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# Anti-bacterial and Anti-oxidant Studies of Extracts from Root of *Prunus africana*

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author TAB designed the study, collected data, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AET and TAB managed the analyses of the study, interpretation of the data, critical revisions of the manuscript and managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

*Prunus africana* belongs to the *Rosaceae* family. It is a geographically wide spread tree to forest habitats of the African continent. *P. africana* is one of the most popular plants in traditional medicine for treating various ailments. It is mainly used to treat benign prostate hyperplasia (BHP). The study was aimed to evaluate the anti-bacterial and anti-oxidant activities of bark of root extract from *P. africana*. The air dried and powdered plant material (200 g) was first soaked with 500 mL of n-hexane for 72 hours and yielded 2.5 g of n-hexane extract. Residue was soaked with 500 mL of ethyl acetate for 72 hours and afforded 3.7 g of ethyl acetate extract. Finally, residue was soaked with 400 mL of methanol and yielded 14.3 g of methanol extract. The methanol extract showed inhibition zones of 18 and 14 mm against *Escherichia coli* and *Staphylococcus aureus,*

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respectively. The extracts also showed encourage results of DPPH radical scavenging activity at various concentrations. The methanol extract of *P. africana* of root showed promising activity against *E. coli*, ATCC25922 and *S.aureus,* ATCC25922. Anti-oxidant activities also were shown prospective result, selectively at lowest concentration and lowest absorbance. This means the result of the study was confirmed that the lowest concentration of 4 mg/mL and absorbance of 0.112 the scavenging activity was 87.9%, while at the highest concentration of 128 mg/mL and absorbance of 0.172 the scavenging activity was 81.5%.

*Keywords: Prunus africana*; *anti-oxidant; anti-bacterial; benign prostate hyperplasia; Escherichia coli; Staphylococcus aureus.*

## 1. INTRODUCTION

### 1.1 *Prunus africana (P. africana)*

Many species from the Rosaceae family have great economic importance. Therefore, they are known as "edible temperate zone fruits". The Rosaceae is the 19$^{th}$ largest family of the plant kingdom, which includes more than 100 genera and 2830-3100 species among which *P. africana* has more medicinal value. *P. africana* is a geographically wide spread tree to forest habitats of the African continent. It is widely distributed in Angola, Mozambique, Zambia, Zimbabwe, Burundi, Congo, Kenya, Rwanda, Nigeria, Sao Tome, and Ethiopia (Northwest and Southeast highlands, Harerge, Illubabor, Kefa, Arsi and Wolega**)** [1,2].

Infectious diseases are the leading causes of death in tropical countries, for approximately one half of all deaths and underlying causes of significant problem, i.e., 8% deaths occurring in developed nations such as USA [3]. Plants have a long history of being used for a wide variety of purposes including therapeutic uses [4]. Prostate cancer has been known to progress slowly and it is crucial to prevent its occurrence to reduce the risk of development of the disease [5-8]. In the past decades, chemopreventive and therapeutic agents for various cancers including prostate cancer have been isolated from plants [9-12]. Over 60% of currently used anticancer agents are claimed to be from natural sources [11].

*Prunus africana* (African cherry) is effective against prostate cancer cell lines. This evergreen plant is found only in sub-Saharan Africa and is highly sought owing to its anticancer phytochemicals [5,6,13]. In fact, the use of *P. africana* in African traditional medicine (ATM) to treat prostate cancer and related conditions is not a new phenomenon across various communities in Africa [5]. The use of *P. africana* has been patented in France for prostate cancer treatment [14]. The bark extract of *P. africana* has been used for the treatment of benign prostatic hyperplasia (BPH). The phytosterols (including *β*-sitosterol) and pentacyclic triterpenoids (including ursolic acid) also have anti-inflammatory effects on the prostate [1,15]. In ATM*, P. africana* has also used to treat myriad of diseases including diarrhea, epilepsy, arthritis, hemorrhage, and hypertension [13,14,16-18]. The novel phytochemicals from *P. africana*, suggested for the treatment of prostate cancer are ursolic acid, oleanolic acid, *β*-amyrin, atraric acid (AA), N-butylbenzene-sulfonamide (NBBS), *β*-sitosterol, *β*-sitosterol-3-O-glucoside, ferulic acid, and lauric acid [17,18]. The study was aimed to evaluate the anti-bacterial and anti-oxidant activities of root extracts of *P. africana*.

## 2. PLANT MATERIALS AND METHODS

The root of *P. africana* was collected from Shero kebele, Misha Woreda, Hadiya Administrative Zone, Southern Nations Nationalities and People Regional State (SNNPR). It was authenticated by Mr.Wege Abebe and a voucher specimen was stored at the National Herbarium of Addis Ababa University (Voucher no.TA002), Addis Ababa, Ethiopia.

### 2.1 Apparatus and Chemicals

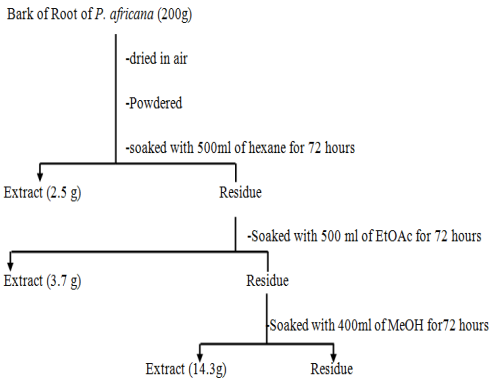
Some of the apparatus were used: Melting point apparatus (Korea) funnels, round bottom flasks, vials, glass wares, refrigerator, Whatman No.1 filter papers, grinder (Ethiopia), drying oven (Germany), measuring cylinders, RV-10-basic Rotavapor (Germany), and others. All the chemicals and solvents for this study were supplied by Hi-Media Co.

### 2.2 Extraction

The air dried and powdered plant material (200 g) was first soaked with 500 mL n-hexane for 72 hours and the extract was collected by filtering and concentrated under reduced pressure using the Rotavapor. The solvent free residue was then

soaked with 500 mL of ethyl acetate for 72 hours and the extract was collected. This filtrate was evaporated under reduced pressure using the Rotavapor. Finally, the solvent free residue was soaked with 400 mL of methanol, and then it was filtrated by using Whatman no.1 filter paper and concentrated under reduced pressure using the Rotavapor.

The scheme of extraction is shown below:




**Scheme 1. Method used to extract bark of root of *P. Africana***

## 2.3 Anti-bacterial Assay

Mueller Hinton agar plates were prepared as per the manufacturer's instructions. The media and the plates were sterilized in an autoclave at 121ºC for 15 minutes. The plates were flamed on the surface using a non-luminous flame to remove air bubble and also ensure sterility of the surface. The cork borer was sterilized using a non-luminous flame. The plates and all the equipment's to be used for the experiment were then transferred in to a germicidal wood. The germicidal lamp was put on for 30 minutes to sterilize the surface of the plates and other equipment. The bacterial suspension was smeared on the media and five wells with a diameter of 6 cm each were drilled in each agar plate using a cork borer. Three of the wells were filled with 0.1 mL of the 500 mg/mL of the extract. The other wells were filled with 0.1 mL of 500 mg/ml of penicillin and 0.1 mL of 100% DMSO positive and negative controls respectively. The plates were labelled on the underside and incubated at 37ºC for between 24-48 hours and the zones of inhibition measured in mm with the aid of a ruler [19].

The crude extracts of *P. africana* also active against some microbes like *Pseudomonas aeruginosa (P. aeruginosa), Staphylococcus aureus (S. aureus)*, *Streptococcus pneumonia (S. pneumoniae)* and *Gonococcus. P. aeruginosa* is Gram-negative rod-shaped bacteria that normally live in human and animal intestine, water, soil, moist environment and in hospitals (sinks, cleaning buckets). It is typically an opportunistic pathogen that seldom causes disease in healthy subjects. Normally, for an infection to occur, some disruptions of the physical barriers like skin [20]. The microbial used as test strain were *S. aureus* ATCC25923 for Gram positives, and *P. aeruginosa* ATCC27853, *E. coli* ATCC25922 and *Proteus mirabilis (P. mirabilis)* ATCC2523, for Gram negatives; Vancomycin was used as the standard drug for Gram positives while Gentamicin for Gram negative.

## 2.4 Anti-oxidant Assay (DPPH) of *P. Africana* Bark of Root Extract

The percentage of antioxidant activity (AA%) of each extract was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams, et al. [21,22]. The samples were reacted with the stable DPPH radical in an ethanol solution. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep violet to light yellow) were monitored at 517 nm after 100 min of reaction using a UV-Vis. spectrophotometer (DU 800; Beckman Coulter, Fullerton, CA, USA). The mixture of ethanol (3.3 mL) and sample (0.5 mL) served as a blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL) [22].

Experiments were performed 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical solution was prepared in methanol, which means 4mg of DPPH mixed with 100 mL of methanol, and the sample solutions were also prepared in 1 mL of methanol; from lowest to highest 4 mg/mL, 8 mg/ml, 16 mg/mL and 32 mg/mL, then 6ml of DPPH solution was mixed with 1 mL of the sample solutions. After incubation for 30 min in the oven at temperature of 40ºC; finally, at the absorbance of 517 nm was measured by using methanol as blank control. Lower absorbance indicates higher anti-oxidant activity. These activities were reported as a percent of DPPH radical scavenging. The mixture of methanol (4 mL) and sample (1 mL) serve as blank. The control solution was prepared by mixing methanol (4.5 ml) and DPPH radical solution (0.4 ml).

## 3. RESULTS AND DISCUSSION

### 3.1 Phytochemical Screening of *P. africana* Bark of Root Extract

The phytochemical analysis of chemical constituents was done using the following procedures.

**Tannins:** About 0.1 g of the extract put in a test tube and 20 mL of distilled water was added and heated to boiling. The mixture was then filtered and 0.1% of $FeCl_3$ was added to the filtrate and observations made. Formation of a brownish green colour or blue-black coloration indicated the presence of tannins.

**Saponins:** About 0.1 g of the extract was mixed with 5 mL of water and vigorously shaken. The formation of stable form indicated the presence of saponins.

**Flavonoids:** About 0.1 g of the extract was added in to a test tube. To the test tube 5mL of dilute ammonia and 2 mL of concentrated sulphuric acid was added and heated for about 2 minutes. The appearance of a yellow colour indicated the presence of flavonoids.

**Terpenoids:** About 0.1g of the extract was taken in a clean test tube; 2 mL of chloroform was added and vigorously shaken, then evaporated to dryness. To this, 2 mL of concentrated sulphuric acid was added and heated for about 2 minutes. Formation of a greyish colour indicated the presence of terpenoids.

**Glycosides:** About 0.1 g of the extract was mixed with 2 mL of chloroform and 2mL of concentrated sulphuric acid was carefully added and shaken gently, then the observations were made. Formation of a red brown colour indicate the presence of steroidal ring (glycone portion of glycoside)

**Alkaloids:** About 0.1 g of the extract was mixed with 1% of HCl in a test tube. The test tube was then heated gently and filtered. To the filtrate a few drops of Wagner's reagents were added by the side of the test tube. A resulting precipitate confirmed the presence of alkaloids.

**Steroids:** About 0.1 g of the extract was put in a test tube and 10 mL of chloroform added and filtered. Then 2mL of the filtrate was mixed with 2 mL of a mixture of acetic acid and concentrated sulphuric acid. Formation of a Bluish green ring indicated the presence of steroids.

**Phenols**: About 0.1 g of the extract was put in a test tube and treated with a few drops of 2% of $FeCl_3$; a formation of blue green or black coloration indicated the presence of phenols.

In this finding, the antimicrobial activities of *P. africana* extracts from bark of root against some microbial strains, namely *Escherichia coli* and *Staphylococcus aureus* were inhibited 18 mm and 14 mm respectively (Table 2). Thus, the effective anti-bacterial activity was observed with methanol extract of root against *Escherichia coli*, which inhibited 18 mm. A significant anti-bacterial activity was also observed against *Staphylococcus aureus* with 14 mm of inhibition zone. The methanol extract of *P. africana* of root showed promising activity against *E. coli*, ATCC25922 and *S. aureus,* ATCC25922. Hence, *P. africana* that proves plant usefulness as therapeutic agent and it is used for the treatment of various diseases.

**Table 1. Results of phytochemical screening bark of root extracts of *P. africana***

| Phytochemical constituents | Bark of root extracts | |
|---|---|---|
| | Hexane | Methanol |
| Tannins | + | + |
| Saponins | + | + |
| Flavonoids | + | + |
| Terpenoids | + | + |
| Glycosides | _ | + |
| Alkaloids | + | + |
| Steroids | _ | + |
| Phenols | + | + |

*[NB: (+) and (-) indicate the presence and absence of Phytochemical Constituents respectively]*

As shown in the Table 3 the concentrations were doubled constantly through all; while the absorbance and scavenging activity were not, they were resulted in the form of inverse proportion relationships. Hence, absorbance was slightly raised while scavenging activity was slightly fall down. For instance, absorbance slightly improved from 0.124 to 0.180, but scavenging activity declined from 86.6% to 80.6%. In spite of the fact, anti-oxidant activities of this study were observed promote results (Table 3) for all concentrations of methanol extract of *P. africana* of root. Moreover, it has shown prospective result, selectively at lowest concentration and lowest absorbance. That means at the lowest concentration of 4 mg/mL and absorbance of 0.112 the scavenging activity was 87.9%, while at the highest concentration of 128 mg/mL and absorbance of 0.172 the scavenging activity was 81.5%.

**Table 2. Anti-bacterial activity screening results of bark of root extract of *P. africana***

| Test of strains | Methanol extract (mg/mL) | Zone of inhibition in diameter (mm) | Standards (mg/mL) | | Zone of inhibition in diameter (mm) | |
|---|---|---|---|---|---|---|
| | | | Vancomycin | Gentamicin | X | Y |
| *P. mirabilis* | 1.5 | 8 | 0.06 | 0.06 | 18 | 19 |
| ATCC2523 | 0.5 | 7 | 0.06 | 0.06 | 27 | 29 |
| *S. aureus* | 0.5 | 14 | 0.06 | 0.06 | 25 | 20 |
| ATCC25923 | 0.5 | 7 | 0.06 | 0.06 | 17 | 19 |
| *E. coli* | 1.5 | 18 | 0.06 | 0.06 | 22 | 25 |
| ATCC25922 | 0.5 | 9 | 0.06 | 0.06 | 23 | 24 |
| *P. aeruginosa* | 1.5 | 9 | 0.06 | 0.06 | 19 | 21 |
| ATCC27853 | 0.5 | 7 | 0.06 | 0.06 | 17 | 19 |

**Table 3. Anti-oxidant activity results of *P. africana* bark of root extract**

| S. No. | Concentrations(mg/mL) | Absorbance | % Scavenging Activity |
|---|---|---|---|
| 1 | 4 | 0.112 | 87.9 |
| 2 | 8 | 0.124 | 86.6 |
| 3 | 16 | 0.180 | 80.6 |
| 4 | 32 | 0.174 | 81.2 |
| 5 | 64 | 0.168 | 81.9 |
| 6 | 128 | 0.172 | 81.5 |

*Calculation Performed: (Standard absorbance-Sample absorbance) ÷Standard absorbance x 100: (0.927 – 0.112) ÷ 0.927 x 100 =87.9%*

Therefore, the anti-oxidant activities of root of *P. africana* were proved that the plant was held valuable medicinal constituents.

## 4. CONCLUSION

Different solvents (hexane and methanol) were used for phytochemical screening of plant materials of bark of root of *P. africana*, which was clearly validated in this study the presence of different phytochemical constituents. For instance, the results shows that methanol extracts were confirmed presence of phytochemical constituents namely, tannins, saponins, flavonoids, terpenoids, glycosides, alkaloids, steroids, and phenols, and also confirms that in hexane extracts of bark of root of *P. africana* absence of glycosides and steroids (in short no glycoside and steroid constituents in the hexane extract).

The results indicate that majority of the secondary metabolites are contained in the extracts of bark of root of *P. africana*. So this medicinal plant holds promises as source of pharmaceutically important phytochemical constituents. The effective anti-bacterial activity was observed in the methanol extract of bark of root against *Escherichia coli*. A significant anti-bacterial activity was also observed within it against the *Staphylococcus aureus*. Moreover, good potential anti-oxidant activity was observed for all concentrations of methanol extract of *P. africana* bark of root. The methanol extract of *P. africana* bark of root showed promising activity against *E. Coli* ATCC25922 and *S. aureus* ATCC25922. Anti-oxidant activities were observed encourage outcome for all concentrations of methanol extract of *P. africana* of root. Moreover, it has shown prospective result, selectively at lowest concentration and lowest absorbance. These findings suggested that *P. africana* bark of root could be a potential source of natural therapeutic agents for treatment of antibacterial and antioxidant ailments. Due to the presence of different phytochemical constituents in the bark of root of *P. africana*. Thus, *P. africana* that proves plant usefulness as therapeutic agent and it is used for the treatment of different ailments.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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