PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.4526

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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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OPEN ACCESS

**ARTICLE HISTORY**

Received: 02 August 2024
Accepted: 29 November 2024

Available online
Version 1.0 : 19 February 2025

Check for updates

**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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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 (Early Access). 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-Ciocalteau 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 $IC_{50}$ 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, snakebites, 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 Elunyeni 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-Ciocalteau 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):

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

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 coworkers (14) was adopted in the determination of the flavonoids, and the following equation was employed in the calculation of the sample concentration:

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

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 $\mu$g/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 $A_o$ 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 positive 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 antibacterial 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 effectwas 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).

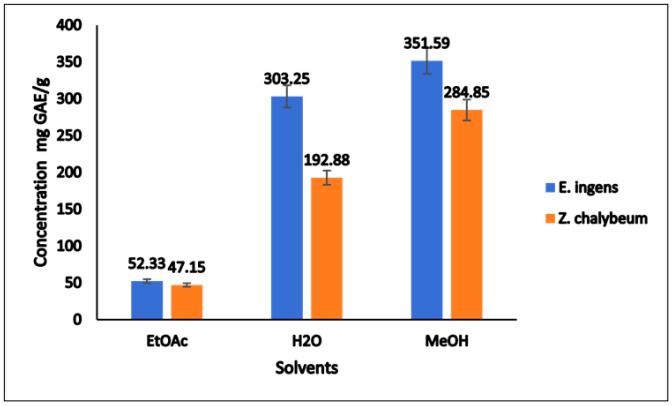


**Fig. 1A.** Concentration of total phenolics of *E. ingens* and *Z. chalybeum* roots extracted with three different solvents. Data represents means (±SD) of three separate measurements.

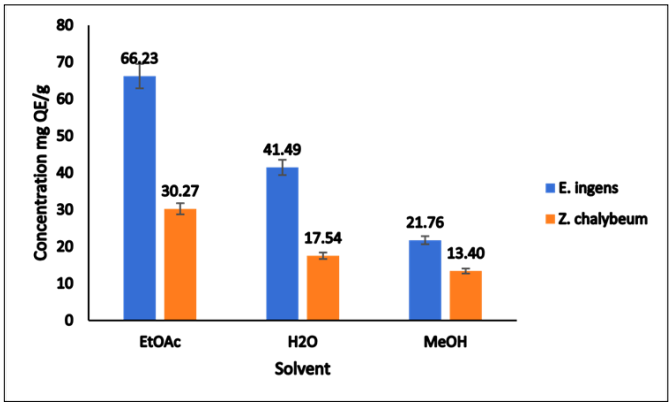


**Fig. 1B**. Total flavonoid content of *E. ingens* and *Z. chalybeum* roots extracted with three different solvents. Data represents means (±SD) of three separate measurements.

The flavonoid content ranged from 21.76 to 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 mgGAE/g) than in ethyl acetate extracts (42.83 mgGAE/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, antibacterial, 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.

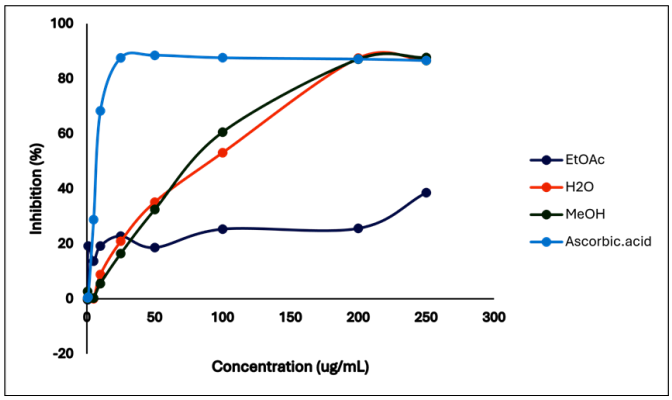


**Fig. 2A.** % Inhibition of DPPH activity of *Z. chalybeum* root using three different solvents at different concentrations. **EtOAc** = ethyl acetate; **$H_2O$** = water; **MeOH** = methanol.

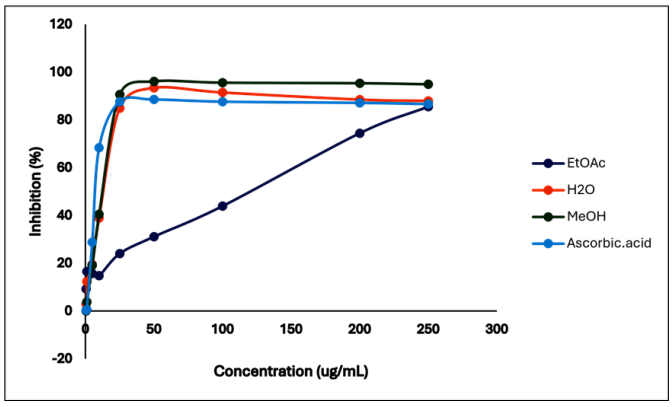


**Fig. 2B**. % Inhibition of DPPH activity of *E. ingens* root using three different solvents at different concentrations. **EtOAc** = ethyl acetate; **$H_2O$** = water; **MeOH** = methanol.

**Table 1**. $IC_{50}$ values of antioxidant activity of *Z. chalybeum* and *E. ingens* extracts

| Plant | Solvent | $IC_{50}$ (μg/mL) |
|---|---|---|
| *E. ingens* | MeOH | 12.85 |
| | $H_2O$ | 11.28 |
| | EtOAc | 119.97 |
| *Z. chalybeum* | MeOH | 81.18 |
| | $H_2O$ | 91.30 |
| Ascorbic acid | | 7.68 |

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

| Solvents | *E. ingens (mg/g)* | *Z. chalybeum* roots (mg/g) |
|---|---|---|
| DCM | NA | 8.93 ± 1.65 |
| EtOAc | 362.73 ± 76.14 | 512.47 ± 54.47 |
| $H_2O$ | NA | 382.79 ± 64.93 |
| MeOH | 2831.77 ± 179.02 | 799.15 ± 32.43 |
| *n*-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.

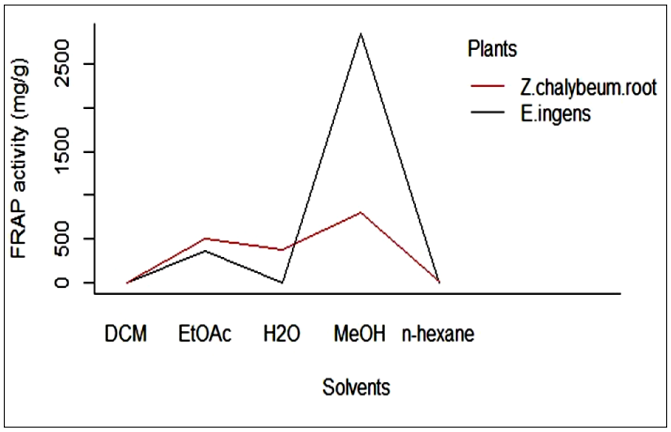


**Fig. 3**. FRAP activity in *E. ingens* and *Z. chalybeum* roots using five different solvents. **DCM** = dichloromethane; **EtoAc** = ethyl acetate; **$H_2O$** = water; **MeOH** = methanol.

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 $IC_{50}$ 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 $IC_{50}$ value is the effective concentration that reduces the DPPH radical by 50%, which indicates its antioxidant efficacy (27). A lower $IC_{50}$ 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 $IC_{50}$ 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 $IC_{50}$ 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 $IC_{50}$ 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 tripyridyltrazine ($Fe^{3+}$-TPTZ) complex and producing a colored ferrous tripyridytriazine ($Fe^{2+}$-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

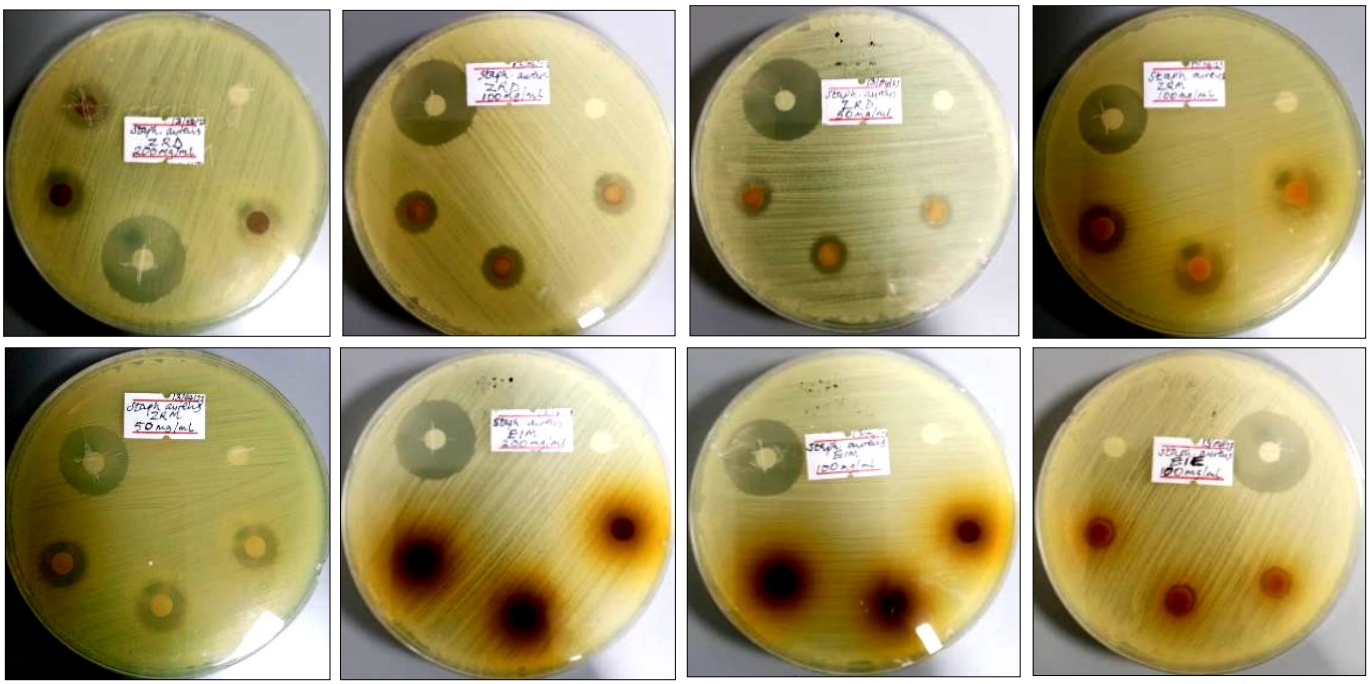

**Fig. 4.** Selected plates showing inhibition zones on *S. aureus*. **ZRM** = *Z. chalybeum* roots methanol extracts ; **ZRD**, *Z. chalybeum* roots dichloromethane extracts; **EIM** = *E. ingens* methanol extracts; **EIE** = *E. ingens* ethyl acetate extracts.

**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

| | | Inhibition zone (mm) | |
|---|---|---|---|
| **Plant** | **Solvent** | ***S. aureus*** | ***P. aeruginosa*** |
| | DCM | NI | NI |
| | EtOAc | 10.53 ± 0.09 | NI |
| *E. ingens* | $H_2O$ | 8.80 ± 0.12 | 7.32 ± 0.17 |
| | MeOH | 7.88 ± 3.04 | 7.65 ± 0.23 |
| | *n*-hexane | NI | NI |
| | DCM | 11.48 ± 0.04 | 7.25 ± 0.16 |
| | EtOAc | 11.03 ± 0.27 | 7.43 ± 0.18 |
| *Z. chalybeum* root | $H_2O$ | 8.37 ± 0.20 | NI |
| | MeOH | 18.32 ± 0.19 | 6.63 ± 0.09 |
| | *n*-hexane | 6.33 ± 0.02 | 7.28 ± 0.21 |
| | *p*-value < 0.001 | | |

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

solvent, and 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

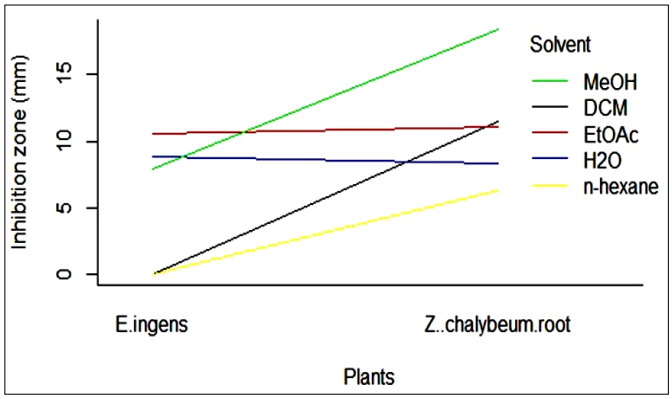


**Fig. 5**. Interactive effect of plant species and type of solvent on inhibition of *A. aureus.* **DCM** = dichloromethane; **EtoAc** = ethyl acetate; **$H_2O$** = water; **MeOH** = methanol.

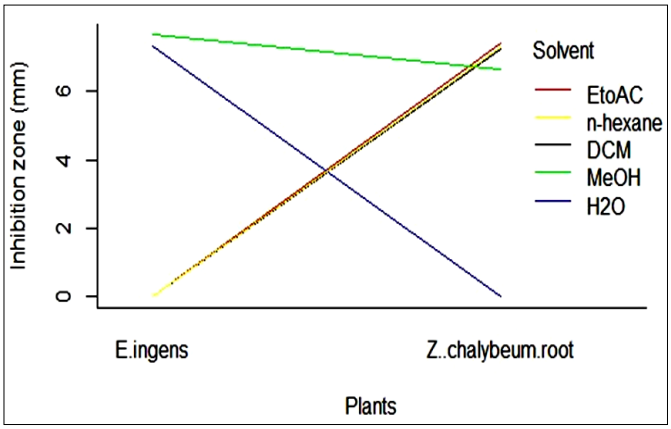


**Fig. 6**. Interactive effect of plant species and type of solvent on inhibition of *P. aeruginosa.* **DCM** = dichloromethane; **EtoAc** = ethyl acetate; **$H_2O$** = water; **MeOH** = methanol.

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. aeruginossa,* 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 | *S. aureus* | *P. aeruginosa* |
|---|---|---|---|
| | | MIC (mg/mL) | MIC (mg/mL) |
| | Dichloromethane | NI | NI |
| | *n*-hexane | NI | NI |
| *E. ingens* | Ethyl acetate | 25 | NI |
| | Methanol | 3.125 | 50 |
| | Water | 3.125 | 50 |
| | Dichloromethane | 1.56 | 200 |
| | *n*-hexane | 200 | 200 |
| | Ethyl acetate | 1.56 | 200 |
| *Z. chalybeum* roots | 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

## References

1. Akram M, Adetunji CO, Laila U, Michael OS, Olerimi Samson E, Kadiri O, et al. 4 - Overview of the traditional systems of medicine in different continents during postwar recovery. In: Mtewa AG, Egbuna C, editors. Phytochemistry, the Military and Health [Internet]. Elsevier; 2021 [cited 2024 Feb 29]: p. 37–52. Available from: https://www.sciencedirect.com/science/article/pii/B9780128215562000098 https://doi.org/10.1016/B978-0-12-821556-2.00009-8
2. Mulaw T, Wubetu M, Dessie B, Demeke G, Molla Y. Evaluation of antimalarial activity of the 80% methanolic stem bark extract of *Combretum molle* against *Plasmodium berghei* in mice. J Evid-Based Integr Med. 2019 Jan 1;24:2515690X19890866. https://doi.org/10.1177/2515690X19890866

3. Nigussie D, Davey G, Tufa TB, Brewster M, Legesse BA, Fekadu A, et al. Antibacterial and antifungal activities of Ethiopian medicinal plants: A systematic review. Front Pharmacol [Internet]. 2021 [cited 2022 Oct 21];12. Available from: https://www.frontiersin.org/articles/10.3389/fphar.2021.633921 https://doi.org/10.3389/fphar.2021.633921
4. Ugboko HU, Nwinyi OC, Oranusi SU, Fatoki TH, Omonhinmin CA. Antimicrobial importance of medicinal plants in Nigeria. Sci World J. 2020 Sep 22;2020:e7059323. https://doi.org/10.1155/2020/7059323
5. Riaz M, Khalid R, Afzal M, Anjum F, Fatima H, Zia S, et al. Phytobioactive compounds as therapeutic agents for human diseases: A review. Food Sci Nutr. 2023 Jun;11(6):2500–29. https://doi.org/10.1002/fsn3.3308
6. Pinto T, Aires A, Cosme F, Bacelar E, Morais MC, Oliveira I, et al. Bioactive (poly)phenols, volatile compounds from vegetables, medicinal and aromatic plants. Foods. 2021 Jan;10(1):106. https://doi.org/10.3390/foods10010106
7. Mguni S, Mashinya F, Khabo-Mmekoa C, Shai LJ. A review of *Zanthoxylum chalybeum* Engl: Ethnomedicinal uses, pharmacology, phytochemistry and toxicology. J Med Plants Econ Dev. 2023 Sep 29;7(1):10. https://doi.org/10.4102/jomped.v7i1.202
8. Mbinile SD, Munishi LK, Ngondya IB, Ndakidemi PA. Conservation and management challenges facing a medicinal plant *Zanthoxylum chalybeum* in Simanjiro area, Northern Tanzania. Sustainability. 2020 Jan;12(10):4140. https://doi.org/10.3390/su12104140
9. Adia MM, Emami SN, Byamukama R, Faye I, Borg-Karlson AK. Antiplasmodial activity and phytochemical analysis of extracts from selected Ugandan medicinal plants. J Ethnopharmacol. 2016 Jun 20;186:14–19. https://doi.org/10.1016/j.jep.2016.03.047
10. Nyirenda CPM. The conservation biology of *Berberis holstii* Engl. in Nyika National Park, Malawi [Internet] [PhD Thesis]. Plymouth; 2008. http://hdl.handle.net/10026.1/1625
11. Ernst M, Grace OM, Saslis-Lagoudakis CH, Nilsson N, Simonsen HT, Rønsted N. Global medicinal uses of *Euphorbia* L. (Euphorbiaceae). J Ethnopharmacol. 2015 Dec 24;176:90–101. https://doi.org/10.1016/j.jep.2015.10.025
12. Okpako IO, Ng'ong'a FA, Kyama CM, Njeru SN. Antiproliferative activity of ethyl acetate fraction of *Euphorbia ingens* against prostate cancer cell line: An *in silico* and *in vitro* analysis. Sci Afr. 2023 Nov 1;22:e01943. https://doi.org/10.1016/j.sciaf.2023.e01943
13. Masoko P, Masiphephethu MV. Phytochemical investigation, antioxidant and antimycobacterial activities of *Schkuhria pinnata* (Lam.) Thell. extracts against *Mycobacterium smegmatis*. J Evid-Based Integr Med. 2019 Jan 1;24:2515690X19866104. https://doi.org/10.1177/2515690X19866104
14. Pokhrel T, Shrestha D, Dhakal K, Yadav PM, Adhikari A. Comparative analysis of the antioxidant and antidiabetic potential of *Nelumbo nucifera* Gaertn. and *Nymphaea lotus* L. var. *pubescens* (Willd.). J Chem. 2022 Mar 16;2022:e4258124. https://doi.org/10.1155/2022/4258124
15. Xiao X, Xu L, Hu H, Yang Y, Zhang X, Peng Y, et al. DPPH radical scavenging and postprandial hyperglycemia inhibition activities and flavonoid composition analysis of hawk tea by UPLC-DAD and UPLC-Q/TOF MSE. Mol J Synth Chem Nat Prod Chem [Internet]. 2017 Oct [cited 2023 Aug 15];22(10). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6151479/ https://doi.org/10.3390/molecules22101622
16. Xiao F, Xu T, Lu B, Liu R. Guidelines for antioxidant assays for food components. Food Front. 2020;1(1):60–69. https://doi.org/10.1002/fft2.10
17. Kosakowska O, Bączek K, Przybył JL, Pióro-Jabrucka E, Czupa W, Synowiec A, et al. Antioxidant and antibacterial activity of roseroot (*Rhodiola rosea* L.) dry extracts. Molecules. 2018 Jul;23(7):1767. https://doi.org/10.3390/molecules23071767
18. Bouslamti M, El Barnossi A, Kara M, Alotaibi BS, Al Kamaly O, Assouguem A, et al. Total polyphenols content, antioxidant and antimicrobial activities of leaves of *Solanum elaeagnifolium* Cav. from Morocco. Molecules. 2022 Jan;27(13):4322. https://doi.org/10.3390/molecules27134322
19. Debalke D, Birhan M, Kinubeh A, Yayeh M. Assessments of antibacterial effects of aqueous-ethanolic extracts of *Sida rhombifolia*'s aerial part. Sci World J. 2018 Dec 18;2018:e8429809. https://doi.org/10.1155/2018/8429809
20. R core team. R: A language and environment for statistical computing_. R Foundation for Statistical Computing [Internet]. Vienna, Austria; 2023 [cited 2023 Dec 15]. https://www.R-project.org/.
21. Prasathkumar M, Raja K, Vasanth K, Khusro A, Sadhasivam S, Sahibzada MUK, et al. Phytochemical screening and *in vitro* antibacterial, antioxidant, anti-inflammatory, anti-diabetic and wound healing attributes of *Senna auriculata* (L.) Roxb. leaves. Arab J Chem. 2021 Sep 1;14(9):103345. https://doi.org/10.1016/j.arabjc.2021.103345
22. Nasiruddin U, Chen G, Li X, Minghui J, Masood T, Safir W, et al. Comparison of phytochemical constituents and pharmacological activities of various solvent extracts obtained from *Millettia speciosa* stem powder. BioMed Res Int. 2022 Nov 16;2022:e2486979. https://doi.org/10.1155/2022/2486979
23. Phuyal N, Jha PK, Raturi PP, Rajbhandary S. Total phenolic, flavonoid contents and antioxidant activities of fruit, seed and bark extracts of *Zanthoxylum armatum* DC. Sci World J. 2020 Jan;2020(1):8780704. https://doi.org/10.1155/2020/8780704
24. Li Y, Li P, Yang K, He Q, Wang Y, Sun Y, et al. Impact of drying methods on phenolic components and antioxidant activity of sea buckthorn (*Hippophae rhamnoides* L.) berries from different varieties in China. Molecules. 2021 Jan;26(23):7189. https://doi.org/10.3390/molecules26237189
25. Kaigongi MM, Lukhoba CW, Musila FM, Taylor M, Mbugua RW, Githiomi J, et al. A versatile untargeted metabolomics-driven technology for rapid phytochemical profiling of stem barks of *Zanthoxylum* species with antioxidant and antimicrobial activities. Adv Tradit Med. 2023 Sep 1;23(3):929–45. https://doi.org/10.1007/s13596-022-00676-w
26. Nantongo JS, Odoi JB, Abigaba G, Gwali S. Variability of phenolic and alkaloid content in different plant parts of *Carissa edulis* Vahl and *Zanthoxylum chalybeum* Engl. BMC Res Notes. 2018 Dec;11(1):1–5. https://doi.org/10.1186/s13104-018-3238-4
27. Hmamou A, Eloutassi N, Alshawwa SZ, Al kamaly O, Kara M, Bendaoud A, et al. Total phenolic content and antioxidant and antimicrobial activities of *Papaver rhoeas* L. organ extracts growing in Taounate region, Morocco. Molecules. 2022 Jan;27(3):854. https://doi.org/10.3390/molecules27030854
28. Khatri D, Chhetri SBB. Reducing sugar, total phenolic content and antioxidant potential of Nepalese plants. BioMed Res Int. 2020 Nov 16;2020:e7296859. https://doi.org/10.1155/2020/7296859
29. Dżugan M, Tomczyk M, Sowa P, Grabek-Lejko D. Antioxidant activity as biomarker of honey variety. Molecules. 2018 Aug;23(8):2069. https://doi.org/10.3390/molecules23082069
30. Mokgehle SN, Tesfay SZ, Makgato MJ, Araya HT. Phytochemical profiling and soluble sugars of African ginger (*Siphonochilus aethiopicus*) from different growing regions in South Africa. South Afr J Plant Soil. 2019 Jun;36(3):157–63. https://doi.org/10.1080/02571862.2018.1548658
31. Spiegel M, Kapusta K, Kołodziejczyk W, Saloni J, Żbikowska B, Hill GA, et al. Antioxidant activity of selected phenolic acids–

ferric reducing antioxidant power assay and QSAR analysis of the structural features. Molecules. 2020 Jan;25(13):3088. https://doi.org/10.3390/molecules25133088

32. Ranabhat K, Regmi KP, Parajuli S, Thapa R, Timilsina AP, Katuwal S, et al. Evaluation of antioxidant, antimicrobial and cytotoxic activities and correlation with phytoconstituents in some medicinal plants of Nepal. J Chem. 2022 Nov 18;2022:e4725801. https://doi.org/10.1155/2022/4725801

33. Unuofin JO, Otunola GA, Afolayan AJ. Polyphenolic content, antioxidant and antimicrobial activities of *Vernonia mespilifolia* Less. used in folk medicine in the Eastern Cape Province, South Africa. J Evid-Based Integr Med. 2018 Jan;23:2515690X18773990. https://doi.org/10.1177/2515690X18773990