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Antimicrobial activity and qualitative phytochemical composition of crude extracts from medicinal plants against selected enteric bacterial pathogens, *Candida albicans*

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Abstract *Opinde HR, Nyamache AK, Gatheri GW. 2018. Antimicrobial activity and qualitative phytochemical composition of crude extracts from medicinal plants against selected enteric bacterial pathogens and* Candida albicans*. Bioteknologi 14: 1-12.* Determining the antimicrobial activity and combined effects of the selected plant leaf extracts of *Tagetes minuta*, *Aloe secundiflora*, *Vernonia lasiopus* and *Bulbine frutescens* against selected clinical isolates of *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Shigella flexineri*, *Enterococcus faecalis* and *Candida albicans* were the aims of this study. Moreover, the determination of qualitative analysis of the phytochemicals present in the extracts was also carried out. Kenyatta University arboretum and voucher specimens deposited in the University herbarium, were the source of plant materials. SAS version 9.1 with ANOVA was used to analyze the collected data. Furthermore, the data was subjected to a post hoc test, with $P<0.05$ being considered significant. In the single use or combination of microorganism test, in tested microorganisms the average inhibition zones were found to be significant at $P<0.05$. In the low concentrations on the tested microorganisms, *V. lasiopus* was more active against *S. flexineri* (MIC 3.3 µg/mL, MBC 7.1 µg/mL), *B. frutescens* against *S. flexineri* (MIC 3.2 µg/mL, MBC 6.2 µg/mL), *A. secundiflora* against *S. flexineri* (MIC 3.7 µg/mL, MBC 8.0 µg/mL) and *T. minuta* against *E. faecalis* (MIC 5.1 µg/mL, MBC 6.3 µg/mL)*. The extract combination also showed a significant increased and decreased antimicrobial activity i.e., $P<0.05$. The average inhibition zone formed by the combination of *A. secundiflora* and *T. minuta* plant leaf extracts (8.67±1.86mm) showed a decrease in antimicrobial activity as compared to *T. minuta* (15.17±2.71 mm) and *A. secundiflora* (17.00±2.10mm) respectively in the usage against *C. albicans*. The presence of four phytochemicals; saponins, tannins, alkaloids, and flavonoids is shown in the qualitative phytochemical analysis. An insight into the antimicrobial activities of the plant extracts and their use in the treatment of bacterial or fungal infections is provided by this study. This information might be used in herbal medicine in making concoctions to maximize their effectiveness. To elucidate the actual compounds in the plant leaf extracts responsible for the antimicrobial activity is needed and this elucidation can be used in drug development.

Keywords: Antimicrobial activity, phytochemical, medicinal plants, enteric bacterial pathogens, *Candida albicans*

INTRODUCTION

Almost 80% of the world's population uses medicinal plants for their basic health care. This is often because of their low cost and ease of availability. From the dawn of civilization, people have developed a great interest in plant-based drugs and pharmaceutical products (Shazadi et al. 2010). In recent decades, many bacterial organisms have continued to show increasing resistance against current antimicrobial agents (Nascimento et al. 2000). Herbal drugs made from medicinal plants have been used from ancient times to treat various diseases and their antimicrobial properties make them a rich source of many potent drugs (Srivastava et al. 2005).

The use of herbal medicinal plants has always played a positive role in the control or prevention of diseases such as diabetes, heart disorders and various cancers (Mohanta et al. 2003). Some medicinal plants have been used in the production of various drugs singly or in combination; even as principal raw material to produce other conventional medicines (Tahir and Khan 2012). *Tagetes minuta* L. is also known as Southern Cone Marigold, Stinking Roger, or black mint. It is a tall upright plant, with small flowers, native to the southern half of South America (Everett 1982). The genus comprises 56 species, of which they grow either annually or perennially, and are mostly herbaceous plants (Soule 1996). Other common species of the family include *Tagetes erecta*, *Tagetes patula* and *Tagetes tenuifolia* (Tereschuk et al. 1997). Most of herbaceous plants are mostly found in North and South America, with some species being naturalized around the world. *T. minuta* extracts have been used as medicinal tea in some areas (Soule 1993). The total extracts from leaves, flowers, stem, and other parts of the plant have shown antibacterial activity against Gram positive and Gram-negative bacteria (Tereschuk et al. 1997). Extracts from the other common species have also been used as medicine in treating various illnesses such as stomach problems and intestinal disorders (Broussalis et al. 1999).

Some of the components contained in *T. minuta* extracts such as flavonoids have been tested and proved to have antimicrobial activity against not only bacteria but also fungi and some nematodes (Cushnie and Lamb 2005). The genus *Aloe* belongs to Aloeaceae (Liliaceae) family which

has around 360 to 400 different species (Newall et al. 1996). Aloe species have antibacterial, antifungal, anticancer, antiviral, and immunomodulatory properties (Holzmuller et al. 2002). *Aloe secundiflora* Engl. is also known as *Aloe floramaculata*, *Aloe engleri* and *Aloe marsabitensis* (Kaingu et al. 2013). Other common species include *Aloe arborescens*, *Aloe chabaudii*, *Aloe turkanensis*, *Aloe greatheadii*, *Aloe cameronii and Aloe excels*. *A. secundiflora* leaf components have been credited for antibacterial, antifungal, and antiviral and anthelmintic medicinal properties (Mwale et al. 2005). *Bulbine* is a genus of plants in the family Xanthorrhoeaceae and subfamily Asphodeloideae and its members are well known for their medicinal value (Acock 1988). They are succulent plants with most species having yellow flowers while others are white, orange, or pink flowers. The most common species is *Bulbine frutescens* which is popularly grown in flower gardens (Van Wyk 2008). Many species have bulb shaped tuber, and they are chiefly found in South Africa with a few species extending to the tropics of Africa and Australia (Coopoosamy et al. 2000). *Vernonia lasiopus* O. Hoffman belongs to the tribe Vernonieae in the family Asteraceae which mostly contains herbaceous plants (Keeley 2007). *Vernonia* is a shrub that grows in tropical Africa and has a height of about 2-5m elliptical leaves of up to 20 cm and a rough bark (Ijeh and Ejike 2011).

The plants in this family usually have a bitter taste and in English, they are called bitter leaf (Ijeh and Ejike 2011). Studies carried out have shown some of the phytochemical components found in their extracts have the antimicrobial capability (Koul et al. 2003). *V. lasiopus* decoctions from the stems and leaves have been traditionally used by herbalists in East Africa to treat, malaria, worms, and gastrointestinal problems (Kareru et al. 2008). The study of contemporary medicine has yielded promising and commendable results on the antibacterial activity of medicinal plant extracts as potential drugs that can be added along with contemporary drugs (Coopoosamy et al. 2007).

The aims of this research were (i) To determine the antimicrobial activity of plant extracts from *T. minuta*, *A. secundiflora*, *V. lasiopus* and *B. frutescens* against bacterial pathogens and *Candida albicans*; (ii) To determine the impact of combined plants extracts against the bacterial pathogens and *C. albicans*; (iii) To determine the qualitative phytochemical present in the plant extracts obtained from *T. minuta*, *A. secundiflora*, *V. lasiopus* and *B. frutescens* were the objectives of this research.

MATERIALS & METHODS

Plants sampling

The plants were randomly collected in densely populated areas in the Kenyatta University arboretum and identified by Taxonomist Prof L.E. Newton. The plants were then placed on a table and the leaves were selected from the plants using the following criteria i.e., they have no dead leaves, they have no flowers, and they have the same height. The plant materials were randomly sampled in densely populated areas namely twenty samples of *T. minuta*, ten samples of *V. lasiopus*, five samples of *A. secundiflora* and five samples of *B. frutescens* plants. Voucher specimens were arranged and stored in the University herbarium in Plant Sciences Department for future reference. The plants were brought to the laboratory and thoroughly washed in running water to remove debris and dust particles, before being rinsed using distilled water and finally air dried.

Preparation of plant extract

The air-dried leaves from the plants were ground into powder and 500 g of them were soaked in 750 mL of Analar grade (AR) methanol in a conical flask for 72 hours. Then they were placed in a Gallenkamp shaker rotating at 65 revolutions per minute. The contents were homogenized and filtered using Whatman filter paper no. 1. The filtrate was poured into a round bottom flask and concentrated using a Buchi Rotavapor R-200 yielding 2.8 g of *B. frutescens*, 3.1 g of *A. secundiflora*, 2.6 g of *T. minuta* and 2.1 g of *V. lasiopus*.

The extracts were then stored in labelled amber glass bottles that were slightly opened, where they were further left to dry in the bottle at room temperature away from light and heat in a laminar flow before being used for antimicrobial efficacy test.

Preparation of media

The media were Muller Hinton agar and Potato dextrose agar (Sharau®) which were prepared following commercially given instructions.

Preparation of potato dextrose agar

One L of distilled water was mixed with 39 mg of potato dextrose agar powder in a flat-bottomed conical flask. To dissolve the potato dextrose agar powder, the stirring was done while it was boiling. The flask was then tightly closed using cotton wool and further covered with aluminum foil. The mixture was autoclaved for 15 minutes at 121ºC, after which it was left to refrigerate down to room temperature. 40mg of tetracycline was added to inhibit bacterial growth, and the media was then stirred before dispensing in Petri dishes. The media was poured in the Petri dishes in a laminar flow to give uniform depth of 3-4 mm. The Petri dishes were left to refrigerate and after which they were placed in a sterile plastic bag and stored at a temperature of 2-8ºC before use.

Preparation of Muller-Hinton agar

One liter of distilled water was mixed with 38 mg of Muller-Hinton agar powder in a flat-bottomed conical flask. The mixture was heated with frequent stirring and boiled for one minute to completely dissolve the media. The flask was then tightly closed using cotton wool and further covered with aluminum foil. The mixture was autoclaved for 15 minutes at 121ºC after which it was left to refrigerate to room temperature. The media was poured in the Petri dishes in a laminar flow to give uniform depth of 3-4 mm. The Petri dishes containing the media were

then placed in a sterile plastic bag and stored at a temperature of 2-8ºC before use.

Preparation of susceptibility test discs

Discs of 6 milliliters were prepared from Whatman no.1 filter paper. This was done by punching the filter papers using a paper punch. The discs filled four McCartney bottles. The discs were then sterilized by autoclaving at 121ºC for 15 minutes, after which the autoclave was left to refrigerate before removing the McCartney bottles containing the discs. The discs were dried in a hot air oven at 50ºC to remove moisture (Arunkumar et al. 2009).

The discs were impregnated with formulated stock solution of the plant leaf extracts of 1000 µg/mL of *T. minuta*, *A. secundiflora*, *B. frutescens* and *V. lasiopus* and they were used for the antimicrobial activity. This was done by taking the sterile discs and the stock solutions into the laminar flow. Forceps, which were sterilized on a spirit lamp and refrigerated, were used to pick the discs and place them in the stock solutions of the plants leaf extracts. The forceps were sterilized after every pick. The disc was then left to stay in the plant leaf extracts stock solution for two hours. The discs were then removed and placed in a sterile Petri dish in a laminar flow and left to dry for 30 minutes. The discs impregnated with leaf extracts from each plant were then picked by sterilized forceps and stored in a sterilized McCartney bottle and stored in a refrigerator at a temperature of 4-8ºC before being used for the antimicrobial susceptibility test.

Test bacterial organisms

The tested microorganisms used in the study were clinical isolates of *Escherichia coli*, *Salmonella typhi*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Shigella flexineri*, *Enterococcus faecalis* and *Candida albicans*. The microorganisms were isolated from samples collected from first-time patients at Kenyatta University Health Centre Laboratory, Nairobi. The patients who showed symptoms associated with enteric bacterial infections such as fever, abdominal pain, diarrhea, vomiting and had no known drug resistance were selected.

Isolation, morphological identification, and biochemical test were carried out. The samples for isolating enteric bacteria were from fecal material. Sterile water was used in cleaning infected wounds to get a sample for isolation of *S. aureus* and high vaginal swab for isolation of *C. albicans*. The samples were used in isolation and of characteristic morphological identification of enteric bacteria based on the type of colonies by streaking of the samples on selected media. The morphologically identified microorganisms were then subjected to a biochemical test for identification on biochemical level.

Isolation, morphological identification

The fecal material was first mixed with sterile distilled water. The blend was serially liquefied up to 10^{-6}. A wire loop was sterilized by heating and left to refrigerate. It was then dipped into the serially liquefied sample of 10^{-6} and streaked on selected and differential media in Petri dishes used to support the growth of each of the tested microorganism. The Petri dishes were then tightly closed with a parafilm and were incubated for 24 hours at 37ºC. The plates were then removed, and the bacteria were identified according to their morphological characteristics.

Escherichia coli was isolated by streaking the sample on Eosin methylene blue and MacConkey agar. The Petri dish was tightly closed with parafilm and was incubated at 37ºC for 24 hours. Later, the plate was observed for growth of microorganism. The growth of metallic green colonies on Eosin methylene blue agar, and pink to red brick colonies on MacConkey agar with or without a zone of precipitated bile were a morphological growth characteristic of *E. coli*.

Salmonella typhi was isolated by streaking sample on *Salmonella-Shigella* agar and Hektoen agar. The Petri dish was tightly closed with parafilm and was incubated at 37ºC for 24 hours. Later, the plate was observed for growth of microorganism. The growth of colonies with or without black centers on *Salmonella-Shigella* agar and blue-green colonies with black centers on Hektoen agar was a morphological growth characteristic of *S. typhi*. *S. flexineri* was isolated by streaking sample on *Salmonella-Shigella* agar. The Petri dish was tightly closed with parafilm and incubated at 37ºC for 24 hours. Later, the plate was observed for growth of microorganism. The growth of transparent and translucent colonies on *Salmonella-Shigella* agar was a morphological growth characteristic of *S. flexineri*.

Enterococcus faecalis was isolated by streaking the sample on Columbia agar with 5% sheep blood. The Petri dish was tightly closed with parafilm and was incubated at 37ºC for 24 hours. Later, the plate was observed for growth of microorganism. The growth of diplococcus colonies with gamma hemolysis on Columbia agar with 5% sheep blood was a morphological growth characteristic of *E. faecalis*. *S. aureus* was isolated by streaking the liquefied sample from infected-free wounds on Mannitol agar. The Petri dish was tightly closed with parafilm and was incubated at 37ºC for 24 hours. Later, the plate was observed for growth of microorganism. The growth of yellow colonies with yellow zones on Mannitol salt agar was a morphological growth characteristic of *S. aureus*.

Candida albicans was isolated by streaking the liquefied sample from high vaginal swabs on Sabourauds agar containing tetracycline to prevent bacterial growth. The Petri dish was tightly closed with parafilm and was incubated at 37ºC for 24 hours. Later, the plate was observed for growth. The growth of white to cream colonies, smooth, glabrous, yeast-like colonies on Sabourauds agar was a morphological growth characteristic of *C. albicans*.

Biochemical identification

The biochemical test depended on the ability of the secluded and morphologically recognizable tested microorganisms to cause fermentation of sugars and oxidation. The biochemical tests were: citrate test, urease test, nitrate test, gelatin test, hydrogen sulphide gas test, arabinose test, fructose test, glucose test, inositol test, lactose test, maltose test, mannitol test, mannose test,

raffinose test, sucrose test and sorbitol test. The media used to complete the test were set up as indicated by the manufacturer guidelines and were poured into Nessler tubes.

Durham tubes were inserted into media containing broth and later were observed for gas production. If there were gas production, the result was marked as positive. However, if there were no gas production, then the result was marked as negative. The tubes were then closed and autoclaved at 121ºC for 15 minutes and left to refrigerate before being stored in a refrigerator for utilization. One mL inoculum of the secluded microbes test from a dissolved sample of 10^{-6} was introduced to the media and incubated at 37ºC, and observation was done on it after 24 hours. The tubes containing solid media were observed for color change. If the color change occurred, the results were marked as positive, but if there were no color changes, the results were marked as negative using standards on http://www.microbiologyinfo.com/biochemical test, 15th June 2014.

Table 1. Standard for biochemical tests on isolated microorganisms

Biochemical test	**Microorganisms**					
	E.coli	*S.typhi*	*S.aureus*	*S.flexineri*	*E.faecalis*	*C.albicans*
Citrate	-	-	+	-	-	+
Urease	-	-	+	-	-	-
Nitrate	+	n/a	+	n/a	+	-
Gelatin	-	-	+	n/a	n/a	n/a
H_2S production	-	+	-	-	-	n/a
Arabinose	+	-	n/a	n/a	-	+
Fructose	-	n/a	+	n/a	+	n/a
Glucose	+	+	+	-	+	+
Inositol	-	-	n/a	n/a	n/a	+
Lactose	+	-	+	-	+	-
Maltose	n/a	-	+	n/a	+	+
Mannitol	+	+	+	+	+	+
Mannose	n/a	+	+	n/a	+	n/a
Raffinose	n/a	-	-	n/a	-	-
Sucrose	n/a	-	+	-	+	+
Sorbitol	+	+	n/a	-	+	+

Note: (+)-Positive, (-)-Negative, (n/a)-Not applicable

Maintenance of the bacterial, fungal cultures

The cultures of the clinical isolates of the tested microorganisms were maintained on agar slants. The agar slants were prepared by making 250 mL of nutrient agar and potato Dextrose agar prepared according to r commercial instructions. The solution of the Medias was divided and placed into a lot of bijou bottles of equal amounts of 10 mL. The bijou bottles were then closed and placed in an autoclave and sterilized for about 15 minutes at 121 ºC. The autoclave was left to refrigerate. The bottle was then placed on a wedge in a laminar flow slanting at around 45ºC and left to refrigerate to form agar slants. The bacterial microorganisms were streaked on nutrient agar slant, whereas *C. albicans* was streaked on potato dextrose agar slant. The streaked agar slants were placed in an incubator at 37 ºC and observed for growth every 24 hours. The tested microorganism was subsequently subcultured every 48 hours to maintain their viability.

Antimicrobial susceptibility testing

The microorganisms (*E. coli, S. typhi, E. faecalis, S. aureus, S. flexineri, E. faecalis and C. albicans*) were concentrated by comparing it with a 0.5 McFarland standard. All the plant extracts were dissolved in 4% dimethyl sulphoxide (DMSO) to form a stock solution of 1000 µg/mL.

The stock solution concentration of each extract was formulated using the formula below:

$$C = S \times M/Q$$

Where:
S: Solute weight (mg)
Q: Dilution solvent (mL)
M: Conversion units (µg)
C: Concentration (µg/mL)

The discs were from Whatman filter paper no.1 and contained different plant leaf extracts (*T. minuta, A. secundiflora, V. lasiopus,* and *B. frutescens*) from the highest concentration (1000 µg/mL) to the lowest concentration (1 µg/mL). This was done by serially halving the concentration in subsequent dilutions and then storing in McCartney bottles away from light (Mangoma et al. 2010).

The antimicrobial efficacy test was carried out with the Kirby-Bauer method (Newall et al. 1996). Muller Hinton agar (Sharau®) was used for the tested microorganism bacteria and potato dextrose agar for *C. albicans* in the spread plate technique and the clinical isolates were spread with sterilized cotton wool swabs. They were exposed to extract discs in mg per microliter from *A. secundiflora, T. minuta, V. lasiopus* and *B. frutescens*. The discs were placed with equal distance between them on agar plates inoculated with the pathogen bacteria and *C. albicans*.

Positive control standard discs containing ciprofloxacin (5 µg/mL) was used for *E. coli, S. typhi, E. faecalis, S. flexineri*; vancomycin (3 µg/mL) for *S. aureus,* and fluconazole (15 µg/mL) for *C. albicans*. A negative control of discs containing 4% Dimethyl sulphoxide and 100% Analar (AR) methanol were also used. The Petri dishes were incubated at 37 ºC for 24 hours, then they were removed and observed to see if there was the formation of any inhibition zones by the extracts from the plant against the tested microbes. The experiment was carried out in duplicates, and the diameter of inhibition zones was measured, and their average was determined.

Minimal inhibitory concentration (MIC) was determined with the broth tube method according to Eloff et al. 1998) namely: 100 µL of 250 mg/mL of methanol extract was added to 100 µL of sterile bacteriological peptone in the first well of the 96 wells microplate and mixed well with a micropipette. 100 µL of this dilution was transferred subsequently to wells, two foldings for each dilution of the original extract. This was done to the extracts of *A. secundiflora, B. frutescens, V. lasiopus,* and

T. minuta. An inoculum of 100 µL (0.5 McFarland standard) of overnight clinical cultures of; *E. coli*, *S. typhi*, *S. aureus*, *S. flexineri*, *E. faecalis* and fungus *C. albicans* were added in each of the wells. Triplicate of each microplate was made, and the procedure repeated for each of the tested organisms. The plates were then incubated at 37ºC for 24 hours. After incubation, 40µL of 0.2 mg/µL of INT was added in each of the wells and the plates examined after an additional 60 minutes of incubation. Red color (conversion of INT to formazan) indicated the growth of organism. The lowest concentration at which the color was apparently invisible, as compared to the next dilution, was taken as the minimum inhibitory concentration (Rabe et al. 2002). Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined according to Rabe et al. 2002 whereby: 100µL of the suspension was taken from microplate wells that demonstrated no growth and inoculated on agar plates. The plates were incubated at 37 ºC for 24 hours. The absence of bacterial growth or the growth, which was not greater than the minimum inhibitory concentration, was used to determine the maximum bacterial concentration and maximum fungicidal concentration.

Combined effect test

The combinations were determined using the permutation formula of P=N!/(N-R)! It produced six combinations namely: *A. secundiflora* and *T. minuta* **(AT)**, *A. secundiflora* and *V. lasiopus* **(AV)**, *B. frutescens* and *T. minuta* **(BT)**, *B. frutescens* and *V. lasiopus* **(BV)**, *A. secundiflora* and *B. frutescens* **(AB)** and *V. lasiopus* and *T. minuta* **(VT)**. The combined effect was determined with ratio of 1:1 using the plants leaf extracts with the concentrations of 1000 µg/mL of each extract. The combined plant leaf extracts were used against the tested microorganisms in replicates and the inhibition zones measured. The average inhibition zones were formed when the extracts which were used in combinations against the tested microbes were compared with the inhibition zones when they are used singly.

Qualitative phytochemical analysis

The method defined by Congesta et al. (2005) was used to determine the presence of saponins, tannins, flavonoids, and alkaloids in the crude extract.

Tannins

Each of the extracts were weighed to 0.5 mg and dissolved in 1 mL of distilled water. Filtration was carried out after 2 mL of $FeCl_3$ was added. If blue or black precipitate was present, it indicated the presence of tannins.

Flavonoids

Each of the extracts were weighed to 0.5 mg and dissolved in 1 mL of ethanol and filtered. 2 mL of 1% HCl and magnesium ribbon was added to the filtrate. If there was the formation of a pink or red color, it indicated the presence flavonoids.

Alkaloids

Each of the extracts were weighed to 0.5 mg and dissolved in 1 mL of methanol and filtered. 1% HCL was added to the filtrate and the solution heated. Mayor`s reagent was added per drop, and if there was the formation of any colored precipitate, it indicated the presence of alkaloids.

Saponins

Each of the extracts were weighed to 0.5 mg and dissolved in 1 mL of methanol and filtered. Distilled water was added and was shaken for a few minutes. If there was persistent frothing, it indicated the presence of saponins.

Statistical analysis

The data was exported to Microsoft excel spreadsheet to carry out descriptive statistics. The data was analyzed with SAS version 9.1 whereby; ANOVA (one way) was carried out to show statistical difference with the varying inhibition zones between the test microbes exposed to plant leaf extracts from *T. minuta*, *A. secundiflora*, *V. lasiopus* and *B. frutescens*. Two-way ANOVA was also carried out to determine if any interaction between the plant extracts and tested microorganism with $P \leq 0.05$ was happened and considered significant. The tests were further subjected to a Tukey's post hoc test to find the difference between the means.

RESULTS AND DISCUSSION

All the bacterial pathogens (*E. coli*, *S. typhi*, *S. aureus*, *S. flexineri* and *E. faecalis*) and *C. albicans* were tested against plant extracts with the concentration of 1000 µg/mL found out on discs. The bacterial pathogens and *C. albicans* cultures of 0.5 McFarland standard were used for the efficacy test.

Efficacy test of the plant extracts on the bacterial pathogens, *Candida albicans*

The expose of all bacterial pathogens (*E. coli*, *S. typhi*, *S. aureus*, *S. flexineri* and *E. faecalis*) and fungus *C. albicans* to the plant extract in replicates showed various antimicrobial activity. The largest average inhibition zone (i.e., 16.67±2.58mm) was produced by *A. secundiflora* extract against *E. coli* in comparison to the other plant extracts (Table 2).

The average inhibition zones formed by *T. minuta* and *A. secundiflora* extracts were significantly different to those formed by *V. lasiopus* ($P<0.05$). Moreover, the average inhibition zones formed by ciprofloxacin, methanol and DMSO (negative control) were significantly different to those formed by the plant extract ($P<0.05$). *T. minuta* extract produced the largest average inhibition zone of 17.17±2.48 mm against *S. typhi* when compared to the other plant extracts. The average inhibition zone formed by an extract from *V. lasiopus* was significantly different from those of *T. minuta* ($P<0.05$). Moreover, the average inhibition zones formed by ciprofloxacin, methanol and DMSO (negative control) were significantly different from

those formed by the plant extract (P<0.05). *T. minuta* extract produced the largest average inhibition zone of 16.67±3.44 mm against *S. aureus* when compared to other plant extracts. The average inhibition zones formed by the plant extracts were not significantly different from each other (P>0.05) (Table 2).

However, the average inhibition zone formed by the plant extracts were significantly different from those formed by vancomycin, methanol and DMSO (negative control) (P<0.05). *Bulbine frutescens* extract produced the largest average inhibition zone of 19.50±1.05mm against *S. flexineri* when compared to other plant extracts. The average inhibition zones formed by the plant extracts were not significantly different from each other (P>0.05). However, the average inhibition zones formed by ciprofloxacin, methanol, and DMSO (negative control) were significantly different from those formed by the plant extract (P<0.05). *T. minuta* extract produced the largest average inhibition zones of 18.67±1.03mm against *E. faecalis* when compared to other plant extracts. The average inhibition zones formed by all the plant extracts were not significantly different (P>0.05). However, the average inhibition zones formed by ciprofloxacin (positive control), methanol, and DMSO (negative control) were significantly different from those formed by the plant extract (P<0.05). *V. lasiopus* extract produced the largest inhibition zone of 20.17±2.71mm against *C. albicans* when compared to other plant extracts. The average inhibition zones formed by *T. minuta* and *V. lasiopus* were significantly different from each other (P<0.05). All average inhibition zones formed by the plant extracts were significantly different from those formed by fluconazole, methanol and DMSO (negative control) (P<0.05) (Table 2).

The average inhibition zone formed by the plants extracts against all the test microorganisms were significantly different from those formed by antibiotics, methanol and DMSO (P<0.05). Moreover, zones formed by *T. minuta* extract against all the tested microorganism was significantly different from those formed by *V. lasiopus* and *B. frutescens* (P<0.05). However, it was not significantly different from zones formed by *A. secundiflora* (P>0.05). The average inhibition zones formed by the plant extracts against *E. faecalis*, *C. albicans*, and *S. flexineri* were not significantly different from each other (P>0.05). However, they were significantly different from those formed by *E. coli*, *S. typhi* and *S. aureus* (P<0.05). The interaction between the plant extracts and tested microorganisms were significant (P<0.05) (Table 3).

Table 3 Interactions between the plants extract and tested microorganism on single use.

Extract	**Zone of inhibition±SEM**
Aloe secundiflora	16.69±0.40cd
Bulbine frutescens	16.06±0.56d
Tagetes minuta	17.19±0.42c
Vernonia lasiopus	15.81±0.61d
Controls	
Antibiotics	23.5±0.43b
Methanol	31.17±0.34a
DMSO	0.00±0.00e
Testing microorganisms	
Candida albicans	18.31±1.45a
Escherichia coli	15.26±1.25c
Enterococcus faecalis	18.26±1.41a
Salmonella typhi	16.60±1.37b
Shigella flexineri	18.69±1.45a
Staphylococcus aureus	16.10±1.51bc
***P values* of the main factors, their interactions**	
Extract	<0.001
tested microorganisms	<0.001
Extract*tested microorganisms	<0.001

Note: The value of average inhibition zones ± standard error of mean (SEM) after two-way ANOVA followed by Tukey`s HSD test. A value followed by the same superscript within the same column are not significantly different (P>0.05). Antibiotics: ciprofloxacin, vancomycin, and fluconazole, DMSO: dimethyl sulphoxide.

Table 2. Average inhibition zones in millimeters when plant extracts are on single-use against bacterial pathogens and *Candida albicans.*

Plant extracts	**Tested microorganisms**					
	E. coli	***S. typhi***	***S. aureus***	***S. flexineri***	***E. faecalis***	***C. albicans***
Tagetes minuta	16.50±1.87c	17.17±2.48c	16.67±3.44c	19.00±1.41bc	18.67±1.03c	15.17±2.71d
Aloe secundiflora	16.67±2.58c	16.50±1.87cd	14.17±2.93c	18.17±1.47c	17.67±1.63c	17.00±2.10cd
Bulbine frutescens	13.33±1.75cd	15.33±2.73cd	11.83±2.48c	19.50±1.05bc	18.50±1.05c	17.83±1.72cd
Vernonia lasiopus	12.33±2.58d	13.17±1.84d	13.00±2.61c	18.17±1.47c	18.00±0.89c	20.17±2.71c
Controls						
Antibiotics	21.67±2.42b	25.67±1.63b	24.83±3.54b	21.50±2.07b	21.67±2.66b	27.17±0.98b
Methanol	28.67±2.34a	30.17±2.71a	31.33±2.94a	31.67±2.88a	33.50±2.56a	31.00±3.20a
4%DMSO	0.00±0.00e	0.00±0.00e	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00±0.00e
P value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Note: The value of average inhibition zones ± standard error after one-way ANOVA followed by Tukey`s HSD test. A value followed by the same superscript within the same column are not significantly different (P>0.05). DMSO-Dimethyl sulphoxide, Plant extracts concentration (1000 µg/mL) Antibiotics standard discs of; Ciprofloxacin (5 µg/mL) for Gram-negative bacteria, Vancomycin (3 µg/mL) for Gram-positive bacteria, Fluconazole (15 µg/mL) for *Candida albicans.*

Table 4 Minimum inhibitory concentration in micrograms/milliliter of plant extracts against bacterial pathogens and *Candida albicans.*

Plant extracts	Tested microorganisms					
	E. coli	***S. typhi***	***S. aureus***	***S. flexineri***	***E. faecalis***	***C. albicans***
Tagetes minuta	8.7	6.1	8.9	7.4	5.1	6.2
Aloe secundiflora	9.1	5.5	10.2	3.7	7.0	8.1
Bulbine frutescens	12.5	8.8	10.4	3.2	6.5	6.9
Vernonia lasiopus	10.0	5.6	12.2	3.3	3.9	4.0
Antibiotics	5.0	5.0	3.0	5.0	5.0	15.0

Note: Antibiotics; Ciprofloxacin (5 µg/mL)-Gram-negative bacteria, Vancomycin (3 µg/mL)-Gram-positive bacteria, Fluconazole (15 µg/mL)-*Candida albicans*).

Table 5. Minimum bactericidal concentration and minimum fungicidal concentration in ug/mL of plant extracts against bacterial pathogens and *Candida albicans.*

Plant extracts	Tested microorganisms					
	E. coli	***S. typhi***	***S. aureus***	***S. flexineri***	***E. faecalis***	***C. albicans***
Tagetes minuta	10	8.2	10	12.6	6.3	8.7
Aloe secundiflora	10.4	7.3	12.9	8.0	9.7	9.0
Bulbine frutescens	14.0	10.9	13.9	6.2	9.1	8.0
Vernonia lasiopus	11.5	7.5	14.2	7.1	5.0	5.5

Tagetes minuta extract was more active against *E. coli* than the other extracts. However, the antibiotic used as a positive control (Ciprofloxacin) was more active against *E. coli* than all other plant extracts. *A. secundiflora* extract had the highest antimicrobial activity against *S. typhi* when compared to other plant extracts, although ciprofloxacin was more active against *S. typhi* than all other extracts (Table 4).

Tagetes minuta extracts were more active against *S. aureus* at low concentration than other plant extracts. The positive control antibiotic (Vancomycin) used against *S. aureus*, showed to be more active in low concentration than all other extracts. *B. frutescens* was more active against *S. flexineri* at low concentration than other plant extracts. Furthermore, the level of concentration of *B. frutescens* extract was lower than standard antibiotic (Ciprofloxacin) against *S. flexineri* (Table 4).

The plant extract from *V. lasiopus* was more active at low concentration against *E. faecalis* than other plant extracts. Its concentration was lower than standard antibiotic (Ciprofloxacin). All plant extracts were more active against *C. albicans* than standard antibiotic (Fluconazole). However, *V. lasiopus* was the most active against *C. albicans* in low concentration among the used plant extracts (Table 4).

Tagetes minuta extract had low concentration bactericidal against *E. coli* when it was compared to the other extracts. The plant extract from *A. secundiflora* was bactericidal against *S. typhi* at low concentration when it was compared to other plant extracts. On the use against *S. aureus*, the extract from *T. minuta* was bactericidal at low concentration when it was compared to the other plant extracts. *B. frutescens* plant extract was bactericidal against *S. flexineri* at low concentration when it was compared to the other plant extracts. On the use against *E. faecalis* and *C. albicans*, *V. lasiopus* was bactericidal and fungicidal respectively at low concentrations on the comparison to the other plant extracts (Table 5).

Combined effect of the plant extracts on the bacterial pathogens, *Candida albicans*

The plant extracts showed to be both more effective and less effective in some instances against all the tested microorganisms in the combination use. *V. lasiopus* and *T. minuta* plant extract combinations formed the largest average inhibition zone of 16.67±1.37mm against *E. coli* when it was compared to the other plant extract combinations. The average inhibition zones formed by the plant extracts combinations were not significantly different ($P>0.05$). However, they were significantly different from those formed by ciprofloxacin, methanol and DMSO (negative control) ($P<0.05$) (Table 6).

Bulbine frutescens and *V. lasiopus*; *B. frutescens* and *T. minuta* plant extract combinations formed the largest average inhibition zones of 16.67±2.58mm and 16.67±2.26mm against *S. typhi* when it was compared to the other plant extract combinations (Table 6). The average inhibition zones formed by all the plant extracts combinations were not significantly different ($P>0.05$). However, the average inhibition zones formed by ciprofloxacin, methanol and DMSO (negative control) were significantly different from those formed by the plant extract ($P<0.05$). *B. frutescens* and *V. lasiopus* plant extract combination formed the largest average inhibition zone of 15.00±2.28mm against *S. aureus* when compared to other plant extract combinations (Table 6). The average inhibition zone formed by all the plant extracts combinations were not significantly different ($P>0.05$). However, the average inhibition zones formed by vancomycin, methanol and DMSO (negative control) were significantly different from those formed by the plant extract ($P<0.05$). *A. secundiflora* and *B. frutescens*; *A. secundiflora* and *T. minuta* plant extract combinations formed the largest average inhibition zones of 18.67±1.03mm and 18.67±0.24mm against *S. flexineri* when compared to other plant extracts combinations (Table 6). The average inhibition zone formed by the plant extract combinations were not significantly different from those formed by ciprofloxacin (positive control) ($P>0.05$) (Table 6).

Table 6. Average inhibition zone in millimeters when extracts are in combinations against the bacterial pathogens and *Candida albicans*

Plant extracts	Tested microorganisms					
	E. coli	***S. typhi***	***S. aureus***	***S. flexineri***	***E. faecalis***	***C. albicans***
AB	16.33±1.97c	14.83±2.14c	14.17±2.32c	18.67±1.03bc	18.00±1.80bc	14.17±2.14b
AV	15.33±2.16c	15.00±3.03c	14.33±1.86c	18.16±1.47c	17.67±1.75c	14.67±2.42b
AT	17.33±1.86c	14.50±2.43c	14.50±1.04c	18.67±0.24bc	17.67±1.51c	8.67±1.86c
BV	16.33±1.75c	16.67±2.58c	15.00±2.28c	17.67±1.63c	17.50±1.64c	13.50±1.52b
BT	15.83±2.32c	16.67±2.26c	14.17±2.17c	17.83±1.47c	16.83±1.47c	16.83±1.47b
VT	16.67±1.37c	14.83±2.64c	14.50±2.17c	18.16±1.47c	16.17±3.19c	14.17±2.99b
Antibiotics	21.67±2.42b	25.67±1.63b	24.83±3.54b	21.50±2.07b	21.67±2.66b	27.17±0.98a
Methanol	28.67±2.34a	30.17±2.71a	31.67±2.88a	31.67±2.88a	33.50±2.26a	30.33±3.20a
4%DMSO	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00±0.00e
P values	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Note: The value of average inhibition zones ± standard deviation after one-way ANOVA followed by Tukey`s HSD test. A value followed by the same superscript within the same column are not significantly different (P>0.05). **AB**-*Aloe* + *Bulbine*; **AV**-*Aloe* + *Vernonia*; **AT**-*Aloe* +*Tagetes*; **BV**-*Bulbine* + *Vernonia*; **BT**-*Bulbine* + *Tagetes*; **VT**-*Vernonia* + *Tagetes*; ± Standard error; DMSO – Dimethyl sulphoxide; Antibiotics standard discs of; Ciprofloxacin (5 µg/mL), Vancomycin (3 µg/mL) and Fluconazole (15 µg/mL); 7mm-10mm Resistant; 11mm-20mm Intermediate; 21mm-30mm Sensitive.

However, methanol and DMSO (negative control) were significantly different from those formed by the plant extract and ciprofloxacin (P<0.05). *A. secundiflora* and *B. frutescens* plant extract combination produced the largest average inhibition zone of 18.00±1.80mm against *E. faecalis* when it was compared to the other plant extracts (Table 6). The zones formed by the plant extracts were not significantly different (P>0.05). Moreover, *A. secundiflora* and *B. frutescens* plant extract combination formed zones that were not significantly different from those formed by ciprofloxacin (positive control) (P>0.05). However, the inhibition zones formed by methanol and DMSO (negative control) were significantly different from those formed by the plant extract combinations and ciprofloxacin (P<0.05). *B. frutescens* and *T. minuta* plant extract combination formed the largest average inhibition zone of 16.83±1.47mm against *C. albicans* when it was compared to the other plant extract combinations (Table 6). The average inhibition zones formed by *A. secundiflora* and *T. minuta* extract were significantly different when it was compared to the other plant extract combinations; (P<0.05). Moreover, it was also significantly different from fluconazole (positive control), methanol and DMSO (negative control) (P<0.05) (Table 6).

The average inhibition zones formed by the plant extracts combinations when they were used against the tested microorganisms were not significantly different (P>0.05). However, they were significantly different from those formed by antibiotics, methanol and DMSO (P<0.05). The average inhibition zones formed by the tested microorganisms; *E. faecalis* and *S. flexineri* were not significantly different (P>0.05). However, they were significantly different from those formed by the other test microorganism (P<0.05) (Table 7).

Table 7. Interactions between the plants extract and tested microorganism in combination uses.

Extract	**Zone of inhibition±SEM**
AB	16.03±0.43c
AT	15.22±0.63c
AV	15.86±0.42c
BT	16.28±0.37c
BV	16.11±0.39c
VT	15.75±0.44c
Controls	
Antibiotics	23.50±0.43b
Methanol	30.83±0.34a
DMSO	0.00±0.00e
Tested microorganisms	
Candida albicans	15.56±1.20b
Escherichia coli	16.20±0.96b
Enterococcus faecalis	17.59±1.11a
Salmonella typhi	16.28±1.08b
Shigella flexineri	18.35±1.13a
Staphylococcus aureus	15.74±1.10b
***P values* of the main factors, their interactions**	
Extract	<0.001
tested microorganisms	<0.001
Extract*tested microorganisms	<0.001

Note: The value of average inhibition zones ± standard error of mean (SEM) after two-way ANOVA followed by Tukey`s HSD test. A value followed by the same superscript within the same column are not significantly different (P>0.05). Antibiotics: ciprofloxacin, vancomycin, and fluconazole, DMSO: dimethyl sulphoxide.

Qualitative phytochemical analysis

The qualitative test was carried out on plant leaf extracts from *T. minuta*, *A. secundiflora*, *B. frutescens,* and *V. lasiopus* to find out for the presence of phytochemicals. All the plant extracts were found to contain saponins, tannins, alkaloids, and flavonoids (Table 8).

Table 8 Qualitative phytochemical tests on the plant extracts.

Name of test	**Plants leaf extracts**			
	T. minuta	***A. secundiflora***	***B. frutescens***	***V. lasiopus***
Saponins test	+	+	+	+
Tannins test	+	+	+	+
Alkaloids test	+	+	+	+
Flavonoids test	+	+	+	+

Note: (+) present

Discussion

The increase of antimicrobial resistance to many available antimicrobial agents has led to the need for the invention of new drugs. The use of plant extracts to test antimicrobial activity has been brought forward as one of the ways of achieving this goal. The plants used in the study have been said to be of medicinal value. This study evaluated the use of the plants in treating selected bacterial pathogens (*E. coli*, *S. typhi*, *S. aureus*, *S. flexineri*, and *E. faecalis*) and fungus *C. albicans* and to test whether they were effective or not when they were used singly and in combinations. Furthermore, qualitative analysis to test the presence of the phytochemicals was also done.

This is because phytochemicals have been said to be responsible for some of the antimicrobial activity by extracts from plants with medicinal value. From the results, the extracts from the plants were found to have antimicrobial activity in the single-use or combination. However, some of the extracts showed activity when they were used singly and in combinations or otherwise. The extracts had shown both increased and decreased antimicrobial activity when they were used in combinations.

Antimicrobial activity

Medicinal plant extracts from various studies have shown that plants from the similar genera with *A. secundiflora* have shown to have antimicrobial activity. In this study, the antimicrobial activity of methanol extracts from medicinal plants was tested against tested bacterial pathogens and fungus *C. albicans*. It was interesting to note that the plant extracts from *A. secundiflora* showed antimicrobial activity against all the tested microorganisms. The findings from this study were the same as Agarry et al. (2005) about activities against medicinal plants. The plant extract from *A. secundiflora* had antimicrobial activity against tested bacterial pathogens; *E. coli*, *S. typhi*, *S. aureus*, *S. flexineri* and *E. faecalis* and fungus *C. albicans*.

The findings were same with those obtained in a study carried out on Gram-negative and Gram-positive bacteria by Robson (1982) in England; on *E. coli* and Agarry et al. (2005) in Nigeria; on *S. aureus* and *C. albicans* who found out that, extracts from *A. secundiflora* had antimicrobial activity against the tested microorganisms. *A. secundiflora* also showed antimicrobial activity which was significant at $P<0.05$ and the inhibition zones formed was ≥ 12.00mm. These findings were also similar to those obtained in a study carried out in Kenya (Mariita et al. 2011), which resulted the methanol extracts of *A. secundiflora* along the lake region in Kenya to be effective against bacterial pathogens such as *E. coli*, *S. typhi*, and *S. aureus* among others. The study also showed that methanol extracts of *A. secundiflora* indicated a significant antimicrobial activity at $P<0.05$ producing inhibition zones of ≥9.00mm against bacterial pathogens including *S. aureus*, *E. coli*, *S. typhi* and a fungus *C. albicans*. This could probably be due to, the use of same part of the plant (leaves) and most likely at the same age. Msoffe and Mbilu (2009) in Tanzania, also found out that extracts from *A. secundiflora* had antimicrobial activity against *C. albicans*. This may be attributed to the use of the same part of the plant.

The plant extracts from *T. minuta* showed antimicrobial activity against the testing microorganism; *E. coli*, *S. typhi*, *S. aureus*, *S. flexineri*, *E. faecalis* and *C. albicans*. Against *E. faecalis*, the antimicrobial activity was significantly higher than *S. aureus*. From the study, the plant extract from *T. minuta* formed an inhibition zone ≥17.00mm against all test microorganisms. The findings like those obtained from a previous study by Panwar and Bhatt (2014), who found out that extracts from *T. minuta* produced inhibition zone of ≥17.00mm against *S. aureus* which is comparable to the one obtained in this study. This may be due to the same method of extraction and testing. Moreover, the plant used could be of the same age. The findings in this study showed that the minimum inhibitory concentration against these tested microorganisms ranged from 3 to 13 µg/mL. The average inhibition zones were ≥ 17.00mm and the standard antibiotic (Ciprofloxacin) produced inhibition zones of ≥ 20.00mm. The findings were the same as Tahir and Khan (2012) who found out that extracts from *T. minuta* had antimicrobial activity against *S. typhi*, *E. coli*, and *S. aureus*. Furthermore, the minimum inhibitory concentration against these tested microorganisms ranged from 4 to 100 µg/mL, the average inhibition zones were ≥ 17.00mm, and the standard antibiotic (Ciprofloxacin) produced inhibition zones of ≥ 20.00mm. This means that if the concentration of *T. minuta* could be standardized, it could be used as an alternative therapy for Ciprofloxacin, against the tested organisms.

Bulbine frutescens extract showed antimicrobial activity against all the tested microorganisms. The extract antimicrobial activity was significantly higher than that on *E. coli* against *S. flexineri*. From the results of study, the minimum inhibitory concentration of *B. frutescens* against *S. aureus* was 10.4 µg/mL. This finding was in contrary to the one in South Africa by Coopoosamy (2011) who found out that the minimum inhibitory concentration against *S. aureus* was 2.0 µg/mL which was low when compared to the one obtained in the study. Furthermore, Coopoosamy (2011) found out that, extract from *B. frutescens* had no antimicrobial activity against *E. coli*. These differences could be due to different geographical and environmental conditions during the growth of the plant, the method of extraction and the age of the plant. In the study, *B. frutescens* showed antifungal activity against *C. albicans* producing an inhibition zone of ≥18.00mm. The findings were the same as Fennel et al. (2004) and Stafford et al. (2005) who found out that extracts from *B. frutescens* were active against *Candida* spp and *C. albicans* producing an *inhibition zone* of ≥10.00mm. Furthermore, they indicated inhibition between very high (41-50mm), high (31-40mm), medium (21-30mm), low (11-20mm) in fungal species of *Candida* sp, including *C. albicans*. The similarity in the activity could be associated with plant's age and the methods of extraction and testing.

The extract from *V. lasiopus* showed antimicrobial activity against tested microorganisms. The extract had a significantly higher antimicrobial activity against *C. albicans* than *E. coli*. In this study, when the plant extract

from *V. lasiopus* was used against *E. coli* and *S. aureus*, the average inhibition zone were ≥ 12.00mm and ≥ 13.00mm respectively. These findings concurred with those of a previous study by Kareru et al. (2008), who found out that leaf extracts from *V. lasiopus* had antimicrobial activity against *E. coli*. However, the findings were in contrary to a study by Kareru et al. (2008) which showed that *E. coli* and *S. aureus* were resistant (≤6.5mm). This could be due to the difference in the age of the plant, the environmental condition and the aqueous method of extraction used in the study.

Combined effect of the plant extracts

When plant extracts were used in combinations, they showed various antimicrobial activity against the tested microorganisms. When the plant extracts were used in combination against *E. coli*, they showed a pronounced antimicrobial activity as compared to when each of them is used separately against it. *B. frutescens* and *V. lasiopus* produced small average inhibition zones when each of them was used separately against the microorganism.

However, the combinations of the plant extracts of *A. secundiflora* with *B. frutescens*, *A. secundiflora* with *V. lasiopus*, *B. frutescens* with *V. lasiopus*, and *B. frutescens* with *T. minuta* produced large average inhibition zones against *E. coli*. These studies concurred with Nascimento et al. (2000) and Alzoreky and Nakahara (2003), which resulted that when the plant extracts were used in combination against *E. coli*, there was an increase in their antimicrobial activity. This could be due to the same method of extraction and testing.

Against *S. typhi*, the combination use of plant extracts of *B. frutescens* with *V. lasiopus*, and *A. secundiflora* with *V. lasiopus* produced larger average inhibition zones than on the single-use against the same microorganism. The findings showed that combining of *B. frutescens*, *A. secundiflora* and *V. lasiopus* with other plant extracts enhanced their antimicrobial activity against *S. typhi*. The findings were similar to studies Cutter (2000) and by Anjeza and Mandal (2012), which showed that combining of the plant extracts increased their antimicrobial activity against *S. typhi*. This could be due to the use of the same part of the plant or the same age of the plant.

The use of plant extracts in combinations against *S. aureus* significantly increased the antimicrobial activity as compared to the use of the extracts singly. The combinations of *B. frutescens* with *V. lasiopus*, *A. secundiflora* with *T. minuta*, and *B. frutescens* with *T. minuta* showed enhanced antimicrobial activity as by producing larger average inhibition zones than when they were used singly against *S. aureus*. The findings were in agreement with Alzoreky and Nakahara (2003) and Adwan and Mhanna (2008), which showed that combining the plant extracts had increased their antimicrobial activity against *S. aureus.* Furthermore, Adwan and Mhanna (2008) found out that their combination with conventionally available antibiotics also increased their antimicrobial activity. This means that if the concentration of the combined extracts can be standardized, it can be used in combination therapy with antibiotics against *S. aureus.*

Shigella flexineri showed either an increase or a decrease in antimicrobial activity when it was exposed to the plant extracts combinations. If each of the plant extracts was used separately against *S. flexineri*, it showed obvious antimicrobial activity with *B. frutescens* extract producing the largest average inhibition zone when it was compared to extracts from other plants. However, the combining of *B. frutescens* with; *T. minuta*, *A. secundiflora*, and *V. lasiopus* each separately against *S. flexineri* showed decreased antimicrobial activity by producing small average inhibition zones as compared to combinations formed using *T. minuta*, *A. secundiflora*, and *V. lasiopus*. The findings concurred with previous studies by Chanda and Rakholiya (2011) and Barkarnga et al. (2015), which showed that combining the plant extracts resulted both an increase and decrease in their antimicrobial activity against *S. flexineri*. This similarity in activity could be associated with the method of extraction and testing, the plant's age and the part of the plant used.

When the combinations of the plant extracts were used against *E. faecalis*, they showed a decrease in antimicrobial activity as compared to when they were used separately against it. From the results of the study, plant extracts of *T. minuta*, *A. secundiflora*, *B. frutescens* and *V. lasiopus* showed obvious antimicrobial activity by producing a large average of inhibition zones when each of them is used separately against *E. faecalis.* Against *E. faecalis*, plant extracts combinations of *A. secundiflora* with *V. lasiopus*, *A. secundiflora* with *T. minuta*, *B. frutescens* with *T. minuta*, *V. lasiopus* with *T. minuta*, and *B. frutescens* with *V. lasiopus*, showed decreased antimicrobial activity as compared to that when each of them was used separately. The findings were similar to previous studies in India by Chanda and Rakholiya (2011) and in South Africa by Olajuyigbe and Afolayan (2012) which showed that combining of the plant extracts resulted in decreased antimicrobial activity against *E. faecalis*. This could be due to the use of the same part of the plant (leaves) and, most likely, the same age of the plants.

Candida albicans showed either an increase or decrease in antimicrobial activity with the latter being more obvious when the plant extracts were used in combinations. The plant extracts had obvious antimicrobial activity when each of them was used separately against *C. albicans* with *V. lasiopus* being the most active by producing the largest average inhibition zone as compared to others. Plant extracts combination of *B. frutescens* with *T. minuta* showed increased antimicrobial activity by producing a larger average inhibition zone against *C. albicans* as compared to when *T. minuta* was used separately against it. The findings were same as those of Bakarnga et al. (2015), which resulted that combining of the plant extracts showed increased antimicrobial activity against *C. albicans*. However, the findings in the study also showed that the combined extracts had a decrease in antimicrobial activity with the combination of *A. secundiflora* with *T. minuta* and it was indicated by the production of smallest average inhibition zone when compared to others. The findings concurred with a previous study carried out by Adwan et al. (2011), which stated that combining of the plant extracts

showed decreased antimicrobial activity against *C. albicans*. This similarity in the activity could be due to the plant's age and the methods of extraction and testing.

Phytochemicals

The extract from *A. secundiflora* showed that the plant contained pharmacologically active components. The extract contained flavonoids, saponins, alkaloids and tannins which may be responsible for the antimicrobial activity. Similar studies carried out have shown that some of the pharmacologically active components have antimicrobial activity. These findings concurred with those obtained in a previous study by Mariita et al. (2011), which after qualitative analysis of phytochemical components of *A. secundiflora* extract used against *E. coli*, *S. typhi*, *S. aureus* and *C. albicans* from plant collected along the Kenya lake region found out that it contained tannins, saponins, flavonoids, and alkaloids. Furthermore, similar findings were obtained in a study carried out in India by Arunkumar and Methuselvan (2009) which confirmed the presence of flavonoids, saponins, tannins, and alkaloids in *Aloe* extract when it was used against *S. aureus* and *E. coli*. Qualitative analysis for the presence of phytochemicals in *T. minuta* extract showed the presence of flavonoids, saponins, alkaloids, and tannins. This active component might be responsible for antimicrobial activity of *T. minuta* extract against the tested microorganisms.

The study results were like those obtained in studies carried out in Pakistan by Tahir and Khan (2012) and in Argentina by Tereschuk (1997) which also confirmed the presence of phytochemicals flavonoids, saponins, tannins and alkaloids in *T. minuta* extract used against Gram-positive and Gram-negative bacteria. The extract from *B. frutescens* contained pharmacologically active compounds namely saponins, tannins, alkaloids, and saponins which could be responsible for antimicrobial activity. This study concurred with that of a previous study by Coopoosamy (2011), which figured out that when the plant extract from *B. frutescens* was used against *S. aureus* and *E. coli*, the qualitative analysis of phytochemicals showed the presence of alkaloids, saponins, tannins, and flavonoids. However, the findings were in contrary to Van Staden et al. (1994), which resulted that the extract from *B. frutescens* did not contain any of the four phytochemicals. This could be due to diverse plant metabolites associated with a geographical and ecological difference and the age of the plant. The extracts of *V. lasiopus* had active pharmacological compound, i.e., flavonoids, saponins, tannins and alkaloids which could be responsible for the antimicrobial activity.

These findings were like those of a previous study by Kareru et al. (2008), which showed that extract from *V. lasiopus* contained alkaloids, flavonoids, saponins, and tannins when it was used against Gram positive and Gram-negative bacteria (*E. coli* and *S. aureus*). Ayoola et al. (2008) in Nigeria also found out that extract from the plant from the family *Vernonia* contained flavonoids, alkaloids, tannins, and saponins. However, the findings were in contrary to those obtained in a study carried out in Southwestern region in Nigeria by Ibrahim et al. (2012) which showed the extract from *V. lasiopus* did not contain alkaloids. This difference could be associated with the geographical and environmental factors of the area from which the plant was collected.

In conclusion, the plant extracts showed antimicrobial activity when they were used against the bacterial pathogens and fungus *C. albicans*. The increase and the decrease in antimicrobial activity were also observed when the plant extracts were combined and used against the tested microorganism. There was the presence of phytochemicals in the plant extracts, i.e., saponins alkaloids, tannins, and flavonoids.

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