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Research Article

Bioassay-Guided Fractionation and Identification of Anti-Inflammatory and Xanthine Oxidase Inhibitory Compounds of *Zanthoxylum tessmannii* Roots

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ABSTRACT

Several bioactive compounds identified in *Zanthoxylum tessmannii* have validated many traditional medicine applications of the plant. Studies have highlighted the anti-inflammatory properties of crude extracts of the plant and the allergenic properties of both extracts and fractions of the plant roots. This study aimed to identify the compounds responsible for these effects in *Z. tessmannii* roots. Using bioassay-guided fractionation, the aqueous extract was partitioned into n-hexane, dichloromethane, ethyl acetate, and butanol fractions, followed by partial purification of the most active fraction with silica gel chromatography. Fractions and sub-fractions were tested for inhibition of protein denaturation, xanthine oxidase (XO), and lipoxygenase activities. The aqueous fraction (AF) showed significantly higher inhibition of protein denaturation (46.74±2.17%) compared to dichloromethane (35.05±1.51%) and butanol (34.78±1.09%) fractions at concentrations of 0.0015 mg/mL and 0.003 mg/mL. AF also demonstrated the highest inhibition of XO activity. Sub-fraction A, from the most active AF, showed 93.19±0.08% inhibition of lipoxygenase. GC-MS analysis of sub-fraction A identified compounds like E-15-Heptadecenal, 9-Octadecenoic acid (Z)-2,3-dihydroxy propyl, trans-13-Octadecenoic acid, Oleic acid, and Tetraacetate 1-Hexanethiol. The findings highlight that *Z. tessmannii* root possesses compounds with known anti-inflammatory effects and those indicating allergenic symptoms, suggesting both therapeutic potential and possible harmful effects.

Keywords: *Inflammation, Xanthine oxidase, Inhibition, Ethnomedicinal, Zanthoxylum tessmannii, Bioactive compounds*

INTRODUCTION

The *Zanthoxylum* genus, with over 220 species identified in locations such as the warm temperate and subtropical regions of the world, has been reported as an excellent source of bioactive compounds (Ombito, 2021). In Africa, this genus also known as *Fagara*, is one of many indigenous plants with varying ethnomedicinal uses (Okagu *et al.*, 2021). Different parts of the species *Zanthoxylum tessmannii*, predominantly found in Central and Western Africa, is traditionally used to treat many health conditions including toothache, cancers, varying types of infections, erectile dysfunctions, tumours and inflammation among others (Djeukeu *et al.*, 2019; Okagu *et al.*, 2021). Many researchers have demonstrated that *Z. tessmannii* possesses

potent antioxidant properties, exhibits anti-proliferative effects against cancer cell lines and demonstrate efficacy in hypolipidemic, cardioprotective, and hepatoprotective activities (Mbaveng *et al.*, 2019; Okagu *et al.*, 2021). Furthermore, numerous bioactive compounds have been isolated from this species, thus providing scientific bases for many reported traditional health application of the plant. Anti-bacterial compounds such as 2,6-dimethoxy-1,4-benzoquinone and 3β-acetoxy-16β-hydroxybetulinic acid (Mbaze *et al.*, 2007; Tankeo *et al.*, 2015) and cytotoxic benphenanthridines have been identified in *Z. tessmannii* (Mbaveng *et al.*, 2019).

Inflammation is a complex process that involves both the activation and regulation of immune cells. This intricate mechanism, if uncontrolled, can lead to cellular and tissue damage, potentially contributing to the development of chronic diseases (Guo *et al.*, 2018). Chronic inflammation has been linked to the development of numerous non-communicable diseases, such as the onset and progression of athritis, cardiovascular diseases, asthma and inflammatory bowel disease (Zhu et al., 2018). Allergies are primarily characterized by an inflammatory response that has become a global clinical health concern (Guo *et al.*, 2018). This often leads to symptoms such as itching, swelling, and respiratory issues due to the body's hypersensitivity to certain substances (Stojković *et al.*, 2015). Likewise, gout disease is a form of inflammatory arthritis. It is caused by the excessive activity of the enzyme, xanthine oxidase (XO) (Bou-Salah *et al.*, 2021). Current treatments for chronic inflammation primarily consist of steroids and non-steroidal anti-inflammatory drugs (NSAIDs), but the long-term use of these medications has resulted in health complications and undesirable side effects (Sohail et al., 2023).

In a previous study, Sohail et al., (2023) demonstrated that crude extracts of *Z. tessmannii* roots possessed anti-inflammatory properties, suggesting the presence of promising bioactive compunds that could contribute to the development of new therapeutic agents. In a separate study, Babarinde *et al.* (2021) found that extracts and fractions of *Z. tessmannii* roots exhibited aggressive stimulation of mast cell degranulation and thus, may possess compounds with allergenic properties. This dual but conflicting properties underscore the dangers associated with unguarded traditional use of indigenous plants. Therefore, it is imperative to identify the compounds responsible for these previously reported properties of the plant towards the development of safer therapeutic applications.

The present study was designed to explore the bioassay-guided identification of compounds responsible for the anti-inflammatory and allergenic properties of *Zanthoxylum tessmannii* roots.

MATERIALS AND METHODS

Collection and identification of plant material

Fresh roots of *Zanthoxylum tessmannii* were collected from Alagbe, Ipetu-Ijesha, Osun state (7°28'43" N 4°53'59" E), Nigeria. The plant roots were collected for identification in February 2018 and were then identified and authenticated with voucher number IFE HERBARIUM 18311 at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State in Nigeria.

Preparation of the *Z. tessmannii* root extracts

The cleaned root samples of *Zanthoxylum tessmannii* were air-dried at room temperature. The dried roots were cut into tiny bits and then ground to powder using a grinding mill. The powdered *Z. tessmannii* root was extracted exhaustively by maceration for 72 hours in aqueous solvent. The *Z. tessmannii* root mixture was decanted, filtered with a muslin cloth before further filtration using a vacuum filter. The aqueous filtrate of the extract was freeze-dried to get the crude aqueous extract. The extract was then stored in a desiccator till further use.

Solvent-solvent partitioning

Solvent-solvent partitioning of the aqueous extract of *Z. tessmannii* root was carried out according to Rédei *et al.* (2017). The aqueous extract was partitioned successively with four solvents of varying polarities (n-hexane, dichloromethane, ethyl acetate and butanol). The aqueous extract previously concentrated to 300 mL was defatted with n-hexane (500 mL x 2), which was not used. Thereafter, the aqueous residue was sequentially partitioned with dichloromethane (500 mL x 2), ethyl acetate (500 mL x 2) and butanol (500 mL x 2). The collected fraction solutions were concentrated using a rotary evaporator or lyophilised to obtain their respective fractions, dichloromethane (DF), butanol (BF), and aqueous (AF) fractions. The ethyl acetate which did not yield any substantial fraction was not used. The resultant fractions were then analysed for their anti-inflammatory activity using the models of protein denaturation and xanthine oxidase inhibitions as described below to determine the most active fraction (MAF).

Inhibition of protein denaturation assay

Inhibition of protein denaturation was carried out according to the method described by Patel and Zaveri (2014) and Kumari *et al.* (2015). The reaction mixture (0.5 mL) contained 0.45 mL of bovine serum albumin (5% aqueous solution) and 0.05 mL of Z. *tessmannii* root fractions (at 0.0015 mg/mL and 0.003 mg/mL respectively). After 20 mins of incubation at 37°C, the samples were heated at 57°C for 3 min. After cooling the samples, 2.5 mL phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm. The control test contained 0.05 mL of distilled water in place of root fractions and excluded bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows:

$$\% \text{ inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abscontrol}} \times 100 \qquad (1)$$

Where ΔAbs is change in Absorbance

The control represents a complete absence of protein, serving as a reference point for comparing the extent of protein denaturation. The results were compared with acetylsalicylic acid (aspirin) (250 mg/mL) treated samples as the standard drug.

In vitro xanthine oxidase inhibition assay of *Z. tessmannii* root fraction

Xanthine oxidase (XO) inhibitory activities of the fractions were evaluated using a spectrophotometer in accordance with Konaté *et al*. (2011) with some modifications. The extracts were directly dissolved in phosphate buffer-MeOH (1%) and tested for xanthine oxidase inhibitory activity. The mixture contained 150 µL of phosphate buffer-methanol (150 mM, pH 7.5), varying concentrations of the fractions and 50 µL of XO solution (0.28 U/mL in phosphate buffer-methanol). The reaction was initiated by adding 250 µL of xanthine solution (0.15 mM in the same buffer The enzyme activity (rate of conversion of xanthine to uric acid by xanthine oxidase) was measured at 295 nm for 2 min. Negative control was prepared containing 1% methanol solution without fraction solutions. Allopurinol, a well-known inhibitor of xanthine oxidase, was used as a positive control at 20 – 100 µg/mL. All experiments were performed in triplicates. Xanthine oxidase inhibitory activity was expressed as the percentage inhibition of xanthine oxidase, calculated as (%) inhibition following:

$$\% \text{ inhibition} = \frac{(\Delta\text{Abs control} - \Delta\text{Abs sample})}{\text{Abscontrol}} \times 100 \quad (2)$$

Where ΔAbs is change in Absorbance

Column fractionation of the most active *z. Tessmannii* root fraction

The aqueous fraction which showed significant anti-denaturation (direct anti-inflammatory activity) and anti-xanthine oxidase (indirect anti-inflammatory activity) effects was selected for column fractionation, as described by Rédei *et al*. (2017). The fraction was submitted to column chromatography (CC) on silica gel (particle size: 200-400 mesh) column (A) using a step gradient eluting solvent system of ethyl acetate and methanol as follows; 10:0 (200 mL), 9: 1 (200 mL), 8: 2 (200 mL), 7:3 (200 mL), 6:4 (200 mL), 5:5 (600 mL), 4:6 (200 mL), 3:7 (200 mL), 2:8 (200 mL), 1:9 (200 mL), and 0:10 methanol (200 mL) at the flow rate of 769 µL per min to yield 146 sub-fractions. Sub-fractions of 10 mL each were collected, monitored by TLC and merged based on similarity of the spots observed on the thin-layer chromatograms. The optimised solvent system for the TLC employed in monitoring the sub-fractions was DCM/EtOAc/MeOH (16:2:1), and a silica gel-coated stationary phase resulting in three partially purified subfractions A, B and C of the MAF. The sub-fractions obtained were bio-assayed for their anti-inflammatory activities using the xanthine oxidase and the lipoxygenase inhibitory assays to determine the most active sub-fraction.

In vitro lipoxygenase inhibitory assay

With adjustments to the procedures as described by Konaté *et al*. (2011) and Olarenwaju *et al*. (2018), the lipoxygenase inhibitory activities of *Z. tessmannii* sub-fractions were assessed using a UV-VIS spectrophotometer and linoleic acid as a substrate. The lipoxygenase inhibitory activities of the sub-fractions were screened using various concentrations (0.1-0.5 mg/ mL). The assay mixture (500 µL) comprised of 150 µL phosphate buffer (0.067 M, pH 7.4) as well as 50 µL each of lipoxygenase enzyme solution (0.28 U/mL in the phosphate buffer) and the sub-fractions. The reaction was initiated by adding 250 µL of substrate solution (0.15 mM) in phophate buffer-EtOH (1%). Enzyme activity (conversion of linoleic acid to conjugated dienes) was monitored at 234 nm, with measurements taken every 15 seconds over a period of 2 minutes. Negative control was prepared and contained 1% (v/v) methanol solution without the sub-fractions. As a standard (positive control), quercetin, known to inhibit lipoxygenase, was utilized at varying concentrations (0.1-0.5 mg/mL). Every experiment was run in triplicate, while the expression below was used to calculate the inhibition of lipoxygenase activity;

$$(\%) \text{ inhibition} = \frac{\text{Absorbance of control - Absorbance of test x 100}}{\text{Absorbance of control}} \quad (3)$$

Lipoxygenase inhibitory activity was expressed as the percentage inhibition of lipoxygenase.

Identification of bioactive compounds using GC-MS spectrometry analysis

The bioactive compounds present in sub-fraction A of *Z. tessmannii* was characterised by GC-MS analysis using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 250 mm in diameter × 0.25 mm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50 –150°C with increasing rate of 3°C /min and holding time of about 10 min. Finally, the temperature was increased to 300°C at 10°C /min. One microliter of the prepared 1% of the partially purified fraction diluted with methanol was injected in a splitless mode. Relative quantity of the constituent compounds present in the fraction of *Z. tessmannii* was expressed as percentage based on peak area produced in the chromatogram.

Statistical analysis

Results from each group were expressed as mean ± standard deviation (SD). The statistical significance of the data was determined using one-way ANOVA which was followed by

the Tukey's post hoc test. $p < 0.05$ was thought to be statistically significant

RESULTS AND DISCUSSION

Inhibition of protein denaturation

The protein denaturation assay conducted on the fractions of *Z. tessmannii* revealed that in the two concentrations (0.0015 mg/mL and 0.003 mg/mL), the aqueous fraction (AF) exhibited a significantly ($p < 0.05$) higher percentage of protein denaturation inhibition (46.74±2.17%) compared to the other fractions from the plant roots (Figure 1). Specifically, the dichloromethane (DF) showed an inhibition of 35.05±1.51%, while the butanol fractions (BF) displayed an inhibition of 34.78±1.09%. Notably, there was no significant difference in the inhibition percentages between the DF and BF.

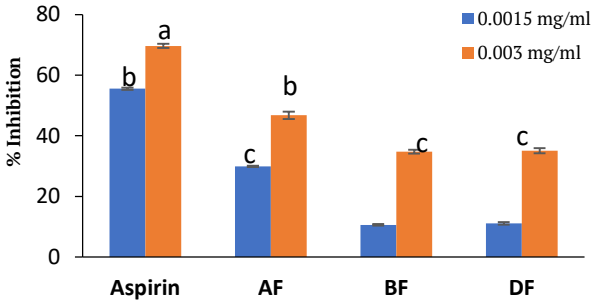


Figure 1. Percentage (%) Inhibition of Protein Denaturation by Various Fractions of *Z. Tessmannii* Roots Extract

The results represent as mean ± SD. a, b and c were used to denote significant difference (p<0.05) when compared with the positive control (aspirin) across the concentrations. AF – Aqueous Fraction; BF – Butanol Fraction; DF – Dichloromethane Fraction.

Xanthine Oxidase Inhibition Activities of *Z. tessmannii* Root Fractions

Results indicate that at both concentrations of 0.0015 mg/mL and 0.003 mg/mL, the AF demonstrated the highest inhibition of XO activity. This inhibition was found to be comparable to that achieved by the standard drug, allopurinol. In contrast, the BF and DF showed poor inhibition of XO activity (Figure 2).

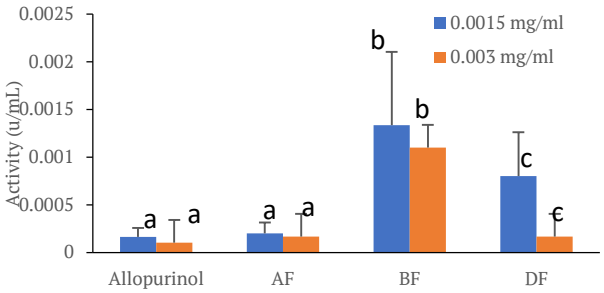


Figure 2. Xanthine Oxidase Activity Following Exposure to Aqueous, Butanol and Dichloromethane Fractions of *Z. Tessmannii* Roots Extract

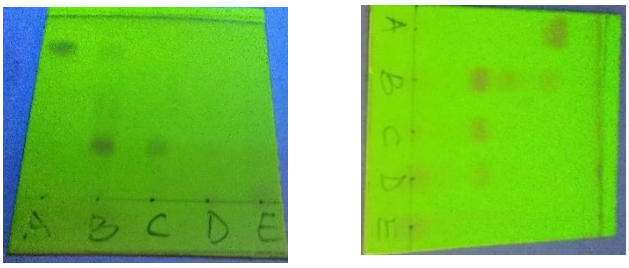
Results represent the mean ± SD. The letters a, b and c denote significant difference (p<0.05) when compared with the standard drug (allopurinol) across the concentrations.
AF – Aqueous Fraction; BF – Butanol Fraction; DF – Dichloromethane Fraction

Column Chromatography of the Most Active (Aqueous) Fraction of *Z. tessmannii* Roots

Based on the protein denaturation XO assays, the aqueous fraction (AF) was used for the column chromatography, yielding three spots from the eluted fractions 14 – 16 (sub-fraction A), 21 – 26 (sub-fraction B) and 30 – 36 (sub-fraction C) as observed by Thin Layer Chromatography (Plate 1).

Xanthine Oxidase Inhibitory Activities of Sub-fractions of *Z. tessmannii* Roots

All sub-fractions of *Z. tessmannii* roots exhibited good xanthine oxidase inhibition in a concentration-dependent manner (Figure 3). Although the result suggests that the xanthine oxidase inhibitory activity of sub-fraction B was similar to the control drug across all concentrations. There was no significant difference ($p > 0.05$) between the three sub-fractions.



(a) (b)

Plate 1. Bulked Eluted Fractions of *Z. tessmannii* Root Showing
(a) Partially Purified Fractions Before Concentration
(b) Partially Purified Fractions After Concentration

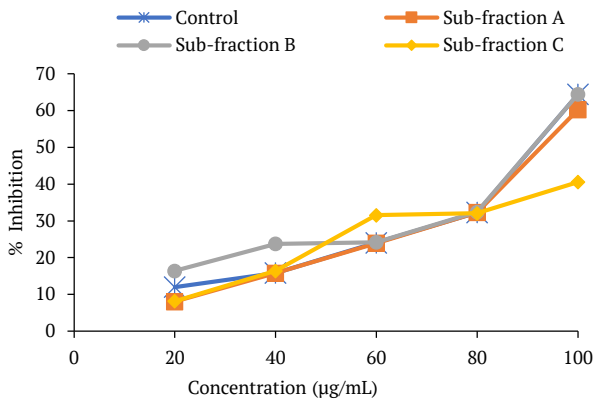


Figure 3. Xanthine Oxidase Inhibitory Activities of Aqueous Sub-fractions A, B, and C of *Z tessmannii* Roots

Values represent the mean ± SD of triplicate readings

Lipoxygenase Inhibitory Activities of *Z. Tessmannii* Root Sub-Fractions

The lipoxygenase inhibitory activities of all the sub-fractions revealed a significant ($p<0.05$) percentage inhibition of the lipoxygenase enzyme in a concentration-dependent manner. It was, however, shown that sub-fraction A exhibited the highest percentage inhibition at 93.19±0.08 % (Table 1).

GC-MS Analysis of the Most Active Sub-fraction of *Z. tessmannii* Roots

Selected compounds identified in the GC-MS analysis with their retention times, peak numbers and percentage compositions are presented in Table 2. Table 3 contains previously reported anti-inflammatory and allergenic activities of some constituents with relatively higher percentage compositions.

Discussion

Babarinde *et al*. (2021) had demonstrated that aqueous crude extracts of Z. *tessmannii* possessed good anti-inflammatory activities while the extracts and fractions exhibited aggressive stimulation of mast cell degranulation. In this study, aqueous crude extracts of *Z. tessmannii* was subjected to solvent/solvent partitioning, and the resulting fractions were evaluated for anti-inflammatory activities using protein denaturation and xanthine oxidase inhibition assays. The fraction with the highest biological activity was further subjected to column chromatography, and the sub-fractions obtained were subjected to bioactivity-guided identification of the bioactive compounds in the most active sub-fraction. Protein denaturation has been documented as a cause of several inflammatory disorders such as rheumatoid arthritis, glomerulonephritis, systemic lupus erythematosus, cancer and diabetes (Aidoo *et al*., 2021; Concha *et al*., 2022). Modi *et al*. (2019) described the mechanism involved in protein denaturation to encompass a disruption of the tertiary and secondary structures of protein which is caused by changes in the electrostatic forces, hydrophilic interactions, hydrogen, and disulphide bonds. Other physical and chemical agents such as acids, alkalis, alcohol, acetone, heavy metal salts, light, heat, and pressure are also responsible for altering the native conformations of proteins. It had been reported previously, that the anti-inflammatory drugs salicylic acid, phenylbutazone, diclofenac sodium, among others, had a dose-dependent inhibition of protein denaturation (Khalid *et al*., 2021; Saleem *et al*., 2020). In the present study, the inhibition of protein denaturation by all fractions of *Z. tessmannii* roots was observed to be moderate. While the percentages of protein denaturation inhibition by dichloromethane (DF) and butanol fractions (BF) was significantly lower ($p < 0.05$) than that of aspirin, the aqueous fraction (AF) demonstrated significantly greater inhibition activity ($p < 0.05$) compared to both DF and BF. These findings align with those of Dhami *et al*. (2019), who found that aqueous extract of *Zanthoxylum armatum* possessed anti-inflammatory activity as demonstrated by its *in vitro* inhibition of protein denaturation. In an earlier investigation, Patel and Zaveri (2014) showed that both the methanolic extract and toluene fraction of *Justicia gendarussa* inhibited protein denaturation by regulating the generation of autoantigens. Additionally, various researchers have indicated that protein denaturation may play a role in the production of autoantigens associated with inflammatory rheumatoid arthritis (Patel and Zaveri 2014). The findings of this study, therefore, suggest that the inhibiting protein denaturation could be a mechanism through which *Z. tessmannii* roots exert anti-inflammatory effects. Xanthine oxidase (XO) plays a crucial role in purine metabolism, leading to the production of uric acid (Tian *et al*., 2021). The enzyme catalyses the hydroxylation of hypoxanthine to xanthine and then finally converts xanthine to uric acid.

Table 1. Lipoxygenase (LOX) Inhibitory Activities of Aqueous Sub-Fractions of *Z. Tessmannii* Roots

Concentrations (mg/mL)	Quercetin control (% inhibition)	Sub-fraction A (% inhibition)	Sub-fraction B (% inhibition)	Sub-fraction C (% inhibition)
1	81.45 ± 0.17^{b}	93.08 ± 0.08^{a}	69.58 ± 2.21^{c}	63.44 ± 1.22^{dc}
0.5	78.27 ± 0.47^{a}	76.53 ± 0.49^{a}	61.31 ± 0.96^{b}	$^{c}57.91 \pm 0.16^{b}$
0.25	60.55 ± 0.41^{ab}	53.29 ± 4.75^{bc}	61.51 ± 0.60^{a}	54.41 ± 0.57^{b}
0.125	43.75 ± 0.41^{b}	50.57 ± 0.73^{a}	56.99 ± 0.50^{a}	54.54 ± 0.24^{a}
0.0625	31.86 ± 0.29^{b}	29.38 ± 0.49^{b}	38.60 ± 0.06^{a}	43.74 ± 1.59^{a}
0.03125	19.97 ± 0.53^{cb}	22.59 ± 0.71^{b}	33.32 ± 0.11^{a}	28.42 ± 0.70^{ab}

Results were expressed as mean ±SD, and $p < 0.05$ was considered significant.
The a,b,c,d superscripts denote significant differences across the row.

Table 2. Select Compounds Identified in the GC-MS Analysis of the Aqueous Sub- fraction A of *Z. tessmannii* Roots

S/N	Retention time (min)	Peak number	Area (%)	Compound
1	12.427	210	0.17	cyclooctadiene]-1,5-Cyclooctadiene
2	12.877	215	0.51	Cyclohexane, 1,2,4,5-tetraethyl- 2-Dodecanol
3	13.415	219	1.07	9-Decenoic acid
4	13.590	220	1.72	E-15-Heptadecenal, trans-13-Octadecenoic acid
5	14.010	221	5.35	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester, Oleic Acid
6	14.247	226	1.54	4-formyl-2-methoxyphenyl ester, 4-Oxo-beta.-damascone
7	14.329	227	1.56	Cyclohexanecarbonitrile

Table 2. Continued

8	14.579	230	2.86	Cyclobutanone, 2-(1,1-dimethylethy l)-Cyclopropane, 1,1-dichloro-2-methyl-2-(1-methylbutyl)-
9	14.917	237	1.16	Methyl alpha-D-glucopyranoside
10	15.067	239	1.96	D-Mannitol, 2,4-di-O-methyl-, tetraacetate, 1-Hexanethiol
11	15.498	250	0.58	3-oxo-, 2,2,2-trichloroethyl Butanoic acid
12	15.592	252	1.19	Benzaldehyde, 4-bromo- Shikimic acid
13	15.786	255	2.05	1-(4-Bromophenyl)-5-(2-diethylaminoethenyl)-1H-tetrazole
14	17.093	285	1.77	Benzoic acid, 4-nitro-, anhydride
15	17.813	301	1.47	Z,Z-8,10-Hexadecadien-1-ol acetate, E,E-10,12-Hexadecadien-1-ol acetate,

Table 3. Reported Activities of Some Compounds Present in Sub-fraction A of *Z. tessmannii* Roots

S/N	Compound	*CAS	Reported Activity	References
1	E-15-Heptadecenal	1000130-97-9	Anti-inflammatory	(Chansiw *et al.*, 2019)
2	trans-13-Octadecenoic acid	000693-71-0	Causes skin irritation, eye irritation and Specific target organ toxicity; Respiratory tract irritation	European Chemicals Agency, http://echa.europa.eu/" as cited by (NLM, National Library of Medicine, 2005d)
3	9-Octadecenoic acid (Z)-2,3-dihydroxy propyl	000111-03-5	Increased the fluidity of human erythrocyte membrane lipids	(NLM, National Library of Medicine, 2005b)
4	Oleic Acid	000112-80-1	Acute pulmonary oedema; lungs, thorax, or respiration changes	(Dickey, Thrall, McCormick, & Ward, 1981 as cited by NLM, National Library of Medicine, 2004)
5	6-Octadecenoic acid (Z)-	000593-39-5	Causes skin irritation, eye irritation and Specific target organ toxicity; Respiratory tract irritation	(ECHA, European Chemicals Agency, N.D as cited by NLM, National Library of Medicine, 2005c)
6	1-Hexanethiol	1000161-21-5	Causes skin irritation, eye irritation and Specific target organ toxicity; Respiratory tract irritation	(ECHA, European Chemicals Agency, N.D as cited by NLM, National Library of Medicine, 2005a)

*CAS – Chemical Abstracts Service, CAS Number also known as CAS Registry Number

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 (Pieczykolan *et al.*, 2021). This hyperuricaemia is a well-known risk factor for debilitating disorders like kidney stones and gout arthritis, which is an inflammatory condition (Aladdin *et al.*, 2020; Ranjana *et al.*, 2019). Furthermore, Ganz *et al.* (2023) reported that while allopurinol is primarily a medication used to lower uric acid levels, it has also been demonstrated to possess anti-inflammatory effects. In adddition, Schlesinger and Brunetti (2019) indicated that reactive oxygen species (ROS) serve as essential signaling molecules in inflammatory diseases and that the anti-inflammatory effects of allopurinol involve a dose-dependent ability to scavenge free radicals. Consequently, XO inhibitors, such as allopurinol, febuxostat, and oxypurinol, are used to treat gout (Ashiq *et al.*, 2021). XO, may therefore, serve as both a target enzyme and a model for evaluating anti-inflammatory efficacy of medicinal plants. The present study found that the AF of Z. *tessmannii* roots inhibited XO at levels comparable to the standard drug, allopurinol. In contrast, the dichloromethane fraction (DF) and butanol fraction (BF) did not show effective inhibition of XO. This finding regarding the AF was consistent with the previous study by Babarinde *et al.* (2021) which reported that aqueous extracts of Z. *tessmannii* roots exhibited a concentration-dependent inhibition of XO. Similarly, Liu *et al.* (2023) demonstrated that aqueouse extratct of *Zanthoxylum Bungeanum* reduced XO activity and serum uric acid levels. They further noted that elevated levels of inflammatory factors are an important cause of the development of gouty arthritis and correlated the ability to reduce XO activity and uric acid levels with the suppression of inflammation associated with gout. The present study, therefore, suggests that the inhibition inhibition of XO is related to its anti-inflammatory effect.

Lipoxygenase (LOX) catalyses the transformation of linoleic, arachidonic, and other polyunsaturated fatty acids (PUFAs) into biologically active compounds associated with inflammation. This involvement of LOX-derived products in both classical and allergic inflammation makes the enzyme one of the important targets in anti-inflammatory interventions. All the sub-fractions of *Z. tessmannii* roots exhibited impressive percentage inhibition of the lipoxygenase enzyme in a concentration-dependent manner. However, sub-fraction A exhibited the highest percentage inhibition. These findings suggest that *Z. tessmannii* may produce anti-inflammatory effects by blocking the synthesis of various leukotrienes, which contribute to the inflammatory response. Tanoh *et al.* (2019) had reported the isolation of essential oils exhibiting potent lipoxygenase inhibitory properties from the roots of two *Zanthoxylum* species found in Côte d'Ivoire (*Z. mezoneurispinosum* and *Z. psammophilum*).

The GC-MS analysis revealed over 300 compounds present in the sub-fraction A of *Z. tessmannii* roots, most of which had minimal percentage compositions. Two of these compounds, E-15-heptadecenal and 9-octadecenoic acid (Z)-

, 2,3-dihydroxy propyl has been reported to possess anti-inflammatory activities (Chansiw *et al*., 2019; Rotimi *et al*., 2014). Chansiw *et al*. (2019) evaluated *Polygonum odoratum*, an indigenous vegetable in Thailand, for its anti-inflammatory and antioxidant activities as well as the active ingredients from the leaf and stem extracts. They identified E-15-heptadecenal as one of the two compounds found in the methanol extracts of the plant and attributed the anti-inflammatory effects to these compounds. In an earlier study, Chansiw *et al*. (2018) revealed that *P. odoratum* possessed anti-haemolytic activity, and E-15-heptadecenal was identified as one of the constituent compounds using GC-MS. Similarly, Rotimi *et al*. (2014) identified octadecenoic acid, its derivatives, and structurally similar compounds (such as hexadecanoic acid, methyl stearate, and phytol) in the ethanolic extracts of other anti-inflammatory plants. Using molecular docking techniques, they demonstrated that 9-octadecenoic acid (z)-, 2,3-dihydroxy propyl ester can bind and inhibit NF-κB, highlighting its potential as an anti-inflammatory agent. Conversely, available reports have indicated that the compounds trans-13-octadecenoic acid, oleic acid, 6-octadecenoic acid (z)-, and tetraacetate 1-hexanethiol exhibit activities that suggest involvement in mast cell-mediated symptoms. The United States national library of medicine (NLM, 2005d) reported that trans-13-octadecenoic acid is an irritant, causing irritation of the skin, eye (severe eye damage), and respiratory tract. Awonyemi *et al*. (2020) also noted that although trans-13-octadecenoic acid possesses anti-inflammatory activity, it is also dermatitigenic, meaning it can cause irritation to the skin. Accordingly, irritation of the skin, eyes, and respiratory tracts are typical allergies mediated by substances released by mast cells. Given that allergens elicit their classical symptoms through mast cell degranulation, it can be inferred that trans-13-octadecenoic acid is a potent mast cell degranulator. In another investigation, Babarinde *et al*. (2021) found that crude extracts and fractions of *Z. tessmannii* roots significantly stimulated mast cell degranulation. Thus, the reported actions of some of the compounds identified from the sub-fraction of *Z. tessmannii* roots in this study validate the dual yet conflicting activities of anti-inflammatory effects and allergenic potential of the root extracts and fractions, as previously demonstrated.

CONCLUSION

This study has demonstrated that *Zanthoxylum tessmannii* contains bioactive compounds with anti-inflammatory potential, as well as previously reported allergenic effects. While there are potential therapeutic benefits for inflammatory conditions, considerations of allergenicity must not be disregarded. Therefore, unguided use of the crude plant roots in ethnomedicinal practices is strongly discouraged, especially for individuals with a history of asthma, due to the presence of compounds implicated in respiratory tract irritation. Future research should focus on improved isolation and purification of beneficial active components. *In vivo* studies are needed to provide further details on mechanisms of action and safety profiles of these compounds to guide the development of safer pharmaceutical products from *Z. tessmannii* roots.

AUTHORS' CONTRIBUTIONS

Conceptualization, SOB and BAA; methodology, SOB and BAA; validation, SOB, GA. and BAA; formal analysis, SOB; investigation, SOB; resources, AEM; data curation, SCN and MVA; writing-original draft preparation, SOB; writing—review and editing, GA, BAA and TAO; supervision, TAO; project administration, SOB and AEM; funding acquisition, SOB and BAA. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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