Asian Journal of Research in Biochemistry

Volume 14, Issue 4, Page 149-156, 2024; Article no.AJRB.119630
ISSN: 2582-0516

Anti-inflammatory Property of Methanolic Extract of *Zanthoxylum leprieurii* Guill. & Perr. Root Bark

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Authors' contributions

This work was carried out in collaboration among all authors. The authors of this article assume full responsibility for any claims related to its content. We acknowledge that the authors named in this specific article are the ones who conducted the work. Author FIE was accountable for the inception, development, and synchronization of the project. Author EJU conducted the laboratory experiments and also drafted the manuscript. Author VCA edited the manuscript and provided significant inputs. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajrb/2024/v14i4303

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/119630

Original Research Article

Received: 02/05/2024
Accepted: 03/07/2024
Published: 05/07/2024

ABSTRACT

Purpose: The purpose of this study was to assess the phytochemical composition and anti-inflammatory effects of an aqueous methanolic extract derived from the root bark of *Zanthoxylum leprieurii*, a tropical plant used traditionally in the treatment of inflammation in Nigeria.

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Cite as: *Ugboma, Evaristus J., Fabian I. Eze, and Victor C. Amaechi. 2024. "Anti-Inflammatory Property of Methanolic Extract of Zanthoxylum Leprieurii Guill. & Perr. Root Bark". Asian Journal of Research in Biochemistry 14 (4):149-56. https://doi.org/10.9734/ajrb/2024/v14i4303.*

Methods: Preliminary phytochemical screening of the aqueous-methanol extract of the root bark of *Zanthoxylum leprieurii* was done to determine the main secondary metabolites. The anti-inflammatory activity of the crude extract of *zanthoxylum leprieurii* root back was determined using the egg albumin-induced model of inflammation, and the results were analyzed using a one-way ANOVA and student t-test.
Results: The yield after extraction was 25g. The phytochemical screening indicates the presence of the following metabolites: tannins, flavonoids, alkaloids, and saponins. The paw edema inhibition percentages at the 3rd and 4th hours were 14.8% and 46.3% for the 200 mg/kg dose, and 26.5% and 70.3% for the 400 mg/kg dose, respectively.
Conclusion: The result of the *in vivo* anti-inflammatory evaluation carried out showed statistical significance and therefore justified the folkloric uses of the plant as an anti-inflammatory agent.

Keywords: Anti-inflammatory; phytochemical screening; methanolic extract; egg albumin-induced model; Zanthoxylum leprieurii.

1. INTRODUCTION

Inflammation is the body's response to disturbed normal homeostasis caused by various stimuli, including bacteria, fungi, viruses, parasites, protozoa, immunological injury, trauma, heat, cold, toxins, or poison, and irradiation [1]. “It is part of the complex biological response of body tissues to harmful stimuli, which can be acute, sub-acute, or chronic” [2]. “The pathological conditions associated with inflammation around the world include gonitis, bursitis, metritis, glossitis, keratitis, stomatitis, duodenitis, blepharitis, salpingitis, sore throat, peritonitis, sialadenitis, encephalitis, oesophagitis, and others” [3]. Inflammatory bowel disease incidence is on the rise on nearly every continent and often surfaces when patients are in their teens, twenties, or thirties, leading to many years of pain and disability as well as costly monitoring [4,5,6,7,8].

“Non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs, and corticosteroids have been the main drugs used to treat inflammation in the past” [9]. However, for socio-economic reasons prevalent, especially in developing countries, factors such as availability, acceptability, affordability, biodegradability, and cultural acceptance of medicinal plants have contributed to this resort to herbal drugs [10]. Plants and their extracts provide significant potential for inflammatory management and treatment due to their affordability and effectiveness, as they are generally safe to use and seldom cause hypersensitivity reactions [11]. “In vivo and in vitro studies have shown a lot of evidence to support the idea that different compounds from plants that have anti-inflammatory properties work by changing the cytokine system” [12]. “*Zanthoxylum leprieurii* Guill and Perr. (Family Rutaceae), also known as *Fagara angolensis* Engl. and *Fagara leprieurii* Engl., has wide distribution across tropical Africa. In southeastern Nigeria, particularly among rural inhabitants in the Nsukka district of Enugu State, people chew the root or stem bark and ingest the juice to treat gum and intestinal inflammations” [13]. Venezuelan traditional medicine commonly uses *Zanthoxylum monophyllum* to treat runny nose or nasal mucosal inflammation, jaundice, ophthalmology, and as an anesthetic [14].

Traditional use of *zanthoxylum leprieurii* as an anti-inflammatory agent lacks scientific assessment of its anti-inflammatory properties. Therefore, the study aims to conduct additional research to ascertain its influence on treating inflammation in vivo.

2. MATERIALS AND METHODS

2.1 Plant Material

The root bark of *Zanthoxylum leprieurii* was collected from a farm in the Nsukka local government area of Enugu State, Nigeria, and authenticated by Mr. A.O. Ozioko of the International Centre for Ethnomedicine and Drug Development, InterCEDD, Nsukka, where a voucher specimen (voucher no: InterCEDD/16017) was kept. The root bark of *Zanthoxylum leprieurii* was collected, washed in clean water, dried under shade at 28 to 30 °C, and pulverized by a mechanical grinder into powder.

2.2 Preparation and Extraction of Plant Material

A total of 500 g of the air-dried plant material was used for successive extractions with 1.5

liters of 95% methanol by the cold maceration method at room temperature. The mixture was stirred intermittently for 48 hours, sieved using a muslin cloth, and then filtered with filter paper. The extract was recovered using a rotary evaporator (Stuar, Stone, UK) at 40°C under minimal pressure, yielding 25 g (5%) crude extract of *Zanthoylum leprieurii*.

2.3 Experimental Animal

Wistar albino rats (18 rats) were collected from the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Science, University of Nigeria and were kept in stainless steel cages and served with a normal commercial rat diet, given water ad libitum, and maintained under laboratory conditions (temperature of 28°C to 30°C, relative humidity of 60%–70%, and normal light-dark cycle). Each animal weighed between 100 g and 250 g. The animals were acclimatized for 7 days with a standard pellet and water before the experiment. This was done to reduce the stress of experimental handling and conditions. Techniques and methods used in this study were performed following the National Institute of Health Guidelines for the Care and Use of Laboratory Animals [15].

2.4 Fractionation of the Plant Extract

The extract weighing 10 g was put in a mortar, and 100 g of silica was introduced into it. It was thoroughly mixed with the aid of a pestle to form a uniform mixture with the plant extract, after which 100 ml of 100% N-hexane was introduced into the separating funnel. The mixture was mixed for 3 minutes and allowed to stand for 24 hours, after which the N-hexane fraction was tapped off and another 100 ml of 100% N-hexane was introduced into the extract. The same procedure was repeated, as described above. The fractionation continued until the color of N-hexane became very clear, with an indication that there was no N-hexane-soluble component in the extract, after which the N-hexane fraction was evaporated using a rotary evaporator and the fraction was used in conducting the experiment. The same procedure was repeated using 100% ethyl acetate, 100% methanol, and water.

2.5 Qualitative Phytochemical Screening

Preliminary phytochemical screening of the aqueous-methanol extract of the root bark of *Z. leprieurii* was done to determine the main secondary metabolites according to the established method [16,17,18], and the total alkaloid, saponins, tannins, flavonoid, and steroid content of the extract was determined according to the existing method [19].

2.6 Acute Toxicity Test

The established procedure for LD_{50} determination was used [20]. This method employs 15 albino mice. The experiment involved a preliminary test in which three groups of mice (n = 3) were orally administered 10 mg/kg, 100 mg/kg, and 1000 mg/kg of *Zanthoxylum leprieurii* extract. The animals were constantly observed for 2 hours, intermittently for the next 4 hours, and then overnight. No deaths of animals per group were recorded at the end of the 24 hours. From the results obtained above, the second stage of the acute toxicity test was performed using doses of 1000, 1600, 2900, and 5000 mg/kg, respectively.

2.7 Anti-inflammatory Activity

Egg albumin-induced rat paw edema: The egg albumin-induced rat paw edema method was used [21]. The crude extract of *Zanthoxylum leprieurii* was administered orally to animal groups of 5 per dose, and 4 dose levels (50 mg/kg, 100 mg/kg, 200 mg/kg, and 400 mg/kg) were used. 30 minutes after administration of the extract fraction, inflammation was induced by a sub-plantar injection of 0.1 ml of fresh, undiluted egg albumin. Edema was assessed in terms of the volume of distilled water displaced before and after 0, 1, 2, 3, and 4 hours after the induction of inflammation using the LETICA Digital Plethysmometer (LE 7500). The average edema level at each interval was determined in terms of the difference in volume displacement after injecting the egg albumin and at zero time volume displacement of the injected paw (Vt - V0).

The level of inhibition of edema was calculated for each dose of the plant extract.

2.8 Statistical Analysis

The results obtained from the above experiment were analyzed with a one-way ANOVA expressed as SEM, and a student T-test was used to test the differences between the mean of the treated and control groups, which were considered significant at $P = .05$.

3. RESULTS

3.1 Phytochemical Composition

The root bark of *Zanthoxylum leprieurii* was extracted by maceration with 95% methanol, filtered, and the solvent recovered using a rotary evaporator. The extract gave a percentage yield of 25g; it was fractionated, and the compositions of the different fractions are listed below in Table 1.

3.2 Acute Toxicity Test (LD_{50})

The acute toxicity study showed no mortality rate in the animal for up to 10,000 mg/kg. This indicates that the LD_{50} of *Zanthoxylum leprieurii* is greater than 8,000 mg/kg. It shows that *Zanthoxylum leprieurii* is safe at high doses.

3.3 Anti-inflammatory Activity

The result in Table 2 shows the doses of 50 mg/kg, 100 mg/kg, 200 mg/kg, and 400 mg/kg at 0, 1st, 2nd, 3rd, and 4th hours, respectively. The effect of the crude extract of *Zanthoxylum leprieurii* root bark was significant (P = .05) with the maximum effect at the 4th hour when compared with the standard.

Table 1. Phytochemical compositions

Phytoconstituents	Results			
	N-hexane	Ethyl acetate	Methanol	Water
Alkaloids	+	+	++	++
Flavonoids	++	+	+++	+
Saponins	+	-	+++	++
Tannins	-	-	++	+

Key
- = absent
+ = Present in small concentration
++ = present in moderate concentration
+++ = present in High concentration

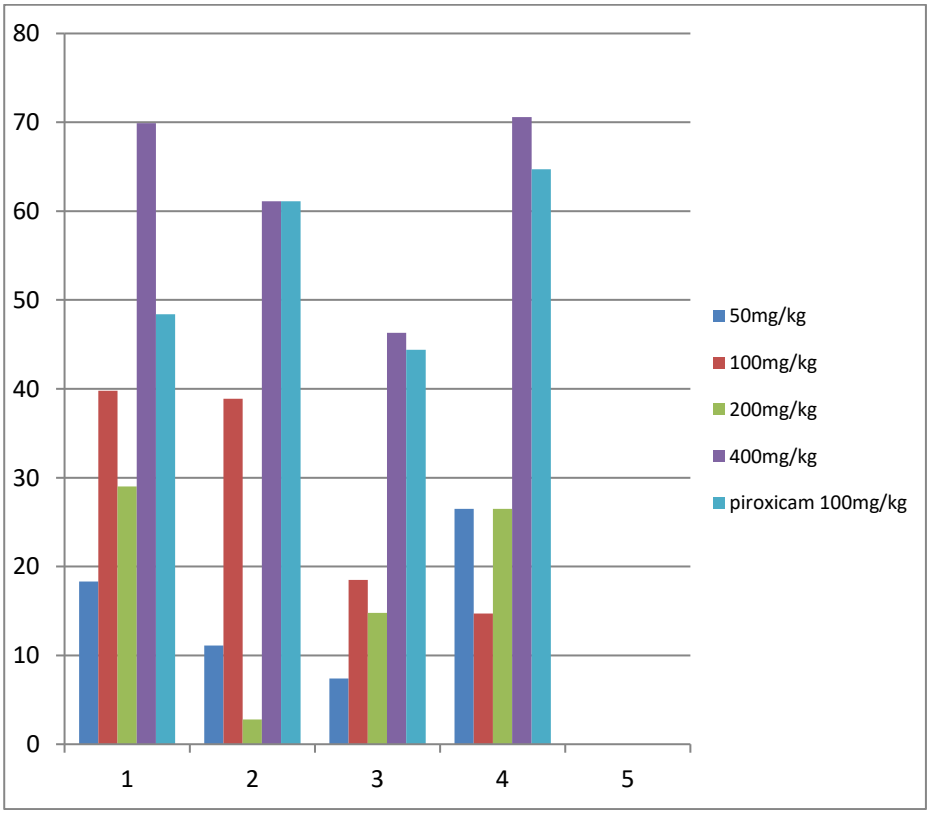

Fig. 1. Graphical representation of the anti-inflammatory activity of *Zanthoxylum leprieurii*

Table 2. Anti-inflammatory activity studies

Group	Dose (mg/kg)	Mean Edema Volume (ml) and Percentage Inhibition at Different Hours				
		Oh	1h	2h	3h	4h
ZL_1	50	0.38±0.033	0.78±0.026(18.3%)	0.73±0.032(11.1%)	0.68±0.041(7.4%)	0.68±0.015(26.5%)
ZL_2	100	0.38±0.017	0.70±0.015(39.8%)	0.65±0.020(38.9%)	0.66±0.040(18.5%)	0.68±0.020(14.7%)
ZL_3	200	0.4±0.029	0.75±0.039(2.8%)	0.76±0.028(29.0%)	0.75±0.057(14.8%)	0.71±0.008(26.5%)
ZL_4	400	0.42±0.044	0.63*±0.008(69.9%)	0.64*±0.020(61.1%)	0.64*±0.011(46.3%)	0.61*±0.029(70.6%)
Piroxicam	100	0.50*±0.029	0.76±0.024(48.4%)	0.67±0.038(61.1%)	0.64*±0.045(44.4%)	0.65±0.025(64.7%)

*Values are Mean ±SEM, n=3, * values are significantly different from the control group at* $P = .05$

4. DISCUSSION

The yield of the crude extract of *Zanthoxylum leprieurii* root bark was expressed in percentage as 5% (25g). The extractive yield was low when compared with the amount of *Zanthoxylym leprieurii* root bark powder (500 g) used for the extraction.

The methanol extract of *Zanthoxylum leprieurii* root bark was phytochemically analyzed and found to have a high concentration of flavonoids and saponins and a moderate concentration of alkaloids and tannins. This is in line with what other research has said about the phytochemical composition of *Zanthoxylum leprieurii* [22]. The ethyl acetate fraction showed low concentrations of flavonoids and alkaloids, while the N-hexane fraction showed a moderate concentration of flavonoids and a low concentration of alkaloids and saponins. The water fraction showed a moderate concentration of alkaloids and saponins, as well as a low concentration of tannins and flavonoids. Plant alkaloids may promote inflammation by increasing cell proliferation [23]. Researchers have proven that tannins and flavonoids in the plant act as free radical scavengers, making their presence particularly significant [24]. Flavonoids and general phenolic compounds act as potent antioxidants that possess significant anti-inflammatory properties by inhibiting the effects of enzymes responsible for the production of inflammatory chemical mediators [25]. Plant flavonoids may improve collagen fibril viability by increasing fiber strength, which promotes DNA synthesis and reduces cell damage [26]. Tannins found in plants can promote inflammation and epithelialization due to their astringent and antibacterial properties [26].

"The lethal dose (LD_{50}) is the dose that produces death in 50% of animals. Lethal dose (LD_{50}) determination is essential because it gives room for safe dosing in subsequent whole animal experiments that will be carried out in the course of the study. Substances more toxic than 1 mg/kg are of no practical interest; LD_{50} values greater than 5000 mg/kg are of no practical interest; an appropriate figure for the LD_{50} is usually adequate to estimate the risk of acute intoxication" [20]. *The experimental animal tolerated high doses of Zanthoxylum leprieurii.*

The anti-inflammatory effect of crude *Zanthoxylum leprieurii* root bark was studied using egg-albumin-induced rat hind paw edema. The characteristic swelling of the paw is due to edema formation and inhibition of increased vascular permeability; hence, the attendant edema modulates the extent and magnitude of the inflammatory reaction. The injection of egg albumin induced paw edema, which peaked between 30 minutes and 1 hour and gradually decreased over time, while the negative control remained almost constant throughout the experiment.

According to the results of the egg-albumin-induced paw edema model, the crude extract showed dose-dependent inhibition of edema, particularly at 3 to 4 hours. The percentage inhibition of paw edema by 50 mg/kg, 100 mg/kg, 200 mg/kg, and 400 mg/kg was 7.4% and 26.5%, 18.5% and 14.7%, 14.8%, and 26.5%, 46.3%, and 70.6% at the 3rd and 4th hours, respectively, while the standard drug (Piroxicam at 100 mg/kg) was 44.4% and 64.7% at the 3rd and 4th hours, as shown in Table 2. This demonstrates that the standard medicine at 100 mg/kg has greater anti-inflammatory action than the extract at 50, 100, and 200 mg/kg; however, at 400 mg/kg, the extract produces a stronger anti-inflammatory response than the standard drug at 100 mg/kg.

5. CONCLUSION

The results of this investigation revealed that the crude extract of *Zanthoxylum leprieurii* root bark is safe at large doses and possesses anti-inflammatory properties. The sample contains phytochemical elements such as alkaloids, saponins, tannins, and flavonoids. The non-polar essential oils in the plant may be responsible for its anti-inflammatory effect. The present study provided evidence supporting the use of the plant *Z. leprieurii* in the management of inflammatory disorders. Thus, it serves as a beneficial alternative to orthodox medicine.

We recommend further research to isolate and characterize the phytochemical constituents responsible for the obtained anti-inflammatory activities.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

The protocols for the study were approved by the ethics committee of the University of Nigeria, Nsukka, as registered by the National Health Research Ethics Committee of Nigeria (ref no. NHREC/05/01/2008B).

ACKNOWLEDGEMENT

We would like to express our gratitude to the Department of Pharmaceutical Science at the University of Nigeria for generously providing a suitable environment for the successful completion of this project. The authors express their gratitude to the Department of Inspection, Monitoring and Quality Assurance, Pharmacy Council of Nigeria for their invaluable technical assistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Guyton AC, Hall JE. A textbook of medical physiology, 11th edition; 2006.
2. Heasman SJ, Giles KM, Ward C. Glucocorticoid mediated regulation of granulocyte apoptosis and macrophage phagocytosis of apoptotic cells: implications for the resolution of inflammation. J Endocrinol. 2003;178:29.
3. Nickrent DL. Satinwood, Encyclopedia of life sciences. 2002;7(7):1-3.
4. Kernouf, Nassima, Hamama Bouriche, Seoussen Kada, Dalila Messaoudi, Areej M. Assaf, and Abderrahmane Senator. Anti-inflammatory and immuno-modulatory effects of capparis spinosa flower bud extract. Annual Research & Review in Biology2018;25(3):1-11. Available:https://doi.org/10.9734/ARRB/2018/40189
5. Ify, Ogbonna Abigail, Adejube Glory Raphael, Okoye Clifford Tochukwu, Ogbonna Ugoy Sonia Amarachi, Njoku Andrew Ikechukwu, Madu Josephine Madukaihe, Yakubu Thomas, and Ogbonna Chike Innocent. The antimicrobial, anti inflammatory and analgesic activities of the rhizome extract of *Curcuma longa* L. (Turmeric). Journal of Advances in Biology & Biotechnology. 2021;24(6):1-16. Available:https://doi.org/10.9734/jabb/2021/v24i630217
6. Naz R, Ayub H, Nawaz S, Islam ZU, Yasmin T, Bano A, Wakeel A, Zia S, Roberts TH. Antimicrobial activity, toxicity and anti-inflammatory potential of methanolic extracts of four ethnomedicinal plant species from Punjab, Pakistan. BMC complementary and alternative medicine. 2017 Dec;17:1-3.
7. Panthong A, Kanjanapothi D, Taesotikul T, Phankummoon A, Panthong K, Reutrakul V. Anti-inflammatory activity of methanolic extracts from *Ventilago harmandiana* Pierre. Journal of ethnopharmacology. 2004 Apr 1;91(2-3):237-42.
8. Charles RC. Lippicott-modern pharmacology with clinical applications, 5th edition. Philadelphia: Lippicotwillim. 2002;303 – 306.
9. Cletus Nze Aguwa. Therapeutic basis of clinical pharmacy in the tropics, 4th edition; 2007.
10. Makata SC. Anti-inflammatory evaluation of crude extract of Combretumobanense stem bark. Faculty of pharmaceutical sciences, University of NgeriaNsukka. 2014;10-12.
11. Raina R, Parwez S, Verma PK, Pankaj NK. Medicinal plants and their role in wound healing. J Vet Res. 2008;3(1):article 21.
12. Ruddle NH, Cuff CA, Sacca R. Mediators of inflammation: Current opinion in Immunology. 1997;9:851.
13. Fabian I Eze1,2, Xavier Siwe-Noundou2,3, Michelle Isaacs3, Srinivas Patnala4, Patience O Osadebe1, Rui WM Krause2,3. Anti-cancer and anti-trypanosomal properties of alkaloids from the root bark of *Zanthoxylum leprieurii* Guill and Perr. Tropical Journal of Pharmaceutical Research. 2020;19(11):2377-2383.
14. Abubakar MS, Musa AM, Ahmed A, Hussaini IM. The perception and practice of traditional medicine in the treatment of cancers and inflammations by the Hausa and Fulani tribe of northern Nigeria. Journal of Ethnopharmacology. 2020;3(3):625-629.
15. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi: proposed standard, NCCLS document M38-P, National

Committee for Clinical Laboratory Standards, Wayne, PA, USA; 1998.
16. Haborne HB. Phytochemical methods: A guide to modern techniques of plant analysis, 3rd edition; London: Chapman and Hall. 1998;302.
17. Edoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol. 2005;4(7):685-688.
18. Evans WC. Trease and Evans Pharmacognosy, 16th ed., Elsevier Limited, China. 2009;225- 356.
19. Agyare C, Koffuor GA, Boamah VE, Adu F, Mensah KB, AduAmoah L. Antimicrobial and anti-inflammatory activities of *Pterygota macrocarpa* and *Cola gigantea* (Sterculiaceae). Evid Based Complement Alternat Med. 2012. Article ID 902394,9 pages.
20. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983;53:275-289.
21. Adeyemi OO, Akindele AJ, Evaluation of anti-inflammatory activity of aqeous leaf extract of *Byrspcarpus coccineus*. Fitoterapia. 2007;78:25-28.
22. Adesina SK. The Nigerian Zanthoxylum; chemical and biological value. Afr J Tradit Complement Altern Med. 2005;2:282-301
23. Barbakadze V, Mulkijanyan K, Gogilashvili L, Amiranashvil L, Merlani M, Novikova Z, Sulakvelidze M. Allantoin and pyrrolizidine alkaloids: free wound healing compositions from *Symphytum asperum*. Bull GA Nat. Acad. Sci. 2009;3:159-164.
24. Marja P, Kahkonen AI, Hopia HJ, Vuorela JR, Kalevi PT, Kujala S, Marina H. Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. 1999;47:3954- 3962
25. Owoyele BV, Oguntoye SO, Dare K, Ogunbiyi BA, Aruboula EA, Soladoye AO. Analgesic, anti-inflammatory and antipyretic activities from flavonoid fraction from *Chromolaena odorata*. J Med Plants Res. 2008;2(9):219-225
26. Panda P, Tripathy G. Wound healing activity of aqueous and methanolic bark extract of *Vernonia arboreus* in wistar rat. Nat Prod Radiance. 2009;8:6-11.

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