



RESEARCH ARTICLE

Study of total phenolic and flavonoid contents, antioxidant, and antibacterial properties of *Zanthoxylum chalybeum* and *Euphorbia ingens* : Medicinal plants from Malawi

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ARTICLE HISTORY

Received: 02 August 2024

Accepted: 29 November 2024

Available online

Version 1.0 : 19 February 2025

Version 2.0 : 07 March 2025



Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

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Kumwenda F D, Sumani J, Jere W, Namandwa B, Nyirenda K K, Mwatseteza J. Study of total phenolic and flavonoid contents, antioxidant, and antibacterial properties of *Zanthoxylum chalybeum* and *Euphorbia ingens* : Medicinal plants from Malawi. Plant Science Today. 2025; 12(1): 1-9. <https://doi.org/10.14719/pst.4526>

Abstract

This study investigated the chemical composition, antioxidant, and antibacterial activities of two medicinal plants from Malawi: *Zanthoxylum chalybeum* and *Euphorbia ingens*. Plant extracts were obtained sequentially macerating the root powder with *n*-hexane, dichloromethane, ethyl acetate, methanol, and water. Total phenolics and flavonoids were determined by the Folin-Ciocalteu and aluminum chloride methods, respectively, while antioxidant activity was assessed by DPPH and FRAP assays. The antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was evaluated using the disc diffusion method. Flavonoids ranged from 13.40 to 30.27 mg/g for *Z. chalybeum* extracts and 21.76 to 66.27 mg/g for *E. ingens*, with the highest amounts in ethyl acetate extracts of both plants. Both plants had the highest amounts of total phenolics in methanol extracts, with *Z. chalybeum* and *E. ingens* exhibiting 284.85 and 351.59 mg/g, respectively. The DPPH radical scavenging activity test showed that *E. ingens*, with IC₅₀ values of 11.28 and 12.85 µg/mL for the aqueous and methanol extracts, respectively, is a stronger antioxidant than *Z. chalybeum*. The FRAP activity was highest in methanol extracts for *E. ingens* (2831.77±179.02 mg/g) and *Z. chalybeum* (799.15±32.43 mg/g). Both plants exhibited more potent antibacterial activity against *S. aureus* compared to *P. aeruginosa*. Dichloromethane and ethyl acetate extracts of *Z. chalybeum* showed significant activity against *S. aureus* with MIC values of 1.56 mg/mL, demonstrating their strong inhibitory potential. The study has revealed that both *E. ingens* and *Z. chalybeum* roots would be excellent natural antioxidants and antibacterial agents.

Keywords

antioxidants; antibacterial; *Euphorbia ingens*; medicinal plants; total phenolic; *Zanthoxylum chalybeum*

Introduction

Plants have been used globally as medicines since time immemorial, and the World Health Organisation (WHO) estimates that about 80% of the world's population uses traditional medicine for their primary healthcare needs (1). In developing countries, where biomedical services are inadequate, people rely heavily on plant-based traditional treatments (2). Traditional medicine is a complement as well as an alternative to modern drugs

and is considered to be more easily accessible and more affordable by the rural population than modern drugs (3).

Medicinal plants contain bioactive compounds called phytochemicals, which usually have no nutritional value (4). Plants use phytochemicals like phenolics, alkaloids, flavonoids, terpenoids, and saponins for their defense. However, these phytochemicals can treat various human diseases (5). A study by Pinto and others (6) reported that phenolic compounds exhibit important properties, including antioxidant, antimicrobial, anticancer, anti-inflammatory, cardioprotective, and immune system-promoting effects. Therefore, there is a need to explore the medicinal value of *Zanthoxylum chalybeum* and *Euphorbia ingens*.

Zanthoxylum chalybeum (Rutaceae family) is traditionally used in the cure of malaria, diabetes, toothache, abdominal pain, diarrhoea, pneumonia, yellow fever, snake bites, asthma, gonorrhoea, sexual impotence, oedema, and anaemia. It is also used to clean respiratory tract airway obstruction and aphrodisiac, among other medicinal uses (7, 8). Research on the phytochemical screening and antiplasmodial properties of *Z. chalybeum* leaves has been conducted in Uganda (9). In Malawi, the traditional medicinal use of the plant has been reported (10), but no chemical composition study on the indigenous plant has been done. *E. ingens* (Euphorbiaceae family) is traditionally used for treating cancer, swelling, fistulas, wounds, snake bites, and mental disorders, among other diseases (11, 12). Despite the many medicinal applications attributed to *Z. chalybeum* and *E. ingens*, there is limited scientific evidence about the phytochemical and antimicrobial properties to inform the folkloric use of the two medicinal plants in Malawi. Therefore, this study was aimed at investigating the chemical composition, antioxidant, and antibacterial activities of *Z. chalybeum* and *E. ingens* from Malawi.

Materials and Methods

Chemicals and reagents

The chemicals used in the study were analytical grade and were bought from LAB ENTERPRISES Ltd., Blantyre, Malawi. The water used in the analyses was double-distilled.

Plant collection and processing

The roots of *Zanthoxylum chalybeum* were collected from the wild at Elunyeneni in Mzimba North, Malawi, at GPS coordinates 11°13'10.71393" S, 33°43'12.33613" E, while the roots of *Euphorbia ingens* were collected from Lilongwe, Malawi, at 14°11'15.828" S, 33°46'38.724" E. The plant specimens were authenticated at the National Herbarium and Botanical Gardens of Malawi (NHBGM), Zomba, using specimen references 89177 and 31742, respectively. The plant materials were taken to the chemistry laboratory at the University of Malawi, where they were processed and analyzed. Separately, the plant roots were rinsed with tap water and distilled water and then air-dried. The root barks were removed from the root using a stainless-steel knife, cut into small pieces, and dried under a shed for 7 days, after which they were ground using a laboratory mill. The

resultant powder was sieved using a 1 mm sieve and kept in Ziploc bags at 4 °C pending analysis.

Plant extraction

The plant extracts were obtained by maceration. A 100 g powder sample was sequentially mixed with 800 mL of solvents for 48 h with regular shaking. The solvents included *n*-hexane, dichloromethane, ethyl acetate, methanol, and water. The mixtures were filtered separately using Whatman No. 42 filter paper, and the organic solvents were evaporated on a rotary evaporator set at 40 °C and 40 rpm under reduced pressure while aqueous extracts were freeze-dried. The residues were weighed and stored at -10 °C. The extracts were dissolved in their respective solvents used for extraction to give a stock solution of 1 mg/mL, which was used in subsequent tests.

Total phenolics

The Folin-Ciocalteu method, as reported by Masoko and others (13), was used to determine the total phenolics of the crude extracts of the plant roots. The following equation was used to calculate the sample concentration as gallic acid equivalent (GAE):

$$\text{Total phenolics (mg GAE/g)} = \frac{R \times DF \times V}{1000 \times m} \dots\dots\dots(\text{Eqn-1})$$

In this equation, R is the value read from the standard curve, DF represents the dilution factor, and V represents the sample stock solution volume. The mass of the sample extract in grams is given by m. All determinations were done in triplicate, and the mean ± SD was reported.

Flavonoids

The aluminum chloride procedure by Pokhrel and co-workers (14) was adopted in the determination of the flavonoids, and the following equation was employed in the calculation of the sample concentration:

$$\text{Flavonoids (mg QE/g)} = \frac{R \times DF \times V}{1000 \times m} \dots\dots\dots(\text{Eqn-2})$$

Where, R is the value read from the standard curve, and DF represents the dilution factor. The sample stock solution volume is represented by V, while the mass of the sample extract in grams is given by m. All determinations were done in triplicate, and the mean ± SD was reported.

Antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity assay

The procedure outlined in literature (15), with some modifications, was used in the determination of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. In triplicate, 5 mL of a 0.2 mM DPPH solution in methanol was mixed with a 5 mL sample dissolved in extracting solvent at eight different concentrations (1, 5, 10, 25, 50, 100, 200, and 250 µg/mL) in separate test tubes. The mixture was vortexed and left in the dark at room temperature for 30 min before reading absorbance on a UV-Vis spectropho-

tometer at 517 nm. Ascorbic acid (1–250 µg/mL) was used as a positive standard, and it was treated similarly to the sample. The negative control was prepared by mixing 5 mL of DPPH solution with 5 mL of extracting solvent. The following formula was used to calculate the radical scavenging activity of the samples:

$$\text{DPPH Scavenging activity \%} = \frac{A_0 - A_1}{A_0} \times 100 \quad \text{.....(Eqn-3)}$$

where A_0 and A_1 are the absorbance values of the control and the sample or standard, respectively. A plot of the inhibition percentages against concentrations produced a regression line ($y = ax + b$), which was used to determine the antioxidant activity of each plant sample expressed as IC_{50} . Since the curve was not straight but curved, the regression line was determined by joining two points enclosing a 50% inhibition (16). The IC_{50} value was calculated by substituting 50 for y in the regression line.

Ferric Reducing Antioxidant Power (FRAP) assay

The Ferric Reducing Antioxidant Power (FRAP) assay was performed using a modified procedure by Kosakowska and others (17). A 3 mL of FRAP reagent freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1 (v/v/v) was added to 100 µL of sample extract (1 mg/mL in extracting solvent) in a falcon tube and vortexed. The mixture was incubated at room temperature for 10 min, and the absorbance was read at 593 nm on a UV-Vis spectrophotometer. Trolox, prepared by dissolving 0.1 g of the solid in methanol and diluting it to 100 mL with distilled water, was used to produce a calibration curve (0–50 µg/mL). The analysis was done in triplicate, and the antioxidant capacity was recorded as Trolox equivalent antioxidant capacity (TEAC) mg/g of extract, expressed as mean ±SD.

Antibacterial activities

Bacterial strains and antibiotics

The bacterial strains were collected from the National Microbiology Reference Laboratory (NMRL) under the Public Health Institute of Malawi (PHIM) in Lilongwe. The strains included the gram-positive *Staphylococcus aureus* (ATCC 25923) and gram-negative *Pseudomonas aeruginosa* (ATCC 27853). A commercial Gentamicin (Ampoules) IV was used as a positive control antibiotic.

Preparation of antibiotic discs

Antibiotic discs were prepared according to Bouslamti and others (18), with some modifications. The blank antibiotic discs were prepared from Whatman No. 1 filter papers using a standard paper punching machine. The filter papers were punched to disc sizes of 6 mm in diameter and sterilised by autoclaving at 121 °C for 15 min before impregnating with the plant extracts. Sterile discs were placed in petri dishes at approximately 5 mm apart in the biosafety cabinet Class II. Each disc was impregnated with 20 µL of the plant extract with a known concentration, using a micropipette. Gentamicin and blank discs were used as posi-

tive and negative controls, respectively. The impregnated discs were dried in an incubator at 37 °C for 4 h, placed in sterile glass vials, and stored at -20 °C until sensitivity testing.

Susceptibility testing

The Kirby-Bauer disc diffusion assay was used for the anti-bacterial susceptibility test according to the National Committee for Clinical Laboratory Standards (NCCLS) Guidelines. The antibacterial assays were prepared by dissolving a known mass of crude plant extracts in their respective extracting solvents to achieve a final concentration of 200 mg/mL and serially diluted to 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.12 mg/mL, 1.56 mg/mL, and 0.78 mg/mL. The Nutrient and Mueller-Hinton agar media used were prepared according to the manufacturer's instructions. The gram-positive strains *S. aureus* and gram-negative *P. aeruginosa* were sub-cultured on nutrient agar plates and incubated for 18–24 h at 37 °C. Using a sterile inoculating loop, well-isolated colonies were suspended in sterile distilled water tubes and vortexed thoroughly to prepare inoculum. The turbidity of the test suspension was standardised to match that of the 0.5 McFarland standard that was alternately formulated as described by Debalke and others (19). The suspension was used within 15 min of its preparation.

Using sterile forceps, the impregnated discs were tested in triplicate on the surface of the Mueller-Hinton agar plate with bacterial lawns. The plates were incubated overnight at 37 °C for 18–24 h, and the diameter of the growth inhibition zones around each impregnated disc was measured in millimetres using a Vernier calliper with a 0.05 mm least count, and the mean values were recorded. Gentamicin (1 mg/mL) served as a positive control, and extracting solvents constituted a negative control.

Statistical analysis

A one-way analysis of variance was used to analyze the effect of using five different solvents on FRAP activity of *E. ingens* and *Z. chalybeum* roots. A three-way analysis of variance was used to analyze the interactive effect of plant extract, solvent, and microorganism species on inhibition at an extract concentration of 200 mg/mL. The separation of the means after a significant main effect was done by the Tukey's test. All tests were analysed using R statistical software (20) at a 5% level of significance.

Results and Discussion

Total phenolics and total flavonoids

Fig. 1A & 1B, respectively, show the total phenolic concentration and the total flavonoid content of *Euphorbia ingens* and *Zanthoxylum chalybeum* root samples extracted with ethyl acetate, methanol, and water. In all solvent extracts, *E. ingens* had a higher phytochemical content than *Z. chalybeum*. The total phenolic content ranged from 52.33 to 351.59 mg GAE/g and from 47.15 to 284.85 mg GAE/g for *E. ingens* and *Z. chalybeum*, respectively (Fig. 1A).

The flavonoid content ranged from 21.76 to

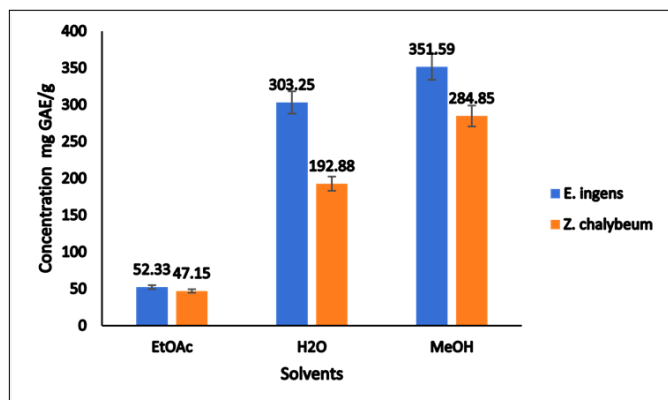


Fig. 1A. Concentration of total phenolics of *E. ingens* and *Z. chalybeum* roots extracted with three different solvents. Data represents means (\pm SD) of three separate measurements.

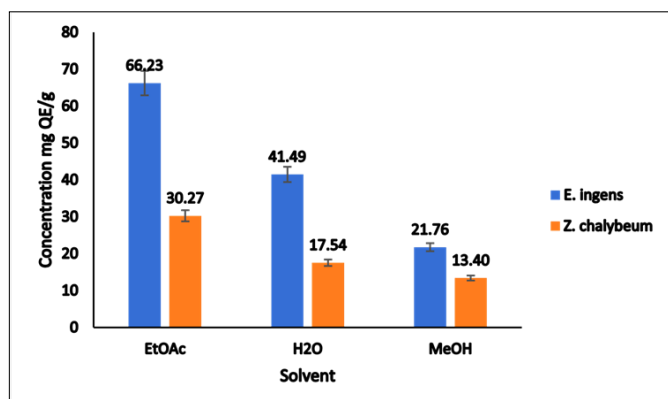


Fig. 1B. Total flavonoid content of *E. ingens* and *Z. chalybeum* roots extracted with three different solvents. Data represents means (\pm SD) of three separate measurements.

66.23 mg QE/g for *E. ingens* and from 13.40 to 30.27 mg QE/g for *Z. chalybeum* (Fig. 1B). In both plants, the highest amounts of total phenolics were found in methanol extracts and the lowest amounts in ethyl acetate extracts, while the reverse was true for the total flavonoid content. Another researcher (21) also found higher amounts of total phenolics in methanol extracts (155.44 mg GAE/g) than in ethyl acetate extracts (42.83 mg GAE/g). The flavonoid contents were highest in ethyl acetate extracts and lowest in methanol extracts. Other researchers have also found that flavonoids were higher in ethyl acetate extracts than in methanol extracts (22). The variation could be due to the polarity of the flavonoids present in the plants, and ethyl acetate, being moderately polar, is able to extract a wide range of flavonoids (22). Apart from ethyl acetate in *E. ingens*, each solvent extracted more total phenolics than flavonoids. This is in agreement with other researchers (23, 24) who reported higher amounts of total phenolics than flavonoids in plant extracts. Generally, methanol was the best solvent for extracting total phenolics, whereas flavonoids were best extracted with ethyl acetate. Our study showed a strong and significant ($p = 0.03$) three-way interaction involving the type of the plant, the solvent, and the chemical type.

A study conducted in Kenya on dichloromethane/methanol extracts of *Zanthoxylum* species barks, which included *Z. chalybeum*, reported total phenolic values of 73.09–145.27 mg TAE/g (25). These values were similar to those found in this study. Another study in Uganda had

total phenolics of 14.58 mg GAE/g (26), which was much lower than the value in this study. Kaigongi and others reported a flavonoid content of 109.42–186.41 mg QE/g for barks of various *Zanthoxylum* species, including *Z. chalybeum* (25). The differences in the phytochemical values could be due to differences in geographical location where the plant was harvested, soil characteristics, differences in extraction solvents and techniques, parts of the plant used, and environmental conditions, among others (27, 28). There were more flavonoids and total phenolics in *E. ingens* than in *Z. chalybeum*. This could be due to genetic factors as well as environmental factors, such as levels of biotic and abiotic stress, among others (26). Studies have shown that total phenolics, which include flavonoids and other secondary metabolites, are responsible for pharmacological activities that include antioxidant, anti-bacterial, anti-malarial, and anti-diarrhoea, among others (13).

Antioxidant activity

The antioxidant activities of *Z. chalybeum* root extracts and *E. ingens* root extracts in various solvents were determined by the DPPH and FRAP assays. Fig. 2A & 2B, respectively, show the %inhibition of DPPH activities of *Z. chalybeum* root extracts and *E. ingens* root extracts in three different solvents, and Table 1 contains the antioxidant activities expressed as IC_{50} determined by the DPPH assay. The FRAP activities of both *Z. chalybeum* root extracts and *E. ingens* root extracts in five different solvents are presented in Table 2 and Fig. 3.

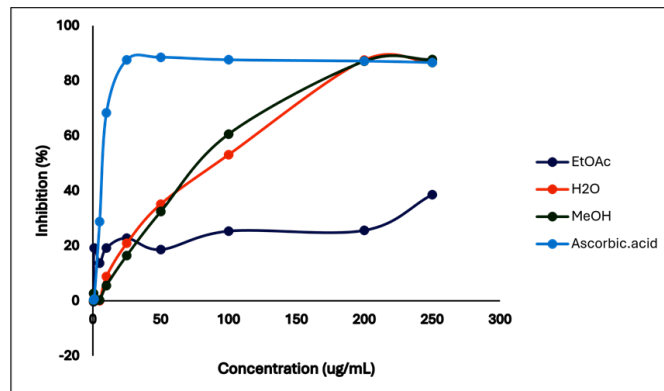


Fig. 2A. % Inhibition of DPPH activity of *Z. chalybeum* root using three different solvents at different concentrations. EtOAc = ethyl acetate; H₂O = water; MeOH = methanol.

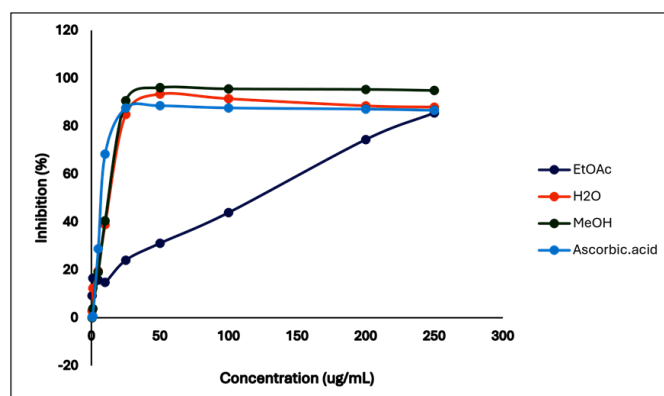


Fig. 2B. % Inhibition of DPPH activity of *E. ingens* root using three different solvents at different concentrations. EtOAc = ethyl acetate; H₂O = water; MeOH = methanol.

Table 1. IC₅₀ values of antioxidant activity of *Z. chalybeum* and *E. ingens* extracts.

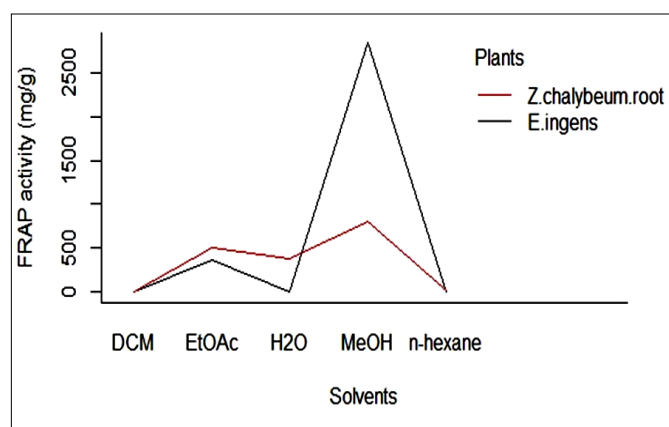
Plant	Solvent	IC ₅₀ (μg/mL)
<i>E. ingens</i>	MeOH	12.85
	H ₂ O	11.28
	EtOAc	119.97
<i>Z. chalybeum</i>	MeOH	81.18
	H ₂ O	91.30
Ascorbic acid		7.68

Table 2. FRAP activity in *E. ingens* and *Z. chalybeum* roots using five different solvents.

Solvents	<i>E. ingens</i> (mg/g)	<i>Z. chalybeum</i> roots (mg/g)
DCM	NA	8.93 ± 1.65
EtOAc	362.73 ± 76.14	512.47 ± 54.47
H ₂ O	NA	382.79 ± 64.93
MeOH	2831.77 ± 179.02	799.15 ± 32.43
<i>n</i> -hexane	NA	20.74 ± 8.51

p-value < 0.001

NA = No Activity. Data represents means (±SD) of three separate measurements. Statistical significance was set at 5% level.

**Fig. 3.** FRAP activity in *E. ingens* and *Z. chalybeum* roots using five different solvents. DCM = dichloromethane; EtOAc = ethyl acetate; H₂O = water; MeOH = methanol.

DPPH is one of the most stable free radicals and is frequently used in the determination of radical scavenging in natural plant extracts (29). Fig. 2A shows that the % inhibition of DPPH activity of *Z. chalybeum* root extracts at different concentrations (1–250 μg/mL) varied depending on the solvent used for extraction. At a concentration of 100 μg/mL, the %inhibition of DPPH activity was 53.12%, 60.60%, and 25.34% for the aqueous, methanol, and ethyl acetate extracts, respectively, while for the standard ascorbic acid was 87.62%.

Kaigongi and co-workers recorded a %inhibition of DPPH activity of 76% for *Z. chalybeum* at 100 μg/mL (25), which was higher than the value in this study. A linear regression for the graph (Fig. 2A) gave IC₅₀ values of 91.30 μg/mL and 81.18 μg/mL for the aqueous and methanol extracts, respectively, while that of the standard ascorbic acid was 7.68 μg/mL (Table 1). The IC₅₀ value is the effective concentration that reduces the DPPH radical by 50%, which indicates its antioxidant efficacy (27). A lower

IC₅₀ value indicates higher DPPH radical scavenging activity of the extract (30). This study shows that the aqueous and methanol *Z. chalybeum* root extracts had moderate antioxidant activity compared to the standard ascorbic acid. The %inhibition of DPPH activity of aqueous, methanol, and ethyl acetate extracts of *E. ingens* was 91.46%, 95.57%, and 43.91%, respectively (Fig. 2B). The aqueous and methanol extracts had strong antioxidant activity, as shown by IC₅₀ values of 11.28 μg/mL and 12.85 μg/mL, respectively, which were comparable to the standard ascorbic acid, while ethyl acetate showed an IC₅₀ value of 119 μg/mL (Table 1). The results show that for both *Z. chalybeum* and *E. ingens*, the ethyl acetate extracts had a very high IC₅₀ value, hence being less efficient radical scavengers compared to aqueous and methanol extracts. The results also show that *E. ingens* is a stronger antioxidant than *Z. chalybeum*, which reflects the abundance of total phenolics and flavonoids in the two plants. Most of the antioxidant activities of plants are due to phenols (23).

FRAP measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe³⁺-TPTZ) complex and producing a colored ferrous tripyridyltriazine (Fe²⁺-TPTZ) (31). Table 2 and Fig. 3 show that there was a significant interaction (*p* < 0.001) between plant species and type of solvent on FRAP activity. The FRAP activity was highest in methanol extracts of both *E. ingens* and *Z. chalybeum* roots among all the other solvents, but it was higher in *E. ingens* than *Z. chalybeum* roots in the extract. When using ethyl acetate and water, FRAP activity was higher in *Z. chalybeum* roots than in *E. ingens*.

There was FRAP activity in *Z. chalybeum* roots when using dichloromethane and *n*-hexane, while there was no activity in *E. ingens* when using the same solvents. It is observed from the results that methanol extracts had the highest total phenolics in both plants, which is reflected in the antioxidant activity values of both DPPH radical scavenging and FRAP assays. The total phenolics and flavonoids influence the antioxidant properties of medicinal plants (32), and *E. ingens* and *Z. chalybeum* roots would make useful natural antioxidants in the pharmaceutical industry.

Antibacteria activity

The inhibition zones for *Z. chalybeum* and *E. ingens* root extracts in various solvents at concentrations ranging from 0.78 to 200 mg/mL were examined on *S. aureus* and *P. aeruginosa*. Fig. 4 displays selected plates showing the inhibition zones at selected extract concentrations, and Table 3 shows the measured zones at a concentration of 200 mg/mL. The extracts exhibited a variety of inhibition zones against *S. aureus* and *P. aeruginosa*. The mean value of inhibition zones for *Z. chalybeum* on *S. aureus* ranged from 6.33 ± 0.02 mm to 18.32 ± 0.19 mm and for *P. aeruginosa* from 6.63 ± 0.09 mm to 7.43 ± 0.18 mm. The *E. ingens* extracts exhibited a mean inhibition value of 7.88 ± 3.04 mm to 10.53 ± 0.09 mm on *S. aureus* and 7.32 ± 0.17 mm to 7.65 ± 0.23 mm on *P. aeruginosa*. The results showed that there was a significant three-way interaction (*p*-value < 0.001) among plant extracts, type of solvent, and

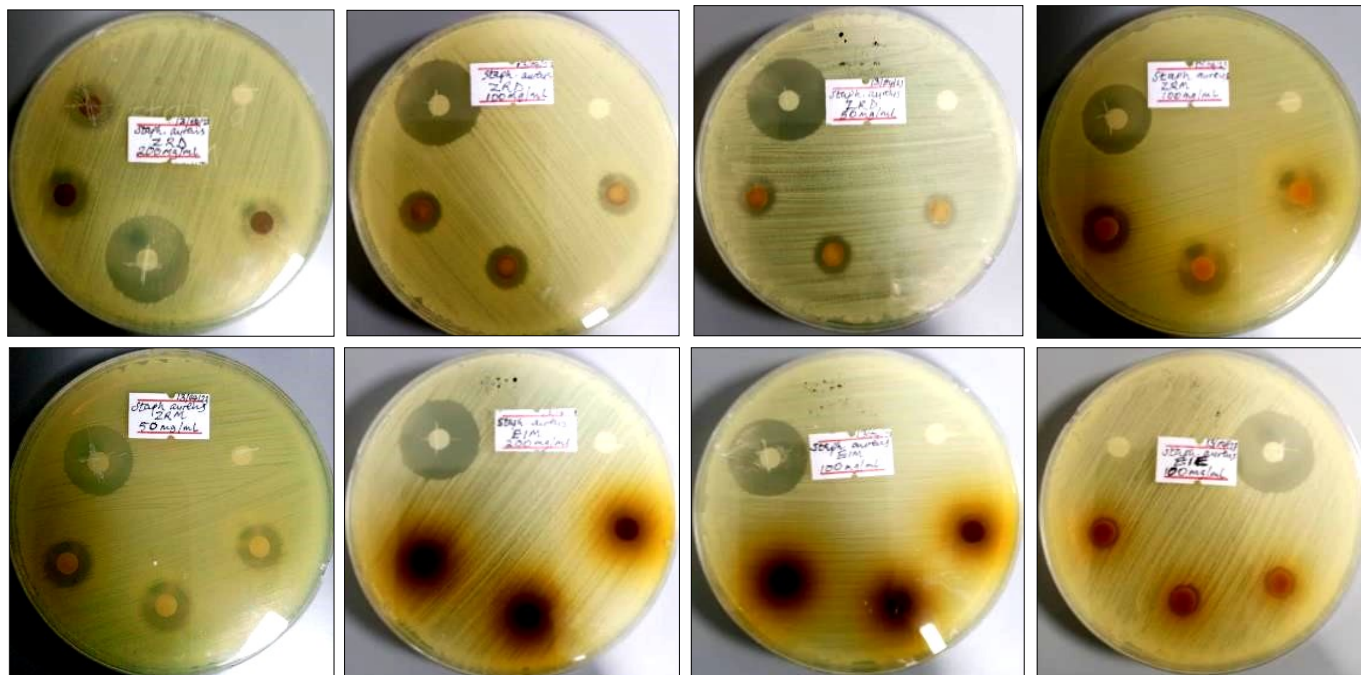


Fig. 4. Selected plates showing inhibition zones on *S. aureus*. **ZRM** = *Z. chalybeum* roots methanol extracts; **ZRD**, *Z. chalybeum* roots dichloromethane extracts; **EIM** = *E. ingens* methanol extracts; **EIE** = *E. ingens* ethyl acetate extracts.

Table 3. Three-way Analysis of variance showing the interactive effect of plant extract, solvent, and microorganism species on inhibition at extract concentration 200 mg/mL.

Plant	Solvent	Inhibition zone (mm)	
		<i>S. aureus</i>	<i>P. aeruginosa</i>
<i>E. ingens</i>	DCM	NI	NI
	EtOAc	10.53 ± 0.09	NI
	H ₂ O	8.80 ± 0.12	7.32 ± 0.17
	MeOH	7.88 ± 3.04	7.65 ± 0.23
	<i>n</i> -hexane	NI	NI
<i>Z. chalybeum</i> root	DCM	11.48 ± 0.04	7.25 ± 0.16
	EtOAc	11.03 ± 0.27	7.43 ± 0.18
	H ₂ O	8.37 ± 0.20	NI
	MeOH	18.32 ± 0.19	6.63 ± 0.09
	<i>n</i> -hexane	6.33 ± 0.02	7.28 ± 0.21
<i>p</i> -value < 0.001			

NI = No Inhibition. Data represents means (±SD) of three separate measurements. Statistical significance was set at 5% level.

type of microorganism in the inhibition zone (Fig. 5 & 6). For *S. aureus*, the inhibition zone was higher with *Z. chalybeum* roots than with *E. ingens* roots when extracted with dichloromethane, ethyl acetate, methanol, and *n*-hexane. The opposite was true when using water as a solvent; inhibition was higher with *E. ingens* roots than *Z. chalybeum* roots. For *P. aeruginosa*, inhibition was higher with *Z. chalybeum* roots than with *E. ingens* when extracted with dichloromethane, ethyl acetate, and *n*-hexane. The opposite was true when using water and methanol as solvents; inhibition was higher with *E. ingens* than *Z. chalybeum* roots. *E. ingens* extracts in *n*-hexane and dichloromethane did not show any inhibition on both *S. aureus* and *P. aeruginosa*, while ethyl acetate extract showed inhibition on *S. aureus* but not on *P. aeruginosa*. This possibly shows the absence of non-polar secondary metabolites in *E. ingens* that would inhibit *S. aureus* and *P. aeruginosa*, as

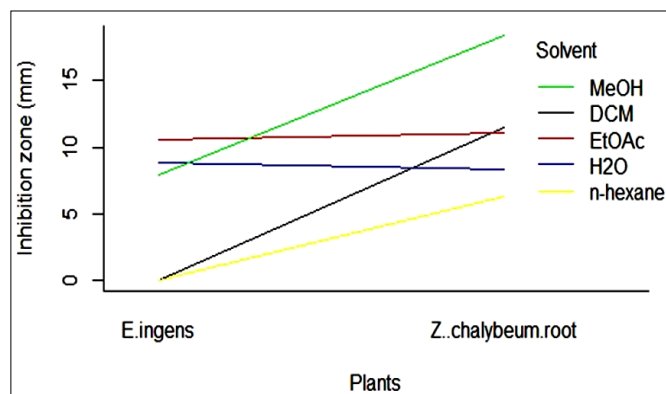


Fig. 5. Interactive effect of plant species and type of solvent on inhibition of *A. aureus*. **DCM** = dichloromethane; **EtoAc** = ethyl acetate; **H₂O** = water; **MeOH** = methanol.

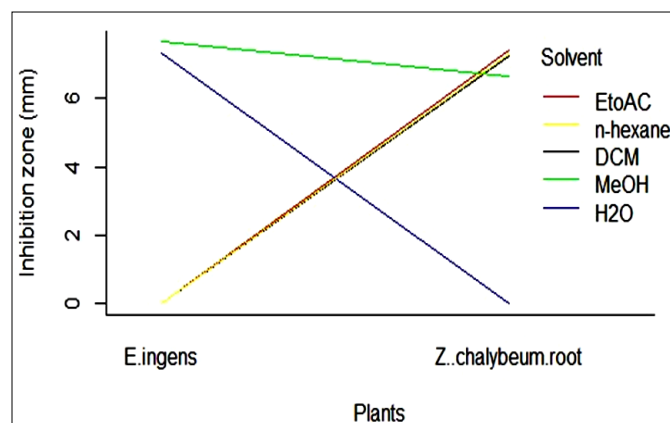


Fig. 6. Interactive effect of plant species and type of solvent on inhibition of *P. aeruginosa*. **DCM** = dichloromethane; **EtoAc** = ethyl acetate; **H₂O** = water; **MeOH** = methanol.

well as moderately polar metabolites that could inhibit *P. aeruginosa*. All *Z. chalybeum* root extracts showed inhibition except for the aqueous extract.

Minimum inhibitory concentrations (MICs) of plant extracts

The serial dilution assay was used to determine the mini-

mum inhibition concentrations (MICs) for the *Z. chalybeum* and *E. ingens* crude extracts on *S. aureus* and *P. aeruginosa*, and the results are presented in Table 4. All the solvent extracts for *Z. chalybeum* roots showed inhibition on *S. aureus*, with MICs ranging from 1.56 mg/mL to 200 mg/mL. The dichloromethane and ethyl acetate *Z. chalybeum* root extracts had the lowest MIC, followed by methanolic, aqueous, and lastly, *n*-hexane extracts. The MIC was identified as the lowest concentration of the bacterial assay, which inhibited the growth of the test strains. The lower the MIC value, the higher the total activity (13). Therefore, dichloromethane and ethyl acetate extracts of *Z. chalybeum* roots have very high antimicrobial activity against *S. aureus* (a gram-positive bacteria), followed by methanol extracts. The aqueous and *n*-hexane extracts showed weak antibacterial activity against *S. aureus*. The weak antimicrobial activity of aqueous and *n*-hexane extracts could be due to the low amounts of active compounds against *S. aureus* in the crude extracts (33). For *P. aeruginosa* (a gram-negative bacteria), all the solvent extracts for *Z. chalybeum* roots showed inhibition with an MIC value of 200 mg/mL except for the aqueous extracts, which did not show any inhibition. All the *Z. chalybeum* root extracts have stronger activity against *S. aureus* than against *P. aeruginosa*. The aqueous and methanol extracts of *E. ingens* showed stronger antibacterial activity against *S. aureus* than ethyl acetate extracts, while *n*-hexane and dichloromethane did not show any activity. The aqueous, methanol, and ethyl acetate extracts of *E. ingens* showed higher activity against *S. aureus* than *P. aeruginosa*. The most imperative observation was that there were fewer or no antibacterial activities on gram-negative bacteria compared to gram-positive bacteria. This observation is in agreement with other studies (33). Gram-negative bacteria have a lipopolysaccharide barrier on their outer membrane, which is not found in gram-positive bacteria, and this makes most medicinal plants ineffective against gram-negative bacteria (33).

Table 4. Minimum inhibition concentration of various solvents on *S. aureus* and *P. aeruginosa* in various plants.

Plant	Solvent	<i>S. aureus</i>	<i>P. aeruginosa</i>
		MIC (mg/mL)	MIC (mg/mL)
<i>E. ingens</i>	Dichloromethane	NI	NI
	<i>n</i> -hexane	NI	NI
	Ethyl acetate	25	NI
	Methanol	3.125	50
	Water	3.125	50
	Dichloromethane	1.56	200
	<i>n</i> -hexane	200	200
<i>Z. chalybeum</i> roots	Ethyl acetate	1.56	200
	Methanol	3.125	200
	Water	100	NI

NI = No inhibition.

Conclusion

The study showed that *Zanthoxylum chalybeum* and *Euphorbia ingens* have strong antibacterial and antioxidant properties, as well as large concentrations of flavonoids and total phenolics. Methanol and ethyl acetate are ideal solvents for extracting total phenolics and flavonoids, respectively. Methanol and aqueous extracts demonstrated the strongest antioxidant properties. The study further found that both plants effectively suppress gram-positive bacteria. *Z. chalybeum* root extracts in ethyl acetate and dichloromethane are the most effective inhibitors. These findings support the use of the two plants in traditional medicine. The results thus serve as a foundation for more investigation into the identification, isolation, and purification of the active compounds present in the two plants, which may potentially lead to the creation of novel pharmaceuticals.

Acknowledgements

The authors would like to thank the Centre for Resilient Agri-Food Systems (CRAFS) and Lilongwe University of Agriculture and Natural Resources for the financial support. Special thanks to Mr. Maurice Mkandawire and Mr. Likiadi Kalioti for their invaluable assistance in the collection of research samples.

Authors' contributions

FK conceptualized the study, developed the methodology, conducted the research, and drafted the manuscript. JS conceptualised the study, supervised the research, reviewed, and edited the manuscript. WJ performed the statistical analysis and reviewed and edited the manuscript. BN developed the methodology and reviewed and edited the manuscript. KN validated the study and reviewed and edited the manuscript. JM conceptualized the study, supervised the research, reviewed, and edited the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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