African Journal of Pharmaceutical Research and Development
Available online at https://ajopred.com
Vol. 18 No.1; pp. 680-688 (2026)

Original Research Article

SUBSTANTIATING THE ANTI-INFLAMMATORY EFFICACY OF *Rhaphiostylis beninensis* ROOT EXTRACT

UDUENEVWO FRANCIS EVUEN[1], SOLOMON UGOCHUKWU OKOM[2*], KESIENA EFE-EYEFIA[3]

1. Department of Biochemistry and Molecular Biology, Faculty of Science, Dennis Osadebay University Asaba, Delta State, Nigeria
2. Department of Medical Biochemistry, Faculty of Basic Medical Sciences, University of Delta, Agbor, Delta State, Nigeria
3. Department of Public Health, Faculty of Allied Medical Sciences, Dennis Osadebay University Asaba, Delta State, Nigeria.

ABSTRACT

There are many chronic diseases that are associated with inflammation across the world today. Remarkably, this represents therapeutic targets. To this extent, synthetic anti-inflammatory drugs have been employed as a panacea for such illnesses. Regrettably, their use in several situations has been linked to unwanted side effects, which have warranted the need to find appropriate substitutes through natural plant resources. Additionally, the pharmacology of most of the plants, such as *Rhaphiostylis beninensis,* is yet to be fully unravelled. Based on this, the methanol root extract of *Rhaphiostylis beninensis* (MRERB) was subjected to an *in vitro*-based assay to evaluate its ability to inhibit membrane stabilisation (haemolysis inhibition) and protein denaturation. This was achieved by employing concentrations of 100-500 mg/mL of the plant extracts against indomethacin as the reference drug. Haemolysis of red blood cells in hypotonic solution was evaluated as the mechanism of haemolysis inhibition, while the protein denaturation was performed through the use of heat. Notable differences ($p \leq 0.05$) were found in the inhibition of haemolysis and protein denaturation by MRERB in a concentration-dependent manner. Nevertheless, haemolyis inhibition by MRERB and indomethacin was found to be equivalent at 300 mg/mL ($p \leq 0.05$). Consequently, the outcomes indicate anti-inflammatory phytoconstituent and pharmacological properties of *R. beninensis,* thereby augmenting and substantiating available literature on the utilisation of the plant as an anti-inflammatory product source.

ARTICLE INFO

Received 15 November, 2025
Accepted 27 April, 2026
Published 30 April, 2026

KEYWORDS

Rhaphiostylis beninensis;
Anti-inflammatory activity;
Natural product,
Pharmacology;
Ethnomedicinal plant.

INTRODUCTION

Inflammation is a multifaceted and essential functional response of the body to infection, injury, or irritation with the purpose of normalisation of tissue by removing injurious stimuli and triggering tissue repair [1]. Nevertheless, in its chronic state, inflammation has been implicated as an integral part of the aetiology of numerous ailments especially, heart disease, diabetes, arthritis, as well as cancer [2]. Inflammation is widely treated with common inflammation-reducing drugs, particularly the NSAIDs (non-steroidal anti-inflammatory drugs). Regardless of their established effectiveness, they are also associated with such adverse conditions as gastric bleeding, kidney or renal failure and can even cause cardiovascular issues [3]. This has intensified the desire to have safer plant-based substitutes with strong anti-inflammatory effects. Recent biomedical studies [4, 5] indicate the effectiveness of plant extract in the regulation of

***Corresponding author:** solookom1996@gmail.com; +234 810 348 1075; orcid.org/0000-0002-7275-9492
https://doi.org/10.59493/ajopred/2026.1.26 ISSN: 0794-800X (print); 1596-2431 (online)

inflammatory processes. This therapeutic effect can be explained by their peculiar phytochemical composition [6]. In perfect harmony, the phytochemicals verified to regulate inflammatory pathways included flavonoids, alkaloids, tannins, and saponins, among others, through multiple mechanisms that encompassed inhibition of cyclooxygenase (COX), lipoxygenase (LOX), and pro-inflammatory cytokines [7, 8]. Nevertheless, red blood cell (RBC) membrane stabilisation and protein denaturation inhibition tests are well established among experiments conducted *in vitro* to determine a plant's ability to inhibit inflammation. Membrane stabilisation mimics lysosomal membrane protection against lysis, the absence of which releases inflammatory mediators. Alternatively, protein denaturation reflects the inflammatory circumstances, where structural proteins are denatured to become antigenic and trigger immunity [9, 10].

Rhaphiostylis beninensis belongs to the family Icacinaceae, famous in West African indigenous therapeutic practices for managing conditions like convulsions, mental disorders, wounds and inflammatory diseases including rheumatism, arthritis to mention a few [11, 12]. *Raphiostylis beninensis* is referred to as *Kpolokoto* in Igbo, *Usuende* in Benin and *Umeni* in Urhobo, speaking tribes of Nigeria. Additionally, the root of the plant is also served as a condiment in the Niger Delta areas of the country [13]. Furthermore, research has been conducted on the stem bark, leaves and root extract of the plant [14]. They demonstrated anti-sickling, antibacterial, growth, cytotoxic, analgesic, aphrodisiac, antioxidant and anti-inflammatory properties [15, 16]. Moreover, the GC-MS (Gas Chromatography Mass Spectroscopy) analysis of *R. beninensis* methanol root extracts identified bioactive anthraquinones, flavonoids and terpenoids, demonstrating inflammation–reducing, anti-bacterial, anti-oxidant activities, owing to the occurrence of anti-inflammatory thiourea compounds [6, 17, 18]. Its root extracts have also been tested *in vivo* with high hepatoprotection and significant antioxidant effects recorded, indicating the presence of anti-inflammatory properties [19, 20].

The pharmacological action, particularly the anti-inflammatory properties of *R. beninensis* that justify its ethnomedicinal importance, have been scientifically researched [21, 22]. Nonetheless, these studies were restricted to *in vivo* research, with the rat hind paw inflammation model being induced with carrageenan and egg albumin. Consequently, to support and supplement previous investigations, this study intends to establish the *in vitro* anti-inflammatory attributes of methanol-based *R. beninensis'* root extract (MRERB) through the assessment of its RBC membrane stabilisation and protein denaturation inhibitory capacity.

MATERIALS AND METHODS

Procurement of Chemicals

Thomas Gold Integrated Resources Limited, Benin City, Nigeria, provided all analytical-grade chemicals used in this study.

Procurement and Identification of the Plant Samples

Root samples of *R. beninensis* were procured from an indigenous market in Oghara, Delta State, Nigeria. They were later validated by a plant taxonomist from the University of Benin's Department of Plant Biology and Biotechnology in Nigeria. A voucher sample of the plant (UBHp0262) was retained in his herbarium.

Plant Extract Preparation

Root samples were dried for two weeks at ambient temperature (27.0 ± 2.0 °C). Subsequently, they were pulverised to yield powdered plant components. Ten grammes (10 g) of the powdery *R. beninensis* was combined with eighty millilitres (80 mL) of methanol. Successively, the resultant solution was allowed to stand for 72 hours with periodic stirring and filtered afterwards with a filter paper (Whatman No.1). The filtered content was then maintained in an incubator at 37°C to dry, resulting in a thick, slurry plant extract that was acquired and weighed at a suitable ratio to prepare a stock solution of the extract.

Drug

Indomethacin, marketed under the brand name indokris by Krishat Pharma Industries Limited in Nigeria, was used as the standard drug.

Choice of dosages

Five dosing levels of *R. beninensis* extract were chosen in increasing concentrations (100-500 µg/mL) to examine its anti-inflammatory effectiveness. The standard drug of choice (indomethacin) was also

given at a comparable dosing level with an increasing concentration (100-500 µg/mL).

Ethical Approval

Ethical approval was sought and received from the constituted research and ethics committee of the Faculty of Basic and Medical Sciences, Delta State University Abraka, with reference number, RBC/FBMC/DELSU/23/219.

Anti-inflammatory Activity Assessment

Human Blood

Five millilitres (5 mL) of blood sample was taken from one of the investigators who had abstained from any form of NSAIDS two weeks preceding the onset of the experiment. The sample was collected in a heparinised vacutainer. Afterwards, it was washed thrice with 0.9 percent saline before being made to undergo centrifugation at 3000 rpm for 10 minutes. Subsequently, a preparation of 0.9% saline, and a 40% v/v suspension, using a hypotonic phosphate-buffered solution (154 mM NaCl in 10 mM sodium phosphate buffer) at pH 7.4, was employed in the washing of the packed cells. This solution was utilised as the stock RBC (red blood cell) suspension.

Hypotonicity-induced haemolysis

With minor adjustments, this test was conducted using the methodology outlined by Padmanabhan and Jangle [23]. The test sample was made up of stock RBC suspension (0.03 mL) combined with five millilitres (5 mL) of the phosphate buffer hypotonic solution that contained 100–500 µg/mL of MRERB. Indomethacin (the standard drug), and the control were prepared in a similar fashion with the test model at concentrations of 100–500 µg/mL but without the plant extract. Three duplicates of the experiment were conducted. After an incubation period (10 minutes) at room temperature and a similar centrifugation duration at three thousand rate per minute (3000 rpm), the respective mixtures' absorbance was determined at 540 nm using an ultra violet spectrophotometer. Thereafter, the percentage of membrane stabilisation or haemolysis inhibition was computed as follows:

Percentage inhibition of haemolysis = $\frac{D_1 - D_2}{D_1} \times \frac{100}{1}$

Where:

D_1 = Absorbance of the hypotonic-buffered solution

D_2 = Absorbance of the test /standard sample in the hypotonic solution

Protein Denaturation

The method adopted by Padmanabhan and Jangle [23], with slight modifications, was used to perform the protein denaturation test. A test solution comprising 1 millilitre of 100–500 µg/mL of MRERB was mixed with 1 mL of 1 mM of a solution of egg albumin. Similarly, 100–500 µg/mL of indomethacin was combined with the same amount and concentration of egg albumin solution. Subsequently, the individual mixtures containing the test and the standard drugs were incubated for 15 minutes at 27 ±1˚C. The reaction mixture was maintained in a water bath at 70˚C for a 10-minute duration to enhance denaturation. Succeeding cooling, the samples' turbidity were ascertained at 660 nm using the ultra violet (UV) spectrophotometer. A control sample, to which no drug was added, was used to calculate the denaturation inhibition in percentage as shown below.

Inhibition of protein denaturation (percentage) = $\frac{R_1 - R_2}{R_1} \times \frac{100}{1}$

Where:

R_1 = Absorbance of hypotonic-buffered solution

R_2 = Absorbance of test /standard sample in the hypotonic solution

Statistical Analysis

The Statistical Package for the Social Sciences, version 21, was employed in the analysis of the data derived from the experiment. The one-way ANOVA (analysis of variance) and the Tukey post hoc test were employed. Statistical significance was deemed to exist at 95% ($p \leq 0.05$). Results were presented as the Mean ± SEM (standard error of the mean) for three separate observations.

RESULTS

3.1 Percentage Inhibition of Haemolysis

The MRERB exhibited concentration-dependent hemolysis inhibitory effect in tandem with that of the standard drug, indomethacin (Fig. 1). Although significant increases ($p \leq 0.05$) in haemolysis inhibition were observed between consecutive concentrations (100 - 500µg/mL), there were moderate inhibitory effects at lower concentrations, increasing with higher concentrations. Thus, the greatest protective efficacy of MRERB against the deleterious impact of

hypotonic solution was seen at the maximum concentration of 500 µg/mL. However, at 300 µg/mL, the plant's haemolysis inhibitory effect was found to be equivalent ($p \leq 0.05$) to that of indomethacin.

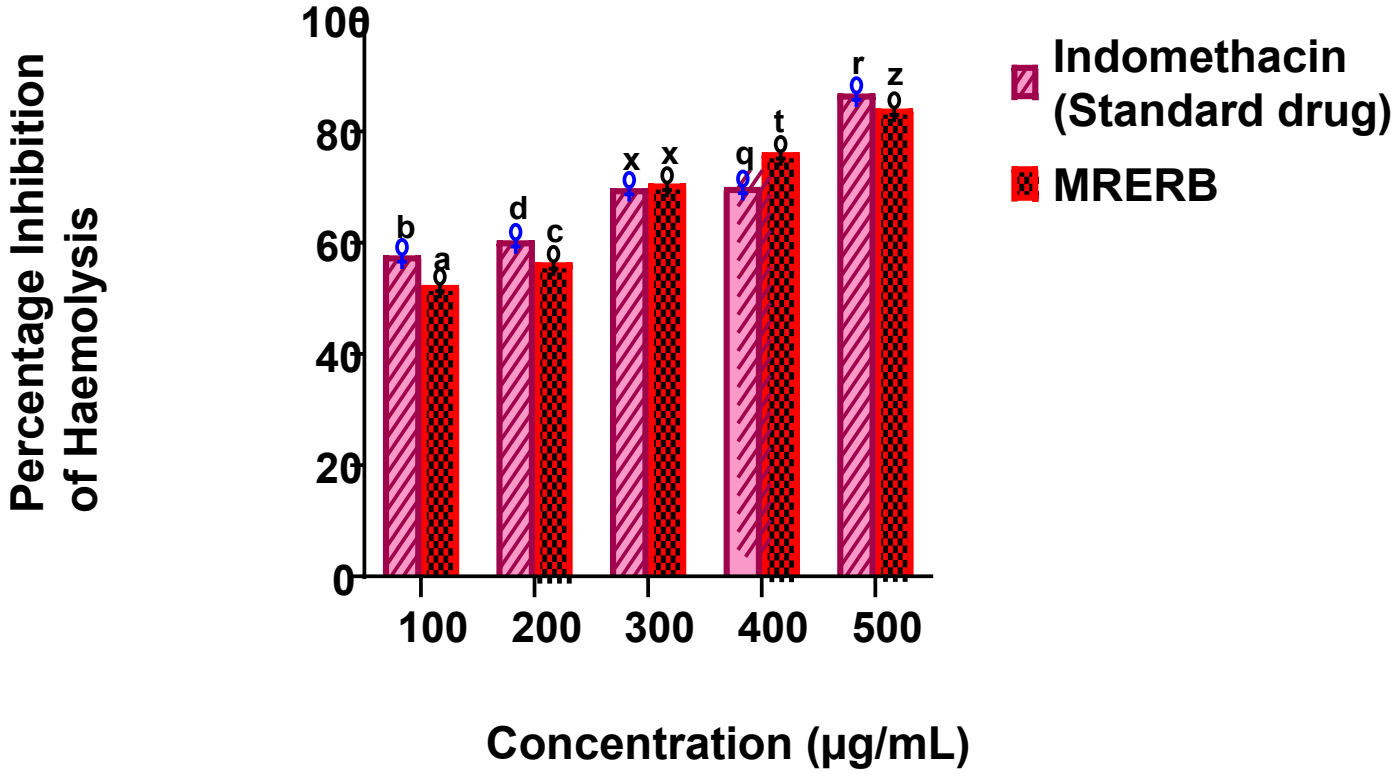

Figure 1. Influence of methanol root extracts of *R. beninensi*s (MRERB) on Haemolysis inhibition. The bars are represented as the Mean ± SEM (standard error of mean) of three replications. Bars marked using dissimilar alphabets are significantly different ($p < 0.05$).

Percentage Protein Denaturation Inhibitory Activity

The protein denaturation inhibition of protein denaturation activity by varying MRERB concentrations is expressed in percentage as illustrated below (figure 2). Significant suppression ($p \leq 0.05$) of denaturation of egg albumin was demonstrated by MRERB and indomethacin (100–500 µg/mL) based on their individual concentrations. However, the standard drug, indomethacin showed a greater inhibitory ability than MRERB at all dose concentrations, but the difference in the degree of inhibition diminishes with increasing dosages. Despite being significantly less effective than Indomethacin, MRERB nevertheless exhibited substantial and notable ($p \leq 0.05$) action.

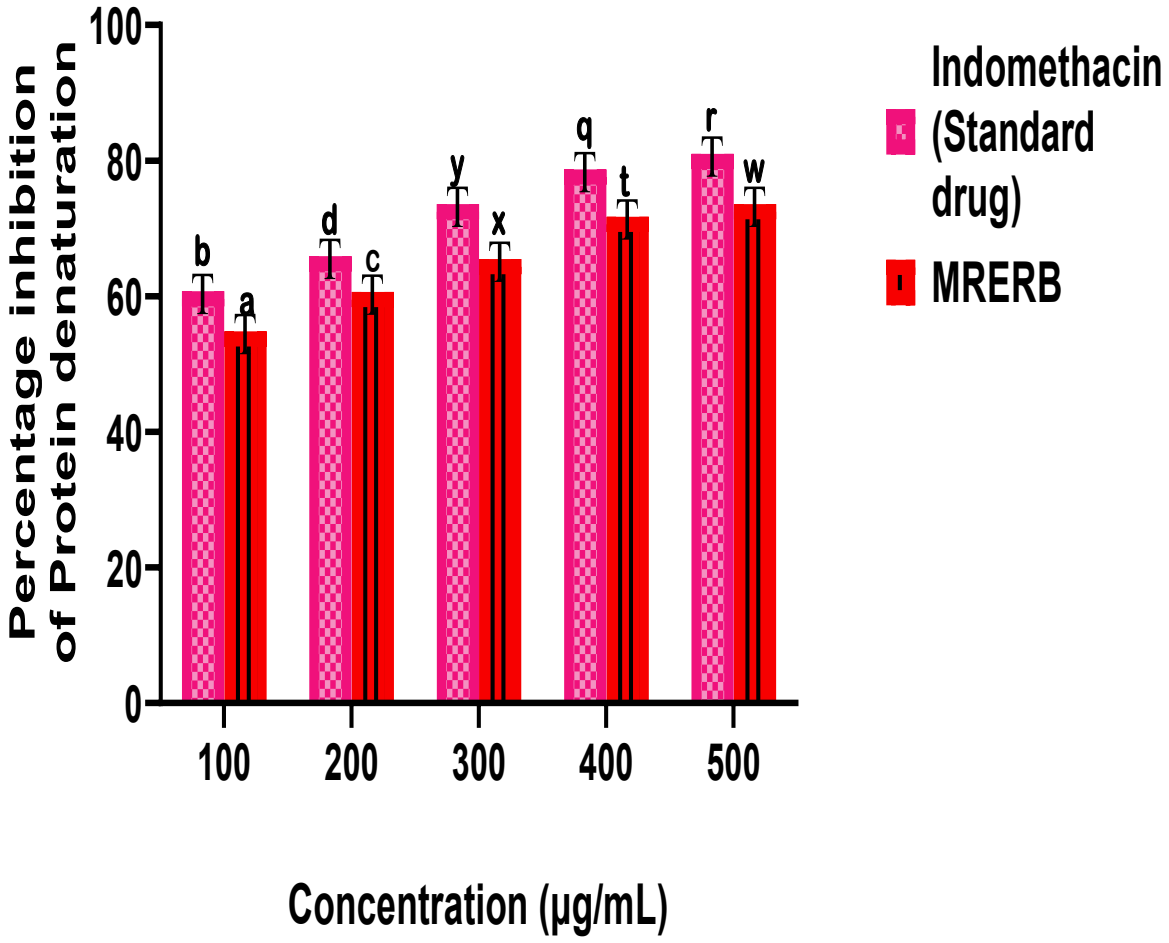

Figure 2. Influence of methanol root extracts of *R. beninensi*s (MRERB) on Protein denaturation activity. The bars are represented as the Mean ± SEM (standard error of mean) of three distinct replications. Bars marked with dissimilar alphabets are significantly different ($p < 0.05$).

DISCUSSION

The traditional medicinal system is based on plants and has been in practice for many centuries [24]. In the current study, anti-inflammatory effects of the MRERB, which were based on their concentrations and evident in haemolysis and protein-denaturation assays, suggest that the extract exerted membrane-stabilising, as well as cytoprotective effects. This two-fold outcome is an indication of a manifold anti-inflammatory profile. Moreover, the similar activity of MRERB, as compared to indomethacin, at the concentration of 300 µg mL^{-1}, in the haemolysis inhibition assay could be a measure that the root extract contains phytoconstituents that preserved erythrocyte integrity under osmotic stress, like the standard drug. In line with this, past research has found that the plant is a source of significant phytochemicals, including Quercetagetin, Apigenin, Thioflavin, Genkwanin, isoflavone, Gingerol, Lupeol, Capsaicin, Dihydrocapsaicin, Squalene, Tocopherol, Thiourea, among others, which were confirmed to possess oxidative and inflammatory reducing properties [6, 17].

The haemolysis inhibition assay has become a consensus as a surrogate of membrane stabilising activity, which is a major mechanism by which NSAIDs inhibit the release of inflammatory mediators [25]. Therefore, the extract can potentially suppress the action of cyclo-oxygenase (COX) since it shows comparable activity to indomethacin at 300 µg mL^{-1} in the haemolysis test [26]. COX inhibitors include flavonoids and phenolic acids, which may impede

prostaglandins production, thus, reducing inflammation [27]. In addition, *R. beninensis* roots have been reported to exert antioxidant activity determined by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays, which supports the idea that reactive oxygen species (ROS) scavenging is also an element that provides the anti-inflammatory effect [28]. In line with this, the stability of cell membranes through flavonoid binding to phospholipid bilayers and free radical scavenging has been reported [29]. This substantiates the report by Kutama et al. [14] on the anti-inflammatory effects of phytochemicals found in certain medicinal plants in Nigeria with a high level of phenolics and flavonoids. Moreover, ethnopharmacological reports of other species of the Icacinaceae family into which *R. beninensis* belongs [3, 30, 31] and studies on other plants with ethnomedicinal anti-inflammatory properties [32-35] are consistent with findings from this study.

It would be worthwhile to mention that at 300 µg/mL, the close proximity of the activity of MRERB to that of the reference drug in the test for haemolysis inhibition indicates that the bioactive compounds in this extract may be operating through the same mechanistic pathway as NSAIDs via maintaining membrane integrity, inhibiting denaturation of proteins and enzymatic activities that support inflammation. Furthermore, the *in vitro* experiment of Lipopolysaccharide-stimulated raw 264.7 macrophages, as reported by Njoya *et al.* [36], found that leaf extracts of *Cassinopsis illicifolia*, another Icacinaceae family member, had a potent anti-inflammatory effect on COX-2, IL-1B, and TNF-2A, which provides molecular evidence of the possible anti-inflammatory effect. Accordingly, the COX-2 and cytokine reductions in *C. ilicifolia* provided a molecular mechanistic basis of the MRERB since the extract could be acting via similar pathways.

The prevention of heat-induced protein denaturation is used as an indicator of an anti-inflammatory outcome, which denotes the capacity of the extract to inhibit protein unfolding during inflammation and pain [37, 38]. In spite of the fact that the performance of MRERB in the protein denaturation test was a little lower than that of indomethacin, it was still significant, particularly at 500 µg mL^{-1} (about 74 percent inhibition). Plausibly, MRERB is a cocktail of bioactive phytochemicals that stabilise protein structures by preventing the structural proteins from denaturing and stabilising their native conformations, thus reducing antigenicity of the damaged tissues [39]. Moreover, the protective effect of MRERB in the assay indicates that it is possible that MRERB disrupts the inflammatory cascade by attaching itself to the structural proteins and thereby stabilizes their native conformations. These results are consistent with an earlier study [40] that had indicated the inflammation-reducing property of a medicinal plants' extract, that is rich in alkaloids, flavonoids, and terpenoids. Moreover, the report of Yesmini *et al.* [41] proved that the ethanol extract of the roots of *Piper chaba* was effective in dose-dependent membrane stabilisation of RBCs and inhibition of albumin (protein) denaturation, which is comparable to NSAIDs, thus validating the applicability of the assays used.

CONCLUSION

This research, to the best of our knowledge, is the first report on the assessment of the plant's root extract anti-inflammatory potency employing the *in vitro-based* techniques, haemolysis inhibition and protein denaturation activities of red blood cells. The results strongly support the fact that the MRERB shows potent *in vitro* anti-inflammatory properties. Such outcomes confirm the ancient use of this plant in treating inflammation and are consistent with other *in vivo* reports of its activity. The dose dependent anti-inflammatory effects in the two assays suggest potential therapeutic index which can be used in the future to optimize dosing in living models. Nevertheless, to completely profile the pharmacological attributes of this plant, it is proposed that future studies provide COX-1/COX-2 inhibition tests to determine the enzyme targets, with complete toxicological studies to determine safety at therapeutic levels.

ACKNOWLEDGMENT

The authors wish to express their profound gratitude to Mr. Efe Ugeh of the Family Medicine Department of the Delta State Teaching Hospital (DELSUTH), Oghara, for his assistance during the collection of blood sample for the investigation.

AUTHORS' CONTRIBUTION

This work was a product of the collaborative efforts of all authors. The authors, UFE and SUO designed the research work, wrote the protocol, and the first draft of the manuscript. EK reviewed and vetted the first draft, while author SUO effected corrections to the first draft. UFE performed the statistical analysis. All

authors read and approved the final draft of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

The research was solely funded by the authors.

REFERENCES

1. Antar SA, Mahmoud AM, Abdo W, Gad C, Al-Karmalawy AA. A Comprehensive overview of organ inflammatory responses: genesis, possible mechanisms and mediators of inflammation. Pharmaceutical Sciences, 29(4), 2023: 397-416. **doi**: 10.34172/PS.2023.9
2. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Zhao L. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget, 9(6), 2018:7204–218. https://doi.org/10.18632/oncotarget.23208.
3. Anjali P, Vimalavathini R, Kavimani S. *In vitro* anti-inflammatory and anti-arthritic activity of ethanolic extract of leaves of *Pyrenacantha Volubilis (Eepv)*. Asian Journal of Pharmaceutical and Clinical Research, 13(8), 2020: 156-58.
4. Chirumamilla P, Taduri S. Assessment of *in vitro* anti-inflammatory, antioxidant and antidiabetic activities of *Solanum khasianum* Clarke. *Vegetos,* 36, 2023: 575–82.
   Krayem N, Jribi F, Alonazi M, Amina M, Horchani H, Karray A, Cherif S, Ben-Bacha A.
5. Unveiling the therapeutic potential: anti-inflammatory and antioxidant properties of selective medicinal plants. Cellular and Molecular Biology (Noisy-le-Grand, France), 71(3), 2025: 88–100. https://doi.org/10.14715/cmb/2025.71.3.11
6. Evuen, UF, Okolie NP, Apiamu A. Evaluation of the mineral composition, phytochemical and proximate constituents of three culinary spices in Nigeria: A Comparative Study. Scientific Reports, 12, 2022: 20705.
7. Nasry WHS, Rodriguez-Lecompte JC, Martin CK. Role of COX-2/PGE2 mediated inflammation in oral squamous cell carcinoma. Cancers, 10(10), 2018: 348. doi:10.3390/cancers10100348.
8. Nisar A, Jagtap S, Vyavahare S, Deshpande M, Harsulkar A, Ranjekar P, Prakash O. Phytochemicals in the treatment of inflammation-associated diseases: the journey from preclinical trials to clinical practice. Frontiers in Pharmacology, 14, 2023: 1177050. doi: 10.3389/fphar.2023.1177050.
9. Sarveswaran R., Jayasuriya WJA, Suresh, TS. In vitro assays to investigate the anti-Inflammatory activity of herbal extracts: a review. World Journal of Pharmaceutical Research, 6(17), 2017: 131-41.
10. Eze FI, Uzor PF, Ikechukwu P, Obi BC, Osadebe PO. *In vitro* and *in vivo* models for anti-inflammation: an evaluative review. INNOSC Theranostics and Pharmacological Sciences, 2 (2), 2019: 3-15.
11. Burkil HM. The useful plants of the net. Tropical Afr. 4th Edition Macmillan Press 1: 34-6 1999.
12. Odugbemi TA. Textbook of Medicinal Plants in Nigeria. Tolu Press Lagos, pp. 23-97. 2008.
13. Evuen UF, Okolie, NP, Apiamu A, Etaware PM, Kpomah ED. Biomodulation of specific biochemical markers linked with oxidative stability in frozen beef. BioMed Central Agriculture, 1, 2025: 1. https://doi.org/10.1186/s44399-024-00001-7.
14. Kutama RM, Abdulkadir S, Kwalli SA., Chiroma G. Phytochemical compositions in some Nigerian medicinal plants and their pharmacological properties: A review. Journal of Anesthesiology 6(1), 2018: 15-25.
15. Ofeimum JO, Mbionwu MI. Cytotoxic and growth inhibitory activity of aqueous extracts of root and leaf of *Rhaphiostylis beninensis* Planch ex Benth and *Pyrenacantha standtii* Engl (Icacinaceae). Journal of Pharmacy and Bioresources, 11(1), 2014: 8–14.

16. Ofeimun, JO, Machie UR. Male Fertility and Aphrodisiac Potential of African Culinary Spices: Exploring the Evidence. Tropical Journal of Natural Product Research, 9(4), 2025:1860 – 867 https://doi.org/10.26538/tjnpr/v9i4.61
17. Ofeimun JO, Ayinde BA, Igbe I, Aderogba M, Adhikari A, Amjad H, Iqbal, MC. Anti-inflammatory constituent from the root of *Rhaphiostylis beninensis*. Research Journal of Phytochemistry, 8, 2014: 127-32. DOI: 10.3923/rjphyto.2014.127.132.
18. Animashaun TQ, Isijola MOA, Naidoo KK, Coopoosamy RM, Olajuyigbe OO. Gas chromatography-mass spectrometry analysis, phytochemical screening, antioxidant and antibacterial activity of methanol root extract of *Rhaphiostylis beninensis* (Hook.f.) planch. [family ICACINACEAE] against uropathogens. Biomedical and Pharmacological Journal, 16(2), 2023: 1055–079.
19. Evuen UF, Apiamu A, Okolie NP, Orji BO. Protective activity of root extract of *Rhaphiostylis beninensis* against Carbon Tetrachloride-induced Hepatotoxicity in Wistar Rats. *Biokemistri* 32(2), 2020: 93–100.
20. Ajayi TO, Akintayo CO, Olaniran DM, Iyobhebhe M, Ojo OA, Owolabi OV. Assessment of the antisickling activities of extracts and isolated compounds from dried leaves of *Rhaphiostylis beninensis* [Planch. Ex Benth]. Global Journal of Pharmacy and Pharmaceutical Science, 8(2), 2024: 555734.
21. Iwueke AV, Okoli CO, Iheanacho KM, Ojiako OA, Muoebonam HM, Nduka R. Anti-inflamatory activity of root extract of *Raphiostylis Beninniensis*. Nigerian Journal of Science and Environment, 10, 2011, 1-12.
22. Ofeimun JO, Ayinde BA, Igbe I, Chouldry MI, Hussain I, Adhikari A. Isolation characterization and anti-inflammatory activity of Rhaphiostylis beninensis Planch. Ex Benth (Icacinaceae). Planta Medica, 78, 2012: I327. DOI: 10.1055/s-0032-1321014.
23. Padmanabhan P, Jangle SN. Evaluation of *in-vitro* anti-inflammatory activity of herbal preparation, a combination of four medicinal plants. International Journal of Basic and Applied Medical Sciences, 2 (1), 2012: 109 - 16.
24. Evuen UF, Apiamu A, Owuzo PO. Comparative Assessment of the Nutritional and Antioxidant Status of *Euphorbia heterophylla (Euphorbiaceae)* and *Morinda lucida (Rubiaceae)* Plants. Nigerian Journal of Pharmaceutical and Applied Science Research, 5(2), 2016: 49 - 57.
25. Ogbomade RS, Asara AA, Eboh A. In vitro anti-inflammatory properties of leaf extract of *Polyalthia longifolia* extract. Pharmaceutical Chemistry Journal, 6, 2019: 80.
26. Idris MH, Mohd Amin SN, Mohd Amin SN, Nyokat N, Khong HY, Selvaraj M, Zakaria ZA, Shaameri Z, Hamzah, AS, The, LK, Salleh MZ. Flavonoids as dual inhibitors of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX): molecular docking and *in vitro* studies. Beni-Suef University Journal of Basic and Applied Sciences, 11, 2022:117. https://doi.org/10.1186/s43088-022-00296-y
27. Ribeiro D, Freitas M, Tomé SM, Silva AMS, Laufa S, Lima, FC, Fernandez E. Flavonoids inhibit COX-1 and COX-2 enzymes and cytokine/chemokine production in human whole blood. Inflammation 38, 2015: 858–70. https://doi.org/10.1007/s10753-014-9995-x
28. Evuen UF, Okolie NP. Antioxidant Prospects of Three African Ethnomedicinal Spices. Western Delta University Journal of Natural and Applied Sciences, 2(1), 2022: 49-64.
29. Okeke ES, Enechi OC, Nkwoemeka NE. Membrane stabilization, albumin denaturation, protease inhibition and antioxidant activity as possible mechanisms for the anti-inflammatory effects of flavonoid-rich extract of *Peltophorum*

pterocarpum (FRE*PP*) stem-bark, in proceedings of the 1st international e-conference on antioxidants in health and disease. MDPI: Basel, Switzerland, 2020. doi:10.3390/CAHD2020-08618.

30. David-Oku E, Akuodor GC, Edet EE, Ogbuji GK, Obiajunwa-Otteh JI, Aja DOJ. Antinociceptive, anti-inflammatory and antipyretic effects of ethanolic root bark extract of *Icacina senegalensis* in rodents. Journal of Applied Pharmaceutical Science, 6 (2), 2016: 104-108.
31. Alawode TT, Lajide L, Owolabi BJ, Olaleye MT. Investigation into the antioxidant and *in vitro* anti-inflammatory use of the leaves and tuber extracts of *Icacina trichantha.* Journal of the Chemical Society of Nigeria, *43*(4), 2018: 699 -706.
32. Oguntibeju OO. Medicinal plants with anti-inflammatory properties from Africa. Journal of Inflammation Research, 11, 2018:307-17.
33. Elgorashi EE, McGaw LJ. African Plants with *in vitro* anti-inflammatory activities: A review. South African Journal of Botany, 126, 2019: 142-69. https://doi.org/10.1016/j.sajb.2019.06.034.
34. Mainka M, Czerwinska ME, Osinska E, Ziaja M, Bazylko A. Screening of antioxidative properties and inhibition of inflammation-linked enzymes by aqueous and ethanolic extracts of plants traditionally used in wound healing in Poland. Antioxidants, 10, 2021:698. doi: 10.3390/antiox10050698.
35. Yoo TK, Jeong WT, Kim JG, Ji HS, Ahn MA, Chung JW, Lim HB. Hyun TK. UPLC-ESI-Q-TOF-MS-based metabolite profiling, antioxidant and anti-inflammatory Properties of Different Organ extracts of Abeliophyllum distichum. Antioxidants, 10, 2021: 70. doi:10.3390/antiox10010070.
36. Njoya E, McGaw LJ, Makhafola TJ. Investigating the phytochemical composition, antioxidant, and anti-inflammatory potentials of *Cassinopsis ilicifolia* (Hochst.) Kuntze extract against some oxidative stress and inflammation molecular markers. Current Issues in Molecular Biology, 46(9), 2024: 9639-658. doi.org/10.3390/cimb46090573.
37. Chaiya P, Senarat S, Phaechamud T, Narakornwit W. *In vitro* anti-inflammatory activity using thermally inhibiting protein denaturation of egg albumin and antimicrobial activities of some organic solvent materials today. Proceedings, 65(4), 2022: 2290-295 https://doi.org/10.1016/j.matpr.2022.04.916.
38. Sudhakaran G, Prathap, P, Guru A, Rajesh R, Sathish S, Madhavan T, Arasu MV, Al-Dhabi NA, Choi KC, Gopinath P, Arockiaraj J. Anti-inflammatory role demonstrated both *in vitro* and in vivo models using nonsteroidal tetranortriterpenoid, Nimbin (N1) and its analogs (N2 and N3) that alleviate the domestication of alternative medicine. Cell Biology International, 46(5), 2022: 771-791.
39. Shahidi F, Dissanayaka CS. Phenolic-protein interactions: insight from in-silico analyses – A review. Food Production, Processing and Nutrition, 5, 2023:2. https://doi.org/10.1186/s43014-022-00121-0.
40. Gonfa YH, Tessema FB, Bachheti A, Rai-Tadesse, MG, Singab AN, Chaubey KK, Bachheti RK. Anti-inflammatory activity of phytochemicals from medicinal plants and their nanoparticles: a review. Current Research in Biotechnology, 6, 2023: 100152. https://doi.org/10.1016/j.crbiot.2023.100152
41. Yesmin S, Paul A, Naz T, Rahman ABM, Akhter SF, Wahed MI, Emran TB, Siddiqui SA. Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Choi (*Piper chaba*). Clinical Phytoscience 6, 2020:59 https://doi.org/10.1186/s40816-020-00207-7