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Original Research Article

Liquid Chromatography-Mass Spectrometric Analyses of Potential Antioxidant Constituents from *Zanthoxylum zanthoxyloides* Leaves: Probing into the Role of Alkaloids

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ABSTRACT

Zanthoxylum zanthoxyloides is a plant used traditionally as food and in medicine, with known antioxidant properties. The study aimed to prepare *n*-butanol fractions of *Z. zanthoxyloides* leaves and evaluate their *in-vitro* antioxidant potential. Vacuum liquid chromatography (VLC) was used to separate the constituents of the *n*-butanol fraction using gradients of binary mixtures of dichloromethane in methanol (1L) sequentially in the ratios; 9:1 (BF1), 7:3 (BF2), 5:5 (BF3), 3:7 (BF4), and 1:9 (BF5). Three standard antioxidant assay models were adopted for the *in-vitro* antioxidant activity. Ascorbic acid (53.60%) exhibited superior antioxidant activity relative to the *n*-butanol sub-fractions; (BF1 14.51%, BF2 24.76%, BF3 10.90%, BF4 6.19% and BF5 23.38%). Meanwhile, BF3 displayed superior reducing potential relative to ascorbic acid using both the TAC and FRAP method (TAC 2.320 ± 0.002; FRAP 2.27 ± 0.002 versus TAC 0.298 ± 0.000; FRAP 0.274 ± 0.002). BF1, BF2, BF4 and BF5 displayed lower reducing potential: TAC 0.202 ± 0.001; FRAP 0.199 ± 0.000, TAC 0.162 ± 0.001; FRAP 0.153 ± 0.001, TAC 0.186 ± 0.002; FRAP 0.174 ± 0.002 and TAC 0.262 ± 0.000; FRAP 0.223 ± 0.003, respectively when compared to ascorbic acid. Several known potential antioxidants were tentatively characterized in the *n*-butanol sub-fractions by liquid chromatography-mass spectrometry (LC-MS) dereplication. However, a significant number of alkaloids and phenolic compounds unknown for any role in radical scavenging activity (RSA) were detected in *n*-butanol sub-fractions. Our findings also further probed the possible role of alkaloids in antioxidant cascade.

Keywords: *Zanthoxylum zanthoxyloides*, Antioxidant, Phytochemical, Dereplication, Liquid Chromatography-mass spectrometry, Radical scavenging activity.

Introduction

Plants are a major source of phytochemicals such as alkaloids, phenols, polyphenols, tannins and terpenes.[1] Research on medicinal plants is centered on phytochemistry, pharmacognosy and horticulture.[2] Research in phytochemistry tends to characterize their possible bioactive compounds, by separating them and subjecting them to detailed structural study.[3,4] These compounds are valuable health-promoting phytoconstituents of herbal preparations and plants with variable biological activities.[2] As most of the medicinal plants are not preserved, suitable analytical techniques are being employed to maintain and preserve the quality of medicinal plants.[5]

Reliable and robust methodology for the analysis of medicinal plant extracts is imperative for quality control as well as drug development of new molecules.[6] Several analytical procedures based on the liquid chromatographic technique have been developed for quality control of plant extracts.[7, 8] High performance liquid chromatographic (HPLC) method coupled with mass spectroscopy (MS) (LC-MS) is one of the most frequently used quality control techniques due to its high reproducibility, selectivity and simplicity and has been used in analyses of bioactive molecules from plants.[7] Specifically, liquid chromatography-mass spectroscopy (LC-MS) facilitates accurate and rapid isolation and identification of chemical compounds and is used extensively for the analysis of antioxidant phenolic compounds in plants.[9-13] Some of these molecules are beneficial in the treatment of diseases and results in the discovery of novel bioactive agents.[14, 15] The choice of solvent and analytical techniques play vital roles in chemical fingerprinting and profiling.[16-20]

Free radicals are highly reactive molecules, possessing one or more unpaired electrons. They are very unstable and possess high energy. They play a vital role in the pathophysiology or etiology of several illnesses.[2] Reactive nitrogen and oxygen production has been connected with metabolic and cellular damage, cancer, aging, diabetes mellitus, cardiovascular and degenerative diseases, neurologic and inflammatory illnesses.[14] Antioxidants serve as guards against oxidative damage.[21,22] Antioxidants are additionally notable to be associated with the repair of tissue and cushioning of physical pressure. The most common sources of antioxidants are phenol and alkaloid-containing fruits and vegetables, which have proven beneficial in reducing the severity of chronic diseases as a result of their antioxidant properties.[14,19] This accounts for the recent interest this group of substances is receiving in drug discovery and research.

Zanthoxylum zanthoxyloides (Lam.) (*Rutaceae*) is a plant known as Senegal prickly ash or artar root or candlewood. It occurs in the savannah of West Africa, dry forest vegetation and coastal areas of

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Senegal, Nigeria and Cameroon.[23] It is an important medicinal plant employed largely in the treatment of various diseases in African traditional medicine.[24] The root and stem decoctions are usually taken for the management of sickle cell anaemia, malaria, fever, tuberculosis, general body weakness, intestinal problems, migraine and to reduce pain during childbirth.[25] The leaves are employed as a food seasoning.[3] The bark, leaf and root extracts are used as pesticides.[25] The root of *Z. zanthoxyloides* has an antimicrobial effect on oral pathogens, thus it has been used as a chewing stick for the management of dental diseases.[3] The methanol extract of the leaves and roots of *Z. zanthoxyloides* has an antioxidant effect.[26]

Based on the antioxidant activity guided LC-MS dereplication process, we evaluated for the first time the antioxidant potential of the various VLC fractions of *Z. zanthoxyloides* leaves. Vacuum liquid chromatography was used to isolate the antioxidant principles of the leaves because it is an established method of extracting phytochemicals from plant materials.[26] Also, this study investigated the chemical constituents of the *n*-butanol fractions using high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) technique. Earlier reports attributed the antioxidant activity of leaf extracts of *Z. zanthoxyloides* to flavonoids, but the oxidation-reduction potential of other phytochemical constituents such as alkaloids and triterpenes were not evaluated.[26-28] Given this, the work aimed to prepare n-butanol extracts of *Z. zanthoxyloides* leaves and isolate phytochemicals.

Materials and Methods

Chemicals

All the chemicals used for this study were of an analytical grade. They included methanol, *n*-hexane, ethyl acetate, *n*-butanol, dichloromethane, silica gel (100-200 mesh), 1,1-diphenyl-2-picrylhydrazyl (DPPH), tetraoxosulphate (iv) acid, ascorbic acid, disodium phosphate, potassium ferricyanide, ammonium molybdate, trichloroacetic acid and ferric chloride. Others are formic acid, acetonitrile. Distilled water was also used for the study. All the chemicals are JHD products purchased from Guangdong Guanghue Science-Tech Co Ltd China. They were used without further purification.

Instruments and equipment

The major instruments used for the study were weighing balance (Mettler HAS, USA), separating funnel (Pyrex, France), chromatographic column (Pyrex, France), 6705UV/Vis-Spectrophotometer (JENWAY, China), refrigerator (Thermocool, China), Dionex Ultimate 3000 RS liquid chromatography System with Dionex Acclaim RSLC 120, C18 column, Bruker Daltonics micrOTOF-MS quadrupole/time-of-flight mass spectrometer, Bruker Daltonics Profile, Analysis 2.0 (Bruker Daltonik GmbH, Germany, 2010), Other common laboratory instruments used included syringe of different capacities (1 mL, 2 mL, 5 mL and 10 mL) (Lifescan, Switzerland), beakers (Pyrex, France), sample bottles, glass funnel (Pyrex, France) and filter cloth.

Plant material

Fresh leafy vegetables of *Z. zanthoxyloides* were collected from farms in Orodo, Mbaitolu L.G.A., Imo State, Nigeria, in November 2019. Identification was done by Prof. Charles N. Mba of the Department of Soil Science, School of Agriculture and Agricultural Technology of Federal University of Technology, Owerri. A voucher specimen (#INTERCEDD/901) of the sample was subsequently kept at the herbarium of the Bioresources Development and Conservation Program (BDCP) research Centre, Nsukka, Enugu State, Nigeria.

Extraction of plant material

The fresh leaves were washed thoroughly with tap water and dried at room temperature. Thereafter, they were ground with a clean grinder into a fine powder. The powdered leaves (100 g) were macerated for 48 h in 1 L of 95% v/v methanol at room temperature, it was then filtered using Whatman filter paper (What Int. Ltd., Maidstone). The filtrate was concentrated to dryness in vacuum at room temperature.

Solvent-solvent extraction

The concentrated methanol extract (10 g) was re-dissolved in 10% aqueous methanol (v/v) (500 mL) and subjected to solvent-solvent extraction successively, in *n*-hexane (2.5 L), ethyl acetate (2 L) and *n*-butanol (1 L) while increasing their polarity. The extracts were separated using a separatory funnel and the filtrates of respective fractionating solvent were concentrated using a rotary evaporator under reduced pressure.[29]

Vacuum liquid chromatographic separation

VLC technique was adopted to further separate the *n*-butanol fraction using the previously described method.[29] The *n*-butanol soluble portion (2 g) was dissolved in 2 mL methanol and introduced to the silica gel ($G_{100\text{-}200}$ mesh size) slurry packed 40 × 3 cm column (sample to silica gel ratio 1:40) at room temperature. The applied fraction was eluted with a gradient of mobile phases, consisting of dichloromethane in methanol (1 L) each starting with 90% DCM and gradually increasing the amount of methanol to 30, 50, 70 and 90% to obtain VLC fractions BF1-BF5, respectively.

High-Performance Liquid Chromatography-Mass Spectrometric analysis (HPLC-MS)

The *n*-butanol fractions (BF1 to BF5) (5 mg/mL) were dissolved in 80% v/v aqueous methanol and analyzed with ultra-high performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometer (UHPLC/ESI-QTOF MS/MS) using the method previously described.[30] UHPLC-MS chromatograms and mass spectra were calibrated with Bruker Daltonics Profile, Analysis 2.0 (Bruker Daltonik GmbH, Germany, 2010). Peak alignment (Join aligner) was employed to complement applicable peaks across several samples under retention time (tR) and m/z tolerances to generate an index of peaks and their partial identification was done by automatically quizzing the required masses of molecular ions $[M\text{-}H]^-$ and common adducts ($[M\text{-}Na]^-$, $[M\text{-}NH_4]^-$, $[M\text{-}K]^-$) (error = 0.005 Da). The molecular formulae of possible compound(s) from each deconvoluted peaks with their corresponding retention time (tR) are shown in Table 1. The deconvoluted peaks were picked based on their relative intensities, as an indicator of the levels of the constituents of the *n*-butanol soluble samples.

Data were interpreted using the database of National Institute Standard and Technology (NIST) and the dictionary of natural products. The retention time, molecular weight, and molecular formula of the sample material were obtained by comparing the spectra of unknown compounds and that of the known compounds in the repositories.

Measurement of antioxidant activity

The *n*-butanol sub-fractions BF1-BF5 were subjected to three types of antioxidant assay.

DPPH radical scavenging assay

DPPH radical scavenging assay was done using ascorbic acid as standard/positive control. Each sample was dissolved in MeOH to make a stock solution of 1000 mg/L. A 5.0 mL of DPPH solution was added to the test tube containing 1.0 mL of each concentration and the solution was evenly mixed. The mixture was incubated for 3 min and observed for colour change (deep violet to yellow). The absorbance was determined spectrophotometrically at 517 nm, using a previously reported method.[31] The concentrations of BF1 to BF5 sub-fractions and ascorbic acid used were 10, 20, 30, 40, and 50 mg/L. The blank was a mixture of DPPH in methanol (5:1). IC_{50} (concentration of the sub-fraction at which there was 50 % inhibition) was calculated using the IC_{50} calculator. Free radical scavenging activity was obtained from;

$$\%\ \text{Inhibition} = ((\text{Ao} - \text{A1}))/\text{Ao} \times 100$$

Where Ao = absorbance of blank and A1 = absorbance of test sample.

Total antioxidant capacity (TAC) by phosphomolybdenum method
Total antioxidant capacity/ free-radical reducing potential was determined using the previously described method.[31] Phosphomolybdenum reagent was prepared by reacting 100 mL each of 0.6 M H_2SO_4, 28 mM disodium phosphate and 4 mM ammonium molybdate. A 3 mL of the reagent was added to 1 mL each of the different concentrations of the *n*-butanol sub-fractions in methanol. The mixture was incubated at 95^{o}C for 90 min and allowed to cool at room temperature for 15 min. The absorbance was read at 695 nm. A blank was prepared by adding 1 mL of methanol to 3 mL of the reagent solution and taken through the same procedure as the test samples. Five different concentrations of the standard drug ascorbic acid were prepared and used to draw a calibration curve of concentration against absorbance. The concentrations of the sub-fractions and ascorbic acid used were 100, 50, 25, 12.5 and 6.25 µg/mL. The concentration at 50% maximal activity (EC_{50}) was calculated using the EC_{50} calculator.

Ferric ion reducing antioxidant power (FRAP) assay
FRAP assay was measured using the previously described method.[31] Different concentrations (100, 50, 25, 12.5 and 6.25 µg/mL) of the BF1 to BF5 sub-fractions were added to 2.5 mL of 20 mM phosphate buffer and 2.5%, w/v potassium ferricyanide. The mixture was then incubated for 30 min at 50°C. Then, 2.5 mL of 10%, w/v trichloroacetic acid and 0.5 mL 0.1%, w/v ferric chloride were added to the mixture. This was left for 10 min. Absorbance was read at 700 nm. All measurements were taken in triplicates and averaged. Ascorbic acid was used as a positive reference standard. The concentrations of BF1 to BF5 sub-fractions at 50% maximal activity (EC_{50}) were calculated using the EC_{50} calculator.

Statistical analysis
SPSS version 16.0 Windows was used for the statistical analysis. Data were expressed as mean ± standard deviation (S.D.) and analyzed using ANOVA with post-hoc Duncan multiple range tests. The difference between the antioxidant activity of BF1 to BF5 sub-fractions at different concentrations was considered significant when $p < 0.05$.[32] GraphPad Prism 8 was used for plotting the graphs.

Results and Discussion

HPLC-MS analysis
The more active VLC sub-fractions were dereplicated by LC-MS, and the potential antioxidant phytoconstituents of *Z. zanthoxyloides* leaves together with their molecular formula and weight, peak area, retention time, and the composition of their bioactive constituents were shown in Table 1 and Figure 1 below. The base peak chromatograms of the active sub-fractions and the compound spectra of the constituents tentatively characterized are presented in Figures S1 and S2 respectively.
Many important potential active compounds such as pimpinellin (**1**), quercitrin (**2**), piperlonguminine (**3**), matairesinol (**4**), datiscin (**5**), savinin (**26**), stigmasterol (**8**), β-sitosterol (**9**), zanthosinamide (**11**), myricetin (**12**), eriocitrin (**13**), diosmin (**14**), magnoflorine (**15**), berberine (**16**), sesamin (hinokinin) (**17**), campesterol (**10**), kaempferol (**18**), dioxamin (**19**), rutin **(30)**, marmesin (**20**), fagaramide (**6**), oxychelerythrine (**28**), scoparone (**27**), quercetin (**7**), arnottianamide (**29**), skimmianine (**25**), herclavine (**24**), lemairamin (**23**), fagaronine (**22**) and lupeol (**21**) have been tentatively characterized from *Z. zanthoxyloides* leaves.
In this study, many phytochemicals such as coumarins (3), flavonoids (8), alkaloids (12), lignans (3) and triterpenes (4) were found in different VLC fractions of *n*-butanol soluble fraction of *Z. zanthoxyloides* leaves (Table 1). Several bioactive compounds including aromatic compounds had earlier been found in the leaves, roots and stem bark of *Z. zanthoxyloides*,[28, 37-39] by LC-MS/MS analysis. Alkaloids, tannins, flavonoids, terpenoids and saponins were found in high proportions in their roots,[27, 40] while flavonoids such as quercitrin, quercetin and datiscin were found in the several extracts of the leaves.[26] These findings had either attributed the antioxidant of *Z. Zanthoxyloides* to the flavonoid constituents only or reported that the array of phytochemicals present in the plant may impact antioxidant properties.[26-28]
However, our antioxidant activity-guided dereplication investigation identified 30 important potential active compounds in *Z. zanthoxyloides* leaves; 17 were found in BF2, 8 in BF3, 1 in BF4 and 4 in BF5. These include compounds with antioxidant, anti-inflammatory, antimalarial, antiviral, antibacterial, antimicrobial, antifungal and anticancer effects. Six (6) flavonoids were found in BF2, as well as 4 alkaloids, 3 lignans, 3 triterpenes and 1 coumarin. In BF3, 4, 2 and 2 alkaloids, flavonoids and coumarins respectively were detected. BF4 has 1 alkaloid, while BF5 has 3 alkaloids and 1 triterpene. Known antioxidants identified are **1, 2, 5, 7, 12** and **17** only; thus opening a new investigation into the antioxidant activity of 24 other compounds identified in this study. The *n*-butanol fraction quantification was found to be rich in phenolic compounds: **2, 5, 7, 12, 13, 14, 18** and **30** and expectedly exhibited good scavenging activity against DPPH probably associated with them. Interestingly, other groups of compounds identified in this study have not been reported to possess antioxidant activity. It is therefore imperative to extend the search for potential antioxidants from phenolic compounds (flavonoids) to alkaloids (**3**, **6**, **11**, **15**, **16**, **19**, **22-25**, **28** and **29**), the next abundant phytochemical and triterpenes (**8-10** and **21**). Terpenoids as antioxidants mop-up free radicals, react with peroxyl radicals, thereby terminating oxidative chain reactions. [19]
Comparing the antioxidant activities of the *n*-butanol sub-fractions with the chemical class of compounds dereplicated (Figure 1) showed an interesting trend. Of the 12 alkaloids identified, four (**3**, **15**, **16** and **19**) were found in the most active fraction, BF2, four (**6**, **11**, **28** and **29**) in BF3, one **(25)** in BF4 and three **(22, 23 and 24)** in BF5. Three triterpenes (**8, 9** and **10**) were identified in BF2 and one (**21)** in BF5. The relative distribution of phytochemicals in the VLC fractions and the abundance of alkaloids in the most active fraction suggest a twist in the roles and contribution of alkaloids to the overall RSA of *Z. zanthoxyloides*.

Antioxidant activity
DPPH radical scavenging assay
All the *n*-butanol soluble fractions (BF1 to BF5) tested positive to qualitative antioxidant activity test. The DPPH free radical scavenging activity of the sub-fractions was expressed as percentage inhibition of free radical activity. The *n*-butanol fractions exhibited a lesser percentage inhibition than the standard, ascorbic acid ($p < 0.05$). Percentage inhibitions at different concentrations of the *n*-butanol fractions are significantly different from each other, with increasing concentration showing significantly increasing scavenging activity.
Sub-fractions with lower IC_{50} values show greater potency. Among the *n*-butanol sub-fractions, BF2 elicited higher DPPH scavenging activity inhibition at 24.76% with an IC_{50} of 21.38 mg/L, while ascorbic acid produced an activity of 53.64% with an IC_{50} of 20.62 mg/L. BF3 produced an inhibitory effect against DPPH scavenging activity at 10.90% with an IC_{50} of 24.78 mg/L. BF1 and BF5 produced an inhibitory effect of 14.51% with an IC_{50} of 26.79 mg/L and 23.38% with an IC_{50} of 23.08 mg/L respectively. The least effect was shown by BF4 (6.19%) having an IC_{50} of 29.43 mg/L (Table 2; Figure 2).

Total antioxidant capacity (TAC) by phosphomolybdenum method
Sub-fractions with lower EC_{50} values are more potent. Using the Phosphomolybdenum method, the reducing potential of the *n*-butanol fractions was significantly different ($p < 0.05$) from each other, with the absorbance significantly concentration-dependent. Similarly, the reducing potential of the *n*-butanol fractions significantly compared favourably with the ascorbic acid ($p < 0.05$). BF3 significantly produced higher antioxidant potential (2.320 ± 0.002) with an EC_{50} of 31.80 µg/mL when compared with the reducing potency of ascorbic acid (0.300 ± 0.001) having an EC_{50} of 32.51 µg/mL. BF1 showed an EC_{50} of 38.69 µg/mL with a reducing potency of 0.200 ± 0.003 while BF5 has an EC_{50} of 33.54 µg/mL with a reducing potency of 0.260 ± 0.002. The reducing potencies of BF2 and BF4 are 0.162 ± 0.001 (EC_{50} 202.03 µg/mL) and 0.186 ± 0.002 (EC_{50} 94.41 µg/mL), respectively (Table 2; Figure 3).

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Ferric ion reducing antioxidant power (FRAP) assay

In the ferric ion reducing antioxidant power (FRAP) assay model, increasing the concentrations of the VLC sub-fractions and the standard, significantly leads to increased absorbance; hence there was a significant difference within the concentrations ($p < 0.05$). The reducing effect of BF3 2.270 ± 0.002 (EC_{50} 36.70 µg/mL) was significantly higher than that of the standard, 0.270 ± 0.002 (EC_{50} 37.22 µg/mL) with a high percentage FRAP. BF4 has an EC_{50} of 41.57 µg/mL with a reducing potency of 0.170 ± 0.002 while BF1 and BF5 have an EC_{50} of 42.05 µg/mL and 38.91 µg/mL, respectively with reducing potencies of 0.199 ± 0.000 and 0.223 ± 0.003, respectively. The reducing effect of BF2 was lowest with 0.15 ± 0.002 (EC_{50} 52.87 µg/mL) (Table 2; Figure 3).

Oxidative stress is due to an imbalance arising from the increased generation of oxidants in cells and tissues against the supply of antioxidants.[33] Antioxidants intercept free radicals, inhibit the generation of free radicals, eliminate hydroperoxides and repair damage.[34] The antiradical properties of the compounds in *Z. zanthoxyloides* have been demonstrated in the works of Chaaib *et al*., (2003) where they worked on the root bark of *Z. zanthoxyloides* reporting the antioxidant and antifungal properties of their phytochemical constituents. The mechanism of antioxidant action of the root extract is by intercepting and inhibiting the generation of free radicals, suggesting their role in the supportive and complementary treatment of degenerative illnesses. In this study *n*-butanol soluble fraction of *Z. zanthoxyloides* leaves exhibited moderate antioxidant activity in the three models used. The scavenging effect of BF2 was lower than ascorbic acid which is the standard used, in the DPPH model, while the reducing effect of BF3 was moderately higher than ascorbic acid in both the TAC and FRAP models. The previous report attributed the antioxidant activity of *Z. zanthoxyloides* to the flavonoids constituents only.[26] The methanol plant extracts of leaves, fruits, stems, trunks, barks and roots were investigated and the leaf and trunk bark extracts were found to have more antioxidant activities than the extracts from other parts.[26] Similar work had also reported moderate inhibition of free-radicals by various extracts of the stem bark of *Z. Zanthoxyloides.*[32]

Table 1: HPLC-MS analysis of *n*-butanol sub-fractions of *Z. zanthoxyloides* leaves

Mass	Sample	t_R(min)	Adduct	Compound	Formula	Class
245.1	BF2	1.1	$[M-H]^-$	Pimpinellin **(1)**	$C_{13}H_{10}O_5$	Coumarin
409.2	BF2	4.4	$[M-39]^-$	Quercitrin **(2)**	$C_{21}H_{20}O_{11}$	Flavonoid
356.2	BF2	5.4	$[M-2H]^-$	Matairesinol **(4)**	$C_{20}H_{22}O_6$	Lignan
373.2	BF2	5.5	$[M-39]^-$	Stigmasterol **(8)**	$C_{29}H_{48}O$	Triterpene
593.2	BF2	6.0	$[M-H]^-$	Datiscin **(5)**	$C_{27}H_{30}O_{15}$	Flavonoid
301.1	BF2	6.9	$[M-H]^-$	Quercetin **(7)**	$C_{15}H_{10}O_7$	Flavonoid
412.3	BF2	8.9	$[M-2H]^-$	β-sitosterol **(9)**	$C_{29}H_{50}O$	Triterpene
299.1	BF2	9.5	$[M-NH_4]^-$	Piperlonguminine **(3)**	$C_{17}H_{19}NO_5$	Alkaloid
317.1	BF2	10.5	$[M-H]^-$	Myricetin **(12)**	$C_{15}H_{10}O_8$	Flavonoid
590.4	BF2	10.8	$[M-NH_4]^-$	Diosmin **(14)**	$C_{28}H_{32}O_{15}$	Flavonoid
319.3	BF2	11.2	$[M-23]^-$	Magnoflorine **(15)**	$C_{20}H_{24}NO_4$	Alkaloid
336.3	BF2	11.2	$[M-NH_4]^-$	Sesamin **(17)**	$C_{20}H_{18}O_6$	Lignan
336.3	BF2	11.2	$[M]^-$	Berberine **(16)**	$C_{20}H_{18}NO_4$	Alkaloid
352.3	BF2	11.5	$[M-2H]^-$	Savinin **(26)**	$C_{20}H_{16}O_6$	Lignan
399.3	BF2	12.8	$[M-H]^-$	Campesterol **(10)**	$C_{28}H_{48}O$	Triterpene
284.3	BF2	13.1	$[M-2H]^-$	Kaempferol **(18)**	$C_{15}H_{10}O_6$	Flavonoid
338.3	BF2	13.8	$[M-H]^-$	Dioxamin **(19)**	$C_{19}H_{17}NO_5$	Alkaloid
609.2	BF3	5.2	$[M-H]^-$	Rutin **(30)**	$C_{27}H_{30}O_{16}$	Flavonoid
244.2	BF3	6.9	$[M-2H]^-$	Marmesin **(20)**	$C_{14}H_{14}O_4$	Coumarin
245.1	BF3	7.5	$[M-2H]^-$	Fagaramide **(6)**	$C_{14}H_{17}NO_3$	Alkaloid
362.3	BF3	8.0	$[M-H]^-$	Oxychelerythrine **(28)**	$C_{21}H_{17}NO_5$	Alkaloid
367.2	BF3	8.8	$[M-2H]^-$	Zanthosinamide **(11)**	$C_{21}H_{23}NO_5$	Alkaloid
205.1	BF3	10.5	$[M-H]^-$	Scoparone **(27)**	$C_{11}H_{10}O_4$	Coumarin
573.4	BF3	10.8	$[M-23]^-$	Eriocitrin **(13)**	$C_{27}H_{32}O_{15}$	Flavonoid
363.2	BF3	11.4	$[M-NH_4]^-$	Arnottianamide **(29)**	$C_{21}H_{19}NO_6$	Alkaloid
241.2	BF4	5.9	$[M-NH_4]^-$	Skimmianine **(25)**	$C_{14}H_{13}NO_4$	Alkaloid
293.2	BF5	8.8	$[M-2H]^-$	Herclavine **(24)**	$C_{19}H_{21}NO_2$	Alkaloid
310.2	BF5	8.8	$[M-H]^-$	Lemairamin **(23)**	$C_{19}H_{21}NO_3$	Alkaloid
327.3	BF5	10.7	$[M-23]^-$	Fagaronine **(22)**	$C_{21}H_{20}NO_4$	Alkaloid
408.3	BF5	14.5	$[M-NH_4]^-$	Lupeol **(21)**	$C_{30}H_{50}O$	Triterpene

BF2-5 = *n*-butanol sub-fractions; t_R = retention time

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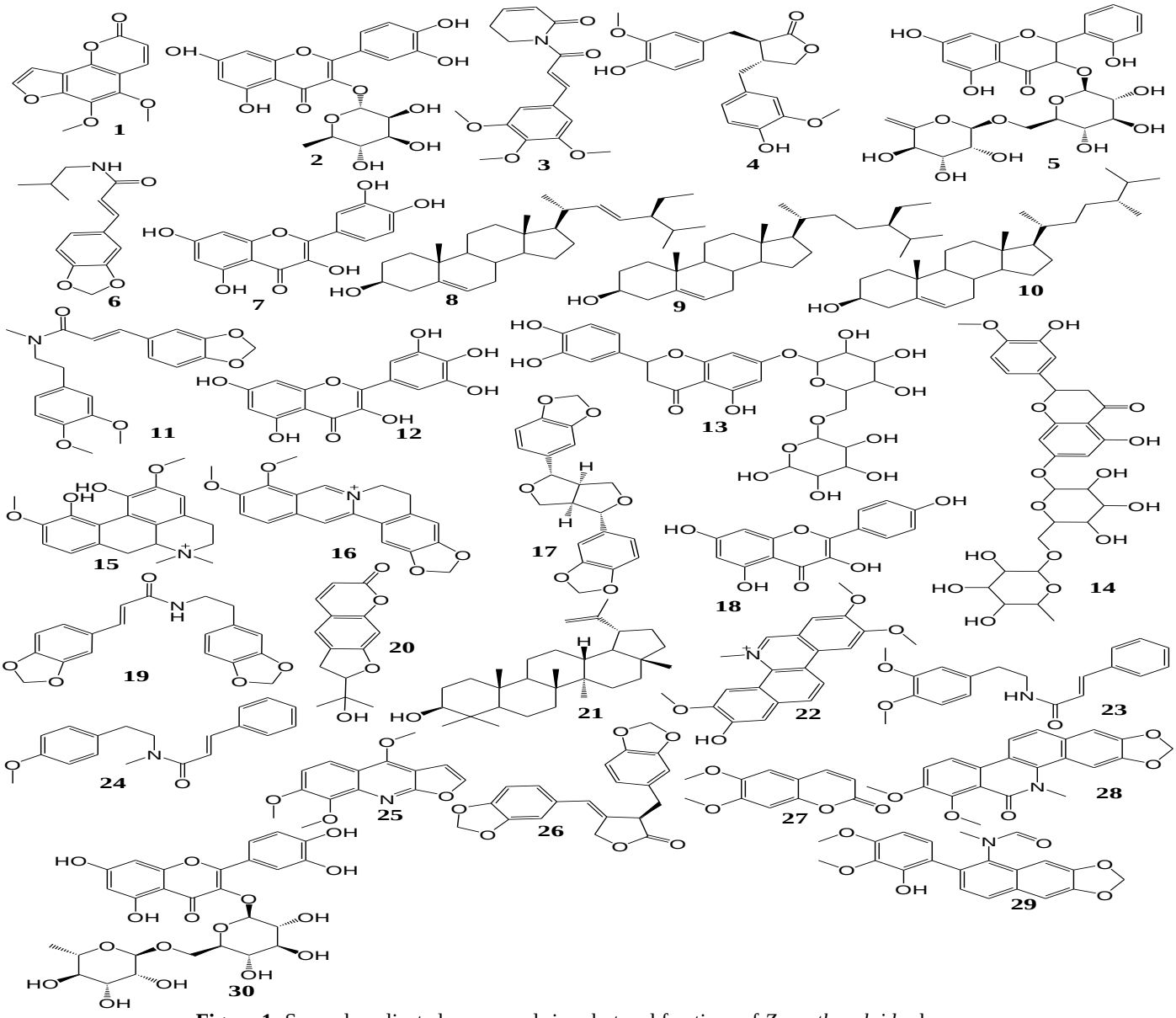

Figure 1: Some dereplicated compounds in *n*-butanol fractions of *Z. zanthoxyloides* leaves

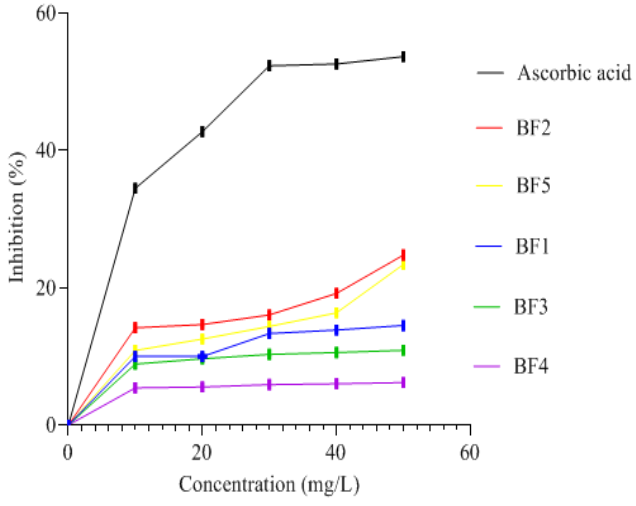

Figure 2: DPPH scavenging activity of the *n*-butanol VLC sub-fractions of *Z. zanthoxyloides* leaves. BF1-BF5= *n*-butanol sub-fractions. Data are mean ± standard deviation, *n* = 3

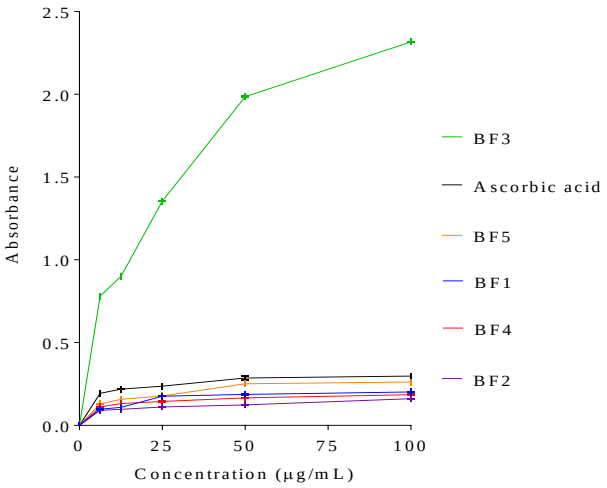

Figure 3: Total antioxidant capacity of the *n*-butanol VLC sub-fractions of *Z. zanthoxyloides* leaves. BF1-BF5= *n*-butanol fractions. Data are mean ± standard deviation, *n* = 3

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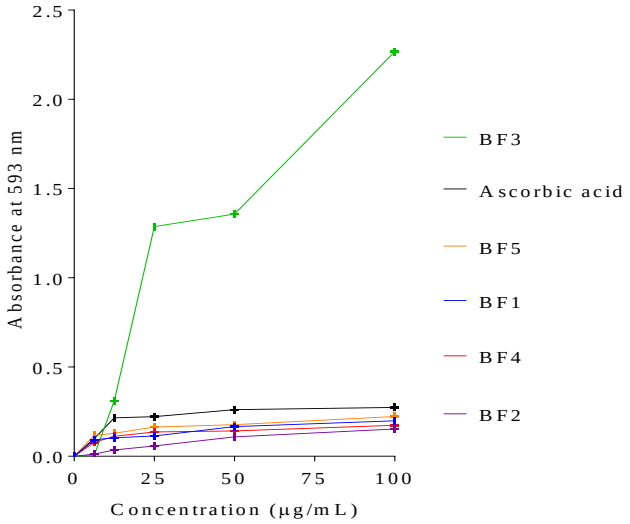

Figure 4: FRAP of *n*-butanol VLC sub-fractions of *Z. zanthoxyloides* leaves. BF1-BF5= *n*-butanol fractions. Data are mean ± standard deviation, $n = 3$

Table 2: IC_{50} and EC_{50} values of the *n*-butanol sub-fractions of *Z. zanthoxyloides* leaves

Fractions	DPPH IC_{50} values (mg/L)	TAC EC_{50} values (µg/mL)	FRAP EC_{50} values (µg/mL)
BF1	26.79 ± 12.07	38.69 ± 0.15	42.05 ± 0.15
BF2	21.38 ± 5.43	202.03 ± 0.21	52.87 ± 0.11
BF3	24.78 ± 9.98	31.80 ± 1.58	36.70 ± 1.33
BF4	29.43 ± 5.85	94.41 ± 0.18	41.57 ± 0.02
BF5	23.08 ± 8.02	33.54 ± 0.20	38.91 ± 0.23
Ascorbic acid	20.62 ± 3.82	32.51 ± 0.25	37.22 ± 0.61

BF1-BF5 = *n*-butanol sub-fractions: (Data are mean ± SD; $n = 3$)

Conclusion

The present study reported *Z. zanthoxyloides* leaves as a potential source of new antioxidant agents. Alkaloids, coumarins, flavonoids and terpenoids were the important bioactive substances responsible for the antioxidant properties of *Z. zanthoxyloides* leaves. The alkaloids present in *Z. zanthoxyloides* confer a superior reducing potential on the *n*-butanol fraction relative to the constituents: flavonoids and terpenoids. In addition to the role of flavonoids as free radical scavengers, our study has opened up the potential of alkaloids as antioxidants.

Conflict of interest

The authors declared no conflict of interest.

Authors' declaration

The authors hereby declare that the work presented in this manuscript is original and that any liability for claims relating to the content of this manuscript will be borne by them.

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