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Phytochemical analysis, antioxidant, antibacterial and combinational effects of medicinal plants used by Bapedi traditional healers to prepare herbal mixtures

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The aim of this study is to evaluate the biological activities of some plant species used in the preparation of herbal mixtures and determine the combinational effects. The plant materials were collected from traders who use them to prepare herbal mixtures claimed to be blood purifiers, pain-relievers, anti-diarrheal and aphrodisiac medicines. An aqueous decoction of each plant species was prepared. Thin layer chromatography (TLC) was used to establish the phytochemical profiles of the decoctions. Standard chemical tests were performed to screen for the presence of phyto-constituent groups. Total polyphenol content of the decoctions was also determined. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay and ferric reducing power were used to assess the antioxidant activities of the extracts. The micro-broth dilution assay was used to determine antimicrobial activity. Fractional inhibitory index was used to determine the consequence of mixing different decoctions together. The phytochemical fingerprints of the decoctions showed that water mainly extracted polar compounds from the plants. Terpernoids, tannins and steroids were detected in all the decoctions. The decoctions of *Delonix elata, Veltheimia capensis*, *Sarcostemma viminale*, and "*Tšhikwana*" had the lowest antioxidant activity. The twigs of *Kirkia wilmsii* exhibited the greatest antioxidant activity (EC_{50} 15.71 µg/mL) and further showed significant broad-spectrum antibacterial activity against diarrheagenic pathogens. The twigs of *K. wilmsii* had the greatest amount of total phenolics followed by *Hypoxis hemerocallidea* (corm) and *Monsonia angustifolia.* Majority of the aqueous extract combinations yielded indifferent and additive interactions. Although synergistic effects are sought after when mixing different plant extracts, this study demonstrated the complexities associated with combinations of aqueous extracts and the importance of appropriate knowledge on which species of plants should be mixed to enhance their therapeutic properties.

Key words: Phytochemical screening, antioxidant, antimicrobial, combinational effects.

INTRODUCTION

Since the earliest of times, plants have been broadly used in various traditional healthcare systems to treat numerous diseases and to promote human health. In addition, they are vital to the development of

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pharmaceutical drugs because half of the drugs in the world are plant derivatives (van Wyk et al., 2013).

Phytochemicals such as phenolic compounds, alkaloids, diterpenoids, steroids, alkaloids and other complex compounds are secreted by plants as a defence mechanism against infections, predators and/or to also survive abiotic factors in the environment. Phytochemicals have been shown to have antioxidant, anticarcinogenic, antimicrobial, antiallergic, antimutagenic, and anti-inflammatory properties (Alesiani et al., 2010). It is, therefore, not entirely startling that traditionally, many medicinal plants were and continue to be used for treatment of diverse ailments (Madikizela et al., 2017).

The utilisation of plants for their therapeutic properties has been a commonplace practice in South Africa. This practice was motivated and accelerated by the abundance of diverse medicinal plant species which are spread across the country. As such, each geographically distal cultural group in the country may have different pharmacopeia for the treatment of similar ailments (Madikizela et al., 2017).

Through experience accumulated over the long use of medicinal plants and the increasing occurrence of health ailments in communities, traditional healers generally no longer prepare herbal remedies using a single plant species. Instead, they combine different plants species in an effort to increase efficacy and the healing potential of the therapies.

Due to the increased demand in plant-based products, the plant trade industry provides job opportunities and thus a much-needed source of income. This benefit brings about an intense competition that may motivate adulteration or the distribution of products of a substandard quality coupled with unsubstantiated claims (Ndhlala and van Staden, 2012).

The Limpopo Province has a high distribution of Bapedi traditional health therapists and they are distributed mainly in the Capricorn, Sekhukhune and Waterberg districts which collectively include more than 17 municipalities in which they are operational (Semenya and Potgieters, 2015). The traditional healers situated at the Capricorn district municipality sell the concoctions use plants such as *Hypoxis hemerocallidea*, *Drimia elata*, *Sarcostemma viminale* and *Kirkia wilmsii* to prepare aqueous herbal mixtures prescribed for immune boosting, diarrhoea and relief of pain. The plants used by these traders have been reported to have numerous biological activities.

Eloff et al. (2010) demonstrated the antimicrobial activity of *K. wilmsii* by using hexane, acetone, methanol and dichloromethane extracts of its leaves against *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Aspergillus fumigatus, Candida albicans, Cryptococcus neoformans, Microsporum canis* and *Sporothrix schenckii*. Katerere and Eloff (2008) reported significant antimicrobial activity of an ethyl acetate extract of the leaf and corm of *H. hemerocallidea* against *S. aureus* and *E. faecalis.* The bulb *D. elata* has been reported to be used for treatment of asthma and pain (Semenya and Potgieters, 2015).

In the Limpopo Province it is mixed with several plants for the treatment of sexually transmitted infections (Semenya et al., 2013).

Although, the named medicinal plants were all used to prepare aqueous herbal mixtures, Matotoka and Masoko, (2018) reported only moderate antibacterial activity of the herbal mixtures against diarrhoeagenic microorganisms. In addition, the antioxidant activity of the herbal mixtures fell short to be comparable to ascorbic acid.

Needless to say, one has to then consider the consequence of plant combinations on the intended therapeutic activity of herbal mixtures. Several studies have reported that phytochemicals from different plant species can interact, and the outcomes of these interactions may be synergistic, antagonistic and/or non-interactive (Orchard and van Vuuren, 2017; van Vuuren and Viljoen, 2011). While the herbal mixtures sold at Ga Maja are prepared to improve biological activity, the underlying interactions of the different plants species is poorly understood. It was therefore, the focus on this study to assess the biological activities of the medicinal plants used by the traders to prepare the herbal mixtures and determine their combinational effects.

MATERIALS AND METHODS

Plant collection

The plants were collected in late summer from traditional healers in Lebowakgomo, Limpopo Province. The plant species in this study were selected for analysis because they have been claimed by the traders and traditional healers to be the ingredients that are used in the preparation of their commercialised herbal mixtures. The plant species were deposited at University of Limpopo's Larry Leach herbarium where voucher specimens were obtained. Table 1 shows the list of the collected plant species. During drying, the plant material was stored at room temperature away from sunlight. Large pieces such as the corms, twigs and stems were cut into smaller pieces to increase the surface area of the parts to allow for a quicker drying period. A commercial blender was used to grind the dried material into fine powders. During the course of this study, the powdered plant material was stored in air-tight glass containers and kept away from sunlight to prevent possible photo-oxidation reactions.

Extraction

The herbal mixtures are normally prepared by the traders by boiling all the plant material together in a pot that is heated over wood fire. The boiling time ranges from 5 to 10 min. Therefore, to gain access to similar phytochemicals as the traders, separate water decoctions of each plant material was prepared. The decoctions were prepared by heating 10 mL of distilled water in a glass beaker for 5 min. Once the water began to boil, 1 g of the ground plant material was added to the water. The plant material was stirred thoroughly with a glass rod throughout the boiling process. After extraction, the extracts were filtered through Whatman No. 1 filter paper into pre-

Table 1. Collected plant species used by traditional healers to prepare herbal mixtures.

Plant material	*Family*	Voucher number	Vernacular name	Ethnopharmacological application
Kirkia wilmsii (leaves)	-	SS 94	*Legaba/Modumela*	
Kirkia wilmsii (corm)	*Kirkiaceae*	SS 94	*Legaba/Modumela*	Hypertension (Semenya and Potgieters, 2015)
Kirkia wilmsii (twigs)	-	SS 94	*Legaba/Modumela*	
Hypoxis hemerocallidea (corm)	*Hypoxidaceae*	SS 115	*Monna maledu*	Testicular tumours, impotency, cardiac diseases (Drewes et al., 2008).
Monsonia angustifolia (leaves)	*Geraniaceae*	121393	*Tee ya thaba*	Aphrodisiac (Fouche et al., 2015)
Drimia elata (corm)	*Hyacinthaceae*	S 18	*Sekanama*	Treatment of human immune-deficiency virus infections (Semenya et al., 2013)
Sarcostemma viminale	*Asclepiadaceae*	121404	*Moema*	Wound healing and treatment of ulcers (Semenya et al., 2013)
Vahlia capensis	-	121394	*Makgonatšohle*	-
"Tšhikwana/Moroto wa tšhwene" (Powder)*	-	-	-	-

Tšhikwana/Moroto wa tšhwene is a powdered plant mixture that is prepared by the traditional healers with claims that it has aphrodisiac and blood purifying properties. Among other unspecified plants species, the plant mixtures were said to be prepared by mixing *D. elata*, *S. viminale*, *V. capensis.* The traditional healers were unable to provide us with the full list of plant species for preparation because they indicated they secrecy of the recipe is essential for them to maintain a good relationship with their ancestral gods.

weighed glass vials and freeze-dried.

Phytochemical analysis

Thin layer chromatography fingerprint profiles

The method used to establish the phytochemical fingerprint profiles of the extracts was that of Kotze and Eloff (2002), detailed by Elisha et al. (2017).

The dried plant extracts were reconstituted with distilled water to a concentration of 10 mg/mL. 10 µL of each of extract was loaded onto thin layer chromatography (TLC) plates (Merck, Silica gel F_{254}). The TLC plates were developed using solvents (Merk, technical grade) which differed in polarities that ranged from non-polar to polar (Table 2).

Once the mobile phase had reached the solvent-front, the chromatograms were removed from the TLC chambers. The mobile phases were prepared as detailed in Table 2. Chromatograms were visualised under ultra-violet (UV) light 254 nm. The TLC plates were sprayed with vanillin spray reagents (0.1 g vanillin [Sigma], 28 ml methanol, 1 ml sulphuric acid [Sigma]) and heated at 110°C for optimal colour development.

Phyto-constituents

Total phenolics

The total phenolic content of the plant decoctions was determined by using the Folin-Ciocalteu reagent method (Tambe and Bhambar, 2014), with minor modifications. The extracts (10 mg/mL) were diluted with 490 µL of distilled water to make up a final volume of 500 µL. This was followed by the addition of 0.25 mL of Folin-Ciocaltleu reagent (Sigma) in each test tube. Sodium carbonate (Na_2CO_3) (Sigma) (1.25 mL) was added and the mixtures were incubated in the dark at room temperature for 30 min. An ultraviolet/visible (UV/VIS) spectrophotometer was used to determine the absorbance of the mixtures at 550 nm. A blank and the standard curves were prepared in a similar manner, except that the plant extracts were replaced by distilled water for the blank. Tannic acid (Sigma) was used as the standard for this procedure; whereby varying concentrations of tannic acid (1.25 - 0.08 mg/mL) were prepared. The results obtained from the linear regression formula of the tannic acid standard curve were expressed as milligram tannic acid equivalence/gram of extract (mg of TAE/g extract). The experiment was conducted in triplicates and independently repeated three times.

Total flavonoids

The total flavonoid content was determined using the aluminium chloride method (Tambe and Bhambar, 2014). Briefly, 100 µL of 10 mg/mL of the decoctions was added to 4.9 mL of distilled water in a clean test tube. To this reaction mixture, 300 µL of 5% sodium nitrite ($NaNO_2$) (Rochelle) dissolved in distilled water was added and the mixture was left at room temperature for 5 min. This was followed by the addition of 300 µL of 10% aluminium chloride ($AlCl_3$) (Rochelle) (dissolved in distilled water). The reaction was allowed to stand for 5 min at room temperature. After the elapsed time, 2 mL of sodium hydroxide (NaOH) (Rochelle) was added to the solution. The mixture in the test tube was then made up to 10 mL with distilled water. Quercetin (Sigma) was used as a standard; whereby different concentrations (500 - 31.5 µg/mL) were prepared. The absorbance of the experimental samples and the standard were determined using a UV/VIS spectrophotometer at a wavelength of 510 nm. The blank was prepared in the same manner as the experimental samples with 100 µL of distilled water added instead of the extracts. The total flavonoid content of the samples was expressed as milligram quercetin equivalence/ gram of extract (mg QE/g extract).

Table 2. Phyto-constituents present in the decoctions of plant species used to prepare the herbal concoctions.

Phyto-constituent	Plant species											
	KwT	KwL	KwC	SV	HH	VC	MA	DE	PM1	PM2	PM3	PM4
Terpenoids	+	+	+	+	+	+	+	+	+	-	-	-
Alkaloids	-	-	-	-	-	+	-	-	-	+	-	-
Saponins	+	-	+	-	+	+	-	+	+	+	+	+
Flavonoids	+	+	+	+	-	+	-	-	+	+	-	-
Phlabatannins	-	-	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides	+	+	+	-	+	+	+	+	+	-	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+	+	+	+

(+): Present, (-): absent, Kw (T, L, C): *K. wilmsii* (twigs, leaves, corm); SV: *S. viminale*; HH: *H. hemerocallidea*; VC: *V. capensis*; MA: *M. angustifolia*; DE: *D. elata;* PM (1, 2, 3, 4): Different *Tšhikwana* powders.

Total tannins

The Folin-Ciocalteu method (Tambe and Bhambar, 2014) was used to determine the tannin content in the concoctions. Briefly, 100 µL of 10 mg/mL of the concoctions and aqueous plant extracts was added to a clean test tube containing 7.5 mL of distilled water. The Folin-Ciocalteu reagent (Sigma) (0.5 mL) was added to the mixture and vortexed. Ten millilitres of a 35% solution of sodium carbonate (Na_2CO_3) was added to the mixture. The mixture in the tube was transferred to a 10 mL volumetric flask and the volume of the mixture was made up to 10 mL with distilled water. The mixture was shaken and kept at room temperature for 30 min in the dark. Gallic acid (Sigma) was used as a standard and reference standard solutions (1.0 - 0.625 mg/mL) were prepared. The absorbance for the solutions was measured against a blank that was prepared in the same manner as the test solutions. A UV/VIS spectrophotometer was used to measure the absorbance at 725 nm. Tannin content was expressed as milligram gallic acid equivalence/ gram of extract (mg GAE/g extract). The experiment was conducted in triplicates and independently repeated three times.

Free radical scavenging activity assay

Thin layer chromatography-DPPH fingerprint profiles

Thin layer chromatography (TLC) coupled with 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma) was used to screen for possible antioxidant compounds in the plant decoctions. Deby and Margotteaux (1970)'s procedure was performed as detailed by Adamu et al. (2014). The chromatograms were prepared and developed in an identical manner to phytochemical fingerprinting. DPPH solution (0.2% w/v) was prepared by dissolving 0.2 g of the DPPH free radical in 100 mL of methanol. This solution was sprayed onto the air-dried chromatograms. The presence of antioxidant activity was indicated by the development of yellow bands against a purple background.

Quantitative free radical scavenging activity

The free radical scavenging activity of the extracts was determined by using the DPPH method (Chigayo et al., 2016), with modifications. Briefly, different concentrations of the extracts (250 - 15.63 µg/mL) were prepared to a volume of 1 mL of the solution. L-ascorbic acid (Sigma) was used as standard by preparing the same concentration range as the extracts. To these solutions, 2 mL of 0.2 mmol/L DPPH solution dissolved in methanol was added and vortexed thoroughly. The solutions were left to stand in the dark for 30 min at room temperature. The control solution was prepared by adding 2 mL of 0.2 mmol/L DPPH to 1 mL of distilled water. After the elapsed time, the solutions were analysed with a UV/VIS spectrophotometer set at a wavelength of 517 nm. The experiment was run in duplicate and repeated three times. Free radical scavenging activity of the extracts was expressed as percentage inhibition of DPPH from the control solution. The percentage was calculated as follows:

$$\%Inhibition = \frac{Ac - As}{Ac} \times 100$$

where Ac is the absorbance of the control solution and As is the absorbance of the extracts.

Ferric reducing power

The antioxidant activity of the decoctions was further evaluated using the ferric reducing power method (Vijayalakshmi and Ruckmani, 2016; Ahmed et al., 2012). Extract stock solutions and L-ascorbic acid (Sigma; 1.25 mg/mL) were serially diluted to obtain five different concentrations of the samples (625 - 39 µg/mL). The different concentrations of the solutions (2.5 mL) were mixed with 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (Rochelle) (1% w/v in distilled water), respectively, in a test tube. Thorough vortexing was done after addition of solutions. The mixtures were incubated at 50°C for 20 min. 2 mL of trichloroacetic acid (Rochelle) (10% w/v in distilled water) was added to the test tubes after incubation. The mixtures were centrifuged at 3000 rpm for 10 min and 5 mL of the resulting supernatant was transferred to a clean test tube. To this solution, 5 mL of distilled water and 1 mL ferric chloride (0.1% w/v in distilled water) were added consecutively with thorough vortexing after each addition. A UV/VIS spectrophotometer was used to read the absorbance of solutions at 700 nm wavelength. The blank for this procedure was prepared by the same procedure as the tests samples whereby in place of the extracts, an equal volume of distilled water was added. L-Ascorbic acid was used as a standard. The experiments were performed in duplicates and repeated three times.

Micro-dilution assay

The antimicrobial activity of the plant decoctions was evaluated by

Table 3. Total phenolic, flavonoid, tannin content and half maximal effective free radical scavenging activity (EC_{50}) of the plant decoctions.

Plant species	Phenolics (mg TAE/g extract) *	Flavonoids (mg QE/g extract)*	Tannins (mg GAE/g extract) *	EC_{50}* (µg/mL)
K. wilmsii (leaves)	61.51 ± 0.07	1.68 ± 0.12	16.62 ± 0.28	18.50 ± 0.242
V. capensis	53.65 ± 0.08	0.46 ± 0.01	47.02 ± 2.57	203.47 ± 4.743
S. viminale	52.84 ± 0.05	0.01 ± 0.04	3.85 ± 0.16	207.25 ± 4.780
K. wilmsii (Twigs)	88.84 ± 0.11	4.90 ± 0.09	22.76 ± 0.22	15.71 ± 0.245
H. hemerocallidea	65.69 ± 0.01	4.57 ± 0.21	8.08 ± 0.31	112.19 ± 0.783
k. wilmsii (Corm)	63.06 ± 0.08	1.45 ± 0.10	6.85 ± 0.09	34.45 ± 0.316
D. elata	53.29 ± 0.06	1.19 ± 1.36	6.97 ± 0.87	329.04 ± 2.116
M. angustifolia	66.84 ± 0.06	4.22 ± 0.05	8.21 ± 0.13	40.87 ± 0.030
Tšhikwana 1	50.18 ± 0.11	9.44 ± 0.49	16.75 ± 0.16	109.67 ± 0.146
Tšhikwana 2	50.83 ± 0.06	1.23 ± 0.07	8.28 ± 0.09	535.91 ± 0.371
Tšhikwana 3	63.16 ± 0.15	3.70 ± 0.05	8.46 ± 0.04	85.85 ± 0.142
Tšhikwana 4	52.5 ± 0.19	1.94 ± 0.20	-	237.53 ± 0.214

(-): No detectable concentration; mg TAE/g: milligram tannic acid equivalence per gram of plant extract; mg QE/g: milligram quercitin equivalence/ gram of plant extract; milligram gallic acid equivalence/ gram of plant extract. *Values are mean of triplicates ± standard deviation (SD) (n=3)

determining the minimal inhibitory concentration against: *E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecalis* and *C. albicans*. These species are common causative agents of infectious diarrhoea (Ahmed et al., 2012). The broth micro-dilution assay by Eloff (1998) and the modified version by Masoko and Eloff (2005) to suit fungal growth requirements were used. Sterile distilled water (100 µL) was added to each well of a round bottom 96 well microtitre plate. The extracts were concentrated to 10 mg/mL using distilled water and 100 µL of the solutions were separately serially diluted to 50% with the distilled water in the wells of the 96 well microtitre plates. Each culture (100 µL) was separately added to each well. The antibiotic Amphotericin B (Sigma) was used as a positive control and sterile distilled water as the negative control. The microtitre plates were covered with laboratory plastic wrap and incubated for 24 h at 37 °C for bacteria and at 25°C for the yeast. After incubation, *p*-iodonitrotetrazolium chloride (INT; Sigma) dissolved in sterile distilled water was used to detect microbial growth. The metabolically active microorganisms reduce the tetrazolium salt to a purple formazan. A volume of 40 µL of INT (0.2 mg/mL) was added to each well of the microtitre plates and further incubated for 30 min (bacteria) and 2 to 3 h (yeast). Minimum inhibitory concentration (MIC) values of the extracts were recorded as the concentrations of the lowest clear wells of each extract that was able to inhibit growth. The assay was repeated three times in duplicate.

Synergistic, additive and antagonistic interactions

The effects of the combination of structurally different bioactive phytochemicals from the various plant decoctions were studied. The same *in vitro* methods as described earlier were used for assessing the combinational effects of the plant decoctions on antioxidant and antibacterial activity respectively. The ratio used for each mixture was a 1:1 of the extracts.

The fractional inhibitory concentration (FIC) of each plant extract was calculated in order to determine types of chemical interactions that occur when they are mixed (van Vuuren and Viljoen, 2011). However, this method was modified to optimise the analysis of the extract combinations towards antioxidant activity. For antioxidant activity, the FIC value for each extract in a combination was calculated by dividing the EC_{50} value of the combination by the EC_{50} value of each plant decoction placed in the combination (equation a). For antibacterial activity, the MIC was used instead of the EC_{50} values (equation b). The fraction inhibitory index ($\sum FIC$) was then calculated by adding the two FIC values of the plant extracts in a combination (equation c).

The interpretations of the fractional inhibitory index to explain the effect of the mixture of the decoctions are detailed in Table 3. Briefly, $\sum FIC$ values ≤ 0.5 demonstrate synergistic interactions. For additive effects of the combinations, $\sum FIC$ are > 0.5 to 1.00. The interpretation for indifference was $\sum FIC$ that are > 1.00 - ≤ 4.00. Antagonistic interactions were interpreted as $\sum FIC$ that is >4.00.

$$FIC = \frac{EC50\,(Combination\,A,B)}{EC50} \quad (1)$$

$$FIC = \frac{MIC\,(Combination\,A,B)}{MIC\,A)} \quad (2)$$

$$FIC\ index = \sum FIC = FICA + FICB \quad (3)$$

where EC_{50} is the concentration of the extract that was about to inhibit 50% of the DPPH. MIC is the minimum inhibitory concentration of an extract towards microbial growth. FICA is the FIC for the first extract in the combination and FICB is of the second extract. Additive interactions occur when the therapeutic effect is of the sum of the individual components. The synergistic effects of a combination refer to when the therapeutic effect of the combination is greater than that of the individual constituents. Antagonistic interactions mainly refer to chemical interactions which result in the reduction or loss of a biological activity compared to the individual Phyto-constituents (Wang et al., 2011). Indifferent interactions indicate the non-interactive reaction of a combination that results in neither an additive nor antagonistic effect (van Vuuren and Viljoen, 2011).

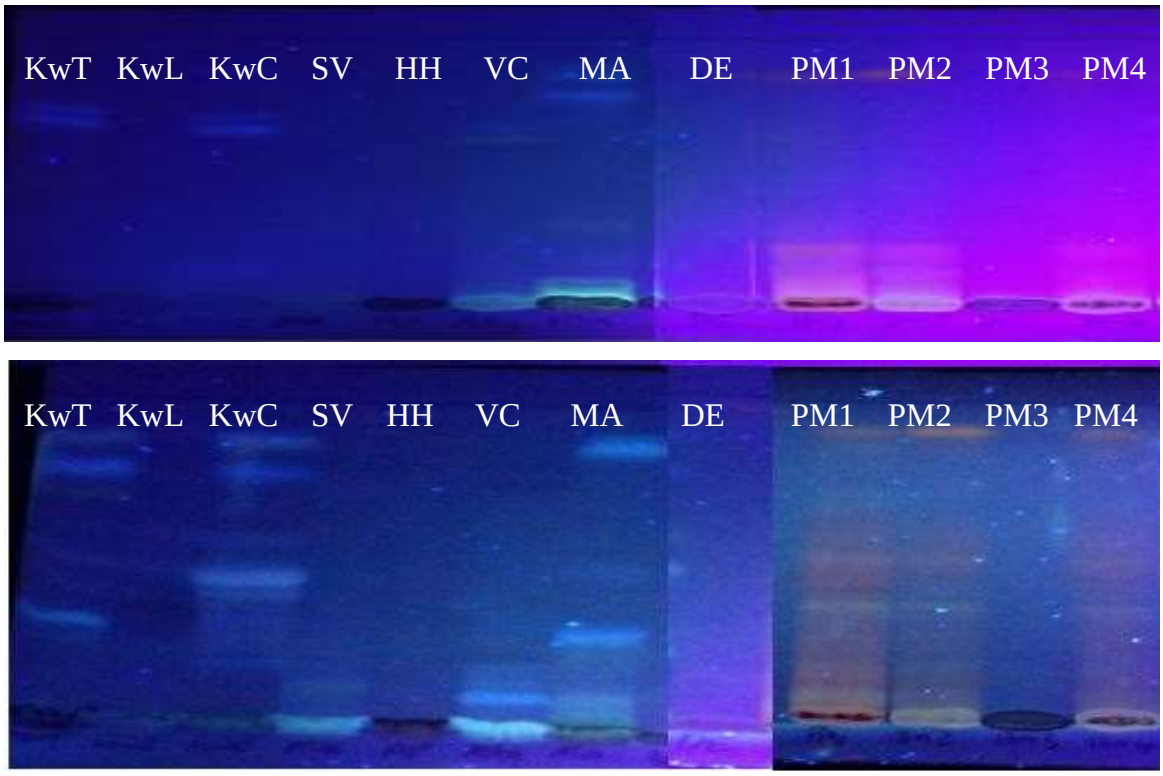

Figure 1. Chromatograms showing a variety of 245 nm UV light fluorescent compounds present in the various plant decoctions. The extracts were eluted with non-polar (BEA) to polar (EMW) mobile systems. Kw (T, L, C): *K. wilmsii* (twigs, leaves, corm); SV: *S. viminale*; HH: *H. hemerocallidea*; VC: *V. capensis*; MA: *M. angustifolia*; DE: *D. elata;* PM (1, 2, 3, 4): Different *Tšhikwana* powders.

RESULTS AND DISCUSSION

Phytochemical analysis

The TLC separated phytochemicals were visualised under ultraviolet (UV) light of 254 nm wavelength. Under this spectrum, electrons in compounds undergo transition from a ground state to an excited state. When the electrons are de-excited to the ground state, they release energy in the form of an electromagnetic radiation wavelength of a lower energy than the UV light that range within the visible spectrum. The latter results in structurally diverse compounds to fluoresce and is visualised in different colours.

The number of coloured bands on a chromatogram reflects the number of fluorescent compounds present in the decoctions. The decoctions of the plants contained most polar compounds because many of them were visible in the chromatogram developed with the polar mobile phase (EMW) followed by the CEF and BEA chromatograms respectively (Figure 1). This observed trend has demonstrated that water extracted mainly polar compounds. This is because water has exhibited extreme polarity and as such it was expected to extract majority of polar compounds and discriminate against non-polar compounds. Barba et al. (2016) have also reported the same principle.

The fluorescence indicated that the compounds consisted of conjugated double bonds and/or extended pi (π) electron configurations (Ahmed et al., 2014) Polyphenols have been reported to have lengthy conjugated aromatic systems in their chemical structures (Dai and Mumper, 2010). Therefore, the various fluorescing compounds in Figure 1 may be due to the presence of polyphenolic compounds or their analogues.

The vanillin-sulphuric acid spray was used to establish the phytochemical profiles of compounds that were otherwise unable for fluoresce. The profile of these non-fluorescent compounds also showed that majority of them were polar (Figure 2). Major chemical classes that were detected were terpenoids (purple or bluish-purple bands), flavonoids (pinkish, yellow or orange) and proanthocyanidins (pink) (Ahmed et al., 2014; Tagana et al., 2011; Dai and Mumper, 2010).

Key phyto-constituents were qualitatively screened from the plant decoctions. The presence of terpernoids, tannins and steroids were common in all the extracts (Table 2). Terpenoids/terpenes were reported to have antibacterial and antioxidant activity (Zengin and Baysal, 2014). Flavonoids and tannins were documented to

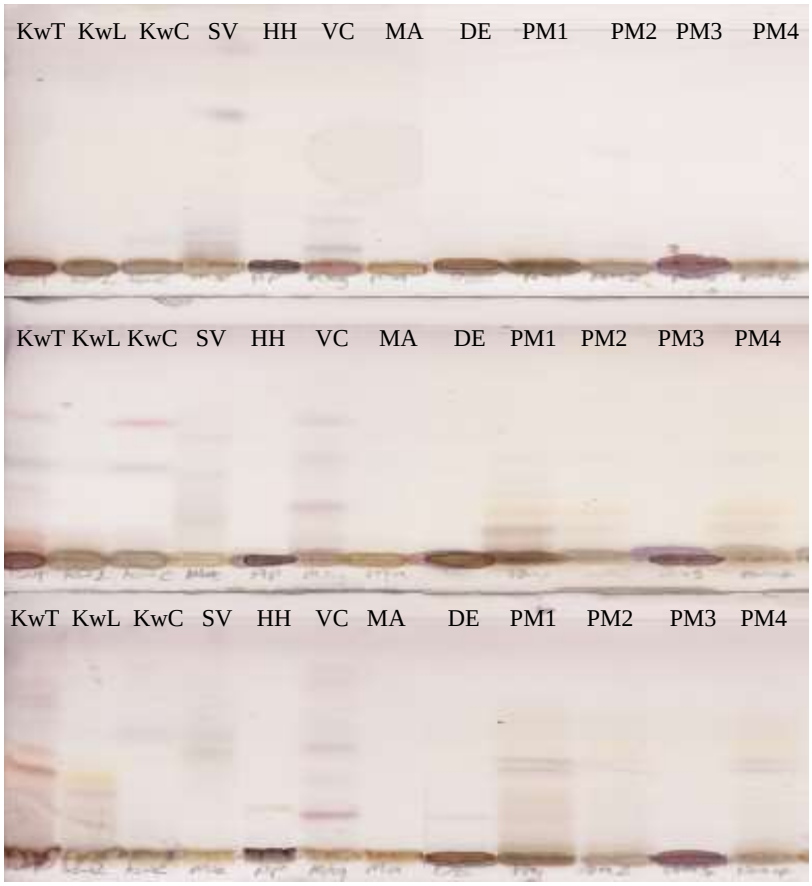

Figure 2. Chromatograms of plant decoctions showing various coloured compounds that reacted with vanillin-sulphuric acid reagent. Different colours developed after heating the sprayed chromatograms at 110°C. Kw (T, L, C): *K. wilmsii* (twigs, leaves, corm); SV: *S. viminale*; HH: *H. hemerocallidea*; VC: *V. capensis*; MA: *M. angustifolia*; DE: *D. elata*; PM (1, 2, 3, 4): Different Tšhikwana powders.

possess antioxidant, anti-allergic, anti-inflammatory, antimicrobial and anticancer properties (Valifard et al., 2014; Balasundram et al., 2006). The biological activities associated with saponins include anti-inflammatory, antimicrobial and cytotoxic effects (Sarikurkcu and Tepe, 2015). The presence of the different phyto-constituents in the plant extracts demonstrated an increased probability for these structurally diverse compounds to chemically interact and affect biological activity by either increasing or decreasing it.

The twigs of *K. wilmsii* (88.84 mg TAE/g extract) had the greatest amount of total phenolics followed by *Monsonia angustifolia* (66.84 mg TAE/g extract) and *H. hemerocallidea* (65 mg TAE/g extract). The flavonoids were highest in "*Tšhikwana*" 1 (PM1) (9.44 mg QE/g extract), followed by the twigs of *K. wilmsii* (4.90 mg QE/g extract) and *H. hemerocallidea* (4.57 mg QE/g extract). Tannin content was greatest in *V. capensis* (47.02 mg GAE/g extract) followed by the twigs of *K. wilmsii* (22.76 mg GAE/g extract) (Table 3). The high concentrations of these polyphenolic constituents may be basis for the biological activity of the plant species.

Higher extraction temperatures were reported to enhance solubility and mass transfer rate of phytochemicals into a solvent. In addition, under such settings, the solvent is able to penetrate the cell membrane and matrices which improves the rate of extraction (Dai and Mumper, 2010). Henceforth, the choice by the traders to use boiling water as an extractant enables them to obtain considerable concentrations of bioactive compounds (phenolics, flavonoids and tannins) across the different parts of the various plant species especially the hard pieces such as the twigs and corms. Phenolics consist of one or more polar hydroxyl functional groups (Dai and Mumper, 2010), thus their high concentrations in the water decoctions was a reasonable outcome.

Studies on phenolics have led to the discovery of the biological and physiological properties, which include anti-microbial, antioxidant, anti-inflammatory, cardio-

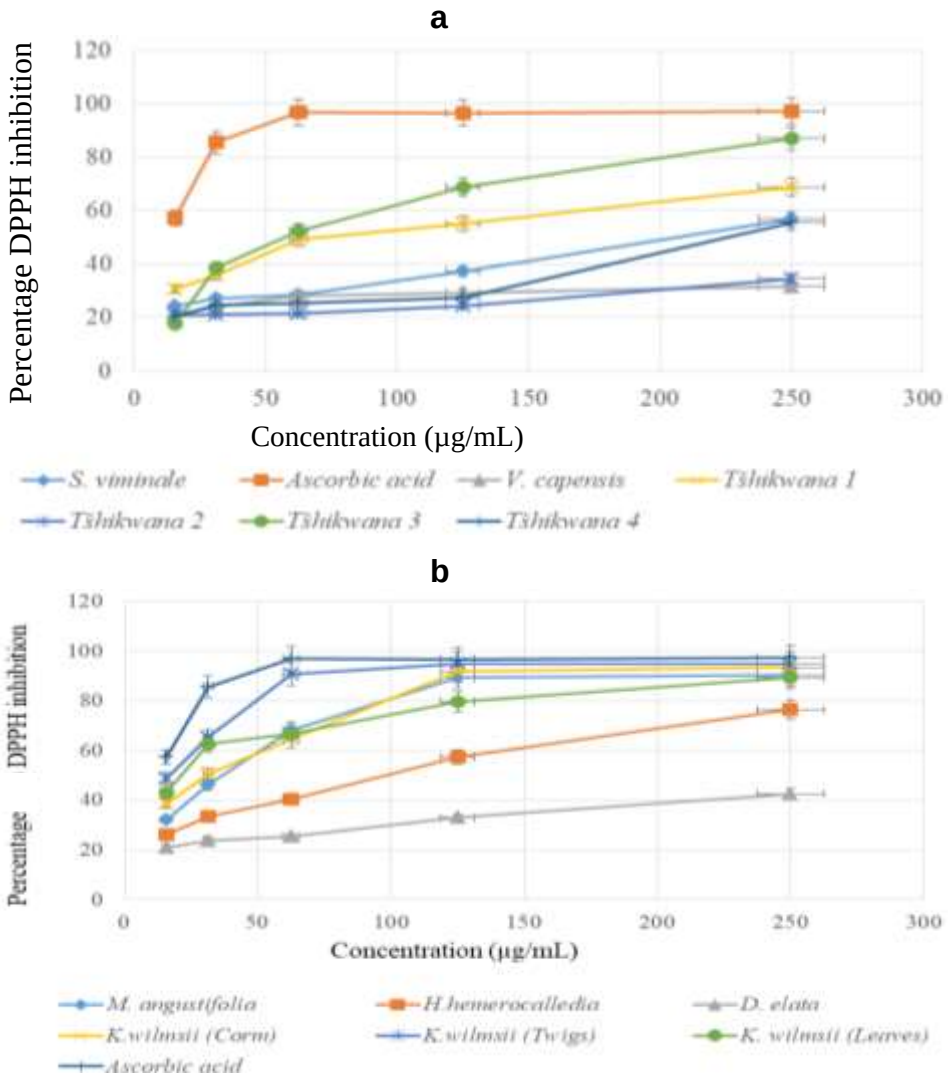

Figure 3. (a) Percentage free radical (DPPH) inhibition by plants and the "*Tšhikwana*" mixtures. L-ascorbic acid was used as a standard to which the activity of the extracts was compared. (b) DPPH inhibition of plant species with higher scavenging activity.

protective and vasodilatory activities (Goszcz et al., 2017; Shanmugapriya et al., 2013). The presence of these polyphenolic phyto-constituents in the plant decoctions displays a host of potential beneficial ways they can be used to improve health.

Free radical scavenging activity

The antioxidant activity of the decoctions was compared with that of the standard, L-ascorbic acid (Figure 3a, b). The decoctions *of D. elata*, *V. capensis*, *S. viminale*, "*Tšhikwana*" powders had the lowest antioxidant activity (Figure 3a). However, the twigs of *K. wilmsii* exhibited the greatest activity among the extracts and was comparable to that of L-ascorbic acid. This high activity was also observed in the extracts from the leaves of *M. angustifolia* (Figure 3b).

The half maximal effective concentration (EC_{50}) to inhibit DPPH was determined to further elucidate the effectiveness of the free radical scavenging activity of the extracts. Low EC_{50} values indicate that a small amount of the extract is required to inhibit half the amount of total the free radical in a solution. Higher EC_{50} values indicate the converse. For example, only 15.71 µg/mL of *K. wilmsii* twig extract was required to inhibit 50% of DPPH in a solution whilst, a greater concentration of "*Tšhikwana*" 2 (535.91 µg/mL) and *D. elata* (329.04 µg/mL) was required (Table 3). Of the plants, *K. wilmsii* displayed noteworthy antioxidant activity.

The species of *Kirkia* has been associated with high levels of fatty acids, flavonols, isocoumarin, lignans,

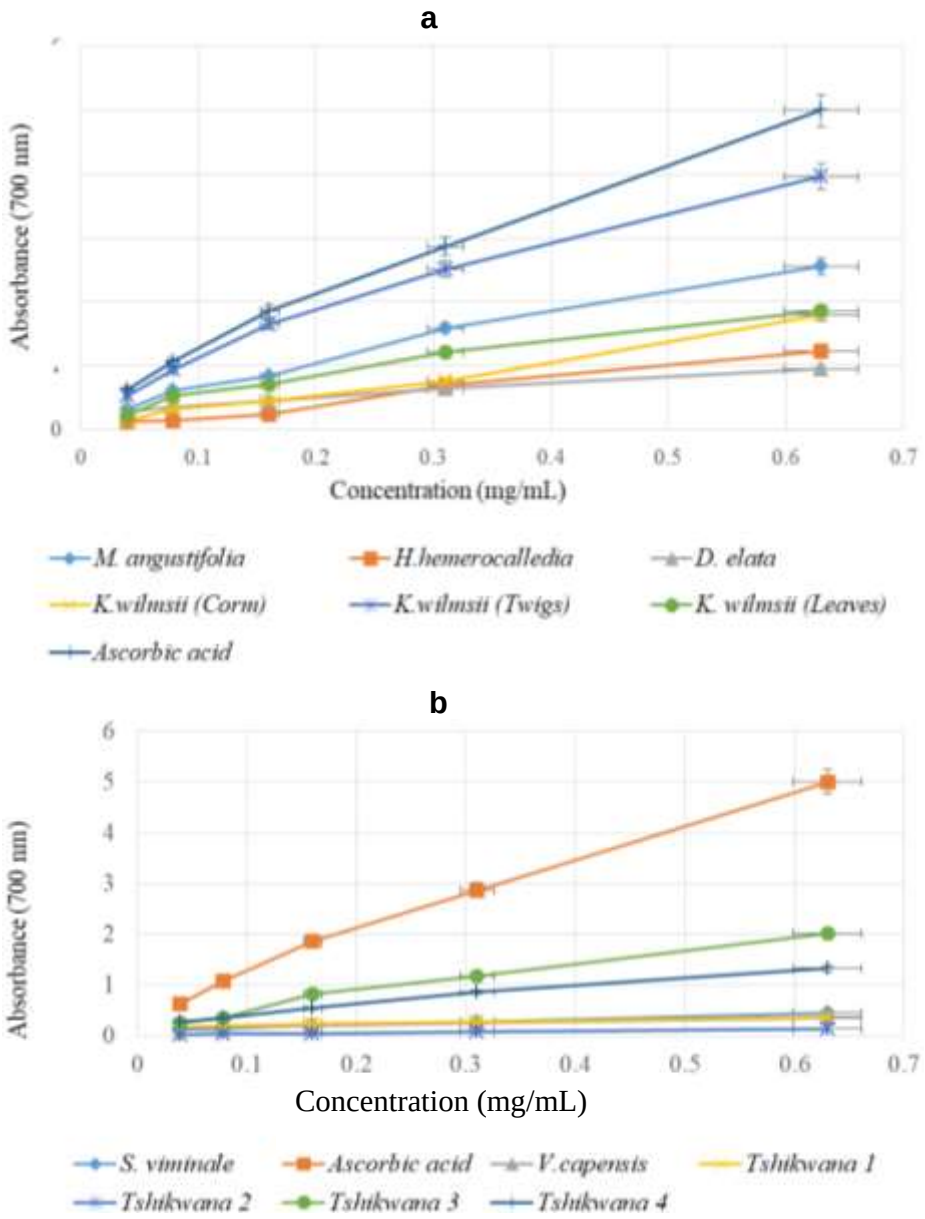

Figure 4. (a) The ferric reducing power of the plant decoctions compared with L-ascorbic acid. (b) The ferric reducing power of plants extracts at different concentrations expressed as absorbance at 700 nm wavelength.

neolignans, carotenoids, phenols and tannins which haven isolated from different parts such as the leaves, bark of the stem and roots (Maroyi, 2016). These compounds may be responsible for the high antioxidant activity of the twigs extract of the plant.

Ferric reducing power

The ferric reducing power of the extracts was compared to that of L-ascorbic acid. As shown in Figure 4a, b, greater absorbance was indicative of higher antioxidant activity. Moreover, the reducing power of the samples showed a concentration-dependent relationship. The twigs of *K. wilmsii* demonstrated the highest activity amongst the plant extracts followed by *M. angustifolia* (Figure 4a).

The ferric reducing power together with the free radical scavenging activity demonstrated that the aqueous extracts have more than one mode of antioxidant activity, that is, they can donate both protons and electrons. Furthermore, they showed that the different modes of

Table 4. Minimum inhibitory concentrations (MIC) of various plant decoctions against diarrheagenic bacteria.

Microorganism	MIC (mg/mL)												
	KWT	KWL	KWC	MA	SV	VC	HH	DE	PM1	PM2	PM3	PM4	Amp (µg/mL)
E. coli	0.16	0.31	1.25	0.04	5	5	0.63	> 5	2.5	2.5	1.25	1.25	0.08
P aeruginosa	0.63	1.25	1.25	1.25	2.5	5	2.5	5	2.5	2.5	2.5	0.63	0.16
E. faecalis	0.63	1.25	2.5	0.63	2.5	> 5	0.63	> 5	1.25	2.5	2.5	2.5	0.13
S. aureus	0.63	2.5	2.5	1.25	5	5	1.25	5	2.5	2.5	0.63	1.25	0.16
Average	0.51	1.33	1.88	0.79	3.75	> 5	2.5	> 5	2.19	2.5	1.72	1.41	0.13

Kw (T, L, C): *K. wilmsii* (twigs, leaves, corm), SV*: S. viminale*, HH: *H. hemerocallidea*, VC: *V. capensis*, MA: *M. angustifolia*, Del: *D. elata,* PM (1, 2, 3, 4): Different "*Tšhikwana*" powder.

activity can vary in strength and efficacy. For example, an extract can have excellent free radical scavenging activity but low reducing power and vice versa. The combined effect of these different modes of action may increase the efficacy of the extracts to reduce oxidative-stress related disorders.

Some of the plant materials used, particularly "*Tšhikwana*" powders, *S. viminale*, and *D. elata* had the lowest reducing powers (Figure 4b) and free radical scavenging activity (Figure 3a). Therefore, these plant species may be omitted when preparing herbal mixtures. This would reduce the rate of harvest of these species and contribute to their conservation.

Antimicrobial micro-dilution assay

The traders claimed that the plant species used to prepare the herbal mixtures had antidiarrheal activity, hence, their inclusion in the recipe. To validate this ethnopharmacological use, four different strains of common diarrhoeagenic bacteria (*E. coli*, *P. aeruginosa*, *S. aureus* and *E. faecalis*) and a yeast isolate (*C. albicans*) were selected to determine the antimicrobial activity of the plant decoctions.

The micro-dilution assay was used to determine the lowest effective concentration of the crude plant decoctions able to inhibit the growth of pathogenic microorganisms. This lowest concentration of the extract responsible for inhibitory effects was taken as the MIC with units of milligram per millilitre (mg/mL).

MIC values equal or less than 1.0 mg/mL were considered as noteworthy/significant antimicrobial activity (Orchard and van Vuuren, 2017). *S. viminale*, *V. capensis*, *M. angustifolia*, *D. elata* and "*Tšhikwana*" powders generally showed weak antibacterial activity. Significant activity against the four bacterial strains was observed with the decoction of the twigs of *K. wilmsii* (KWT) followed by the leaves of *M. angustifolia* (MA) with average MICs of 0.51 and 0.79 mg/mL, respectively across the Gram positive and negative strains (Table 4).

It was also remarkable that the Gram-negative bacteria (*E. coli* and *P. aeruginosa*) were generally more susceptible to the plant decoctions. The antimicrobial activity of the extracts against the Gram-negative bacterial strains may be attributed to their abilities to inactivate microbial adhesion mechanisms, enzymes (peptidyl transferase) and cell membrane transport proteins (Ahmed et al., 2012). The plant species that have significant antibacterial activity against the diarrhoeagenic pathogens demonstrated their potential to be used to treat infectious diseases such as diarrhoea as indicated by the traders.

Further studies are required to isolate and characterise the antibacterial compounds in the extracts to investigate in-depth the mechanism of action that would lead to pharmacological effects. Active compounds isolated from plant resources may be used for further toxicological studies and pharmaceutical development (Gado et al., 2018).

Combinational effects

A large number of combinations resulted in indifferent outcomes (Table 5). Despite this, some combinations which include *K. wilmsii* and *M. angustifolia* extracts demonstrated significant activity (MIC < 1 mg/mL). The only synergistic interaction detected was from the combination of *M. angustifolia* and *H. hemerocallidea* against *P. aeruginosa* (MIC of 0.63 mg/mL). The combinations that consisted of *V. capensis*, *S. viminale* and *D. elata* generally resulted in antagonistic interactions. From Table 4, it was shown that these three species of plants had weak antibacterial activities against the diarrhoeagenic pathogens. Therefore, the presence of these plants in the combinations decreases the antimicrobial activity because they may be diluting active compounds in the solution.

There were also no synergistic effects of the combinations towards improving activity of the individual extracts. Most of the interactions were antagonistic and indifferent. Even so, the combinations exhibited significant growth inhibitory potential against *C. albicans* because the MIC values were lower than 1 mg/mL (Table 5). The combinational effects of the different plant

Table 5. Combinational effects of the plant decoctions towards antimicrobial activity.

Combination	*E. faecalis*	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*
	FIC index ($\sum FIC$)				
KWT + KWL	0.75	2,5	6	3	4
KWT + KWC	1.25	2,5	6	4,5	3
KWT + MA	2	3	6	20	3
KWT + HH	2	3	5	5	2.25
KWL + KWC	0.75	2	2	2,5	3
KWL + MA	1.5	3	4	9	0,75
KWL + HH	3	1,5	4,5	3	18
KWC + MA	0.63	4	2	8.25	2
KWC + HH	2.5	3	3	3	10
MA + HH	2	4	0.38	17	1.25
SV + VC	1.5	1	1.5	1	4.97
SV + DE	1.5	1	1.5	1	4.97
SV +KWT	5	2.25	2.5	4.14	9.79
SV + KWL	3	1.5	3	4.28	9.79
SV + KWC	2	1.5	3	2.5	12.03
SV + MA	2.5	2.5	3	16.16	3.03
SV + HH	5	2.5	2	4.47	5.97
VC + DE	1	1	1	1	2
VC + KWT	4.5	2.25	4.5	8.06	8.31
VC + KWL	2.5	1.5	2.5	8.56	16.63
VC + KWC	1.5	1.5	2.5	2.5	9.06
VC + MA	4.5	2.5	2.5	32.3	9.06
VC + HH	4.5	2.5	1.5	4.5	3
DE + KWT	4.5	4.5	4.5	8.06	8.31
DE + KWL	2.5	1.5	2.5	4.28	16.63
DE + KWC	1.5	1.5	2.5	2.5	9.06
DE + MA	4.5	2.5	2.5	16.28	9.06
DE +HH	4.5	2.5	1.5	4.47	3

Kw (T, L, C): *K. wilmsii* (twigs, leaves, corm), SV*: S. viminale*, HH: *H. hemerocallidea*, VC: *V. capensis*, MA: *M. angustifolia*, DE: *D. elata,* PM (1, 2, 3, 4): Different "*Tšhikwana*" powders, $\sum FIC$: Sum of fractional inhibitory concentrations, $\sum FIC \leq 0.5$: Synergistic, $\sum FIC > 0.5 - 1.00$: Additive, $\sum FIC > 1.00 - \leq 4.00$: Indifferent, $\sum FIC > 4.00$: Antagonistic.

species suggested that the decoctions of the plants would be more efficacious when used singularly than in combinations.

Conclusion

The noteworthy biological activities exhibited by some of the plant species validate their potential for use for therapeutic purposes. Although synergistic effects are sought after when mixing different plant species, this study demonstrated the complexities that are associated with mixing plant decoctions. Moreover, adequate knowledge is required to know which species of plants should be mixed to enhance a therapeutic response because diverse chemical groups are prone to interact and may alter the strength of biological activity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Adamu S, Naidoo V, Eloff JN (2014). The antibacterial activity, antioxidant activity and selectivity index of leaf extracts of thirteen South African tree species used in ethnoveterinary medicine to treat helminth infections. BMC Veterinary Research 10(1):52.

Ahmed AS, Esameldin E, Elgorashi EE, Moodley N, McGaw LJ, Naidoo V, Eloff JN (2012). The antimicrobial, antioxidative, anti-inflammatory activity and cytotoxicity of different fractions of four South African Bauhinia species used traditionally to treat diarrhoea. Journal of Ethnopharmacology 143(3):826-839.

Ahmed AS, McGaw LJ, Elgorashi E, Naidoo V, Eloff JN (2014). Polarity of extracts and fractions of four Combretum (Combretaceae) species used to treat infections and gastrointestinal disorders in southern African traditional medicine has a major effect on different relevant in vitro activities. Journal of Ethnopharmacology 154(2):339-350.

Alesiani D, Canini A, D'abrosca B, Dellagreca M, Fiorentino A, Mastellone C, Monaco P, Pacifico S (2010). Antioxidant and antiproliferative activities of phytochemicals from Quince (Cydonia vulgaris) peels. Food Chemistry 118(2):199-207.

Balasundram N, Sundram K, Samman S (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence and potential uses. Food Chemistry 99(1):191-203.

Barba FJ, Zhu Z, Koubaa M, Sant'Ana AS, Orlien V (2016). Green alternative methods for the extraction of antioxidant bioactive compounds from winery wastes and by-products: A review. Trends in Food Science and Technology 49:96-109.

Chigayo K, Mojapelo PEL, Moleele SM (2016). Phytochemical and antioxidant properties of different solvent extracts of Kirkia wilmsii tubers. Asian Pacific Journal of Tropical Biomedicine 6(12):1037-1043.

Dai J, Mumper RJ (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules 15(10):7313-7352.

Deby C, Margotteaux G (1970). Relationship between essential fatty acids and tissue antioxidant levels in mice. Comptes Rendus des Seances de la Societe de Biologie et de ses Filiales 164(12): 2675-2681.

Elisha IL, Botha FS, McGaw LJ, Eloff JN (2017). The antibacterial activity of extracts of nine plant species with good activity against *Escherichia coli* against five other bacteria and cytotoxicity of extracts. BMC Complementary and Alternative Medicine 17(1):133.

Eloff JN (1998). A sensitive and quick method to determine the minimum inhibitory concentration of plant extracts for bacteria. 64(08):711-713.

Eloff JN, Suleiman MM, McGaw LJ, Naidoo V (2010). Evaluation of several tree species for activity against the animal fungal pathogen Aspergillus fumigatus. South African Journal of Botany 76:64-71.

Fouche G, Afolayan AJ, Wintola OA, Khorombi TE, Senabe J (2015). Effect of the aqueous extract of the aerial parts of Monsonia angustifolia E. Mey. Ex A. rich on the sexual behaviour of male Wistar rats. BMC Complementary and Alternative Medicine 15(1):343.

Gado D, Abdalla MA, Ahmed AS, Madikizela B, Mcgaw L (2018). Isolation and characterization of antibacterial compounds from *Loxostylis alata* A. Spreng. ex Rchb leaf extracts against *Salmonella serovars.* South African Journal of Botany 115:285-286.

Goszcz K, Duthie GG, Stewart D, Leslie SJ, Megson IL (2017). Bioactive polyphenols and cardiovascular disease: chemical antagonists, pharmacological agents or xenobiotics that drive an adaptive response? British Journal of Pharmacology 174(11):1209-1225.

Katerere DR, Eloff JN (2008). Anti-bacterial and anti-oxidant activity of Hypoxis hemerocallidea (Hypoxidaceae): Can leaves be substituted for corms as a conservation strategy? Journal of Ethnopharmacology 74(4):613-616.

Kotze M, Eloff JN (2002). Extraction of antibacterial compounds from Combretum microphyllum (Combretaceae). South African Journal of Botany 68(1):62-67.

Madikizela B, Ndhlala AR, Rengasamy KRR, McGaw LJ, van Staden J (2017). Pharmacological evaluation of two South African commercial herbal remedies and their plant constituents. South African Journal of Botany 111:291-298.

Maroyi A (2016). Ethnomedicinal uses, phytochemistry and pharmacological properties of the genus, Kirkia. Tropical Journal of Pharmaceutical Research 15(11):2507-2516.

Masoko P, Eloff JN (2005). The diversity of antifungal compounds of six South African Terminalia species (Combretaceae) determined by bioautography. African Journal of Biotechnology 4:1425-1431.

Matotoka MM, Masoko P (2018). Phytochemical screening and pharmacological evaluation of herbal concoctions sold at Ga Maja, Limpopo Province. South African Journal of Botany 117:1-10.

Ndhlala AR, van Staden J (2012). Smokescreens and mirrors in safety and quality of herbal medicines: A case of commercialized herbal preparations. South African Journal of Botany 82:4-10.

Orchard A, van Vuuren SF (2017). Commercial essential oils as potential antimicrobials to treat skin diseases. Evidence-Based Complementary and Alternative Medicine.

Sarikurkcu C, Tepe B (2015). Biological activity and phytochemistry of firethorn (*Pyracantha coccinea* M.J. Roemer). Journal of Functional Foods 19:669-675.

Semenya SS, Potgieter MJ, Erasmus LJC (2013). Indigenous plant species used by Bapedi healers to treat sexually transmitted infections: Their distribution, harvesting, conservation and threats. South African Journal of Botany 87:66-75.

Semenya SS, Potgieters MJ (2015). Kirkia wilmsii: A Bapedi treatment for hypertension. South African Journal of Botany 100:228-232.

Shanmugapriya K, Nityanandi D, Saravana PS (2013). Evaluation of antioxidant, anti-inflammatory and antimicrobial activities of two different extracts of *Camellia sinensis* (L.) O. Kuntze. International Journal of Drug Development and Research 5(3):155-168.

Tagana JC, Quanico JP, Perono RMG, Amor EC, Rivera WL (2011). Tannin-rich fraction from Terminalia catappa inhibits quorum sensing (qs) in Pseudomonas aeruginosa. Journal of Ethnopharmacology 134(3):865-871.

Tambe VD, Bhambar RS (2014). Estimation of total phenol, tannin, alkaloid and flavonoid in Hibiscus Tiliaceus Linn. wood extracts. Journal of Pharmacognosy and Phytochemistry 2(4):2321-6182.

Valifard M, Mohsenzadeh S, Kholdebarin B, Rowshan (2014). Effects of salt stress on volatile compounds, total phenolic content and antioxidant activities of Salvia mirzayanii. South African Journal of Botany 93:92-97.

van Vuuren S, Viljoen A (2011). Plant-based antimicrobial studies--methods and approaches to study the interaction between natural products. Planta Medica 77(11):1168-1182.

van Wyk B-E, Oudshoorn B, Gericke N (2013). Medicinal Plants of South Africa. (Second edition). Pretoria: Briza pp. 336.

Vijayalakshmi M, Ruckmani K (2016). Ferric reducing anti-oxidant power assay in plant extract. Bangladesh Journal Pharmacology 11(3):570-572.

Wang S, Meckling KA, Marcone MF, Kakuda Y, Tsao R (2011). Synergistic, additive, and antagonistic effects of food mixtures on total antioxidant capacities. Journal of Agricultural and Food Chemistry 59(3):960-968.

Zengin H, Baysal AH (2014). Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. Molecules 19:17773-17798.