Journal of Medicinal Plants for Economic Development
ISSN: (Print) 2519-559X

Antioxidant activity and antimicrobial properties of *Entada leptostachya* and *Prosopis juliflora* extracts

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Dates:
Received: 11 Aug. 2017
Accepted: 15 Jan. 2018
Published: 27 Feb. 2018

How to cite this article:
Ruto, M.C., Ngugi, C.M., Kareru, P.G., Cheruiyot, K., Cheruiyot, K., Rechab, S.O. et al., 2018, 'Antioxidant activity and antimicrobial properties of *Entada leptostachya* and *Prosopis juliflora* extracts', *Journal of Medicinal Plants for Economic Development* 2(1), a31. https://doi.org/10.4102/jomped.v2i1.31

Background: Natural phytoconstituents produced by plants for their sustenance have been reported to reduce disease.

Objectives: This study determined the phytoconstituents, antioxidant and antimicrobial activity of crude methanolic extracts of *Entada leptostachya* and *Prosopis juliflora* extracts.

Methodology: Antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl and β-carotene assays; the total phenolic and flavonoid were estimated using Folin–Ciocalteau and aluminium chloride, whereas antimicrobial activity was determined using the zone of inhibition method.

Results: Screening of the extracts revealed the presence of terpenoids, flavonoids, saponins and phenols. Fourier transform infrared spectra of the extracts revealed presence of hydrogen bonded –OH functional group. *E. leptostachya* barks had the highest antioxidant activity followed by *P. juliflora* roots, *E. leptostachya* roots and *P. juliflora* leaves [µg/mL]. *Prosopis juliflora* (roots) had the highest bleaching effect, whereas *E. leptostachya* (barks) had the lowest bleaching effect. The total flavonoids were determined to be 0.15 ± 0.02 mg/g, 1.18 ± 0.18 mg/g, 0.39 ± 0.05 mg/g and 0.64 ± 0.03 mg/g for *E. leptostachya* roots, *E. leptostachya* barks, *P. juliflora* leaves and *P. juliflora* roots extracts, respectively. The total phenols were determined to be 0.93 ± 0.18 mg/g, 2.69 ± 0.41 mg/g, 0.62 ± 0.08 mg/g and 0.62 ± 0.08 mg/g for *E. leptostachya* roots, *E. leptostachya* barks, *P. juliflora* roots and *P. juliflora* leaves extracts. All plant extracts exhibited moderate activity against the growth of selected microorganisms.

Conclusion: Antimicrobial and antioxidant activity of the two plants was as a result of secondary metabolites found in the crude extracts.

Introduction

Antioxidants play a significant role in protecting the health of individuals. There is scientific evidence that suggests that antioxidants reduce the risk of chronic diseases such as cancer and heart disease (Dai & Mumper 2010). Plant-sourced antioxidants such as vitamin E, vitamin C, phenolic acids, carotenes, phytate and phytoestrogens have been recognised as having the potential to reduce disease risk (Pandey & Rizvi 2009).

Most antioxidants derived from plant sources belong to various classes of compounds with a wide variety of chemical and physical properties (Brewer 2011). The main characteristic of antioxidants is their ability to trap free radicals. In most biological systems, there is a wide variety of highly-reactive free radicals and oxygen species from different plant parts. These free radicals may oxidise nucleic acids, proteins, lipids or DNA and can lead to development of degenerative disease. Antioxidants such as phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and in the process inhibit the oxidative mechanisms that lead to degenerative diseases (Prakash, Rigelhof & Miller 2017). The major antioxidative plant phenolics can be divided into four general groups: phenolic diterpenes (carnosic acid and carnosol), phenolic acids (gallic, caffeic, protocatechuic and rosmarinic acids), volatile oils (eugenol, carvacrol, thymol and menthol) and flavonoids (catechin and quercetin) (Dai & Mumper 2010). Phenolic acids generally act as antioxidants by trapping free radicals, whereas flavonoids can also scavenge free radicals and chelate metals (Gheldof & Engeseth 2002). Antioxidant activity of plant extracts can be determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH), which is a rapid, simple and inexpensive method that involves the use of the free radical, DPPH, which tests the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity. Antioxidants may be insoluble, water soluble, fat soluble or bound to cell walls, and

are thus not necessarily freely available to react with DPPH; hence, they react at different rates, that is, differing kinetics, and the reaction will often not go to completion in a reasonable assay time (Prakash et al. 2017). Medicinal plants also contain active components which can be used as an alternative to cheap and effective herbal drugs against common bacterial infections. Kenya is endowed with a wide variety of indigenous medicinal plants that are used widely by the local herbalists for treating a number of bacterial and non-bacterial diseases (Kareru et al. 2007). With the ever-rising cases of antimicrobial resistance and the rising cases of cancer in the country, there is an urgent need to come up with active agents that will inhibit the growth of microorganism and also act as antioxidants.

With a wide diversity of medicinal plants, focus has now been turned onto African medicinal plants in search of these agents. Various parts of *P. juliflora* and *E. leptostachya* have been used as remedies for many ailments; hence, the main objective of this study was to evaluate the antioxidant activity and antimicrobial activity, and to quantitatively determine the total phenolic and flavonoid contents of the two plants. In this study, phytochemical analysis, antimicrobial activity and antioxidant activity of *P. juliflora* (leaves and roots) and *E. leptostachya* (roots and barks) were determined to ascertain the ability of these plants to inhibit the growth of microorganisms and their potential as antioxidants.

Materials and methods

Sample preparation

Prosopis juliflora leaves and roots were collected from Marigat, Baringo County, Kenya, whereas *E. leptostachya* roots and barks were collected from Chuka, Meru South District, Tharaka-Nithi County, Kenya. The samples were then transported to Jomo Kenyatta University of Agriculture and Technology (J.K.U.A.T.), where they were identified with the help of a taxonomist from the Department of Botany. The plant samples were separated into leaves, barks and roots; washed with distilled water; chopped; and shade dried for three weeks. The dried samples were then ground into fine powder using an in-house mechanical grinder.

Extraction of plant material

Cold sequential extraction was carried out using methanol as the extracting solvent (Chang-Geun et al. 2011). An amount of 100 g of the fine powders of every plant sample was macerated in 1000 mL of methanol at room temperature. The extracts were filtered using Whatman filter paper no. 1 and concentrated using a Rota evaporator (Rotavapor R-200, BÜCHI Labortechnik AG, Switzerland) at 40°C. The crude extracts were left in a fume chamber to dry, after which they were stored at 4°C until required for phytochemical screening and antioxidant activity (Ghasemzadeh et al. 2015).

Characterisation of plant extracts

The plant extracts were characterised using a Fourier transform infrared (FT-IR) spectrophotometer (Shimadzu FTS-8000, Shimadzu, Kyoto, Japan). The potassium bromide (KBr) pellets of samples were prepared by grinding 10 mg of 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}.

Total phenolic content

The total phenolic content of the extracts was determined using the Folin–Ciocalteau method with some modifications (Baba & Malik 2015; Mburu et al. 2016). About 100 mg of the sample was reconstituted in methanol and filtered with Whatman filter paper no. 1. Then, 0.5 mL of the sample was added to 2.5 mL of 0.2 N Folin–Ciocalteau reagent and incubated for 5 min. Then, 2 mL of 75 g/L of Na_2CO_3 was added and the total volume made up 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 measured at 760 nm using a Shimadzu 1800 UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan). Tannic acid (0 mg/L–800 mg/L) was used to produce a series of solutions that were used to obtain the standard calibration curve and the total phenolic content was expressed in milligram of tannic acid equivalents (TAE) per gram of extract (Baba & Malik 2015; Mburu et al. 2016).

Total flavonoid content

Total flavonoid content was determined using the aluminium chloride method (Baba & Malik 2015; Mburu et al. 2016). An amount of 5 mL 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 515 nm using a Shimadzu 1800 UV-VIS spectrophotometer were taken after 10 min against a blank sample consisting of a 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, and the total flavonoid content was expressed as milligram of catechin equivalents (CE) per gram of extract (Mburu et al. 2016).

Estimation of antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity method

The DPPH free radical method is based on the evaluation of the concentration of DPPH in a methanol solution after addition of methanolic plant extracts. 2,2-Diphenyl-1-picrylhydrazyl absorbs at 517 nm but its concentration is greatly reduced by the presence of an antioxidant. By using a Shimadzu 1800 UV–VIS spectrophotometer, the quantity of the plant extracts needed to reduce the initial DPPH concentration by 50% was evaluated (Ramamoorthy & Bono 2007). This characteristic parameter is called the

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 Equation 1 (Baba & Malik 2014):

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

β-carotene-reducing assay

The antioxidant capacity of the methanolic plant extracts was determined according to a method described by Indrianingsih et al. (2015). In brief, 0.1 g β-carotene, 20 mg linoleic acid and 100 mg Tween 40 were separately dissolved in chloroform. After dissolution, the three solutions were then mixed together and chloroform removed by using a Buchi R-200 Rotavapor. Then, 50 mL of distilled water was added to the solution to form an emulsion, after which 4.8 mL of the emulsion was transferred to tubes containing 2 mL of the crude extracts with a concentration range of 320 mg, 160 mg/L, 80 mg/mL, 40 mg/mL, 20 mg/mL and 10 mg/mL, respectively. Once added, absorbance reading was taken using a Shimadzu 1800 UV-VIS spectrophotometer set at 454 nm (Bljajic et al. 2017). Then, samples were incubated at 50°C for 2 h, after which the absorbance reading was measured at the same wavelength. Standards were prepared in the same concentration range and measured at the same wavelength (Indrianingsih et al. 2015). The degradation rate of the samples was evaluated according to the following first-order kinetics equation (see Equation 2) (Maisarah et al. 2013):

$$dr\ of\ sample = \frac{ln\left(\frac{A_0}{A_t}\right)}{t} \quad [Eqn\ 2]$$

where ln = natural log, A_0 = initial absorbance at time 0, A_t = absorbance at 120 min incubation, t = 120 min and dr = degradation rate. Antioxidant activity (AA) was expressed as percent of inhibition relative to the control using the equation (Bljajic et al. 2017):

$$AA\ (\%) = \frac{dr\ control - dr\ sample}{dr\ control} \times 100 \quad [Eqn\ 3]$$

Determination of antimicrobial properties

The crude extracts were screened for anti-*Escherichia coli*, anti-*Pseudomonas aeruginosa*, anti-*Staphylococcus aureus*, anti-*Bacillus subtilis* and anti-*Candida albicans* activity by using the disc diffusion method in the Department of Botany, J.K.U.A.T. (Zaidan et al. 2005). Six millimeter disc that were sterilized at 120 C for 15 minutes were loaded with inoculum suspension which were spread over the nutrient agar surface and gentamycin used as the positive controls. The crude extracts were loaded onto the surface of inoculated plates with flamed forceps. The plates were then incubated at 37°C for 24 h. The zone of inhibition (ZOI) around the disc was measured in millimetres, using a ruler; low activity (1 mm–6 mm), moderate activity (7 mm–10 mm), high activity (11 mm–15 mm), very high activity (16 mm and above) and no activity (-) (Zaidan et al. 2005).

Statistical analysis

Statistical analysis of the data was evaluated using Statistical Package for the Social Sciences from IBM corporation Version 20.0 64 bit, and the results were expressed as mean ± SD (standard deviation). The effective concentration (EC_{50}) values were obtained from linear regression curves. Pearson's correlation test was used to assess correlations between means.

Results and discussions

Phytochemical screening of plant extracts

Phytochemical screening serves as the initial step in predicting the types of potentially active compounds from plants. Previous studies of these plants have revealed the presence of saponins, alkaloids, terpenoids, steroids, cardiac glycosides and tannins and are already reported in our previous manuscript (Cheruiyot et al. 2015; Kareru et al. 2007; Mutembei et al. 2015; Recharb et al. 2014; Wamburu et al. 2013).

Fourier transform infrared characterisation of plant extracts

From FT-IR spectra of *E. leptostachya* (Figure 1, Table 1) roots and bark extracts, the broad absorption band at around 3340.5 and 3319.3 was attributed to the presence of OH stretching vibrations (Harborne 1998), CH_2 stretching vibrations at 2922.0 cm^{-1}, C=O stretching vibrations at 1700 cm^{-1} and CH bending vibrations at 1461.9. Vibrations because of glycosidic linkage (C–O–C) were observed at 1074.3 cm^{-1} and 1039.7 cm^{-1} for root and bark extracts, respectively. All extracts exhibited the presence of a broad peak for hydrogen bonded –OH stretching in the functional group region. Presence of this functional groups can be attributed to the presence of alkaloids, flavonoids and polyphenols-containing phytochemicals in the root and bark extracts of *E. leptostachya* (Poojary, Vishnumurthy & Adhikari 2015).

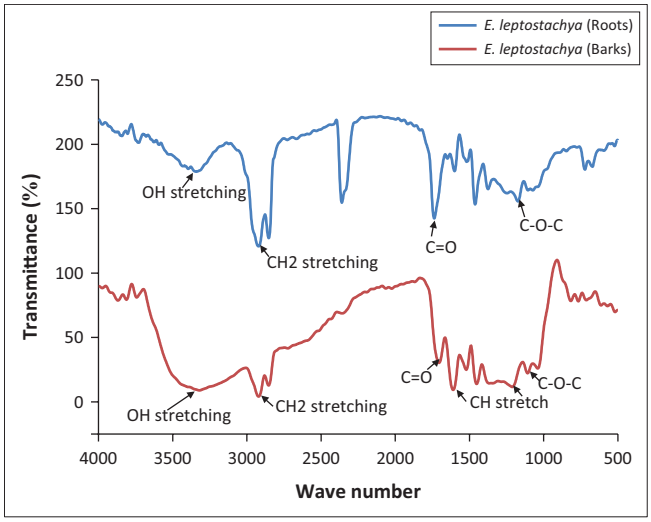

FIGURE 1: Fourier transform infrared spectra of roots and bark extracts of *Entada leptostachya*.

TABLE 1: Major bands observed in the Fourier transform infrared spectra of *Entada leptostachya*.

Peak	Roots	Barks	Probable phytochemicals
OH alcohol or phenol	3340.5	3319.3	Alkaloids, flavonoids, polyphenol, tannins, sterols, terpenoids
CH_2 alkyl stretch	2922.0	2922.0	Alkaloids, flavonoids, polyphenol, tannins, sterols, terpenoids
C=O ester stretch	1737.7	1705.0	Alkaloids, flavonoids, polyphenol, tannins, sterols, terpenoids
Alkenyl C=C stretch	1600.8	1610.5	Alkaloids, flavonoids, polyphenol, tannins, sterols, terpenoids
CH bending	1461.9	1452.3	Alkaloids, flavonoids, polyphenol, tannins, sterols, terpenoids
Esters	1174.6	1213.1	Alkaloids, flavonoids, polyphenol, tannins, sterols, terpenoids
C–O–C	1074.3	1039.7	Alkaloids, flavonoids, polyphenol, tannins, sterols, terpenoids

TABLE 2: Major band observed in Fourier transform infrared spectra of *Prosopis juliflora* roots.

Peak	Roots	Leaves	Probable phytochemicals
OH alcohol or phenol	3174.6	3174.6	Alkaloids, flavonoids, polyphenol, tannins, sterols, terpenoids
CH_2 alkyl stretch	2922.0	2922.0	Alkaloids, flavonoids, polyphenol, tannins, sterols, terpenoids
C=O ester stretch	1732.2	1732.0	Alkaloids, flavonoids, polyphenol, tannins, sterols, terpenoids
Alkenyl C=C stretch	1618.2	1652.9	Alkaloids, flavonoids, polyphenol, tannins, sterols, terpenoids
CH bending	1446.5	1454.2	Alkaloids, flavonoids, polyphenol, tannins, sterols, terpenoids
Esters	1174.6	1264.3	Alkaloids, flavonoids, polyphenol, tannins, sterols, terpenoids
C–O–C	1060.5	1068.5	Alkaloids, flavonoids, polyphenol, tannins, sterols, terpenoids

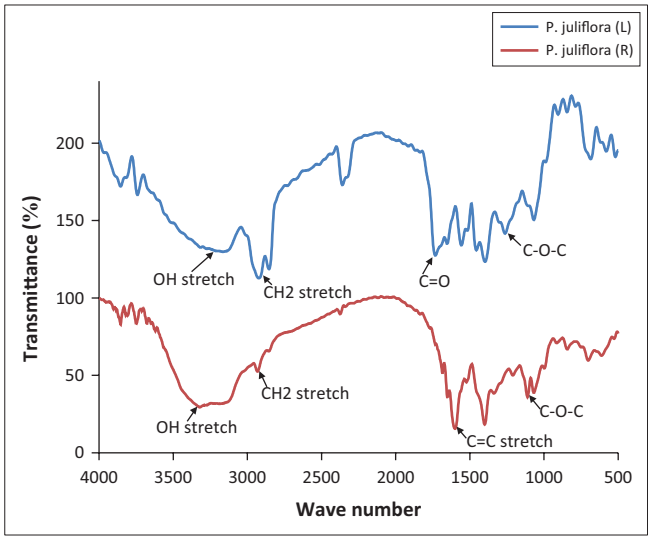

FIGURE 2: Fourier transform infrared spectra of methanolic roots (R) and leaves (L) extracts of *Prosopis juliflora.*

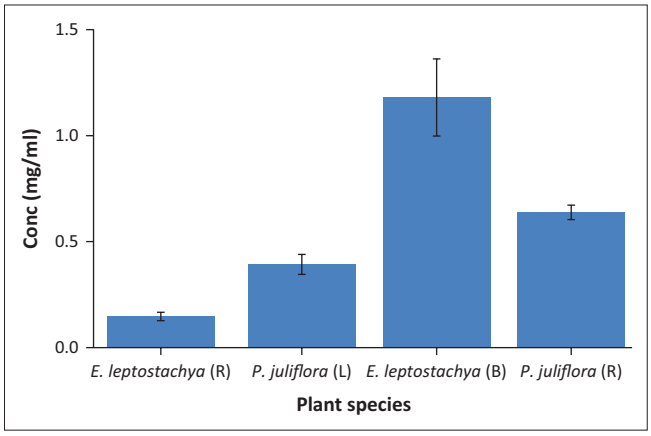

FIGURE 3: Bar graph indicating the concentration of total flavonoids (mg/g) in *Entada leptostachya* and *Prosopis juliflora* extracts.

Fourier transform infrared spectra of methanolic roots and leaves extracts of *P. juliflora* (Figure 2, Table 2) revealed the presence of OH stretching vibrations at 3174.6 cm^{-1}, aldehydic CH_2 stretching vibrations at 2922.0 cm^{-1}, C=O stretching vibrations at 1732.0 cm^{-1}, alkenyl C=C stretching vibrations at 1618.2 cm^{-1}, CH bending vibrations at 1446.5 cm^{-1} and C–O–C stretching vibrations at 1060.5 cm^{-1}. Presence of these functional groups can be attributed to the presence of alkaloids, flavonoids and polyphenol-containing phytochemicals in the leaf extracts of *P. juliflora* (Kareru et al. 2007; Poojary et al. 2015). In this study, FT-IR spectral analysis of the root bark extracts of *P. juliflora* and *E. leptostachya* showed the presence of phytochemicals carrying a hydrogen bonded –OH functional group. Most of the phenolic phytochemicals such as tannins and flavonoids have the hydroxyl functional group (Poojary et al. 2015). Recent studies show that several plant products, including polyphenolic substances (e.g. flavonoids and tannins) and various herbal extracts, show antioxidant and anti-inflammatory activities (Diaz et al. 2012).

Quantification of total flavonoids and total phenols

The concentration of total flavonoids in *E. leptostachya* and *P. juliflora* extracts was determined and is depicted in Figure 3.

From the results obtained, *E. leptostachya* bark extracts had the highest concentration of flavonoids as compared to all other extracts followed by *P. juliflora* (roots and leaves). The total flavonoid content in this study was calculated to be 0.15 ± 0.02 mg/g, 1.18 ± 0.18 mg/g, 0.39 ± 0.05 mg/g and 0.64 ± 0.03 mg/g for *E. leptostachya* (roots), *E. leptostachya* (barks), *P. juliflora* (leaves) and *P. juliflora* (roots) extracts, respectively. Secondary metabolites from plants are natural antioxidants or phytochemical antioxidants.

Flavonoids such as quercetin have been reported to have anticancer activities by inhibiting the development of malignant tumours. Gallic acid has also been reported to be a free radical scavenger and an inducer of differentiation and apoptosis in leukaemia, colon and lung cancer (Ghasemzadeh, Jaafar & Rahmat 2010). In a similar study, Ghasemzadeh et al. (2010) reported that there was a positive relationship between high flavonoid content and high antioxidant activity of Malaysian young ginger (*Zingiber officinale* Roscoe) extract.

Quantitative analysis of the different extracts revealed the presence of total phenolic compounds in all the plant extracts (Figure 4). From the results obtained, the concentration of total phenols was calculated to be 0.93 ± 0.02 mg/g, 2.69 ± 0.04 mg/g, 0.62 ± 0.01 mg/g and 0.62 ± 0.01 mg/g for *E. leptostachya* (roots), *E. leptostachya* (barks), *P. juliflora* (roots) and *P. juliflora* (leaves) extracts, respectively (Figure 4). The activity of the extracts can be attributed to the presence of

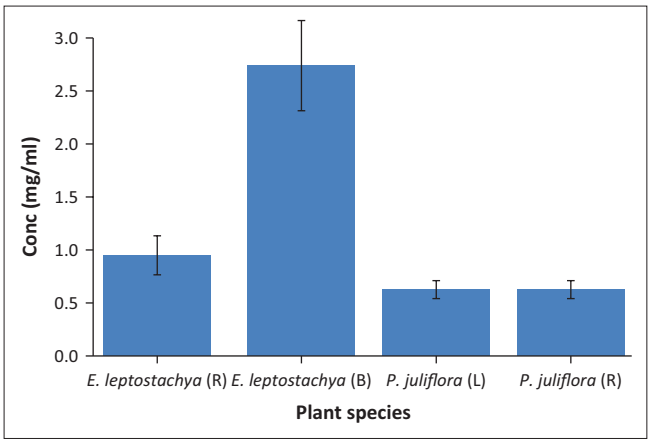

FIGURE 4: Bar graph indicating the concentration of total phenols (mg/g) in *E. leptostachya* and *P. juliflora* extracts.

phenolic compounds which have been reported to have antioxidant activity (Ghasemzadeh et al. 2010).

Antioxidant activity of crude methanolic extracts

2,2-Diphenyl-1-picrylhydrazyl scavenging

The results for the antioxidant activity of both *P. juliflora* and *E. leptostachya* extracts are illustrated in Figure 5, and EC_{50} values are presented in Table 3.

From the results obtained (Figure 5), *P. juliflora* leaves had the lowest antioxidant activity when compared to *E. leptostachya* roots, *E. leptostachya* barks and *P. juliflora* roots. The highest antioxidant activity was observed for *E. leptostachya* barks, followed by *P. juliflora* roots and *E. leptostachya* roots. This is attributed to the difference in proportions of the active components that were responsible for antioxidant activity. In Table 3, the EC_{50} of the extracts (µg antioxidant/mg DPPH) required to scavenge 50% of DPPH radical is presented. As compared to the positive control, the EC_{50} was in the order *P. juliflora* (leaves) > *E. leptostachya* (roots) > *P. juliflora* (roots) > *E. leptostachya* (barks) µg/mL of DPPH used. A study carried out by Wangia et al. (2016) on methanolic extracts of *Ruellia linearibracteolata* and *R. bignoniiflora* showed IC_{50} value of 2.7 µg/mL and 24.4 µg/mL, respectively. Thus, extracts from these plants had a relatively higher antioxidant activity compared with the extracts used in this study. Plant extracts have strong H-donating activity, thus making them extremely effective antioxidants. This antioxidant activity is most often because of phenolic acids (gallic, caffeic, protocatechuic and rosmarinic acids), phenolic diterpenes (carnosol, rosmanol, carnosic acid and rosmadial), flavonoids (quercetin, naringenin, catechin and kaempferol) and volatile oils (eugenol, thymol, carvacrol and menthol) (Brewer 2011; Harborne 1998).

β-carotene–linoleate bleaching assay

The results of β-carotene bleaching assay of the control and the methanolic extracts of *E. leptostachya* and *P. juliflora* are shown in Figure 6. The β-carotene–linoleate bleaching assay

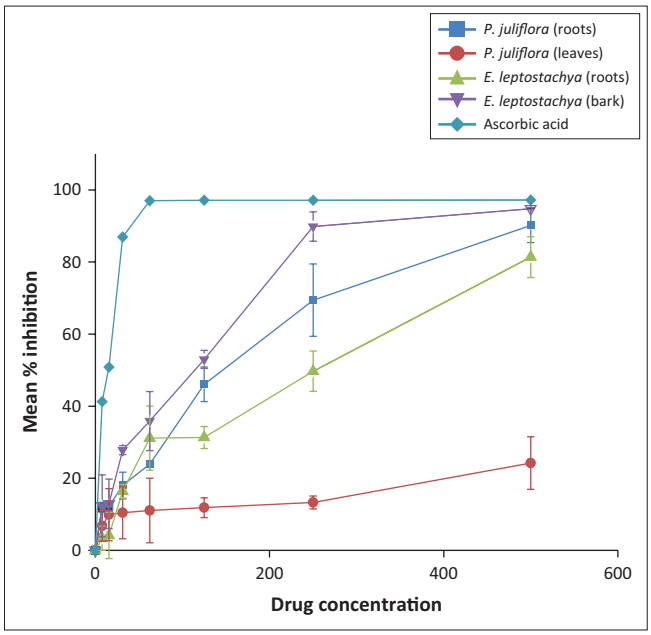

FIGURE 5: A graph of mean per cent inhibition against plant extract concentration (µg/mL).

TABLE 3: Effective concentration of extracts required to scavenge 50% 2,2-diphenyl-1-picrylhydrazyl.

Extract	EC_{50} (µg/mL)
P. juliflora (leaves)	133071.25
P. juliflora (roots)	115.13
E. leptostachya (roots)	185.04
E. leptostachya (barks)	72.717
Ascorbic acid	10.135

EC_{50}, *effective concentration.*

is usually conducted because most plants consist of a lipid–water system with some emulsifier. Hence, an aqueous emulsion system consisting of β-carotene–linoleic acid can be used to determine the antioxidant activity of plant extracts (Indrianingsih et al. 2015). A free peroxy radical is usually formed in these systems upon oxidation of linoleic acid which then attacks β-carotene molecules, thereby triggering rapid decolourisation of β-carotene.

As depicted in Figure 6, the antioxidant activity of the plant extracts differed between the methanolic extracts. *Prosopis juliflora* (roots) extracts had the highest antioxidant activity, whereas *E. leptostachya* (barks) extracts had the lowest antioxidant activity. As compared to the DPPH method in which *E. leptostachya* (barks) extracts had the highest antioxidant activity, *P. juliflora* (roots) extracts had the highest inhibition activity in the β-carotene assay, followed by *P. juliflora* (leaves) then *E. leptostachya* (roots) and then *E. leptostachya* (barks) extracts. This can be attributed to the fact that the mechanism of action of DPPH and β-carotene is different, and there is no correlation between the results obtained in both assays. In the absence of an antioxidant, β-carotene undergoes rapid discoloration, but the presence of phenolic compounds inhibits the extent of β-carotene destruction through neutralising the linoleate free radical formed in the system (Chaouche et al. 2014; Ghasemzadeh et al. 2015). Results in this study indicate

that *E. leptostachya* and *P. juliflora* extracts efficiently inhibited the oxidation of linoleic acid, thereby inhibiting bleaching of β-carotene. In a similar study, Indrianingsih et al. (2015) evaluated inhibition of β-carotene bleaching by the leaf extracts of *C. esculenta*. It was reported that the leaf extracts of *C. esculenta* were the most active extracts among the plants under study with an ability to protect β-carotene bleaching of 32.7%.

This was attributed to the presence of anthocyanins, flavonols and flavanols in the plant extracts that have been reported to be active in the β-carotene bleaching test (Indrianingsih et al. 2015; Javanmardia et al. 2003). In this study, the methanolic extracts of *E. leptostachya* and *P. juliflora* mostly protected the oxidation of emulsified linoleic acid which was confirmed by the presence of flavonoids and phenolic compounds found in the plants (Figures 3 and 4).

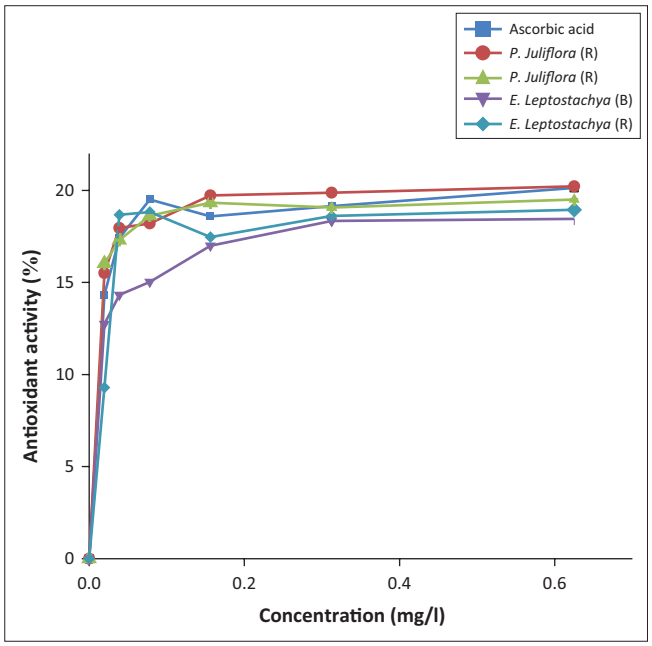

FIGURE 6: β-carotene bleaching assay of *P. juliflora* (roots and leaves) and *E. leptostachya* (barks and roots).

Antimicrobial activity of the crude extracts

Plants have the ability to synthesise aromatic substances, most of which are phenols or their oxygen derivatives that serve as defence mechanism against microorganisms, insects and herbivores. Flavonoids are hydroxylated phenolic substance known to be synthesised by plants in response to microbial infection (Cowan 1999; Murugan, Wins & Murugan 2013). The results of the minimum inhibitory concentration of the plant extracts are presented in Tables 4 and 5. The methanolic extracts of *P. juliflora* and *E. leptostachya* showed various degrees of inhibition against the microorganisms under study. The antibacterial efficacy of the *P. juliflora* and *E. leptostachya* extracts can be attributed to the presence of phenolic compounds (Figure 5); flavones and flavonoids (Figure 6); tannins and terpenoids (Ciocan & Ioan 2007). Terpenoids have been found to have excellent activity against *B. subtilis* and *S. aureus* and lesser activity against gram negative bacteria as well as *C. albicans* (Hufford et al. 1993). In a similar study, Batista et al. (1994) reported that some diterpenes are active against *Staphylococcus aureus*, *Vibrio cholerae*, *Pseudomonas aeruginosa* and *Candida albicans*.

Pearson's correlation

Calculation of Pearson's correlation coefficient of EC_{50}, total phenols and total flavonoids data is depicted in Table 6. From the data obtained, there was a strong negative correlation between EC_{50}, total phenols and total flavonoids. As the EC_{50} value increased, total phenols and total flavonoids decreased;

TABLE 4: Zone of inhibition of *P. juliflora* (leaves and roots).

Plant extract	Conc.(ppm)	Gram positive		Fungus	Gram negative	
		S. aureus	*B. subtilis*	*C. albicans*	*P. aeruginosa*	*E. coli*
P. juliflora (R)	100	7.50 ± 0.71	8.00 ± 0.00	7.00 ± 0.00	9.00 ± 2.82	8.5 ± 2.12
	10	Nil	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	8.5 ± 0.71
	1	Nil	7.00 ± 0.00	7.00 ± 0.00	Nil	8.5 ± 0.71
	0.1	Nil	Nil	7.00 ± 0.00	Nil	8 ± 1.41
P. juliflora (L)	100	8.50 ± 0.71	13.00 ± 0.00	9.00 ± 0.00	10.00 ± 2.83	11.5 ± 0.71
	10	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	8.00 ± 1.41	8.5 ± 0.71
	1	Nil	7.00 ± 0.00	7.00 ± 0.00	8.00 ± 1.41	7.5 ± 0.71
	0.1		Nil	7.00 ± 0.00	7.00 ± 0.00	Nil

S. aureus, Staphylococcus aureus; B. subtilis, Bacillus subtilis; C. albicans, Candida albicans; P. aeruginosa, Pseudomonas aeruginosa; E. coli, Escherichia coli; Conc.(ppm), concentration in parts per million.

TABLE 5: Zone of inhibition of *E. leptostachya* (roots and barks).

Plant extract	Conc.(ppm)	Gram positive		Fungus	Gram negative	
		S. aureus	*B. subtilis*	*C. albicans*	*P. aeruginosa*	*E. coli*
E. leptostachya (R)	100	7.50 ± 0.05	8.00 ± 0.66	9.00 ± 0.06	7.00 ± 0.00	9.00 ± 2.82
	10	7.50 ± 0.95	Nil	7.00 ± 0.00	7.00 ± 0.00	8.00 ± 0.00
	1	Nil	Nil	7.00 ± 0.00	7.00 ± 0.00	7.50 ± 0.71
	0.1	Nil	Nil	Nil	Nil	7.00 ± 0.00
E. leptostachya (L)	100	8.00 ± 1.41	7.50 ± 0.71	8.00 ± 1.41	8.50 ± 0.71	7.00 ± 0.00
	10	Nil	7.00 ± 0.00	8.00 ± 1.41	7.50 ± 0.71	7.50 ± 0.71
	1	Nil	7.00 ± 0.00	8.00 ± 0.00	7.50 ± 0.71	7.00 ± 0.00
	0.1	Nil	Nil	7.00 ± 0.00	9.00 ± 2.82	7.50 ± 0.71

S. aureus, Staphylococcus aureus; B. subtilis, Bacillus subtilis; C. albicans, Candida albicans; P. aeruginosa, Pseudomonas aeruginosa; E. coli, Escherichia coli; Conc.(ppm), concentration in parts per million.

TABLE 6: Pearson's correlation coefficient values for EC_{50}, total phenols and total flavonoids.

	EC_{50}	Total phenols	Flavonoids
EC_{50}	1		
Total phenols	-0.4007764	1	
Flavonoids	-0.29775019	0.822107114	1

thus, the antioxidant activity was strongly correlated with the concentration of phenols and flavonoids in the plant extract (Ghasemzadeh et al. 2010).

Conclusion

The results of this study showed that *E. leptostachya* (barks) extracts had the highest antioxidant activity, total phenolic content and total flavonoid content as compared to the other extracts. The high antioxidant activity of *E. leptostachya* (barks) extracts was as a result of high total phenolic and flavonoid contents which are responsible for antioxidant activity. Like the DPPH method, β-carotene bleaching assay is another popular antioxidant assay that measures the ability of plant extracts to inhibit bleaching of β-carotene by oxidised linoleic acid. Presence of antioxidants in plant extracts can delay the extent of bleaching by neutralising the linoleate free radical in the system. As such, degradation of β-carotene is dependent on the antioxidant activity of the plant extracts (Maisarah et al. 2013). The results obtained in this study revealed a positive relationship between total phenol content, total flavonoid content and the high antioxidant activity of the extracts. Maisarah et al. (2013) reported a strong positive correlation between total phenolic content, total flavonoid content and antioxidant activity by DPPH radical scavenging assay (r = 0.846) of *Carica papaya* plant extracts. This correlation supports the fact that the mode of action and the antioxidant activity of *P. juliflora* and *E. leptostachya* extracts may be identical and related to total phenolic content and flavonoid compounds and their free radical scavenging activity. Further investigation needs to be performed to isolate and identify the phytoconstituents responsible for the antioxidant activity of *P. juliflora* and *E. leptostachya*. The presence of phenolic compounds such as tannins and flavonoids in *P. juliflora* and *E. leptostachya* plant extracts may be responsible for the antimicrobial properties of the plant extracts. These compounds have been reported to protect plants from microbial infections through disruption of bacterial cell membranes, thereby forming complexes with bacterial cell walls and thus inactivating bacterial adhesins, enzymes and transport proteins (Atienza et al. 2016; Savoia 2012).

Acknowledgements

The research was jointly funded by the authors. No funding body was involved.

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

M.C.R., C.M.N., C.K. and S.O.R. did the analysis and species identification. E.S.M., P.K.K., E.G.M., J.M.K. and P.G.K. did data interpretation and analysis.

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