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The evaluation of cytotoxic effects, antimicrobial activity, antioxidant activity and combination effect of *Viscum rotundifolium* and *Mystroxylon aethiopicum*

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ABSTRACT

The high rate of infections and antibiotic resistance causes serious difficulties to the healthcare system. Therefore there is a need to develop new antimicrobial agents with little side effects. The aim of the study was to investigate the impact of different solvents on extraction yields, antioxidant and antimicrobial potential, cytotoxic and combination effects of different leaf extracts of *Viscum rotundifolium* (parasite) and *Mystroxylon aethiopicum* (host). The leaves of *V. rotundifolium* and *M. aethiopicum* were collected, dried and ground into fine powder. The powdered plant leaves were extracted using solvents of varying polarity namely: hexane, ethyl acetate, acetone, methanol and water. The standard chemical tests were used to screen the phyto-constituents present in the plant leaves. The antioxidant activity was determined using 2, 2-Diphenyl-1-pycrylhydryzyl (DPPH) assay and ferric reducing power assay. The antimicrobial activity of extracts was tested against bacterial strains using serial micro-dilution assay. The cytotoxic effects of the plant extracts were determined using cell viability assay. Methanol was the best extractant compared to the other extractants with the percentage yield of 31% (*M. aethiopicum)* and 27% (*V. rotundifolium*). The occurrence of phyto-constituents (terpernoids, flavonoids, phlabotannins, tannins steroids and cardiac-glycosides, alkaloids and saponins) was similar in both plant leaves. The quantitative DPPH and ferric reducing power assay revealed the presence of antioxidant activity in all plant species. The minimum inhibitory concentration (MIC) values ranged from 0.31 mg/mL to 2.5 mg/mL. The combination of hexane, ethyl acetate and acetone extracts showed synergistic effects in inhibiting the growth of *S. aureus*. The cytotoxicity study reveals that plants are nontoxic to human cells. The study demonstrated that the extracts of the selected plants have antibacterial potential against the tested microorganisms which may be ascribed to the phytochemicals present in the plants.

1. Background

The high rate of microbial infections has fostered the generation of new antimicrobial agents, since numerous available synthetic drugs have unpleasant side effects and are ineffective against emerging microbial infections. The infectious diseases are now the second leading killers in the world and the third in the developing countries (Anthony, 2001). Around the world, a total of 17 million people die annually from bacterial infections (Butler and Buss, 2006). Antibiotic resistance is influenced by the overuse and misuse of antibiotics including the overprescribing antibiotics for minor and self-limiting bacterial infections which increases the rate of mutations in microorganisms (Straand et al., 2017). This incidence has increased the dependence on traditional medicine as primary health care. About 60% of the population in South Africa consults traditional health practitioners since traditional herbal medicine is affordable, readily available and contains fewer side effects (McGaw and Eloff, 2008).

Plants represent the greatest source of active substances that can be used in medical therapy ascribed to the large structural variation they exhibit (Cragg and Newman, 2013). These active substances are the secondary metabolites that demonstrate many biological activities and are recently the main focus for many scientific research studies. These biological activities include antimicrobial, antifungal, anticancer and anti-inflammatory (Vaghasiya et al., 2011). Most occurring secondary metabolites are aromatic substances where the major types are phenols, terpenes and nitrogen/sulfer containing compounds (Madiha et al., 2018). According to Newman and Cragg (2016) 10 out of 44 approved small molecule drugs are natural products derivatives which accounts for 25% of the 44 approved natural products.

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In different regions around the world, medicinal plants are used as a single herb or combination of herbs. However, when herbs are used in combination, the interactions that can occur as a result of the combination are complex. The most expected outcome when using the combination of herbs is the one that result in an added therapeutic benefit. However, the presence of various compounds within the herbs and their products, the herb combination is unpredictable (Gurly *et al*., 2012). Some traditional health practitioners believe that medicinal plants have no side effects because they come from nature. However, studies have indicated that some plants produce toxic reactions, mutagenic effects and allergic reactions (Senior, 1998). Therefore, safety evaluation of traditional medicinal plants is of great importance.

The two plants selected for the study based on the information from literature are: *Mystroxylon aethiopicum* (plant host) and *Viscum rotundifolium* (parasitic plant). *Mystroxylon aethiopicum* (Thumb.) Loes subsp. *schlechteri* (loes.) R.H. Archer commonly known as bushveld kooboob berry (English) is a plant that belongs to Celastracea family. *M. aethiopicum* is mostly abundant in Ethiopia, Sudan, South Africa, Namibia, Angola, Cameroon, Madagascar, Seychelles and Comoro. (Curtis and Mannheimer, 2005). Traditionally, the plant's root bark infusions are consumed in Africa to treat hemorrhagic diarrhea, stomach and respiratory tract infections, coughs and anemia (Iwu, 2014) Kilonzo et al. (2016a). reported on *in vitro* antifungal and cytotoxicity of the leaves, stem and root barks. *Viscum rotundifolium* is a parasitic plant that belongs to the Santalaceae family. *Viscum rotundifolium* is a red-berry mistletoe (English) of southern Africa that is adapted to attach to branches of host trees and access the vascular tissue of the host tree using their specialised structure called haustorium (Glatzel and Geils, 2009). *V. rotundifolium* is used traditionally to treat various ailments such as epilepsy, infertility, hypertension, arthritis and cancer (Pearden, 2001). Not much scientific information on the proper use of indigenous medicinal plants has been documented (Gaoue and Ticktin, 2018). Therefore, the aim of this study was to validate the use of the selected plants in traditional medicine by evaluating their antioxidant activity, antimicrobial activities, synergistic and antagonistic effects of host-parasite combination and cytotoxic effects on human cells. Although studies have been conducted on some of the plants, there are no studies conducted on the antibacterial activity of the parasites and hosts altogether.

2. Methodology

2.1. Plant collection and storage

The leaves of *Mystroxylon aethiopicum* (UNIN 121,992) and *Viscum rotundifolium* (UNIN 122,441) were collected during summer and deposited at Larry Leach Herbarium for identification. The leaves were dried at room temperature for about a week until the leaves were easy to break using hands. The dried plant leaves were ground to a fine powder using a blender and stored in air tight bottles in the dark until extraction.

2.2. Extraction

For successful extraction of diverse phytochemical compounds from plants, solvents with different polarities were used. Finely ground leaf material (1 g) was extracted with 10 mL of different solvents, namely hexane, acetone, ethyl acetate, methanol and water. Each solvent (100%) was allowed to extract the ground plant material for 30 min on a shaking incubator (Labotec model 20.2). The extracts were filtered through Whatman no. 1 filter paper using a Buchner funnel. The resulting filtrates were evaporated under vacuum using Buchi rotavaporator R-114 (Labotec). The concentrated extracts were poured into pre-weighed glass vials. The extraction procedure was repeated twice to exclusively extract the plant material. The filtered solvent was allowed to dry under a stream of cold air (at room temperature). The masses of the extracts yield were determined and the extracts were reconstituted in acetone (100%) to a final concentration of 10 mg/mL. The reconstituted extracts (10 mg/mL) were used as stock solutions to prepare the working solutions of different concentrations while performing the quantitative phytochemical assay, antioxidant assay and antibacterial assay).

2.3. Screening of phyto-constituents

The presence of the following phytochemicals in the plant materials was performed using standard chemical tests: saponins, terpenoids, cardiac glycosides, alkaloids (Odebiyi and Sofowora, 1978). Phlobatannin, tannins, flavonoids and steroids were tested using the methods reported by Borokini and Omotayo (2012).

2.4. Quantitative phytochemical analysis

2.4.1. Total phenolic content

The quantity of phenolics present in each plant extract was determined by using the Folin-Ciocaltleu reagent method as Velioglu et al. (1998). The extract (10 μL) was diluted with 490 μL of distilled water, followed by the addition of 0.25 mL of folin-ciocaltleu reagent. To stop the reaction, sodium carbonate (1.25 mL) was added and the mixture was 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 725 nm. A blank and the standard curve were prepared in a similar manner, except that the plant extracts were replaced by distilled water and various concentration of gallic acid (1.25, 0. 63, 0.31, 0.16, 0.08 mg/mL), respectively. The results obtained from the linear regression formula of the tannic acid standard curve were expressed as milligram gallic acid equivalence/gram of extract (mg of GAE/g extract). The experiment was conducted in triplicates and independently repeated three times.

2.4.2. Total tannin content

The Folin–Ciocalteu method described by Tambe and Bhambar (2014) was used to determine the tannin content in the plant extracts. Briefly, 100 μL of 10 mg/mL extract was added to a clean test tube containing 7.5 mL of distilled water. The Folin–Ciocalteu reagent (0.5 mL) was added to the mixture and vortexed. Ten milliliter of a 35% solution of sodium carbonate (Na_2CO_3) was added to 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 by distilled water. The mixture was shaken and kept at room temperature for 30 min in the dark. Gallic acid was used as a standard and reference standard solutions (1–0.625 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 without adding any extract. 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.

2.4.3. Total flavonoid content

Total flavonoid content in each plant extract was determined by the aluminum chloride colorimetric assay described by Tambe and Bhambar (2014). Briefly, 100 μL of 10 mg/mL extract was added to 4.9 mL of distilled water in a clean test tube. To this reaction mixture, 300 μL of 5% sodium nitrite ($NaNO_2$) dissolved in distilled water was added and the mixture was left at room temperature for 5 min. After the 5 min, 300 μL of 10% aluminum chloride ($AlCl_3$) (dissolved in distilled water) was added to the reaction mixture. The reaction was allowed to stand for 5 min at room temperature, after which 2 mL of sodium hydroxide (NaOH) was added to the solution. The mixture in

the test tube was made up to 10 mL with distilled water. Quercetin was used as a standard. Different concentrations (500–31.5 μg/mL) of the quercetin were prepared in the same method as the extracts. 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 and standard samples, however, 100 μL of distilled water was added instead of the concoctions. The total flavonoid content of the samples was expressed as milligram quercetin equivalence/gram of extract (mg QE/g extract).

2.5. Quantitative antioxidant assay

2.5.1. Quantitative DPPH free radical scavenging assay

Free radical scavenging activity of the concoctions was quantified and compared using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma) method reported by Brand-Williams (1995). Briefly, different concentrations of the concoctions (250–15.63 μg/mL) were prepared to a volume of 1 mL of the solution. L-Ascorbic acid was used as standard by preparing the same concentration range as the concoctions. To this 1 mL solutions, 2 mL of 0.2 mmol/L DPPH solution dissolved in methanol was added and vortexed thoroughly. All the prepared mixtures were left to stand in the dark for 30 min. 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. The absorbance of the solutions was read at 517 nm and the percentage antioxidant potential was calculated using the formula:

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

2.5.2. Ferric reducing power assay

The ferric reducing power of the plant extracts was determined using the methods of Oyaizu (1986). Five different concentrations of the plant extracts (625–39 μg/mL) were prepared by serially diluting a stock solution of 1250 μg/mL. The different concentrations (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 (1% w/v in distilled water) respectively, in a test tube. The mixtures were vortexed after addition of solutions and were incubated at 50 °C for 20 min. Two mL of trichloroacetic acid (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. L-Ascorbic acid (625–39 μg/mL) was used as a positive control. The experiments were performed in triplicates and repeated three times.

2.6. Antibacterial activity assay

2.6.1. Microorganisms used in this study

The pathogenic bacterial species selected were: Gram-positive (*Staphylococcus aureus* ATCC 29,213 and *Enterococcus faecalis* ATCC 29,212) and Gram-negative (*Pseudomonas aeruginosa* ATCC 27,853 and *Escherichia coli* ATCC 25,922). These microorganisms were selected based on the recommendation of the Clinical Laboratory Standards Institute (CLSI) (NCCLS, 1990). These bacterial species were maintained on a nutrient agar at 4 °C and later inoculated in Müller-Hinton (MH) broth and incubated at 37 °C for 12 h prior to the screening procedures.

2.6.2. Quantitative antibacterial activity by micro-dilution assay

Antibacterial activity was evaluated by the determination of the minimum inhibitory concentration (MIC) for each extract on *E. faecalis, E. coli, P. aeruginosa* and *S. aureus* following the micro-dilution method developed by Eloff (1998). Sterile distilled water (100 μL) was added to each well of a 96 well microtitre plate using a multichannel micropipette. The plant extracts (100 μL) were separately serially diluted to 50% with the distilled water in the wells of the 96 well microtitre plates. The culture (100 μL) was aseptically added to each well. The antibiotic chloramphenicol was used as a positive control for bacteria. Sterile distilled water served as a negative control. The microtitre plates were covered with laboratory plastic wrap and incubated for 24 h at 37 °C for bacteria. After incubation, 40 μL of 0.2 mg/mL of p-iodonitrotetrazolium chloride (INT) (Sigma) dissolved in distilled water was added to each well of the microtitre plates and further incubated for 30 min. INT, served as a growth indicator, whereby the growth of the microorganism reduced the tetrazolium salt to a purple formazan. MIC was determined as the lowest concentration of the plant extract that was able to inhibit bacterial growth *i.e.* MIC values were recorded as the concentrations of the lowest clear wells of each extract. Microbial growth in the wells was indicated by a violet-purple color, whereas clear wells indicated growth inhibition. The assay was repeated three times in duplicate.

2.6.3. Antibacterial interaction activity

The stock solutions (10 mg/mL) of hexane, ethyl acetate, acetone and methanol extracts of each plant were prepared by reconstituting the extracts in acetone (100%). Three different host and parasite combinations were performed. For 1:1 test combinations, 50 μL of each of the two extracts were mixed to make up a volume of 100 μL in the first wells of a 96-well microtitre plate. Each extract contributed 33.3 μL and 66.6 μL for the 1:2 and 66.6 μL and 33.3 μL for the 2:1 combination respectively, to make up 100 μL in the first wells of a 96-well microtitre plate. MIC values were determined for each of these combinations to establish any interaction effect following the antibacterial assay described in the section above. Following investigations of the independent MIC of the selected plants, the synergistic or antagonistic interactions between the plants were investigated. This was achieved by determining the MIC of the combinations exhibiting antibacterial activity to establish any interaction effect. The fractional inhibitory concentration (FIC) was calculated for the 1:1 combinations of the plants. This was determined with the equation below, where (i) and (ii) represented the different 1:1 plant combinations (Mabona et al., 2013). The FIC index was expressed as the sum of FIC (i) and FIC (ii) and this was used to classify the interaction as either synergistic (≤0.50), additive (0.50–1.00), indifferent (>1.00–4.00) or antagonistic (>4.00) (van Vuuren and Viljoen, 2008).

$$\mathrm{FIC}(i) = \frac{\text{MIC of (a)in combination with (b)}}{\text{MIC of (a)independently}}$$

$$\mathrm{FIC}(ii) = \frac{\text{MIC of (b)in combination with (a)}}{\text{MIC of (b)independently}}$$

2.7. Cytotoxicity assay

The toxic effects of the selected plants (acetone extracts) on the human liver (C3A) cells obtained from the culture collection of the Department of Veterinary Tropical Diseases (University of Pretoria) was determined by the 3-(4, 5-dimethylthiazol-2-yl)−2, 5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983). The cells were maintained in Minimum Essential Medium (MEM, Whitehead Scientific) supplemented with 0.1% gentamicin (Virbac) and 5% foetal calf serum (Highveld Biological). The cell suspension (5 × 104 cells/mL) was seeded in a sterile 96-well microtitre plate and incubated for 24 h at 37 °C in 5% CO2 for the cells to attach. The MEM was aspirated

and the cells were washed with 150 μL phosphate buffered saline (PBS, Whitehead Scientific). The cells were treated with different concentrations of the extracts (100–1000 μg/mL) prepared in MEM. The microtitre plates were incubated for 48 h with the extracts in the same conditions as described earlier. Untreated cells were included as a negative control. After treatment, the treatment medium was aspired and replaced with 200 μL of fresh MEM and then 30 μL of MTT (5 mg/mL) in PBS (Sigma) and the plates were incubated further for 4 h at 37 °C. The medium was removed and replaced with 50 μL of DMSO to dissolve the MTT formazan crystals. The absorbance was measured in a microplate reader (BioTek Synergy) at 570 nm. Cytotoxicity was expressed as the concentration of test sample resulting in a 50% reduction of absorbance compared to untreated cells (LC50 values). All the analysis was made in quadruplicate. The selectivity index (SI) was expressed as LC50/ MIC value.

2.8. Statistical analysis

Each experiment was performed in triplicates and the results were expressed as mean values ± the standard deviation. Microsoft Excel® was used to enter and capture data. Various graphs and tables were extracted from this data. Data was then exported to SPSS for further analysis. The statistical comparisons were carried out by analysis of variance (ANOVA) Tukey's multiple comparison test. P value $<$ 0.05 was considered as significant. SPSS 25.0 was employed for statistical analysis.

3. Results

3.1. Extraction

The mass extracted from each plant material using the different solvents are represented in Fig. 1. The results showed a significant difference in the extraction yield of *V. rotundifolium* and *M. aethiopicum* plants (Fig. 1). Methanol, among all tested solvents, resulted in high mass extracted, followed by water in both plants. In *M. aethiopicum*, acetone was not different from hexane and hexane was not different to ethyl acetate. However, acetone was higher than ethyl acetate. Whereas in *V. rotundifolium* plant, hexane was higher than acetone. Ethyl acetate was not different from hexane and acetone in both plants.

Table 1
Phyto-constituents of *V. rotundifolium* and *M. aethiopicum* leaf extracts.

	Plants	
Phyto-constituents	*V. rotundifolium*	*M. aethiopicum*
Terpenoids	+	+
Alkaloids	–	–
Saponins	–	–
Flavonoids	+	+
Phlabotannins	+	+
Cardiac- glycosides	+	+
Tannins	+	+
Steroids	+	+

Key: (+) present, (-) absent.

3.2. Screening of phyto-constituents

Preliminary phytochemical screening (Table 1) revealed the presence of terpernoids, flavonoids, phlabotannins, tannins steroids and cardiac-glycosides and the absence of alkaloids and saponins all the plants.

3.3. Quantitative phytochemical analysis

The estimated concentration of the total phenolic content ranged from 28.324 to 893.210 mgGAE/g extract, the total tannin content ranged from 2.135 to 18.438 mgGAE/g of extract and the total flavonoid content ranged from 0.048 to 10.315 mgQE/g of extract (Table 2). Solvents had high significant effect on the total phenolic, flavonoid and tannin content. The ethyl acetate extract of *M. aethiopicum* had the highest concentration of phenolic content (893.210 ± 3.016 mgGAE/g of extract) and tannin content (18.438 ± 0.074). While the hexane extract of *V. rotundifolium* had high flavonoid content (10.315 ± 0.871). In *V. rotundifolium* plant, the methanol extract was significantly higher in phenolic content. While, the hexane extract was significantly high in flavonoid and tannin

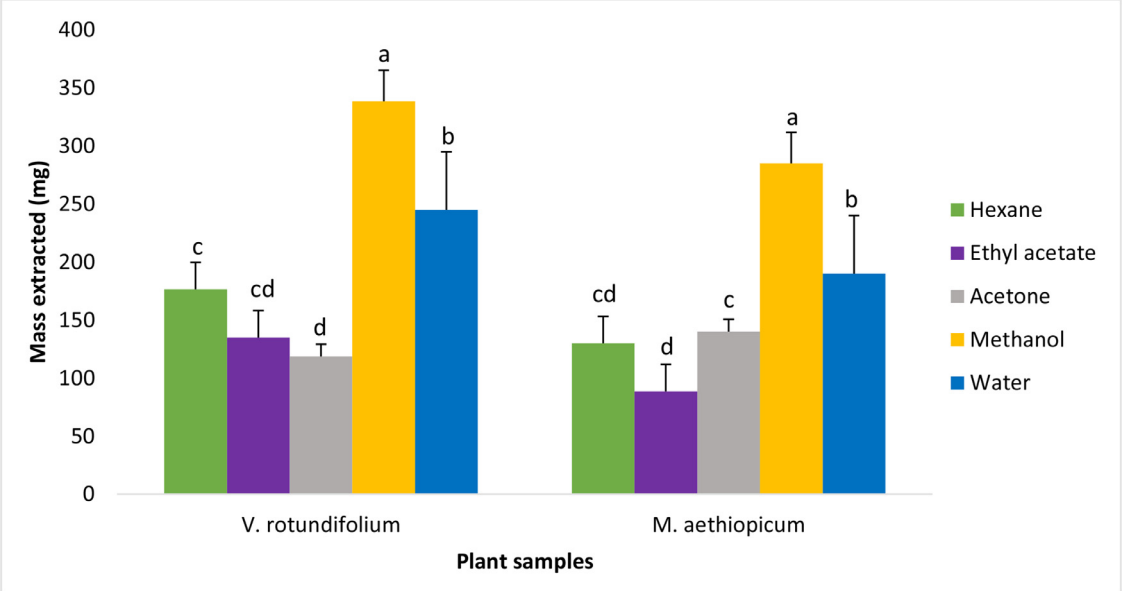

Fig. 1. The mass of *V. rotundifolium* and *M. aethiopicum* dried leaves extracted with hexane, ethyl acetate, acetone, methanol and water. Vertical error bars illustrate standard deviation (n = 3). Different lowercase characters represent significant difference at p<0.05 by Tukey's test.

Table 2
The total phenol, tannin and flavonoid content of *M. aethiopicum* and *V. rotundifolium* leaf extracts.

Solvents	Phenolic content (mg GAE/g extract)	Flavonoid content (mg QE/g extract)	Tannin content (mg GAE/ g extract)
V. rotundifolium			
Hexane	$702.426^{b} \pm 3.661$	$10.315^{a} \pm 0.871$	$16.757^{a} \pm 0.358$
Ethyl acetate	$187.310^{d} \pm 2.494$	$6.088^{b} \pm 0.777$	$15.514^{b} \pm 0.441$
Acetone	$338.959^{c} \pm 3.016$	$6.631^{b} \pm 0.823$	$7.770^{d} \pm 0.477$
Methanol	$748.899^{a} \pm 3.170$	$2.869^{c} \pm 0.405$	$10.874^{c} \pm 0.246$
Water	$28.324^{e} \pm 3.170$	$2.394^{c} \pm 0.241$	$7.272^{d} \pm 0.521$
M. aethiopicum			
Hexane	$553.713^{b} \pm 4.320$	$3.984^{b} \pm 0.692$	$13.181^{b} \pm 0.074$
Ethyl acetate	$893.210^{a} \pm 3.016$	$6.099^{a} \pm 0.117$	$18.438^{a} \pm 0.074$
Acetone	$191.224^{d} \pm 2.075$	$2.278^{c} \pm 0.559$	$3.619^{c} \pm 0.140$
Methanol	$322.816^{c} \pm 1.830$	$0.048^{d} \pm 0.049$	$4.048^{c} \pm 0.658$
Water	$86.048^{e} \pm 3.016$	$0.852^{d} \pm 0.109$	$2.135^{d} \pm 0.239$

Key: GAE - garlic acid equivalence, QE - quercetin equivalence.
Results are represented as mean of triplicates ± standard deviation. Values with different subscript letters in the same column are significantly different ($p<0.05$, one-way ANOVA followed by Tukey's test).

content. In the *M. aethiopicum* plant, the ethyl acetate extract was significantly higher in phenolic content. While, the ethyl acetate extract was significantly higher in both flavonoid and tannin content.

3.4. Quantitative antioxidant assay

The antioxidant activity of the plant extracts was expressed as the percentage inhibition where it is observed that the antioxidant activity increases with increase in concentration of the plant extract (Fig. 2A and 2B). The methanol extract of *V. rotundifolium* (Fig. 3A) had the highest antioxidant activity while the acetone and water extract had the lowest activity. The ethyl acetate extract of *M. aethiopicum* (Fig. 3B) had the highest activity while the methanol extract had the lowest activity.

3.5. Antibacterial activity assay

The results of antibacterial assay exhibited that the MIC values range from 0.31 to 2.5 mg/mL (Table 3). The lowest average MIC of the plant extracts was against *E. faecalis* (1.14 mg/mL) followed by *P. aeruginosa* (1.28 mg/mL) and lastly *E. coli* (1.50 mg/mL) and *S. aureus* (1.69 mg/mL). The tested bacteria were susceptible to the treatment of *V. rotundifolium* and *M. aethiopicum* extracts. The highest total activity (Table 4) was observed with the hexane extract of *V. rotundifolium* against *P. aeruginosa* (594 mL/g) followed by ethyl acetate extract of *M. aethiopicum* against *E. faecalis* (500 mL/g) and the hexane extract of *V. rotundifolium* against *E. coli* (302 mL/g) and the ethyl acetate extract of *M. aethiopicum* against *S. aureus* (238 mL/g).

3.6. Antibacterial interaction activity

The hexane, ethyl acetate, acetone and methanol extracts of all the plants exhibited the highest activity against the tested bacteria therefore, they were selected for synergistic and antagonistic study. The hexane, ethyl acetate, acetone and methanol extract of the host and parasite were combined to determine the effect of the combination on the antibacterial activity (Table 5). The hexane and ethyl acetate extracts had notable antibacterial activity against all tested microorganisms with the average MIC of 2.35 mg/mL and 0.39 mg/mL respectively. The 1:1 combination of acetone extracts also had the potent antibacterial activity against all tested microorganisms with the average MIC of 0.52 mg/mL. When the *V. rotundifolium* and *M. aethiopicum* were combined in the 1:1, 1:2 and 2:1 ratio, the hexane, ethyl acetate, acetone and methanol extracts showed potent antibacterial activity against *S. aureus* (Table 5). There was no notable difference between the antibacterial activity of the 1:1, 1:2 and 2:1 combinations of the *V. rotundifolium* and *M. aethiopicum* plant extracts against *E. faecalis* when compared to the individual MIC. The 1:1 combination decreased the activity of the plant extracts against *P. aeruginosa* while 1:2 combination showed highly significant antibacterial activity but the opposite was observed in the 2:1 combination. There was no significant difference in the antibacterial activity of the plant extracts in all combinations against *E. coli*.

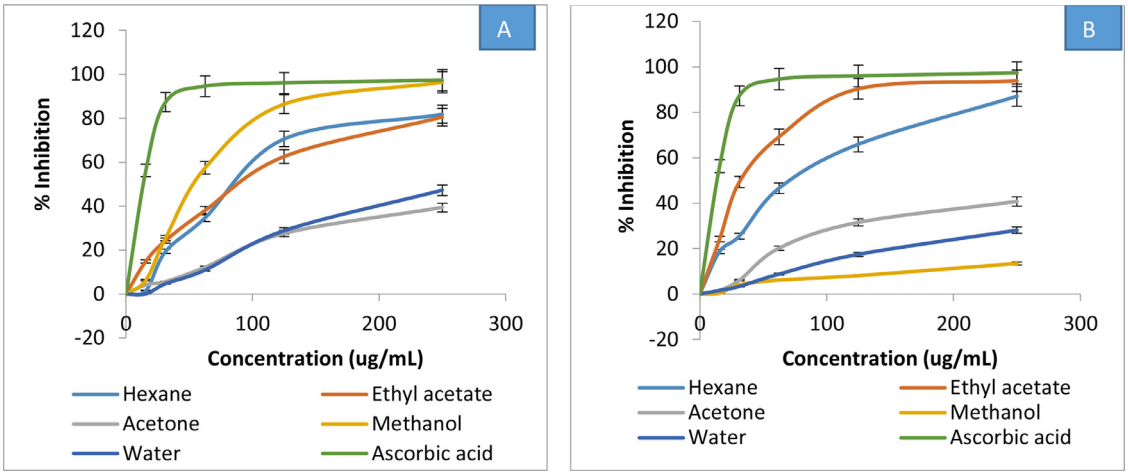

Fig. 2. The percentage of free radical (DPPH) inhibition of *V. rotundifolium* (A) and *M. aethiopicum* (B). The values used are mean triplicates ± standard deviation.

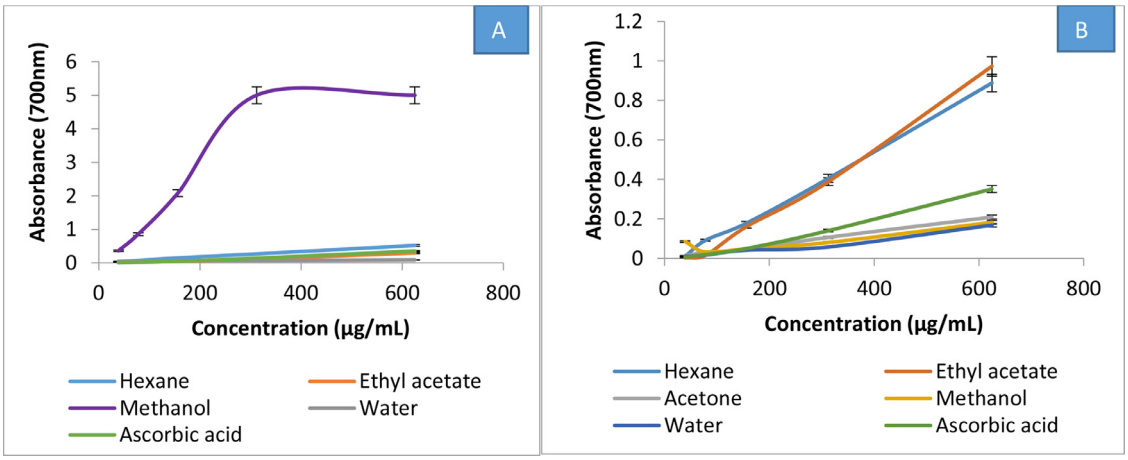

Fig. 3. The percentage of free radical (DPPH) inhibition of *V. rotundifolium* (A) and *M. aethiopicum* (B). The values used are mean triplicates ± standard deviation.

The combination of hexane, ethyl acetate and acetone extracts of *V. rotundifolium* and *M. aethiopicum* showed synergistic effects in inhibiting the growth of *S. aureus*. While the combination of hexane and acetone extracts showed antagonistic effects in inhibiting the growth of *E. coli*.

3.7. Cytotoxicity assay

Similar results were observed in both *V. rotundifolium* and *M. aethiopicum* plants where different concentration had highly significant effects on the percentage viability of cells (Table 7). The concentrations 400, 600, 700 and 1000 μg/mL had high percentage viability in both plants. In *M. aethiopicum* plant, 600 μg/mL reported higher viability than 100, and 200 μg/mL concentrations. Furthermore, the 100 μg/mL concentration reported significantly low viability in all the plants. However, there was no significant difference in the viability of untreated cells and cells treated with 100 μg/mL. The results of the study showed no significant difference in cell viability at the concentration of 400 and 1000 μg/mL in both plants. The 100 μg/mL concentration reported the lowest viability in both plants respectively. However, the viability was higher in *V. rotundifolium* plant than in *M. aethiopicum* plant.

4. Discussion

The extraction procedure is the most important step in processing the bioactive constituents from the plant material. The various organic solvents used during extraction vary from polar to non-polar and the choice of solvents depends on what is intended with the extract. The extraction yield and biological activity of the extracts are affected by both the extraction technique and the types of selected solvents. The different biologically active compounds present within the plant materials have the different solubility properties, hence solvent of differing polarity are used during the extraction process (Mahdi-Pour et al., 2012). Methanol had a highly significant effect on the extraction of the plant materials, therefore, it is considered a good extractant resulting in high amount of mass extracted. The results are in agreement with the study Truong et al. (2019). Methanol has the ability to extract both the polar and non-polar compounds present in the plant materials; hence it has the highest extraction yield (Eloff, 1998). Previous research studies on plants indicated that various secondary metabolites are potential antimicrobial agents although in many cases they serve as defense mechanisms against microorganisms in plants (Vaghasiya et al., 2011). Therefore, it was important to screen the different secondary metabolites. It was observed that similar phytochemicals are present (terpernoids, flavonoids, phlabotannins, tannins steroids and cardiac-glycosides, alkaloids and saponins) in both the parasite and host plants, this may be because the pharmacologically active compounds pass from the host tree to the parasite plants (Khwaja et al., 1986).The flavonoids and tannins are known to exhibit antioxidant activity (Złotek et al., 2016) while terpenoids are known to exhibit the antimicrobial activity. The presence of these compounds makes the plants (*V. rotundifolium* and *M. aethiopicum*) a potential source of antibacterial agents. *M. aethiopicum* ethyl acetate extract had the highest concentration of phenolic content (893.210 ± 3.016 mgGAE/g of extract) and tannin content (18.438 ± 0.074). However, in the study by Ahreen *et al.* (2010) and Ao *et al.* (2007), ethyl acetate was found to have lower concentrations. The hexane extract of *V. rotundifolium* was high in flavonoid content. The total phenolic content in all the plants was higher than the flavonoid and tannin content. This is because the phenolic phytochemicals include the flavonoids and tannins (Stankovic, 2011).

The antioxidant activity of the plant extracts was also evaluated using the ferric reducing power method. The ferric reducing power measures the reduction of ferric ion (fe^{3+}) to ferrous ion (fe^{2+}) in the presence of antioxidants (Meir et al., 1995). The methanol of *V. rotundifolium* had the highest reducing power as compared to ascorbic acid and other extracts (Fig. 3A). The reducing power of the leaves of *M. aethiopicum* was found remarkable where the ethyl acetate and hexane extracts had high reducing ability as compared to ascorbic

Table 3
The MIC values of *V. rotundifolium* and *M. aethiopicum* leaf extracts (mg/mL) against the test microorganisms.

Microorganisms	*V. rotundifolium*					*M. aethiopicum*						
	H	E	A	M	W	H	E	A	M	W	Avg	PC
S. aureus	2.5	1.25	2.5	2.5	1.25	0.63	1.25	1.25	1.25	>2.5	**1.69**	0.02
E. faecalis	0.63	0.31	0.63	>2.5	>2.5	0.31	0.16	0.63	1.25	>2.5	**1.14**	0.02
P. aeruginosa	0.31	0.63	1.25	2.5	1.25	0.31	0.31	1.25	2.5	>2.5	**1.28**	1.25
E. coli	0.63	0.63	>2.5	>2.5	>2.5	0.63	0.63	0.63	2.5	>2.5	**1.50**	2.50
Average	**1.02**	**0.71**	**1.72**	**2.50**	**1.88**	**0.47**	**0.59**	**0.94**	**2.19**	**2.19**		

Key: H- Hexane, E- Ethyl acetate, A- acetone, M- Methanol, W- Water, Avg- Average, PC- Positive control.

Table 4
Total activity of *V. rotundifolium* and *M. aethiopicum* leaf extracts (mL/g).

Microorganisms	*V. rotundifolium*					*M. aethiopicum*					
	H	E	A	M	W	H	E	A	M	W	Avg
S. aureus	76	112	48	124	208	238	64	104	216	72	**126**
E. faecalis	302	452	190	124	104	484	500	206	216	72	**265**
P. aeruginosa	594	222	96	124	208	484	258	104	108	72	**227**
E. coli	302	222	48	124	104	238	127	206	108	108	**159**
Average	**318**	**252**	**96**	**124**	**156**	**361**	**237**	**155**	**162**	**81**	

Key: H- Hexane, E- Ethyl acetate, A- Acetone, M- Methanol, W- Water, Avg- Average, PC- Positive control.

Table 5
The MIC (mg/mL) values of *V. rotundifolium* and *M. aethiopicum* plant extracts in combination.

Microorganisms	VR + MA (1:1)				VR + MA (1:2)				VR + MA (2:1)				
	H	E	A	M	H	E	A	M	H	E	A	M	Avg
S. aureus	0.16	0.16	0.31	0.63	0.16	0.16	0.31	0.63	0.16	0.31	0.31	0.63	**0.85**
E. faecalis	0.31	0.31	0.63	1.25	0.63	0.31	0.63	1.25	0.31	0.31	0.63	1.25	**0.71**
P. aeruginosa	0.63	0.63	0.63	0.63	0.16	0.16	0.31	1.25	0.31	0.31	0.63	0.63	**0.77**
E. coli	2.5	1.25	2,5	1.25	1.25	1.25	2.5	2.5	0.63	0.63	1.25	1.25	**1.47**
Average	**0.9**	**0.59**	**0.52**	**0.94**	**0.55**	**0.47**	**0.94**	**1.41**	**0.35**	**0.39**	**0.71**	**0.94**	

Key: *H*= hexane, *E*= Ethyl acetate, *A*= acetone, *M*= Methanol, *W*= Water, Avg= average, VR=*Viscum rotundifolium*, MA= *Mystroxylon aethiopicum*.

acid and other extracts (Fig. 3A and 3B). Since the phenolic compounds including the flavonoid are known to have antioxidant activity, it is likely that the antioxidant activity of these plant extracts is attributed by these compounds (Tepe et al., 2006).

According to Gupta et al. (2017), the plant extracts with MIC value of less than 1 mg/mL are highly recommended in drug discovery. The hexane and ethyl acetate extracts of both *V. rotundifolium* and *M. aethiopicum* showed high activity against the tested microorganisms. These results are similar to those found by Nemudzivhadi and Masoko (2015). The antibacterial activity of these plants may be due to the presence of various bioactive compounds which includes tannins, flavonoids and terpenoids which were reported to exhibit various pharmacological activities, including antibacterial activity (McGaw and Eloff, 2008).

The total activity indicates the volume in which an extract from 1 g of the plant material can be diluted and still inhibit the growth of the test organism (Eloff, 2004). The highest total activity was observed with the hexane extract of *V. rotundifolium* against *P. aeruginosa* (594 mL/g) followed by ethyl acetate extract of *M. aethiopicum* against *E. faecalis* (500 mL/g) and the hexane extract of *V. rotundifolium* against *E. coli* (302 mL/g) and the ethyl acetate extract of *M. aethiopicum* against *S. aureus* (238 mL/g). Therefore, in case of the total activity of *V. rotundifolium* (594 mL/g), it means that the quantity extracted from 1 g of the *V. rotundifolium* material with acetone can be diluted to 594 mL and still have the ability to inhibit the growth of *P. aeruginosa*.

The 1:1 combination of acetone extracts also had the potent antibacterial activity against all tested microorganisms with the average MIC of 0.52 mg/mL. When the *V. rotundifolium* and *M. aethiopicum* were combined in the 1:1, 1:2 and 2:1 ratio, the hexane, ethyl acetate, acetone and methanol extracts showed potent antibacterial activity against *S. aureus* (Table 5). There was no notable difference between the antibacterial activity of the 1:1, 1:2 and 2:1 combinations of the *V. rotundifolium* and *M. aethiopicum* plant extracts against *E. faecalis* when compared to the individual MIC. The 1:1 combination decreased the activity of the plant extracts against *P. aeruginosa* while 1:2 combination showed highly significant antibacterial activity but the opposite was observed in the 2:1 combination. There was no significant difference in the antibacterial activity of the plant extracts in all combinations against *E. coli*.

The fractional inhibitory concentration index (FIC_i) was used to evaluate the combination effect of the plant extracts on the antibacterial activity (Table 6). Synergy occurs as a result of the interaction

Table 6
The fractional inhibitory concentration of *V. rotundifolium* and *M. aethiopicum*.

Microorganisms	Combination	MIC (mg/mL)	FIC values	FIC index	Outcome	
			VR	MA		
S. aureus	Hexane	0.16	0.06	0.25	0.32	Synergistic
	Ethyl acetate	0.16	0.13	0.13	0.26	Synergistic
	Acetone	0.31	0.12	0.25	0.37	Synergistic
	Methanol	0.63	0.25	0.5	0.76	Additive
E. faecalis	Hexane	0.31	0.49	1	1.49	Indifferent
	Ethyl acetate	0.31	1	1.94	2.94	Indifferent
	Acetone	0.63	1	1	2.00	Indifferent
	Methanol	1.25	0.5	1	1.50	Indifferent
P. aeruginosa	Hexane	0.63	1.97	2.03	4.00	Indifferent
	Ethyl acetate	0.63	1	2.03	3.03	Indifferent
	Acetone	0.63	0.5	0.5	1.01	Indifferent
	Methanol	0.63	0.25	0.25	0.50	Synergistic
E. coli	Hexane	2.5	3.97	3.97	7.94	Antagonistic
	Ethyl acetate	1.25	1.98	1.98	3.97	Indifferent
	Acetone	2.5	1	3.97	4.97	Antagonistic
	Methanol	1.25	0.5	0.5	1.00	Additive

Key: FIC= Fractional inhibitory concentration.

Table 7
The effect of *V. rotundifolium* and *M. aethiopicum* acetone extracts on the viability of human liver (C3A) cells.

Concentration	*V. rotundifolium*	*M. aethiopicum*
0	$100.00^{ab}\pm0.00$	$100.00^{cd}\pm0.00$
100	$83.33^{c}\pm4.11$	$94.00^{d}\pm3.27$
200	$86.67^{bc}\pm0.94$	$106.00^{bc}\pm0.82$
300	$99.67^{ab}\pm8.18$	$112.00^{ab}\pm4.55$
400	$103.00^{a}\pm1.41$	$113.00^{ab}\pm4.55$
500	$100.33^{ab}\pm6.34$	$116.00^{a}\pm2.16$
600	$108.67^{a}\pm6.94$	$120.00^{a}\pm2.16$
700	$105.67^{a}\pm2.05$	$114.00^{ab}\pm2.16$
800	$97.00^{ab}\pm3.74$	$117.67^{a}\pm3.09$
900	$100.67^{ab}\pm5.44$	$115.00^{ab}\pm2.16$
1000	$108.33^{a}\pm4.50$	$112.33^{ab}\pm1.89$

Values with different subscript letters in the same column are significantly different ($p<0.05$, one-way ANOVA followed by Tukey's test).

between two or more compounds in ways that mutually enhance each other's effect more significantly than individual contributions (Williamson, 2001). The combination of hexane, ethyl acetate and acetone extracts showed synergistic effects in inhibiting the growth of *S. aureus w*hile, the combination of hexane and acetone extracts showed antagonistic effects in inhibiting the growth of *E. coli*. The presence and distribution of the phytochemicals on the parasite plants are partially dependent on the host plant (Deeni and Sadiq, 2002), there is no notable difference in the overall antibacterial activity of the combination of the parasite and host extracts relative to the individual activities.

The findings from this study indicate that *M. aethiopicum* shows no signs of toxicity on the human liver (C3A) cells at a concentration up to 1000 μg/mL. The similar results were found in the toxicity study by Kilonzo et al. (2016b) on the root bark aqueous extract of *M. aethiopicum* tested in albino mice were it was found that the plant is safe when administered orally up to the concentration of 5000 mg/kg.

5. Conclusion

The study demonstrated significant difference between the extraction yields of the different solvents. Among the solvents, methanol was the best extractant. The extracts of the selected plants have antibacterial potential against the tested microorganisms which may be ascribed to the phytochemicals present in the plants. This is the first report on the antibacterial activity of the host and parasite combination (*Viscum rotundifolium* and *Mystroxylon aethiopicum*). There was no notable difference on the antibacterial activity of the plants in combination as compared to the individual activity. The synergistic effects of the extracts in combination were observed against *S. aureus* whereas antagonistic effects were observed in hexane and acetone extracts against *E. coli*. Furthermore, the evaluation to determine toxicity and safety of the plant extracts revealed that the plants may be nontoxic to human cells, hence there was no significant difference between untreated and treated cells. Further research is still ongoing on the identification and isolation of active compounds.

6. Author contributions

PM and MMM were involved conception and design of the study. PMM carried out the experiments, analysed the data and drafted the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that there is no conflict of interest

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