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Original Research

In Vitro Antibacterial Activity, Preliminary Phytochemical Screening Profile, and *in Vivo* Toxicity of Seven Traditional Medicinal Plants in Ethiopia

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Abstract

The continuous threat of antibiotic-resistance calls for novel antibacterial agents. This study was aimed at screening medicinal plants for their antibacterial properties, phytochemical content and safety. Leaves of *Allophylus abyssinicus* (Hochst.) Radlk., *Dicliptera laxata* C.B.Clarke, *Ligustrum vulgare* L., *Solanecio gigas* (Vatke) c. Jeffrey and *Gymnanthemum myrianthum* (Hook.f.) H.Rob.; leaf and stem-bark of *Olinia rochetiana* A. Juss. and the seed of *Cucurbita pepo* L. were used. Chloroform and ethanol were used to extract *G. myrianthum*, *D. laxata* and *O. rochetiana*; ethyl acetate and methanol for the rest, and water for all. The extracts were tested against clinical/standard strains of *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus* by the agar-diffusion method. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined. Acute toxicity to mice was checked and preliminary phytochemical screening was done. Thirteen extracts, out of 24, were active (inhibition zone >7 mm) at differing levels (9.67±0.33-25.66 ± 0.57 mm) against at least one bacterial strain. The MICs and MBCs were 1.95-15.6 mg/mL and 7.8-125 mg/mL respectively. The aqueous extract of *S. gigas*, methanol extracts of *L. vulgare* and *A. abyssinicus*, and ethanol extract of *O. rochetiana* leaf were the most active (MIC 1.95 mg/ml) against *S. aureu*s. Ethyl acetate extracts of *A. abyssinicus*, *L. vulgare* and *S. gigas*; aqueous of *C. pepo*, *O. rochetiana* and *G. myrianthum*; and all *D. laxata* had no antibacterial activity. *P. aeruginosa* was the least susceptible to any extract, although the methanol and aqueous extracts of *S. gigas* performed better against it. Preliminary phytochemical screening of selected extracts for phenols, flavonoids, tannins,

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steroids, terpenoids, steroidal glycosides, alkaloids, saponins, resins and glycosides showed positivity at least for four of these phytochemicals with glycoside and terpenoids in nearly all extracts and resin in none. The plants were not toxic to mice at 2000 mg/kg. Further consideration of *S. gigas*, *L. vulgare*, *A. abyssinicus* and *O. rochetiana* is recommended in light of their promising potential and safety.

Keywords: Antibiotic-resistance; Antibacterial agent; Minimum inhibitory concentration (MIC); Minimal bactericidal concentration (MBC); Agar-diffusion

Introduction

Infectious diseases remain a global health challenge with bacterial diseases constituting a major portion [1]. For various reasons, bacterial diseases continue to be disproportionately prevalent among the least-developed countries [2]. *Escherichia coli*, *Salmonella* species, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* stand among the most common causes of bacterial diseases in man [3]. Around 63% of children with persistent diarrhea in low- and middle-income countries are positive for invasive *E. coli* strains [4]. About 27 million cases and 200,000 deaths are recorded annually due to *S. typhi* enteric fever [5]. An estimated 9-10% of nosocomial infections are caused by *P. aeruginosa* [6]. Mortality from *P. aeruginosa* bacteremia remains high and there is a 33-61% mortality rate among patients with *P. aeruginosa* bacteremia [7]. It was also recognized that *P. aeruginosa* bacteremia is associated with higher mortality than other gram-negative bacteremias [8]. *S. aureus* is another nosocomial infection, which is a leading cause of bacteremia among the gram-positives. It causes endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections throughout the globe [9].

Currently, the control and management of bacterial diseases is challenged by the continuous emergence and rapid spread of drug-resistant strains. Although an estimated 23,000 and 25,000 additional deaths occur by antibiotic-resistant bacteria each year in the United States [10] and the European Union [11] respectively, the problem is matchlessly higher in the least-developed countries [12]. The World Health Organization (WHO) pathogen priority list categorized *P. aeruginosa, E. coli, S. aureus*, and *Salmonella* spp. as multidrug-resistant strains [13].

Thus, novel antibiotics are required to combat the threat of drug-resistance. Unless this objective is achieved urgently, the success history of containing bacterial diseases following the discovery of antibiotics can easily be reversed shortly. Screening traditional medicinal plants for the development of novel therapeutics is one option. Medicinal plants have been a source of many modern drugs as well as for traditional management of bacteria-related ailments throughout history. Currently, over 30% of modern pharmacological drugs are derived directly or indirectly from plants and their extracts [14].

The plants *Allophylus abyssinicus* (Hochst.) Radlk, *Cucurbita pepo* L., *Dicliptera laxata* (Vatke) c. Jeffrey, *Ligustrum vulgare* L., *Olin*

ia rochetiana A. Juss*, Solanecio gigas* (Vatke) c. Jeffrey and *Gymnanthemum myrianthum* (Hook.f.) are used to treat various ailments in Ethiopia and globally. In Ethiopia, beyond its role as a food source, *C. pepo* is used traditionally to treat abdominal pain in general. *A. abyssinicus* is used treat helminths, wounds, burns, skin diseases, and to stop bleeding in Ethiopian folk medicine [15]. *L. vulgare* leaf was well-known in Mediterranean historical medicine and has been used for the treatment of oropharyngeal inflammation or as an antirheumatic, diuretic, and hypotensive agent in folk medicine in southern Europe; and in Azerbaijan, the leaves are used to treat hypertension [16]. Moreover, water infusions from *L. vulgare* leaves have shown a high antimutagenic effect [17]. Several extracts of this plant have also been proven to act as a dual angiotensin-converting enzyme [18]. *G. myrianthum, O. rochetiana* and *D. laxata* are commonly used traditional medicines in Kambata and Hadiyya Zones of southern Ethiopia. The people in these areas reported that they use the leaf juice of *G. myrianthum* for healing wounds and blood clotting. *D. laxata* is used for the treatment of acne and skin allergies and *O. rochetiana* to treat toothache and as a toothbrush or mouth freshener.

However, there is little work on the bioactivity, phytochemical profile as well as acute toxicity of these plants in Ethiopia. Particularly, the safety margin of herbal medicines needs serious attention. Several authors addressed this subject critically [19-21]. Thus, this study aimed to investigate the antibacterial activity of the crude extracts of these plants against clinical/standard strains of *E. coli, P. aeruginosa, S. typhi* and *S. aureus,* and their acute oral toxicity in Swiss albino mice.

Methods

Plant materials

Matured fresh leaves of *S. gigas, A. abyssinicus*, and *L. vulgare* were collected from the premises of the College of Natural and Computational Sciences, Addis Ababa University (AAU). The leaves of *G. myrianthum*, *D. laxata*, and leaves and stem-bark of *O. rochetiana* were obtained from Hadiyya Zone, 241km to the southwest of Addis Ababa. The seed fruits of *C. pepo* were purchased from a local market (*Merkato*) in Addis Ababa. The plant materials were identified and authenticated by Mr. Melaku Wondaferash in the National Herbarium, Department of Plant Biology and Biodiversity Conservation (AAU), where voucher specimens were deposited as EM001, EM002, ENM001, ENM002, AE001, AE002, and AE003 for *C. pepo, S. gigas, A. abyssinicus, L. vulgare, O. rochetiana, G. myrianthum, and D. laxata* respectively.

Extract preparation

The plant materials were washed with tap water thrice and rinsed with sterile distilled water once, and allowed to air-dry, under shade, in the Biomedical Laboratory of the *Department of Microbial, Cellular and Molecular Biology* (AAU) at room temperature for two weeks. Each plant was powdered by an electric grinder and sieved (sieves with 500 μm opening) through a fine mesh to obtain a fine powder. The

powders were packed in glass bottles and were kept at room temperature until used for aqueous and solvent extract preparation [22].

Aqueous extract preparation

The powder of each plant material was soaked in distilled water (50 g powder to 500 mL distilled water - 1:10) in a 1000 mL Erlenmeyer flask and placed on a shaker (GFL, model 3020, Germany) rotating for 72 h at 120 rotations per minute at room temperature. The macerates were first filtered through a four-fold muslin cloth, and the supernatant was re-filtered using Watman №.1 filter paper (Whatman Ltd., England). The filtrates were kept in a deep freezer for 24 hours and then concentrated using a lyophilizer (CHRIST ALPHA1-4, Germany) at -40°C with vacuum pressure. The dry and concentrated extracts were placed in a bottle covered with aluminum foil at -20°C for further use.

Solvent extracts

Each plant material was soaked in 99.0% ethyl acetate (Joseph Mills (Denaturants) Ltd, UK), 99.8% methanol (Sigma-Aldrich, Germany), 97.0% ethanol (Sigma-Aldrich, Germany), or 99.9% chloroform (Honeywell Riedel-de Haen™, Germany) in 1:10 solute/solvent (w/v) ratio in 1000 mL Erlenmeyer flasks, and placed on a shaker (GFL, model 3020, Germany) shaking at 120 rotations per minute for 72 hours at room temperature. Ethyl acetate and methanol was used for *C. pepo, S. gigas, A. abyssinicus, L. vulgare*, and ethanol and chloroform for *O. rochetiana, G. myrianthum* and *D. laxata*. The macerates first filtered through a four-fold muslin cloth and the supernatants were filtered using Whatman № 1 filter paper (Whatman Ltd., England). The filtrates were concentrated in a rotary evaporator (BUCHI, Germany) at 45°C and 60 rotations per minute and the extracts were further allowed to fully dry in an oven (45°C). The yield of each extract was measured, the percentage yield was calculated, and finally preserved (-20°C) in a bottle covered with aluminum foil for future tests. Percent yield was calculated using equation 1 [22].

Equation 1.

Percentage extract yield = Weight of dry crude extract / Weight of dry powder sample × 100

Bacterial strains

The test involved standard American Type Culture Collection (ATCC) and clinical isolates of gram-negative and gram-positive bacterial strains. The standard strains were *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *S. aureu*s (ATCC 25923) and *S. typhi* (ATCC 13311), and the respective clinical isolates.

Inoculants preparation

The bacterial strains were streaked on the corresponding differential or selective media using an inoculating wire loop following aseptic conditions in a safety cabinet, and were incubated (37°C) for 18-24 h. The media used were Salmonella-Shigella (SS) agar (Himedia®, India) for *S. typhi*, mannitol salt agar (Himedia®, India) for *S. aureus*, Pseudomonas agar (Himedia®, India) for *P. aeruginosa*, and eosin methylene blue agar (Himedia®, India) for *E.*

coli. Saline solution bacterial suspensions were prepared using the direct suspension method in the following manner. Three to five colonies of each bacterium were picked up by a sterile wire loop from fresh agar plates of the respective culture, aseptically transferred into pre-labeled test tubes containing 5mL sterile 0.9% saline solution, vortexed and homogenized, and swabbed on an appropriate antimicrobial susceptibility test agar.

The bacterial turbidity of each culture was prepared and standardized following the guideline of *Clinical and Laboratory Standard Institute* [23]. The turbidity of the inoculum tube was adjusted visually with a white background and contrasting black lines in the presence of adequate light by adding either bacterial colonies or sterile normal saline to that of the already prepared 0.5 McFarland standard. McFarland standard [24] was prepared by adding a 0.5 mL aliquot of 0.48 mol/L dihydrated barium chloride - 1.175% w/v $BaCl_2.2H_2O$ (Himedia®, India) added to 99.5 mL of 0.18 mol/L H2SO4 (1% v/v) (Himedia®, India) which is assumed to contain a bacterial concentration of $1x10^8$ colony-forming unit (CFU) per mL.

Antibacterial susceptibility assay

Agar well diffusion

The inhibitory activities of the crude plant extracts were determined using the agar-well diffusion method [25]. Sterile Müller Hinton agar (Himedia®, India) plates were prepared by pouring 25 mL of molten media into 90 mm diameter sterile Petri-dish and plates were allowed to solidify for 5 min. Then, fresh inoculants of each bacterium were prepared as described above and using a sterile swab, the inoculum suspensions swabbed uniformly on the media and allowed to dry for 5 min.

Five equidistant wells were bored into the medium using a sterile 6mm diameter cork borer. Fresh inoculums of each bacterium uniformly swabbed on the medium using a sterile swab and allowed to dry for 5 min. Each three of the wells was filled with 80 µL (500 mg/mL) of one of the three specific extract types of the seven plants. Chloramphenicol (30 µg) (Addis Ababa Pharmaceutical, Addis Ababa, Ethiopia) and distilled water was loaded on the remaining two wells as positive and negative controls respectively. After loading the extracts, the plates were left undisturbed for 2 h at room temperature to give time for pre-diffusion. The plates were incubated for 24 h at 37°C, and inhibition zones measured with a ruler (in mm) including the diameter of the well. The experiment was performed in a triplicate for each extract and bacterial strain. This was a preliminary screening and intended to eliminate extracts/plants having no antibacterial activity at this extremely high concentration. Accordingly, the active extracts (zone of inhibition > 7 mm) were identified and concentration halved to have a stock solution of 250 mg/mL for minimum inhibitory concentration (MIC) determination.

Minimum inhibitory concentration (MIC)

The MIC of the extracts on the test bacteria strains were determined by the serial broth macrodilution method. The MIC was defined as

the lowest concentration of antimicrobial agent that was able to inhibit visible growth [26]. The stock solution (250 mg/mL) of extracts that showed antibacterial activity by agar diffusion subjected to twofold serial dilutions and different concentrations (125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.95, 0.97 and 0.48 mg/mL) prepared. The bacterial concentration was made to be approximately 1×10^6 CFU/mL by diluting the 0.5 McFarland standard turbidity equivalent bacterial suspensions in 1:150 ratio in Müller Hinton broth (MHB) (Himedia®, India) next to this within 15 min 1 mL of diluted bacterial suspension was added to each pre-labeled sterile test tubes except the negative control. The test tube containing only MHB was considered negative control and the tube containing MHB and inoculum was considered positive control. Then, all tubes were incubated (37°C) for 24 h, and the presence and absence of bacterial growth were checked by turbidity and the clear solution respectively. The average value of triplicate was taken as the MIC.

Minimum bactericidal concentration

Minimum bactericidal concentration (MBC) is the lowest concentration of an antimicrobial agent that completely killed the growth of the culture [27]. MBC was determined by sub-culturing loop full tests from last MIC results for each bacterium on nutrient agar and incubated at 37°C for 24 h. The least concentration that prevented the growth of the test bacteria was determined and the average of triplicates was taken as the MBC.

Ethics

The College of Natural and Computational Sciences Institutional Review Board (CNS-IRB), AAU granted ethical approval (Code: CNS-IRB/036/2019) for the toxicity study on mice. The animals were handled humanely including proper feeding and accommodation, and post-experiment euthanizing [28].

The experiment

The test was conducted following the *Organization for Economic Cooperation and Development* (OECD) Guideline 420 [29]. Female Swiss albino mice weighing 20-25 g and aged 6-8 weeks were used for this experiment. The mice were infection-free and drug or test naïve. The animals were randomly grouped into four groups each containing five mice. Before the experiment, the mice were acclimatized for one week. After acclimatization, the mice's weight was measured by a sensitive digital electronic beam balance (A & D Company, Japan). The mice were left to fast for three hours and then, administered orally in different concentrations of the plant extract solutions. The extracts that demonstrated more promising *in vitro* antibacterial activity were delivered orally based on the average bodyweight (BW) of the mice in each group, at doses of 1000, 1500, and 2000 mg/kg for three treatment groups. Distilled water was administrated for the control group. After oral administration, the animals were inspected within 30 min, 4 h, and after 24 h checking for signs of behavioral, neurological, and autonomic manifestations. The mice were further moni

tored for 14 days for any signs of acute toxicity. BW of the mice was further measured on days 7 (D7) and 14 (D14) to notice any extract-related effect. Percent average BW changes were calculated as [(BW on D14 − BW on D0)/BW on D0] × 100 [30,31]. The packed cell volume (PCV) of each mouse was measured before extract administration and on D14 post-administration. For this purpose, tail-blood samples were drawn from each mouse in a heparinized microhematocrit capillary tube up to 3/4th length. The tubes were sealed by sealer and placed in a microhematocrit centrifuge (Hawksley, England) with the sealed ends outwards. The samples were centrifuged at 12,000 rpm minute for 4 min. The PCV of the experimental mice was calculated using equation 2 [32].

Equation 2.
PCV= Volume of erythrocyte in a given blood/Total blood volume ×100

Preliminary qualitative phytochemical screening
Promising extracts (methanol and aqueous *S. gigas, A. abyssinicus* and *L. vulgare*; ethyl acetate and methanol *C. pepo*; ethanol and chloroform *G. myrianthum* and *O. rochetiana*) were qualitatively screened for the presence of alkaloids, flavonoids, glycoside, saponins, tannins, steroids, terpenoid, phenol [22].

Data analysis
The data were analyzed using SPSS software version 23 (IBM SPSS Statistics, United States). The results were presented as the mean ± standard error of the mean (SEM) and statistical significance was considered at 95% confidence interval ($p < 0.05$). The toxicity and antimicrobial susceptibility tests were compared using one-way ANOVA followed by the Duncan test.

Results

Extraction yield
The solvents used for the extraction of the different plants produced differing yields. The aqueous extract of *G. myrianthum* was the highest (18.0%) followed by *O. rochetiana* ethanol leaf extract (15.0%). The least yield (5.5%) was the chloroform extract of *O. rochetiana* stem-bark. Similarly, ethyl acetate, aqueous and methanol, respectively, yielded 4.7, 12.6, and 15.4% for *A. abyssinicus*; and 3.84, 11.35, and 13.8% for *L. vulgare* respectively. The highest yield was from the methanol extract and the least from ethyl acetate for both *S. gigas* and *C. pepo*. The methanol, ethyl acetate, and aqueous extract yields of *C. pepo* were 7.7, 10.2, and 6.2%, and that of the corresponding *S. gigas* was 11.8, 10.5, and 8.4% respectively (Table 1).

Table 1. Gram and percentage yield from crude extracts of *C. pepo*, *S. gigas*, *A. abyssinicus*, *L. vulgare*, *O. rochetiana*, *G. myrianthum* and *D. laxata*

Plant	Solvent	Dry powder (g)	Solvent (mL)	Yield (g)	Yield (%)
C. pepo	M	200	2000	15.30	7.65
	EA	200	2000	20.40	10.20
	A	200	2000	12.30	6.15
S. gigas	M	200	2000	23.50	11.75
	EA	200	2000	21.00	10.50
	A	200	2000	16.70	8.35

A. abyssinicus	M	200	1000	15.35	15.35
	EA	200	1000	4.69	4.69
	A	200	1000	12.63	12.63
L. vulgare	M	200	1000	13.78	13.78
	EA	200	1000	3.84	3.84
	A	200	1000	11.39	11.39
O. rochetiana leaf	E	100	1000	15.0	15.0
	C	100	1000	11.5	11.5
	A	100	1000	9.0	9.0
O. rochetiana stem-bark	E	100	1000	8.0	8.0
	C	100	1000	12.0	12.0
	A	100	1000	5.5	5.5
G. myrianthum	E	100	1000	14.0	14.0
	C	100	1000	18.0	18.0
	A	100	1000	8.0	8.0
D. laxata	E	100	1000	7.0	7.0
	C	100	1000	10.0	10.0
	A	100	1000	6.5	6.5

Abbreviations: M: methanol; EA: ethyl acetate; A: aqueous; E: ethanol; C: chloroform; mL: milliliter g: gram

Antibacterial activity

Overall, 13 extracts out of 24 from seven plant species were active (inhibition zone > 7 mm) at differing levels (9.67 ± 0.33 - 25.66 ± 0.57 mm) against at least one bacterial strain. Among these active extracts, the highest inhibition zone was recorded for *O. rochetiana* leaf chloroform extract against *S. typhi* standard strain and the lowest for ethyl acetate extract of *S. gigas* against the clinical strain of the same bacterial species. Some of the activity differences between the extracts against a particular bacterial strain were statistically significant others not (Table 2). Five extracts inhibited all test bacteria. These were the aqueous and methanol extracts of *S. gigas*; chloroform and ethanol of *O. rochetiana* leaf and ethanol of *O. rochetiana* stem-bark. *S. gigas* seemed to have the broadest activity as all its extracts were active against all the bacteria except its ethyl acetate extract against *E. coli* which was also active to some extent but below the cutoff and nil to *P. aeruginosa*. No extract could produce a higher than or even comparable inhibition zone to that of the positive control (chloramphenicol). The only comparable value was chloramphenicol activity against the standard strain of *P. aeruginosa*. In all cases, the activity of the positive control was significantly higher than any extract.

*S. aureu*s was the most inhibited bacterium by a wide range of extracts (13 extracts) followed by *S. typhi* [12], *E. coli* [11], and *P. aeruginosa* the

least [5]. Furthermore, it was against *S. aureus* that most extracts had their highest values with respect to the magnitude of the inhibition zone. The least was *P. aeruginosa*, which was not inhibited by at least six extracts that inhibited all the other test bacteria.

Table 2. Mean bacterial growth inhibition zones (mm) in the agar well diffusion method treated with plant extracts

Plant	Sol	*Bacteria*							
		E. coli		*S. typhi*		*S. aureus*		*P. aeruginosa*	
		Stan	Clin	Stan	Clin	Stan	Clin	Stan	Clin
C. pepo	A	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
	EA	14.67±0.33^{c}	13.33±0.33^{c}	17.67±0.33^{c}	16.33±0.66^{c}	20.33±0.33^{c}	19.33±0.33^{c}	00.00±0.00	00.00±0.00
	M	17.33±0.33^{b}	15.67±0.33^{b}	19.67±0.33^{b}	18.67±0.33^{b}	22.33±0.33^{b}	20.67±0.33^{b}	00.00±0.00	00.00±0.00
S. gigas	A	16.67±0.33^{b}	15.67±0.33^{b}	22±0.57^{b}	20.67±0.33^{b}	21.67±0.33^{b}	19.67±0.33^{c}	14.67±0.33^{b}	12.67±0.33^{b}
	EA	6.33±0.33^{d}	5.33±0.33^{d}	11±0.57^{d}	9.67±0.33^{d}	11.33±0.66^{d}	10.33±0.33^{d}	00.00±0.00	00.00±0.00
	M	17±0.57^{b}	15.67±0.88^{b}	22.33±0.33^{b}	21.33±0.33^{b}	22 ±1.15^{b}	21±0.577^{b}	15.67±0.33^{a}	14.33±0.33^{c}
L. vulgare	A	16.33±0.57^{b}	14.66±0.57^{b}	21.33±0.57^{b}	20.66±0.57^{b}	20.33±0.57^{b}	19.33±0.57^{b}	00.00±0.00	00.00±0.00
	EA	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
	M	16.66±0.57^{b}	15±1.0^{b}	20.66±0.57^{b}	19.66±1.52^{b}	22±0.00^{b}	21±00^{b}	00.00±0.00	00.00±0.00
G. myrianthum	A	00.00±0.00	00.00±0.00	00.00±0.00	-	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
	E	00.00±0.00	00.00±0.00	00.00±0.00	-	21.00±1.73^{b}	22.00±0.00^{b}	00.00±0.00	00.00±0.00
	C	00.00±0.00	00.00±0.00	00.00±0.00	-	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
O. rochetiana leaf*	A	00.00±0.00	00.00±0.00	00.00±0.00	-	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
	E	24.33±0.57^{e}	21.66±2.88^{e}	25.66±0.57^{e}	-	24.33±1.15^{b}	22.66±1.15^{b}	20.66±1.15^{a}	20.33±0.57^{d}
	C	25.33±0.57^{e}	20.33±0.57^{e}	23.00±1.00^{e}	-	20.00±1.00^{c}	22.66±2.51^{b}	18.00±0.00^{b}	19.00±1.73^{d}
O. rochetiana stem-bark*	A	00.00±0.00	00.00±0.00	00.00±0.00	-	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
	E	20.66±1.15^{f}	18.00±0.00^{e}	23.33±2.08^{e}	-	19.33±0.57^{e}	19.00±1.08^{e}	20.66±1.15^{e}	18.00±0.00^{d}
	C	00.00±0.00	00.00±0.00	00.00±0.00	-	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
Chloramph		31.33±1.15^{a}	29.33±1.15^{a}	30.66±1.15^{a}	-	31.33±1.52^{a}	31.66±1.52^{a}	21.66±3.05^{a}	31.33±1.52^{a}

Abbreviations: A: aqueous, C: chloroform, Clin: clinical, Chloramph: chloramphenicol: EA: ethyl acetate, E: ethanol, M: methanol, Sol: extraction solvent, Stan: Standard, SEM: standard error of the mean. Treatment means in the same column having the same superscript have no significant difference; *Not tested against clinical *S. typhi* strain.

No activity was observed for ethyl acetate extract of *A. abyssinicus* and *L. vulgare*; aqueous extracts of *C. pepo* and *O. rochetiana* (both leaf and bark) and *G. myrianthum* (all except the ethanol extract against *S. aureus*). Similarly, none of the extracts of *D. laxata* was potent against any of the test bacteria.

The MIC and MBC values for the screened and selected extracts ranged from 1.95-15.6 (at most 7.8) and 7.8-125 mg/mL, respectively. The extracts with the best performance, having the lowest MIC value (1.95 mg/mL), were *S. gigas* aqueous, *L. vulgare* methanol, *A. abyssinicus* methanol and *O. rochetiana* leaf ethanol extract (Table 3). The most susceptible bacterium, against which the lowest MIC value was obtained, also appeared to be *S. aureus*. The highest MIC value for the different extracts against this bacterium was 3.9 mg/mL except for the ethyl acetate extract of *C. pepo*, which was 7.8 mg/mL.

Table 3. MIC and MBC (mg/mL) values of crude extracts of different plants tested against pathogenic bacteria in the broth dilution method

Plant species		Bacterial strain															
		E. coli				*S. typhi*				*S. aureus*				*P. aeruginosa*			
	S	Stan		Clin		Stan		Clin		Stan		Clin		Stan		Clin	
		MI	MB	MI	MB	MI	MB	MI	MB	MI	MB	MI	MB	MI	MB	MI	MB
C. pepo	EA	15.6	125.0	15.6	125.0	15.6	62.5	15.6	62.5	7.8	31.25	7.8	31.25	NA	NA	NA	NA
	M	15.6	62.59	15.6	62.59	7.8	62.5	15.6	62.5	3.9	31.25	3.9	31.25	NA	NA	NA	NA
S. gigas	A	15.6	62.5	15.6	62.59	15.6	31.25	15.6	31.25	1.95	31.25	1.95	31.25	15.6	31.25	15.6	62.5
	M	7.8	62.5	7.8	62.59	3.9	62.5	3.9	62.5	3.9	31.25	3.9	31.25	7.8	31.25	15.6	62.5
A. abyssinicus	A	7.8	31.25	7.8	31.25	7.8	31.25	7.8	31.25	3.9	15.6	3.9	15.6	NA	NA	NA	NA
	M	3.9	31.25	15.6	31.25	3.9	15.6	3.9	15.6	1.95	7.8	1.95	7.8	NA	NA	NA	NA
L. vulgare	A	7.8	62.5	15.6	62.5	7.8	62.5	15.6	62.5	3.9	62.5	3.9	31.25	NA	NA	NA	NA
	M	7.8	31.25	7.8	31.25	3.9	31.25	3.9	31.25	1.95	31.25	1.95	15.6	NA	NA	NA	NA
O. rocheti-ana leaf*	E	3.9	62.5	3.9	62.5	3.9	62.5	NA	NA	1.95	31.25	1.95	31.25	7.8	62.5	15.6	62.5
	C	7.8	62.5	7.8	62.5	7.8	62.5	NA	NA	3.9	62.5	3.9	62.5	15.6	125.0	7.8	125.0
O. rocheti-ana stem-bark*	E	7.8	62.5	7.8	62.5	7.8	62.5	NA	NA	3.9	62.5	3.9	62.5	15.6	125.0	15.6	125.0
*G. myrian-thum**	E	NA	NA	NA	NA	NA	NA	NA	NA	31.25	125.0	31.25	125.0	NA	NA	NA	NA

Abbreviations: A: aqueous, C: chloroform, Clin: clinical, EA: ethyl acetate, E: ethanol, M: methanol, MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration, S: solvent, Stan: Standard, *No test done for *S. typhi* clinical strains; NA: Not applicable

Acute toxicity

None of the plant extracts showed acute toxicity in mice. This was demonstrated by the absence of any behavioral and physical changes among the mice. The experimental mice did not show any clinical symptoms of toxicity including hair erection, loss of appetite, restlessness within 30 min, 4 h and 24 h post oral administration of methanol/ethanol crude extracts. No mortality was found among the mice at the dose of 2000 mg/kg. After 14 days, the mice did not show any significant BW reduction compared to the control group (Table 4).

Table 4. Effect of different plant crude extracts on bodyweight of experimental mice

Plant species			Mean bodyweight ± SEM			
	S/control	Dose (mg/kg)	D0	D7	D14	% Change b/n D14 and D0
S. gigas	A	1000	24.66±0.83	26.98±0.78	30.44±0.71	23.43
		1500	25.06±0.34	27.66±0.48	31.3±0.83	24.90
		2000	22.00±0.64	26.3±0.45	28.2±0.50	28.18
	Control		24.86±0.64	27.24±0.5	31.54±0.32	26.87
A. abyssinicus	M	1000	24.56±0.80	27.14±0.71	30.24±0.79	24.12
		1500	24.74±0.45	27.24±0.86	30.70±0.79	24.09
		2000	25.06±1.00	27.30±1.05	30.64±0.58	22.26
	Control		24.46±0.72	27.24±0.81	30.74±0.66	25.67
L. vulgare	M	1000	24.66±1.26	26.98±0.90	30.44±1.36	23.43
		1500	25.06±0.98	27.66±0.95	31.28±0.6	24.82
		2000	24.84±1.18	27.24±1.05	30.54±0.66	22.94
	Control		24.62±1.08	27.28±0.77	30.96±1.21	25.75

O. rochetiana leaf	E	2000	24.50±0.60	27.00±0.80	30.80±0.71	25.71
	C	2000	26.30 ±0.40	28.20±0.90	32.50±0.85	23.57
	Control		26.80±0.60	28.00 ±0.80	33.70±0.90	25.74

Abbreviations: A: aqueous, M: methanol, E: ethanol, C: chloroform, SEM: standard error of the mean, S: solvent, D0: first day, D7: day seven, D14: day fourteen, b/n: between

Although there were reductions in the mean PCV of the experimental mice after the administration of the extracts, the difference was not statistically significant (Table 5).

Table 5. Effect of different plant crude extracts on packed cell volume (PCV) of experimental mice

Plant species	Dose (mg/kg)	Mean PCV ± SEM		
		D0	D14	% Change (D14-D0)
S. gigas	1000	50.23±0.29	47.94±0.42	- 4.55
	1500	48.76±0.3	45.6±0.25	-6.48
	2000	51.8±0.31	48.2±0.51	-6.94
	Control	50.16±0.22	47.92±0.5	-4.46
A. abyssinicus	1000	51.66±1.17	51.66±1.17	-6.07
	1500	49.74±1.46	46.38±1.14	-6.75
	2000	48.68±2.30	44.12±2.6	-9.36
	Control	50.23±1.30	47.18±1.6	-6.00
L. vulgare	1000	49.24±1.28	46.8±1.8	-4.95
	1500	48.12±1.21	45.58±0.65	-5.27
	2000	49.26±1.72	46.3±0.58	-6.00
		48.04±1.36	45.76±1.36	-4.70

PCV: packed cell volume, D0: first day, D14: day fourteen, SEM: standard error of the mean

Preliminary phytochemical screening

The selected extracts were screened for ten phytochemical classes: phenol, flavonoid, tannin, steroids, terpenoids, a steroidal glycoside, alkaloid, saponin, resin, and glycoside. The extracts were positive at least for four of these phytochemicals. The methanol extracts of *A. abyssinicus* and *L. vulgare* tested positive for all target phytochemicals except resin. Glycoside and terpenoid were detected in all except the ethanol extract of *O. rochetiana* leaf and aqueous of *A. abyssinicus* respectively. On the other hand, no extract turned out positive for resin (Table 6). No preliminary phytochemical screening was done for those extracts with apparently little or no antibacterial activity.

Table 6. Preliminary phytochemical screening profile of the different plant extracts of various solvents

Phytochemical	*Cp*		*Sg*		*Aa*		*Lv*		*Or**		*Or*+		*Gm*	
	M	EA	M	A	M	A	M	A	E	C	E	C	E	C
Phenol	-	-	x	x	x	x	x	x	x	-	x	-	-	-
Flavonoid	x	x	x	x	x	x	x	x	-	-	-	-	x	x
Tannin	x	-	-	-	x	x	x	x	x	x	x	x	-	-

Steroids	x	-	-	x	x	x	x	x	x	x	x	x	-	-
Terpenoids	x	x	x	x	x	-	x	x	x	x	x	x	x	x
St glyc	x	-	x	x	x	-	x	-	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*
Alkaloids	x	x	x	x	x	x	x	x	-	-	-	-	x	x
Saponin	x	x	x	x	x	x	x	x	-	-	-	x	-	x
Resin	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glycoside	x	x	x	x	x	x	x	x	-	x	x	x	x	x

Abbreviations: x: detected; -: not detected, St glyc: steroidal glycoside, *leaf, +stem-bark, Cp: *C. pepo*, Sg: *S. gigas*, Aa: *A. abyssinicus*, Lv: *L. vulgare*, Or: *O. rochetiana*, Gm: *G. myrianthum*, M: methanol, EA: ethyl acetate, A: aqueous, E: ethanol, C: chloroform, nc: not checked

Discussion

From the data, it is possible to conclude that the leaf of *G. myrianthum* contained more polar compounds compared to *O. rochetiana* and *D. laxata*. Similarly, the highest (15.0%) and the lowest (8.0%) yields were obtained from ethanol extracts of *O. rochetiana* leaf and stem-bark respectively, showing that more polar compounds exist in the leaf than stem-bark of *O. rochetiana*. Extraction yield is primarily a measure of solvent efficiency to extract specific components from the original material. Water dissolved relatively more bioactive compounds from the leaf and stem-bark of the plants. In general, the yield obtained from these plants is reasonable, and considering them for antibacterial research appears economically feasible.

Methanol dissolved more active components of *A. abyssinicus* and *L. vulgare* than either ethyl acetate or water. Similarly, a relatively higher yield was obtained from *S. gigas* methanol extract and the minimum from aqueous extract of *C. pepo*. From this, it is possible to conclude that methanol could dissolve more components of these plants. The nature of crude extracts was also different from solvent to solvent. Ethyl acetate and methanol crude extracts of the two plants were moist semisolid while the aqueous extracts were freeze-dried powder.

The ethanol leaf extract of other species of the genus *Vernonia* (now *Gymnanthemum*), *V (G). amygdalin*, is positive for alkaloid, saponin, and tannin but lacked flavonoids, steroids, phenols and terpenoids [33]. Preliminary phytochemical screening of another species of the same genus, *V. auriculifera*, was positive for saponins, tannins, alkaloids, flavonoids, terpenoids, and phenolic compounds [34]. These two reports are in agreement with the phytochemical content of *G. myrianthum* noted in the current study. The detection of these phytochemicals in *G. myrianthum* may justify its use in traditional medicine for healing wounds and stopping bleeding as claimed by the local people. The presence of saponins, phenols, and alkaloids could confer antibiotic properties on the plant. The antimicrobial tendency of each of these phytochemicals, in general, has been extensively studied [35-37]. Eugenol, caffeic acid, catechol, and pyrogallol had antibacterial and antifungal effects through the reaction of sulfhydryl groups or more non-specific reactions with proteins is thought to be the possible mechanism for phenolic effect on microorganisms [38].

Flavonoids and flavonoid-derived phytochemicals are antimicrobial agents against a broad spectrum of microorganisms [39]. Intake of tannin comprising beverages, particularly green teas, suggested healing or precluding varieties of microbial infections [36]. Tannins can cause complexes of proteins through hydrogen bonding, hydrophobic effects, and the formation of covalent bonds. A review of the antimicrobial properties of tannins indicated that they inhibit growth and protease activity in many luminal bacteria. It is reported that tannins bind to cell coat polymers in several strains of bacteria [35]. They also cause morphological changes in the organisms indicating that the cell wall is the main target of tannin toxicity [39]. Terpenoids and essential oils are other groups of compounds reported to have antimicrobial activities. Studies indicate that terpenes and terpenoids were active against bacteria and fungi [40]. The diterpenoids and sesquiterpenes obtained from *Salvia sclarea* were active against *S. aureus* and *Candida albicans*. Two terpenoid constituents, capsaicin and petalostemumol, showed excellent activity against various strains of bacteria and fungi [40].

Whereas the detection of tannins and saponins in the methanol extract of *C. pepo* seed in this study agrees with Chonoko and Rufai 2011 [41] that alkaloids contradict. The presence of flavonoids, tannins, alkaloids, steroids, saponins, and glycosides in *C. pepo* seed extract in this study is in line with other similar studies [42].

Except for a single study [43] that reported the presence of a high amount of alkaloids in *S. gigas*, little data is available on the leaf phytochemical constituents of this plant. A different species in the genus, *S. biafae*, has tannins, saponins, flavonoids, alkaloids, and glycosides [44] corroborating the current study.

As reported by Tadege et al. [45], the methanol extract of *O. rochetiana* inhibited *S. aureus*, *E. coli*, and *P. aeruginosa* well. In another study, a comparable result was recorded for the methanol extract of this plant on clinical and standard strains of *S. aureus*, *P. aeruginosa* and *E. coli* [46]. The findings are in agreement with the current study as both ethanol and chloroform extracts of the plant performed against these same bacterial strains.

Although there is no published data on the antimicrobial activity of *A. abyssinicus* leaf in particular and the plant's other parts in general, the current result is comparable to other similar studies on another species of the same genus, *A. cobbe*, in which aqueous extracts had lower antimicrobial activity compared to methanol extracts [47]. The data indicated that both the methanol and aqueous extracts of this plant were ineffective against both clinical and standard strains of *P. aeruginosa.*

Ethyl acetate extract of *A. abyssinicus* was ineffective against all test organisms and the reason may be due to the inefficacy of the solvent to dissolve secondary metabolites. In line with this study, *S. aureus* and *E. coli* were susceptible to the methanol extracts of *S. oleo* seed of the same family [48]. Antibacterial activity of the same genus *A. cobbe* on the test organisms might be due to the availability of alkaloid, saponin, flavonoid, terpenoid, and tannin that demonstrated strong antimicrobial effect [47].

Ethyl acetate extract of *L. vulgare* leaf was also ineffective against all test organisms probably for the same reason suggested above for the *A. abyssinicus* extract.

The positive control chloramphenicol showed significantly higher inhibitory activity against all bacterial strains than that of all active extracts of all plants. *P. aeruginosa* exhibited a considerable level of resistance to chloramphenicol in other studies [48].

The higher antibacterial activity of *C. pepo* methanol extract compared to the ethyl acetate, and impotence of the aqueous extract demonstrated in this study are similar to a study by Ibrahim et al. 2010 [49]. The current data also confirms and extends the work of Abd EI-Aziz and Abd EI-Kalek 2011 [50] where *C. moschata* seed methanol extract inhibited *S. aureus* and *E. coli* but not *P. aeruginosa.* Moreover, the susceptibility of *S. aureus* and *S. typhi* to the methanol extracts of *C. pepo* was documented [41]. Antibacterial activity of *C. pepo* is because of the presence of various phytochemicals including terpenoids, flavonoids, and tannins that are known for their antimicrobial effect [51].

There is little information on the antimicrobial activity of *S. gigas* directly. Nevertheless, *S. biafae*, a species in the same genus, exhibited fair antibacterial activity against *S. aureus* and *E. coli* [44]. Most of the phytochemicals detected in *S. gigas* leaf have antibacterial, anti-malarial, anti-inflammatory and antioxidant activities [52-53]. Thus, it is possible to conclude that *S. gigas* leaf extracts can be considered as a potential source for antibacterial agent search.

As noted by Tadege et al. 2005 [45], 80% of methanol crude extract of *O. rochetiana* leaf exhibited MIC values on *S. aureus* 5mg/mL, *P. aeruginosa* 2.5 mg/mL and *E. coli* 10mg/ml. Muguweru et al. 2016 [54] reported that the MIC values of *O. usambarensis* extract against *S. typi, E. coli* and *P. aeruginosa* were 50 mg/mL, 6.25 mg/mL and 250 mg/mL respectively. The same study reported MBC values against the same bacteria were 75 mg/mL, 12 mg/mL, and 75 mg/mL respectively. Compared with the current study, the MIC against *S. aureus* and *E. coli* was higher but that against *P. aeruginosa* was lower.

The plants tested for their acute toxicity in the mice were safe. This is promising as phytochemicals, in general, are potential sources of toxins some of which are associated with the occurrence of more serious diseases such as cancer [55]. Studies on the safety profile of the plants are scarce severely limiting comparative discussion of the current findings. Thus, further acute, as well as chronic toxicity studies, are required to establish the safety of the plants, and then to discourage or recommend their use in traditional medicine and consider them as potential sources of antimicrobial agents.

Conclusions

S. aureus was the most inhibited bacterium by all extracts, probably because of its unique outer membrane. It is gram-positive which makes it more susceptible to the extract than the gram-negative strains. The gram-negative *P. aeruginosa* exhibited a considerable level of resistance to most otherwise active extracts.

The relatively higher inhibitory effect of methanol or ethanol extracts suggests the power of the solvents in extracting secondary metabolites responsible for the antibacterial property. The apparent discrepancy between different *in vitro* plant crude extract antimicrobial activity studies with the current one may be due to differences in the specific procedures followed, solvents used, and agro-ecology of the plants and their age or season of collection. The bacterial strains tested by the different authors may also account for the variations of the data. The plants particularly those that showed relatively superior antibacterial activity could be further investigated for identification of lead compound(s) as an antibacterial agent(s) in light of their more promising potential and safety.

Conflict of Interest

None.

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