

Phytochemical composition and antibacterial activity of *Ocotea usambarensis* Engl. and *Secamone africana* (Oliv.) Bullock, medicinal plants used in Bushenyi district, Uganda

Stephen Onkalith

onekalithstephen@gmail.com

Mbarara University of Science and Technology

Godfrey Magyezi Begumisa

Mbarara University of Science and Technology

Justine Nakintu

Mbarara University of Science and Technology

Research Article

Keywords: Apocynaceae, Bactericidal, Lauraceae, Minimum Inhibitory Concentration, Phytochemical profile, Plant extracts

Posted Date: October 21st, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-5256552/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

Ocotea usambarensis, lately reclassified as *Kuloa usambarensis* (Engl.) Trofimov & Rohwer, and *Secamone africana* are extensively utilized medicinal plants in Bushenyi district, southwestern Uganda. These plants traditionally complement the conventional drugs in the treatment of various ailments including malaria, cough, measles, anemia, syphilis, gonorrhea, and diarrhea. The purpose of this study was to evaluate the phytochemical content and antibacterial activity of extracts from the two plants against common pathogenic bacteria; *Escherichia coli*, *Streptococcus pneumoniae* and *Staphylococcus aureus*.

Methods

The stem bark and leaves of the two medicinal plants were harvested from Kalinzu Forest Reserve and subsequently air dried at ambient temperature. Phytochemical extraction was performed using cold maceration, decoction, and infusion procedures with ethanol, ethyl acetate, and water respectively serving as solvents. The extracts from the stem bark and leaves of *O. usambarensis* and leaves of *S. africana* were subjected to qualitative and quantitative phytochemical profiling following standard analytical protocols. The antibacterial properties of the plant extracts against *E. coli*, *S. pneumoniae* and *S. aureus* were assessed basing on the zones of inhibition, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and total antibacterial activity. ANOVA and Tukey's post hoc multiple comparison tests were used to determine statistical differences among zones of inhibition of different extract concentrations (7.812-100mg/ml).

Results

The extracts from the two plants contained a diverse spectrum of phytochemical components, including flavonoids, phenols, saponins, and tannins among others. Extracts from both plants demonstrated inhibitory effects on the growth of *E. coli*, *S. aureus*, and *S. pneumoniae*. All extracts were bactericidal at a concentration of 500mg/ml although, a few extracts had bactericidal concentrations as low as 31.25mg/ml.

Conclusion

The diversity of phytochemicals and in vitro antibacterial activity results against *E. coli*, *S. aureus* and *S. pneumoniae* of the extracts of *O. usambarensis* and *S. africana*, validate the usage of the two plants in herbal remedies.

Introduction

Traditional medicinal therapies, including the use of medicinal plants have continuously evolved along human history [1]. Ancient records reveal medicinal plant usage since 2600 BC in Egypt, 100 BC in China and 100 AD by the Greek physicians [2]. Traditional medicines have played a crucial role not only in human healthcare but in veterinary applications too. They were the earliest remedies employed to treat diseases in domestic animals [1, 3]. The advancement of contemporary medical science is significantly indebted to pharmaceuticals originally sourced from plant and animal materials [1].

The increased incidence of diseases such as COVID-19 and cancer, for which there is lack of specific curative treatments, along with the limited accessibility and high cost of conventional drugs, as well as shortage of professional healthcare workers, have collectively driven a surge in the demand for and use of traditional remedies [4]. Consequently, the 21st century has witnessed an overwhelming rise in the reliance on traditional medicine, with approximately 80% of the global population [5] and 90% of the population in rural areas of Uganda [3] relying on these treatments for disease prevention, detection, and management. Traditional medicine encompasses a variety of practices, including spiritual therapies, plant, animal and mineral-based remedies. Among these, plant-based remedies are the most widely utilized [6] and the World Health Organization (WHO) endorses their integration into national healthcare systems [7].

Plant-based remedies present a golden opportunity in combating antimicrobial resistance to conventional drugs [8]. Unlike most allopathic medications, which have narrow anti-pathogenic spectra, phytochemicals present in medicinal plants exhibit synergistic effects and wide anti-pathogenic activities [9]. Although there is substantial evidence that higher plants are excellent sources of antibiotics and other therapeutic agents, and have for long been used to treat a variety of ailments, only approximately 15% of them have been formally examined for bioactivity [10]. *O. usambarensis* and *S. africana* are among the higher plants of medical importance in Uganda whose bioactivities are insufficiently studied.

O. usambarensis is a medium to large evergreen tree typically reaching a height of about 15–30 meters. The tree features a stout, straight trunk that can extend up to 10 meters, with a diameter ranging from 2 to 3 meters, and is often buttressed at the base [11]. This species predominantly thrives in mountain forest ecosystems known as *Ocotea* forests. Its ecological niche is primarily situated within the wet forests of African countries,

including Kenya, Tanzania, the Democratic Republic of Congo, Rwanda, northern Malawi, northern Zambia, and Uganda. In Uganda, *O. usambarensis* is mainly found in Bwindi Impenetrable, Kalinzu and Kasyoha-Kitomi Forests [12].

Local communities in African countries including Uganda believe that the stem bark and roots of *O. usambarensis* contain therapeutic compounds, thus use these parts in traditional medicine [13]. The roots and stem bark are used for deworming and for the treatment of malaria, fever, backache, headache, stomachache, and tumors. When the roots and stem bark of *O. usambarensis* are blended with meat soup, *Brucea* species, and *Myrica salicifolia*, they serve as a remedy for abscesses, coughs, and measles. Externally, the pounded barks of the roots and stem are applied to treat swellings, boils, and wounds [11, 14]. These uses indicate antimicrobial, antiparasitic, and antitumor properties of *O. usambarensis*. Previous studies have demonstrated the cytotoxic activity of *O. usambarensis* against various human cancer cell lines, as well as its antifungal activity against *Cladosporium cucumerinum* [15].

S. africana on the other hand is a small climbing shrub characterized by twining stems approximately 4.5 meters in length [16]. It is extensively distributed across the dense tropical forests of Africa, including the Kalinzu Forest Reserve, Bwindi Impenetrable Forest and Budongo Forest Reserve in Uganda. *S. africana* is widely recognized for its medicinal properties and usage in the treatment of a variety of diseases [16, 17]. In Uganda, it is traditionally employed as a therapeutic agent for malaria, syphilis, constipation, menstrual pain, pediatric swelling, and antenatal conditions [18]. Ethanol and aqueous extracts of *S. africana* have demonstrated significant inhibitory effects on worm motility in addition to notable anti-plasmodial and anthelmintic activities against *Ascaris suum* [18].

Despite the substantial evidence demonstrating the cytotoxic activity of *O. usambarensis* against cancer cells, its antifungal properties, and the antiplasmodial activity of *S. africana*, there is limited empirical evidence for their phytochemical composition and antimicrobial efficacy against common pathogenic bacterial species. This study focused on qualitative and quantitative profiling of the phytochemical constituents of *O. usambarensis* and *S. africana*, as well as their antimicrobial activity against common bacterial species. The findings of this study provide critical evidence essential for informing decisions about the antimicrobial medicinal application of *O. usambarensis* and *S. africana*, and support the potential integration of these plants into conventional healthcare systems.

Materials and methods

Collection of plant materials

Plant material samples for laboratory analysis were collected from Kalinzu Forest Reserve, a natural tropical rainforest situated in the Bushenyi district, Kalinzu forest is approximately 10 kilometers west of Ishaka Town along the Mbarara-Kasese Highway in southwestern Uganda, at coordinates 0°17'S and 30°07'E (Fig. 1). This forest is located atop an escarpment on the eastern edge of the Great Albertine Rift Valley and spans an area of 147 square kilometers, adjacent to Queen Elizabeth National Park and the Imaramagambo Forest. Kalinzu Forest is among the most biodiverse forests in Uganda, with more species diversity than anywhere else [19]. The forest was purposively selected as the source of plant samples due to the availability of the specific plant species of interest for the study in addition to its status as the primary source of the two medicinal plants (*O. usambarensis* and *S. africana*) for the local inhabitants of Bushenyi district.

Selection of plant parts

Survey respondents from three selected sub-counties (Kyamuhunga, Bumbaire, and Nyakabirizi) in Bushenyi District provided information on the medicinal uses of *O. usambarensis* and *S. africana*. Based on their responses, the leaves and stem bark of *O. usambarensis* and the leaves of *S. africana* were the most utilized plant parts. As such, these parts were selected for subsequent phytochemical and antibacterial activity analyses.

Plants selection and identification

From a highly conserved area in Kalinzu Forest Reserve, *O. usambarensis* and *S. africana* plants were identified with the assistance of a field botanist. Mature and healthy specimens of *O. usambarensis* and *S. africana* were selected, ensuring that the chosen plants exhibited no dead stems and were free from visible pests and diseases [20]. The geographical coordinates of each sampling site were recorded using a handheld Global Positioning System (GPS) device, facilitating the creation of a detailed map (Fig. 1). Formal identification of the plant materials was done by Dr. Eunice Apio Olet, a plant systematic specialist at Mbarara University of Science and Technology. Voucher specimens of the plant species studied were deposited in the national herbarium at Makerere University for further confirmation and allocation of the voucher numbers; MHU51301(*O. usambarensis*) and MHU51302(*S. africana*).

Collecting and processing of plant samples

The stem bark of *O. usambarensis* was cut using a sterile panga following the vertical alternate strip bark two quarters procedures [10]. The stem bark was then carefully detached from the cambium by cutting square wounds from two opposing ends of the tree's circumference, taking only the

outer and middle bark ensuring the inner bark remained intact. The harvested stem bark samples were placed in labeled zip lock bags. Sterilized pruning shears were used to harvest the leaves of *S. africana* and the leaves of the trees from which the stem bark of *O. usambarensis* was extracted. The collected leaves were immediately placed in separate zip lock bags labelled with the plant name, collection date, and GPS location. Excess air was expelled from the bags, and water was sprinkled inside the bags to keep the collected plant samples moist [21]. All the samples were rinsed with distilled water to get rid of any residual dirt, air dried for a month and subsequently ground for phytochemical extraction.

Phytochemical extraction

Phytochemical extraction was done using three solvents: water, ethyl acetate and ethanol. The solvents were selected due to their different polarities [22] to facilitate optimal extraction of both polar and non-polar phytochemical compounds. The inclusion of water was particularly imperative given that it is a major constituent of local herbal concoctions [23], thereby rendering its extraction efficiency highly relevant to the study. Phytochemicals were extracted from 200 g of powdered samples of the leaves of *S. africana* and the stem bark and leaves of *O. Usambarensis* following three extraction protocols: cold maceration, decoction, and infusion. Cold maceration extracted phytochemicals from the stem bark and leaf samples of both medicinal plants using ethyl acetate and ethanol as solvents for a period of 24 and 72 hours, respectively [24, 25]. The decoction technique was utilized for extracting phytochemicals from the stem bark of *O. usambarensis*, using water as the solvent for 15 minutes [26]. The infusion technique extracted phytochemicals from the leaves of both *S. africana* and *O. usambarensis*, with water as the solvent for 45 minutes [27]. The extracts were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated using a rotary evaporator at 70°C, followed by air-drying. The air-dried extracts were transferred into tightly stoppered labelled sterile glass bottles and stored in a refrigerator at 4°C until further analysis.

Qualitative and quantitative phytochemical profiling

Qualitative screening for alkaloids, anthraquinones, anthocyanins, cardiac glycosides, chalcones, flavonoids, phenols, phlobatannins, saponins, steroids, tannins, terpenoids, quinones and polyphenols in the ethanol, water and ethyl acetate extracts was done following standard methods described by [28, 29]. Quantification of total phenolic, flavonoid, saponin and tannin contents in the various plant extracts was done by Folin-Ciocalteu [30], aluminium chloride colorimetric [31], vanillin–sulphuric acid assay [32] and vanillin assay [33] methods respectively.

Antibacterial activity of individual plant extracts and extract combinations

Individual extracts and extract combinations of *O. usambarensis* and *S. africana* were examined for their antibacterial properties. The individual extracts assessed included the ethanol extracts from the stem bark of *O. usambarensis* (S_1), leaves of *O. usambarensis* (S_2), and leaves of *S. africana* (S_3); aqueous extracts from the stem bark of *O. usambarensis* (S_4), leaves of *O. usambarensis* (S_5), and leaves of *S. africana* (S_6); and ethyl acetate extracts from the stem bark of *O. usambarensis* (S_7), leaves of *O. usambarensis* (S_8), and leaves of *S. africana* (S_9). Following the promising antibacterial activities observed in individual extracts, various combinations were prepared by mixing 1 gram of each respective plant extract and the composite examined for antibacterial efficacy and potential synergistic effects. The combinations included S_2S_4 , S_1S_8 , S_5S_9 , S_1S_5 , S_1S_7 , S_3S_6 , S_8S_5 , S_2S_8 , and S_3S_5 .

Standardization of test bacteria stock cultures and culture media

Standard cultures of bacteria strains from the American Type Culture Collection (ATCC), particularly *E. coli*, *S. pneumoniae*, and *S. Aureus* were used in antibacterial activity experiments [34]. Standard stock cultures of *E. coli* and *S. Aureus* were sub cultured on Muller Hinton Agar (MHA) and incubated at 37°C for two days [35] while *S. pneumoniae* was cultured on the Chocolate Agar [36]. Fresh primary cultures of bacteria were preserved in a refrigerator at 40°C for use in antibacterial activity studies. The standard cultures were diluted separately in sterile distilled water to achieve the necessary cell density of 1.5×10^8 cells/ml for comparison with the 0.5 McFarland turbidity standard.

The 0.5 McFarland turbidity standard was prepared by reacting 0.05ml of 1.175% Barium chloride dehydrate solution to 9.95ml of 1% sulphuric acid solution[37]. Media preparation strictly adhered to manufacturer's instructions as follows: 38g and 37g of the Muller Hinton Agar and Brain Heart Infusion Broth respectively were separately weighed and suspended separately in one litre of distilled water. The obtained mixture was heated with continuous agitation until vigorous boiling and sterilized by autoclaving at 121°C for 15 minutes [38]. To prepare the Chocolate Agar, 36.0g of the powder was suspended in 500ml of distilled water. The Chocolate agar-water mixture was also heated to boiling, mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes. The mixture was cooled to 50°C and 500ml of separately autoclaved Hemoglobin solution was added to obtain a final volume of 1 litre [39]. Portions of 25ml of the sterile Muller Hinton Agar and Chocolate Agar were separately poured on sterile glass petri plates and left to solidify. The prepared media were then incubated for 12 hours to test for sterility. Sterile media plates were stored in a refrigerator until further use in antibacterial activity investigations.

Rationale for selection of the bacteria

Three bacterial strains used in the study included *E. coli* ATCC 25922, *S. pneumoniae* ATCC 49619 and *S. aureus* ATCC 25923 which are standard laboratory cultures whose susceptibility on commonly used antibiotics is already established. These bacteria species are also responsible for wide range of human diseases. For instance, *E. coli* is a suitable test microorganism as a representative of the pathogenic enteric bacteria since it is one of the causative agents of gastrointestinal infections and represents the critical priority group of antibiotic resistant bacteria [40]. *S. aureus* represents the high priority category of antibiotic resistant bacteria [40] and the leading cause of bacteremia, skin and soft tissue infections with high resistance to a number of antibiotics. *S. pneumoniae*, a member of the medium priority group, is a common colonizing bacterium in the respiratory tract accounting for 20–43% of bacterial infections and a primary cause of respiratory diseases worldwide responsible for over one million deaths every year [41]. Moreover, *S. pneumoniae* and *S. aureus* have been widely used in antibacterial activity studies as representatives of Gram-positive bacteria [42] while *E. coli* represents the Gram-negative group of bacteria [43].

Agar well diffusion assay

The Agar well diffusion assay experiment was employed in assessing the antibacterial activity of the individual extracts from the two plants; *O. usambarensis* and *S. africana* and selected combinations [10, 44]. To prepare the plant extract stock solutions (1000 mg/ml), 2.0g of the individual extract or combination were dissolved in 2ml of the respective extraction solvent. The stock solutions were subsequently subjected to serial dilutions to achieve the following concentrations: 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.625 mg/ml, and 7.8125 mg/ml.

The test microorganisms were inoculated onto the surface of the sterile MHA (for *S. aureus* and *E. coli*) and Chocolate Agar (for *S. pneumoniae*). The spread plate technique was employed to ensure a uniform distribution of the inoculum across the agar surfaces. Using a sterile 1 ml cork, borer, wells of 8 mm diameter, were created in the culture media on each glass plate. A volume of 200 µL of individual plant extract or combinatory extract solutions, at known concentrations of 1000 mg/mL, 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL, 15.62 mg/mL, and 7.8125 mg/mL, was introduced into each well. Ciprofloxacin solution at a known concentration of 1 mg/mL served as an antibacterial positive control, distilled water, ethanol and ethyl acetate for the solvent controls and an empty well as a negative control for the antibacterial assay. The MHA plates and Chocolate Agar plates (in 5% carbon dioxide) were incubated at 37°C for 24 hours. The experiments were carried out in quadruplicates and upon completion of incubation, the diameters of zone of inhibition were measured using a ruler and recorded in millimeters.

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentrations (MIC) of the plant extracts against the three strains of bacteria were ascertained by macro broth dilution method [44]. Individual plant extracts and combinations stock solutions (1000mg/ml) were prepared. Using a micropipette, 1ml of Brain heart infusion broth was placed into seven sterile glass Bijou Bottles labeled 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.62mg/ml and 7.81mg/ml respectively. This was followed by pipetting a known volume of the stock solution (1ml) into the 1ml of Brain heart infusion broth in the Bijou bottle labeled 500mg/ml and mixed thoroughly to make an overall extract dilution of 500mg/ml. Similarly, other lower dilutions of 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.62mg/ml and 7.81mg/ml were obtained in the brain heart infusion broth. Every diluted extract in triplicates, separately received 10µl of the standard microorganism inoculum (*E. coli*, *S. pneumoniae* and *S. aureus*) and incubated at 37°C for 24 hours. Thereafter, Bijou bottles were removed and visually inspected for turbidity. The lowest extract concentration at which there was no visible growth was taken to be the MIC. To determine the MICs of the selected extract combinations, the procedure was repeated separately using the plant extract combinations instead of the individual extracts.

Minimum Bactericidal Concentration (MBC)

The bacteria inoculants without visible growth after determination of the MIC were sub-cultured separately on fresh MHA (for *E. coli* and *S. aureus*) and Chocolate Agar (for *S. pneumoniae*) at 37°C for 48 hours. The lowest concentrations of the plant extracts that did not yield any colony on the MHA and Chocolate Agar after incubation for the two days were the MBCs. The absence of growth from sub cultured dilutions was indicative of bactericidal activity [45].

Total Antibacterial Activity (TAA) of plant extracts

Total Antibacterial Activity (TAA) of the extracts was determined by dividing the mass of extract yielded per gram of dry plant powder by the minimum inhibitory concentration of the extract against the selected microorganisms [46] as shown in the equations below.

Mass of extract yielded from 1g of dry plant powder (mg/g) = Mass of extract yield (mg)

Mass of powder (g)

The Total Antibacterial Activity was then calculated using the formula:

TAA (ml/g) = Mass of extract from 1gram of powder (mg/g)

Minimum Inhibitory Concentration (mg/ml)

Fractional Inhibitory Concentration Index of plant extract combination effect

The Fractional Inhibitory Concentration Index ($\sum FICI$) was used to investigate the combination effect of selected plant extracts on bacterial strains [47]. The Fractional Inhibitory Concentration Index ($\sum FICI$) was calculated as the sum of the Fractional Inhibitory Concentration (FIC) of the two individual extracts using the formula:

$$\sum FICI = FIC (A) + FIC (B)$$

Where FIC (A) and FIC (B) are the Fractional Inhibitory Concentrations for plant extracts A and B respectively calculated using the formulae:

$$FIC (A) = \frac{MIC (A+B) \text{ in combination}}{MIC (A) \text{ Alone}}$$

$$FIC (B) = \frac{MIC (A+B) \text{ in combination}}{MIC (B) \text{ Alone}}$$

The combination effect was interpreted from the fractional inhibitory concentration index as follows; Synergy was defined by $\sum FICI \leq 0.5$. The $\sum FICI$ between 0.5 and 1.0 was interpreted as additive interaction, $\sum FICI$ between 1.0 and 4.0 as indifferent and antagonist by $\sum FICI > 4.0$ [48].

Data analysis and presentation

Descriptive Statistics (mean and standard deviation of zones of inhibition) and inferential statistical tests were carried out in the Statistical Package for Social Scientists (SPSS) version 26. Quantitative phytochemical data was presented graphically using GraphPad Prism Version 5 [49]. The data on zones of inhibition of the different extracts and extract combinations against the bacterial strains was subjected to one-way ANOVA and Tukey's multiple comparison tests. All tests were performed at 5% level of significance.

Results and discussion

Percentage yield of extracts from *O. usambarensis* and *S. africana*

The percentage yields of aqueous, ethanol and ethyl acetate extract from the stem bark and leaves of *O. usambarensis* and leaves of *S. africana* ranged from 2.450–20.745% (Table 1). The individual solvents produced varying ranges of extracts (ethanol = 9.975–20.745%, water = 2.450–14.625%, ethyl acetate = 5.100–8.265%) attributed to differences in their polarities [50]. Polar solvents such as ethanol and water are capable of extracting secondary metabolites with polar properties such as flavonoids, glycosides, tannins, anthocyanins, terpenoids, saponins as well as phenolic compounds with low molecular weights and moderate polarity [51]. Non-polar solvents such as ethyl acetate however, mainly dissolve lipophilic compounds including waxes, coloured pigments and sterols [52]. The substantial extract yields achieved with ethanol and water (Table 1) are suggestive of significant proportions of phytochemicals present in the two plants to be of moderate to high polarity.

Further comparisons of the two solvents reveal higher percentage yields of phytochemical in ethanol than water. Similarly, previous studies have reported higher phytochemical extract diversity in ethanol than water [53]. Due to the intermediate polarity of ethanol (0.654) coupled with high penetration potential of plant cell membranes interacting with both hydrophilic and hydrophobic parts of the plant cells, ethanol is a versatile solvent with a broader spectrum of phytochemicals extraction of both polar and non-polar compounds. Water is highly polar (1.000) and more selective for polar and hydrophilic compounds [54] which narrow its phytochemical extraction potential. Ethanol is also more volatile than water [55] enabling concentration of extracts at lower temperature giving ethanol a higher retention capacity of temperature-sensitive phytochemicals than water.

The variation in phytochemicals among parts of the same plants as observed in this study with the stem bark of *O. usambarensis* producing higher percentage yields of the extracts in the three solvent than the leaves maybe attributed to differences in functionality and the ages of the plant tissues [56]. Unlike the leaves, whose main function is synthesis, some stems serve as storage sites, transport routes and defense barriers against biotic and abiotic stress. The stem functions coupled with the frequent replacement of leaves, give the stem bark an upper hand for accumulating phytochemical compounds over time. These findings align with results of previous studies that reported the stem as the most phytochemically enriched part of the plant with the order stems > leaves > flowers > roots [57].

Variations in extraction temperature and time may also be responsible for the differences in extract yields observed. Higher temperatures coupled with longer extraction time, render plant tissues more soft and allow extend contact between the plant tissue and the solvent increasing the diffusion of phytochemicals through the cell membrane into the solvent [58]. Elevated temperatures may account for the higher percentage yield of the aqueous leaf extract of *S. africana* obtained through hot water infusion compared to ethyl acetate extracts of the same plants attained at room temperature by cold maceration. Similarly, the longer extraction time (72 hours) allowed for maceration could explain the higher percentage yield of stem bark extracts of *O. usambarensis* compared to the extract yield of stem bark of the same plant by decoction done for 15 minutes [59]. Genetic

variances can as well account for the different extract yields obtained from the two plants using similar plant parts, same extraction solvent and method [60].

Table 1
Percentage yield of *O. usambarensis* and *S. africana* stem bark and leaf extracts

Sample Extract	Mass of powder(g)	Mass of extract (g)	Percentage yield (%)
S ₁	200	41.49	20.745
S ₂	200	19.95	9.975
S ₃	200	37.33	18.665
S ₄	200	18.53	9.265
S ₅	200	4.90	2.450
S ₆	200	29.25	14.625
S ₇	200	16.53	8.265
S ₈	200	10.78	5.390
S ₉	200	10.2	5.100
KEY: S ₁ -S ₃ : - ethanol extracts [stem bark of <i>O. usambarensis</i> (S ₁); leaves of <i>O. usambarensis</i> (S ₂); leaves of <i>S. africana</i> (S ₃)], S ₄ -S ₆ : - aqueous extracts [stem bark of <i>O. usambarensis</i> (S ₄); leaves of <i>O. usambarensis</i> (S ₅); leaves of <i>S. africana</i> (S ₆)], S ₇ -S ₉ : - ethyl acetate extracts (stem bark of <i>O. usambarensis</i> (S ₇); leaves of <i>O. usambarensis</i> (S ₈); leaves of <i>S. africana</i> (S ₉)). Phytochemical profile of extracts from the stem bark and leaves of <i>O. usambarensis</i> and leaves of <i>S. africana</i>			

Qualitative phytochemical profile

The leaves of *S. africana* and leaves and stem bark of *O. usambarensis* were screened for flavonoids, phenols, saponins, alkaloids, cardiac glycosides, glycosides, terpenoids, tannins, anthocyanins, anthraquinones, chalcones, phlobatannins, quinones, steroids and polyphenones as shown in Table 3. The distribution of the phytochemicals may be related to their functions in plant systems and biochemical reactions. For instance, all the plant parts studied had flavonoids, saponins, tannins, terpenoids and quinones.

Given that the leaves and some stem barks are active photosynthetic and respiratory organs, the presence of quinones in these parts maybe related to their active role as electron carriers for the two biological systems [61]. Similarly, flavonoids and saponins protect plants against UV radiations, microorganism and herbivores [62, 63]. Due to their location on the plants, leaves and the stem barks are exposed and are vulnerable to UV radiations, microorganism attack and herbivory activity dictating the presence of protective bioactive compounds such as flavonoids and saponins to accord these parts the necessary protection. Tannins, terpenoids and cardiac glycosides are not much different from saponins and flavonoids in terms of their functions. They also offer plants antibacterial, anti-inflammatory, antioxidant services in addition to protection against other pathogens, harmful insects, herbivores and abiotic stress such as high temperature, functions needed in all parts of a plant, hence their presence in the all parts studied [64]. Cardiac glycosides are also confined to the angiosperms and widely present in family Apocynaceae and Lauraceae to which *S. africana* and *O. usambarensis* belong [65]. The presence of these phytochemicals in the extracts of *O. usambarensis* and *S. africana*, corroborate the use of these plants in herbal medicine among Ugandan communities.

Phenols and cardiac glycosides were present in all the extracts of *S. africana* and *O. usambarensis* except in the ethyl acetate acetate extracts of the two-plant species. Ethyl acetate might have failed to extract the phenols and cardiac glycosides due to its lower polarity and limited hydrogen bonding capacity compared to water and ethanol. These properties render ethyl acetate less suitable for dissolving and extracting phenols and cardiac glycosides which are typically more polar and depend on hydrogen bonding for their solubility [66]. Alkaloids and anthocyanins were however absent in extracts of the leaves of *S. africana* and leaves and stem bark of *O. usambarensis* (Table 3). Despite their protective roles, some plant families do not produce alkaloid and they occur in about 20% of known plants [67]. The two-plant species studied may be among the species that are genetically incapacitated from producing alkaloids. Alternatively, alkaloids may be produced in other parts of these two species such as the roots or seeds as earlier reported for *Sophora flavescens* [68]. The observed absence of anthocyanins among all the extracts could as well be attributed to a genetic inability of the plant species to produce them [69]. The two plants may also contain betalains instead of anthocyanins given that the two phytochemicals have antagonistic pathways and never occur in the same plant [70]. Despite the wide distribution of steroids among plants [71] with a wide range of solubility values that enable their extraction with both polar and non-polar solvents [72], the ethyl acetate leaf extract of *S. africana* (S₉) tested negative. Given that the solvent was able to extract, the same compounds from *O.usambarensis*, their absence in the ethyl acetate extract of *S. African* may be due to low quantities below the detection limit or any other factor that we could not explained from

the settings of our study. All extracts from the leaves of *S. africana* (S₃, S₆ and S₉) tested negative for anthraquinones pointing to the possible absence of these compounds from the species. Moreover, anthraquinones exist in limited groups of angiosperm families such as Fabaceae, Liliaceae, Polygonaceae, Rhamnaceae, Rubiaceae and Scrophulariaceae [73]. Previous attempts with other species of the family Apocynaceae to which *S. africana* belongs, equally revealed negative results for anthraquinones [74, 75]. Chalcones were absent in ethanol extracts of the stem bark and leaves of *O. usambarensis*, aqueous and ethyl acetate extracts of leaves of *O. usambarensis* and *S. africana*. This could be attributed to the conversion of chalcones into flavonoids by the enzyme chalcone isomerase[76].

Table 3
Phytochemical profile of the leaf and stem bark extracts of *O. usambarensis* and *S. africana*

Phytochemical	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉
Alkaloids	-	-	-	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	-	-
Saponins	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	-	-	-
Glycosides	+	-	-	-	+	-	-	-	-
Terpenoids	+	+	+	+	+	+	+	-	+
Tannins	+	+	+	+	+	+	+	+	+
Anthocyanins	-	-	-	-	-	-	-	-	-
Steroids	+	+	+	+	+	+	+	+	-
Polyphenols	+	+	+	+	+	+	+	+	+
Anthraquinones	+	-	-	-	-	-	+	-	-
Chalcones	-	-	+	+	-	-	+	-	-
Phlobatannins	+	-	+	+	+	-	+	+	-
Quinones	+	+	+	+	+	+	+	+	+

KEY: +: - Present, -: - Absent, S₁-S₃: - ethanol extracts [stem bark of *O. usambarensis*(S₁); leaves of *O. usambarensis* (S₂); leaves of *S. africana* (S₃)], S₄-S₆: - aqueous extracts [stem bark of *O. usambarensis* (S₄); leaves of *O. usambarensis*(S₅); leaves of *S. africana* (S₆)], S₇-S₉: - ethyl acetate extracts (stem bark of *O. usambarensis* (S₇); leaves of *O. usambarensis* (S₈); leaves of *S. africana* (S₉)).

Quantitative Phytochemical Composition of extracts of *O. usambarensis* and *S. Africana*

The quantities of phytochemical compounds varied among the aqueous, ethanol and ethyl acetate extracts of *O. usambarensis* and *S. africana* (Fig. 2). The total phenolic content varied significantly among the extracts of *O. usambarensis* and *S. africana* (p < 0.001) (Fig. 2A). The aqueous and ethyl acetate stem bark extract of *O. usambarensis* (S₄ and S₇ respectively) had the highest amount of total phenolics. The total phenolic content was however below detectable limits in the ethyl acetate leaf extracts of *O. usambarensis* (S₈) and *S. africana* (S₉). In conjunction with the qualitative results, the variations in total phenolic contents among extracts could as well be accounted for by the solubility of phenols which varies with the families to which the phenols belong [77] and differences in polarities of extraction solvents [78]. Differences in physiological states of the plant parts at the time of collection coupled with their respective functions may explain the variations observed in the phenolic content of the extracts of the stem bark and leaves of *O. usambarensis* in the same solvent [79]. The results could also be explained by the extraction temperature and duration [80]. For instance, the aqueous extract of the stem bark of *O. usambarensis* (S₄) was extracted by decoction at higher temperature which could have increased the rate of diffusion and solubility of the phenolic compounds from the crude extract into the extraction solvent.

Generally, the total flavonoid content was higher in the aqueous and ethyl acetate extracts than ethanol extracts (Fig. 2B). Total flavonoid content was highest in ethyl acetate leaf extracts of *S. africana* (S₉) and lowest in the ethanol extract of the stem bark of *O. usambarensis* (S₁). This could be explained by factors such as genetic variation between the two plants and the amount of sunlight received by the plant parts as well as the polarity of extraction solvent used [81]. Flavonoids absorb UV wavelengths and protect plant parts against exposure to UV radiation. Therefore, leaves which are usually more exposed to sunlight have higher flavonoid content to accord them more protection than the stem bark. The highly polar water and non-polar ethyl acetate extracted more flavonoids than ethanol depicting presence of very highly polar and non-polar flavonoids in the two plants. This could account for the high flavonoid content in the water and ethyl acetate extract than in the ethanol with moderate polarity.

Being a large group of secondary metabolites that occurs in significant amounts in many plant species [82], considerable quantities of total saponins in the range of 25–90 µg/mg were obtained in the extracts (Fig. 2C). Although saponins are known to be polar compounds and mainly extracted by water and alcohols [83], the ethyl acetate extracts in this study, had the second highest amounts of saponins after the aqueous extracts. Relatedly, previous fractionation experiments recovered more saponins in the ethyl acetate fraction than other solvent fractions [84]. The higher quantities of saponins recorded in the aqueous extracts than the other two solvents may be attributed to the relatively higher temperature at which the aqueous extracts were obtained using the decoction method. For instance, an increase in temperature from 30°C to 70°C increased the solubility of gypsophia saponin in water from 7.4 g/100 ml to 18.0 g/100 ml [83]. Given that the samples from the two plants were obtained from the same location /environment, the variations in saponin quantities among extracts of the two plants obtained with the same solvent can only be attributed to their genetic differences [85].

Despite the fact that tannins were present in all ethanol, aqueous and ethyl acetate extracts of *O. usambarensis* and *S. africana*, the content was more pronounced in the leaf than stem extracts (Fig. 2D). The leaves are more exposed to various environmental stresses such as herbivory, pathogenic infections and harmful UV radiations requiring more protection than the stem. As such, the higher tannin content gives the leaves more protection by making them unpalatable and through its antimicrobial properties [86]. The presence of tannins in the two plant extracts gives them the antimicrobial and antioxidant properties which support their medicinal usage [86]

Antibacterial activity of the extracts from leaves and stems bark of *O. usambarensis*, leaves of *S. africana* and their combinations against *E. coli*, *S. pneumonia* and *S. aureus*

Antibacterial Zones of Inhibition

The phytochemical composition of any plant extract has an impact on the plant's bioactive potential including aspects such as its antibacterial activity [87]. The ethanol, aqueous and ethyl acetate extracts and extract combinations of *O. usambarensis* and *S. africana* all inhibited the growth of *E. coli*, *S. aureus* and *S. pneumoniae* demonstrating their antibacterial activity on both Gram-positive and Gram-negative bacteria. The antibacterial properties of the extracts are attributed to phytochemical compounds such as flavonoids, phenols, saponins, terpenoids, tannins, quinones, and steroids among others detected in the extracts of the two medicinal plants [88]. These phytochemicals have been previously reported to possess antibacterial, antiviral, antifungal and antioxidant properties which are vital for human health [88, 89]. The phytochemical compounds disrupt the bacterial cell structure and increase its permeability and leakage of the cell constituents, alter the bacterial cell wall and membrane, inhibit protein synthesis in the bacteria, lead to ATP loss, intracytoplasmic damage and pH disturbance. They also inhibit peptidoglycan synthesis and impair the bacterial membrane structure by modifying its surface hydrophobicity [90].

The antibacterial activities of the plant extracts increased with increase in concentration against the three strains of bacteria used in this study with the maximum zones of inhibition attained at 1000mg/ml and the minimum zones of inhibition at 31.25mg/ml. This is because as the concentration of the plant extracts increase, the concentrations of phytochemical compounds with antibacterial activities increase. Similar studies have reported related findings, portraying positive correlation between antibacterial activity and plant extract concentration [91].

Various extracts including the ethanol and aqueous leaf extracts of *S. africana* as well as the aqueous and ethyl acetate extracts of leaves and the stem bark of *O. usambarensis* had high antibacterial activities against *S. aureus* at concentrations of 1000mg/ml and 500mg/ml. The highest antibacterial activity against *S. aureus* was attained with the aqueous extract of the leaves of *O. usambarensis* (S5) (Table 4). This could be due to the presence of high diversity of phytochemical compounds such as terpenoids, tannins, steroids, quinones, flavonoids, phenols, and saponins among others in the respective plant extracts. The availability of flavonoids, tannins and phenols which have been proved to have antibacterial activity against *S. aureus* including the resistant strains [92, 94] in the various extracts from the two plants explains the sensitivity of *S. aureus*. Studies involving the treatment of *S. aureus* with tannins and phenols show that these phytochemicals change the properties of its cell membranes. Phenols and tannins exert their activity on *S. aureus* in three ways, that is bacterial cell membrane attachment, cell membrane fluidity modification and cell membrane structure disruption [95, 96]. Flavonoids on the other hand, act on *S. aureus* by disrupting the membrane structure and inhibiting the efflux pumps and beta lactamases [94].

S. aureus exhibited intermediate to low sensitivity against most plant extract combinations (Table 9) which could be attributed to indifferent or antagonistic effects of the combinations [97]. The intermediate activity of the plant extract combinations compared to the activity of individual extracts of the two-plant species can be attributed to the fact that the concentration of each individual extract in a combination was lower than the concentration of single individual extract acting against *S. aureus* or reactions between the extracts produced weaker compounds [98].

Table 4
Mean $\pm$ SD Zone of inhibition of individual extracts *O. usambarensis* and *S. africana* and extract combinations against *S. aureus*

Mean $\pm$ SD Zone of inhibition of <i>S. aureus</i>									
Extract	1000mg/ml	500 mg/ml	250 mg/ml	125 mg/ml	62.5mg/ml	31.25mg/ml	15.625mg/ml	7.812mg/ml	Ciprofloxacin
S ₁	14.67 $\pm$ 0.58 ^a	13.33 $\pm$ 1.53 ^{ab}	11.67 $\pm$ 1.53 ^a	9.333 $\pm$ 0.58 ^a	6.667 $\pm$ 1.155 ^e	0.0	0.0	0.0	17.33 $\pm$ 1.155
S ₂	22.67 $\pm$ 0.58 ^b	16.00 $\pm$ 1.00 ^a	12.67 $\pm$ 1.16 ^a	10.67 $\pm$ 1.53 ^a	8.000 $\pm$ 1.00 ^e	6.000 $\pm$ 1.0025 ^h	0.0	0.0	26.00 $\pm$ 1.00
S ₃	22.00 $\pm$ 1.00^b	11.33 $\pm$ 0.58 ^b	10.00 $\pm$ 1.00 ^a	7.000 $\pm$ 1.00 ^b	0.0	0.0	0.0	0.0	21.00 $\pm$ 1.00
S ₄	20.00 $\pm$ 1.00 ^b	17.00 $\pm$ 1.00 ^a	15.67 $\pm$ 0.58 ^c	15.00 $\pm$ 0.00 ^c	11.33 $\pm$ 0.58 ^g	9.667 $\pm$ 0.58 ⁱ	0.0	0.0	32.67 $\pm$ 0.58
S ₅	20.67 $\pm$ 0.58 ^b	16.67 $\pm$ 0.58 ^a	14.67 $\pm$ 0.58 ^c	13.00 $\pm$ 1.00 ^a	11.00 $\pm$ 1.00 ^g	0.0	0.0	0.0	32.00 $\pm$ 1.00
S ₆	26.67 $\pm$ 0.58 ^c	25.33 $\pm$ 0.58 ^h	14.33 $\pm$ 0.58 ^{a c}	10.67 $\pm$ 0.58 ^a	5.333 $\pm$ 0.58 ^f	0.0	0.0	0.0	35.33 $\pm$ 0.58
S ₇	26.00 $\pm$ 1.00^c	16.00 $\pm$ 1.00 ^{ab}	13.00 $\pm$ 1.00 ^{a c}	8.667 $\pm$ 0.58 ^{a b}	6.000 $\pm$ 1.00 ^{ef}	4.667 $\pm$ 0.58 ^j	0.0	0.0	20.33 $\pm$ 0.58
S ₈	18.67 $\pm$ 0.58 ^b	15.67 $\pm$ 0.58 ^{a c}	12.67 $\pm$ 0.58 ^{a c}	8.000 $\pm$ 1.00 ^{a b}	0.0	0.0	0.0	0.0	24.67 $\pm$ 0.58
S ₉	29.00 $\pm$ 1.00^c	11.33 $\pm$ 0.58 ^{ab}	9.000 $\pm$ 1.00 ^b	7.000 $\pm$ 1.00 ^{ab}	0.0	0.0	0.0	0.0	21.00 $\pm$ 1.00
S ₂ S ₄	18.67 $\pm$ 1.53 ^b	16.00 $\pm$ 1.00 ^{ac}	11.33 $\pm$ 0.58 ^{abc}	8.333 $\pm$ 1.16 ^{ab}	7.000 $\pm$ 1.00 ^{ef}	0.0	0.0	0.0	28.67 $\pm$ 0.58
S ₁ S ₈	17.00 $\pm$ 1.00 ^{ab}	15.00 $\pm$ 1.732 ^{a d e}	11.33 $\pm$ 0.58 ^{a b}	8.333 $\pm$ 1.16 ^{ab}	7.333 $\pm$ 0.58 ^{ef}	0.0	0.0	0.0	19.00 $\pm$ 1.00
S ₅ S ₉	17.00 $\pm$ 1.00 ^{ab}	15.67 $\pm$ 1.155 ^{a d e}	14.00 $\pm$ 1.00 ^{a c}	13.00 $\pm$ 1.00 ^{ac}	0.0	0.0	0.0	0.0	25.67 $\pm$ 0.58
S ₁ S ₅	21.67 $\pm$ 0.58 ^b	18.00 $\pm$ 1.00 ^{ae}	14.67 $\pm$ 1.53 ^{a c}	10.00 $\pm$ 1.00 ^{ad}	7.000 $\pm$ 1.00 ^{ef}	0.0	0.0	0.0	30.00 $\pm$ 1.00
S ₁ S ₇	36.00 $\pm$ 1.00^d	29.67 $\pm$ 0.58 ⁱ	24.67 $\pm$ 0.58 ^e	22.33 $\pm$ 0.58	17.67 $\pm$ 1.53	14.00 $\pm$ 1.00	0.0	0.0	26.00 $\pm$ 1.00
S ₃ S ₆	20.67 $\pm$ 0.58 ^b	17.33 $\pm$ 0.58 ^{ae}	15.67 $\pm$ 0.58 ^{a c}	0.0	0.0	0.0	0.0	0.0	22.67 $\pm$ 0.5
S ₈ S ₅	19.67 $\pm$ 0.58^b	14.00 $\pm$ 1.00 ^{ab}	11.33 $\pm$ 1.16 ^{a d}	9.333 $\pm$ 0.58 ^{ab}	8.333 $\pm$ 0.58 ^e	7.667 $\pm$ 0.58	0.0	0.0	18.00 $\pm$ 1.00
S ₂ S ₈	16.33 $\pm$ 0.58 ^{ab}	13.00 $\pm$ 1.00 ^{abf}	12.00 $\pm$ 0.00 ^{a c}	9.667 $\pm$ 0.58 ^a	8.667 $\pm$ 0.58 ^{eg}	7.333 $\pm$ 0.58	0.0	0.0	29.67 $\pm$ 0.58
S ₃ S ₅	15.50 $\pm$ 0.71 ^{ab}	11.67 $\pm$ 0.58 ^{abg}	10.00 $\pm$ 1.00 ^{a b}	10.33 $\pm$ 0.58 ^a	8.333 $\pm$ 0.58 ^{eh}	0.0	0.0	0.0	28.00 $\pm$ 1.00
ANOVA P-value	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	*	*	*

^{a-j} in columns: mean values sharing a superscript letter are not significantly different ($p > 0.05$) as based on Tukey's multiple comparison test, bolded values are above and significantly differently from those of Ciprofloxacin ($p < 0.001$). S₁-S₃: - ethanol extracts [stem bark of *O. usambarensis*(S₁); leaves of *O. usambarensis* (S₂); leaves of *S. africana* (S₃)], S₄-S₆: - aqueous extracts [stem bark of *O. usambarensis* (S₄); leaves of *O. usambarensis*(S₅); leaves of *S. africana* (S₆)], S₇-S₉: - ethyl acetate extracts (stem bark of *O. usambarensis* (S₇); leaves of *O. usambarensis* (S₈); leaves of *S. africana* (S₉)). *- No ANOVA p-value.

All extracts and combinations at concentrations of 1000mg/ml, 500mg/ml and 250mg/ml inhibited the growth of *E. coli* (Table 5). The ethanol extract of the stem bark of *O. usambarensis* (S₁) showed exceptional antibacterial activity by inhibiting the growth of *E. coli* at the lowest concentration of 31.25mg/ml. Although majority of the extracts required high concentrations to show sensitivity against *E. coli*, some extracts and combinations including the ethanol extract of the stem of *O. usambarensis* (S₁), aqueous extract of leaves of *S. africana* (S₆) and the ethyl acetate extracts of the stem bark (S₇) and leaves (S₈) of *O. usambarensis* showed greater activity on *E. coli* than Ciprofloxacin, the standard drug. These results depict the presence of bioactive compounds with antibacterial activity against Gram-negative bacteria including *E. coli* [99, 100]. Some extract combinations; the ethanol and aqueous extracts of leaves of *O. usambarensis* (S₂S₄), ethanol and aqueous extracts of leaves of *S. africana* (S₃S₆) had higher activities compared to individual extracts against *E. coli* which could be attributed to cumulative effects of the constituents in the extracts of the combinations [101].

Table 5
Mean $\pm$ SD Zone of inhibition of individual extracts of *O. usambarensis* and *S. africana* and extract combinations against *E. coli*

Mean $\pm$ SD Zone of inhibition of <i>E. coli</i>									
Extract	1000mg/ml	500mg/ml	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml	15.625mg/ml	7.812mg/ml	Ciprofloxacin
S ₁	20.00 $\pm$ 1.00^a	12.67 $\pm$ 1.53 ^a	11.00 $\pm$ 1.00 ^a	9.667 $\pm$ 0.58 ^c	6.667 $\pm$ 0.58 ^d	5.333 $\pm$ 0.58 ⁱ	0.0	0.0	19.67 $\pm$ 0.58
S ₂	20.00 $\pm$ 1.00 ^a	16.00 $\pm$ 1.00 ^a	14.33 $\pm$ 1.53 ^b	11.67 $\pm$ 0.58 ^c	10.33 $\pm$ 0.58 ^f	0.0	0.0	0.0	26.00 $\pm$ 1.00
S ₃	25.00 $\pm$ 1.00 ^c	19.00 $\pm$ 1.00 ^a	16.00 $\pm$ 1.00 ^b	12.00 $\pm$ 1.00 ^c	10.33 $\pm$ 0.58 ^e	0.0	0.0	0.0	28.00 $\pm$ 1.00
S ₄	18.00 $\pm$ 1.00 ^a	17.33 $\pm$ 1.53 ^a	14.67 $\pm$ 0.58 ^b	13.00 $\pm$ 1.00 ^c	11.00 $\pm$ 1.00 ^{fe}	0.0	0.0	0.0	32.00 $\pm$ 1.00
S ₅	22.33 $\pm$ 0.58 ^c	16.00 $\pm$ 1.00 ^a	12.00 $\pm$ 1.00 ^{ab}	12.00 $\pm$ 1.00 ^c	8.000 $\pm$ 1.00 ^d	0.0	0.0	0.0	35.00 $\pm$ 1.00
S ₆	24.33 $\pm$ 0.58^c	19.00 $\pm$ 1.00^a	13.00 $\pm$ 1.00 ^{ab}	11.33 $\pm$ 0.58 ^c	8.000 $\pm$ 1.00 ^d	0.0	0.0	0.0	22.00 $\pm$ 1.00
S ₇	25.33 $\pm$ 0.58^c	20.80 $\pm$ 1.00^{ac}	14.67 $\pm$ 0.58 ^b	12.67 $\pm$ 1.00 ^c	0.0	0.0	0.0	0.0	15.00 $\pm$ 1.00
S ₈	22.00 $\pm$ 1.00^{ac}	20.67 $\pm$ 0.58 ^a	18.67 $\pm$ 0.58	14.67 $\pm$ 0.58 ^c	10.67 $\pm$ 0.58 ^{fe}	0.0	0.0	0.0	19.00 $\pm$ 1.00
S ₉	19.67 $\pm$ 0.58 ^a	11.00 $\pm$ 1.00 ^a	9.667 $\pm$ 0.58 ^{ac}	7.000 $\pm$ 1.00 ^f	6.000 $\pm$ 0.58 ^d	0.0	0.0	0.0	22.67 $\pm$ 0.58
S ₂ S ₄	25.33 $\pm$ 1.53^{cd}	21.67 $\pm$ 0.58^{ad}	10.00 $\pm$ 1.00 ^{ab}	7.667 $\pm$ 0.58 ^{cf}	0.0	0.0	0.0	0.0	19.67 $\pm$ 0.58
S ₁ S ₈	33.67 $\pm$ 0.58 ^e	19.67 $\pm$ 0.58 ^a	13.33 $\pm$ 0.58 ^{abc}	0.0	0.0	0.0	0.0	0.0	36.00 $\pm$ 1.00
S ₅ S ₉	32.67 $\pm$ 0.58 ^f	18.67 $\pm$ 3.055 ^{ab}	12.67 $\pm$ 0.58 ^{ab}	9.000 $\pm$ 1.00 ^{cdf}	8.333 $\pm$ 1.155 ^{efd}	0.0	0.0	0.0	37.00 $\pm$ 1.00
S ₁ S ₅	16.33 $\pm$ 0.58 ^{ab}	13.67 $\pm$ 0.58 ^a	0.0	0.0	0.0	0.0	0.0	0.0	24.67 $\pm$ 1.53
S ₁ S ₇	34.33 $\pm$ 3.79 ^f	26.33 $\pm$ 0.58	13.67 $\pm$ 0.58 ^b	11.00 $\pm$ 1.00 ^{ce}	0.0	0.0	0.0	0.0	35.67 $\pm$ 0.58
S ₃ S ₆	35.67 $\pm$ 0.58^{ef}	27.00 $\pm$ 1.00 ^a	13.33 $\pm$ 0.58 ^{ab}	0.0	0.0	0.0	0.0	0.0	30.67 $\pm$ 0.58
S ₈ S ₅	16.00 $\pm$ 1.00 ^a	11.67 $\pm$ 0.58 ^{ad}	10.00 $\pm$ 1.00 ^{ad}	8.000 $\pm$ 1.00 ^{df}	6.000 $\pm$ 1.00 ^{dg}	0.0	0.0	0.0	26.67 $\pm$ 1.53
S ₂ S ₈	12.67 $\pm$ 0.58 ^{ab}	11.67 $\pm$ 0.58 ^a	10.00 $\pm$ 1.00 ^a	8.667 $\pm$ 0.58 ^{cfe}	6.667 $\pm$ 0.58 ^d	0.0	0.0	0.0	28.33 $\pm$ 0.58
S ₃ S ₅	18.67 $\pm$ 1.53 ^a	16.00 $\pm$ 1.00 ^{ae}	11.33 $\pm$ 0.58 ^{ab}	8.333 $\pm$ 1.155 ^{cfg}	7.000 $\pm$ 1.00 ^{dh}	0.0	0.0	0.0	28.33 $\pm$ 0.58
ANOVA p-value	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	*	*	*

^{a-i} In columns: mean values sharing a superscript letter are not significantly different ($p > 0.05$) as based on Tukey's multiple comparison test, bolded values are above and significantly different from those of Ciprofloxacin ($p < 0.001$). S₁-S₃: - ethanol extracts [stem bark of *O. usambarensis*(S₁); leaves of *O. usambarensis* (S₂); leaves of *S. africana* (S₃)], S₄-S₆: - aqueous extracts [stem bark of *O. usambarensis* (S₄); leaves of *O. usambarensis*(S₅); leaves of *S. africana* (S₆)], S₇-S₉: - ethyl acetate extracts (stem bark of *O. usambarensis* (S₇); leaves of *O. usambarensis* (S₈); leaves of *S. africana* (S₉)). *: - No ANOVA p-value.

Like *S. aureus* and *E. coli*, all individual extracts and extract combinations showed antibacterial activity against *S. pneumoniae* at extract concentrations of 1000mg/ml and 500mg/ml with mean zones of inhabitation ranging from 11.67-29.00mm (Table 6). This could probably be a

result of the high concentrations of bioactive compounds such as flavonoids and quinones with previous reports of antibacterial activity on various bacterial strains, *S. pneumoniae* inclusive [102]. At concentration below 250mg/ml however, *S. pneumoniae* was generally resistant with inhibition zones below 16.00mm. Therefore, the plant extracts and their combinations at concentrations of 1000mg/ml and 500mg/ml had the capacity to cause significant antimicrobial susceptibility to *S. pneumoniae* just like standard ciprofloxacin while at lower concentrations, the extracts lost this activity. The higher activities of some extract combinations such as S3S5 against *S. pneumoniae* than ciprofloxacin justify the use of more than one plant for development of herbal medicine by traditional healers and herbal medicine practitioners. Generally, the leaf extracts of *O. usambarensis* and *S. africana* had stronger inhibitory activity against *S. pneumoniae* compared to the stem bark extracts. The stem bark and leaves carry out different functions in the plant accounting for the variation in their phytochemical composition and antimicrobial activities.

Table 6

Mean $\pm$ SD Zone of inhibition of individual extracts *O. usambarensis* and *S. africana* and combinations against *S. pneumoniae*

Mean $\pm$ SD Zone of inhibition of <i>Streptococcus pneumoniae</i>									
Extract	1000mg/ml	500mg/ml	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml	15.62mg/ml	7.81mg/ml	Ciprofloxacin
S ₁	22.00 $\pm$ 1.00 ^a	13.33 $\pm$ 0.58 ^b	9.667 $\pm$ 0.58 ^c	8.000 $\pm$ 1.00 ^d	0.0	0.0	0.0	0.0	36.33 $\pm$ 0.58
S ₂	21.33 $\pm$ 0.58 ^a	16.33 $\pm$ 0.58 ^c	13.00 $\pm$ 1.00 ^c	11.67 $\pm$ 0.58 ^g	9.00 $\pm$ 1.00 ^e	0.0	0.0	0.0	29.67 $\pm$ 0.58
S ₃	22.67 $\pm$ 5.86 ^a	19.00 $\pm$ 1.00 ^d	16.00 $\pm$ 1.00 ^f	11.67 $\pm$ 1.53 ^g	8.667 $\pm$ 0.58 ^e	0.0	0.0	0.0	36.00 $\pm$ 1.00
S ₄	26.67 $\pm$ 0.58 ^a	21.33 $\pm$ 0.58 ^d	17.67 $\pm$ 0.58 ^f	15.67 $\pm$ 0.58	0.0	0.0	0.0	0.0	33.67 $\pm$ 0.58
S ₅	16.00 $\pm$ 1.00 ^d	12.67 $\pm$ 0.58 ^b	11.00 $\pm$ 1.00 ^c	7.667 $\pm$ 0.58 ^d	0.0	0.0	0.0	0.0	16.00 $\pm$ 1.00
S ₆	23.67 $\pm$ 0.58 ^a	17.33 $\pm$ 0.58 ^{ce}	13.00 $\pm$ 1.00 ^c	10.67 $\pm$ 0.58 ^{eg}	0.0	0.0	0.0	0.0	27.33 $\pm$ 0.58
S ₇	19.00 $\pm$ 1.00 ^{ad}	11.67 $\pm$ 0.58 ^b	9.000 $\pm$ 1.00 ^{cg}	8.333 $\pm$ 2.52 ^{de}	0.0	0.0	0.0	0.0	28.33 $\pm$ 1.155
S ₈	13.67 $\pm$ 0.58 ^e	11.67 $\pm$ 0.58 ^b	8.000 $\pm$ 1.00 ^c	7.333 $\pm$ 0.58 ^d	0.0	0.0	0.0	0.0	13.33 $\pm$ 0.58
S ₉	22.00 $\pm$ 1.00^a	12.00 $\pm$ 1.00 ^b	8.000 $\pm$ 1.00 ^c	0.0	0.0	0.0	0.0	0.0	16.67 $\pm$ 1.53
S ₂ S ₄	18.67 $\pm$ 0.58^{ade}	15.33 $\pm$ 0.58 ^{bcf}	13.33 $\pm$ 0.58 ^{cf}	6.333 $\pm$ 0.58 ^d	4.333 $\pm$ 0.58 ^f	0.0	0.0	0.0	17.67 $\pm$ 0.58
S ₁ S ₈	27.00 $\pm$ 1.00 ^{ac}	16.33 $\pm$ 1.53 ^c	0.0	0.0	0.0	0.0	0.0	0.0	29.67 $\pm$ 0.58
S ₅ S ₉	29.00 $\pm$ 1.00 ^c	18.67 $\pm$ 0.58 ^{cd}	13.00 $\pm$ 1.00 ^c	10.00 $\pm$ 1.00 ^{fgd}	0.0	0.0	0.0	0.0	32.67 $\pm$ 0.58
S ₁ S ₅	19.33 $\pm$ 0.58 ^{ad}	13.67 $\pm$ 0.58 ^b	11.33 $\pm$ 0.58 ^c	0.0	0.0	0.0	0.0	0.0	25.33 $\pm$ 0.58
S ₁ S ₇	17.67 $\pm$ 0.58 ^{ade}	15.33 $\pm$ 0.58 ^{bcg}	12.67 $\pm$ 0.58 ^c	0.0	0.0	0.0	0.0	0.0	18.33 $\pm$ 0.58
S ₃ S ₆	26.00 $\pm$ 1.00 ^{abc}	17.33 $\pm$ 0.58 ^{cd}	13.33 $\pm$ 0.58 ^c	0.0	0.0	0.0	0.0	0.0	27.33 $\pm$ 0.58
S ₈ S ₅	18.67 $\pm$ 1.53 ^{abde}	13.00 $\pm$ 1.00 ^{bh}	10.67 $\pm$ 0.58 ^{cd}	0.0	0.0	0.0	0.0	0.0	29.00 $\pm$ 1.00
S ₂ S ₈	17.00 $\pm$ 1.00 ^{ade}	16.00 $\pm$ 1.00 ^{cb}	11.67 $\pm$ 0.58 ^{ch}	8.667 $\pm$ 0.58 ^d	0.0	0.0	0.0	0.0	28.67 $\pm$ 0.58
S ₃ S ₅	21.67 $\pm$ 1.53^a	18.33 $\pm$ 0.58 ^{cd}	15.33 $\pm$ 0.58 ^{efc}	12.67 $\pm$ 1.53 ^g	0.0	0.0	0.0	0.0	19.67 $\pm$ 0.58
ANOVA	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	*	*	*	*
P-value									

^{a-l} In columns: mean values sharing a superscript letter are not significantly different ($p > 0.05$) as based on Tukey's multiple comparison test, bolded values are above and significantly different from those of Ciprofloxacin ($p < 0.001$). S₁-S₃: - ethanol extracts [stem bark of *O. usambarensis*(S₁); leaves of *O. usambarensis* (S₂); leaves of *S. africana* (S₃)], S₄-S₆: - aqueous extracts [stem bark of *O. usambarensis* (S₄); leaves of *O. usambarensis*(S₅); leaves of *S. africana* (S₆)], S₇-S₉: - ethyl acetate extracts (stem bark of *O. usambarensis* (S₇); leaves of *O. usambarensis* (S₈); leaves of *S. africana* (S₉)). *: - No ANOVA p-value.

Minimum Inhibitory Concentration (MIC)

The extracts from the two-plant species were active against the three species of bacteria studied with MICs ranging from 31.25 to 500mg/ml (Table 7). The activities of the extracts of the two against Gram positive and Gram-negative bacteria depicts the presence of broad-spectrum antibacterial compounds with activities against both types of bacteria. Gram-positive and Gram-negative bacteria [103]. The *Escherichia coli* and *Staphylococcus aureus* were more sensitive to the plant extracts than *Streptococcus pneumoniae* whose inhibition required relatively higher concentrations of the same extracts. This points to the possibility that phytochemicals in the two-plant investigated are more effective against *E. coli* and *S. aureus* than *S. pneumoniae* [46, 104]. Similar findings were reported by Elisha et al. [46] where Gram Negative *E. coli* was more sensitive to plant extracts than the Gram-positive bacteria. Although there were some minor variations in the minimum inhibitory concentrations of the extracts from the plants attributed to differences in concentrations of the different phytochemicals, the two plants worked almost in a similar manner because screening of phytochemicals responsible for the antibacterial activity[103], produced similar results.

Table 7
The Minimum Inhibitory Concentration (MIC) of individual extracts of *O. usambarensis* and *S. africana* and their combinations

Minimum Inhibitory Concentration (mg/ml)			
Extract	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>
S ₁	31.25	62.50	125.00
S ₂	62.50	31.25	62.50
S ₃	62.50	125.00	62.50
S ₄	62.50	31.25	125.00
S ₅	62.50	62.50	125.00
S ₆	62.50	62.50	125.00
S ₇	125.00	31.25	125.00
S ₈	62.50	125.00	125.00
S ₉	62.50	125.00	250.00
S ₂ S ₄	125.00	62.50	62.50
S ₁ S ₈	250.00	62.50	500.00
S ₅ S ₉	62.50	125.00	125.00
S ₁ S ₅	500.00	62.50	250.00
S ₁ S ₇	125.00	31.25	250.00
S ₃ S ₆	250.00	250.00	250.00
S ₈ S ₅	62.50	31.25	250.00
S ₂ S ₈	62.50	31.25	125.00
S ₃ S ₅	62.50	62.50	125.00

Key: S₁-S₃: - ethanol extracts [stem bark of *O. usambarensis*(S₁); leaves of *O. usambarensis* (S₂); leaves of *S. africana* (S₃)], S₄-S₆: - aqueous extracts [stem bark of *O. usambarensis* (S₄); leaves of *O. usambarensis*(S₅); leaves of *S. africana* (S₆)], S₇-S₉: - ethyl acetate extracts (stem bark of *O. usambarensis* (S₇); leaves of *O. usambarensis* (S₈); leaves of *S. africana* (S₉)).

Minimum Bactericidal Concentration (MBC) of individual extracts of *O. usambarensis* and *S. africana* and their combinations

The individual extracts from both plants and the combinations investigated were bactericidal to the three strains of bacteria studied with MBCs ranging from 31.25 to 500mg/ml (Table 8). Similar bactericidal findings have been reported against *E. coli*, *Pseudomonas aeruginosa*, *S. aureus* and *Listeria monocytogenes* with gallic and ferulic acids that caused irreversible changes in membrane properties of these pathogens [105]. The ethyl acetate leaf extracts of the two plants species had the lowest MBCs demonstrating their high antibacterial potential compared to the other extracts. The high amounts of tannins and flavonoids and moderate amounts of saponins recorded in the ethyl acetate leaf extracts of the two plants may be responsible for their bactericidal properties [103, 106, 107]. These findings further demonstrate the high antibacterial potency of the leaf extracts

compared to the stem which may be attributed higher phytochemical content in the leaves [108]. Given that leaves are renewable parts of a plant, the antibacterial activity results of leaves of *O. usambarensis* and *S. africana* present a sustainable means of utilizing the two plant medicinal plants without damaging the bark [109].

Table 8
The Minimum Bactericidal Concentration (MBC) of individual extracts of *O. usambarensis* and *S. africana* and their combinations

Minimum Bactericidal Concentration mg/ml			
Extract	Escherichia coli	Staphylococcus aureus	Streptococcus pneumoniae
S ₁	500.00	250.00	125.00
S ₂	125.00	125.00	62.50
S ₃	125.00	500.00	500.00
S ₄	250.00	500.00	125.00
S ₅	500.00	500.00	250.00
S ₆	250.00	125.00	250.00
S ₇	62.50	62.50	62.50
S ₈	31.25	31.25	62.50
S ₉	31.25	31.25	31.25
S ₂ S ₄	250.00	500.00	500.00
S ₁ S ₈	250.00	125.00	500.00
S ₅ S ₉	250.00	125.00	250.00
S ₁ S ₅	500.00	250.00	250.00
S ₁ S ₇	250.00	125.00	500.00
S ₃ S ₆	500.00	250.00	250.00
S ₈ S ₅	250.00	500.00	250.00
S ₂ S ₈	500.00	500.00	250.00
S ₃ S ₅	125.00	500.00	125.00

Key: S₁-S₃: - ethanol extracts [stem bark of *O. usambarensis*(S₁); leaves of *O. usambarensis* (S₂); leaves of *S. africana* (S₃)], S₄-S₆: - aqueous extracts [stem bark of *O. usambarensis* (S₄); leaves of *O. usambarensis*(S₅); leaves of *S. africana* (S₆)], S₇-S₉: - ethyl acetate extracts (stem bark of *O. usambarensis* (S₇); leaves of *O. usambarensis* (S₈); leaves of *S. africana* (S₉)).

Total Antibacterial Activity (TAA) of extracts of the stem bark and leaves of *O. usambarensis* and leaves of *S. africana*

Total Antibacterial Activity (TAA) of the extracts of the two plants were calculated to measure the antibacterial efficacy of plants taking into consideration their respective antibacterial activities and quantities of extracts obtained from the plants [46]. TAA depicted that the plants extract had varying effectiveness on the three pathogens. Three out of the nine extracts (S₁, S₈ and S₉) registered higher efficacy on *E. coli*, the Gram-negative bacteria than the Gram-positive species, *S. aureus* and *S. pneumonia* (Table 9). Pauw & Eloff [110], reported similar results for plant extracts obtained from five orders of trees (Celastrales, Rosales, Myrtales, Sapindales and Proteales) from Southern Africa. Makhafola [111] also found *E. coli* to be more sensitive to extracts from *Ochna* species than Gram-positive bacteria. These findings reveal the promising potential of *O. usamarensis* and *S. Africana* as sources of antibiotics against resistant bacterial strains since Gram negative bacteria have previously showed antibiotic resistant traits [112]. Five plant extracts (S₂, S₃, S₅, S₆ and S₇) had equal TAA attained against *E. coli* and one of the Gram-Positive bacterial species (*S. aureus* or *S. Pnuemonia*) portraying the presence of broad-spectrum phytochemicals in the two-plant species capable of producing antibiotics for a wide range of bacterial strains regardless of their Gram-staining properties [90].

Table 9
Total Antibacterial Activity (TAA) of extract of the stem bark and leave of *O. usambarensis* and leaves of *S. africana*

Total Antibacterial Activity (ml/g)			
Extract	E. coli	S. aureus	S. pneumonia
S ₁	6.638 x 10 ⁰	3.319 x 10 ⁰	1.660 x 10 ⁰
S ₂	1.596 x 10 ⁰	3.192 x 10 ⁰	1.596 x 10 ⁰
S ₃	2.986 x 10 ⁰	1.493 x 10 ⁰	2.986 x 10 ⁰
S ₄	1.482 x 10 ⁰	2.965 x 10 ⁰	7.412 x 10 ⁻¹
S ₅	3.920 x 10 ⁻¹	3.92 x 10 ⁻¹	1.960 x 10 ⁻¹
S ₆	2.350 x 10 ⁰	2.340 x 10 ⁰	1.170 x 10 ⁰
S ₇	6.612 x 10 ⁻¹	2.645 x 10 ⁰	6.612 x 10 ⁻¹
S ₈	8.624 x 10 ⁻¹	4.314 x 10 ⁻¹	4.312 x 10 ⁻¹
S ₉	8.160 x 10 ⁻¹	4.080 x 10 ⁻¹	2.040 x 10 ⁻¹

KEY: S₁-S₃: - ethanol extracts [stem bark of *O. usambarensis*(S₁); leaves of *O. usambarensis* (S₂); leaves of *S. africana* (S₃)], S₄-S₆: - aqueous extracts [stem bark of *O. usambarensis* (S₄); leaves of *O. usambarensis*(S₅); leaves of *S. africana* (S₆)], S₇-S₉: - ethyl acetate extracts (stem bark of *O. usambarensis* (S₇); leaves of *O. usambarensis* (S₈); leaves of *S. africana* (S₉)).

Fractional Inhibitory Concentration (FIC) of plant extract combinations of *O. usambarensis* and *S. africana*

Fractional Inhibitory Concentrations of combinations of the extracts from the two plants mainly depicted indifferent or antagonistic interactions without any synergism (Table 10). Similar findings were reported by Odds [113] whereby the plant extract combinations registered 98.2% indifferent action. Extract combinations with indifferent actions did not work any better than the individual extracts. On the other hand, the antagonist combinations had lower efficacy than individual extracts. These findings could be due to plant extracts containing many bioactive compounds and the chemical reactions between the two combined extracts resulting in products with weaker antibacterial activity. Antagonism could also be caused by the bioactive compounds in one of the extracts in the combination inhibiting the components the other extract [114]. Based on these findings, combining extracts for these the two plants (*O. usambarensis* and *S. Africa*) against the three pathogens does not seem to produce promising results and it is not recommended. The only combination (S₈S₅) that produced an additive effect against *S. aureus* was obtained by combining the aqueous and ethyl acetate leaf extracts of *O.usambarensis* (S₅ and S₈ respectively). This could be due to accumulated polar and non-polar bioactive compounds extracted by the highly polar solvent (water) and ethyl acetate with low polarity. The polar and non-polar components should have complemented each other leading to a summation effect [115].

Table 10
Fractional Inhibitory Concentration of plant extract combinations of *O. usambarensis* and *S. africana* against *E. coli*, *S. aureus* and *S. pneumoniae*

Microorganisms	Extract A	Extract B	FIC(A)	FIC(B)	Σ FIC	Combination Effect
<i>E. coli</i>	S2	S4	2.00	1.000	3.00	Indifferent
	S1	S8	8.00	4.00	12.00	Antagonistic
	S5	S9	1.00	1.00	2.00	Indifferent
	S1	S5	16.00	8.00	24.00	Antagonistic
	S1	S7	4.00	1.00	5.00	Antagonistic
	S3	S6	1.00	1.00	2.00	Indifferent
	S8	S5	1.00	1.00	2.00	Indifferent
	S2	S8	1.00	1.00	2.00	Indifferent
	S3	S5	1.00	1.00	2.00	Indifferent
	S2	S4	2.00	2.00	4.00	Indifferent
	S1	S8	1.00	0.5	1.50	Indifferent
	S5	S9	2.00	1.00	3.00	Indifferent
	S1	S5	1.00	1.00	2.00	Indifferent
	S1	S7	0.50	1.00	1.50	Indifferent
	S3	S6	2.00	4.00	6.00	Antagonistic
<i>S. aureus</i>	S8	S5	0.25	0.5	0.75	Additive
	S2	S8	1.00	0.5	1.50	Indifferent
	S3	S5	0.5	1.00	1.50	Indifferent
	S2	S4	1.00	2.00	3.00	Indifferent
	S1	S8	4.00	4.00	8.00	Antagonistic
	S5	S9	1.00	0.5	1.50	Indifferent
	S1	S5	2.00	2.00	4.00	Indifferent
<i>S. pneumoniae</i>	S1	S7	2.00	2.00	4.00	Indifferent
	S3	S6	4.00	2.00	6.00	Antagonistic
	S8	S5	2.00	2.00	4.00	Indifferent
	S2	S8	2.000	1.00	3.00	Indifferent
	S3	S5	2.00	1.00	3.00	Indifferent

Key: Combination effect scale: Σ FIC = ≤ 0.5 : - Synergistic, $> 0.5-1$: - Additive, $> 1-4$: - Indifferent, > 4 : - Antagonistic. S₁-S₃: - ethanol extracts [stem bark of *O. usambarensis*(S₁); leaves of *O. usambarensis* (S₂); leaves of *S. africana* (S₃)], S₄-S₆: - aqueous extracts [stem bark of *O. usambarensis* (S₄); leaves of *O. usambarensis*(S₅); leaves of *S. africana* (S₆)], S₇-S₉: - ethyl acetate extracts (stem bark of *O. usambarensis* (S₇); leaves of *O. usambarensis* (S₈); leaves of *S. africana* (S₉)).

Conclusion

The percentage extract yield varies according to the plant species, plant part being extracted and the extraction solvent used during the phytochemical extraction process. Ethanol and water showed significantly higher phytochemical extraction efficiency than ethyl acetate in this study.

O. usambarensis and *S. africana* contain a high diversity of phytochemical compounds including flavonoids, phenols, saponins, cardiac glycosides, glycosides, terpenoids, tannins, anthraquinones, chalcones, phlobatannins, quinones, steroids and polyphenones which are responsible for the antibacterial activity of their extracts and responsible for the medicinal use of the two-plant species by the local communities.

O. usambarensis leaf and stem bark extracts, and *S. africana* leaf extracts have *in-vitro* antibacterial activity against *S. pneumoniae*, *S. aureus* and *E. coli*. This implies that the plant part extracts contain broad spectrum of bioactive compounds which not only inhibit the growth but with bactericidal properties against both Gram-positive and Gram-negative bacteria.

Although a few extract combinations showed positive effects, majority had indifferent or antagonistic interactive effects. As such, combination results for these two plants are not good and it is better to use each plant separately.

The leaves of *O. usambarensis* had higher Total Antibacterial Activity (TAA) than the stem bark. This reduces the risk of over exploitation of the plant for herbal medicine since plant leaves replenish faster than the stem bark.

Abbreviations

ANOVA Analysis of Variance

MUST Mbarara University of Science and Technology

GPS Global Positioning System

S₁ *O. usambarensis* ethanol stem bark extract

S₂ *O. usambarensis* ethanol leaf extract

S₃ *S. africana* extract

S₄ *O. usambarensis* aqueous stem bark extract

S₅ *O. usambarensis* aqueous leaf extract

S₆ *S. africana* aqueous extract

S₇ *O. usambarensis* Ethyl acetate stem bark extract

S₈ *O. usambarensis* Ethyl acetate leaf extract

S₉ *S. africana* ethyl acetate extract.

MIC Minimum Inhibitory concentration

MBC Minimum Bactericidal concentration

TAA Total Antibacterial Activity

FICI Fractional Inhibitory Concentration Index

SPSS Statistical Package for Social Scientists

Declarations

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The research protocol was approved by the Institutional Research Ethics Committee of Mbarara University of Science and Technology, under reference number MUST 2022-470. All methods used in sample collection and processing were sustainable and in compliance with the Uganda National Council of Science and Technology, Convention on the Trade in Endangered Species of Wild Fauna and Flora guidelines for experimental research and field studies on plants and IUCN Policy Statement on Research Involving Species at Risk of Extinction.

Consent for publication

Not applicable.

Availability of data

All data sets used or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there are no competing interests.

Author details

¹ Department of Biology, Mbarara University of Science and Technology, P.O.

Box 1410, Mbarara, Uganda.

Funding

This study received no external funding.

Authors' contributions

This manuscript is a product of a master's research conducted by Stephen Onekalith under the supervision of Dr. Justine Nakintu and Mr. Godfrey Begumisa. Stephen Onekalith conducted the study experiments and also drafted the manuscript. Dr. Justine Nakintu and Mr. Godfrey Magyezi Begumisa supervised the experiments, edited and approved the final version of the manuscript.

Acknowledgements

We acknowledge and appreciate Kalinzu Forest management for the permission and support during the collection of the plant samples. Appreciations also go to the Department of Biology, Department of Microbiology and Parasitology and Department of Pharmacy, Mbarara University of Science and Technology for the provision of laboratory equipment used in this study. Acknowledgement also goes to Mbarara University of Science and Technology REC for reviewing the proposal. Lastly, we thank Mr. Mwesigye James and Dr. Olusoji Ajayi Clement for their assistance during the laboratory work.

References

1. Šantić Ž, Pravdić N, Bevanda M, Galić K. The historical use of medicinal plants in traditional and scientific medicine. *Psychiatr Danub.* 2017;29:4:69–74.
2. Devkota HP, Paudel KR, Khanal S, Baral A, Panth N, Adhikari-Devkota A, et al. Stinging nettle (*Urtica dioica* L.): Nutritional composition, bioactive compounds, and food functional properties. *Molecules.* 2022;27:5219.
3. Kamatenesi MM, Acipa A, Oryem-Origa H. Medicinal plants of Otwal and Ngai sub counties in Oyam district, Northern Uganda. *J Ethnobiol Ethnomed.* 2011;7:1–14.
4. Pekar JE, Magee A, Parker E, Moshiri N, Izhikevich K, Havens JL et al. The molecular epidemiology of multiple zoonotic origins of SARS-CoV-2. *Science* (80-). 2022;377:960–6.
5. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol.* 2014;4:177.
6. Chaachouay N, Douira A, Zidane L. Herbal medicine used in the treatment of human diseases in the Rif, Northern Morocco. *Arab J Sci Eng.* 2022;47:131–53.
7. World Health Organisation. WHO traditional medicine strategy: 2014–2023. World Health Organization; 2013.
8. Arip M, Selvaraja M, Tan LF, Leong MY, Tan PL, Yap VL, et al. Review on plant-based management in combating antimicrobial resistance-mechanistic perspective. *Front Pharmacol.* 2022;13:879495.
9. Muthamil S, Balasubramaniam B, Balamurugan K, Pandian SK. Synergistic effect of quinic acid derived from *Syzygium cumini* and undecanoic acid against *Candida* spp. biofilm and virulence. *Front Microbiol.* 2018;9:2835.
10. Lukubye B, Ajayi CO, Wangalwa R, Kagoro-Rugunda G. Phytochemical profile and antimicrobial activity of the leaves and stem bark of *Symphonia globulifera* L.f. and *Allophylus abyssinicus* (Hochst.) Radlk. *BMC Complement Med Ther.* 2022;22:1–20.
11. Bussmann RW, Paniagua-Zambrana NY, Njoroge GN. *Ocotea usambarensis* Engl. Lauraceae. *Ethnobotany of the Mountain Regions of Africa.* Springer; 2021. pp. 753–60.
12. Tropical Plants Database. *Ocotea usambarensis*. <https://tropical.theferns.info/viewtropical.php?id=Ocotea+usambarensis>. Accessed 23 Sep 2024.
13. Oloya B, Namukobe J, Ssengooba W, Afayoa M, Byamukama R. Phytochemical screening, antimycobacterial activity and acute toxicity of crude extracts of selected medicinal plant species used locally in the treatment of tuberculosis in Uganda. *Trop Med Health.* 2022;50.
14. Mutua A, Kindt R, Jamnadass R, Simons A. *Agroforestry Database: a tree reference and selection guide version 4.0.* 2009. <http://www.worldagroforestry.org/af/treedb/>. Accessed 23 Sep 2024.

15. Terreaux C, Maillard M, Hostettmann K, Lodi G, Hakizamungu E. Analysis of the fungicidal constituents from the bark of *Ocotea usambarensis* Engl.(Lauraceae). *Phytochem Anal.* 1994;5:233–8.
16. Tropical Plants Database. *Secamone Africana*. <https://tropical.theferns.info/viewtropical.php?id=Secamone+africana>. Accessed 23 Sep 2024.
17. Kacholi DS. Density and aboriginal uses of wild tree species in Milawilila forest reserve in Morogoro region, Tanzania. *Tanzan J Sci.* 2020;46:85–100.
18. Sekandi P, Namukobe J, Byamukama R, Akala HM, Yeda RA, Heydenreich M. Bioactive secondary metabolites from the leaves of *Secamone africana* (Olive.) Bullock. *Int J Biol Chem Sci.* 2020;14:1820–30.
19. Musinguzi D. Medicinal plants use in and around Kalinzu central forest reserve. Western Uganda. 2017.
20. Khond M, Bhosale JD, Arif T, Mandal TK, Padhi MM, Dabur R. Screening of Some Selected Medicinal Plants Extracts for In-vitro. *Antimicrob Activity.* 2009;4:271–8.
21. Silva DM, DA COSTA PA, Ribon AOB, Purgato GA, Diaz-Muñoz G, Diaz MAN. Plant extracts display synergism with different classes of antibiotics. *Acad Bras Cienc.* 2019;91.
22. Widyawati PS, Budianta TDW, Kusuma FA, Wijaya EL. Difference of solvent polarity to phytochemical content and antioxidant activity of *Pluchea indica* less leaves extracts. *Int J Pharmacogn Phytochem Res.* 2014;6:850–5.
23. Tugume P, Nyakoojo C. Ethno-pharmacological survey of herbal remedies used in the treatment of paediatric diseases in Buhunga parish, Rukungiri District, Uganda. *BMC Complement Altern Med.* 2019;19:1–10.
24. Onyegbule AF, Anowi CF, Gugu TH, Uto-Nedosa AU. Evaluation of antimicrobial properties of ethyl acetate extract of the leaves of *Napoleoneae imperialis* family *Lecythiaceae*. *Int J Drug Res Tech.* 2011;1:45–51.
25. Fiardilla F, Warsiki E. The experiment of activity and stability of antioxidant extracted from *Senduduk* (*Melastoma malabathricum* L) leaves at various conditions of concentration, pH values, and temperatures. In: *IOP Conference Series: Earth and Environmental Science*. IOP Publishing; 2020. p. 12003.
26. Mathews A, Arbal AV, Kaarunya A, Jha PK, Le-Bail A, Rawson A. Conventional vs modern extraction techniques in the food industry. *Extraction processes in the food industry*. Elsevier; 2024. pp. 97–146.
27. Bitwell C, Indra S, Sen, Luke C, Kakoma MK. A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. *Sci Afr.* 2023;19:e01585.
28. Bakir Çilesizoğlu N, Yalçın E, Çavuşoğlu K, Sipahi Kuloğlu S. Qualitative and quantitative phytochemical screening of *Nerium oleander* L. extracts associated with toxicity profile. *Sci Rep.* 2022;12:21421.
29. Balamurugan V, Fatima S, Velurajan S. A guide to phytochemical analysis. *Int J Adv Res Innov Ideas Educ.* 2019;5:236–45.
30. Kalpoutzakis E, Chatzimitakos T, Athanasiadis V, Mitakou S, Aligiannis N, Bozinou E, et al. Determination of the total phenolics content and antioxidant activity of extracts from parts of plants from the Greek Island of Crete. *Plants.* 2023;12:1092.
31. Sharma T, Gamit R, Acharya R, Shukla VJ. Quantitative estimation of total tannin, alkaloid, phenolic, and flavonoid content of the root, leaf, and whole plant of *Byttneria herbacea* Roxb. *AYU (An Int Q J Res Ayurveda).* 2021;42:143–7.
32. Le V, Parks AE, Nguyen SH, Roach MD. Improving the vanillin-sulphuric acid method for quantifying total saponins. *Technologies.* 2018;6:84.
33. Herald TJ, Gadgil P, Perumal R, Bean SR, Wilson JD. High-throughput micro-plate HCl–vanillin assay for screening tannin content in sorghum grain. *J Sci Food Agric.* 2014;94:2133–6.
34. Bonko MDA, Lompo P, Tahita MC, Kiemde F, Karama I, Some AM, et al. Antibiotic susceptibility of *staphylococcus aureus* and *streptococcus pneumoniae* isolates from the nasopharynx of febrile children under 5 years in Nanoro, Burkina Faso. *Antibiotics.* 2021;10:444.
35. Balboa SJ, Hicks LM. Revealing AMP mechanisms of action through resistance evolution and quantitative proteomics. *Methods in enzymology*. Elsevier; 2022. pp. 259–71.
36. Quintero Moreno B, Araque M, Mendoza E. Evaluation of Two Supplemented Culture Media for Long-Term, Room-Temperature Preservation of *Streptococcus pneumoniae* Strains. *Biomed Res Int.* 2017;2017:1218798.
37. Gayathiri E, Bharathi B, Priya K. Study of the enumeration of twelve clinical important bacterial populations at 0.5 McFarland standard. *Int J Creat Res Thoughts.* 2018;6:880–93.
38. Otegwu TC, Abdullahi FB. Microbial Contamination of Mobile Phones Amongst Pharmacy Students in Gombe State University. *BIMA J Sci Technol.* 2024;8:56–64.
39. Hardy Diagonistics. Criterion GC gar Base for the preparation of Chocolate Agar. 1996. <https://biotrading.com/assets/productinformatie/hardy/ifu/c5790-5794.pdf>. Accessed 19 Sep 2022.
40. Organization WH. WHO publishes list of bacteria for which new antibiotics are urgently needed. 2017.
41. Loughran AJ, Orihuela CJ, Tuomanen EI. *Streptococcus pneumoniae*: invasion and inflammation. *Microbiol Spectr.* 2019;7:10–1128.
42. Rajagopal M, Walker S. Envelope structures of Gram-positive bacteria. *Protein sugar Export Assem Gram-positive Bact.* 2017;;1–44.
43. Muñoz KA, Hergenrother PJ. Facilitating compound entry as a means to discover antibiotics for gram-negative bacteria. *Acc Chem Res.* 2021;54:1322–33.

44. Balouiri M, Sadiki M, Ibensouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal.* 2016;6:71–9.
45. Sykes JE, Rankin SC. Isolation and identification of aerobic and anaerobic bacteria. *Canine feline Infect Dis.* 2013;6:17–28.
46. Elisha IL, Botha FS, McGaw LJ, Eloff JN. The antibacterial activity of extracts of nine plant species with good activity against *Escherichia coli* against five other bacteria and cytotoxicity of extracts. *BMC Complement Altern Med.* 2017;17:1–10.
47. Ni WenTao NW, Shao XiaoDi SX, Di XiuZhen DX, Cui JunChang CJ, Wang Rui WR, Liu YouNing LY. In vitro synergy of polymyxins with other antibiotics for *Acinetobacter baumannii*: a systematic review and meta-analysis. 2015.
48. Botelho MG. Fractional inhibitory concentration index of combinations of antibacterial agents against cariogenic organisms. *J Dent.* 2000;28:565–70.
49. Motulsky H, Beutler E. Graphpad software Prism 5 for windows. 2009.
50. Ghaffar N, Perveen A. Solvent polarity effects on extraction yield, phenolic content, and antioxidant properties of Malvaceae family seeds: a comparative study. *New Zeal J Bot.* 2024;:1–11.
51. Kumar A, P N, Kumar M, Jose A, Tomer V, Oz E, et al. Major phytochemicals: recent advances in health benefits and extraction method. *Molecules.* 2023;28:887.
52. Rajauria G. In-vitro antioxidant properties of lipophilic antioxidant compounds from 3 brown seaweed. *Antioxidants.* 2019;8:596.
53. Cieniak C, Walshe-Roussel B, Liu R, Muhammad A, Saleem A, Haddad PS, et al. Phytochemical comparison of the water and ethanol leaf extracts of the Cree medicinal plant, *Sarracenia purpurea* L. (Sarraceniaceae). *J Pharm Pharm Sci.* 2015;18:484–93.
54. Omara T, Kiprop AK, Kosgei VJ. Intraspecific Variation of Phytochemicals, Antioxidant, and Antibacterial Activities of Different Solvent Extracts of *Albizia coriaria* Leaves from Some Agroecological Zones of Uganda. *Evidence-based Complement Altern Med.* 2021;2021.
55. Esteban-Decloux M, Distillation. The behaviour of volatile compounds during distillation of hydro-alcoholic solutions and during hydro-distillation. *Handbook of Molecular Gastronomy.* CRC; 2021. pp. 213–20.
56. Nobossé P, Fombang EN, Mbofung CMF. Effects of age and extraction solvent on phytochemical content and antioxidant activity of fresh *Moringa oleifera* L. leaves. *Food Sci Nutr.* 2018;6:2188–98.
57. Sikolia SF, Omondi S. Phytochemical Analysis of Some Selected Plants and Families in the University Botanic Garden Of Maseno, Kenya. *IOSR J Pharm Biol Sci.* 2017;12:31–8.
58. Weldegebrieal GK. Synthesis method, antibacterial and photocatalytic activity of ZnO nanoparticles for azo dyes in wastewater treatment: A review. *Inorg Chem Commun.* 2020;120:108140.
59. Shi L, Zhao W, Yang Z, Subbiah V, Suleria HAR. Extraction and characterization of phenolic compounds and their potential antioxidant activities. *Environ Sci Pollut Res.* 2022;29:81112–29.
60. Ajayi OE, Yoon SY, Moon S, Kim KH, Kim JH, Chung J-W, et al. Variability in Phytochemical Contents and Biological Activities among *Adenophora triphylla* Genotypes. *Appl Sci.* 2023;13:11184.
61. Nowicka B, Kruk J. Occurrence, biosynthesis and function of isoprenoid quinones. *Biochim Biophys Acta (BBA)-Bioenergetics.* 2010;1797:1587–605.
62. Roy A, Khan A, Ahmad I, Alghamdi S, Rajab BS, Babalghith AO, et al. Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. *Biomed Res Int.* 2022;2022:5445291.
63. Hussain M, Debnath B, Qasim M, Bamisile BS, Islam W, Hameed MS, et al. Role of saponins in plant defense against specialist herbivores. *Molecules.* 2019;24:2067.
64. Barbehenn RV, Constabel CP. Tannins in plant–herbivore interactions. *Phytochemistry.* 2011;72:1551–65.
65. Morsy N. Cardiac glycosides in medicinal plants. *Aromat Med Plants–Back to Nature*; Intechopen London, UK. 2017;:29–45.
66. Galanakis CM, Goulas V, Tsakona S, Manganaris GA, Gekas V. A knowledge base for the recovery of natural phenols with different solvents. *Int J Food Prop.* 2013;16:382–96.
67. Richard T, Tamsamani H, Cantos-Villar E, Monti J-P. Application of LC–MS and LC–NMR techniques for secondary metabolite identification. *Advances in botanical research.* Elsevier; 2013. pp. 67–98.
68. Zhang S, Li W, Nie H, Liao M, Qiu B, Yang Y, et al. Five new alkaloids from the roots of *Sophora flavescens*. *Chem Biodivers.* 2018;15:e1700577.
69. Khusnutdinov E, Sukhareva A, Panfilova M, Mikhaylova E. Anthocyanin biosynthesis genes as model genes for genome editing in plants. *Int J Mol Sci.* 2021;22:8752.
70. Pucker B, Walker-Hale N, Dzurlic J, Yim WC, Cushman JC, Crum A, et al. Multiple mechanisms explain loss of anthocyanins from betalain-pigmented Caryophyllales, including repeated wholesale loss of a key anthocyanidin synthesis enzyme. *New Phytol.* 2024;241:471–89.
71. Heftmann E. Functions of steroids in plants. *Phytochemistry.* 1975;14:891–901.
72. Schmidt G, Steinhart H. Impact of extraction solvents on steroid contents determined in beef. *Food Chem.* 2002;76:83–8.
73. Wang D, Wang X-H, Yu X, Cao F, Cai X, Chen P, et al. Pharmacokinetics of anthraquinones from medicinal plants. *Front Pharmacol.* 2021;12:638993.

74. Osuntokun OT, Faniyi IT, Curative. Suppressive and Prophylactic Phyto-Medicinal Therapy/Synergistic Efficacy of *Alstonia boonei*/Capsicum frutescens Ethanolic Extracts against Plasmodium berghei (NK 65)/Salmonella typhi (ATCC 35723) Infected Swiss Albino Mice. Asian J Biotechnol Genet Eng. 2020;3:19–34.
75. Chandran A, Saj OP. Phytochemical studies on the medicinal herb Catharanthus pusillus. World J Pharm Res. 2015;5:152–71.
76. Rudrapal M, Khan J, Dukhyil AA, Bin, Alarousy RMII, Attah EI, Sharma T, et al. Chalcone scaffolds, bioprecursors of flavonoids: Chemistry, bioactivities, and pharmacokinetics. Molecules. 2021;26:7177.
77. Lama-Muñoz A, Contreras M. del M. Extraction systems and analytical techniques for food phenolic compounds: a review. Foods. 2022;11:3671.
78. Nouioura G, El Fadili M, El Barnossi A, Loukili EH, Laaroussi H, Bouhrim M, et al. Comprehensive analysis of different solvent extracts of Ferula communis L. fruit reveals phenolic compounds and their biological properties via in vitro and in silico assays. Sci Rep. 2024;14:8325.
79. Liebelt DJ, Jordan JT, Doherty CJ. Only a matter of time: The impact of daily and seasonal rhythms on phytochemicals. Phytochem Rev. 2019;18:1409–33.
80. Mokrani A, Madani K. Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (Prunus persica L.) fruit. Sep Purif Technol. 2016;162:68–76.
81. Sabindo NH, Yatim RM, Thirumulu PK. Phytochemical composition of Clinacanthus nutans based on factors of environment, genetics and postharvest processes: A review. BioMedicine. 2024;14:1.
82. Press JB, Reynolds RC, May RD, Marciani DJ. Structure/function relationships of immunostimulating saponins. Stud Nat Prod Chem. 2000;24:131–74.
83. Güçlü-Üstündağ Ö, Mazza G. Saponins: properties, applications and processing. Crit Rev Food Sci Nutr. 2007;47:231–58.
84. Segaran A, Chua LS. Saponins Rich Fractions From Eurycoma longifolia Extract. In: Third International Conference on Separation Technology 2020 (ICoST 2020). Atlantis Press; 2020. pp. 57–61.
85. Mohammadi Bazargani M, Falahati-Anbaran M, Rohloff J. Comparative analyses of phytochemical variation within and between congeneric species of willow herb, Epilobium hirsutum and E. parviflorum: Contribution of environmental factors. Front Plant Sci. 2021;11:595190.
86. Iqbal N, Poór P. Plant Protection by Tannins Depends on Defence-Related Phytohormones. J Plant Growth Regul. 2024;:1–18.
87. Hochma E, Yarmolinsky L, Khalfin B, Nisnevitch M, Ben-Shabat S, Nakonechny F. Antimicrobial effect of phytochemicals from edible plants. Processes. 2021;9:2089.
88. Patra AK. An overview of antimicrobial properties of different classes of phytochemicals. Diet Phytochem microbes. 2012;:1–32.
89. Djeussi DE, Noumedem JAK, Seukep JA, Fankam AG, Voukeng IK, Tankeo SB, et al. Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. BMC Complement Altern Med. 2013;13:1–8.
90. Khameneh B, Eskin NAM, Iranshahy M, Fazly Bazzaz BS. Phytochemicals: a promising weapon in the arsenal against antibiotic-resistant bacteria. Antibiotics. 2021;10:1044.
91. Haghighoo R, Mehran M, Afshari E, Zadeh HF, Ahmadvand M. Antibacterial effects of different concentrations of Althaea officinalis root extract versus 0.2% chlorhexidine and penicillin on Streptococcus mutans and Lactobacillus (in vitro). J Int Soc Prev Community Dent. 2017;7:180–5.
92. Hatano T, Kusuda M, Inada K, Ogawa T, Shiota S, Tsuchiya T, et al. Effects of tannins and related polyphenols on methicillin-resistant Staphylococcus aureus. Phytochemistry. 2005;66:2047–55.
93. Kyaw BM, Lim CS. Bactericidal antibiotic-phytochemical combinations against methicillin resistant Staphylococcus aureus. Brazilian J Microbiol. 2012;43:938–45.
94. Xu S, Kang A, Tian Y, Li X, Qin S, Yang R et al. Plant Flavonoids with Antimicrobial Activity against Methicillin-Resistant Staphylococcus aureus (MRSA). ACS Infect Dis. 2024.
95. Olchowik-Grabarek E, Sekowski S, Bituicki M, Dobrzynska I, Shlyonsky V, Ionov M et al. Inhibition of interaction between Staphylococcus aureus α -hemolysin and erythrocytes membrane by hydrolysable tannins: Structure-related activity study., 2020, 10. DOI <https://doi.org/10.1038/s41598-020-68030-1> PMID <https://www.ncbi.nlm.nih.gov/pubmed/32636484>.:11168
96. Mikłasińska-Majdanik M, Kępa M, Wojtyczka RD, Idzik D, Wąsik TJ. Phenolic compounds diminish antibiotic resistance of Staphylococcus aureus clinical strains. Int J Environ Res Public Health. 2018;15:2321.
97. Moussaoui F, Alaoui T. Evaluation of antibacterial activity and synergistic effect between antibiotic and the essential oils of some medicinal plants. Asian Pac J Trop Biomed. 2016;6:32–7.
98. Plaskova A, Mlcek J. New insights of the application of water or ethanol-water plant extract rich in active compounds in food. Front Nutr. 2023;10:1118761.
99. Barbieri R, Coppo E, Marchese A, Daglia M, Sobarzo-Sánchez E, Nabavi SF, et al. Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. Microbiol Res. 2017;196:44–68.
100. Bittner Fialová S, Rendeková K, Mučaji P, Nagy M, Slobodníková L. Antibacterial activity of medicinal plants and their constituents in the context of skin and wound infections, considering European legislation and folk medicine—a review. Int J Mol Sci. 2021;22:10746.

101. Ayaz M, Ullah F, Sadiq A, Ullah F, Ovais M, Ahmed J, et al. Synergistic interactions of phytochemicals with antimicrobial agents: Potential strategy to counteract drug resistance. *Chem Biol Interact.* 2019;308:294–303.
102. Khare T, Anand U, Dey A, Assaraf YG, Chen Z-S, Liu Z, et al. Exploring phytochemicals for combating antibiotic resistance in microbial pathogens. *Front Pharmacol.* 2021;12:720726.
103. Adem Y, Getachew S. Phytochemical property and antimicrobial activity of *Ficifolius A.* Rich root extract: Advancing Ethiopian indigenous wart curing medicinal plant. *Heliyon.* 2024;10.
104. Odongo EA, Mutai PC, Amugune BK, Mungai NN, Akinyi MO, Kimondo J. Evaluation of the antibacterial activity of selected Kenyan medicinal plant extract combinations against clinically important bacteria. *BMC Complement Med Ther.* 2023;23:100.
105. Borges A, Ferreira C, Saavedra MJ, Simões M. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microb drug Resist.* 2013;19:256–65.
106. Prihambodo TR, Sholikin MM, Qomariyah N, Jayanegara A, Batubara I, Utomo DB, et al. Effects of dietary flavonoids on performance, blood constituents, carcass composition and small intestinal morphology of broilers: a meta-analysis. *Anim Biosci.* 2021;34:434.
107. Farha AK, Yang Q-Q, Kim G, Li H-B, Zhu F, Liu H-Y, et al. Tannins as an alternative to antibiotics. *Food Biosci.* 2020;38:100751.
108. Drabińska N, Jež M, Nogueira M. Variation in the accumulation of phytochemicals and their bioactive properties among the aerial parts of cauliflower. *Antioxidants.* 2021;10:1597.
109. Chen S-L, Yu H, Luo H-M, Wu Q, Li C-F, Steinmetz A. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. *Chin Med.* 2016;11:1–10.
110. Pauw E, Eloff JN. Which tree orders in southern Africa have the highest antimicrobial activity and selectivity against bacterial and fungal pathogens of animals? *BMC Complement Altern Med.* 2014;14:1–12.
111. Makhafola TJ. Five *Ochna* species have high antibacterial activity and more than ten antibacterial compounds. *S Afr J Sci.* 2012;108:1–6.
112. Breijyeh Z, Jubeh B, Karaman R. Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules.* 2020;25:1340.
113. Odds FC. Synergy, antagonism, and what the checkerboard puts between them. *J Antimicrob Chemother.* 2003;52:1.
114. Caesar LK, Cech NB. Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2. *Nat Prod Rep.* 2019;36:869–88.
115. Vaou N, Stavropoulou E, Voidarou C, Tsakris Z, Rozos G, Tsigalou C, et al. Interactions between medical plant-derived bioactive compounds: focus on antimicrobial combination effects. *Antibiotics.* 2022;11:1014.

Figures

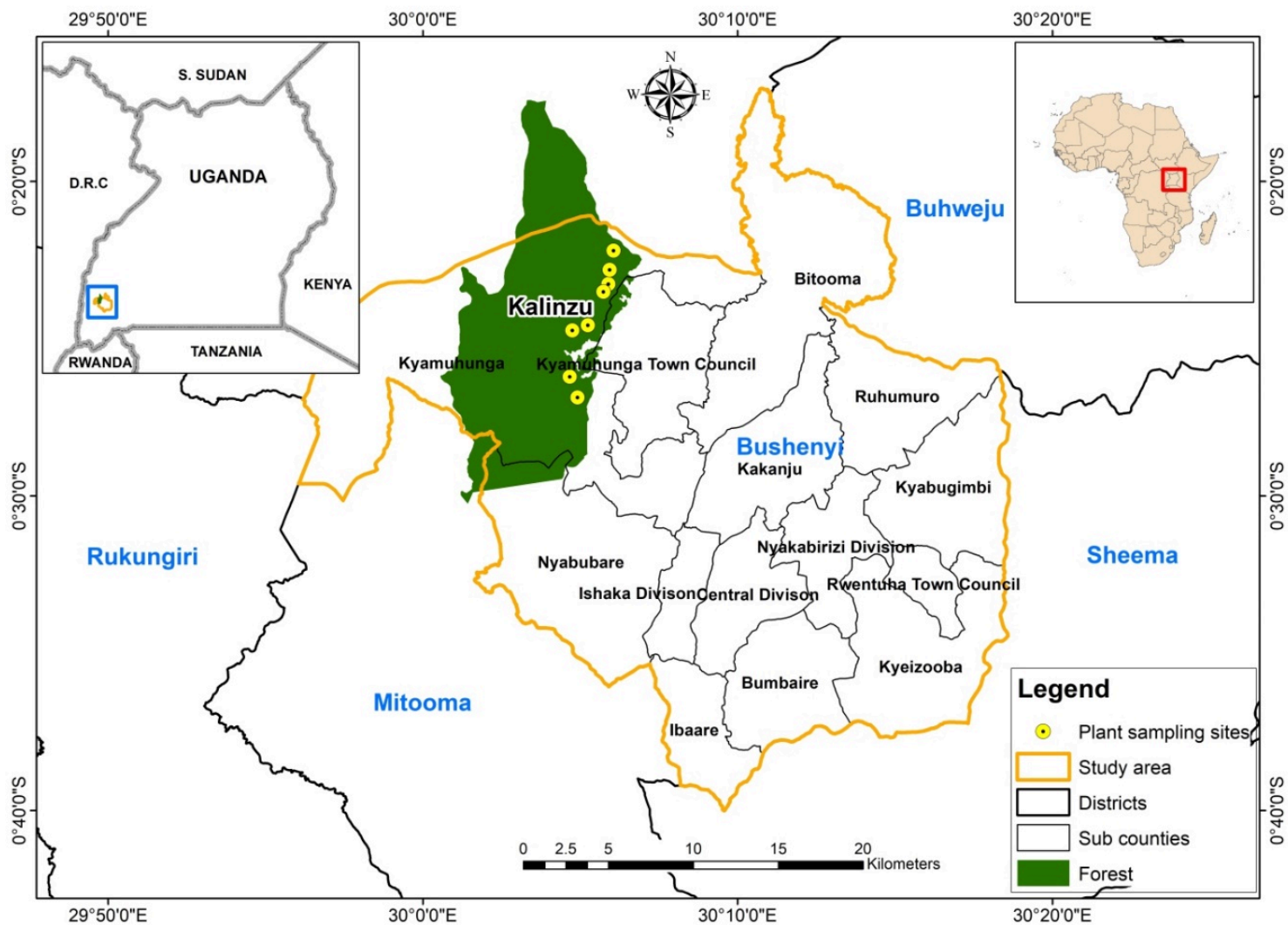


Figure 1

Map of Bushenyi district showing the location of Kalinzu forest and sampling sites. Generated using QGIS 3.34.1.

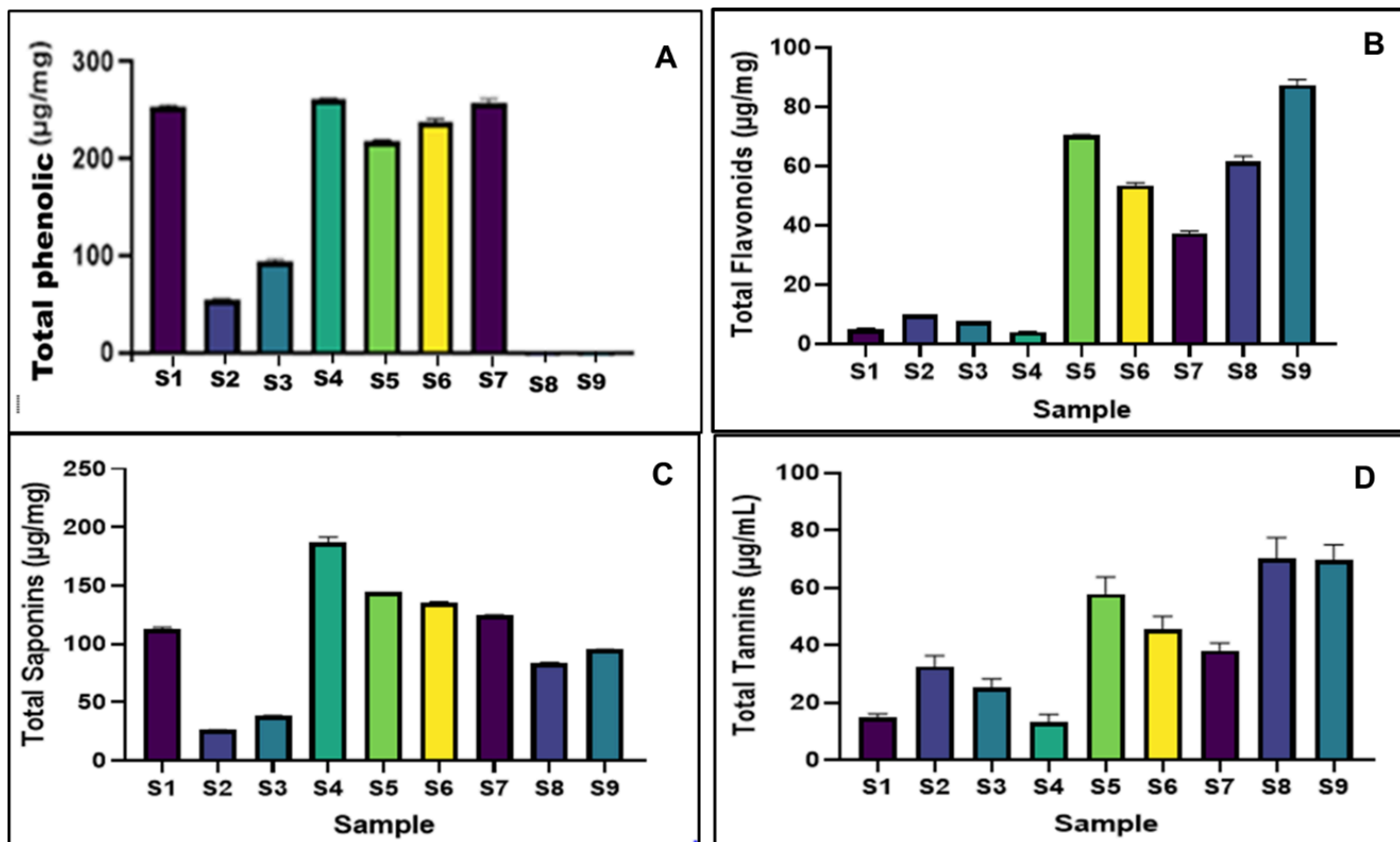


Figure 2

A) Total phenolic content; B) Total flavonoid content; C) Total saponin content and; D) Total tannin content in the extracts of the stem bark and leaves of *O. usambarensis* and leaves of *S. africana*. S₁-S₃: - ethanol extracts [stem bark of *O. usambarensis*(S₁); leaves of *O. usambarensis* (S₂); leaves of *S. africana* (S₃)], S₄-S₆: - aqueous extracts [stem bark of *O. usambarensis* (S₄); leaves of *O. usambarensis*(S₅); leaves of *S. africana* (S₆)], S₇-S₉: - ethyl acetate extracts (stem bark of *O. usambarensis* (S₇); leaves of *O. usambarensis* (S₈); leaves of *S. africana* (S₉)]