Advances In Multidisciplinary & Scientific Research
A Multidisciplinary & Interdisciplinary Journal
Volume 7, No 1, March, 2021 Edition

Membrane Stabilising and Xanthine oxidase Inhibitory Potentials of *Z. tessmannii* Root Extracts on Stressed Bovine Erythrocytes

***[1]Babarinde, S. O., [1]Adekoya E. S., [1]Abdulwasiu S., [2]Akinpelu, B. A., and [1]Oyedepo, T. A.**
[1]Department of Biochemistry, Adeleke University, Ede, Osun State, Nigeria.
[2]Department of Biochemistry and Molecular Biology, Obafemi Awolowo University, Ile-Ife, Nigeria.
Corresponding Author: *samuelbabarinde@adelekeuniversity.edu.ng

ABSTRACT

The persistence of the inflammatory response gives rise to chronic inflammation, which has been implicated in the pathogenesis of many chronic diseases, including arthritis, asthma, and cardiovascular diseases. Ethnomedicinal uses of different parts of *Zanthoxylum tessmannii* has been reported for tumours, inflammation and gonorrhoea. This study investigated the anti-inflammatory potentials of *Zanthoxylum tessmannii* roots, thereby providing scientific evidence for the reported traditional use of the plant. Erythrocyte membrane stabilisation and xanthine oxidase inhibitory activities of polar (aqueous, A.E. and ethanol, E.E.) and non-polar (ethyl acetate, EA.E and dichloromethane, DCM.E) extracts of *Z. tessmannii* roots were used to evaluate the anti-inflammatory property. Results showed that the four extracts of the plant roots exhibited varied membrane stabilisation profiles on hypotonic and heat-stressed erythrocytes. A.E. was shown to significantly ($p < 0.05$) stabilise the stressed erythrocyte membranes at 0.25 mg/ml compared to other extracts at different concentrations. The xanthine oxidase inhibitory activities of the extracts also showed that A.E. exhibited strong % inhibition of xanthine oxidase in a concentration-dependent manner. The highest % inhibition was 99.9 % at 0.4 mg/ml. EA.E, on the contrary, showed a declining % inhibition of xanthine oxidase with increasing concentration. Results from this study revealed that the aqueous extract of *Zanthoxylum tessmannii* root possess promising anti-inflammatory property and may provide lead compounds that would aid in better treatment of inflammatory-related diseases.

Keywords— Anti-inflammatory, erythrocytes, inflammation, membrane stabilisation, xanthine oxidase, *Zanthoxylum tessmannii*.

Aims Research Journal Reference Format:

Babarinde, S. O., Adekoya E. S., Abdulwasiu S., Akinpelu, B. A. & Oyedepo, T. A. (2021): Membrane Stabilising and Xanthine oxidase Inhibitory Potentials of *Z. tessmannii* Root Extracts on Stressed Bovine Erythrocytes". Advances in Multidisciplinary and Scientific Research. Vol. 7. No. 1, Pp 67–74 Article DOI: dx.doi.org/10.22624/AIMS/V6N1P6

1. INTRODUCTION

Inflammation is the body's natural defensive response to various stimuli, which may be endogenous or exogenous. Endogenous stimuli which activate the multiple events that characterise inflammation arise from damaged cells and tissue. Similarly, exogenous stimuli arise from microbial infection, chemical irritation and physical injury [1]. This immune response can be classified into two types, acute and chronic inflammation.

The function of acute inflammation is to identify, neutralise and eliminate the threat to the body. Therefore, acute inflammation is a beneficial process that protects the body from any harmful invasion [2]. The typical progression of inflammation results in a resolution phase after the threat has been effectively eliminated. The failure of this resolution usually constitutes a problem since the mechanisms activated to eliminate the harmful threat are still active, causing damage to nearby healthy cells and tissues; hence, disease conditions are triggered.

Chronic inflammation has been implicated in the onset of various non-communicable diseases including, arthritis, asthma, inflammatory bowel disease, cardiovascular diseases and even diabetes [3]. The release of lysosomal enzymes is one of the response mechanisms to eliminate invading pathogens, and their activities are associated with acute and chronic inflammation [4]. Xanthine oxidase is also an important pro-inflammatory enzyme whose product (uric acid) and by-products (reactive oxygen species, ROS) are known to trigger the inflammatory response [5]. Existing treatments of inflammation rely on steroids and non-steroidal anti-inflammatory drugs (NSAIDs). However, the prolonged use of these drugs has led to further health complications with unintended side effects. The phytodiversity has been relied upon for many centuries by different world populations to treat various disease conditions successfully. The scientific community has increasingly explored the plant biodiversity for alternative pharmacological products that may treat diseases with fewer adverse reactions in the least [6].

Zanthoxylum tessmannii is among the many indigenous plants of Africa, and its root is commonly used as a chewing stick. In addition, many scientific documentations have reported that different parts of the plant have been employed in traditional remedies for ailments like tumours, inflammation and gonorrhoea [7]. However, there is a dearth of information on the anti-inflammatory potentials of *Z. tessmannii* root, hence this study

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Fresh roots of *Zanthoxylum tessmannii* were collected from Alagbe, Ipetu-Ijesha, Osun state (7o28'43" N 4o53'59" E), Nigeria, with the help of a local hunter, Mr. Samuel. The plant roots, leaves and seeds were collected for identification in the month of February 2018 and were then identified and authenticated at the IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State in Nigeria.

2.2 Preparation of the Z. tessmannii Root Extracts

The cleaned root samples were air-dried at room temperature until they are free from moisture. The dried roots were cut into tiny bits and then ground to powder using a grinding mill. Approximately 130 g of the powdered *Z. tessmannii* root was extracted exhaustively by maceration for 72 hours in respective polar (aqueous and ethanolic) and non-polar (dichloromethane and ethyl acetate) solvents. The *Z. tessmannii* root solutions were separately decanted, filtered with a muslin cloth, and filtered with filtration apparatus. Each solution was concentrated using a rotary evaporator at 40 °C except for the aqueous solution of the extract that was freeze-dried to get the crude extracts. The extracts were stored in a desiccator till further use. The *Z. tessmannii* root samples and crude extracts were weighed, and the percentage yield calculated accordingly.

2.3 Preparation of Bovine Erythrocytes

Bovine blood was collected in anticoagulant (trisodium citrate 3.8% w/v) from the Adeleke University Cafeteria in Osun state. The freshly collected blood samples poured into clean, dry centrifuge tubes were centrifuged at 3000 rpm for 10mins in a refrigerated centrifuge. The supernatants obtained were discarded with the aid of dried and clean Pasteur pipettes. The packed cells of red blood cells left were re-suspended in fresh isosaline (0.85%), mixed carefully and gently to prevent lysis of the cells and then centrifuged at the same speed for 10 mins. The supernatant was then discarded again using a Pasteur pipette. This process was repeated till a clear supernatant was obtained. 500 ml of 2% (v/v) red blood cell was then prepared (i.e., 10ml of the packed erythrocytes, made up to 500 ml with isosaline) [4, 8].

2.4 Bovine Erythrocytes Membrane Stabilization Assay

The assay mixture consisted of hyposaline (1ml), 0.1M phosphate buffer pH 7.4 (0.5ml), varying concentration of aqueous, ethyl-acetate, ethanolic and dichloromethane extracts of the roots of *Z. tessmannii* (100mg/ml, 150mg/ml, 200mg/ml, 250mg/ml, and 300mg/ml respectively) and 0.5ml of 2% (v/v) erythrocyte suspension, making a total volume of 3ml. The drug control was prepared as stated above without the erythrocyte suspension. The standard anti-inflammatory drug used for the assay is prednisolone. The reaction mixtures were then incubated at 56^{o}C for 30mins. After incubating, the absorbance of the released haemoglobin was be read at 560 nm against the reagent blank. The percentage membrane stability was estimated using the expression:

$$= \frac{100 - \text{Abs. of test drug} - \text{Abs. of drug control}}{\text{Abs. of blood control}} \times 100 \quad \text{.....(1)}$$

Where

Abs. represents absorbance and the blood control represented 100% lyses.

2.5 In vitro Xanthine Oxidase Inhibition Assay of Z. tessmannii Root Extracts

The extracts were directly dissolved in phosphate buffer-MeOH (1%) and screened for xanthine oxidase inhibitory activity [9]. The mixture assay consisted of 150 µl phosphate buffer (150 mM, pH 7.5), 50 µl of the extract solutions at varying concentrations (0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml, and 0.5 mg/ml) and 50 µl enzyme solution (0.28 U/ml in phosphate buffer). The reaction was initiated by adding 250 µl of substrate solution (0.15 mM in the same buffer). Enzymatic kinetic was recorded at 295nm for 2 min. Negative control was prepared and contained 1% methanol solution without extract solution. Allopurinol, a well-known inhibitor of xanthine oxidase, was used as a positive control at varying concentrations. All experiments were performed in triplicates. Xanthine oxidase inhibitory activity was expressed as the percentage inhibition of xanthine oxidase, calculated as (%) inhibition following:

$$\% \text{ inhibition} = ((\Delta\text{Abs. control} - \Delta\text{Abs. sample})/\Delta\text{Abs. control}) \times 100 \text{.....(2)}$$

Where

ΔAbs. represents change in absorbance.

2.6 Statistical Analysis

Results within the groups were expressed as mean ± SEM for triplicate independent experiments. The statistical significance of the data was analysed with a one-way ANOVA followed by the Post hoc-tukey analysis. P-values of less than 0.05 was considered to be statistically significant [10, 11].

3. Results and discussion

3.1 Bovine RBC Membrane Stabilising Activity of Crude Extracts of Z. tessmannii Roots

All the extracts of *Z. tessmannii* roots at different concentrations showed variable stabilisation of the membranes of stressed erythrocytes compared with the standard anti-inflammatory drug used (Table 1). While the aqueous and dichloromethane extracts were consistent in comparing favourably with the standard drug used across the range of concentrations, the ethanolic extract was less consistent. Conversely, ethyl acetate extract compared poorly with the standard drug over the range of concentrations.

The statistical multiple comparisons of the membrane stabilising profiles of all the extracts of *Z. tessmannii* roots across the concentrations showed that the aqueous extract at 0.25 mg/ml was statistically significant ($p<0.05$) membrane stabilisation with other extracts at different concentrations. Lysosomal constituents of activated neutrophils are often released as part of the inflammatory response. These constituents, including bactericidal enzymes and proteases, are released to destroy invading pathogens [12].

However, a persistent inflammatory response further destroys nearby healthy cells and tissues. Therefore, the stabilisation of the lysosomal membrane, which prevents the spilling of its constituents, is a crucial mechanism of anti-inflammatory drugs [4]. Since the structural architecture of the erythrocyte membrane is analogous to that of the lysosome, the stabilisation of erythrocyte membranes has been employed extensively by investigators as a model for evaluating anti-inflammatory activity [13].

Table 1: Multiple Comparison of Membrane Stabilising activities of crude extracts of *Z. tessmannii* Across a Range of Concentrations on Bovine Erythrocytes Exposed to both Heat and Hypotonic Induced Lyses.

	Volume (ml)							
		0.05	0.1	0.15	0.2	0.25	0.3	
0.1 mg/ml	Pred	55.97±0.62	23.27±2.67	45.82±0.84	52.83±0.47	54.2±1.78	59.63±0.97	p>0.05
	A.E	44.54±0.10	82.18±1.94	76.10±1.26	77.77±0.73	73.27±2.2	76.41±0.94	p>0.05
	E.E	25.47±1.41	33.96±0.00	38.67±1.41	11.32±0	6.81±1.04	5.13±0.73	p<0.05
	EA.E	24.05+0.22	4.72±0.31	40.09±0.25	3.30±0.00	9.43±0.02	3.30±0.01	p<0.05
	DCM.E	84.591±0.29	52.58±0.35	40.25±0.23	75.47±0.12	46.54±0.36	34.59±0.23	p<0.05
0.15 mg/ml	Pred	27.67±3.46	62.89±2.04	64.77±0.16	63.31±0.56	73.58±1.88	55.66±0.94	p>0.05
	A.E	59.44±0.94	44.34±0	70.44±0.16	77.98±0.31	48.11±0.00	77.39±1.43	p>0.05
	E.E	29.24±0.94	21.17±3.98	22.32±2.51	24.84±2.20	-42.14±1.73	-3.77±0.00	P<0.05
	EA.E	28.30±0.32	31.13±0.63	19.81±0.20	22.64±0.32	-43.86±0.21	-3.77±0.02	p>0.05
	DCM.E	81.45±0.52	35.37±0.35	45.75±0.84	49.5285	4.71±0.03	-7.54±0.05	p<0.05
0.2 mg/ml	Pred	71.7±0.00	67.29±1.57	61.01±1.73	62.26±0.00	93.41±2.83	51.89±2.36	p>0.05
	A.E	85.85±0.00	66.98±0.47	84.90±0.94	78.30±0.94	80.18±0.94	73.17±0.73	p>0.05
	E.E	88.68±1.88	97.27±2.41	87.1±1.73	95.89±0.16	88.99±0.16	95.60±1.73	p>0.05
	EA.E	90.56±1.33	99.68±2.32	88.67±1.23	95.26±0.93	92.80±1.23	99.68±0.48	p>0.05
	DCM.E	89.93±0.89	94.96±1.31	102.83±2.23	104.40±1.45	105.66±2.21	105.66±3.72	p>0.05
0.25 mg/ml	Pred	65.73±0.78	84.90±1.88	82.45±0.33	63.52±1.73	83.33±1.1	72.32±0.16	p>0.05
	A.E	85.53±2.04	92.24±0.84	98.11±0	95.59±0.31	96.54±0.16	93.4±1E-14	p>0.05
	E.E	62.57±3.61	27.76±3.17	-55.97±4.08	-67.92±3.30	81.44±0.78	66.04±0.00	p>0.05
	EA.E	58.96±0.43	24.59±0.32	-51.88±0.84	-64.62±0.34	80.66±0.38	58.01±0.32	p>0.05
	DCM.E	40.53±0.38	82.70±0.23	69.49±0.39	94.33±0.92	99.68±0.49	101.41±024	p>0.05
0.3 mg/ml	Pred	125.69±0	60.38±0	-32.39±3.29	-65.09±0	62.79±1.15	59.11±2.51	p>0.05
	A.E	70.76±0	59.12±0.78	72.95±0.16	64.46±2.67	61.32±0	50.96±0.70	p>0.05
	E.E	60.06±2.04	61.00±1.57	89.62±0.47	86.79±3.77	81.13±0.47	-33.01±1.42	p>0.05
	EA.E	66.667±0.22	59.43±0.82	89.15±0.69	83.01±0.24	80.66±0.03	-31.60±0.43	p>0.05
	DCM.E	65.0945±0.32	54.57±0.74	92.45±0.92	83.01±1.05	96.23±0.02	58.02±0.34	p>0.05

Each value represented the mean ± SEM of 3 readings, and $p < 0.05$ was considered significant. Pred - Prednisolone; A.E – Aqueous Extract; E.E – Ethanol Extract; EA.E – Ethyl acetate Extract; DCM.E – Dichloromethane Extract.

The erythrocyte membrane stabilisation activities of all *Z. tessmannii* root extracts showed that the four extracts of the plant roots exhibited varied membrane stabilisation profiles on hypotonic and heat-stressed erythrocytes. Although the aqueous and dichloromethane extracts compared favourably with the standard anti-inflammatory drug used across the range of tested concentrations, the aqueous extract at 0.25 mg/ml exhibited statistically significant ($p>0.05$) inhibition to other extracts at different concentrations.

By implication, it is considered that *Z. tessmannii* root possesses anti-inflammatory activity since it can stabilise the lysosomal membrane and, therefore, mitigate the destructive actions of proteases resident in the lysosomes on healthy tissues. *Zanthoxylum macrophylla* roots had been reported to have some roles in stabilising erythrocyte membranes which justified its use in managing sickle cell disease [14].

3.2 Xanthine Oxidase Inhibitory Activity

The xanthine oxidase inhibitory activities of all the extracts of Z. *tessmannii* roots varied across the range of concentrations (Table 2). The aqueous extract exhibited the highest inhibitory activity in a dose-dependent manner. While dichloromethane compared favourably with the standard drug used, ethanol extract was only comparable at high concentrations. The inhibitory activity of ethyl acetate extract decreased as concentrations increased.

Table 2 Xanthine Oxidase Inhibitory Activity of the Crude Extracts of *Z. tessmannii* Roots

S/N	Concentration (mg/ml)	Aqueous Extract (% inhibition)	Ethyl acetate Extract (% inhibition)	Dichloromethane Extract (% inhibition)	Ethanol Extract (% inhibition)	Allopurinol (% inhibition)
1	0.1	46.92±0.44	70.00±0.44	88.46±2.22	--	^{a,b}82.41±0.16
2	0.2	73.08±2.22	68.81±0.24	73.08±2.22	42.30±6.66	^{b,d}72.54±0.17
3	0.3	**95.38**±0.44	30.00±0.44	77.69±0.44	75.77±0.66	^{a,b,c,d}67.53±0.17
4	0.4	**99.99**±0.00	23.46±0.22	63.46±1.11	69.23±4.44	^{a,b,c}54.39±0.34
5	0.5	**99.81**±0.11	16.12±0.22	52.69±0.67	99.88±0.07	^{a,b,c,d}69.55±0.02

Each value represented the mean ± SEM of 3 readings, and $p < 0.05$ was considered significant.
a,b,c,d denote significant differences compared with aqueous, ethyl acetate, ethanol and dichloromethane extracts, respectively. Xanthine oxidase catalyses the hydroxylation of hypoxanthine to xanthine and then converts xanthine to uric acid. Excessive production of uric acid, hyperuricemia results in its supersaturation and crystallisation in circulation. Subsequently, the crystals are deposited in joints, leading to inflammation with pain [15]. Thus, Xanthine oxidase is a target enzyme and a model in the assessment of anti-inflammatory activities. The results of the inhibition of xanthine oxidase revealed that the aqueous extract of *Z. tessmannii* roots presented statistically significant inhibition of the xanthine oxidase enzyme in a concentration-dependent manner.

Therefore, *Z. tessmannii* root aqueous extract can exert anti-inflammatory activity via preventing the generation of reactive oxygen species (ROS) and production of uric acid by xanthine oxidase. Both products are implicated in triggering inflammatory responses. This mechanism reveals that *Z. tessmannii* roots possess active components that may aid the treatment and management of gout arthritis. Similarly, it has been reported that active compounds in *Zanthoxylum armatum* fruits inhibited xanthine oxidase and may serve as a source of products that could be better in the management of gout [16].

4. Conclusion

This study revealed that the aqueous extract of *Zanthoxylum tessmannii* root exhibited good erythrocyte membrane stabilisation and significant inhibition of xanthine oxidase. Hence, *Z. tessmannii* roots possess promising anti-inflammatory properties and may provide lead compounds that would aid in better treatment of inflammatory-related diseases. Therefore, further studies are recommended to identify bioactive constituents in *Z. tessmannii* roots responsible for these activities.

REFERENCES

[1] Samuel, B. B., Oridupa, O. A., and Gbadegesin, F. (2019). Anti-inflammatory and Analgesic Potential of Acetone Leaf Extract of Combretum Sordidum and its Fractions, *Acta Pharmaceutica Sciencia*, 57(2), 147-159. doi:10.23893/1307-2080.APS.05709.

[2] Aghasafari, P., George, U., and Pidaparti, R. (2019). A Review of Inflammatory Mechanism in Airway Diseases. *Inflammation Research*, 68: 59-74. doi:10.1007/s00011-018-1191-2.

[3] Zhu, F., Du, B., and Xu, B. (2018). Anti-inflammatory Effects of Phytochemicals from Fruits, Vegetables, and Food Legumes: A review. *Critical Reviews in Food Science and Nutrition*, 1260-1270.

[4] Kumari, C. S., Yasmin, N., Hussain, M. R., and Babuselvam, M. (2015). In vitro Anti-inflammatory and Anti-arthritic Property of Rhizopora Mucronata Leaves. *International Journal of Pharma Sciences and Research*, 6(3), 482-485.

[5] Olanrewaju, O., Apata, J., Akinpelu, B., Akomolafe, R., Oyemitan, I., Asaolu, F., Ologe M., and Iwalewa, E. (2018). Anti-inflammatory Potentials, Membrane Stabilising and Xanthine Oxidase Inhibitory Activities of *Clerodendrum volubile* Ethanolic Extract on Carragenaan-induced Inflammation in Rats. *International Journal of Pharmacology and Toxicology*, 6(1), 7-11. doi:10.14419/ijpt.v6i1.8410.

[6] Akinpelu, L., Olawuni, I., Ogundepo, G., Adegoke, A., Olayiwola, G., and Idowu, T. (2019). Spectroscopic Analysis and Anti-Inflammatory Effects of *Milicia excelsa* (*Moraceae*) Leaf and Fractions. *GSC Biological and Pharmaceutical Sciences*, 6(3), 51-60.

[7] Patiño, L., Prieto, R., and Cuca, S. (2012). "Zanthoxylum Genus as Potential Source of Bioactive Compounds, Bioactive Compounds in Phytomedicine." *Bioactive Compounds in Phytomedicine* Prof. I. Rasooli (Ed.), Rijeka: *InTech*, 185-218.

[8] Oyedapo, O., Akinpelu, B., Akinwunmi, K., Adeyinka, M., and Sipeolu, F. (2010). Red Blood Cell Membrane Stabilising Potentials of Extracts of *Lantana camara* and its Fractions. *International Journal of Plant Physiology and Biochemistry*, 2(4), 46-51.

[9] Konaté, K., Souza, A., Thérèse, K., Dibala, I., Barro, N., Raso-lodimby, J., and Nacoulma, O. (2011). Phytochemical Composition, Antioxidant and Anti-inflammatory Potential of Bioactive Fractions from Extracts of three Medicinal Plants Traditionally used to Treat Liver Diseases in Burkina Faso. *International Journal of Phytomedicine*, 3: 406-415.

[10] Liu, X., Xie, J., Jia, S., Huang, L., Wang, Z., Li, C., and Xie, M. (2018). Immunomodulatory Effects of an Acetylated *Cyclocarya paliurus* Polysaccharide on Murine Macrophages RAW264.7. *International Journal of Biological Macromolecules*, 2018, 98: 576–581. doi:10.1016/j.ijbiomac.2017.02.028.

[11] Saravanan, S., V.I.H., I., David, D., Sundaram, R., Chellappandian, M., Balakrishna, K., Rajendran R., Vijayaraghavan P., Paulraj M.G., and Ignacimuthu S. (2015). Bioassay-guided Fractionation and Identification of Active Anti-inflammatory Constituents from *Delonix elata* Flowers using RAW 264.7 Cells. *Pharmaceutical Biology*, 53(2), 174-184. doi:10.3109/13880209.2014.913067.

[12] Parvin, M. S., Das, N., Nusrat, J., Akhter, M. A., Nahar, L., and Islam, M. E. (2015). Evaluation of *In vitro* Anti-inflammatory and Antibacterial Potential of *Crescentia cujete* Leaves and Stem Bark. *BMC Research Notes*, 8(412), 1-7. doi:10.1186/s13104-015-1384-5.

[13] Akinpelu, B. A., Makinde, A. M., Isa, M. O., Taiwo, P. O., Ojelabi, M. O., and Oyedapo, O. O. (2015). In vitro Evaluation of Membrane Stabilizing Potential of Selected Bryophyte Species. *European Journal of Medicinal Plants*, 6(3), 181-190. doi:10.9734/EJMP/2015/7629.

[14] Elekwa, I., Monanu, M. O., and Anosike, E. O. (2005). Effects of Aqueous Extracts of *Zanthoxylum macrophylla* Roots on Membrane Stability of Human Erythrocytes of Different Genotypes. *Biokemistri*, 17(1), 7-12.

[15] Pieczykolan, A., Pietrzak, W., Gawlik-Dziki, U., and Nowak, R. (2021). Antioxidant, AntiInflammatory, and Anti-Diabetic Activity of Phenolic Acids Fractions Obtained from *Aerva lanata* (L.) *Juss*. *Molecules*, 26(3484), 1-17. doi:10.3390/molecules26123486.

[16] Ranjana, Nooreen, Z., Bushra, U., Jyotshna, Bawankule, D. U., Shanker, K., Ahmad A., and Tandon, S. (2019). Standardization and Xanthine Oxidase Inhibitory Potential of *Zanthoxylum armatum* Fruits. Journal of Ethnopharmacology, 230: 1-8. doi:10.1016/j.jep.2018.10.018.