Phytochemical profile and antimicrobial activity of the leaves and stem bark of *Symphonia globulifera* L.f. and *Allophylus abyssinicus* (Hochst.) Radlk

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Abstract

Introduction

Symphonia globulifera and *Allophylus abyssinicus* are used in the management of skin rashes and sores, cough, malaria, digestive diseases, stomach ache, wounds, helminthic infections among others in Uganda, Kenya, Ethiopia, Cameroon. This study aimed at determining the phytochemical profile and antimicrobial activity of these two plants.

Methods

The stem bark and leaves of both plants were collected from Bwindi Impenetrable National Park, and air dried under shade at room temperature. Cold maceration, decoction and infusion with methanol, water and ethyl acetate as solvents were used in phytochemical extraction. Preliminary qualitative screening and thin layer chromatography were used for phytochemical profiling. Antimicrobial activity was analysed by Agar well diffusion assay, macro broth dilution assay and fractional inhibition concentration index.

Results

Leaves and stem bark of both plants have a diverse set of phytochemical compounds of variable polarity including, tannins, alkaloids, flavonoids, saponins, quinones, anthraquinones among others. Generally, methanol and water extracts of *S. globulifera* and *A. abyssinicus* had *in-vitro* bactericidal activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* but weak fungistatic activity against *Candida albicans*. *Allophylus abyssinicus* Leaf water and *Symphonia globulifera* Leaf methanol extract combination had a synergistic activity ($\Sigma FICI=0.37$) against *S. aureus*. Similarly, *Allophylus abyssinicus* stem bark water extract and *A. abyssinicus* leaf water extract combination had an additive effect ($\Sigma FICI=1$) against *P. aeruginosa*.

Conclusion

The leaves and stem bark extracts of *S. globulifera* and *A. abyssinicus* possess antimicrobial bioactive compounds against both gram positive and gram-negative bacteria. They could therefore offer potential sources of novel antibiotics.

1.0 Introduction

The usage of natural products of plant or animal origin for medicine dates back to prehistoric times at least 60,000 years ago (Yuan et al., 2016). Currently, 149 (88%) of the 170 member states of the world health organization acknowledge usage and regulation of traditional medicine including herbal medicines for primary health care (World Health Organization, 2019) greatly higher than the 64 (37%) member states as of 2000 (WHO, 2002). This could indicate the growing popularity of traditional medicine and its usage among the people. The past two decades have witnessed an incredible increase in acceptance and public interest in natural therapies both in developing and developed countries. This is attributed to several factors including, limited availability of the pharmaceutical products, high prices of the drugs, side effects associated with the pharmaceutical drugs and the society's general disapproval of the modern pharmaceutical drugs (Oyebode et al., 2016). In developing countries, the limited number of modern health care workers has contributed greatly to the continued usage of traditional medicine, for instance, the ratio of traditional healers to population in Africa is 1:500 whereas the ratio of medical doctors to population is 1:40000 (Qi, 2015). Due to this the WHO has advocated for the incorporation of herbal medicine into the public health care systems of the different countries through authentication of the herbal extracts using modern science techniques. With the rapidly growing drug resistance especially among microorganisms, plants are potential sources of novel antibiotics and drugs but among the higher plants only 15% of the world higher plants had been systematically investigated for bioactivity (Newman et al., 2000).

Symphonia globulifera L.f. belongs to a large family Clusiaceae and is widely distributed along the swamps of low land tropical rainforests of tropical countries like Madagascar, Cameroon, Uganda, Gabon, Nigeria, Panama, Brazil and Colombia (Fromentin et al., 2015). In Uganda, *Symphonia globulifera* mainly occurs in the Rwenzori mountains and Sango bay area (Katende et al., 2000; Ssegawa & Kasenene, 2007). *Symphonia globulifera* L.f. has been used to treat a wide range of diseases including Skin rashes and sores, cutaneous Leishmaniasis, cough, malaria, digestive diseases, diarrhea, stomach ache (Fromentin et al., 2015; Simoben et al., 2018; Ssegawa & Kasenene, 2007). This shows the anti-microbial and antiparasitic potential of this plant. Interestingly invitro

antiparasitic studies have shown the potential of extracts and isolated compounds from the stem bark *Symphonia globulifera* to significantly inhibit the growth of *Plasmodium falciparum*, *Trypanosoma brucei* and *Leishmania donovani* with the activity Benzophenones and Xanthenes (Fromentin et al., 2013; Bruno Ndjakou Lenta et al., 2007; Simoben et al., 2018). However, the phytochemical profile and the antimicrobial activity of the leaves and stem bark of this potential medicinal plant have not been investigated hence the focus of the current study.

On the other hand, *Allophylus abyssinicus* (Hochst.) Radlk. is a medium to large sized tree that belongs to family *Sapindaceae* and a large genus *Allophylus* with about 255 species distributed worldwide in the tropical and subtropical regions (Chavan & Gaikwad, 2016). The leaves of *A. abyssinicus* are used in the management of helminthic infections, sexually transmitted diseases, boils (Yesuf & Asres, 2013) and skin diseases in Ethiopia (Aydagnehum & Girma, 2014). The bark is used for treatment of diarrhea, and wounds in Ethiopia (Gijan & Dalle, 2019). The roots of *A. abyssinicus* are used in the treatment of cough in Kenya. In addition, the value of the plant is not restricted to humans alone. The leaves of *Allophylus abyssinicus* are often eaten by mountain gorillas in Bwindi forest impenetrable national park hence hypothesized to be an anthelmintic self-deworming avenue (Rothman et al., 2006). Despite the above mentioned medicinal uses of the plant, only the anti-inflammatory and wound healing activity of the plant have been investigated with results showing genuine wound healing activity (Yesuf & Asres, 2013). However, no study has been carried out to investigate the phytochemical profile and the antimicrobial activity efficacy of *Allophylus abyssinicus* stem bark and leaf extracts.

Determination of the phytochemical profile of extracts from *S. globulifera* and *Allophylus abyssinicus* plant parts is key towards development of monograph for usage in the incorporation of into modern public health care systems. In this study we test the hypotheses that *Allophylus abyssinicus* and *Symphonia globulifera* leaves and stem bark have potential antimicrobial activity against pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) and fungi (*Candida albicans*).

2.0 Materials And Methods

2.1 Plant material collection area

The leaves and stem bark of both *Symphonia globulifera* and *Allophylus abyssinicus* were collected from the Ruhija sector, Bwindi Impenetrable National Park (BINP), located in south western Uganda at coordinates between latitude 0°53'S and 1°8'S and between longitude 29°35'E and 29°50'E on the eastern edge of the Albertine rift valley (Babaasa et al., 2004) as shown in Figure 1 below. Permission to access the Bwindi Impenetrable National Park for sample collection and transportation was obtained from the Uganda Wildlife Authority (UWA) under reference number COD/96/05. Bwindi Impenetrable national park was chosen purposively as the source of samples because of the availability of the selected plants but also due to the great conservation efforts.

2.2 Plant material collection

Symphonia globulifera and *Allophylus abyssinicus* plants were identified and confirmed with the help of a field botanist from the Institute of Tropical Forest Conservation (ITFC) from a highly conserved area with no or limited human interference. Mature healthy plants were selected as sampling units according to (Delvaux, 2009; Ingram et al., 2015). The stem bark was harvested using the vertical alternate strip bark two quarters technique (Delvaux, 2009; Khumalo, Fröde, & Sola, 2006; Pandey, Yadav, & Sahu, 2011). Pruning shears was used to cut the leaves from the same trees from which stem bark was obtained. Furthermore, confirmation of the plant identity was performed by Dr. Eunice Apio Olet Alele (plant specialist, Mbarara University of science and Technology) and herbarium specimen submitted to the national herbarium at Makerere University under Accn. No. MHU51049 and MHU51050 for *Symphonia globulifera* and *Allophylus abyssinicus* respectively.

2.3 Preparation of extracts

Air dried leaves and stem bark of *Symphonia globulifera* and *Allophylus abyssinicus* were independently crushed into powder using an electric grinder and extracted using water, methanol (70%) and ethyl acetate (100%) as solvents. Cold maceration was used for the extraction using ethyl acetate (100%) and methanol (70%) as solvents with extraction time of 24 hours and 48 hours respectively. Decoction technique was used in the extraction of phytochemicals from the stem bark of the two plants with water as

a solvent for 15 minutes. Infusion technique was used in the extraction of phytochemicals from the leaves with water as a solvent for 45 minutes (Balakrishna et al., 2016; Handa et al., 2008). The extracts were filtered using the Whatman No. 1 filter paper and the filtrates concentrated using a Rotary evaporator at 70 °C and air-dried. Air dried extracts were placed into tightly stoppered sterile glass bottles, labelled and then stored in a refrigerator at 4 °C for further analysis.

2.4 Phytochemical screening

Qualitative phytochemical screening for saponins, alkaloids, tannins, flavonoids, terpenoids, cardiac glycosides, phlobatannins, anthraquinones, steroids, phenols, anthocyanins, quinones and chalcones in the methanol, water and ethyl acetate extracts was done following standard methods as described by (Agber et al., 2019; Evans, 2002; Isebe, 2015; Jacqueline, 2015; Mariita et al., 2010; Tiwari et al., 2011; Uboh et al., 2018).

2.5 Phytochemical Profiling As Per Thin Layer Chromatography (Tlc)

Plant extracts were dissolved in their respective extraction solvents and spotted onto the TLC Analytica plate (20×20 cm) and left to dry. The TLC plate was then placed into the TLC tank with Hexane, Chloroform and Methanol 6:3:1 (v/v) as the solvent system and monitored for 2 hours. The different phytochemical spots were visualized under Normal light and under a UV- lamp at 254 nm and 365 nm separately. For visualization of phenolic compounds, the TLC plate was sprayed with concentrated sulphuric acid and then heated for 4 minutes for better visualization (Abubakar et al., 2016; Agber et al., 2019). Retention factor of the different components (R_f) was computed using the formula below.

$$RetentionFactor(R_f) = \frac{Distance\ moved\ by\ the\ Phenolic\ spot\ (cm)}{Solvent\ Front\ (cm)}$$

2.6 Antimicrobial activity of individual plant extracts and extract combinations

Plant individual extracts namely: *Symphonia globulifera* leaf water extract (SGLW), *Allophylus abyssinicus* leaf water extract (AALW), *Allophylus abyssinicus* stem bark water extract (AASW) and *Symphonia globulifera* leaf methanol extract (SGLM) and plant extract combinations including (SGLW and AALW, SGLW and SGLM, AASW and AALW, SGLM and AASW, SGLW and AASW, AALW and SGLM) were investigated for antimicrobial activity and their combination effect. For each extract combination for example SGLW and AALW, 1 gram of each extract was measured separately into the same sample bottle and dissolved to make the extract combination sample.

Standards stock cultures of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231 were obtained from the Microbiology laboratory, Mbarara University of Science and Technology. The bacteria strains were sub cultured on Mueller Hinton Agar (MHA) and incubated at 37 °C for 48 hours. *Candida albicans* ATCC 10231 was sub cultured on Sabouraud Dextrose agar at 37 °C for 72 hours. Fresh sub-cultures were used in the antimicrobial activity assays.

2.6.1 Agar well diffusion assay

Agar well diffusion assay (Reshmi et al., 2012; Roger et al., 2015) was used to determine the zone of inhibition for the different plant extracts and extract combinations. Plant extract stock solutions (1000 mg/ml) was prepared by dissolving, 2 grams of the extract or combination into 2 ml of water for water extracts or DMSO (1%) for methanolic extracts and consequently diluted further to obtain doubling dilutions 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.625 mg/ml, 7.8125 g/ml, 3.9062 mg/ml and 1.9531 mg/ml. The test microorganism was inoculated onto the surface of the sterile MHA (Bacteria strains) and SDA (*Candida albicans*). Using a micropipette, 200 µl of plant extract and extract combination solutions of known concentration (1000 mg/ml, 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.625 mg/ml, 7.8125 g/ml, 3.9062 mg/ml and 1.9531 mg/ml) were put into different wells on freshly prepared sterile MHA (Bacteria strains) and SDA (*Candida albicans*) plates.

Fluconazole (2 mg/ml) and 200 µl of Ciprofloxacin (1 mg/ml) were used as positive controls for the anti-fungal and antibacterial assays respectively. Furthermore, 200 µl of DMSO (1%) or distilled water was placed into a free well as a solvent control and an empty well was left to act as a negative control in each assay. The plates were then incubated at 37 °C for 24 hours for

antibacterial activity assays and at 37 °C for 72 hours for anti-fungal activity assays. After incubation, the diameter of zone of inhibition was then measured using a ruler. The experiments were repeated in quadruplicates.

2.6.2 Minimum Inhibitory Concentration (MIC)

The minimum Inhibitory concentration of extracts and extract combinations was determined using the macro broth dilution method (Guarro et al., 1995; Pujol et al., 1996). Stock plant extract or combination solution (1 g/ml) was prepared as described above. Stock extract or combination solution (1 ml) was placed into a Bijou bottle with Brain heart infusion broth (1 ml) to make a concentration of 500 mg/ml and then diluted into doubling dilutions using brain heart infusion broth to make extract concentrations of 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.625 mg/ml, 7.8125 mg/ml, 3.9062 mg/ml and 1.9531 mg/ml respectively in different Bijou bottles. These were each inoculated with 100 µl of 1.5×10^8 (CFU/ml) of a specific test microorganism (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*). Bijou bottles inoculated with bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*) and *Candida albicans* were incubated at 37 °C for 24 hours and at 37 °C for 72 hours. Bijou bottles were removed and visually inspected for turbidity and growth. MIC was defined as the lowest extract concentration at which there was no visible growth. The above procedure was repeated in duplicates. The media, microorganism viability and negative control were done for each assay.

2.6.3 Minimum Bactericidal Concentration And Minimum Fungicidal Concentration

Bijou bottles with no visible growth after determination of the MIC as described above, their bacteria or fungal inoculum was sub cultured separately on fresh Muller Hinton Agar (MHA) at 37°C for 48 hours for bacteria and Sabouraud dextrose agar and incubated at 37°C for 72 hours for *Candida albicans*. The lowest concentration of the plant extracts that did not yield any colony on the solid agar after incubation was taken as the Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC). The presence of growth from all sub cultured doubling dilutions was indicative of fungistatic or bacteriostatic activity (Samie et al., 2010). The above procedure was repeated in duplicates.

Total antibacterial activity and total antifungal activity (TAA) was calculated following a standard formular (Eloff, 2004; Famuyide, Aro, Fasina, Eloff, & Mcgaw, 2019).

$$TAA(ml/g) = \frac{\text{Mass of extract from 1 gram of powder (mg per gram)}}{MIC(mg per ml)}$$

The combination effect of the selected plant extracts on the selected microbial strains was studied using fractional inhibitory concentration index (ΣFIC) (Van Vuuren & Viljoen, 2011; Zonyane et al., 2013). The fractional inhibitory concentration (FIC) for each extract was calculated using the formulae below.

$$FIC^B = \frac{MIC(A + B)_{\text{combination}}}{MIC(B)_{\text{Independently}}}$$

$$FIC^A = \frac{MIC(A + B)_{\text{combination}}}{MIC(A)_{\text{Independently}}}$$

Where A and B represent different plant extracts

The fractional inhibitory concentration index (ΣFIC) was then calculated as the sum of the fractional inhibitory concentration (FIC) of both extracts using the formulae below

$$\Sigma FIC = FIC^A + FIC^B$$

Combination effect was interpreted following the fractional inhibitory concentration index scale i.e ΣFIC = ≤ 0.5 was considered as synergistic interaction, and ΣFIC = > 0.5–1.0 was considered as additive interaction and ΣFIC > 1.0– ≤ 4 was considered as noninteractive and ΣFIC > 4.0 was considered as antagonistic (Van Vuuren & Viljoen, 2011).

2.7 Data analysis

The data was analysed for descriptive statistics and presented as mean $\pm$ SD in form of tables. One-way ANOVA and LSD Post-hoc test were used to analyse for statistical differences in the mean zones of inhibition of the different extracts and combinations against the test microorganisms. All statistical tests were carried out at 5 % level of significance in Statistical Package for Social Scientists (SPSS) version 23.

3.0 Results And Discussion

3.1 Percentage extract yield of *Symphonia globulifera* and *Allophylus abyssinicus* extracts

The percentage yield of methanol, water and ethyl acetate extracts from the stem bark and leaves of *Symphonia globulifera* and *Allophylus abyssinicus* ranged from 0.75–25.82% with *Allophylus abyssinicus* stem bark methanol extract (AASM) showing the highest percentage yield (25.82%) as shown in Fig. 2. Generally, ethyl acetate showed the lowest extraction capacity (2.99%, 0.75%, 2.04% and 5.04%) compared to methanol and water for both leaves and stem bark. The difference in the percentage yield of the extracts is mainly caused by difference in polarity of the extracting solvent. Polar solvents like water have high affinity for polar compounds compared to non-polar bioactive compounds (Wakeel et al., 2019). The observed higher percentage yield of water and methanolic leaf and stem bark extracts from both plants in this study indicates that polar phytochemicals and bioactive compounds are readily available in the leaves of these plants compared to the non-polar compounds. Methanol has also demonstrated a higher extraction percentage yield among other plants like *Severinia buxifolia* (Rutaceae) (Truong et al., 2019).

3.2 Phytochemical profile of *Symphonia globulifera* and *Allophylus abyssinicus*.

Qualitative screening showed a wide range of phytochemical compounds including saponins, steroids, terpenoids, alkaloids, flavonoids, tannins, phenol, polyphenols, quinones among others occurred in higher amounts within one of the extracts of the two plants as shown in Table 1. Tannins, polyphenols and phenols were present in all methanol, ethyl acetate and water extracts of both *Symphonia globulifera* and *Allophylus abyssinicus*. However, saponins were only present in the methanol and water extracts of both extracts but absent in all the ethyl acetate extracts of the two plants. The phytochemical profile in any plant extract is significantly affected by the type of solvent due to differences in the polarity of the different phytochemical compounds (Iloki-Assanga et al., 2015). Therefore, extraction solvents should always be selected carefully considering the polarity of the targeted bioactive compounds and that of the solvent.

Table 1

Phytochemical screening of the leaves and stem bark extracts of *Symphonia globulifera* and *Allophylus abyssinicus*

Phytochemical Class	SGSM	AASM	SGSE	AASE	SGSW	AASW	SGLM	AALM	SGLE	AALE	SGLW	AALW
Anthraquinone	++	+	-	++	-	++	-	+	+	-	++	++
Saponins	+++	+++	-	-	++	+++	+++	+++	-	-	++	++
Steroids	++	++	+	+++	++	+++	++	+	-	-	++	++
Chalcones	++	+	+++	-	+	+	+	-	-	-	+	+
Phlobatannins	+	+	-	+	-	+	-	++	+	-	++	++
Terpenoids	++	++	+	+++	++	+++	+++	++	-	-	++	++
Alkaloids	+++	++	-	+++	+	++	+++	++	+	+	+++	+++
Cardiac glycosides	+	-	-	++	+	+++	++	+++	+	-	+++	++
Flavonoids	++	-	+++	+++	+	-	-	++	+++	+++	+	-
Tannins	+++	++	+++	++	+++	+++	+++	+++	++	++	+++	+++
Phenol	+	+	++	+++	+	+++	+	+++	+++	+++	+	+++
Polyphenols	++	++	+++	+++	++	+++	+	+++	+++	+++	+	+++
Anthocyanins	-	-	++	-	-	-	-	-	+	-	-	-
Quinones	-	+	++	-	-	+	++	+++	++	+	++	+++
Key: +++:-High concentration, ++:-Moderate concentration, +:-Low Concentrations, - :- Absent, SGSM:-Symphonia globulifera stem bark methanol extract, AASM:-Allophylus abyssinicus stem bark methanol extract, SGSE:- Symphonia globulifera stem bark ethyl acetate extract, AASE:-Allophylus abyssinicus stem bark ethyl acetate extract, SGSW:- Symphonia globulifera stem bark water extract, AASW:- Allophylus abyssinicus stem bark water extract, SGLM:-Symphonia globulifera leaf methanol extract, AALM:-Allophylus abyssinicus leaf methanol extract, SGLE:- Symphonia globulifera leaf ethyl acetate extract, AALE:-Allophylus abyssinicus leaf ethyl acetate extract, SGLW:-Symphonia globulifera leaf water extract, AALW:-Allophylus abyssinicus leaf water extract												

Methanol has higher extraction capacity for tannins compared to other solvents (Xuan Cuong et al., 2020) and this was also proven by the observed high concentrations of tannins in methanolic extracts of leaves and stem bark of *S. globulifera* and leaves of *A. abyssinicus* (Table 1).

It was not surprising that anthraquinones were generally in low concentrations or absent in the leaves and stem bark of both *Symphonia globulifera* and *Allophylus abyssinicus* as they are not common within family Sapindaceae and family Clusiaceae to which *Symphonia globulifera* and *Allophylus abyssinicus* respectively belong. Anthraquinones exist in limited groups of angiosperm families such as Fabaceae, Liliaceae, Polygonaceae, Rhamnaceae, Rubiaceae, and Scrophulariaceae (Bartnik & Facey, 2017).

On comparing the phytochemical profile of the two plants, there were notable differences for example Anthocyanins only occurred in the leaves and stem bark of ethyl acetate extracts of *Symphonia globulifera* but not in *Allophylus abyssinicus*, as shown in Table 1. This was not surprising as the composition of these secondary metabolites in plants varies from species to species (Hussain & Deeni, 1991). The inability of methanol and water to extract the anthocyanins compounds from *Symphonia globulifera* was due to the high polarity of these solvents which increases the polarity difference between anthocyanins and the solvent (Hutabarat et al., 2019).

Quinones were mainly concentrated in the leaf extracts of *Symphonia globulifera* and *Allophylus abyssinicus* compared to the stem bark. This concentration can be attributed to their role as electron carriers during the process of photosynthesis hence the concentration in the leaves an organ specialized to synthesis food for the plant (El-Najjar et al., 2011).

Thin layer chromatography (TLC) also showed a wide diversity of secondary metabolite compounds in the extracts of both *A. abyssinicus* and *S. globulifera* leaves and stem bark as determined using Hexane: Chloroform: Methanol 6:3:1 (v/v) solvent system. Generally normal light visualization revealed a low number of compounds compared to ultra-violet light. The increase in number of spots was due to fluorescence of the separated compounds when hit by ultra-violet light hence making them more visible. Under normal light, *Allophylus abyssinicus* leaves ethyl acetate extract showed the highest number of compounds (8 compounds) with retention factor (R_f) values ranging from 0.347 to 0.972. However, *Symphonia globulifera* leaves ethyl acetate extract had the highest number of compounds upon visualization with 245 nm (10 compounds) and 365 UV-light (11 compounds), with retention factor (R_f) values ranges from 0.083 to 0.889 and 0.097 to 0.903 respectively. The wide range of retention factor values is indicative of the presence of compounds of different polarity in the same extract i.e. highly polar, highly non polar and moderately polar. Other studies (Agber et al., 2019; Asante et al., 2016; Udeozo et al., 2016) have also showed presence of compounds of different polarity within plant extracts. The variation in the retention factor is very important in selecting the most appropriate solvent system for the further purification and isolation of the bioactive compounds within the plant extract.

TLC also revealed the two plants shared common phytochemical compounds for example *Allophylus abyssinicus* leaf methanol extract and *Symphonia globulifera* leaves ethyl acetate extract both had compounds with retention factor 0.069, 0.097, 0.333, 0.903 (Table 2). Similarly, *Symphonia globulifera* stem bark water extract and *Allophylus abyssinicus* stem bark ethyl acetate extract both had compound with 0.082 despite them belonging to different species. This may be attributed to the fact that both plants are higher plants and share many metabolic pathways hence yielding the same compounds. It is also important to note that both plants were collected from the same tropical ecological and climatic conditions of Bwindi Impenetrable forest National park. The *Allophylus abyssinicus* stem bark methanol extract and *Allophylus abyssinicus* stem bark water extract upon visualization under 365 nm revealed homogenous spots contrarily to the many phytochemical groups reported present according to preliminary phytochemical screening (Table 1). Homogenous spots have also been reported from extracts of *Lecuas aspera* (Dutta, 2013), *Cordia milleni* (Udeozo et al., 2016).

Table 2

Thin layer chromatography finger print of the stem bark and leaves' extracts of *Symphonia globulifera* and *Allophylus abyssinicus*

Extract Number (Code)	Under Normal light			Under 245 nm		Under 365 nm	
	Spots	Spot Color	Retention factor (R _f)	Spots	Retention factor (R _f)	Spots	Retention factor (R _f)
1 (SGLM)	1	Yellowish Brown	0.085	2	0.085	2	0.056
					0.887		0.099
4 (AALM)	5	Light Green	0.389	5	0.097	8	0.069
		Yellowish Green	0.431		0.403		0.097
					0.431		0.167
		Green	0.458		0.472		0.333
		Yellowish Green	0.847		0.861		0.417
							0.472
		Light Green	0.889				0.875
							0.903
2 (SGLE)	7	Yellow Brown	0.306	10	0.083	11	0.069
					0.222		0.097
		Light Green	0.375		0.333		0.208
		Yellowish Green	0.417		0.361		0.333
					0.417		0.389
		Green	0.444		0.444		0.444
		Light Yellow	0.667		0.569		0.514
		Yellowish Green	0.875		0.583		0.583
					0.861		0.681
		Green	0.889		0.889		0.889
							0.903
5 (AALE)	8	Yellow Brown	0.347	8	0.333	8	0.056
		Green	0.375		0.361		0.083
		Dark Green	0.417		0.417		0.361
		Yellow	0.667		0.444		0.403
		Light Orange	0.778		0.583		0.431
		Yellow green	0.861		0.861		0.639
		Dark green	0.903		0.903		0.861

Key: SGLW:-*Symphonia globulifera* leaf water extract, SGLE:- *Symphonia globulifera* leaf ethyl acetate extract, SGLM:- *Symphonia globulifera* leaf methanol extract, SGSW:- *Symphonia globulifera* stem bark water extract, SGSE:- *Symphonia globulifera* stem bark ethyl acetate extract, SGSM:- *Symphonia globulifera* stem bark methanol extract, AALW:-*Allophylus abyssinicus* leaf water extract, AALM:-*Allophylus abyssinicus* leaf methanol extract, AALE:-*Allophylus abyssinicus* leaf ethyl acetate extract, AASW:- *Allophylus abyssinicus* stem bark water extract, AASM:-*Allophylus abyssinicus* stem bark methanol extract, AASE:-*Allophylus abyssinicus* stem bark ethyl acetate extract

Extract Number (Code)	Under Normal light			Under 245 nm		Under 365 nm	
	Spots	Spot Color	Retention factor (R _f)	Spots	Retention factor (R _f)	Spots	Retention factor (R _f)
		Yellow	0.972		0.958		0.903
3 (SGLW)						4	0.083
							0.139
							0.292
							0.486
6 (AALW)				1	0.083	2	0.056
							0.083
1' SGSM)				1	0.083	4	0.069
							0.111
							0.417
							0.556
4' AASM)						1	0.083
2' (SGSE)	4	Orange brown	0.384	3	0.055	9	0.055
		Orange brown	0.493		0.466		0.096
		Yellow Green	0.671		0.630		0.219
		Light Yellow	0.890				0.397
							0.466
							0.630
							0.699
							0.822
							0.890
5' (AASE)	1	Light Green	0.890	3	0.055	4	0.082
					0.438		0.425
					0.877		0.548
							0.781
3' SGSW)						2	0.082
							0.438
6' AASW)				1	0.055	1	0.096
Key: SGLW:-Symphonia globulifera leaf water extract, SGLE:- Symphonia globulifera leaf ethyl acetate extract, SGLM:- Symphonia globulifera leaf methanol extract, SGSW:- Symphonia globulifera stem bark water extract, SGSE:- Symphonia globulifera stem bark ethyl acetate extract, SGSM:-Symphonia globulifera stem bark methanol extract, AALW:-Allophylus abyssinicus leaf water extract, AALM:-Allophylus abyssinicus leaf methanol extract, AALE:-Allophylus abyssinicus leaf ethyl acetate extract, AASW:- Allophylus abyssinicus stem bark water extract, AASM:-Allophylus abyssinicus stem bark methanol extract, AASE:-Allophylus abyssinicus stem bark ethyl acetate extract							

Upon spraying of the TLC plate with concentrated sulphuric acid and heating the TLC plate, it confirmed the presence of phenolic compounds in all plant extracts as shown in Table 3. This was agreeing with the preliminary phytochemical screening which showed the presence of phenols in all extracts and phenolic compounds like flavonoids, tannins among others as in Table 1.

Allophylus abyssinicus leaves ethyl acetate extract had the highest number of phenolic compounds (11 compounds) with Rf values ranging from 0.083 to 0.958 followed by *Symphonia globulifera* stem bark ethyl acetate (9 compounds) Rf values 0.055 to 0.959. Homogenous spots were observed for *Symphonia globulifera* stem bark water extract, *Allophylus abyssinicus* stem bark water extract, *Allophylus abyssinicus* stem bark methanol extract, *Symphonia globulifera* stem bark methanol extract and *Symphonia globulifera* leaves methanol extract. The concentrations and composition of phenolic metabolites in plants are influenced by many factors, including soil and climatic conditions (Chandra et al., 2014). It has been reported that highly polar solvents like water and methanol and water are more effective in extraction of a large amount of phenolic contents plants (Altemimi et al., 2017), however in this study we note the high ability of ethyl acetate, a moderately polar solvent in extracting a high number of phenolic compounds with different retention factor values compared to the highly polar methanol and water.

The various identified phytochemical compounds have been recorded to have antimicrobial activity (Chung et al., 1998; Mabhiza et al., 2016; Mbaveng et al., 2014; Siddiquee, 2014; Sparg et al., 2004). It was therefore expected that all extracts of *Symphonia globulifera* and *Allophylus abyssinicus* should have antimicrobial activity.

3.4 Antimicrobial activity of *Symphonia globulifera* and *Allophylus abyssinicus* plant extracts and extract combinations

3.4.1 Antimicrobial Zones Of Inhibition

All methanol and water extracts of *S. globulifera* and *A. abyssinicus* and selected combination showed inhibition against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* as summarised in Tables 3, 4 and 5 respectively. The antimicrobial activity of these methanolic and water extracts of the leaves and stem bark of *S. globulifera* and *A. abyssinicus* is attributed to the presence of bioactive compounds like tannins, flavonoids, alkaloids among others. Plant secondary metabolites have therapeutic action against pathogens and parasites (Bhalodia & Shukla, 2011; Murugan et al., 2013). *Allophylus abyssinicus* leaf water extract had the highest mean zone of inhibition against *S. aureus* at concentrations 1000 mg/ml, 500 mg/ml, 250 mg/ml, 125 mg/ml and 62.5 mg/ml (31.25 ± 0.50 mm, 30.00 ± 0.00 mm, 27.25 ± 0.50 mm, 24.00 ± 1.15 mm, 19.00 ± 0.00 mm respectively), followed by *Allophylus abyssinicus* stem bark water extract while extract combination (*S. globulifera* leaf water extract and *A. abyssinicus* leaf water extract) had the lowest mean zone of inhibition at each of the same concentrations against *Staphylococcus aureus*. The high activity of *Allophylus abyssinicus* leaf water extract compared to other extracts could be due to the presence for quinones, alkaloids, polyphenols, and phenols in high concentrations in this extract and the other phytochemical groups in moderate concentrations. This observed high activity of water extracts also justifies the usual practice of traditional medicine practitioners of preparing herbal medicine using water as the main solvent. Quinones exert interesting antibacterial activity against *S. aureus* by donating free radicals which form irreversible complexes with amino acids in proteins hence easily attacking the cell walls and membrane enzymes of gram positive bacteria like *Staphylococcus aureus* to inactivation (Campanini-Salinas et al., 2018; Cowan, 1999). Crude extracts from other *Allophylus* species like *Allophylus cobbe* and *Allophylus serratus* showed the maximum zone of inhibition in the range of 20–23 mm, comparatively less than standard antibiotic Cefotaxime against *Staphylococcus aureus* (Chavan & Gaikwad, 2013). Oliveira et al., (2013) also demonstrated larger zone of inhibition of aqueous extracts of *Symphonia globulifera* against *S. aureus* as shown in this study (Tables 3). The significantly higher zones of inhibition of the aqueous leaf extracts of both *Allophylus abyssinicus* and *Symphonia globulifera* than the stem bark extracts respectively against *Staphylococcus aureus* is highly advantageous in sustainable utilization and conservation of the two plants. Collection of leaves for herbal medicine causes less damage to plant compared to harvesting of the stem bark because the stem bark takes long periods to heal. Increasing demand and destructive harvesting of stem bark rather than leaves has led to the depletion of several valuable medicinal trees and threatens the continued availability of the ethnomedicinal value of these trees to the public (Pandey et al., 2011).

Table 4

Mean $\pm$ SD Zone of Inhibition of *Symphonia globulifera* and *Allophylus abyssinicus* extracts and combinations against *Escherichia coli*

Extract	Mean $\pm$ SD Zone of Inhibition (mm) of <i>Staphylococcus aureus</i>										
	1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.62 mg/ml	7.81 mg/ml	3.90 mg/ml	1.95 mg/ml	Ciproflo xacin
AALW	31.25 $\pm$ 0.50 ^a	30.00 $\pm$ 0.00 ^a	27.25 $\pm$ 0.50 ^a	24.00 $\pm$ 1.15 ^a	19.00 $\pm$ 0 ^a	7.25 $\pm$ 0.50 ^{cd}	3.75 $\pm$ 0.50 ^{cd}	0	0	0	20.50 $\pm$ 3.10
AALM	19.00 $\pm$ 0.00 ^{de}	14.50 $\pm$ 1.00 ^{ghi}	12.25 $\pm$ 0.96 ^{fg}	11.00 $\pm$ 0 ^{df}	8.50 $\pm$ 0.56 ^e	7.00 $\pm$ 0.00 ^d	3.00 $\pm$ 0.82 ^{de}	0.75 $\pm$ 0.96 ^g	0	0	24.00 $\pm$ 0.81
AASW	26.75 $\pm$ 1.50 ^b	24.50 $\pm$ 0.58 ^b	21.75 $\pm$ 0.50 ^b	19.75 $\pm$ 1.70 ^b	15.00 $\pm$ 0.00 ^b	9.75 $\pm$ 0.50 ^a	7.00 $\pm$ 0.82 ^a	3.25 $\pm$ 0.96 ^{ab}	0	0	20.25 $\pm$ 0.50
AASM	17.75 $\pm$ 0.96 ^{eg}	15.00 $\pm$ 0.82 ^{fh}	12.00 $\pm$ 0 ^{fg}	10.75 $\pm$ 0.96 ^{ef}	8.75 $\pm$ 0.96 ^e	7.25 $\pm$ 0.50 ^{cd}	4.75 $\pm$ 0.96 ^b	1.00 $\pm$ 1.41 ^{fg}	0	0	27.00 $\pm$ 0.82
SGLW	22.75 $\pm$ 0.96 ^c	17.25 $\pm$ 0.50 ^c	14.75 $\pm$ 0.50 ^d	12.00 $\pm$ 0 ^d	10.50 $\pm$ 0.58 ^d	8.00 $\pm$ 0 ^{bc}	6.25 $\pm$ 0.96 ^a	2.75 $\pm$ 0.50 ^{bd}	0	0	32.50 $\pm$ 1.00
SGLM	20.25 $\pm$ 0.50 ^d	16.00 $\pm$ 0.00 ^{def}	12.00 $\pm$ 0 ^{fg}	10.00 $\pm$ 0.82 ^{fg}	5.00 $\pm$ 0.00 ^g	3.00 $\pm$ 0.82 ^g	0	0	0	0	27.00 $\pm$ 0.00
SGSW	18.25 $\pm$ 0.96 ^e	15.25 $\pm$ 0.96 ^{fg}	12.75 $\pm$ 0.96 ^{ef}	10.00 $\pm$ 1.41 ^{fg}	7.00 $\pm$ 0.00 ^f	5.25 $\pm$ 0.50 ^f	3.50 $\pm$ 0.58 ^{ce}	1.25 $\pm$ 1.26 ^{fg}	0	0	24.00 $\pm$ 0
SGSM	18.00 $\pm$ 0.82 ^{ef}	16.50 $\pm$ 0.58 ^{ce}	12.50 $\pm$ 0.58 ^{eg}	10.00 $\pm$ 0 ^{fg}	7.00 $\pm$ 0.82 ^f	3.75 $\pm$ 0.96 ^g	3.00 $\pm$ 0.82 ^{de}	0	0	0	24.75 $\pm$ 0.96
SGLW + AALW	15.00 $\pm$ 0.82 ⁱ	12.25 $\pm$ 0.96 ^k	10.25 $\pm$ 0.96 ^h	6.75 $\pm$ 0.50	5.00 $\pm$ 0.00 ^g	3.00 $\pm$ 0.82 ^g	0	0	0	0	18.25 $\pm$ 0.96
SGLW + SGLM	18.25 $\pm$ 0.96 ^e	14.75 $\pm$ 0.96 ^{gh}	12.75 $\pm$ 0.50 ^{ef}	9.25 $\pm$ 0.50 ^{gh}	6.75 $\pm$ 0.50 ^f	6.00 $\pm$ 0 ^{ef}	4.25 $\pm$ 0.96 ^{bc}	1.75 $\pm$ 0.50 ^{deg}	0	0	22.25 $\pm$ 1.71
AASW + AALW	15.50 $\pm$ 1.29 ^{hi}	13.00 $\pm$ 0.82 ^k	12.00 $\pm$ 0.00 ^{fg}	11.25 $\pm$ 0.96 ^{de}	10.00 $\pm$ 8.62 ^d	8.75 $\pm$ 0.50 ^b	7.00 $\pm$ 0.00 ^a	4.25 $\pm$ 0.50 ^a	1.50 $\pm$ 0.57 ^{ab}	0	22.75 $\pm$ 0.97
SGLM + AASW	15.25 $\pm$ 1.50 ⁱ	12.75 $\pm$ 0.96 ^{jk}	10.25 $\pm$ 0.50 ^h	8.25 $\pm$ 0.50 ^h	7.00 $\pm$ 0.00 ^f	5.75 $\pm$ 0.96 ^f	3.50 $\pm$ 0.58 ^{ce}	2.00 $\pm$ 0.82 ^{cdef}	0.75 $\pm$ 0.50 ^c	0	25.75 $\pm$ 1.26
SGLW + AASW	17.75 $\pm$ 0.96 ^{eg}	16.75 $\pm$ 0.50 ^{cd}	15.75 $\pm$ 0.50 ^c	14.00 $\pm$ 0 ^c	11.50 $\pm$ 1.29 ^c	7.25 $\pm$ 0.96 ^{cd}	4.25 $\pm$ 0.50 ^{bc}	2.50 $\pm$ 0.58 ^{be}	1.00 $\pm$ 0.82 ^{bc}	0	22.00 $\pm$ 0
AALW + SGLM	16.75 $\pm$ 0.50 ^{fgh}	13.50 $\pm$ 1.29 ^{ij}	13.00 $\pm$ 0 ^e	11.25 $\pm$ 0.96 ^{de}	9.75 $\pm$ 0.50 ^d	6.75 $\pm$ 0.50 ^{de}	5.00 $\pm$ 0.82 ^b	3.00 $\pm$ 0.82 ^{bc}	2.00 $\pm$ 0.82 ^a	0	23.50 $\pm$ 1.00

Extract	Mean $\pm$ SD Zone of Inhibition (mm) of <i>Escherichia coli</i>										
	1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.62 mg/ml	7.81 mg/ml	3.90 mg/ml	1.95 mg/ml	Cipro floxacin
AALW	26.75 $\pm$ 0.96 ^a	22.00 $\pm$ 0 ^a	20.00 $\pm$ 0 ^a	15.25 $\pm$ 0.96 ^a	10.25 $\pm$ 0.50 ^b	8.00 $\pm$ 0.00 ^b	4.50 $\pm$ 0.58 ^a	1.00 $\pm$ 0.82 ^a	0	0	37.00 $\pm$ 0
AALM	20.25 $\pm$ 0.50 ^d	13.75 $\pm$ 0.96 ^e	12.00 $\pm$ 0 ^d	9.00 $\pm$ 0 ^f	8.50 $\pm$ 0.58 ^c	7.00 $\pm$ 0.82 ^b	4.00 $\pm$ 0 ^{ab}	1.25 $\pm$ 0.50 ^a	0	0	36.50 $\pm$ 1.00
AASW	22.00 $\pm$ 0 ^{bc}	17.00 $\pm$ 0 ^d	11.25 $\pm$ 0.96 ^{de}	6.75 $\pm$ 0.50 ^g	4.00 $\pm$ 0 ^f	1.75 $\pm$ 0.50	0	0	0	0	37.00 $\pm$ 0.00
AASM	13.00 $\pm$ 0.82 ^h	8.75 $\pm$ 0.96 ^h	6.75 $\pm$ 0.50 ^g	3.50 $\pm$ 0.58 ^h	2.25 $\pm$ 0.50 ^g	0	0	0	0	0	32.75 $\pm$ 0.96
SGLW	26.00 $\pm$ 0.82 ^a	20.25 $\pm$ 0.50 ^b	16.25 $\pm$ 0.50 ^b	14.75 $\pm$ 0.50 ^a	12.00 $\pm$ 0 ^a	9.25 $\pm$ 0.96 ^a	4.50 $\pm$ 0.58 ^a	0	0	0	34.00 $\pm$ 2.45
SGLM	22.75 $\pm$ 0.96 ^b	19.00 $\pm$ 0.82 ^c	8.00 $\pm$ 1.83 ^g	0	0	0	0	0	0	0	32.00 $\pm$ 0.82
SGSW	15.75 $\pm$ 0.96 ^{ef}	9.75 $\pm$ 0.96 ^g	4.50 $\pm$ 1.29 ⁱ	2.25 $\pm$ 0.50 ⁱ	0	0	0	0	0	0	32.50 $\pm$ 1.0
SGSM	13.25 $\pm$ 0.50 ^{gh}	6.75 $\pm$ 0.50 ⁱ	4.25 $\pm$ 0.50 ⁱ	0	0	0	0	0	0	0	32.75 $\pm$ 0.96
SGLW + AALW	16.25 $\pm$ 0.96 ^e	13.25 $\pm$ 0.50 ^e	12.00 $\pm$ 0 ^d	10.25 $\pm$ 0.50 ^{cd}	7.50 $\pm$ 1.00 ^d	4.25 $\pm$ 2.50 ^{def}	0	0	0	0	34.00 $\pm$ 2.45
SGLW + SGLM	20.75 $\pm$ 1.50 ^{cd}	16.75 $\pm$ 0.96 ^d	13.75 $\pm$ 1.26 ^c	9.25 $\pm$ 0.50 ^{ef}	5.75 $\pm$ 1.50 ^e	0	0	0	0	0	38.50 $\pm$ 2.38
AASW + AALW	14.50 $\pm$ 0.58 ^{fg}	12.25 $\pm$ 0.50 ^f	11.00 $\pm$ 0.82 ^{df}	10.00 $\pm$ 0.82 ^{de}	7.00 $\pm$ 0.82 ^d	5.00 $\pm$ 0.82 ^{ce}	4.00 $\pm$ 0 ^{ab}	1.25 $\pm$ 0.96 ^a	0	0	35.00 $\pm$ 2.45
SGLM + AASW	20.00 $\pm$ 0.82 ^d	18.50 $\pm$ 0.58 ^c	14.75 $\pm$ 0.96 ^c	11.25 $\pm$ 0.50 ^b	7.75 $\pm$ 0.50 ^{cd}	5.50 $\pm$ 0.58 ^c	3.50 $\pm$ 0.58 ^b	0	0	0	32.75 $\pm$ 0.96
SGLW + AASW	20.25 $\pm$ 1.26 ^d	18.25 $\pm$ 0.50 ^c	14.00 $\pm$ 0 ^c	11.00 $\pm$ 0.82 ^{bc}	8.50 $\pm$ 0.58 ^c	5.25 $\pm$ 0.96 ^{cd}	2.75 $\pm$ 1.26 ^c	0	0	0	36.00 $\pm$ 1.83
AALW + SGLM	16.50 $\pm$ 1.29 ^e	13.00 $\pm$ 0.82 ^{ef}	10.50 $\pm$ 1.00 ^{ef}	7.25 $\pm$ 0.50 ^g	5.25 $\pm$ 0.96 ^e	3.50 $\pm$ 0.58 ^f	1.75 $\pm$ 0.50	0	0	0	34.50 $\pm$ 3.00
^{a-j} In columns mean values sharing a superscript letter are not significantly different ($p > 0.05$) as based on the LSD multiple Comparison test. AALW:-Allophylus abyssinicus leaf water extract, AALM:-Allophylus abyssinicus leaf methanol extract, AASW:-Allophylus abyssinicus stem bark water extract, AASM:-Allophylus abyssinicus stem bark methanol extract, SGLW:-Symphonia globulifera leaf water extract, SGLM:-Symphonia globulifera leaf methanol extract, SGSW:- Symphonia globulifera stem bark water extract, SGSM:-Symphonia globulifera stem bark methanol extract											

Table 5
Mean $\pm$ SD Zone of Inhibition of *Symphonia globulifera* and *Allophylus abyssinicus* extracts and combinations against *Pseudomonas aeruginosa*

Extract	Mean $\pm$ SD Zone of Inhibition (mm) of <i>Pseudomonas aeruginosa</i>										
	1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.62 mg/ml	7.81 mg/ml	3.90 mg/ml	1.95 mg/ml	Cipro floxacin
AALW	24.75 $\pm$ 1.89 ^{ac}	15.00 $\pm$ 2.16 ^{efg}	10.75 $\pm$ 0.50 ^d	6.75 $\pm$ 0.50 ^g	4.25 $\pm$ 0.50	2.50 $\pm$ 0.58 ^d	0.75 $\pm$ 0.50 ^d	0	0	0	21.75 $\pm$ 1.26
AALM	19.75 $\pm$ 0.96 ^{ef}	15.75 $\pm$ 0.50 ^{df}	10.75 $\pm$ 0.50 ^d	4.25 $\pm$ 1.50 ^{hi}	3.00 $\pm$ 0.00	1.00 $\pm$ 0.82 ^e	0	0	0	0	16.75 $\pm$ 0.50
AASW	20.25 $\pm$ 0.96 ^e	16.50 $\pm$ 1.00 ^d	14.00 $\pm$ 0.82 ^b	12.00 $\pm$ 0 ^b	9.00 $\pm$ 0.82 ^c	5.50 $\pm$ 1.29 ^b	2.25 $\pm$ 0.50 ^c	0.75 $\pm$ 0.50 ^b	0	0	21.00 $\pm$ 0.82
AASM	19.50 $\pm$ 1.00 ^{eg}	15.50 $\pm$ 0.58 ^{dg}	13.00 $\pm$ 0 ^{bc}	10.75 $\pm$ 0.96 ^{cd}	8.75 $\pm$ 0.50 ^c	5.00 $\pm$ 0.82 ^b	4.00 $\pm$ 1.63 ^a	3.25 $\pm$ 0.96 ^a	0.50 $\pm$ 0.00 ^a	0	21.75 $\pm$ 0.96
SGLW	24.00 $\pm$ 1.41 ^{bcd}	21.00 $\pm$ 0.82 ^a	16.75 $\pm$ 0.96 ^a	14.25 $\pm$ 0.96 ^a	11.25 $\pm$ 0.50 ^a	3.50 $\pm$ 0.58 ^{cd}	0	0	0	0	17.00 $\pm$ 0.82
SGLM	16.25 $\pm$ 1.26 ^h	11.00 $\pm$ 0.82 ^h	6.50 $\pm$ 0.58 ^e	4.00 $\pm$ 0.82 ^{hj}	2.00 $\pm$ 0.82 ^f	1.25 $\pm$ 0.50 ^e	0	0	0	0	20.75 $\pm$ 0.96
SGSW	16.25 $\pm$ 0.96 ^h	13.25 $\pm$ 0.50	7.00 $\pm$ 0.82 ^e	3.25 $\pm$ 0.50 ^{ij}	0	0	0	0	0	0	17.50 $\pm$ 1.29
SGSM	16.00 $\pm$ 0.82 ^h	10.75 $\pm$ 0.96 ^h	7.00 $\pm$ 0.82 ^e	4.50 $\pm$ 0.58 ^h	1.75 $\pm$ 0.96 ^f	0.75 $\pm$ 0.50 ^e	0	0	0	0	20.75 $\pm$ 0.96
SGLW + AALW	25.75 $\pm$ 1.50 ^a	20.75 $\pm$ 0.96 ^{ab}	16.25 $\pm$ 0.50 ^a	12.75 $\pm$ 0.96 ^b	10.25 $\pm$ 0.50 ^b	7.00 $\pm$ 1.41 ^a	3.75 $\pm$ 0.50 ^{ab}	1.50 $\pm$ 0.58 ^b	0	0	19.00 $\pm$ 0.82
SGLW + SGLM	23.00 $\pm$ 0.82 ^d	15.75 $\pm$ 0.96 ^{df}	12.25 $\pm$ 0.50 ^c	9.25 $\pm$ 0.50 ^e	6.25 $\pm$ 0.96 ^{de}	3.50 $\pm$ 0.58 ^{cd}	0	0	0	0	17.75 $\pm$ 0.50
AASW + AALW	20.25 $\pm$ 0.96 ^e	16.00 $\pm$ 0 ^{de}	13.75 $\pm$ 0.50 ^b	11.75 $\pm$ 0.50 ^{bc}	9.00 $\pm$ 0.82 ^c	5.00 $\pm$ 1.16 ^b	3.00 $\pm$ 0 ^{bc}	1.50 $\pm$ 0.56 ^b	0.50 $\pm$ 0.58 ^a	0	21.00 $\pm$ 0.82
SGLM + AASW	23.00 $\pm$ 0.00 ^d	19.25 $\pm$ 0.96 ^c	13.75 $\pm$ 1.50 ^b	10.00 $\pm$ 0.00 ^{de}	7.00 $\pm$ 0.82 ^d	3.75 $\pm$ 0.50 ^c	1.25 $\pm$ 0.96 ^d	0	0	0	20.75 $\pm$ 0.96
SGLW + AASW	18.25 $\pm$ 0.50 ^{fg}	15.50 $\pm$ 1.00 ^{dg}	12.00 $\pm$ 0.82 ^c	8.00 $\pm$ 0.82 ^f	5.75 $\pm$ 0.50 ^e	3.50 $\pm$ 0.58 ^{cd}	1.25 $\pm$ 0.96 ^d	0	0	0	20.25 $\pm$ 0.50
AALW + SGLM	25.25 $\pm$ 2.22 ^{ab}	19.50 $\pm$ 1.00 ^{bc}	16.75 $\pm$ 0.96 ^a	12.25 $\pm$ 0.96 ^b	10.75 $\pm$ 0.50 ^{ab}	0	0	0	0	0	21.25 $\pm$ 0.96
a-j In columns mean values sharing a superscript letter are not significantly different (p > 0.05) as based on the LSD multiple Comparison test. AALW:-Allophylus abyssinicus leaf water extract, AALM:-Allophylus abyssinicus leaf methanol extract, AASW:-Allophylus abyssinicus stem bark water extract, AASM:-Allophylus abyssinicus stem bark methanol extract, SGLW:-Symphonia globulifera leaf water extract, SGLM:-Symphonia globulifera leaf methanol extract, SGSW:- Symphonia globulifera stem bark water extract, SGSM:-Symphonia globulifera stem bark methanol extract											

The methanolic, water extracts and selected combinations of *S. globulifera* and *A. abyssinicus* also inhibited the growth of *E. coli* as shown in Table 4. At concentrations 1000 to 250 mg/ml *Allophylus abyssinicus* leaf water extract showed the largest zone of inhibition against *E. coli* (26.75 $\pm$ 0.96 mm, 22.00 $\pm$ 0 mm and 20.00 $\pm$ 0 mm). This shows the presence of bioactive compounds with activity against gram negative bacteria like *E. coli*. However, AALW showed a smaller zone of inhibition against *E. coli* compared to *S. aureus*. This reduced activity against gram negative *E. coli* was not surprising as plant extracts are usually more active against gram-positive bacteria than gram-negative bacteria due to structural differences in the cell wall. Gram-negative

bacteria possess an outer membrane with hydrophilic surface that functions as a permeability barrier against many natural compounds (Silva & Nascimento, 2016).

This study presents novel evidence of growth inhibition of *Pseudomonas aeruginosa* by *Symphonia globulifera* and *Allophylus abyssinicus*. Extract combinations (*Symphonia globulifera* leaf water extract and *Allophylus abyssinicus* leaf water extract, *Allophylus abyssinicus* leaf water extract and *Symphonia globulifera* leaf methanol extract) generally showed the largest zones of inhibition against *P. aeruginosa* compared to the individual single extracts (Table 5). *Pseudomonas aeruginosa* is highly resistant and easily acclimates to new environment, which probably explains its minimal susceptibility to individual plant extracts. *P. aeruginosa* has a large genome size ranging between 5.5–7 Mbp which enables it encode for a large proportion of regulatory enzymes important for metabolism, transportation and efflux of organic compounds hence metabolic versatility and high adaptability potential to environmental stresses (Pang et al., 2019). Among the individual extracts, at 1000 mg/ml, *Allophylus abyssinicus* leaf water extract showed the highest zone of inhibition (24.75 ± 1.89 mm) against *P. aeruginosa* but this was not significantly different from the mean zone of inhibition by *Symphonia globulifera* leaf water extract (24.00 ± 1.41 mm), this trend may be attributed to common phytochemical compounds like tannins and alkaloids in higher concentrations in both extracts. Tannins have a high selective action for *Pseudomonas aeruginosa* with a bacteriostatic mode of action through impairing its adhesion onto surfaces (Trentin et al., 2013). Alkaloids from leaves of the *Callistemon citrinus* had ability of inhibiting ATP-dependent transport efflux pumps in *Pseudomonas aeruginosa* (Mabhiza et al., 2016).

The plant extracts of *Allophylus abyssinicus* and *Symphonia globulifera* showed small zones of inhibition at high concentrations (1000 mg/ml and 500 mg/ml) against the growth of *Candida albicans* as shown in Table 6. At 1000 mg/ml of extracts, SGSM had the highest zone of inhibition (13.00 ± 1.41 mm) against *Candida albicans* and AASW had the lowest mean zone of inhibition (5.75 ± 6.28 mm) against *Candida albicans*. This minimal anti-*Candida albicans* activity from the extracts of *Allophylus abyssinicus* and *Symphonia globulifera* may be caused by low and moderate concentrations of anthraquinones, chalcones and anthocyanins within the extracts. Anthraquinones, chalcones, anthocyanins possess antifungal activity properties (Seleem et al., 2017). All four methanolic extracts of the two plants inhibited the growth of *Candida albicans* unlike the water extracts, this is indicative of the high capacity of methanol to extract antifungal phytochemicals compared to water. Methanolic extracts of plants like Lamiaceae species, *Papaver rhoeas* L. have showed strong antifungal activity against *Candida* species, *Fusarium* species, *Aspergillus* species (Askun et al., 2009; Othman et al., 2019).

3.4.2 Antimicrobial Minimum Inhibitory Concentration (Mic)

The Minimum Inhibitory Concentrations (MICs) of extracts against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* ranged from 7.81 mg/ml to 31.25 mg/ml, 7.81 mg/ml to 250 mg/ml and 3.90 mg/ml to 125 mg/ml respectively as shown in Table 8. Generally, extract combinations showed the lowest MICs values against *S. aureus*, *E. coli* and *P. aeruginosa* ranging from 3.90 mg/ml to 31.25 mg/ml, 7.81 mg/ml to 31.25 mg/ml and 3.90 mg/ml to 6.25 mg/ml respectively (Table 7) indicative of possible synergistic activity between the extracts. Extract combinations (AASW and AALW, SGLM and AASW, SGLW and AASW, AALW and SGLM) had a homogenous, lowest MIC (3.90 mg/ml) against *Staphylococcus aureus*. Similarly, combination of *Allophylus abyssinicus* stem bark water extract and *Allophylus abyssinicus* leaves water extract (AASW & AALW) had the lowest MIC (7.81 mg/ml and 3.90 mg/ml) against both *E. coli* and *P. aeruginosa* respectively. The higher anti-bacterial activity of extract combinations than single extracts justify the practice of using more than one plant for development of herbal therapeutics by herbal medicine practioners. The therapeutic value of synergistic interactions is relied upon by herbal healing systems to obtain enhanced efficacy against ailments (Van Vuuren & Viljoen, 2011). The MIC of the methanol extracts of *Symphonia globulifera* leaves and stem bark (15.62 mg/ml) against *S. aureus* in the current study were relatively higher than that of the methanol extract of the seeds of *Symphonia globulifera* (MIC = 0.3 mg/ml) as reported by Lenta,et al, (2004). This low antimicrobial activity of the *Symphonia globulifera* extracts of the current study compared to Lenta et al., (2004) can be associated to differences in phytochemical composition as a result of differences in soil types and climate between the Bwindi Impenetrable forest, Uganda and North-west province of Cameroon. *Symphonia globulifera* has a variety of isolated compounds with good anti- *Staphylococcus aureus* activity compared to the crude methanol extracts in the current study, for example Biflavonoid GB2, Manniflavanone GB3, Globulixanthone F, have lower MICs' 8.50 µg/ml, 8.50 µg/ml and 4.50µg/ml respectively against *Staphylococcus aureus* (Mkougaa et al., 2009). However, the mode of action whether bacteriostatic or bactericidal of these compounds still remains uncertain.

Table 8

The minimum bactericidal and fungicidal concentration of *Allophylus abyssinicus* and *Symphonia globulifera* extracts

The minimum bactericidal and fungicidal concentration of <i>Allophylus abyssinicus</i> and <i>Symphonia globulifera</i> extracts				
Extract		MBC (mg/ml)		MFC (mg/ml)
	Staphylococcus aureus ATCC 25923	Escherichia coli ATCC 25922	Pseudomonas aeruginosa ATCC 27853	Candida Albicans ATCC 10231
AALW	31.25	250.00	500.00	N/A
AALM	62.50	250.00	250	*
SGLW	15.62	500.00	250	N/A
SGLM	125.00	500.00	250	*
AASW	31.25	*	250	N/A
SGSM	250.00	250.00	250	*
SGSW	500.00	500.00	250	N/A
AASM	250.00	250.00	*	*
Key: N/A: - No antifungal Activity, *- Bacteriostatic or fungistatic extract, AALW:-Allophylus abyssinicus leaf water extract, AALM:-Allophylus abyssinicus leaf methanol extract, SGLW:-Symphonia globulifera leaf water extract, SGLM:-Symphonia globulifera leaf methanol extract, AASW:- Allophylus abyssinicus stem bark water extract, SGSM:-Symphonia globulifera stem bark methanol extract, SGSW:- Symphonia globulifera stem bark water extract, AASM:-Allophylus abyssinicus stem bark methanol extract				

Table 7

The minimum inhibitory concentration (MIC) of singular *Symphonia globulifera* and *Allophylus abyssinicus* extracts and their combinations

Extract	Mean $\pm$ SD Diametric Zone of Inhibition (mm) of <i>Candida albicans</i>										
	1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.62 mg/ml	7.81 mg/ml	3.90 mg/ml	1.95 mg/ml	Fluconazole
AALW	0	0	0	0	0	0	0	0	0	0	32.75 $\pm$ 0.96
AALM	11.25 $\pm$ 0.96 ^{ac}	3.25 $\pm$ 0.96 ^a	0	0	0	0	0	0	0	0	32.00 $\pm$ 0.00
AASW	5.75 $\pm$ 6.28 ^e	4.00 $\pm$ 4.28 ^a	0	0	0	0	0	0	0	0	33.00 $\pm$ 0.83
AASM	12.00 $\pm$ 0.82 ^{ab}	2.75 $\pm$ 1.50 ^a	0	0	0	0	0	0	0	0	33.50 $\pm$ 1.00
SGLW	0	0	0	0	0	0	0	0	0	0	35.00 $\pm$ 2.45
SGLM	7.25 $\pm$ 0.50 ^{de}	0	0	0	0	0	0	0	0	0	45.00 $\pm$ 0
SGSW	0	0	0	0	0	0	0	0	0	0	37.75 $\pm$ 0.96
SGSM	13.00 $\pm$ 1.41 ^a	0	0	0	0	0	0	0	0	0	43.25 $\pm$ 2.50
SGLW + AALW	0	0	0	0	0	0	0	0	0	0	33.25 $\pm$ 0.96
SGLW + SGLM	0	0	0	0	0	0	0	0	0	0	33.00 $\pm$ 0.82
AASW + AALW	0	0	0	0	0	0	0	0	0	0	33.25 $\pm$ 0.96
SGLM + AASW	0	0	0	0	0	0	0	0	0	0	33.25 $\pm$ 1.26
SGLW + AASW	9.00 $\pm$ 0.82 ^{bcd}	5.25 $\pm$ 0.96 ^a	0	0	0	0	0	0	0	0	33.50 $\pm$ 0.58
AALW + SGLM	9.00 $\pm$ 0.82 ^{bcd}	5.25 $\pm$ 0.96 ^a	0	0	0	0	0	0	0	0	34.00 $\pm$ 0.00

Extract	Minimum Inhibitory Concentration (mg/ml)			
	<i>Staphylococcus aureus</i> ATCC 25923	<i>Escherichia coli</i> ATCC25922	<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Candida albicans</i> ATCC 10231
AALW	15.62	7.81	7.81	N/A
AALM	7.81	7.81	31.25	500.00
AASW	7.81	31.25	7.81	500.00
AASM	7.81	62.50	3.90	500.00
SGLW	7.81	15.62	31.25	N/A
SGLM	31.25	250.00	31.25	1000.00
SGSM	15.62	250.00	31.25	1000.00
SGSW	7.81	125.00	125.0	N/A
SGLW + AALW	31.25	31.25	7.81	N/A
SGLW + SGLM	7.81	15.62	31.25	500.00
AASW + AALW	3.90	7.81	3.90	N/A
SGLM + AASW	3.90	15.62	15.62	N/A
SGLW + AASW	3.90	15.62	15.62	1000.00
AALW + SGLM	3.90	15.62	62.50	500.00
Ciprofloxacin (µg/ml)	0.26	0.02	0.02	N/A
Fluconazole (µg/ml)	N/A	N/A	N/A	4.00
Key: N/A:- No antimicrobial activity, AALW:-Allophylus abyssinicus leaf water extract, AALM:-Allophylus abyssinicus leaf methanol extract, AASW:- Allophylus abyssinicus stem bark water extract, AASM:-Allophylus abyssinicus stem bark methanol extract, SGLW:-Symphonia globulifera leaf water extract, SGLM:-Symphonia globulifera leaf methanol extract, SGSM:-Symphonia globulifera stem bark methanol extract, SGSW:- Symphonia globulifera stem bark water extract.				

All extracts and combination except AALM had lower MIC against *Staphylococcus aureus* than *Escherichia coli* indicating a stronger antimicrobial activity against *Staphylococcus aureus* than *Escherichia coli* possibly due to the presence of an outer membrane in the cell wall of *E. coli*, that acts as a permeability barrier preventing the penetration of the bioactive compound into *E. coli* cells. Lower MIC values of plant extracts against *S. aureus* compared to *Escherichia coli* have also been reported by Atef et al., (2019) and Debalke et al., (2018).

Surprisingly, AALM had the same MIC (7.81 mg/ml) for both *E. coli* and *S. aureus*, this is evocative of broad-spectrum antibiotic compounds with activity against both gram positive and gram-negative bacteria.

AASM and extract combination (AASW and AALW) had the highest antimicrobial activity (MIC = 3.90 mg/ml) against *Pseudomonas aeruginosa* as shown in Table 8. AASM and AASW had a high concentration of saponins which have bactericidal activity and inhibit formation of biofilms, which exposes *Pseudomonas aeruginosa* cells to the activity of other bioactive compounds (Ana et al., 2019). Combinations SGLM and AASW, SGLW and AASW had the same MIC values for *E. coli* and *P. aeruginosa* probably because both gram-negative bacteria with many common structures as drug targets.

The extracts and combinations had high minimum inhibitory concentration values (1000 mg/ml and 500 mg/ml) (Table 7) against *Candida albicans* interpretable as low antifungal activity just as indicated by the zone of inhibitions (Table 6). This is probably due to limited concentrations of antifungal compounds like anthraquinones and anthocyanins that have been reported as antifungal (de Barros et al., 2011; Sakunpak et al., 2009; Wen et al., 2016) with in the extracts. AALW had no anti-fungal activity however its combination with SGLM showed activity (MIC = 500mg/ml) compared to that of SGLM (MIC = 1000 mg/ml) as a result of

synergistic interaction between the two species. It has been earlier reported that plant extracts with no antimicrobial activity when mixed with antimicrobial active compounds, may induce a much stronger antimicrobial activity (Nascimento et al., 2000). For example chlorhexidine's zone of inhibition against *C. albicans* was 30.3–19.3 mm, but in combination with the ethyl acetate extract (100 mg/mL) of *Tanacetum vulgare*, there was an increase in the inhibition (32.7–30 mm), indicating synergistic effect (Kameri et al., 2019).

3.4.3 Minimum Bactericidal Concentration (Mbc)

The MBC of the extracts against *Staphylococcus aureus* ATCC 2592, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 ranged between 15.62 mg/ml to 500 mg/ml, 250 mg/ml to 500 mg/ml and 250 mg/ml to 500 mg/ml respectively as shown in Table 8. These results in Table 8, indicate that the plant extracts had variable bactericidal activity. *Symphonia globulifera* leaf water extract had the lowest minimum bactericidal concentration (15.625 g/ml) against *Staphylococcus aureus*.

There was less variation in the MBC values of the extracts against *E. coli*. *Allophylus abyssinicus* leaves water extract, *Allophylus abyssinicus* leaves methanol extract, *Allophylus abyssinicus* stem bark methanol extract and *Symphonia globulifera* stem bark methanol extract showed the lowest but same MBC (250 mg/ml) against *Escherichia coli*. Similarly, five of the extracts (AALM

SGLW, SGLM, AASW, SGSM, SGSW) had the lowest MBC (250 mg/ml) against *Pseudomonas aeruginosa*. *Allophylus abyssinicus* stem bark water extract and *Allophylus abyssinicus* stem bark methanol extracts had a bacteriostatic activity against *E. coli* and *P. aeruginosa* respectively. The mode of action of the plant extracts that leads to death of the bacterial cells is not well understood. However, it has been proposed that plant extracts induce bacterial cell death through interaction of antimicrobial components of the plant extracts with enzymes and proteins of the microbial cell membrane. Hence causing the disruption of microbial cell membrane to disperse high amounts of protons towards the cell exterior which induces cell death (Burt, 2004; Mostafa et al., 2018). Alternatively, it has been thought that hydrophobicity characters of plant extracts react with proteins of microbial cell membrane and mitochondria which affects their permeability resulting into cell death (Friedman et al., 2004; Tiwari et al., 2009).

None of the extracts or combinations with antifungal activity (AALM, SGLM, SGSM, AASM, AASW) had fungicidal activity against *Candida albicans*. This further supports the earlier reported low anti *Candida albicans* activity.

3.4.4 Total Antibacterial Activity And Total Antifungal Activity (Taa)

The total antimicrobial activity of the extracts is a pharmacologically useful measure to compare the antimicrobial efficacy of different plants since it takes into account both the anti-microbial activity and the quantity extracted from the plant material. The total activity of the extract is expressed in ml/g and is the volume of solvent that can added to the extract obtained from 1 g of plant material that will still inhibit the growth of the specific pathogen (Elisha et al., 2017). Generally, TAA of the extracts was highest when tested against *Staphylococcus aureus*, followed by *Escherichia coli* and least with *Candida albicans* as shown in Table 10. AASM had the highest TAA against *Staphylococcus aureus* (33.04×10^0 ml/g), *Pseudomonas aeruginosa* (1.35×10^{-1} ml/g) and *Candida albicans* (2.71×10^{-4} ml/g). AALM had the highest TAA (1.13×10^0 ml/g) against *Escherichia coli* as shown in Table 9. Basing on TAA, *Allophylus abyssinicus* leaves and stem bark powder had a high concentration of antimicrobial compounds and would require a high volume of solvent to dissolve it before it loses its antimicrobial activity compared to the *Symphonia globulifera*. Therefore, *Allophylus abyssinicus* stem bark would be the best choice for treatment and development of drugs for ailments caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Elisha et al., (2017) reports higher TAA values for *Bolusanthus speciosus*, *Calpurnia aurea*, *Maesa lanceolata*, *Hypericum roeperianum* and *Cremaspora triflora* against *Escherichia coli* compared to those of *Symphonia globulifera* and *Allophylus abyssinicus* reported by this study. Total antibacterial activity of plant extract against any pathogen significant for publication should at least be 100 ml/g (Eloff, 2004), however all TAA values of the current study were all below 100 ml/g hence not significant. However, they inform us on the variable overall antimicrobial activity of the leaves and stem bark of *Symphonia globulifera* and *Allophylus abyssinicus*.

Table 10

Fractional inhibitory concentration indices of extract combinations of *Symphonia globulifera* and *Allophylus abyssinicus* against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*

Microorganism	Extract 1	Extract 2	FIC (1)	FIC (2)	ΣFIC	Combination Effect
<i>Staphylococcus aureus</i>	SGLW	AALW	4	2	6	Antagonistic
	SGLW	SGLM	1	0.2478	1.2478	Indifferent
	AASW	AALW	0.5	0.25	0.75	Additive
	SGLM	AASW	0.1239	0.5	0.6239	Additive
	SGLW	AASW	0.5	0.5	1	Additive
	AALW	SGLM	0.25	0.1239	0.3739	synergistic
<i>Escherichia coli</i>	SGLW	AALW	2	4	6	Antagonistic
	SGLW	SGLM	1	0.0625	1.0625	Additive
	AASW	AALW	0.2478	1	1.2478	Indifferent
	SGLM	AASW	0.0625	0.4957	0.5582	Additive
	SGLW	AASW	1	0.4957	1.4957	Indifferent
	AALW	SGLM	2	0.0625	2.0625	Indifferent
<i>Pseudomonas aeruginosa</i>	SGLW	AALW	0.2478	1	1.2478	Indifferent
	SGLW	SGLM	0.9914	0.9914	1.9828	Indifferent
	AASW	AALW	0.5	0.5	1	Additive
	SGLM	AASW	0.4957	2	2.4957	Indifferent
	SGLW	AASW	0.4957	2	2.4957	Indifferent
	AALW	SGLM	8	1.9828	9.9828	Antagonistic
Combination Scale ΣFIC = ≤ 0.5:- Synergistic, > 0.5-1:-Additive, > 1-4:- Indifferent/Non interactive, > 4:-Antagonistic (Van Vuuren & Viljoen, 2011), SGLW:-Symphonia globulifera leaf water extract, AALW:-Allophylus abyssinicus leaf water extract, SGLM:-Symphonia globulifera leaf methanol extract, AASW:- Allophylus abyssinicus stem bark water extract.						

Table 9
Total antimicrobial activity of *Symphonia globulifera* and *Allophylus abyssinicus* extracts

Extract	TAA (ml/g)			
	Staphylococcus aureus	Escherichia coli	Pseudomonas Aeruginosa	Candida albicans
AALW	7.08×10^0	9.06×10^{-1}	1.16×10^{-1}	N/A
AALM	8.83×10^0	1.13×10^0	3.59×10^{-2}	7.17×10^{-5}
AASW	18.34×10^0	5.82×10^{-1}	7.45×10^{-2}	1.49×10^{-4}
AASM	33.04×10^0	5.29×10^{-1}	1.35×10^{-1}	2.71×10^{-4}
SGLW	11.83×10^0	7.57×10^{-1}	2.40×10^{-2}	N/A
SGLM	2.04×10^0	8.15×10^{-3}	2.59×10^{-4}	3.00×10^{-7}
SGSM	5.56×10^0	2.22×10^{-2}	7.06×10^{-4}	7.00×10^{-7}
SGSW	10.16×10^0	8.13×10^{-2}	6.51×10^{-4}	N/A
Key: N/A:- No Antifungal Activity, SGLW:-Symphonia globulifera leaf water extract, SGLM:-Symphonia globulifera leaf methanol extract, SGSW:- Symphonia globulifera stem bark water extract, SGSM:-Symphonia globulifera stem bark methanol extract, AALW:-Allophylus abyssinicus leaf water extract, AALM:-Allophylus abyssinicus leaf methanol extract, AASW:- Allophylus abyssinicus stem bark water extract, AASM:-Allophylus abyssinicus stem bark methanol extract				

3.4.5 Fractional Inhibitory Concentration Indices

The selected *Symphonia globulifera* and *Allophylus abyssinicus* extract combinations observed in the current study had antagonistic, indifferent, additive and synergistic interactions against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* as shown in Table 10. *Allophylus abyssinicus* leaves water extract and *Symphonia globulifera* leaves methanol extract combination showed a synergistic effect ($\Sigma FICI = 0.3739$) against *Staphylococcus aureus*. Synergism arises as a result of mutual interaction between the extracts that each enhances the activity of the other or as a result of reaction between the two extracts forming a new component with a much high activity against the pathogen (Ndhlala et al., 2011). Furthermore, combination of plant extracts increases the diversity of bioactive compounds which increases the number of drug targets that the combination can attack hence strong synergistic antimicrobial activity (Al-bayati, 2008). Interestingly extract combinations with AASW i.e. (AASW and AALW, SGLM and AASW, SGLW and AASW) had an additive combination effect against *Staphylococcus aureus* which suggests that AASW could have a contributory effect in enhancing the potency and the activity of other extracts against *Staphylococcus aureus*.

Both extract combinations SGLW and SGLM, SGLM and AASW had an additive effect against the growth of *E. coli* indicative of extract mutually exclusive antimicrobial activity against the bacteria with no chemical interaction between the extracts (Van Vuuren & Viljoen, 2011). Focusing on *Pseudomonas aeruginosa*, AASW and AALW combination had an additive effect with the lowest fractional inhibitory concentration index ($\Sigma FICI = 1$) against *Pseudomonas aeruginosa* and all other combinations had either antagonistic or indifferent activity. Plant extract combination studies (Moussaoui & Alaoui, 2016; Njimoh et al., 2015; Olajuyigbe & Afolayan, 2013) mainly assume that each extract is a single bioactive compound yet phytochemical composition of any plant extract is naturally diverse with high chemical complexity hence a range of pharmacological effects (Bruneton, 2012). It is worth noting that revelations of either synergy, additive, non-interactive and antagonism are all equally important in phytotherapy. Plant extract combinations with synergistic and additive effect can be chosen for development of combination phytotherapies.

Table 10 : **Fractional inhibitory concentration indices of extract combinations of *Symphonia globulifera* and *Allophylus abyssinicus* against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa***

Combination Scale $\Sigma FIC = \leq 0.5$:- Synergistic, > 0.5 -1:-Additive, > 1 -4:- Indifferent/Non interactive, > 4 :-Antagonistic (Van Vuuren & Viljoen, 2011), SGLW:-Symphonia globulifera leaf water extract, AALW:-Allophylus abyssinicus leaf water extract, SGLM:-

4.0 Conclusion

S. globulifera and *A. abyssinicus* stem bark and leaves contain a wide range of bioactive compounds with antimicrobial activity for example tannins, alkaloids, flavonoids, quinones, anthraquinones among others. This is suggestive of the potential usage of their crude extracts or their constituents in phyto-chemotherapy. Generally, antimicrobial assays proved that the water and methanolic extracts of both *S. globulifera* and *A. abyssinicus* stem bark and leaves had stronger in vitro bactericidal activity against *S. aureus*, *E. coli*, and *P. aeruginosa* but low anti-*Candida albicans* activity. This justifies their continued usage in the management of ailments caused by bacteria but unsuitability for treatment of ailments due to *Candida albicans*. They also offer potential natural sources of broad-spectrum antibiotics for both gram positive and gram-negative bacteria. Interestingly, the leaves of both plants had higher total antibacterial activity (TAA) than the stem bark, therefore leaves rather than the stem bark should be utilized for large scale development of phyto-remedies from these plants. Due to the varied extract combination effect, we recommend that only those extracts combinations with empirical synergistic or additive activity be used in management of any ailment. With this reported in vitro activity evidence, in vivo antimicrobial activity of these extracts ought to be conducted. Furthermore, isolation, structure determination and antimicrobial activity of the phytochemical compounds present in the extracts of the *S. globulifera* and *A. abyssinicus* would offer more insight in understanding their phytochemistry and bioactivity.

Declarations

Ethics approval and consent to participate

The research protocol was approved by Uganda Wildlife Authority (UWA) under reference number COD/96/05 and Institutional Research Ethics committee at Mbarara University of Science and Technology, under reference number MUREC 1/7.

Consent for publication

All authors approved the publication of these findings.

Availability of data and material

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

BL, RW and GRK conceptualized and designed the study. The study experiments were conducted by BL, RW and COA. GRK and RW supervised the study. BL drafted the manuscript. All authors read and approved the final version of the manuscript.

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References

1. Abubakar, N., Shehu, K., Yahaya, M. M., Tafinta, I. Y., & Imonikhe, M. A. (2016). Phytochemical Screening and Thin Layer Chromatographic Studies of *Guierasenegalensis* G. F Gmel (Egyptian mimosa). *Annals of Biological Sciences*, *4*(1), 26–30.
2. Agber, C. T., Sewuese, S., & Akacha, L. U. (2019). Preliminary Phytochemical Screening and Thin Layer Chromatography Analysis of Stem Bark Extracts of African Mistletoe Parasitic on *Vitellaria paradoxa*, *Piliostigma thonningii* and *Combretum fragrans*. *Asian Journal of Applied Chemistry Research*, *3*(2), 1–6. <https://doi.org/10.9734/ajacr/2019/v3i230087>
3. Al-bayati, F. A. (2008). *Synergistic antibacterial activity between Thymus vulgaris and Pimpinella anisum essential oils and methanol extracts*. *116*, 403–406. <https://doi.org/10.1016/j.jep.2007.12.003>
4. Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, *6*(42), 2,23. <https://doi.org/10.3390/plants6040042>
5. Ana, C., Stankovic, J., Gođevac, D., & Tešević, V. (2019). Antibacterial and Antibio fi lm Activity of Flavonoid and Saponin Derivatives from *Atriplex tatarica* against *Pseudomonas aeruginosa* . *J. Nat. Prod*, *82*. <https://doi.org/10.1021/acs.jnatprod.8b00970>
6. Asante, I., Owusu, E., Essilfie, M., & Amuzuah, O. (2016). Phytochemical investigation and thin layer chromatography of methanolic extracts of some selected grain legumes. *Journal of Pharmacognosy and Phytochemistry JPP*, *5*(53), 240–244.
7. Askun, T., Tumen, G., Satil, F., Kilic, T., Askun, T., Tumen, G., Satil, F., & Kilic, T. (2009). Effects of Some Lamiaceae Species Methanol Extracts on Potential Mycotoxin Producer Fungi Effects of Some Lamiaceae Species Methanol Extracts on. *Pharmaceutical Biology*, *46*(10–11), 688–694. <https://doi.org/10.1080/13880200802215792>
8. Atef, N. M., Shanab, S. M., Negm, S. I., & Abbas, Y. A. (2019). Evaluation of antimicrobial activity of some plant extracts against antibiotic susceptible and resistant bacterial strains causing wound infection. *Bulletin of the National Research Centre*, *43*(144). <https://doi.org/https://doi.org/10.1186/s42269-019-0184-9>
9. Aydagnehum, S. G., & Girma, Z. (2014). An ethnobotanical study of medicinal plants in Debre Libanos Wereda, Central Ethiopia. *African Journal of Plant Science*, *8*(7), 366–379. <https://doi.org/10.5897/AJPS2013.1041>
10. Babaasa, D., Eilu, G., Kasangaki, A., Bitariho, R., & Mcneilage, A. (2004). Gap characteristics and regeneration in Bwindi Impenetrable National Park, Uganda. *African Journal of Ecology*, *42*(3), 217–224.
11. Balakrishna, T., Vidyadhara, S., Sashidhar, R., Ruchitha, B., & Venkata Prathyusha, E. (2016). A review on extraction techniques. *Indo American Journal of Pharmaceutical Sciences*, *3*(8), 880–891. <http://www.iajps.com>
12. Bartnik, M., & Facey, P. C. (2017). Glycosides. In *Pharmacognosy: Fundamentals, Applications and Strategy* (pp. 101–161). Elsevier Inc. <https://doi.org/10.1016/B978-0-12-802104-0.00008-1>
13. Bhalodia, N. R., & Shukla, V. J. (2011). Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* L.: An ethnomedicinal plant. *Journal of Advanced Pharmaceutical Technology & Research*, *2*(2), 104–109. <https://doi.org/10.4103/2231-4040.82956>
14. Bruneton, J. (2012). Principles of herbal pharmacology. In *Principles and Practice of Phytotherapy: Modern Herbal Medicine* (pp. 45–82). Elsevier. <https://doi.org/DOL: http://dx.doi.org/10.1016/B978-0-443-06992-5.00002-5>
15. Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*, *94*(3), 223–253. <https://doi.org/https://doi.org/10.1016/j.ijfoodmicro.2004.03.022>
16. Campanini-Salinas, J., Andrades-Lagos, J., Rocha, G. G., Choquesillo-Lazarte, D., Dragnic, S. B., Faúndez, M., Alarcón, P., Silva, F., Vidal, R., Salas-Huenuleo, E., Kogan, M., Mella, J., Gajardo, G. R., & Vásquez-Velásquez, D. (2018). A new kind of quinonic-antibiotic useful against multidrug-resistant *S. aureus* and *E. faecium* Infections. *Molecules*, *23*(7). <https://doi.org/10.3390/molecules23071776>

17. Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M. H., Elsohly, M. A., & Khan, I. A. (2014). Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. *Evidence-Based Complementary and Alternative Medicine*, 2014. <https://doi.org/10.1155/2014/253875>
18. Chavan, R. B. B., & Gaikwad, D. K. (2016). The Ethnobotany, Phytochemistry and Biological Properties of *Allophylus* Species Used in Traditional Medicine: a Review. *World Journal of Pharmacy and Pharmaceutical Sciences*, 5(11), 664–682. <https://doi.org/10.20959/wjpps201611-8039>
19. Chavan, R. B., & Gaikwad, D. K. (2013). Antibacterial activity of medicinally important two species of *Allophylus*- *Allophylus* *cobbe* (L.) Raeusch. and *Allophylus* *serratus* (Roxb.) Kurz. *Journal of Pharmacognosy and Phytochemistry*, 2(1), 1–7.
20. Chung, K. T., Wong, T. Y., Wei, C. I., Huang, Y. W., & Lin, Y. (1998). Tannins and human health: A review. *Critical Reviews in Food Science and Nutrition*, 38(6), 421–464. <https://doi.org/10.1080/10408699891274273>
21. Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12(4), 564–582. <https://doi.org/10.1128/0893-8512.1999.00400-10>
22. de Barros, I. B., de Souza Daniel, J. F., Pinto, J. P., Rezende, M. I., Filho, R. B., & Ferreira, D. T. (2011). Phytochemical and antifungal activity of anthraquinones and root and leaf extracts of *Coccoloba mollis* on phytopathogens. *Brazilian Archives of Biology and Technology*, 54(3), 535–541. <https://doi.org/10.1590/s1516-89132011000300015>
23. Debalke, D., Birhan, M., Kinubeh, A., & Yayeh, M. (2018). Assessments of Antibacterial Effects of Aqueous-Ethanol Extracts of *Sida rhombifolia*'s Aerial Part. *Scientific World Journal*, 2018. <https://doi.org/10.1155/2018/8429809>
24. Delvaux, C. (2009). *Responses to bark harvesting of medicinal tree species from Forêt Classée des Monts Kouffé, Benin* (Issue September). [http://www.africamuseum.be/publication_docs/PhD Thesis Delvaux 2009.pdf](http://www.africamuseum.be/publication_docs/PhD%20Thesis%20Delvaux%202009.pdf)
25. Dutta, J. (2013). Phytochemicals Analysis and Tlc Fingerprinting of Methanolic Extracts of Three Medicinal Plants. *International Research Journal of Pharmacy*, 4(6), 123–126. <https://doi.org/10.7897/2230-8407.04627>
26. El-Najjar, N., Gali-Muhtasib, H., Ketola, R. A., Vuorela, P., Urtti, A., & Vuorela, H. (2011). The chemical and biological activities of quinones: Overview and implications in analytical detection. *Phytochemistry Reviews*, 10(3), 353–370. <https://doi.org/10.1007/s11101-011-9209-1>
27. Elisha, I., Jambalang, A. R., Botha, F. S., Buys, E. M., McGaw, L. J., & Eloff, J. N. (2017). Potency and selectivity indices of acetone leaf extracts of nine selected South African trees against six opportunistic Enterobacteriaceae isolates from commercial chicken eggs. *BMC Complementary and Alternative Medicine*, 17(90). <https://doi.org/10.1186/s12906-017-1597-3>
28. Elisha, I. L., Botha, F. S., McGaw, L. J., & Eloff, J. N. (2017). The antibacterial activity of extracts of nine plant species with good activity against *Escherichia coli* against five other bacteria and cytotoxicity of extracts. *BMC Complementary and Alternative Medicine*, 17(1), 133. <https://doi.org/10.1186/s12906-017-1645-z>
29. Eloff, J. N. (2004). Quantification the bioactivity of plant extracts during screening and bioassay guided fractionation. *Phytomedicine*, 11(4), 370–371. <https://doi.org/10.1078/0944711041495218>
30. Evans, W. C. (2002). *Trease and Evans Pharmacognosy* (15th ed.). Saunders Elsevier.
31. Famuyide, I. M., Aro, A. O., Fasina, F. O., Eloff, J. N., & McGaw, L. J. (2019). Antibacterial and antibiofilm activity of acetone leaf extracts of nine under-investigated south African *Eugenia* and *Syzygium* (Myrtaceae) species and their selectivity indices. *Complementary and Alternative Medicine*, 19(141). <https://doi.org/10.1186/s12906-019-2547-z>
32. Fromentin, Y., Cottet, K., Kritsanida, M., Michel, S., Gaboriaud-Kolar, N., & Lallemand, M. C. (2015). *Symphonia globulifera*, a widespread source of complex metabolites with potent biological activities. *Planta Medica*, 81(2), 95–107. <https://doi.org/10.1055/s-0034-1396148>
33. Fromentin, Y., Gaboriaud-kolar, N., Ndjakou, B., Duplex, J., Buisson, D., Mouray, E., Grellier, P., Loiseau, P. M., Lallemand, M., & Michel, S. (2013). European Journal of Medicinal Chemistry Synthesis of novel guttiferone A derivatives: In-vitro evaluation toward *Plasmodium falciparum*, *Trypanosoma brucei* and *Leishmania donovani*. *European Journal of Medicinal Chemistry*, 65, 284–294. <https://doi.org/10.1016/j.ejmech.2013.04.066>
34. Gijan, M., & Dalle, G. (2019). Ethnobotanical Study of Medicinal Plants in Nagelle Arsi District, West Arsi Zone of Oromia, Ethiopia. *Journal of Natural Sciences Research*, 9(13), 1–19. <https://doi.org/10.7176/JNSR>
35. Guarro, J., Soler, L., & Rinaldi, M. G. (1995). Pathogenicity and antifungal susceptibility of *Chaetomium* species. *European Journal of Clinical Microbiology & Infectious Diseases*, 14(7), 613–618. <https://doi.org/10.1007/BF01690737>

36. Handa, S. S., Khanuja, S. P. S., Longo, G., & Rakesh, D. D. (2008). *Extraction Technologies for Medicinal and Aromatic Plants*. United Nations Industrial Development Organization. International Centre for Science and High Technology.
37. Huang, Y., Xiao, D., Burton-freeman, B. M., & Edirisinghe, I. (2016). Chemical Changes of Bioactive Phytochemicals during Thermal Processing. In *Reference Module in Food Science*. Elsevier. <https://doi.org/10.1016/B978-0-08-100596-5.03055-9>
38. Hussain, H. S. N., & Deeni, Y. Y. (1991). Plants in Kano Ethnomedicine; Screening for Antimicrobial Activity and Alkaloids. *International Journal of Pharmacognosy*, 29(1), 51–56. <https://doi.org/10.3109/13880209109082849>
39. Iloki-Assanga, S. B., Lewis-Luján, L. M., Lara-Espinoza, C. L., Gil-Salido, A. A., Fernandez-Angulo, D., Rubio-Pino, J. L., & Haines, D. D. (2015). Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*. *BMC Research Notes*, 8(1), 396. <https://doi.org/10.1186/s13104-015-1388-1>
40. Ingram, V., Loo, J., Vinceti, B., Dawson, I., Muchugi, A., Duminil, J., Awono, A., Asaah, E., & Tchoundjeu, Z. (2015). *Ensuring the future of the pygeum tree (Prunus africana) A multiple-use tree* (Issue April). LEI Wageningen UR.
41. Isebe, T. I. (2015). Phytochemical Composition And Antibacterial Activity Of *Eichhornia Crassipes* In Lake Victoria Kisumu. *International Journal of Scientific & Technology Research*, 4(8), 45–52.
42. Jacqueline, I. (2015). *Efficacy of some medicinal plants used in various parts of Kenya in treating selected bacterial and fungal pathogens*. KENYATTA UNIVERSITY.
43. Kameri, A., Koçani, F., Hashani, Z., Kurteshi, K., Kamberi, B., Kurti, A., & Haziri, A. (2019). Antifungal and Synergistic Effects of the Ethyl Acetate Extract of *Tanacetum vulgare* (L.) Against *Candida albicans*. *Medical Science Monitor Basic Research*, 25, 179–186. <https://doi.org/10.12659/MSMBR.917394>
44. Katende, A. B., Birnie, A., & Tengnäs, B. (2000). *Useful Trees and Shrubs for Uganda* (1st ed.). Regional Land Management Unit (RELMA). <http://books.google.co.jp/books?id=sZYfAQAAIAAJ>
45. Khumalo, S. G., Fröde, A., & Sola, P. (2006). *Guidelines for the Sustainable Harvesting of Traditional Medicinal Plants in Zimbabwe*. Southern Alliance for Indigenous Resources.
46. Larayetan, R., Ololade, Z. S., Ogunmola, O. O., & Ladokun, A. (2019). Phytochemical Constituents, Antioxidant, Cytotoxicity, Antimicrobial, Antitrypanosomal, and Antimalarial Potentials of the Crude Extracts of *Callistemon citrinus*. *Evidence-Based Complementary and Alternative Medicine*, 2019, 5410923. <https://doi.org/10.1155/2019/5410923>
47. Lenta, Bruno N., Ngouela, S., NOUNGOU, D. T., Tsamo, E., & Connolly, J. D. (2004). Symphonin: A new prenylated pyranoxanthone with antimicrobial activity from the seeds of *Symphonia globulifera* (Guttiferae). *Bulletin of the Chemical Society of Ethiopia*, 18(2), 175–180. <https://doi.org/10.4314/bcse.v18i2.61439>
48. Lenta, Bruno Ndjakou, Vonthron-Sénécheau, C., Weniger, B., Devkota, K. P., Ngoupayo, J., Kaiser, M., Naz, Q., Choudhary, M. I., Tsamo, E., & Sewald, N. (2007). Leishmanicidal and cholinesterase inhibiting activities of phenolic compounds from *Allanblackia monticola* and *Symphonia globulifera*. *Molecules*, 12(8), 1548–1557. <https://doi.org/10.3390/12081548>
49. Mabhiza et al. (2016). Antibacterial Properties of Alkaloid Extracts from *Callistemon citrinus* and *Vernonia adoensis* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *International Journal of Medicinal Chemistry*, 2016. <https://doi.org/https://doi.org/10.1155/2016/6304163>.
50. Mariita, R. M., Ogo, C. K. P. O., Ogo, N. O., & Okemo, P. O. (2010). Antitubercular and phytochemical investigation of methanol extracts of medicinal plants used by the samburu community in Kenya. *Tropical Journal of Pharmaceutical Research*, 9(4), 379–385. <https://doi.org/10.4314/tjpr.v9i4.58935>
51. Mbaveng, A. T., Hamm, R., & Kuete, V. (2014). Harmful and Protective Effects of Terpenoids from African Medicinal Plants. In *Toxicological Survey of African Medicinal Plants* (pp. 557–576). Elsevier Inc. <https://doi.org/10.1016/B978-0-12-800018-2.00019-4>
52. Mkoungaa, P., Fomuma, Z. T., Meyer, M., Bodob, B., & Nkengfack, A. E. (2009). Globulixanthone F, a New Polyoxygenated Xanthone with from the Stem Bark of *Symphonia globulifera* ¶. *Natural Product Communications*, 4(6), 803–808.
53. Mostafa, A. A., Al-askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., & Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences*, 25(2), 361–366. <https://doi.org/10.1016/j.sjbs.2017.02.004>

54. Moussaoui, F., & Alaoui, T. (2016). Evaluation of antibacterial activity and synergistic effect between antibiotic and the essential oils of some medicinal plants. *Asian Pacific Journal of Tropical Biomedicine*, 6(1), 32–37. <https://doi.org/https://doi.org/10.1016/j.apjtb.2015.09.024>
55. Murugan, T., Wins, J., & Murugan, M. (2013). Antimicrobial Activity and Phytochemical Constituents of Leaf Extracts of *Cassia auriculata*. *Indian Journal of Pharmaceutical Sciences*, 75(1), 122–125. <https://doi.org/10.4103/0250-474X.113546>
56. Nascimento, G. G. F., Locatelli, J., Freitas, P. C., & Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, 31(4), 247–256. <https://doi.org/10.1590/S1517-83822000000400003>
57. Ndhlala, A. R., Stafford, G. I., Finnie, J. F., & Van Staden, J. (2011). Commercial herbal preparations in KwaZulu-Natal, South Africa: The urban face of traditional medicine. *South African Journal of Botany*, 77(4), 830–843. <https://doi.org/10.1016/j.sajb.2011.09.002>
58. Newman, D. J., Cragg, M., Snader, K. M., & December, C. (2000). The influence of natural products upon drug discovery. *Natural Product Reports*, 17(3), 215–234. <https://doi.org/10.1039/a902202c>
59. Ngouela, S., Lenta, B. N., Nongoue, D. T., Ngoupayo, J., Boyom, F. F., Tsamo, E., Gut, J., Rosenthal, P. J., & Connolly, J. D. (2006). Anti-plasmodial and antioxidant activities of constituents of the seed shells of *Symphonia globulifera* Linn f. *Phytochemistry*, 67(3), 302–306. <https://doi.org/10.1016/j.phytochem.2005.11.004>
60. Njimoh, D. L., Assob, J. C. N., Mokake, S. E., Nyhalah, D. J., Yinda, C. K., & Sandjon, B. (2015). Antimicrobial Activities of a Plethora of Medicinal Plant Extracts and Hydrolates against Human Pathogens and Their Potential to Reverse Antibiotic Resistance. *International Journal of Microbiolog*, 2015, 1–15. <https://doi.org/http://dx.doi.org/10.1155/2015/547156>
61. Olajuyigbe, O. O., & Afolayan, A. J. (2013). Evaluation of combination effects of ethanolic extract of *Ziziphus mucronata* Willd. subsp. *mucronata* Willd. and antibiotics against clinically important bacteria. *The Scientific World Journal*, 2013. <https://doi.org/10.1155/2013/769594>
62. Oliveira, A. A., Segovia, J. F. O., Sousa, V. Y. K., Mata, E. C. G., Gonçalves, M. C. A., Bezerra, R. M., Junior, P. O. M., & Kanzaki, L. I. B. (2013). Antimicrobial activity of amazonian medicinal plants. *SpringerPlus*, 1–6.
63. Othman, L., Sleiman, A., & Abdel-Massih, R. M. (2019). Antimicrobial activity of polyphenols and alkaloids in middle eastern plants. *Frontiers in Microbiology*, 10(MAY). <https://doi.org/10.3389/fmicb.2019.00911>
64. Oyeboode, O., Kandala, N. B., Chilton, P. J., & Lilford, R. J. (2016). Use of traditional medicine in middle-income countries: A WHO-SAGE study. *Health Policy and Planning*, 31(8), 984–991. <https://doi.org/10.1093/heapol/czw022>
65. Pandey, A. K., Yadav, S., & Sahu, S. K. (2011). Sustainable bark harvesting and phytochemical evaluation of alternative plant parts in *Holarrhena antidysenterica* R. Br. Sans (Kutaj). *International Journal of Green Pharmacy*, 5(2), 107–112. <https://doi.org/10.4103/0973-8258.85166>
66. Pang, Z., Raudonis, R., Glick, B. R., Lin, T. J., & Cheng, Z. (2019). Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. *Biotechnology Advances*, 37(1), 177–192. <https://doi.org/10.1016/j.biotechadv.2018.11.013>
67. Pujol, I., Guarro, J., Llop, C., Soler, L., & Fernández-Ballart, J. (1996). Comparison study of broth macrodilution and microdilution antifungal susceptibility tests for the filamentous fungi. *Antimicrobial Agents and Chemotherapy*, 40(9), 2106–2110. <https://doi.org/10.1128/aac.40.9.2106>
68. Qi, Z. (2015). The WHO traditional medicine strategy 2014–2023: Background and progress in the last decade. *Global Health History Seminar on Traditional Medicine and Ayurveda*, March, 1–28.
69. Reshmi, S. K., Aravinthan, K. M., & Devi, P. S. (2012). Antimicrobial activity of *Basella alba* fruit. *International Journal of Pharmaceutical Sciences and Research*, 3(12), 4757.
70. Roger, T., Pierre-marie, M., Igor, V. K., & Patrick, V. D. (2015). Phytochemical screening and antibacterial activity of medicinal plants used to treat typhoid fever in Bamboutos division, West Cameroon. *Journal of Applied Pharmaceutical Science Vol.*, 5(06), 34–49. <https://doi.org/10.7324/JAPS.2015.50606>
71. Rothman, J. M., Dierenfeld, E. S., Molina, D. O., Shaw, A. V, Hintz, H. F., & Pell, A. N. (2006). Nutritional chemistry of foods eaten by gorillas in Bwindi Impenetrable National Park, Uganda. *American Journal of Primatology: Official Journal of the American Society of Primatologists*, 68(7), 675–691.

72. Sakunpak, A., Sirikatitham, A., & Panichayupakaranant, P. (2009). alata extract and its stability Preparation of anthraquinone high-yielding Senna alata extract and its stability. *Pharmaceutical Biology*, 0209. <https://doi.org/10.1080/13880200802434757>
73. Samie, A., Tambani, T., Harshfield, E., Green, E., Ramalivhana, J. N., & Bessong, P. O. (2010). Antifungal activities of selected venda medicinal plants against candida albicans, Candida krusei and cryptococcus neoformans isolated from South African AIDS patients. *African Journal of Biotechnology*, 9(20), 2965–2976. <https://doi.org/10.5897/AJB2010.000-3129>
74. Seleem, D., Pardi, V., & Murata, R. M. (2017). Review of flavonoids: A diverse group of natural compounds with anti-Candida albicans activity in vitro. *Archives of Oral Biology*, 76, 76–83. <https://doi.org/https://doi.org/10.1016/j.archoralbio.2016.08.030>
75. Siddiquee, S. (2014). Recent Advancements on the Role and Analysis of Volatile Compounds (VOCs) from Trichoderma. In *Biotechnology and Biology of Trichoderma* (pp. 139–175). Elsevier. <https://doi.org/10.1016/B978-0-444-59576-8.00011-4>
76. Silva, A., & Nascimento, S. (2016). Antimicrobial activity and phytochemical analysis of organic extracts from Cleome spinosa Jacq. *Frontiers in Microbiology*, 7(JUN), 1–10. <https://doi.org/10.3389/fmicb.2016.00963>
77. Simoben, C. V., Ntie-Kang, F., Akone, S. H., & Sippl, W. (2018). Compounds from African Medicinal Plants with Activities Against Selected Parasitic Diseases: Schistosomiasis, Trypanosomiasis and Leishmaniasis. *Natural Products and Bioprospecting*, 8(3), 151–169. <https://doi.org/10.1007/s13659-018-0165-y>
78. Sparg, S. G., Light, M. E., & van Staden, J. (2004). Biological activities and distribution of plant saponins. *Journal of Ethnopharmacology*, 94(2–3), 219–243. <https://doi.org/10.1016/j.jep.2004.05.016>
79. Ssegawa, P., & Kasenene, J. M. (2007). Medicinal plant diversity and uses in the Sango bay area, Southern Uganda. *Journal of Ethnopharmacology*, 113(3), 521–540. <https://doi.org/10.1016/j.jep.2007.07.014>
80. Tiwari, P., Bimlesh, K., Kaur, M., Gurpreet, K., & Kau, H. (2011). Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Scientia*, 1(1).
81. Trentin, D. S., Silva, D. B., Amaral, M. W., Zimmer, K. R., Silva, M. V., Lopes, N. P., Giordani, R. B., & Macedo, A. J. (2013). Tannins Possessing Bacteriostatic Effect Impair Pseudomonas aeruginosa Adhesion and Biofilm Formation. *PLoS ONE*, 8(6). <https://doi.org/10.1371/journal.pone.0066257>
82. Truong, D. H., Nguyen, D. H., Ta, N. T. A., Bui, A. V., Do, T. H., & Nguyen, H. C. (2019). Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of severinia buxifolia. *Journal of Food Quality*, 2019. <https://doi.org/10.1155/2019/8178294>
83. Uboh, I., Ituen, A., Chidozie, E., & Uchendu, A. (2018). Phytochemical and Antibacterial Activities of Some Selected African Medicinal Plant Extracts on Methicillin-Resistant and Methicillin-Sensitive Staphylococcus Aureus. *Journal of Dental and Medical Sciences (IOSR-JDMS)*, 17(10), 72–78. <https://doi.org/10.9790/0853-1710017278>
84. Udeozo, I., Ejikeme, C. ., Eboatu, A. ., Arinze, R. ., & Kelle, H.. (2016). An Assay of Characteristics, Chemical Constituents and Functional Group Analysis of Cordia Milleni : A Tropical Timber. *International Journal of Life Sciences Research*, 4(2), 29–36.
85. Van Vuuren, S., & Viljoen, A. (2011). Plant-based antimicrobial studies methods and approaches to study the interaction between natural products. *Planta Medica*, 77(11), 1168–1182. <https://doi.org/10.1055/s-0030-1250736>
86. Wakeel, A., Jan, S. A., Ullah, I., Shinwari, Z. K., & Xu, M. (2019). Solvent polarity mediates phytochemical yield and antioxidant capacity of Isatis tinctoria. *PeerJ*, 2019(10), 1–19. <https://doi.org/10.7717/peerj.7857>
87. Wen, H., Kang, J., Li, D., Wen, W., Yang, F., Hu, H., & Liu, C. (2016). Antifungal Activities of Anthocyanins from Purple Sweet Potato in the Presence of Food Preservatives. *Food Science and Biotechnology*, 25(1), 165–171. <https://doi.org/10.1007/s10068-016-0025-7>
88. WHO. (2002). WHO Traditional Medicine Strategy 2002–2005. In *World Health Organisation Geneva*. World Health Organization. http://www.wpro.who.int/health_technology/book_who_traditional_medicine_strategy_2002_2005.pdf
89. World Health Organization. (2019). WHO Global report on traditional and complementary medicine 2019. In *World Health Organization*. <https://apps.who.int/iris/bitstream/handle/10665/312342/9789241515436-eng.pdf?ua=1>
90. Yesuf, A., & Asres, K. (2013). Wound healing and antiinflammatory properties of Allophylus abyssinicus (Hochst.) Radlk. *Phytopharmacology*, 4(2), 442–453.
91. Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules*, 21(5). <https://doi.org/10.3390/molecules21050559>

92. Zonyane, S., Van Vuuren, S. F., & Makunga, N. P. (2013). Antimicrobial interactions of Khoi-San poly-herbal remedies with emphasis on the combination; *Agathosma crenulata*, *Dodonaea viscosa* and *Eucalyptus globulus*. *Journal of Ethnopharmacology*, 148(1), 144–151. <https://doi.org/10.1016/j.jep.2013.04.003>

Figures

Figure 1

A Map of Uganda showing Location of Bwindi Impenetrable Forest National Park and plant Sample collection sites

Figure 2

Percentage Yield of Extracts.

Key: AASM:-Allophylus abyssinicus Stem Bark Methanol Extract, AASW:- Allophylus abyssinicus Stem Bark Water Extract, AALW:- Allophylus abyssinicus Leaf Water Extract, SGLW:-Symphonia globulifera Leaf Water extract, SGSM:-Symphonia globulifera Stem Bark Methanol extract, SGSW:- Symphonia globulifera Stem Bark Water extract, AALM:-Allophylus abyssinicus Leaf Methanol Extract, SGLM:-Symphonia globulifera Leaf Methanol extract, SGSE:- Symphonia globulifera Stem Bark Ethyl Acetate extract, SGLE:- Symphonia globulifera Leaf Ethyl Acetate extract, AASE:-Allophylus abyssinicus Stem Bark Ethyl Acetate Extract, AALE:- Allophylus abyssinicus Leaf Ethyl Acetate Extract