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In vitro antioxidant and antimicrobial activity of *Prunus africana* (Hook. f.) Kalkman (bark extracts) and *Harrisonia abyssinica* Oliv. extracts (bark extracts): A comparative study

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Background: Plants are new sources of antibacterial agents, hence the need to determine and evaluate the antibacterial properties, antioxidant activity and gas chromatography – mass spectrometer (GC-MS) profile of medicinal plants.

Methodology: In this study, sequential extraction of *Prunus africana* and *Harrisonia abyssinica* was used to obtain ethyl acetate and methanol extracts. Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH), whereas the total phenolic and total flavonoid contents were estimated using Folin-Ciocalteu and aluminium chloride, respectively. Antibacterial properties of the extracts against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* were estimated using the disc diffusion method and compared against streptomycin.

Results: Screening of crude methanolic extracts revealed the presence of secondary metabolites which was further confirmed by Fourier transform infrared (FT-IR) characterisation that revealed the presence of H-bonded OH functional groups. The extracts revealed that *P. africana* had a higher total phenolic and total flavonoid contents compared to *H. abyssinica*. Methanolic extracts of both plants had moderate activity against selected microorganisms and both inhibited DPPH radical scavenging activity. GC-MS analysis of *P. africana* and *H. abyssinica* extracts revealed the presence of several phytochemicals that have been reported to have medicinal uses. Total phenolic and flavonoid contents showed positive correlations with the DPPH radical scavenging activity and negative correlations with EC_{50}.

Conclusion: *Prunus africana* and *H. abyssinica* extracts had moderate antimicrobial properties against the selected microorganisms because of the presence of secondary metabolites.

Introduction

Antibacterial agents inhibit the growth of bacteria and may rapidly kill them by disrupting one or more of their essential cellular functions. For example, depending on the type of antibacterial agent, the mechanism of activity may result in inhibition of the production of proteins or cell wall materials, inhibition of DNA replication or disruption of cell membrane activities that maintain chemical balance (World Health Organization 2014). Moreover, bacteria have the ability to acquire resistance to one or more antibacterial agents to which they would normally be susceptible. Because of mutation during gene replication or gene encoding, bacteria can also acquire resistance (Courvallin 2008). The ease with which resistance can be acquired varies between bacterial types. Unfortunately, some strains of bacteria which are normally susceptible to antibacterial agents can also acquire resistance (Laxminarayan et al. 2013). In extreme cases, bacteria can show resistance to most, if not all, of the medicinal agents that would commonly be used to treat them (Courvallin 2008). Moreover, 70% of the world's HIV and/or AIDS are found in sub-Saharan Africa where oral candidiasis is a very common occurrence amongst these patients, which makes the management of candida infections challenging. This can be attributed to the limited number of antifungal agents, increased resistance against antifungal agents, relapse of candida infections and toxicity of the available antifungal agents among many other challenges (Runyoro et al. 2006). Natural products derived from plants may offer potential lead to a class of new compounds. The search for antimicrobial agents has accelerated in recent years because of the increase in antimicrobial resistance (Lim 2012). Currently, 25% – 50% of active pharmaceutical ingredients are derived from plants, yet none are used as antimicrobials. Plant extracts have traditionally been used by traditional medical practitioners to prevent or treat infectious diseases; thus, Western medication is trying to duplicate their successes.

Secondary metabolites such as tannins, phenols, terpenoids, alkaloids and flavonoids which are derived from plants have been found to have in vivo antimicrobial properties (Cowan 1999).

The plant commonly called African cherry, *Prunus africana*, is an evergreen canopy tree species typically reaching 25 m–30 m in height and occurs primarily in montane and middle elevation forests (Fashing 2004). Its bark is commonly administered to treat fevers, wound management, stomach pain, malaria, arrow poisoning, kidney disease, as an appetite stimulant, and to treat insanity and gonorrhoea (Stewart 2003). *Prunus africana* (Hoolh f.) Kalkman is a useful timber tree, and the bark is used for liver problems and constipation. *Prunus africana* extracts have been reported to have antibacterial and antifungal activity (Mwitari et al. 2013). In a study to determine bioactive constituents in *P. africana*, Kadu et al. (2012) found that the extracts contained lauric acid, myristic acid, n-docosanol, ferulic acid, sitostenone, sitosterol and ursolic acid. Because of its medicinal properties that have been exploited over the last 35 years, it is one of the most over-exploited plants on the continent (Stewart 2003). *Harrisonia abyssinica,* which is a native plant to east, central and southern Africa, grows to approximately 6 m tall. It is commonly used in traditional medicine for the treatment of menstrual problems, stomach pains and infertility. The tree has been reported to contain antibacterial, antifungal, antimalarial, anticancer and antiviral activities (Balde et al. 1995). In a study to determine its activity against *Candida albicans*, Runyoro et al. (2006) found that methanolic extracts of *H. abyssinica* had an average moderate activity against *C. albicans* of 10 mm. In a similar study, Bene et al. (2017) reported that the aqueous and ethanolic extracts of *H. abyssinica* had good antioxidant activity, with ethanolic extracts having the highest activity.

Plants have been known to be a major source of therapeutic agents; hence, there is need to determine the phytochemical constituents of medicinal plants that have been reported to be used in ethno medicine. This study was carried out to determine phytochemical constituent, antibacterial properties, antioxidant activity and GC-MS profile of *P. africana* and *H. abyssinica* extracts.

Materials and methods

Sample preparation

Prunus africana and *H. abyssinica* plant samples were collected from Chuka, Meru-South District, Tharaka Nithi County. The samples were then transported to Jomo Kenyatta University of Agriculture and Technology where they were identified with the help of a taxonomist from the Department of Botany, and voucher specimens were kept in Botany Department Herbarium. The plant samples were washed in water, chopped and dried under shade for three weeks. The dried samples were then ground into fine powder using a mechanical grinder (locally assembled, no model number).

Extraction of plant material

Cold sequential extraction was carried out using ethyl acetate and methanol as extracting solvents to obtain respective extracts. Cold extraction was carried out by weighing 100 g of the fine powders of every plant sample and macerating in 1000 mL ethyl acetate. After ethyl acetate extraction, the plant samples were dried in an oven, and then methanol was used to obtain methanolic extracts. The extracts were filtered using Whatman Filter Paper No. 1 (Whatman international, England) and concentrated using a Rota evaporator (BUCHI R 200) at 40ºC. The crude extracts were then left to dry in the fume chamber, after which they were stored at 4°C awaiting analysis.

Secondary metabolite screening of plant extracts

The screening for secondary metabolites was carried out qualitatively on the ethyl acetate and methanol extracts using standard established procedures for identifying plant constituents as described by Harborne (1998). An aliquot of every plant extract was analysed for the presence of saponins, alkaloids, terpenoids, flavonoids and tannins.

Characterisation of plant extracts

The plant extracts were characterised using a Shimadzu Fourier transform infrared spectrometer, Model FTS-8000. The KBr pellets of samples were prepared by mixing finely grounded 10 mg of the samples with 250 mg KBr (FT-IR grade). The 13 mm KBr pellets were prepared in a standard device under a pressure of 75 kN cm^{-2} for 3 min. The spectral resolution was set at 4 cm^{-1} and the scanning range from 400 cm^{-1} to 4000 cm^{-1}.

Gas chromatography – mass spectrometer analysis of *Prunus africana* and *Harrisonia abyssinica* extracts

An amount of 5 g of the powdered plant samples was extracted with acetonitrile and then solvent exchanged with 2, 2, 4-trimethylpentane before GC-MS analysis. Gas chromatographic analysis was carried out on Agilent 5975 GC-MS operating in EI mode at 70 eV. A capillary column of 30 m × 0.25 mm (id) was used, and helium gas was used as a carrier gas with flow rate of 1.2 mL/min and oven temperature of 60°C. Various compounds were identified by their retention time and NIST library search.

DPPH radical scavenging activity

DPPH radical scavenging was used to determine the antioxidant activity of the methanolic extracts of *P. africana* and *H. abyssinica*. Different concentrations (0.109 mM–3.5 mM) of crude methanolic extracts were prepared to make 100 µL. Five millilitres of 0.1 mM methanolic solution of DPPH was then added into the extracts and incubated for 20 min at room temperature for 20 min. A Shimadzu 1800 UV–VIS spectrometer was used to determine the quantity of plant extracts needed to reduce the initial DPPH concentration by 50% (Ramamoorthy & Bono 2007).

This characteristic parameter is called efficient concentration (EC_{50}) or oxidation index. The lower the EC, the higher the antioxidant activity of the examined plant extract. The DPPH radical scavenging activity in terms of percentage was calculated using the Equation 1 (Baba & Malik 2014):

$$\text{DPPH scavenging activity}\,(\%) = 1 - \frac{Abs_{517}\,sample}{Abs_{517}\,DPPH\ solution} \times 100\% \quad \text{[Eqn 1]}$$

Total phenolic content

The total phenolic content of the extracts was determined using Folin–Ciocalteu (Baba & Malik 2015; Mburu et al. 2016). Five grams per 50 mL of sample was filtered with Whatman no.1 filter paper. Then, 0.5 mL of the sample was added to 2.5 mL of 0.2 N Folin–Ciocalteu reagent and incubated for 5 min; 2 mL of 75 g/L of Na_2CO_3 was then added and the total volume made to 25 mL using distilled water. The above solution was then kept for incubation at room temperature for 2 h (Ramamoorthy & Bono 2007). Absorbance was read at 760 nm using a Shimadzu 1800 UV–VIS spectrometer. Tannic acid (0 mg/L–800 mg/L) was used to produce a standard calibration curve. The total phenolic content was expressed in milligrams of tannic acid equivalents (TAE)/gram of extract (Baba & Malik 2015; Mburu et al. 2016).

Total flavonoid content

Total flavonoid content was determined using aluminium chloride (Baba & Malik 2015; Mburu et al. 2016). Five millilitres of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution (0.4 mg/mL). Absorption readings at 415 nm using Shimadzu 1800 UV–VIS spectrometer were read after 10 min against a blank sample consisting of 5 mL extract solution with 5 mL methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with catechin (0 mg/L–100 mg/L) as the standard. Total flavonoid content was expressed as milligrams of catechin equivalents (CE)/gram of extract (Mburu et al. 2016).

Disc diffusion assay

The plant extracts were screened for their activity against *Staphylococcus aureus* (ATCC-25923), *Bacillus subtilis*, *Pseudomonas aeruginosa* (ATCC-27853), *Escherichia coli* (ATCC-25922) and *C. albicans* (ATCC 90028) using disc diffusion method (Zaidan et al. 2005). Inoculum suspension was spread over the nutrient agar surface by sterile collection swab, and 6 mm discs sterilised at 120°C for about 15 min were then loaded with streptomycin (20 mg/mL) as positive controls. The paper discs were prepared by dipping Whatman filter paper in plant extracts and placed onto the surface of inoculated plates with flamed forceps. The plates were labelled and incubated at 37°C for 24 h. The zones of inhibition (ZOI) were measured using a ruler in millimetres around the disc and interpreted as low activity (1 mm–6 mm), moderate activity (7 mm–10 mm), high activity (11 mm–15 mm), very high activity (16 mm–) and no activity (–) (Zaidan et al. 2005).

Results and discussions

Phytochemical screening

Antimicrobial properties of plants have been linked to the presence of biologically active secondary metabolites in plants. Screening for secondary metabolites present in *P. africana* and *H. abyssinica* extracts revealed the presence of alkaloids, saponins, terpenoids, tannins and phenols (Table 1).

Characterisation of crude extracts

The FT-IR spectrometer data for the crude extracts revealed the presence of multiple functional groups in the extracts (Figure 1 and Figure 2). FT-IR spectra of the extracts confirmed the presence of various bioactive functional groups such as OH, NH, CHO, COOH and –COOR (Tables 2 and 3). A broad absorption peak at around 3207.4 cm^{-1} was attributed to the presence of hydrogen bonded -OH stretching (Poojary, Vishnumurthy & Adhikari 2015).

Gas chromatography – mass spectrometer analysis of *Prunus africana* and *Harrisonia abyssinica* crude extracts

Knowledge of chemical constituents of plants is essential because such information is important for the possible synthesis of these chemical substances. From the GC-MS results obtained in this study (Tables 4 and 5), crude extracts of *P. africana* and *H. abyssinica* revealed the presence of phytochemicals that have been reported in literature (Figure 3) (Kumar, Kumaravel & Lalitha 2010). The results showed the presence of stigmast-4-en-3-one (Alexander-Lindo, Morrison & Nair 2004), ergosta-4,6,8(14),22-tetraen-3-one (Nguyen et al. 2015), squalene (Rudzinska et al. 2017), 4,8,12,16-tetramethylheptadecan-4-olide (Gobalakrishnan, Manikandan & Bhuvaneswari 2014), 9,12-octadecanoic acid (Z,Z) (Abubakar & Majinda 2016; Vijayakumar & Sumathi 2016), 10,13-tetradecanoic acid methyl ester (Abubakar & Majinda 2016), thymol (Figure 4), phthalic acid cyclobutyl

TABLE 1: Phytochemical screening of crude extracts of *Prunus africana* and *Harrisonia abyssinica.*

Plant species	Saponins	Alkaloids	Terpenoids	Flavonoids	Tannins
P. africana	+	+	++	++	++
H. abyssinica	+	++	++	+	+

+, Present; ++, Present in high concentration.

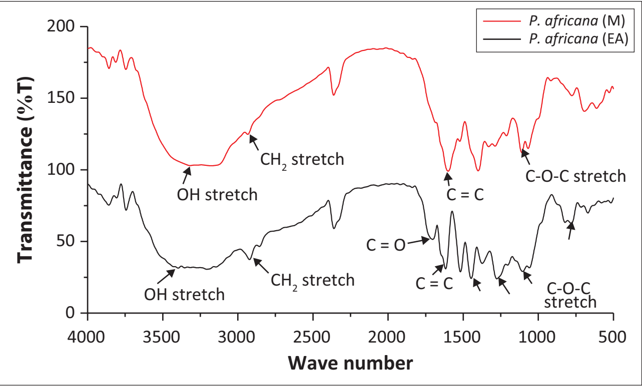

FIGURE 1: Fourier transform infrared spectra of *Prunus africana* extracts.

isobutyl ester (Sigh et al. 2006), 5-hydroxyl-7-methoxy-2-methyl 4H-1-benzopyran-4-one, 2(4H)-benzofuranone, 5,6,7,7a-trimethyl-,(R) (Figure 5) (Abarca-Vargas, Pena Malacara & Petricevich 2016), 1, 3-dioxolane-2-heptanenitrile (Yue et al. 2017), a-methyl-e-oxo-2-phenyl (Ramakrishnan & Venkataraman 2011), pentanedioic acid monoester (Figure 6), O-methyl (+)-a-tocopherol (Takeoka & Dao 2003), ethyl oleate (Mahmood, Ahmed & Kosar 2009), hexadecanoic acid methyl ester (Sharma, Satpathy & Gupta 2014), phosphonic acid, methyl-, bis (trimethylsilyl) ester (Liu et al. 2007) and 9-octadecanoic acid methyl ester (Abubakar & Majinda 2016). Some of these compounds have been reported to have medicinal properties (Table 4). 9,12,15-Octadecanoic acid (Z,Z) belonging to linoleic acid group has been reported to act as anti-inflammatory, hypocholesterolemic, antihistaminic, anti-arthritic and antimicrobial agent. O-methyl (+)-a-tocopherol was also identified in the extracts, and it belongs to vitamin E which has antioxidant activity and anti-inflammatory activity (Reiter, Jiang & Christen 2007) and is able to control neutrophil oxidative bursts (Gobalakrishnan et al. 2014).

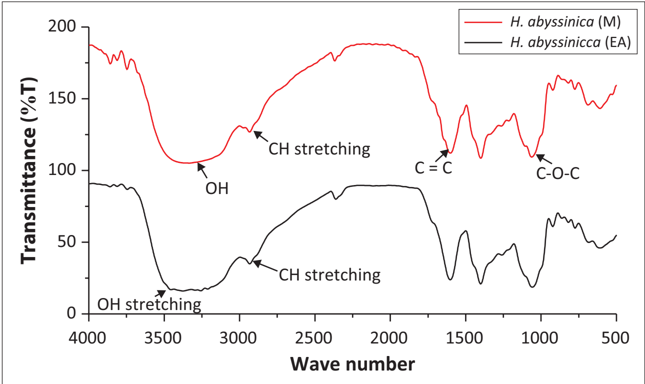

FIGURE 2: Fourier transform infrared spectra of *Harrisonia abyssinica* extracts.

TABLE 2: Major peaks in Fourier transform infrared spectra of *Prunus africana* bark extracts.

Peak	Functional group	Probable phytochemical
3207.4	OH alcohol or phenol	Alkaloids, flavonoids, polyphenol, saponins, tannins
2922.0	CH_2 alkyl stretch	Alkaloids, flavonoids, polyphenol, saponins, tannins
1703.0	C = O ester stretch	Alkaloids, flavonoids, polyphenol, saponins, tannins
1618.2	Alkenyl C = C stretch	Alkaloids, flavonoids, polyphenol, saponins, tannins
1446.5	CH bending	Alkaloids, flavonoids, polyphenol, saponins, tannins
1276.8	Esters	Alkaloids, flavonoids, polyphenol, saponins, tannins
1060.8	C-O-C	Alkaloids, flavonoids, polyphenol, saponins, tannins

TABLE 3: Major peaks on Fourier transform infrared spectra of *Harrisonia abyssinica* ethanolic extracts.

Peak	Functional group	Probable phytochemical
3332.8	OH stretch	Alkaloids, flavonoids, polyphenol, saponins, tannins
2933.5	CH_2 stretch	Alkaloids, flavonoids, polyphenol, saponins, tannins
1602.7	C = C stretch	Alkaloids, flavonoids, polyphenol, saponins, tannins
1402.2	O – H bending	Alkaloids, flavonoids, polyphenol, saponins, tannins
1064.6	C – O stretch	Alkaloids, flavonoids, polyphenol, saponins, tannins

Antioxidant activity of the crude extracts

Radical scavenging activity (DPPH assay)

The radical scavenging activity of the crude methanolic extracts of *P. africana* and *H. abyssinica* are depicted in Figure 7 and Table 6. Based on the results obtained in Figure 7, it was observed that both *P. africana* bark and *H. abyssinica* roots exhibited antioxidant activity comparable to ascorbic acid. However, *P. africana* bark showed a higher antioxidant

TABLE 5: Phytochemical compounds of *Harrisonia abyssinica* identified by gas chromatography – mass spectrometer.

Compound identified	MF	R_t	Nature of compound	Activity
Caryophellene	$C_{15}H_{24}$	8.14	Bicyclic sesquiterpene	Anti-inflammatory, (Fidyt et al. 2016; Urbizu-González et al. 2017)
Trans-calamenene	$C_{15}H_{22}$	9.95	Terpenoid	(Goore et al. 2017)
a-calacorene	$C_{15}H_{20}$	10.36	Terpenoid	(Babushok, Linstrom & Zenkevich 2011)
Spiro[4.5] decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-	$C_{15}H_{24}O_2$	11.39	-	Anti-inflammatory (Hussein, Hadi & Hameed 2016)
Aromadendrene oxide 2	$C_{15}H_{24}O_2$	13.61	sesquiterpenes	Anti-tumour Invalid source specified.
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	17.74	palmitic acid ester	Anti-oxidant (Krishnamoorthy & Subramaniam 2014)
9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	21.99	Unsaturated alcohol	Anti-microbial (Krishnamoorthy & Subramaniam 2014)
Pregnane-3, 11, 20-trione, (5a)-	$C_{21}H_{30}O_3$	23.33	-	(Mamadalieva et al. 2013)
9, 12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	24.45	Linoleic acid	Anti-histaminic (Stewart 2003)
2H-Pyran-2-one, tetrahydro-6-octyl-	$C_{13}H_{24}O_2$		Flavonoid	Antimicrobial (Dey, Dutta & Chaudhuri 2015)
26,27-Dinorergost-5-ene-3,24-diol, (3a)-	-	27.12	-	(Paritala et al. 2015)
Estra-1,3,5(10)-trien-17-one,	$C_{18}H_{22}O_2$	28.67	Steroids	Anti-osteoporosis (Dey et al. 2015)

TABLE 4: Phytochemical compounds of *Prunus africana* identified by gas chromatography – mass spectrometer.

Compound	Molecular formulae	R_t	Nature of compound	Activity
Benzofuran, 2,3-dihydro	C_8H_8O	5.23	Phenolic	-
1,3-Dioxolane-2-heptanenitrile, a-methyl-e-oxo-2-phenyl	$C_{17}H_{21}NO_3$	16.49	Aromatic nitrile	Antimicrobial (Ramakrishnan & Venkataraman 2011)
4H-1-Benzopyran-4-one, 5-hydroxyl-7-methoxy-2-methyl	$C_{10}H_8O_4$	16.78	Flavonol	Antioxidant (Brown 2010)
Tetradecanoic acid, 10,13 dimethyl-, methyl ester	$C_{17}H_{34}O_2$	17.73	Myristic acid	Lavicidal and repellant activity (Sivakumar et al. 2011), antioxidant activity (Murugan & Iyer 2014)
9,12-Octadecanoic acid (Z, Z)-, methyl ester	$C_{19}H_{34}O_2$	21.83	Linoleic acid	Anticancer, anti-inflammatory (Abubakar & Majinda 2016)
4,8,12, 16-tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	25.28	isoprenoid γ-lactone	-
Squalene	$C_{30}H_{50}$	32.17	Triterpenoid	Anticancer, antimicrobial, antioxidant (Rudzinska et al. 2017)
O-methyl (+)-a-tocopherol	$C_{29}H_{50}O_2$	37.99	Vitamin E	Antioxidant (Reiter et al. 2007)
Ergosta-4,6,8(14),22-tetraen-3-one	$C_{28}H_{40}O$	39.48	Ergosterol	Renal injury (Zhao et al. 2011)
Stigmast-4-en-3-one	$C_{29}H_{48}O$	40.26	Steroid	Hypoglycaemic (Alexander-Lindo et al. 2004) Antibacterial (Ulubelen 2003)

activity as compared to *H. abyssinica* roots. The antioxidant activity increased with increase in the extract concentrations because of the ability of these plants to donate electrons to neutralise free radicals and form stable products (Lobo et al. 2010). Antioxidants generally work as scavengers of free radicals, hydrogen donors, peroxide decomposers, enzyme inhibitors, electron donors, synergist and metal-chelating agents (Choe & Min 2009). Phenolic compounds such as polyphenols, lignans, tocopherol and phenolic acids are widely distributed in plants and are the most important natural antioxidants (Tsao 2010).

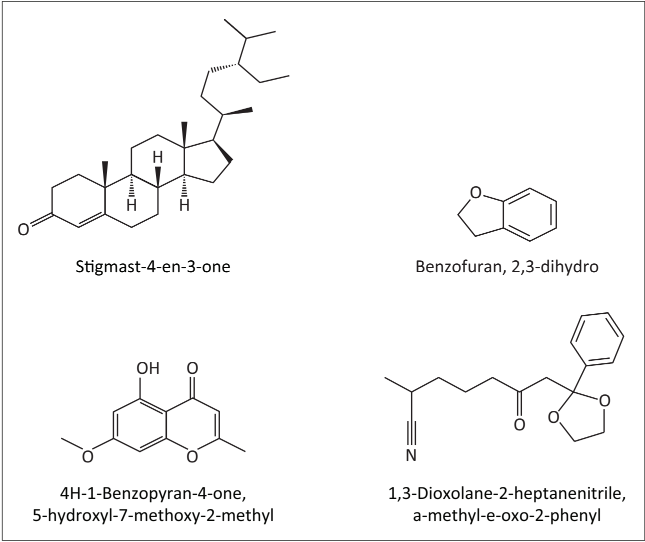

FIGURE 3: Chemical structure of some of the compounds identified by gas chromatography – mass spectrometer.

Total flavonoid content

Total flavonoid content of the crude methanolic extracts of *P. africana* and *H. abyssinica* was determined by aluminium chloride method based on quercetin standard (Al-Jadidi & Hossain 2015). Total flavonoid content in crude methanolic extracts of *P. africana* and *H. abyssinica* was found to be 2.15 mg/g ± 0.37 mg/g and 0.12 mg/g ± 0.02 mg/g, respectively (Figure 8). Flavonoids are secondary plant phenolics that are capable of inhibiting lipid peroxidation, chelate redox-active metals and reduce the effects of processes involving reactive oxygen species (Heim, Tagliaferro & Bobilya 2002). In plants, they act as antioxidants, antimicrobials, photoreceptors, visual attractors and feeding repellants and for light screening (Pietta 2000).

FIGURE 4: Gas chromatography – mass spectrometer spectra of thymol.

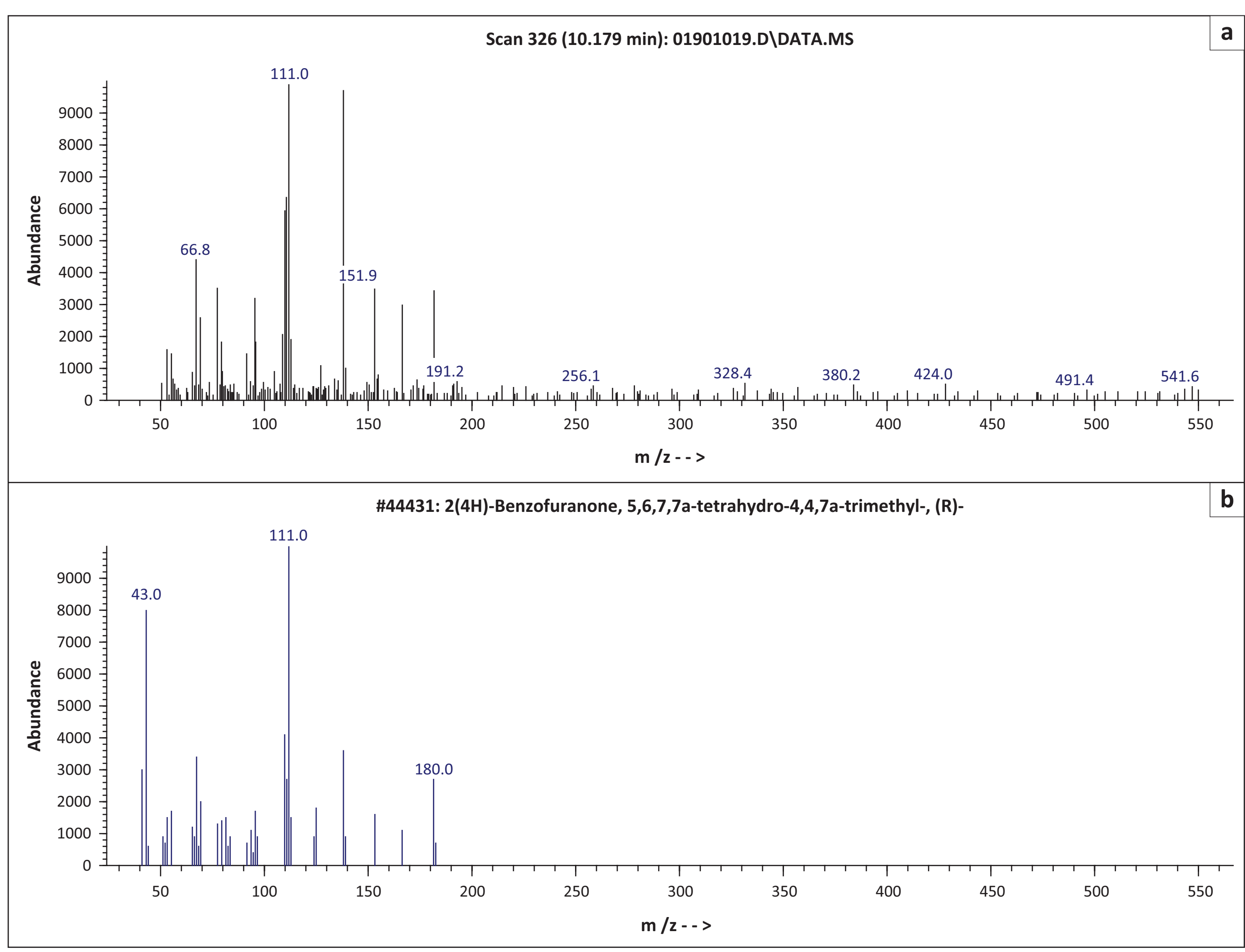

FIGURE 5: Gas chromatography – mass spectrometer spectra of 2(4H)-benzofuranone, 5,6,7,7a-trimethyl-,(R).

Total phenolic content

Crude methanolic extracts of *P. africana* and *H. abyssinica* were used to estimate the total phenolic content of the plants using Folin–Ciocalteu reagent (Al-Jadidi & Hossain 2015). The results of the total phenolic content using Folin–Ciocalteu reagent are depicted in Figure 9. From the results obtained, *P. africana* had the highest phenolic content of 0.26 mg/g ± 0.04 mg/g as compared to *H. abyssinica* with a total phenolic content of 0.08 mg/g ± 0.01 mg/g. Phenols are secondary metabolites that are produced by plants to protect themselves against organisms. Studies have shown that dietary polyphenols play an important role in human health and they have been associated with lowered risks of chronic diseases such as cancer (Zhou et al. 2016), cardiovascular diseases (Quinones, Miguel & Aleixandre 2013) and many degenerative diseases (Sambanthamurthi et al. 2011), because of their ability to neutralise free radicals by electron donation or hydrogen atom (Tsao 2010).

Antimicrobial screening of plant extracts

Medicinal plants are considered new sources for producing antimicrobial agents that could act as alternatives to antibiotics in the treatment of antibiotic-resistant bacteria (Al-Mariri & Safi 2014). Table 7 demonstrates that *P. africana* and *H. abyssinica* had good antibacterial activities against selected microorganisms. The crude extracts of *H. abyssinica* showed moderate activity against *S. aureus* (11 mm), *B. subtilis* (7.8 mm), *P. aeruginosa* (7.0 mm), *E. coli* (8.5 mm) and the fungus *C. albicans* (8.0 mm). *Prunus africana* extracts showed moderate activity against *S. aureus* (11.0 mm), *B. subtilis* (10.7 mm), *P. aeruginosa* (9.7 mm), *E. coli* (8.0 mm) and the fungus *C. albicans* (11.3 mm). Antimicrobial properties of *H. abyssinica* and *P. africana* can be attributed to the presence of secondary metabolites in the plant extracts such as phenols and flavonoids (Cheruiyot, Olila & Kateregga 2009; Goyal et al. 2012). Because of the proven effectiveness, these secondary metabolites have been reported to be alternatives to antibiotics resistance (Wintola & Afolayan 2015). In a similar study, Wintola and Afolayan (2015) reported that the presence of flavonoids and high phenolic content in the crude extract of *Hydnora africana* was responsible for antimicrobial, anti-inflammatory, analgesic, anti-allergic, antioxidant, anti-trypanosomal and anti-leishmanial properties of the plant. It has also been reported that the presence of alkaloids and flavonoids in *Mammea suriga* was responsible for the plant's anti-bacterial and anti-fungal activities (Poojary et al. 2015).

FIGURE 6: Gas chromatography – mass spectrometer spectra of pentanedioic acid monoester.

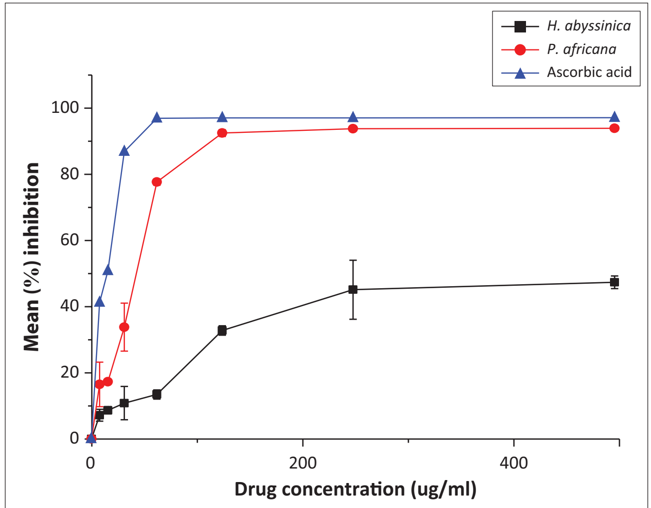

FIGURE 7: Antioxidant activity of *Prunus africana* and *Harrisonia abyssinica* methanolic extracts.

TABLE 6: EC_{50} values for *Prunus africana* and *Harrisonia abyssinica*.

Plant species	EC_{50} (µg/mL)
P. africana	35.87
H. abyssinica	493.94
Ascorbic acid	10.14

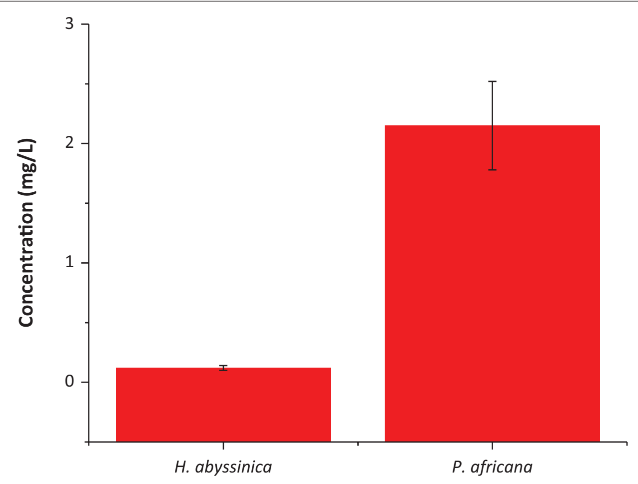

FIGURE 8: Flavonoid content of methanolic extracts of *Prunus africana* and *Harrisonia abyssinica*.

Pearson's correlation

Pearson's correlation coefficients between total phenolics, total flavonoids and antioxidant activity are depicted in Table 8. As shown in the table, there was a strong positive

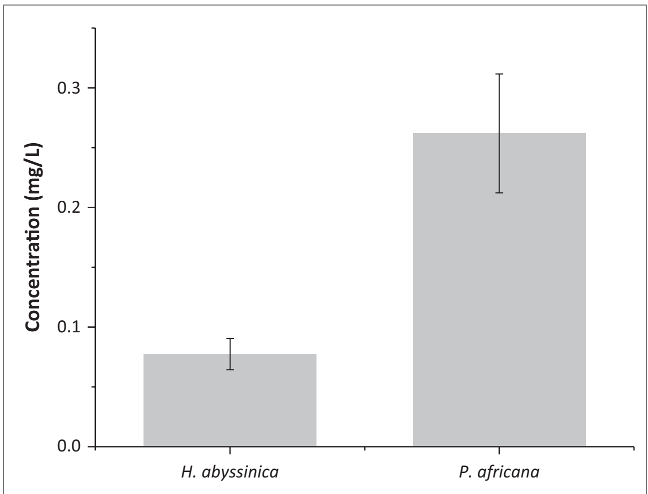

FIGURE 9: Phenolic content of methanolic extracts of *Prunus africana* and *Harrisonia abyssinica*.

TABLE 7: Inhibition zone diameters (mm) for methanolic extracts of *Harrisonia abyssinica* and *Prunus africana* at different concentrations.

Plant extract	Gram-positive bacteria		Fungus	Gram-negative bacteria	
	Escherichia coli	*Pseudomonas aeruginosa*	*Candida albicans*	*Staphylococcus aureus*	*Bacillus subtilis*
P. africana	8.0 ± 1.0	9.7 ± 0.2	11.3 ± 0.2	11 ± 1.0	10.7 ± 0.5
H. abyssinica	8.5 ± 0.7	7.0 ± 0.1	8.0 ± 1.0	9.0 ± 0.0	7.8 ± 1.0

TABLE 8: Pearson's correlation coefficient values for EC_{50}, total phenols and total flavonoids.

Variable	EC_{50}	Total Phenols	Total flavonoids
EC_{50}	1	-	-
Total phenols	-1	1	-
Total flavonoids	-1	1	1

correlation between total phenolic and flavonoid contents and DPPH radical scavenging and a high negative correlation between EC_{50} and the variables. This implies that the radical scavenging ability of DPPH depends on the flavonoid and phenolic contents of the extracts and high phenolic and flavonoid contents indicate that the effective concentration of the extracts is low. Several studies have shown that high antioxidant activity can be correlated with high total phenolic and flavonoid contents. The presence of flavonoids and phenolic compounds is one of the most effective antioxidant properties of the plant extracts (Farasat et al. 2014; Priya, Prakasan & Purushothaman 2017).

Conclusion

Prunus africana exhibited high total flavonoid and total phenolic contents as compared to *H. abyssinica* extracts and hence the high antioxidant activity of *P. africana* extracts as compared to *H. abyssinica* extracts. A strong positive correlation between DPPH radical scavenging activity and flavonoid and phenolic contents indicated that the presence of phenolic compounds including flavonoids in *P. africana* and *H. abyssinica* extracts was responsible for the antioxidant activity and antimicrobial properties of the plant extracts. The secondary metabolites present in the crude extracts were identified with GC-MS and are responsible for the antioxidant activity and antimicrobial properties of the extracts as reported in other literature.

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Competing interests

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

Authors' contributions

As the corresponding author, E.S.M. mainly dealt with the development of the manuscript, proof reading and part of characterisation of the extracts. E.M.G., P.K.K., M.K.M. and J.O.N. dealt with GC-MS profiling of the extracts, while C.K. dealt with phytochemical screening and quantification. P.K.K. and J.K.O. were responsible for the identification of the species and microbial analysis of the extracts.

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