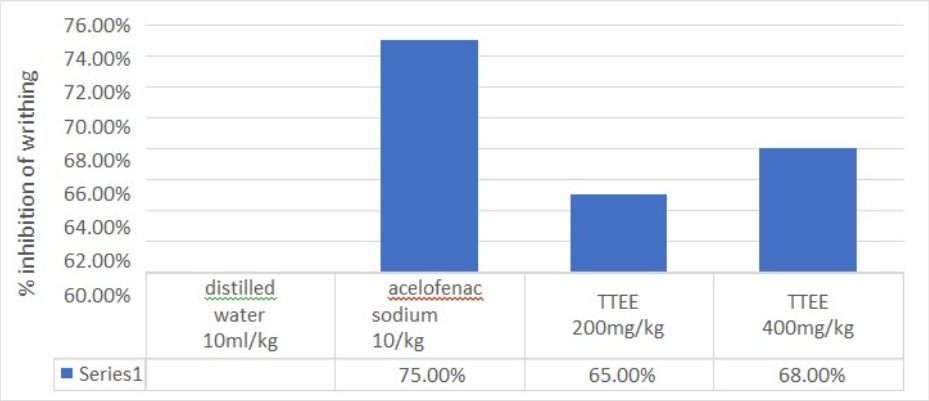

Fig 1: Effects of ethanolic extract of *Talinum triangulare* on acetic acid–inducedwrithing response

Anti-inflammatory Activity of ethanolic extract of *Talinum triangulare*

The effect of ethanolic extract of *Talinum triangulare* on carrageenan-induced edema in rats is shown in Table 3. The results obtained indicate that theethanolic extract of *Talinum triangulare* had significant anti-inflammatory activity in rats. The ethanolic extract of *Talinum triangulare* reduced the edema induced by carrageenan by 55.40% and 58.16% on oral administration of 200 and 400 mg/kg, as compared to the untreated control group. Indomethacin at 10 mg/kg inhibited the edema volume by 62.05%. The effect of ethanolic extract of *Talinum triangulare* on carrageenan-induced pleurisy in rats is shown in Table 2. The volume of pleural exudates in the toxic control group was 0.44±0.17 ml. Animals treated with the ethanolic extract of *Talinum triangulare* (200 and 400 mg/kg, p.o.) decreased the pleural exudates to 0.29±0.13 ml and 0.24±0.11. Treatment with Indomethacin (10 mg/kg, p.o.) produced the exudates of 0.21±0.08 ml. The leukocyte count for the control group was found to be $4.35\pm0.40\times10^3$ cells/ml. Animals treated with the ethanolic extract of *Talinum triangulare* and standard produced a leukocyte migration of $0.68\pm0.15\times10^3$,$0.60\pm0.12\text{x}10^3$ and $0.54\pm0.08\times10^3$ cells/ml, respectively.

Table 3: Effect of ethanolic extract of *Talinum triangulare* on Carrageenan Induced RatPaw Edema

Treatment	Dose (mg/kg, p.o.)	Mean increasein paw volume (ml)	% Decreasein paw volume
control	10ml/kg saline	0.92 ± 0.09	-
Carrageenan	0.1 ml, 1% carrageenan	4.35 ± 0.48*a	-
Standard	10mg/kg	1.65 ± 0.24*b	62.05%

	Indomethacin		
Treatment	200mg/kg ethanolicextract of *Talinum triangulare*	1.94 ± 0.36*b	55.40%
Treatment	400mg/kg ethanolicextract of *Talinum triangulare*	1.82 ± 0.30*b	58.16%

- Values are expressed as mean ± SEM.
- Values were compared by using analysis of variance (ANOVA) followed byNewman-Keul's multiple range tests.
- (a)Values are significantly different from normal control G1 at P<0.01.
- (b) Values are significantly different from Toxic control G2 at P<0.01.

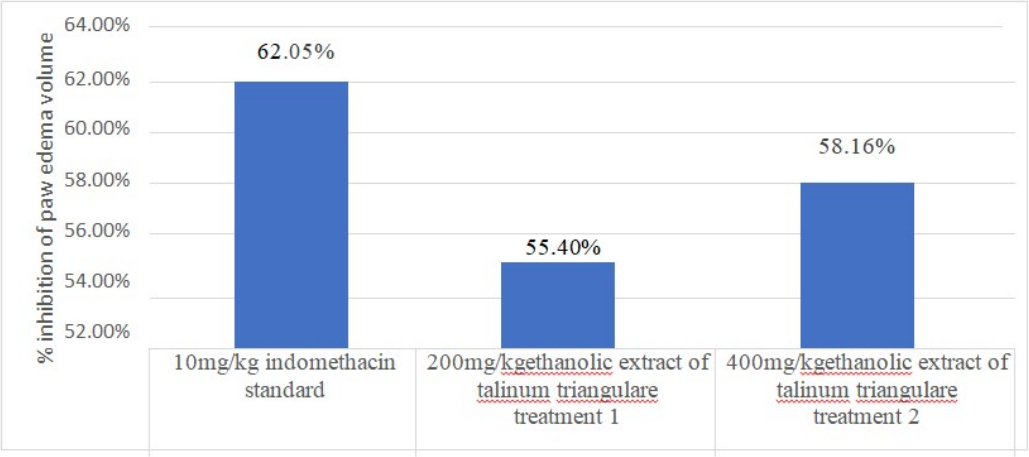

Fig 2: Effect of ethanolic extract of *Talinum triangulare* on Carrageenan Induced RatPaw Edema

Table 4: Effect of ethanolic extract of *Talinum triangulare* on Carrageenan InducedPleurisy in Rats.

Treatment	Dose (mg/kg, p.o.)	Pleural exudates (ml)	Leukocytes ($\times 10^3$ cells/ml)
Normal	10ml/kg saline	0.14±0.04	0.38±0.04
Carrageenan	0.1 ml, 1% carrageenan	0.44±0.17*a	4.35±0.40*a
Standard	10mg/kg Indomethacin	0.21±0.08*b	0.54±0.08*b
Treatment	200mg/kg ethanolic extract of *Talinum triangulare*	0.29±0.13*b	0.68±0.15*b
Treatment	400mg/kg ethanolic extract of *Talinum triangulare*	0.24±0.11*b	0.60±0.12*b

- Values are expressed as mean ± SEM.
- Values were compared by using analysis of variance (ANOVA) followed byNewman-Keul's multiple range tests.
- (a)Values are significantly different from normal control G1 at P<0.01.
- (b) Values are significantly different from Toxic control G2 at P<0.01.

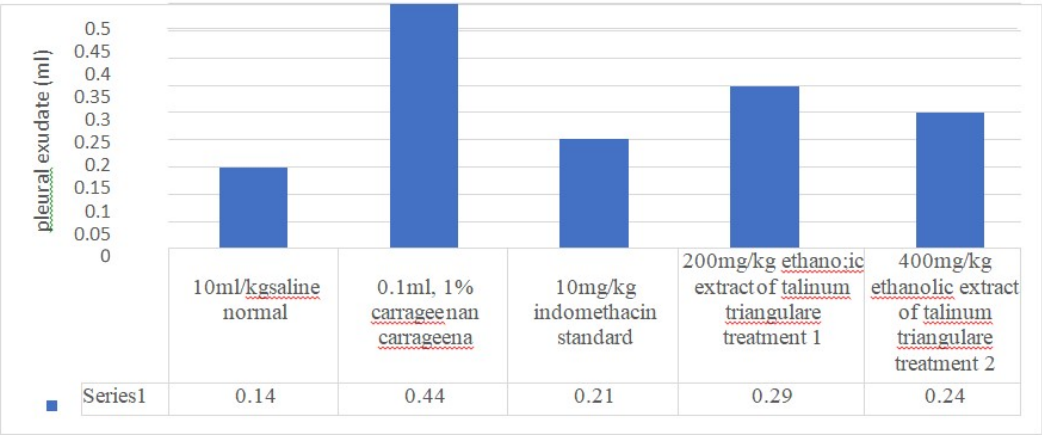

Fig 3: Effect of ethanolic extract of *Talinum triangulare* on Carrageenan InducedPleurisy in Rats.

DISCUSSION

The study investigated the analgesic properties of the ethanol leaf extract derived from *Talinum triangulare* in mice, particularly focusing on its effects on both peripheral and central nociception.The acetic acid-induced writhing test was employed as a model to evaluate peripheral-mediated analgesic activity.[16][17] The results demonstrated a significant reduction in writhing responses, indicating the extract's potential to alleviate peripheral pain. This finding is consistent with previous studies by Williamson et al[18] and Koster et al[19], which highlighted the efficacy of the writhing test in assessing peripherally-acting analgesics.

Furthermore, the extract exhibited a notable increase in reaction latency to thermally-induced painon the hot plate test, suggesting its involvement in central nociception modulation. This observation aligns with earlier research emphasizing the specificity of the hot plate test for centrally mediated pain perception reported by Florence et al.[20] Thus, the ethanol leaf extract of *Talinum triangulare* appears to possess a dual mechanism of action, targeting both peripheral andcentral pathways to alleviate pain.The anti-nociceptive activity of the extract was found to be dose-dependent, indicating a potentialcorrelation between dosage and efficacy. This suggests that certain bioactive constituents within the extract, such as flavonoids, saponins, or phenolic compounds, may contribute to its analgesic effects. Notably, acetic acid-induced pain is associated with an increase in prostaglandin levels inperitoneal fluid, implying a possible mechanism of action involving the inhibition of prostaglandinsynthesis by the extract.[21] Moreover, the study underscores the importance of further investigating specific chemical components responsible for the observed analgesic effects. Particularly, terpenoids, especially saponins, are implicated as potential candidates warranting further exploration. By elucidating the molecular mechanisms underlying the analgesic properties of *Talinum triangulare* extract, future research can pave the way for the development of novel therapeutics for pain management and inflammatory disorders. The ethanolic leaf extract of *Talinum triangulare* demonstrates promising anti-nociceptive properties, acting through both peripheral and central mechanisms. Its ability to modulate pain perception suggests potential therapeutic applications in managing various painful conditions, necessitating further investigationinto its molecular constituents and mechanisms of action. The escalating use of NSAIDs and their accompanying side effects prompt a critical exploration of plant extracts with potentially fewer adverse effects. Thus, there's an ongoing pursuit of indigenous remedies offering anti-inflammatory relief. Carrageenan-induced inflammation, characterized by a biphasic process involving histamine, 5-hydroxytryptamine, kinin-like substances, and prostaglandin-like substances, underscores the complexity of inflammatory pathways.[22] Understanding these mediators is pivotal for elucidating the mechanisms of action of potential therapeutic agents. In our investigation, the ethanolic extract of Talinum triangulare exhibited promising results in a pleurisy model, demonstrating its ability to inhibit leukocyte migration and pleural exudate formation when administered orally.[23]These findings align with priorresearch, indicating the extract's potential as an anti-inflammatory agent. Furthermore, our anti- inflammatory studies revealed a significant reduction in edema in the hind paws of rats treated with the leaf extract. The observed anti-inflammatory effects may be attributed to the extract's modulation of the cyclooxygenase (COX) pathway of arachidonate metabolism, leading to the production of prostaglandins which play crucial roles in various inflammatory processes.[24]Additionally, the extract may inhibit the synthesis of inflammatory mediators, including polypeptide kinins and prostaglandins, thereby contributing to its anti-inflammatory properties.

Existing literature reports the analgesic and anti-inflammatory effects of flavonoids, steroids, andtannins, suggesting their potential involvement in mediating the effects of *Talinum triangulare* extract.[25]Therefore, it's plausible that the observed analgesic and anti-inflammatory effects of the *Talinum triangulare* leaf extract may be

attributed, either individually or synergistically, to these bioactive constituents. In summary, our study underscores the significant anti-inflammatory activity of the ethanolic extract of *Talinum triangulare* in rats.

CONCLUSION

The ethanolic extract of Talinum triangulare demonstrated a dose-dependent, significant anti- nociceptive activity in animal models of pain in mice, and the ethanolic extract of *Talinum triangulare* possesses significant anti-inflammatory activity in rats. Further exploration, including purification and elucidation of biochemical pathways, holds promise for developing a potent anti-inflammatory agent with a favorable safety profile and an improved therapeutic index. This highlights the potential of *Talinum triangulare* extract as a valuable natural remedy for managinginflammation and associated conditions, offering insights into its intricate mechanisms of action within the inflammatory cascade.

ACKNOWLEDGMENTS

We would like to give thanks to Sri Vijay Vidyalaya College of Pharmacy, Department of Pharmacology, Nallampalli, Dharmapuri, Tamil Nadu for providing laboratory facilities and necessary reagent during this study.

REFERENCES

1. Kumar, Abbas, Faristo, Mitchell. Robbin's Basic Pathology. 8th edition. chapter 2;31-58
2. Undewood JCE. General & systematic pathology,4th edition. Churchill Livingstone. Chapter 10: Inflammation, 2005;202.
3. USDA,Talinum triangulare [online]. Natural resource conservation service,plant Database,available at:https//plants.usda.gov/jawa/classification servlet? Source=display &classide=TATR2[Accessed on; 09.04.2020]
4. ITIS Standard Report page,talinum triangulare(Jacq).wild[online].Available at;hppts://www.itis.gov.
5. AOAC, 2005. International official methods of analysis. 18th ed. Gaithersburg, M.D.:AOACInternational.
6. Harbourne, N. V., 1993. Phytochemical method: A guide to modern technique of plant analysis. 2nd ed. London: Fakenhamn Press Ltd.
7. Sofowora, A. E., 1993. Medicinal plants and traditional medicines in Africa. 2nd ed. Nigeria:Spectrum Books, Ibadan. p. 289.
8. Tiamiyu, A. M., 2019. Feed additive potentials and antibacterial effects of allium sativum, Chromolaena odorata and Talinum triangulare against Pseudomonas aeruginosa infection in clarias gariepinus burchell, 1822. PhD Thesis. University of Ibadan, Post Graduate School.
9. Liang D, Zhou Q, Gong W, Wang Y, Nie Z, He H, Li J, Wu J, Wu C, Zhang J. (2011). Studieson the antioxidant and hepatoprotective activities of polysaccharides from Talinum triangulare. Journal of Ethnopharmacol; 136(2):316–321. https://www.sciencedirect.com/science/article/pii/S0378874111002996
10. Ezekwe, C. I., Chidinma, R. U. and Okechukwu, P. C. U., The Effect of Methanol Extract of Talinum Triangulare (Water Leaf) on the Hematology and Some Liver Parameters of Experimental Rats, Global Journal of Biotechnology & Biochemistry. 2013; 8(2):51-60. https://www.academia.edu/download/80568839/3.pdf
11. Mensah J. K., Okoli R. I., Ohaju-Obodo J. O. and Eifediyi K. Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. African Journal of Biotechnology, 2008; 7(14): 2304-2309. https://www.ajol.info/index.php/ajb/article/view/58988
12. Afolabi OB, Oloyede OI. Antioxidant Properties of the Extracts of Talinum Triangulare and its Effect on Antioxidant enzymes in Tissue Homogenate of Swiss Albino Rat. Toxicol Int. 2014 Sep-Dec;21(3):307-13. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4413415/
13. C.A. Winter, E.A. Risley, G.W. Nuss, Carrageenan-induced oedemain the hind paw of rat as an assay for anti-inflammatory activity, Proc. Soc. Exp. Biol. Ther. 1962;111: 544–547.
14. Tomlinson A, Appleton I, Moore AR, Gilroy DW, Willis D,Mitchell JA, Willoughby DA. Cyclo-oxygenase and nitric oxidesynthase isoforms in rat carrageenin-induced pleurisy. Br JPharmacol 1994;113:693–98. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1510410/
15. Vinegar R, Truax JF, Selph JL, Voelker FA. Pathway of onset,development and decay of carrageenan pleurisy in the rat. FedProc 1982;41:2588-95. https://europepmc.org/article/med/6806127
16. Abdollahi M, Karimpour H and Monsef-Esfehani HR. Anti-nociceptive effects Of Teucrium polium L. total extract and essential oil in mouse writhing test. Pharmacol. Res. 2003; 48: 31-35. https://europepmc.org/article/med/6806127

17. Golshani S, Karamkhani F, Monsef-Esfehani HR and Abdollahi M. Anti-nociceptive effects of the essential oil of Dracocephalum kotschyi in the mouse writhing test. J. Pharm. Pharm. Sci. 2004;7: 76-79.
18. Williamson EM, Okpako DT, Evans FJ. Selection, Preparation and Pharmacological Evaluation of Plant Materials. Vol. 1. Chichester: John Wiley; 1996. Pharmacological Methods in Phytotherapy Research; pp. 184–186.
19. Koster R, Anderson M, Debeer E J. Acetic acid for analgesic screening. *Federation Proceeding.* 1959;18:412–417 https://www.sid.ir/paper/540037/en
20. Florence N, Claire S, Olga V, Rafaël M, Pascale C, Serge T, Marie C, Fournié Z, Bernard P. Pain-suppressive effects on various nociceptive stimuli (thermal, Chemical, electrical and inflammatory) of the first orally active enkephalin-Metabolizing enzyme inhibitor RB 120. *Pain.* 1997;73(3):383–391. https://www.sciencedirect.com/science/article/pii/S0304395997001255
21. Francis G, Kerem Z, Makkar HP, Becker K. The biological action of saponins in animal systems: a review. British journal of Nutrition. 2002 Dec;88(6):587-605.
22. Vinegar R, Schreiber W, Hugo RJ. Biphasic development of carrageenin edema in rats. *J Pharmacol Exp Ther* 1969; 166(1): 96- 103. https://jpet.aspetjournals.org/content/166/1/96.short
23. Mikami T, Miyasaka K. Effects of several anti-inflammatory drugs on the various parameters involved in the inflammatory response in rat carrageenin-induced pleurisy. European Journal of Pharmacology. 1983 Nov 11;95(1-2):1-2. https://www.sciencedirect.com/science/article/pii/0014299983902613
24. Tomlinson A, Appleton I, Moore AR, Gilroy DW, Willis D, Mitchell JA, Willoughby DA. Cyclo-oxygenase and nitric oxide synthase isoforms in rat carrageenin-induced pleurisy. British journal of pharmacology. 1994 Nov;113(3):693. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1510410/
25. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Proceedings of the society for experimental biology and medicine. 1962 Dec;111(3):544-7. https://journals.sagepub.com/doi/abs/10.3181/00379727-111-27849