International Journal of Biochemistry, Biophysics & Molecular Biology
2018; 3(4): 52-59
http://www.sciencepublishinggroup.com/j/ijbbmb
doi: 10.11648/j.ijbbmb.20180304.11
ISSN: 2575-5889 (Print); ISSN: 2575-5862 (Online)

Antibacterial Screening of Extracts of Stem Bark of *Prunus africana* and Its Heavy Metal Determination

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To cite this article:
Desta Dirbeba, Ahmed Hussen. Antibacterial Screening of Extracts of Stem Bark of *Prunus africana* and Its Heavy Metal Determination. *International Journal of Biochemistry, Biophysics & Molecular Biology*. Vol. 3, No. 4, 2018, pp. 52-59. doi: 10.11648/j.ijbbmb.20180304.11

Received: April 16, 2018; **Accepted**: May 16, 2018; **Published**: February 26, 2019

Abstract: In Ethiopia, the traditional healers use fusions of leaves and decoctions of stem barks of the Prunus africana to treat diarrhea, wound dressing and other bacterial diseases. This provided us an impetus to investigate the antibacterial activities of crude extracts of the plant. In line with, the bioactive molecules from the plant were extracted using Soxhlet and maceration extraction techniques with solvents of different polarities. The Soxhlet technique showed relatively better extraction efficiency in extracting the secondary metabolites of the plant than maceration technique. Among the different solvents examined for their better extraction yield potential, methanol was found to be the best extractant with extraction yield of 22.5%. The antimicrobial activities of crude extracts of petroleum ether, diethyl ether, chloroform, acetone, ethanol and methanol were investigated using agar well disc diffusion and agar dilution antimicrobial susceptibility testing methods. The methanolic extract showed antibacterial activity against both Staphylococcus aereus and Bacillus subtilis with 18.00±1.53 mm and 19.33±2.08 mm diameter of zones of inhibition, respectively. Similarly, diethyl ether extract showed antibacterial activity against Bacillus subtilis with 17.00±1.15 mm of zones of inhibition. The levels of seven heavy metals (Zn, Cu, Mn, Cr, Ni, Pb and Cd) were determined using Flame Atomic Absorption Spectroscopy (FAAS) and microwave digester at optimized conditions. Concentrations of Cr, Ni, Pb and Cd were below the method detection limit while Zn, Mn and Cu metal ions were detected and found to be in the Permissible Levels (World Health Organization standards). The concentration of Mn was the highest as compared to that of metals in both stem and leaves the plant.

Keywords: *Prunus africana*, Agar Well Diffusion Assay, Gar Dilution Assay, Heavy Metals, FAAS

1. Introduction

Prunus africana (Hook. f.) Kalkman (Rosaceae) is a multiple-use tree species with local and international economic and medicinal value. The species is popular for its bark whose extract is used to treat benign prostate hyperplasia which is a non-cancerous enlargement of prostate common in men over the age of 50 [1]. The plant is widely used in traditional medicine in southern, eastern and central Africa countries [2]. Traditional healers across Africa use the plant as a medicine to treat diarrhea, dysmenorrheal, epilepsy, impotence, infertility, irregular menstruation, kidney disease, mental illness, eye disorders, fevers, obesity, pneumonia, arthritis, hemorrhage, hemorrhoids, hyper menorrhea, hypertension and prostate gland enlargement. Its stem and leaf extracts have also been used as antibacterial, anti-inflammatory, antimalaria, antiparasitic and antirheumatic [3, 4].

The plant is known by different local names in Ethiopia as "Tikurinchet" in Amharic language and "gurayu" in Afan Oromo language; the dominant language spoken in Ethiopia. The plant is also known by a local name "garbe" in Gedeo language where the plant was collected [5, 6]. The plant is known as red ivory, iron wood or red stinkwood in English [5]. *P. africana* is a geographically widespread tree restricted to Afromontane forest habitats of the African continent. *P. africana* is widely distributed in Angola, Mozambique, Nigeria, Zambia, Zimbabwe, Burundi, Congo, Kenya, Rwanda, Sudan, Tanzania, Uganda, Cameroon, Sao Tome, and Ethiopia [6].

In Ethiopia, the traditional healers use infusions of leaves, decoctions of stem and root barks of the plant to treat

diarrhea, wound dressing and other bacterial diseases [5]. Consequently, antibacterial screening of different extracts of the plant against *Escherichia coli*, *Staphylococcus aereus,* and *streptococcus sp* is very invaluable in providing scientific proof for this claimed traditional therapeutic use.

Previous studies on the antimicrobial activity of the plant showed that its crude extracts are active against some bacteria like *Pseudomonas aeruginosa, Staphylococcus aereus* and *Streptococcus pneumoniae.* It was also reported that crude extracts of *Prunus africana* have antifungal activities against *Trichophytonmentagropytes, microsporumgypseum*, *Cryptococcus neoformans* and *Candida albicans* [7].

On the other hand, the traditional users of medicinal plant do not know the exact of the amount to be taken to cure the ailment for which it is intended. This is the prominent weakness of traditional use of traditional remedies as the active principles may be toxic at certain concentration level and become fatal [8]. In addition to this inherent problem, contaminations of the herbal drugs from external agents exacerbate the toxicity problems related to the usage of herbal remedies. The World Health Organization (WHO) database showed myriads of case reports related to toxicities of herbal drugs either due the over dosage of active ingredient or due to external contaminants including pesticide residue, toxic heavy metals, mycotoxins as well as adulterants added to increase potency [9].

For the purpose of overcoming or mitigating the aforementioned inconvenient issues, World Health Organization has developed a series of technical guidelines and documents in relation to the safety and the quality assurance of medicinal plants and herbal drug preparations [8]. Determination of the levels of heavy metals is one of such quality control methods for medicinal plant materials. In this context, determination of the levels of heavy metals in the *P. africana*, important medicinal plant, plays a paramount importance in checking the safety of traditional use of medicinal the plant [10].

2. Materials and Methods

2.1. Sample Collection and Preparation

The stem bark of *P. africana* was collected from Wonago district of Gedeo Zone in South Nations, Nationalities and People's Regional State in Ethiopia. The leaves were pressed and authenticated by Addis Ababa University National Herbarium and the specimen was reserved in the National Herbarium with specimen code of DD-001. Since the plant is among the threatened species in Africa, the debarking procedure was carried out as recommended by [11]. The stem bark of *P. africana* was collected from WonagoWoreda of Gedeo Zone in South Nations and Nationality People's Front (SNNPR) in Ethiopia from four particular sites, namely; Balebuksa, Tikrese, Sokcha and Sugale areas. The Wonago Woreda was selected mainly based on ethno botanical literature survey that the plant has been used for medicinal purpose in the area.

The sample for phytochemical analysis was collected from mature plants and it was washed with tap water and then with distilled water before it was cut into smaller pieces with stainless still knife so as to remove dust and other particulates. The pieces were air dried in a dark and well ventilated room. The dried plant materials from the four sites were thoroughly homogenized and grinded into fine powder using electrical grinder. The leaf and stem samples were collected for heavy metal analysis from the same sites as samples for phytochemical analysis.

The sites are approximately one to four kilometers apart. Healthy and unaffected leaves were collected from a total of 12 mature plants, three plants per site starting from the bottom to the tips. The stem bark was debarked from the same sites and same plants as for leaves using stainless steel knife. Both samples were washed with tap water and then rinsed with distilled and finally with deionized water so as to remove adsorbed particulates before cutting them into smaller pieces.

Each sample was put on acid-washed porcelain crucibles and dried in oven at 75°C until it became brittle. The dried samples were grinded in to fine powder with mortar and pestle which was finally sieved with 2 mm mesh sieves.

The powdered sample was then placed in polyethylene container and stored in a desiccator so as to keep the dry weight remains constant until digestion. The plant samples were made ready for analysis after digestion using microwave assisted digestion system.

2.1.1. Extraction

Both maceration and Soxhlet extraction techniques were used for extraction of the phyto-constituents of stem bark of the plant for the purpose of comparison of their extraction efficiencies.

2.1.2. Maceration

Ninety milliliters of each of petroleum ether, diethyl ether, chloroform, acetone, ethanol and methanol were added to separate 250 mL conical flask to which 6 g of the powdered stem bark was macerated with. The conical flask containing the macerated sample was sealed with rubber stopper wrapped completely with aluminum sheet which was then continuously shaken with electrical shaker for 72 hours at 180 revolutions per minute. The marc of each crude extract was separated from the solvent containing the extract using Whatman No.1 filer paper. The solvents were removed from the crude extracts under reduced pressure. The extraction was done in triplicate with methanol only. The crude extracts obtained after the removal of the extraction solvents were stored separately at 6°C until they were used for antibacterial assay.

2.1.3. Direct Soxhlet Extraction

Twenty grams of the powdered plant material were placed in paper thimble which was in turn placed in Soxhlet extractor. Sufficient amount of each solvent was transferred to separate 500 mL round bottom flask which was in turn

placed in heating mantle. The extraction was conducted for a period of 8 hours at rate of 5 cycles per hour with petroleum ether and methanol; for a period of 9 h at rate of 4 cycles per hour with ethanol; and for a period of 7 h at rate of 6 cycles per hour with chloroform and acetone. The extraction of crude extracts with diethyl ether, however, was found difficult to go to completion without excessive loss of the solvent as diethyl ether is very volatile. Thus, it was concluded that the extraction of the stem bark of the plant with diethyl ether using Soxhlet extraction was inefficient even with reasonable amount of the solvent due to high volatility nature of diethyl ether. The extraction was thus done in triplicate with methanol only.

2.2. Microbial Strains

In the present study, the cultures of four bacteria species were used. Three Gram positive species (*Staphylococcus aereus*, *Streptococcus sp., and Bacillus subtilis*) and one Gram negative (*Escherichia coli*) bacterial species were used. All bacteria were obtained from Dilla University Microbiology Research Laboratory and they were cultured in nutrient.

2.2.1. Preparation of Media for Inoculation

The nutrient broth was prepared according to manufacturer's guide line for the growth of bacterial species. The indicated amount of nutrient broth was taken and suspended in the indicated amount of distilled water. The suspension was then heated on a Bunsen burner until it was completely dissolved. The clear solution was transferred into test tubes and sterilized at 121°C for 15 minutes under a pressure of 15 lb using autoclave. These sterilized solutions were inoculated independently with the *Escherichia coli*, *Staphylococcus aereus, Streptococcus sp., and Bacillus subtilis* within safety cabinet. The bacterial strains were then incubated at 37°C for 24 hours for their proper growth. Ten grams of nutrient agar was suspended in 500 ml of distilled water and heated on a Bunsen burner until the suspension became clear solution. The clear solution obtained was autoclaved at 121°C for 15 minutes under a pressure of 15 lb. About 20 ml of this sterilized solution was poured into each sterile Petri dish in a laminar flow of cabinet where the nutrient agar was allowed to settle and solidify for about one hour. The plates were then kept for subsequent inoculation with *Staphylococcus aereus, Escherichia coli*, *Streptococcus sp.* and *Bacillus subtilis*.

2.2.2. Antibacterial Assay

The antimicrobial susceptibility testing was done using agar well diffusion method to detect the presence of antibacterial activities of the plant extract as recommended by Delahaye and co-authors with a little modification [12]. After overnight incubation, the bacteria suspension was prepared in 0.5 ml of the broth medium. The turbidity was then adjusted to match that of 0.5 McFarland standards to obtain approximately the organism number of 1x 10^6 colony forming units (CFU) per mL. Then a sterile swab was dipped into the suspension and then the swab was applied to the center of the agar plate from which it was evenly spread on the whole medium.

Wells were made on the semi-solidified Petri dishes with sterile cork borer of approximately 8mm diameter into which 40% (V/V) of each crude extract and the isolates were added with micropipette. Three wells were made with sterile cork borer on each Petri dish into which the isolates and the crude extracts of methanol, acetone, chloroform, diethyl ether and petroleum ether were poured with micropipette. Each extract was dissolved in dimethyl sulfoxide which was used as negative control while gentamycin was used as a positive control for bacteria. The inoculated and loaded Petri plates with plant extracts were incubated for 24 h at temperature of 37°C for bacteria after which they were examined for inhibition zones. Antibacterial activity was indicated when clear inhibition of zones were noticed around the wells. The diameters of zone of inhibitions were measured with scale and the results were expressed as mean of three independent experiments. The zones of inhibition the extracts and the isolates were measured in triplicate.

2.2.3. Determination of Minimum Inhibitory Concentration (MIC)

The determination of the MIC was carried out using agar dilution method as recommended by Esimone and co-authors with slight modification [13]. Forty milligram of diethyl ether extract and 30 mg of methanolic extract were dissolved in 20 ml of 25% (v/v) DMSO to get a stock solution with concentration of 2 mg/ml of diethyl ether and 1.5 mg/ml of methanolic crude extracts. From these stock solutions 2, 1, 0.5, 0.25, 0.125 and 0.0625 mg/ml of diethyl ether extract and 1.50, 0.75, 0.375, 0.1875 and 0.09375 mg/ml of methanolic extract were prepared by serial two fold dilution. One milliliter of each extract dilution was added to 19 ml of sterile molten nutrient agar and mixed thoroughly before pouring into sterile Petri dishes. The dishes were allowed to solidify and then labeled appropriately. Plain nutrient agar (agar without the extract) was also streaked and served as negative controls. Based the agar well diffusion assay results, the dishes containing diethyl ether dilute extracts were inoculated with *Bacillus subtilis* while those containing methanolic dilute extracts were inoculated with *Staphylococcus aereus* then incubated at 37°C for 24 hours. MIC was taken as the lowest concentration of extract that resulted in no visible growth on the surface of the agar. The final concentration was calculated from the equation, $C_f = C_i/20$ where C_f is the final concentration of extract in the agar and C_i is the initial concentration of extract in the solution. Thus, the final concentrations of diethyl ether extract in labeled plates were 100, 50, 25, 12.5, 6.25 and 3.125 µg /mL while that of that methanolic crude extract in plates were 75, 37.5, 18.75, 9.375 and 4.686 µg /mL.

2.3. Optimization of the Working Procedures for Heavy Metal Analysis

To prepare a clear and colorless sample solution that is

suitable for the analysis of the metals by FAAS different working procedures for the digestion of stem and leaf samples were assessed using the HNO_3 and H_2O_2 mixtures by varying parameters such as volume of the reagent mixture, digestion time and digestion temperature. This was done by keeping all parameters constant while only one was varying. By examining the nature of the final digests obtained by varying the above parameters, the optimum procedure selected was the one that gave a clear digests, a less time, a less reagent volume and a less temperature for digestion. Based on this, digestion temperature of 190°C, digestion time of 25 minutes and mixture of HNO_3 and H_2O_2 in the ratio of 4:3 was found to be the optimum parameters for digestion of stem and leaf samples of *P. africana.*

2.3.1. Instrument Calibration

Calibration curves were prepared to determine the concentration of the metals in the sample solution. Thus, five series of working standard solutions were prepared carefully from the 10 mg/L intermediate standard solutions of their respective metals. The working standard solutions of each metal were prepared freshly by diluting the intermediate standard solutions. The correlation coefficient obtained for each metal from the corresponding standard metal solution was determined and found to be in the range of 0.998 to 0.999, which is in the acceptable range for the determination of the heavy metals.

2.3.2. Digestion of the Plant Samples for Heavy Metal Analysis

Exactly 200 mg of powdered stem and 250 mg of powdered leaf samples were transferred quantitatively into a 60 ml microwave digestion vessels to which 7 ml of mixture of conc. HNO_3 and H_2O_2 in the ratio of 4:3 was added. The samples were swirled gently to homogenize and then placed on turntable motor shaft to rotate the sample vessels in microwave field. The samples were digested in the field for 25 minutes at a maximum temperature of 190°C and maximum power of 90%. The digest gave a clear and colorless solution and it was quantitatively transferred to a 10 ml volumetric flask and made up to the mark with deionized water. Each sample was digested in triplicate and hence a total of 6 digests were made for the plant samples. Digestion of seven reagent blank samples were also performed in parallel with the samples keeping all digestion parameters the same to determine the method detection limit which was determined as three times the standard deviation of the blank plus the average of the blank signal.

2.3.3. Evaluation of Analytical Method

In this study, efficiency of the digestion method was tested using spiking experiment. In the spiking experiment, 200 µL of solution containing 0.08 mg/L of each of the metals (Cd, Cr, Pd) and 500 µL of solution containing 0.1 mg/L of each of the metals (Mn, Zn, Cu, Ni) was added to 200 mg of stem and 250 mg leaf samples, respectively. The percent recovery from the spiking experiment was determined as:

$$\%R = \frac{C_M \text{in spiked sample} - C_M \text{ in non-spiked sample}}{C_M \text{added for spiking}} \text{x} 100$$

C_M = concentration of metal

3. Results

Solvents have different extraction efficiency in extracting phytochemicals of a given plant. Consequently, the extraction efficiencies of different solvents using maceration and Soxhlet extraction techniques were compared to see the differences. In both techniques, the extraction yields showed an increase with increase in the order of polarities of solvents employed for the extraction of the phyto-constituents of the plant. As it is evident from Table 1, the general trend is the same except that the extraction efficiency of each solvent in Soxhlet is slightly greater compared to the maceration technique.

Table 1. *Comparison of efficiencies of maceration and direct Soxhlet extraction techniques.*

Types of solvent	Mass taken (g)	Mass obtained (g)		Percent yield (%)	
		maceration	Soxhlet	maceration	Soxhlet
Petroleum ether	6.00	0.24	0.45	4.00	7.50
Chloroform	6.00	0.92	1.13	15.30	18.80
Acetone	6.00	1.00	1.14	16.67	19.00
Ethanol	6.00	1.05	1.18	17.50	19.67
Methanol	6.00	1.14±0.01	1.35±0.02	19.00±1.05	22.50±0.84

Statistical test analysis using t-test at 95% confidence level showed that there is significant difference between maceration and Soxhlet extraction techniques for extraction of the methanolic crude extract from the stem bark of *P. africana*. The result indicated that chloroform, acetone and ethanol have similar extraction efficiencies better than petroleum ether while methanol showed superior extraction efficiency. The result also showed the majority of phyto-constituents of *P.* africana are polar compounds as the largest yield was obtained with methanol.

In the antimicrobial evaluation of the plant extracts, all the extracts were screened against four bacterial strains namely *Staphylococcus aereus* (*S. aereus*), *Bacillus subtilis* (*B. subtilis*), *streptococci* sp. and *Escherichia coli* (*E. coli)* using agar well disk diffusion assay. The result showed that methanolic crude extract has antibacterial activity against *S. Aereus* and *B. subtilis* while the diethyl ether extract showed antibacterial activity against *B. subtilis* (Figure 1, Table 2). Both diethyl ether and methanolic crude extracts, however, did not show any antibacterial activity against *E. coli* and *streptococci sp.* The remaining crude extracts did not show any antibacterial activity at all. The minimum inhibitory

concentration of methanolic extract against *Staphylococcus aereus* and that of diethyl ether crude extract against *Bacillus subtilis* were found to be 9.375µg/ml and 12.5 µg/ml, respectively.

Table 2. *Zones of inhibition (mm) of the active extract against the susceptible microbes.*

Microbial strains	ZI of diethyl ether extract	ZI of MeOH extract	ZI of positive controls	Name of the positive control
S. aereus	NI	18.00±1.53	20.00±1.00	Gentamycin
B. subtilis	17.00±1.15	19.33±2.08	19.00±1.06	Gentamycin
E. coli	NI	NI	22.00±2.05	Gentamycin
Streptococcus sp.	NI	NI	30.00±2.25	Chloramphenicol

NI = no inhibition, ZI = zone of inhibition

As it is shown in the above Table 2, the zones of inhibition of the above susceptible bacteria caused by positive control (Gentamicin) appear to be greater than that caused by crude extracts. However, statistical t-test at 95% confidence level (P = 0.05) indicated that there is no significant difference between the zones of inhibitions by crude extracts and that of the positive control. Similarly, no significant difference was observed between the two zones of inhibition caused by the two diethyl ether and methanolic crude extract. On the other hand, one-way ANOVA analysis at 95% confidence level (P = 0.05) was performed to examine differences among zones of inhibition of *B. subtilis* caused by methanolic crude extract, diethyl ether crude extract and the positive control. The result of the analysis of variance revealed insignificant difference between zones of inhibition caused the three antimicrobials. This indicates that the crude extracts are potential antimicrobial agents.

The report of [7] showed the methanolic crude extract of stem bark of *Prunus africana* had bioactivity against *Staphylococcus aereus* with diameter of zone of inhibition of 20 mm which is virtually the same with zone of inhibition of the microbe by methanolic crude extract of the same plant in the current work.

Phongpaichit *et al* [14], Dewanjee *et al.* [15], Peni *et al.* [16], Naing *et al*. [17] Prasannabalaji *et al.* [18] and many others reported the antibacterial activity of methanolic plant stem bark of *Prunus africana* had bioactivity against *Staphylococcus aereus* with diameter of zone of inhibition of 20 mm which is virtually the same with zone of inhibition of the microbe by methanolic crude extract of the same plant in the current work.

Phongpaichit *et al* [14], Dewanjee *et al.* [15], Peni *et al.* [16], Naing *et al*. [17] Prasannabalaji *et al.* [18] and many others reported the antibacterial activity of methanolic plant crude extract from other plants against *B. subtilis and S. aereus*. The diameters of zone inhibition of *B. subtilis and S. aereus* caused by methanolic crude extract of stem of *Parinari curatellifolia* in the work of Peni *et al.* [16] were 21 mm and 20 mm, respectively, which are similar to that of present work. However, the diameters of zone of inhibition of *B. subtilis* (8 mm) and *S. aereus* (9.03 mm) caused by crude extract of *Diospyros peregrina*as reported by Dewanjee *et al.* [15] are smaller as compared to that of present work.

crude extract from other plants against *B. subtilis and S. aereus*. The diameters of zone inhibition of *B. subtilis and S. aereus* caused by methanolic crude extract of stem of *Parinari curatellifolia* in the work of Peni *et al.* [16] were 21 mm and 20 mm, respectively, which are similar to that of present work. However, the diameters of zone of inhibition of *B. subtilis* (8 mm) and *S. aereus* (9.03 mm) caused by crude extract of *Diospyros peregrina*as reported by Dewanjee *et al.* [15] are smaller as compared to that of present work.

Figure 1. *ZI of methanolic extract against S. aereus and against B. subtilis and that of diethyl ether extract against B. subtilis from left to right, respectively.*

The validity of the optimized digestion procedures for determination of heavy metal in both stem and leaf samples were tested by spiking the samples with standard analytes of known concentrations; the results of which indicated that the percent recoveries for both stem and leaf samples were in the range of 86-108% which are within the tolerance levels based on World Health Organization standards for all metals analyzed showing the optimization procedure efficient for determination of the heavy metals in the samples. Table 3 and 4 present the percent recovery of each of the metal in stem and leaf of *P. africana* samples.

Table 3. *Recovery test results for leaf sample (all data are in mg/kg).*

Metal	Concentration in sample	Amount Added	Conc. in spiked sample	Amount recovered	Recovery (%)
Zn	13.15±0.02	4.00	17.14±0.02	3.99	99.75±0.58
Cu	7.37±0.05	4.0	11.25±0.09	3.88	97.00±0.52
Ni	ND	4.00	5.73±0.02	4.12	103.00±1.50
Cr	ND	3.20	4.51±0.03	3.16	98.75±0.18
Pb	ND	3.20	6.00 ±0.14	3.40	106.25±1.53
Mn	33.59±0.12	4.00	37.07±0.12	3.48	87.00±0.02
Cd	ND	3.20	4.29±0.09	3.41	106.56±0.75

Table 4. *Recovery test results for stem sample (all data in mg/kg).*

Metal	Conc. in sample	Amount added	Conc. in spiked	Amount recovered	Recovery (%)
Zn	6.56±0.12	5.00	11.50±0.03	4.94	98.80±1.40
Cu	8.17±0.55	5.00	13.14±0.01	4.97	99.40±1.08
Ni	ND	5.00	7.50±0.08	5.23	104.60±1.08
Cr	ND	4.00	5.67±0.06	3.97	99.25±1.80
Pb	ND	4.00	8.58±0.03	4.26	106.50±1.44
Mn	42.07±0.12	5.00	46.37±0.14	4.30	86.00±2.60
Cd	ND	4.00	6.00±0.10	4.35	108.75±1.53

In the current study, the concentration of the seven heavy metals (Mn, Cu, Zn, Cr, Ni, Pb, Cd) in the stem and leaves of *P. africana* were determined by FAAS using five point calibration curves. Among the analyzed seven elements, Zn, Cu and Mn were found to be above the method detection limit for both stem and leaf samples while Cr, Ni, Cd and Pb were found to below the method detection limit for all samples and thus they were not detected with this method.

As it is evident from Figure 2, Cu and Mn had greater concentrations in stem as compared to their concentrations in the leaf. The concentration of zinc was, however, greater in leaf as compared to that of stem. Statistical t-test at 95% confidence levels showed copper concentration differences in the stem and leaf is not significant; however, concentration differences of both Mn and Zn in stem and leaf of the plant showed significant differences. The permissible limit of these heavy metals in medicinal plant has not yet set by FAO/WHO. However, their concentrations in the current work are much less than their corresponding permissible limit in the dietary food.

Table 5. *Mean concentrations (X ± SD, mg/kg dry weight, SD = standard deviation) of the detected metals in Prunus africana samples.*

Metal	concentration in leaf	concentration in stem
Zn	13.15±0.02	6.56±0.12
Cu	7.37±0.05	8.17±0.55
Mn	33.59±0.12	42.07±0.12

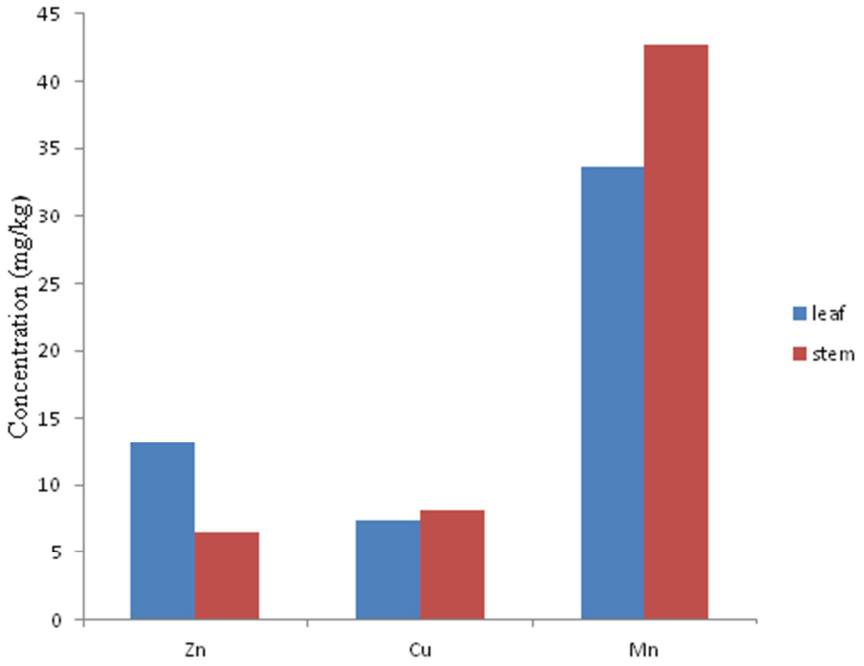

Figure 2. *Comparison of concentrations of the detected metals in stem and leaf samples.*

4. Discussion

Zinc is an essential trace element for plant growth and also plays an important role in various cell processes including normal growth, brain development, behavioral response, bone formation and wound healing. Higher concentration of zinc, however, has adverse effect [10, 19]. The report of Adongo *et al.* [19] is in similar trend with this present work. Adongo *et al.* [19] analyzed the concentration of heavy metals in both stem bark and leaf of *P. africana.* The result of the report indicated that 1.05 mg/L of Zn in leaf and 0.86 mg/L in stem of *P. africana.* These results are similar to those of the present work in that the concentration of zinc in leaf (0.329 mg/L) is greater than the concentration of zinc in stem (0.164 mg/L). However, the concentrations of zinc in both stem and leaf of *P. africana* in the current work is less than those of Adongo *et al.* [19].

Manganese is considered to be the 12th most abundant element in the biosphere. It is widely distributed in soil, sediment, water and in biological materials. Although manganese is essential for humans and other species of animal kingdom as well as for plants, it is toxic above its permissible levels. In man, chronic exposure to manganese affects the central nervous system with the symptoms resembling those of Parkinson's disease [20]. In this current study, the concentrations of manganese both in stem and leaf samples were found to the highest among the concentrations of detected metals. However, the concentration of manganese in stem (42.07 mg/kg) is greater than concentration of the same metal in the leaf (33.59 mg/kg). This current work is consistent with Idris *et al* [21] and Khan *et al.* [22] reports where greater concentration of manganese was obtained in stem as compared to that of the leaf. Idris *et al.* [21] determined the concentration of heavy metals in both stem and leaf of *Ocimum gratissimum* and found a greater concentration of manganese in the stem (19.17 mg/kg) and leaf (7.67 mg/kg). Similarly, Khan *et al.* [23] carried out comparative assessment of heavy metals in a medicinal plant, *Euphorbia helioscopia* L and reported 12.53 mg/kg and 9.77 mg/kg of manganese concentrations in stem and the leaf of the plant respectively. On the other hand, Hussain and Khan [24] did a comparative study of heavy metal levels in stem and leaf of *Taraxacum officinale* and found 1.38 mg/kg and 2.68 mg/kg of manganese respectively.

Copper is indeed essential but in high doses it can cause anaemia, liver and kidney damage, and stomach and intestinal irritation. Akan *et al* [22] determined the heavy metal levels in leaf and stem bark of neem tree collected from seven different sites. Unlike the results of this study, the results of their analysis indicated higher concentration of copper (2.64 mg/kg) in leaf and lower concentration in stem (1.43 mg/kg) of neem tree. The concentration levels of copper in stem (8.17 mg/kg) and in leaf (7.37 mg/kg of *P. africana* are appreciably greater than those determined by Akan *et al* [22] in stem and the leaf of neem tree. On the other hand, a comparative assessment of heavy metals in *Euphorbia helioscopia* L. performed by Khan *et al* [23] showed a similar trend with this current work. The result of their work indicated 1.46 mg/kg of copper concentration in the stem of the tree and 1.12 mg/kg concentration in the leaf of the plant.

5. Conclusion

In this work, the methanolic extract of stem bark of *Prunus africana* was found to have antibacterial activities against two bacterial strains, *staphylococcus aereus* and *Bacillus subtilis*, among the screened bacteria. Its activity against *Staphylococcus aereus* may be a promising potential source of antimicrobial for wound dressing. The traditional healer in the study uses water decoction of the stem bark of the plant for would dressing. Thus, the activity of methanolic extract against *Staphylococcus aereus* supports its traditional uses and provides scientific proof of polar solvent extract claimed efficacy for wound dressing. On the other hand, diethyl ether extract was also observed to have antibacterial activity against *Bacillus subtilis* bacterium.

The plant has higher concentration of Mn in both stem and leaf of the plant as compared to all other detected heavy metals. Although the World Heal Organization has not set the tolerance limit for Mn in medicinal plant, its concentration is within the permissible limit WHO [25] for dietary food. Toxic heavy metals Pb, Cr and Cd were not detected as their concentrations were below the detection limit of the method. Significant difference was observed between the concentrations of Mn and Zn in stem and leaf of the plant but no significant difference was seen between concentrations of copper in stem and leaf of the plant.

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