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Research Article
Antimicrobial Activities of *Vernonia amygdalina* Del and *Prunus africana* Extracts against Multidrug Resistant Clinical Strains

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Abstract

Background and Objective: Many plant-derived compounds have been used as drugs, either in their original or semi-synthetic forms. Plant derived metabolites can also serve as a lead compounds, which may be used as templates for the development of new drugs. Therefore, the aim of this study was to evaluate the antimicrobial activities of solvent fractions of *Vernonia amygdalina* shoot apex and *Prunus africana* bark against multidrug resistant bacterial strains. **Materials and Methods:** The plant materials were obtained through successive extractions using solvents of different polarity such as petroleum ether, chloroform, acetone and methanol. The antibacterial activities of the fractions were then evaluated by the hole-agar-well diffusion method. The minimum inhibitory concentration of the solvent fraction was determined against the isolated microorganism by agar dilution method. Then, the data were analyzed using Statistical Package for Social Science (SPSS) version 16. $p<0.05$ based on one-way ANOVA was used to indicate statistically significant differences. **Results:** All the *Prunus africana* bark solvent fractions showed antimicrobial activities which were found to exhibit significant antimicrobial activity differences among fractions against the clinical strains. The methanol extract demonstrated the strongest activities against the majority of clinical strains, whereas the acetone extract of *Prunus aricana* was the most effective with minimal inhibitory concentration of 0.65 mg mL^{-1} against *Citrobacter fruindi* and *Staphylococcus pyogenes*. On the other hand, the methanol extract of *Vernonia amygdalina* shoot apex, inhibited majority of strains at tested concentrations while other solvent fractions had limited antimicrobial activities. **Conclusion:** In general, *Prunus africana* extracts exhibited more antimicrobial activities than *Vernonia amygdalina* extracts. Comparatively, the methanol fraction of the plant showed the strongest antibacterial activities mainly against *E. coli*, *C. koseri*, *E. aerogenes*, *S. aureus* and *E. cloacae* clinical strains.

Key words: Antimicrobial activity, solvent fraction, *Vernonia amygdalina*, *Prunus africana*, multidrug resistance

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antimicrobial drug resistance has received increased attention from several international bodies and is more generally recognized as a threat to global health[1]. Moreover, many of the bacterial pathogens associated with epidemics of human disease have evolved into multidrug-resistant (MDR) forms subsequent to antibiotic use, which render therapy more precarious, costly and sometimes unsuccessful[2,3]. Therefore, appropriate measures should be taken through a comprehensive approach to minimize drug resistance. Moreover, a search for new drugs should be carried out incessantly to overcome this precarious public problem[4].

Numerous methods have been utilized to acquire compounds for drug discovery, including isolation from plants and other natural sources, synthetic chemistry, combinatorial chemistry and molecular modeling[5,6]. From the history of drug development, it is evident that many drugs have been derived from medicinal plants[7]. Many plant-derived compounds have been used as drugs, either in their original or semi-synthetic forms. Plant derived metabolites can also serve as a lead, which may be used as templates for the development of new drugs[8]. Moreover, the demand of drugs from plants has increased in recent times, as many plants or herbs are scientifically proven to contain bioactive compounds[9].

However, there are still a number of medicinal plants in which their activities are not yet confirmed scientifically even though they are traditionally used by the local communities[10]. The present study involved *Vernonia amygdalina* Del shoot apex and *Prunus africana* bark which are grown and used traditionally for treatment of different diseases in Ethiopia.

Vernonia amygdalina commonly called bitter leaf is a perennial shrub belonging to the family Asteraceae[11]. It is a small shrub that grows in the tropical Africa with a petiolate leaf of about 6 mm diameter and elliptic shape[12]. Traditionally, this plant is used for treatment of stomach disorder, skin wound, swelling, diarrhea, scabies, hepatitis, ascarasis, tonsillitis, fever, mastitis, tapeworm and worms infection[13-17]. On the other hand, *Prunus africana* belongs to the Rosaceae family. It is a widespread evergreen tree, growing at an altitude of 1500-2000 m, usually 10-25 m high with alternate leaves and small white or cream fragrant flowers[18]. *Prunus africana* is employed to treat various diseases such as fever, stomach pain, kidney disease, urinary symptoms, diarrhea, wound and hemorrhoids[14,15,19].

Thus, the present study was aimed to evaluate antimicrobial activities of different solvent extracts of these medicinal plants against antibiotic resistant clinical isolates.

MATERIALS AND METHODS

Chemicals and solvents: Petroleum ether, chloroform, acetone, methanol, ethanol, dimethyl sulphoxide (DMSO), Mueller Hinton agar, nutrient agar, nutrient broth and standard antibiotic discs were used. Analytical grade reagents/chemicals were also used in this experiment.

Collection and identification of plant materials: Fresh plant materials of *Prunus africana* bark (Rosaceae) and *Vernonia amygdalina* Del shoot apex (Asteraceae) were collected from Ilu-Ababor zone, Didesa district, South west Ethiopia in March, 2015. The collected plants were identified and the specimens were deposited with voucher specimens (No. DK24 and DK35) in the natural herbarium, Department of Biology, Addis Ababa University.

Preparation of plant fractions: The plant materials were air dried under shade at room temperature. The dried plant materials were separately powdered and sequentially extracted using different solvents (pet-ether, chloroform, acetone and methanol). Then the solvents were removed by evaporation using Rotavapor at no more than 40°C. The resulting dried masses were packed and stored in desiccators.

Test microorganisms: The multi-drug resistant strains isolated from clinical samples of patients from Jimma University Teaching Referral Hospital such as *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Enterobacter cloacae*, *Citrobacter fruindi*, *Klebsela pneumonia*, *Enterobacter* spp., *Citrobacter koseri*, *Enterobacter aerogenes* and *Staphyloccus pyogenes* were used.

Antibiotic sensitivity test: An antibiogram was generated by a disk diffusion method in Mueller-Hinton (MH) agar with commonly used antibiotics[20].

Antimicrobial activity test: The antibacterial activity test of the crude plant extracts was carried out by the agar-well diffusion method[21]. Accordingly, 0.2 mL of the standardized inoculums (0.5 Mc Far land standard turbidity) was mixed with 20 mL of sterile Mueller Hinton agar (maintained at 45°C in a molten state) and then poured into sterilized petri dishes and set aside. The seeded agar was punched out with a sterile hole borer at specified positions to make holes (8 millimeter in diameter). The holes were filled with 0.1 mL of the test sample solution (concentrations of 50 mg mL^{-1}) of the extracts, while

the fourth with 0.1 mL of 1% of the solvent (used to dissolve extracts). The antibacterial activity was evaluated by measuring the diameter of the zone of inhibition (including the diameter of holes).

Determination of minimum inhibitory concentration: The MIC of the solvent extracts was determined against the selected microorganism by agar dilution method[22]. Dilutions of the extract were prepared in 1% dimethyl sulfoxide, which has been confirmed to be devoid of antimicrobial activity against the test organisms. Two fold dilutions of extracts were prepared and 2 mL aliquots of various concentrations of the solution were added to 18 mL presterilized Mueller Hinton agar at the 50°C to produce a final concentrations ranging from 20-0.312 mg mL^{-1} which was then poured into pre-labeled sterile petri dishes on a level surface. Additional petri dishes containing only the growth media were prepared in the same way in order to serve for comparison of the growth of the respective organisms. The lowest concentration which inhibited the growth of the respective organisms was taken as MIC.

Ethical considerations: The research project was reviewed and approved by research ethics committee of Jimma University. Informed consent of the study participants was taken part in the study voluntarily after adequate explanation about the purpose, importance and potential discomforts of the study.

Data analysis: The data were analyzed using Statistical Package for Social Science (SPSS) version 16. The data were interpreted based on the standard interpretive results of inhibition zone diameter (mm) of extracts and drugs for each bacterial isolate. $p<0.05$ based on one-way ANOVA was used to indicate statistically significant differences and the results were presented using tables[23].

RESULTS AND DISCUSSION

Most clinical strains were resistant to more than 2 antimicrobial agents. The most sensitive bacteria was *E. aerogenes* while the most resistant microbes were *E. cloacae* and *S. aureus* against a number of drugs (Table 1). This finding could be an evidence for evolvement of multidrug resistant strains (MDR) forms which renders the treatment difficult and urges for the development of new drugs[2,4].

All the *Prunus africana* bark solvent extracts exhibited antimicrobial activities against various gram positive and gram negative multi drug resistant clinical strains. There is a significant difference among the activities of fractions against the microbial strains ($p<0.05$). The methanol fraction significantly ($p<0.05$) inhibited microbial strains such as *E. coli*, *C. koseri*, *E. aerogenes*, *S. aureus* and *E. cloacae* as compared with other solvent fractions. On the other hand, the acetone extract exhibited significant activities against *P. mirabilis*. In contrary of this, petroleum ether extract demonstrated the least activity towards the majority of strains except against *E. coli* strains where in the methanol, ether and acetone extracts exhibited similar antimicrobial activities (Table 2). In line with this finding, the antimicrobial activities of *Prunus africana* extracts against some microbial strains were reported in previous studies[24-26]. Moreover, it was reported that the plant extracts contain various secondary metabolites such as tannins, saponins, flavonoids, terpenoids, alkaloids and phenols[25,27,28]. Therefore, the antimicrobial activities of the extracts could be due to these constituents as different researchers explored that these metabolites possess antimicrobial activities: Phenols and falovonoids[29]; alkaloids[30,31]; tannins, terpenoids and phenols[32].

Table 1: Multidrug resistance pattern of clinical isolates

Microbial strains	Phenotype resistance
E. coli	A Ax Ag Co Am Ctr Ctz Dx Pi
C. koseri	A Ax Ctr Ctz Ce Pi
S. aureus	A Ax Ag Cp Co Ctr Ctz Ch Ce Cf Pi
E. aerogenes	A Ax Co Ctz Pi
E. cloacae	A Ax Ag Cp Co Ctr Ctz Ch Ce Cf Pi
Enterobacter sp.	A Ax Ag Cp Co Ctr Ctz Ch Ce Pi
P. mirabilis	A Ax AgCo Ctr Ctz Ch Ce Cf Pi
C. fruindi	A Ax AgCo Am Ctr Ctz Ce Dx Pi
K. pneumonia	A Ax Ctz Ch Ce Cf Pi
S. pyogenes	A Ax Ctr Ch Ce Cf Pi

A: Ampicillin, Ax: Amoxicillin, Ag: Amox-clavulinic acid, Ch: Chloramphenicol, Ctr: Ceftriaxone, Ctz: Ceftazidime, Ce: Cephalotin, Cf: Cefoxitin, Pi: Piperacillin, Am: Amikacin, Co: Cotrimoxazole, Cp: Ciprofloxacin, Dx: Doxycyclin, R: Resistance, S: Susceptible

The antimicrobial activity test of the apex shoot of *Vernonia amygdalina* extract showed activities against some strains. The methanol extracts inhibited most of strains at tested concentrations while the ether extract was active only against *S. pyogenes* (Table 3). Hence, the activities of the extracts, especially the methanol fraction could confirm the traditional use of the plants against Eczema[14], wounds[15] and typhoïd fever[33]. The previous studies also verified the antimicrobial activities of the plant extracts against *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Salmonella typhi* and *Pseudomonas aeruginosa*[34].

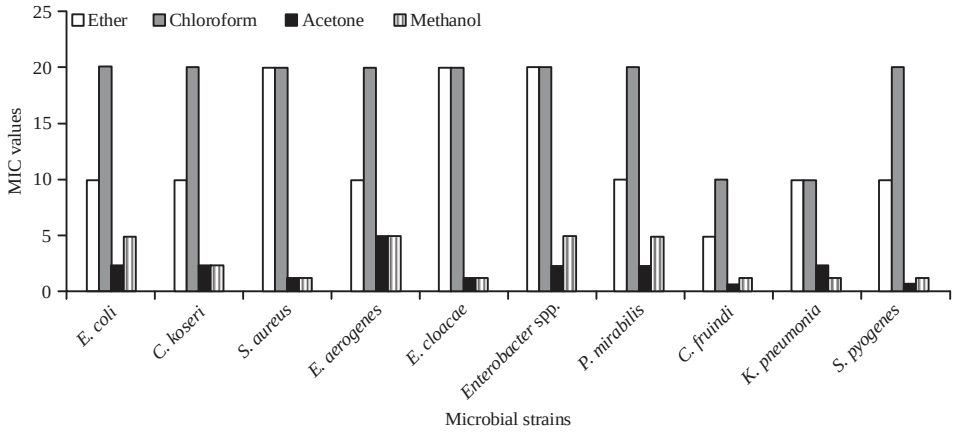

Fig. 1: MIC of different solvent fractions of *Prunus africana* bark against multidrug resistant clinical isolates

Table 2: Antimicrobial activities of various solvent extracts of *Prunus africana* bark (50 mg mL) against multidrug resistant clinical isolates

	Zone of inhibition (mm)			
Microbial strains	Pet. Ether	Chloroform	Acetone	Methanol
E. coli	18.3±0.58	11.0±1.00	18.3±1.53	20.3±0.58[b]
C. koseri	13.3±0.58	13.7±0.58	15.8±0.76	18.3±0.58[a,b,c]
S. aureus	9.2±0.27	11.8+0.76	11.7±1.53	16.2±1.04[a,b,c]
E. aerogenes	8.7±0.58	10.7±0.58	13.3±0.58	15.0±1.00[a,b,c]
E. cloacae	11.3±1.53	10.3±0.58	15.3±1.53	20.0±1.00[a,b,c]
Enterobacter spp.	11.7±0.58	9.0±0.00	12.3±1.53	12.3±0.58[b]
P. mirabilis	11.8±0.29	11.0±1.00	15.0±1.00	13.3±2.08
C. fruindi	13.2±0.76	10.0±1.00	22.3±1.53	21.3±1.15[a,b]
K. pneumonia	12.7±1.53	13.3±0.58	17.7±1.15	18.7±1.53[a,b]
S. pyogenes	9.8±1.26	11.3±0.58	15.7±0.58	15.7±0.58[a,b]

Values are Means±Standard Deviation, n = 3; Statistical analysis: one-way ANOVA, $p<0.05$ indicates significant difference. [a]Methanol extract showed significant activities compared to petroleum fraction, [b]Methanol extract showed significant activities compared to chloroform fraction, [c]Methanol extract exhibited significant activities compared to acetone fraction

Table 3: Antibacterial activities of the extracts of *Vernonia amygdalina* apex shoot (50 mg mL^{-1}) against multidrug resistant clinical isolates

	Zone of inhibition (mm)			
Microbial strains	Pet. Ether	Chloroform	Acetone	Methanol
E. coli	-	-	-	16.0±1.00
C. koseri	-	-	-	12.3±0.58
S. aureus	-	14.3±0.58	16.3±0.58	12.3±0.58
E. aerogenes	-	-	-	13.1±0.00
E. cloacae	-	-	-	-
Enterobacter spp.	-	-	-	11.0±1.00
P. mirabilis	-	-	-	-
C. fruindi	-	13.3±0.58	-	15.0±1.15
K. pneumonia	-	11.5±0.87	13.3±0.58	12.7±0.29
S. pyogenes	9±0.0	13.0±0.00	-	12.3±0.29

Values are Means±Standard Deviation, n = 3

The bark extracts of *Prunus africana* exhibited variable minimum inhibitory concentration with the lowest MIC values of 0.65 mg mL^{-1}. The acetone fraction was the most effective with MIC values of 0.65 for *C. fruindi* and *S. pyogenes*. However, the same extract demonstrated the highest MIC value of 5 mg mL^{-1} against *E. aerogenes*. On the other hand, *K. pneumonia* was the most sensitive strains to methanol extract compared to others fraction whereas the ether and chloroform fractions were found to be the least effective with more than MIC of 5 mg against tested bacterial strains except against *C. fruindi* (Fig. 1). The acetone and methanol extracts exhibited MIC at 1.25 mg mL^{-1} against *S. aurous*. This finding

is fairly consistent with previous studies conducted on antimicrobial activities of the methanol extracts against methicillin resistant *Staphylococcus aurous* (MRSA) and *Staphylococcus aureus* standard strains[26,33].

CONCLUSION AND RECOMMENDATION

The *Prunus africana* solvent fractions exhibited stronger antimicrobial activities than *Vernonia amygdalina* extracts. The methanol fractions of *Prunus africana* exhibited remarkable antibacterial activities and the extracts significantly inhibited the multidrug resistant clinical strains such as *E. coli*, *C. koseri*, *E. aerogenes*, *S. aureus* and *E. cloacae* when compared with other solvent fractions. Hence, medium to polar plant secondary metabolites are accountable for the strongest antimicrobial activities of the methanol extracts. Therefore, it is suggested that further studies should be conducted on the methanol fraction of *Vernonia amygdalina* to isolate and characterize the potential compounds and evaluate their antibacterial activities.

SIGNIFICANCE STATEMENT

This study discovers potential activities of the methanol fractions of *Prunus africana bark* against multidrug resistant strains that can be beneficial for new antimicrobial drug discovery. This study will help the researcher to reveal the critical areas of multidrug resistance and the need for discovery of new drugs from of *Prunus africana* bark extracts that many researchers were not able to explore. Thus, new antimicrobial drugs might be developed from the secondary metabolites obtained from the plant extracts.

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