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Chemical composition, antioxidant potential and antimicrobial activities of *Ixora scheffleri* subspecies *keniensis* essential oil

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Background: Essential oil (EO) obtained from *Ixora scheffleri* subspecies (subsp.) *keniensis* has valuable biological properties and can play a possible therapeutic role because of its antimicrobial and antioxidant nature.

Aim: In this article, the chemical constituents, antimicrobial activity and antioxidant capacity of the EO obtained from *Ixora scheffleri* subsp. *keniensis* were determined using standard procedures.

Setting: Plant material collection, essential oils extractions and antimicrobial assays were performed at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Department of Botany Laboratories. Gas Chromatography Mass Spectrophotometry (GC/MS) analysis was carried out at the Physical Science Laboratory, MacEwan University, Canada.

Methods: The chemical constituents were determined using GC/MS. The total phenol content was evaluated using the Folin–Ciocalteu procedure. The antioxidant activity was determined using 2,2-diphenyl-1- picrylhydrazyl (DPPH) and β-carotene linoleic acid assays. The antimicrobial assay was performed using the disc diffusion method.

Results: The total phenol content of the EO was estimated to be 91.6 $\mu g/mg \pm 0.5\ \mu g/mg$. The antioxidant activity of EO obtained from *Ixora* relative to ascorbic acid was found to be 52% using the β-carotene linoleic acid assay. The DPPH scavenging activity of 30 $\mu g/mL$ EO obtained from *Ixora* and 30 $\mu g/mL$ ascorbic acid was 81.65% and 97.0%, respectively. The EO was found to have significant susceptibility against *Escherichia coli*, and intermediate susceptibility against *Candida albicans*. However, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* showed complete resistance.

Conclusion: The *in vitro* chemical compositional analysis of EO obtained from *Ixora scheffleri* subsp. *keniensis* confirms the presence of phenolic compounds attesting to the antioxidant properties and antimicrobial properties.

Keywords: *Ixora scheffleri*; essential oils; phenolic compounds; antioxidant; antimicrobial.

Introduction

Ethnopharmacological surveys show that there has been a tremendous demand for the use of indigenous medicinal plant extracts as health enhancers and herbal therapies. Essential oils (EOs) obtained from plant extracts contain chemical compounds, such as vitamins, flavonoids, polyphenols, flavones, isoflavones, flavonoids anthocyanin, coumarin lignans, catechins and isocatechins, which are believed to have useful antioxidant and therapeutic properties (Antolovich et al. 2000; Mollica et al. 2016). Antioxidants are unique compounds having the ability to trap highly reactive free radicals and reactive oxygen species (ROS). These free radicals and ROS may oxidise nucleic acids, proteins, lipids or deoxyribonucleic acid (DNA) and can initiate degenerative diseases such as atherosclerosis, stroke, diabetes, Alzheimers, and cancer in humans (Ushio-Fukai & Nakamura 2008). Antioxidants are believed to be prophylactic for the mentioned diseases. Living cells possess an inherent ROS scavenging mechanism, but this becomes inefficient with age and under environmental stresses; hence, dietary supplementation with synthetic antioxidants is necessary (Barros et al. 2011). There are, however, toxicological and mutagenic concerns related to synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in foods and this has exacerbated the need to source and screen for naturally occurring antioxidants (Amiri 2014; Gholivand et al. 2010; Peschel et al. 2006).

Several methodologies have been used to monitor and compare the antioxidant activity of foods, natural and synthetic compounds and extracts of various natural sources including medicinal herbs (Antolovich et al. 2002; Politeo, Jukic & Milos 2006; Terpinc, Bezjak & Abramovic 2009; Topal et al. 2016). All these various methods used to measure antioxidant activity can give varying results depending on the specificity of the free radical being used as a reactant and as such it is ideal to use a number of methods in combination for more representative and comparative outcomes.

Herbal drugs made from medicinal plants have been used from ancient times to treat various diseases and their antimicrobial properties make them a rich source of many potent drugs (Srivastava, Lambert & Vietmeyer 2005). Plants with medicinal value produce phytochemicals that have antimicrobial activity. In the last few decades, many bacterial organisms have continued to show increasing resistance to current antimicrobial agents (Nascimento et al. 2000). Therefore, more plant extracts need to be screened for useful antimicrobial components of therapeutic value. Currently, there is very little information available on this aspect for the *Ixora scheffleri* subspecies (subsp.) *keniensis* EO extract. The selected bacterial pathogenic members used for this study are associated with various infections in humans while *Candida albicans* is a yeast fungus that can cause systemic infections in immune-compromised individuals.

Ixora, belonging to the family Rubiaceae, is a large rain forest genus comprising shrubs and small trees. Estimates of species number range between 300 and 400 (Bridson 1988). Two sub species of *I. scheffleri* have been identified: *I. scheffleri* subsp. *keniensis* and *I. scheffleri* subsp. *scheffleri. I. scheffleri* subsp. *keniensis* is a small tree (Figure 1), up to 8 m tall with opposite long narrow leaves. Flowers are whitish-pink with a long corolla tube. The *I. scheffleri* subsp. *keniensis* plant (called

Source: Photo taken by author Peter K. Njenga.
FIGURE 1: The *Ixora scheffleri* subspecies *keniensis* plant.

mutichuma or Muthioya (kikuyu) is rare, vulnerable and endangered (Beentje, Adamson & Bhanderi 1994; Bridson 1988). The reason for this status is that logging has seriously depleted the camphor forests within the Mt Kenya forest reserve, Kenya to which the plant is endemic. This tree has very dense wood which produces a special charcoal for smelting (Gachathi 2007). Its designation in the Red list category and criteria is CR B1 + 2 (International Union for Conservation of Nature [IUCN] 2004). A group of botanists from Jomo Kenyatta University of Agriculture and Technology (JKUAT) discovered about 17 plants in 1999 in the Mt Kenya region and embarked on successful tissue culture studies of this species. The botanists have been able to reintroduce the plant in a number of areas in Kenya and are actively involved in its cultivation and conservation.

The aim of this study was to evaluate the chemical composition, antimicrobial activities and antioxidant properties of EO from the leaves of *I. scheffleri* subsp. *keniensis.*

Materials and methods
Materials
Plant material
Leaves were gathered from *I. scheffleri* subsp. *keniensis* originally planted for the study in the botanical garden of JKUAT, Thika Central Kenya. A voucher specimen number 3000 has been deposited at the herbarium at JKUAT's Department of Botany for future reference.

Solvents and chemicals
Methanol (American Chemical Society [ACS] grade), chloroform (ACS grade), linoleic acid, ascorbic acid, *β*-carotene, 2,2-diphenyl-1-picrylhydrazyl (DPPH, 95%), dimethyl sulphoxide (DMSO), anhydrous sodium sulphate, sodium carbonate, Folin–Ciocalteu reagent, gallic acid, Tween 40, deionised water (DI) and Whatman filter paper no. 3, 6-mm diameter were purchased from Sigma-Aldrich, Oakville, Ontario, Canada. The microbes including, *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 2785), *C. albicans* (ATCC 90028) were all obtained from the Department of Botany at JKUAT, Juja, Kenya.

Extraction of essential oil
Fifty grams of *I. scheffleri* subsp. *keniensis* leaves were pulverised and then steam distilled for 6 hours using a Clevenger-type apparatus as recommended by British pharmacopoeia, 1988 to yield 0.77% weight/volume (w/v) waxy EO. The obtained EO was dried over anhydrous sodium sulphate and then stored in sealed vials at +4 °C prior to chemical composition and antimicrobial activity analysis. For antioxidant determination assays, stock solutions of samples were prepared by dissolving 50 mg of the EO extract in 25 mL of respective solvents, to obtain a final concentration of 2 μg/μL. From this stock solution, different volumes of samples were taken for various

experiments. The antimicrobial activity stock sample was prepared in a concentration of 100 mg/mL in DMSO.

Gas chromatography mass spectrometry analysis

Gas chromatography mass spectrometry analyses were performed on an Agilent capillary gas chromatograph directly coupled to the mass spectrometer system (model GC). A HP-5MS non-polar fused silica capillary column (60 m × 0.25 mm, 0.5 μm film thickness) was used under the following conditions: oven temperature programmed from 50 °C (6 minutes) to 250 °C at 3 °C/min and the final temperature retained for 20 min; injector temperature, 250 °C; helium carrier gas, flow rate 10 mL/min; the volume of the injected sample was 1 μL of oil diluted in hexane; splitless injection technique; ionisation energy 70 eV, in the electronic ionisation mode; ion-source temperature 200 °C; a scan mass range of mass-to-charge ratio (m/z) 30–450 and interface temperature 300 °C. The constituents of EOs were identified based on their Kovats index, calculated in relation to the retention times of a series of linear alkanes (C4–C28), in comparison with Kovats index values of reference products of the appropriate chemical components gathered based on the Adams table (Derwich et al. 2009; Politeo et al. 2006; Vemin, Lageot & Parkanyi 1998). Identification was also realised by comparing their mass spectra with those gathered in a library of National Institute of Standards and Technology (NIST)-Mass Spectrum (MS) type and with those reported in the literature.

Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl assay

The DPPH colorimetric assay was conducted as described in literature (Akowuah et al. 2005; Okoh, Sadimenko & Afolayan 2011; Terpinc et al. 2009) to test the ability of *I. scheffleri* subsp. *keniensis* EOs to act as free radical scavengers or hydrogen donors. Briefly, 1.7 mL of 0.5 mM DPPH solution in methanol was added to a set of six vials of different concentrations (0 μg/mL, 10 μg/mL, 20 μg/mL, 30 μg/mL, 40 μg/mL, 50 μg/mL, 60 μg/mL, 70 μg/mL and 80 μg/mL) of EO obtained from *I. scheffleri* subsp. *keniensis* prepared using methanol as a solvent. The mixtures were thoroughly mixed and left in the dark at room temperature for 60 min. The absorbance of each solution was measured at 517 nanometer (nm) using a Perkin-Elmer Lambda 45 spectrophotometer. The procedure was repeated with the same varying concentrations of ascorbic acid (0 μg/mL – 80 μg/mL) in methanol as a reference antioxidant standard. Each sample was measured in triplicate and averaged. The free radical scavenging activity (FRSA %) was calculated using the formula:

$$\text{FRSA \%} = \left[\left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100 \right] \qquad \text{[Eqn 1]}$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound (Hatano et al. 1988). The sample concentration required to reduce the absorbance of DPPH by 50% (IC$_{50}$) was determined by plotting inhibition

percentages against the concentration of the sample. A lower IC$_{50}$ value indicates a greater antioxidant potency.

β-carotene linoleic acid assay

Briefly, 500 μL of β-carotene solution (1 mg/mL in chloroform), 1 mL of linoleic acid solution (10 mg/mL in chloroform) and 333 μL of Tween 40 solution (300 mg/mL in chloroform) were combined in a 100 mL flask with vigorous shaking. The chloroform was removed by rotary evaporation at 40 °C for 5 min. Fifty millilitres of deionized (DI) was added to the residue and mixed thoroughly to create an emulsion. Fifty grams of *I. scheffleri* subsp. *keniensis* EO samples were dissolved in 25 mL DMSO to obtain a final concentration of 2 μg/μL, and a 350-μL aliquot of the solution was added to 2.5 mL of the above-prepared reagent mixture and mixed thoroughly. The absorbance was immediately measured at 490 nm against a blank, consisting of an emulsion without β-carotene. The tubes were incubated for 2 h at 50 °C incubation and the absorbance was noted at 490 nm. The procedure was repeated with BHT as a positive control and a 350-μL methanol blank was prepared for background correction. Tests were carried out in triplicate. Relative antioxidant activities (RAA%) activity was calculated by dividing the absorbency of the sample by the absorbency of butylated hydroxytoluene (BHT) using the following equation:

%Antioxidant activity = (β-carotene content after 2 h of assay/initial beta-carotene content) × 100 [Eqn 2]

Determining the total phenol content

Total phenol content was measured using the Folin–Ciocalteu colorimetric procedure described previously (Taga, Miller & Pratt 1984), which determines how much of the test sample is necessary to repress the oxidation of the Folin–Ciocalteu reagent. A 50-μL (2 μg/μL in methanol) aliquot of *I. scheffleri* subsp. *keniensis* EO was mixed with 0.45 mL of DI and 2.5 mL of 0.2 N Folin–Ciocalteu reagent. After 5 min, 2 mL of saturated sodium carbonate (75 g/L) was added. The solution was incubated for 90 min at 30 °C with intermittent shaking. The absorbance was measured at 765 nm after dilution (at 3 parts sample to 57 parts DI water). A standard curve was established for the assay using gallic acid in 80% methanol. The same procedure was repeated for all the standard gallic acid solutions (0, 50, 100, 150, 200 and 250 mg/25 mL solutions of gallic acid in 80% methanol) and a standard curve equation was found:

Absorbance = 0.0002 × gallic acid (mg) + 0.0035 [Eqn 3]

The total phenolic content of the extract as gallic acid equivalents were determined by entering the absorbance value of the sample reaction into the above calibrated equation to find mg gallic acid equivalents. This was further used to calculate mg gallic acid equivalents per mL of the oil. All tests were carried out in triplicate and the results were averaged, and phenolic contents as gallic acid equivalents were reported as a means of triplicate determinations.

Antimicrobial assay methodology

Antimicrobial assay test organisms

The disk diffusion method as described by Korir et al. (2012) and Tepee, Akpulat and Sokmen (2011) was used to evaluate the antimicrobial activity of EO obtained from the leaves of *I. scheffleri* subsp. *keniensis*. The following microorganisms were used as test organisms to represent several major groups: *S. aureus* (American Type Culture Collection [ATCC] 25923), a facultative anaerobic Gram-positive coccal bacterium frequently found as part of the normal skin flora on the skin and nasal passages, *B. subtilis* (ATCC 6633), a Gram-positive obligate aerobe commonly found in soil, *E. coli* (ATCC 25922), a Gram-negative, rod-shaped bacterium commonly found in the lower intestine of endotherms, *P. aeruginosa* (ATCC 2785), a Gram-negative, aerobic rod-shaped bacterium and *C. albicans* (ATCC 90028), a diploid fungus that grows both as yeast and filamentous cells.

All the bacteria were obtained from the Department of Botany at JKUAT, Juja, Kenya and standard isolates were used. The bacteria were maintained at 4 °C on nutrient agar plates. Mueller-Hinton agar was prepared under sterile conditions as per manufactures instructions. Base plates were prepared by pouring 10 mL of the prepared sterilised Mueller-Hinton agar cooled to 45 °C into sterile Petri dishes and allowed to solidify. About 15 mL of Molten Mueller-Hinton agar kept at 45 °C was inoculated with each of the broth culture. The turbidity was then adjusted to equal the turbidity of the 0.5 McFarland standard giving a final inoculum of 1.5×10^8 colony forming units (CFU)/mL of the test bacterial suspension of the medium and poured over the base plates forming a homogenous top layer. Filter paper discs (Whatman no. 3, 6 mm diameter) were sterilised by autoclaving. *I. scheffleri* subsp. *keniensis* EOs present in vials were re-dissolved in 10% dimethyl sulfoxide (DMSO). About 10 µL of the *Ixora* extract was applied per filter paper disc. The discs were air-dried and placed onto the seeded top layer of the Mueller-Hinton agar plates. Air-dried discs saturated with 10% DMSO were used as negative controls. Filter paper discs loaded with 5 mg of Gentamycin in 10% DMSO were used as positive controls. The plates were evaluated after incubation at 37 °C for 24 h after which the zones of inhibition were measured using Vernier calipers. The size of the inhibition zone in (mm) produced by the EO was recorded and considered to express the antibacterial activity. *In vitro* antifungal activity was performed on *C. albicans* as described for bacterial assays except that Sabourands dextrose Chloramphenicol agar was used to test antifungal bioactivity and incubation was conducted at 25 °C for 120 h for yeasts and filamentous fungi. Each of these assays was analysed in triplicate. The results of the disk diffusion test were 'qualitative', in that a category of susceptibility (i.e., susceptible, intermediate or resistant) is derived from the test rather than an minimun inhibition concentration (MIC).

Ethical considerations

This article followed all ethical standards for research without direct contact with human or animal subjects.

Results and discussion

Chemical composition of the essential oils and the phenolic compounds

The hydrodistillation of the *I. scheffleri* subsp. *keniensis* leaves delivers a light yellowish oil with a yield of 0.77% (weight by weight [w/w]). Gas chromatography mass spectrometry analysis of the EO revealed that a total of 29 compounds have been identified representing around 90% of the total oil. The identified compounds have been summarised in Table 1. The major chemical constituents (> 5%) of the oil were: 2,3-dihydroxybenzaldehyde (4.5%), 6-methoxycoumaran-3-one (1.3%), ethyl 7,7-diformylhepta-2,4,6-trienoate (3.0%), 3-O-methyl-d-glucose, (5.3%), 7-quinolinol (2.0%), n-butyric acid 2-ethylhexyl ester (7.2%), benzofuran-2-carboxaldehyde (22.0%), 2,3,4-trihydroxybenzaldehyde (10.3%), hydroquinone (7.5%), 2,6-dimethyl-1,7-Octadien-3-ol (2.1%), 2,3-dihydrobenzofuran (1.0%) and 1,2-benzenediol (5.5%), 2,3-dihydroxybenzaldehyde (4.5%).

Clearly, the oil is rich in phenolic compounds and as such the obvious next step was to test the antioxidant properties of the oil.

Antioxidant activity

Figure 3 shows a graph of the DPPH scavenging activities of increasing amounts of *I. scheffleri* subsp. *keniensis* EO and ascorbic acid solution expressed as a percentage.

TABLE 1: Essential oil composition of *Ixora scheffleri* subsp. *keniensis*.

Compound	Area (%)	RI	Method of identification
Methyl-cyclopentane	1.4	-	RI, MS
1-Hydroxy-2-propanone†	1.4	-	RI, MS
2-Propanone, 1,3-dihydroxy-	0.4	-	RI, MS
Pentanol, 5-amino-	0.1	-	RI, MS
2-Cyclopenten-1-one, 2-hydroxy-	0.2	-	RI, MS
Carbamic acid, phenyl ester	0.7	-	RI, MS
2-Hydroxy-gamma-butyrolactone	0.4	-	RI, MS
γ-Guanidinobutyric acid	0.4	-	RI, MS
Cyclopropyl carbinol	0.2	-	RI, MS
2-Phenylethanol	0.7	-	RI, MS
2,3-Dihydroxybenzaldehyde†	4.5	839.2	RI, MS
2,6-Dimethyl-3,7-Octadiene-2,6-diol	0.2	866.2	RI, MS
1,2-Benzenediol†	5.5	879.9	RI, MS
2,3-Dihydro-benzofuran	1.0	897.1	RI, MS
Hydroquinone†	7.5	952.4	RI, MS
2,6-Dimethyl-1,7-Octadien-3-ol†	2.1	1005.9	RI, MS
2,6-Dimethyl- 2,7-Octadiene-1,6-diol	0.4	1012.7	RI, MS
2,3,4-Trihydroxybenzaldehyde†	10.3	1042.1	RI, MS
3-Hydroxy-5-methoxy-benzenemethanol	0.6	1060.1	RI, MS
Benzofuran-2-carboxaldehyde†	22.0	1114.5	RI, MS
1,6-Octadiene, 3-ethoxy-3,7-dimethyl-†	1.9	1168.5	RI, MS
n-Butyric acid 2-ethylhexyl ester†	7.2	1286.8	RI, MS
7-Quinolinol†	2.0	1302.7	RI, MS
d-Mannose	1.5	1343.4	RI, MS
3-O-Methyl-d-glucose†	5.3	1388.6	RI, MS
Ethyl 7,7-diformylhepta-2,4,6-trienoate†	3.0	1465.0	RI, MS
6-Methoxycoumaran-3-one†	1.3	1484.9	RI, MS

RI, retention index; MS, mass spectrum.

†, Major components that may be significant in inducing antioxidant activity.

The β-carotene-linoleate method is a hydrogen atom transfer assay where antioxidant capacity is determined by measuring the inhibition of the linoleic acid oxidation products especially the conjugated diene hydroperoxides from attacking the β-carotene, resulting in the bleaching of its characteristic yellow colour (Kulisic et al. 2004; Tepe et al. 2011; Terpinc et al. 2009). The DPPH method is not specific and applies to the overall antioxidant capacity of the sample and as such provides a good measure of the functional properties of the oil. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in colour. After reduction by an antioxidant, the colour turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of the DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H (Molyneux 2004). The reaction schematic is shown in Figure 2. The resulting decolorisation is stoichiometric with respect to the number of electrons captured.

A DPPH inhibition activity of 16.6 μg/mL ascorbic acid was determined to be 96% with no increase in inhibition activity with an increase in concentration. For the EO there was a gradual increase between 0 μg/mL and 30 μg/mL in inhibition activity reaching a maximum of about 92% with
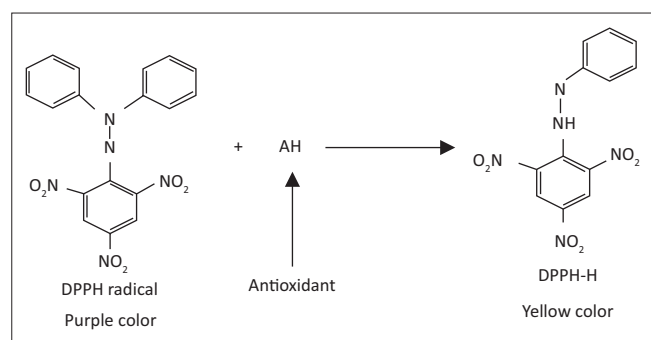
N, Nitrogen; O₂N, 5-{[(2-{[bis(4-methoxyphenyl)methyl]carbamoyl}benzyl)(prop-2-en-1-yl)amino]methyl}-1,3-benzodioxole-4-carboxylic acid; NO₂, Nitrogen dioxide; AH, Antioxidant; DPPH, 1,1-diphenyl-2-picrylhydrazyl; DPPH-H, 1-diphenyl-2-picrylhydrazyl-Hydrogen.

FIGURE 2: Hydrogen atom transfer reaction schematic of the 2,2-diphenyl-1-picrylhydrazyl free radical with an antioxidant.
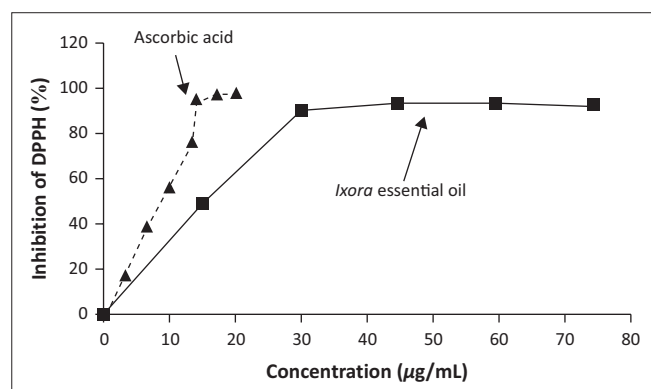
DPPH, 1,1-diphenyl-2-picrylhydrazyl.

FIGURE 3: Comparison of 2,2-diphenyl-1-picrylhydrazyl radical scavenging activities of *Ixora scheffleri* subsp. *keniensis* essential oil and ascorbic acid at different concentrations.

TABLE 2: Antimicrobial activity of essential oil of 10-μL of *Ixora scheffleri* subsp. *keniensis* essential oil using the agar diffusion method.

Microbial strain	Mean diameter zones of inhibition (mm)	Susceptibility
Bacillus subtilis	6.0 ± 0.0	Resistant
Escherichia coli	10.5 ± 0.3	Susceptible
Pseudomonas aeruginosa	6.0 ± 0.0	Resistant
Candida albicans	6.7 ± 0.0	Intermediate
Staphylococcus aureus	6.0 ± 0.0	Resistant

the addition of 30 μg/mL EO, beyond which the inhibition did not increase with concentration. The IC$_{50}$ for *I. scheffleri* subsp. *keniensis* EO was determined to be 15.1 μg/mL ± 0.1 μg/mL while that of ascorbic acid was 8.5 μg/mL ± 0.2 μg/mL, as was reported by Khalaf et al. (2008). It is notable that *I. scheffleri* subsp. *keniensis* EO has higher antioxidant activity than commonly used herbs such as *Cinnamomum zeylanicum* leaves (53 μg/mL), clove buds (36 μg/mL), jasmine absolute (1297 μg/mL) and thyme red (1563 μg/mL) (Wang, Wang & Yih 2008). Additionally, the IC$_{50}$ value for green tea was 6.7 μg/mL and for black tea it was 9.7 μg/mL as determined by Khalaf et al. (2008). The same authors determined the IC$_{50}$ value of the synthetic antioxidant BHT to be 19.8 μg/mL. The IC$_{50}$ values of other common herbs such as peppermint, spearmint and rosemary were determined by Gharib and Teixeira da Silva (2013) to be 59.19 μg/mL, 63.80 μg/mL and 62.49 μg/mL, respectively. It is clearly evident therefore that the antioxidant potency of EO from *Ixora* leaves is significantly high compared to these common herbs. The RAA% of *I. scheffleri* subsp. *keniensis* EO was found to be 52% of that of BHT.

The total phenolic content of *I. scheffleri* subsp. *keniensis* EO was found to be 91.6 μg ± 0.5 μg of GA/mg. The high content of phenolic compounds that the main active agents that can donate hydrogen to free radicals (scavenging effect) and thus break the chain reaction of lipid oxidation, is clearly indicative of the high antioxidant activity of the *Ixora* EO.

While it was clearly evident that *Ixora* EO demonstrated significant antioxidant activity as the three chemical assays tests determined, it was important to evaluate if it could have added benefits of bioactivity. As mentioned in the 'Methods' section, the bioactivity of *Ixora* EO was screened against several microbial strains and the mean diameter zones of inhibition were determined using the disc diffusion method. The antimicrobial susceptibility test results against the test organisms are summarised in Table 2. From the table, it is evident that *Ixora* EO had significant activity against *E. coli* with a mean diameter zone inhibition of 10.5 mm. The EO also had some activity against *C. albicans* (6.7 mm) and no activity (6.0 mm) against *B. subtilis*, *Pseudomonas aeruginosa* and *S. aureus.* The diameter of the disc was 6 mm and so any value above this indicated some degree of inhibition.

Conclusion

The antioxidant, antibacterial and antifungal activity of EO of *I. scheffleri* subsp. *keniensis* found in Central Kenya is

presented here for the first time and extends knowledge in the range of valuable biological activities and possible roles in therapy associated with this medicinal herb.

Results from both DPPH and *β*-carotene linoleic acid assays established that *I. scheffleri subsp. keniensis* EO possesses antioxidant and radical scavenging potency. However, the EO antioxidant properties were found to be slightly lower compared to the standard antioxidant ascorbic acid. Despite having lower activity compared to standard ascorbic acid, the antioxidant activity of *I. scheffleri* subsp. *keniensis* EO was found to be significant for potential applications in the food, cosmetic and pharmaceutical industries and could act as a potential alternative to more toxic synthetic antioxidants. Because of the toxic nature of EOs, further studies on the toxicity and other biological properties of the extract are needed prior to possible applications. Also electrochemical antioxidant activity, another good indicator of the antioxidant nature of the EOs would be a useful study. It is also notable that the EO may be a good source of bioactive compounds especially against microbes such as *E. coli* (susceptible) and against *C. albicans* fungi which showed intermittent susceptibility.

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Competing interests

The authors have declared that no competing interests exist.

Authors' contributions

P.K.N. collected the plant samples, carried out extractions and was also involved in drafting the manuscript. S.M.M. conducted the data analysis, interpretation and drafting of the manuscript.

Funding information

Data availability statement

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

References

Akowuah, G.A., Ismail, Z., Norhayati, I. & Sadikun, A., 2005, 'The effects of different extraction solvents of varying polarities on polyphenols of *Orthosiphon stamineus* and evaluation of the free radical-scavenging activity', *Food Chemistry* 93(2), 311–317. https://doi.org/10.1016/j.foodchem.2004.09.028

Amiri, H., 2014, 'Chemical composition and antioxidant activity of essential oil and methanolic extracts of *Ferula microcolea* (Boiss.) Boiss (Apiaceae)', *International Journal of Food Properties* 17(4), 722–730. https://doi.org/10.1080/10942912.2012.665403

Antolovich, M., Prenzler, P. & Patsalides, E., 2002, 'Methods for testing antioxidant activity', *Analyst* 127(1), 183–198.

Antolovich, M., Prenzler, P., Robards, K. & Ryan, D., 2000, 'Sample preparation in the analysis of phenolic compounds in fruits', *Analyst* 125(5), 989–1009. https://doi.org/10.1039/b000080i

Barros, L., Cabrita, L., Boas, M.V., Carvalho, A.M. & Ferreira, I.C.F.R., 2011, 'Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants', *Food Chemistry* 127(4), 1600–1608. https://doi.org/10.1016/j.foodchem.2011.02.024

Beentje, H.J., Adamson, J. & Bhanderi, D., 1994, *Kenya trees, shrubs and lianas*, p. 722, National Museums of Kenya, Nairobi. ISBN: 9789966986108.

Bridson, D.M., 1988, 'Ixora', in D.M. Bridson & B. Verdcourt (eds.), *Flora of East Tropical Africa*, pp. 610–617, Crown Agents Publishing, London.

Derwich, E., Benziane, Z., Boukir, A. & Benaabidate, L., 2009, 'GC-MS analysis of the leaf essential oil of *Mentha rotundifolia*, a traditional herbal medicine in Morocco', *Chemical Bulletin of Politehnica University of Timisoara* 54(68), 85–88.

Gachathi, F.N., 2007, *Kikuyu botanical dictionary: A guide to plant names, uses and cultural values*, p. 151, English Press, Kenya.

Gharib, F.A.E.L. & Teixeira da Silva, J.A., 2013, 'Composition, total phenolic content and antioxidant activity of the essential oil of four lamiaceae herbs', *Medicinal and Aromatic Plant Science and Biotechnology* 7, 19–27.

Gholivand, M.B., Rahimi-Nasrabadi, M., Batooli, H. & Ebrahimabadi, A.H., 2010, 'Chemical composition and antioxidant activities of the essential oil and methanol extracts of *Psammogeton canescens*', *Food and Chemical Toxicology* 48(1), 24–28. https://doi.org/10.1016/j.fct.2009.09.007

Hatano, T., Kagawa, H., Yasuhara, T. & Okuda, T., 1988, 'Two New Flavonoids and Other Constituents in Licore Root: Their Relative Astringency and Radical Scavenging Affects', *Chemical and Pharmaceutical Bulletin* 36(6), 2090–2097. http://dx.doi.org/10.1248/cpb.36.2090

International Union for Conservation of Nature (IUCN), 2004, *2004 IUCN red list of threatened species*, viewed 10 January 2018, from www.redlist.org.

Khalaf, N.A., Shakya, A.K., Al-Othman, A., El-Agbar, Z. & Farah, H., 2008, 'Antioxidant activity of some common plants', *Turkish Journal of Biology* 32(1), 51–55.

Korir, R., Kimani, C., Gathirwa, J. & Wambura, M., 2012, 'In-vitro antimicrobial properties of methanol extracts of three medicinal plants from Kilifi District – Kenya', *African Journal of Health Sciences* 20, 4–10.

Kulisic, T., Radonic, A., Katalinic, V. & Milos, M., 2004, 'Use of different methods for testing antioxidative activity of oregano essential oil', *Food Chemistry* 85(4), 633–640. https://doi.org/10.1016/j.foodchem.2003.07.024

Mollica, A., Locatelli, M., Macedonio, G., Carradori, S., Sobolev, A.P., De Salvador, R.F. et al., 2016, 'Microwave-assisted extraction, HPLC analysis, and inhibitory effects on carbonic anhydrase I, II, VA, and VII isoforms of 14 blueberry Italian cultivars', *Journal of Enzyme Inhibition and Medicinal Chemistry* 31(sup 4), 1–6. https://doi.org/10.1080/14756366.2016.1214951

Molyneux, P., 2004, 'The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity', *Songklanakarin Journal of Science and Technology* 26(2), 211–219.

Nascimento, G.G., Locatelli, J., Freitas, P.C. & Silva, G.L., 2000, 'Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria', *Brazilian Journal of Microbiology* 31(4), 247–256. https://doi.org/10.1590/S1517-83822000000400003

Okoh, O.O., Sadimenko, A.P. & Afolayan, A.J., 2011, 'Antioxidant activities of *Rosmarinus officinalis* L. essential oil obtained by hydro-distillation and solvent free microwave extraction', *African Journal of Biotechnology* 10(20), 4207–4211.

Peschel, W., Sanchez-Rabaneda, F., Diekmann, W., Plescher, A., Gartzia, I., Jiménez, D. et al., 2006, 'An industrial approach in the search of natural antioxidants from vegetable and fruit wastes', *Food Chemistry* 97(1), 137–150. https://doi.org/10.1016/j.foodchem.2005.03.033

Politeo, O., Jukic, M. & Milos, M., 2006, 'Chemical composition and antioxidant activity of essential oils of twelve spice plants', *Croatica Chemica Acta* 79(4), 545–552.

Srivastava, J., Lambert, J. & Vietmeyer, N., 2005, 'Medicinal plants: An expanding role in from Western India for potential antimicrobial activity', *Indian Journal of Pharmacology* 37, 406–409.

Taga, M.S., Miller, E.E. & Pratt, D.E., 1984, 'Chia seeds as a source of natural lipid antioxidants', *Journal of the American Oil Chemists' Society* 61(5), 928–931. https://doi.org/10.1007/BF02542169

Tepe, B., Akpulat, H.A. & Sokmen, M., 2011, 'Evaluation of the chemical composition and antioxidant activity of the essential oils of *Peucedanum longifolium* and *P. Palimbiooides*', *Records of Natural Products* 5, 108–116.

Terpinc, P., Bezjak, M. & Abramovic, H., 2009, 'A kinetic model for evaluation of the antioxidant activity of several rosemary extracts', *Food Chemistry* 115(2), 740–744. https://doi.org/10.1016/j.foodchem.2008.12.033

Topal, M., Gocer, H., Topal, F., Kalin, P., Köse, L.P., Gülçin, İ. et al., 2016, 'Antioxidant, antiradical, and anticholinergic properties of cynarin purified from the Illyrian thistle (*Onopordum illyricum* L.)', *Journal of Enzyme Inhibition and Medicinal Chemistry* 31(2), 266–275. https://doi.org/10.3109/14756366.2015.1018244

Ushio-Fukai, M. & Nakamura, Y., 2008, 'Reactive oxygen species and angiogenesis: NADPH oxidase as target for cancer therapy', *Cancer Letters* 266(1), 37–52. https://doi.org/10.1016/j.canlet.2008.02.044

Vemin, G., Lageot, C. & Parkanyi, C., 1998, 'GC-MS (EI, PCI, NCI, SIM, ITMS) data bank analysis of flavors and fragrances, Kovats indices', *Developments in Food Science* 39, 245–301. https://doi.org/10.1016/S0167-4501(98)80012-5

Wang, H.F., Wang, Y.K. & Yih, K.H., 2008, 'DPPH free-radical scavenging ability, total phenolic content, and chemical composition analysis of forty-five kinds of essential oils', *Journal of Cosmetic Science* 59(6), 509–522. https://doi.org/10.1111/j.1468-2494.2009.00531_5.x

Whitney, H.A.K., 1989 , Book Review: British pharmacopoeia 1988, *Sage*, 23(5), 433. https://doi.org/10.1177/106002808902300525