https://dx.doi.org/10.22161/ijmpd.4.3.2 *ISSN: 2456-8015*

Anti-bacterial and Anti-oxidant Studies on the Stem Bark Extracts of *Prunusafricana*

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***Abstract*— *Prunusafricana 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 stem bark extract from P. africana. The air dried and powdered plant material (300 g) was first soaked with 400mLofn-hexane for 72 hours and yielded 1.5g of n-hexane extract. Residue was soaked with 400mL of ethyl acetate for 48hours and afforded 4.5g of ethyl acetate extract. Finally, residue was soaked with 400mLof methanol and yielded 12.5g of methanol extract. The ethyl acetate extract showed inhibition zones of 16mm and 11mm against Escherichia coli and Staphylococcus aureus, respectively.The extracts also showed encourage results of DPPH radical scavenging activity at various concentrations. The ethyl acetate extract of P. africana of stem bark showed promising activity against E. coli, ATCC24534 and S.aureus,ATCC26452. 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 12.5mg/mL and absorbance of 0.208the scavenging activity was 77.6%, while at the highest concentration of 200mg/mL and absorbance of 0.288the scavenging activity was 68.9%.*

Keywords*— *stem bark; medicine; ailments;anti-bacterial; anti-oxidant; inhibition;Escherichia coli; Staphylococcus aureus.

I. INTRODUCTION

Prostate cancer is one of the most common non-skin cancers in men. It is caused by unregulated prostate cell division, which leads to abnormal growth, with the potential to spread to other parts of the body [1]. These neoplastic cells originate from highly specialized cells through a process of regression to an advanced stage. Unlike the normal parent cells, these cells divide continuously, resulting in a tumour. Approximately, 9–11% of men are at risk of clinically suffering from prostate cancer in their life time [2–5]. Prostate cancer is typically androgen-dependent during its initial stages when the hormone androgen binds to the androgen receptor (AR) and then trans activates target genes [6, 7].

Androgen and AR-mediated signalling are therefore crucialfor the development and functioning of both the normal prostate and prostate cancer. The importance of androgen in prostate cancer is further supported by the fact that prostate cancer rarely occurs in men with the deficiency in 5α-reductase, an enzyme that converts testosterone to its active metabolite 5α-dihydrotestosterone (DHT) [8]. Currently, one of the main approaches to the treatment of prostate cancer is down regulation of androgens by ant androgenic agents [5, 6, 9,10]. For years, prostate cancer, similar to other forms of cancer, has been managed through the conventional treatment modalities such as surgery, radiation therapy, cryosurgery, and hormone therapies [11].

However, there is still no effective treatment for advanced stages of prostate cancer. 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. Chemoprevention and chemotherapy, including the administration of one or more naturally occurring antiprostate cancer agents [1, 3, 4, 10] have been identified as approaches by which the prevalence of such diseases as prostate cancer can be reduced, suppressed, or reversed. In the last decades, several plants have been confirmed to contain chemopreventive and therapeutic agents for various cancers including prostate cancer [10, 12-14]. More importantly, over 60% of currently used anticancer agents are estimated to be from natural sources [13].

https://dx.doi.org/10.22161/ijmpd.4.3.2 *ISSN: 2456-8015*

Among plants with enormous antiprostate cancer potential is *Prunusafricana* (African cherry), which belongs to the plant family Rosaceae.

This evergreen miraculous plant is only found in sub-Saharan Africa and is highly sought after owing to its unique anticancer phytochemicals [1, 2, 15]. 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 [1]. More importantly, the use of *P. africana* has been patented in France for prostate cancer treatment [16].

In addition to prostate cancer, the bark extract of P. africana has for many years been used for the treatment of benign prostatic hyperplasia (BPH). Recent studies by Nyamai*et al*. [17] and Jena *et al*. [18] confirmed the effectiveness of the bark extract of *P. africana* in BPH treatment and attributed this to the synergistic effects of pentacyclictriterpenoids, ferulic esters of long-chain fatty alcohols, and phytosterols contained in *P. Africana* bark. The phytosterols (including *β*-Sitosterol) and pentacyclictriterpenoids (including ursolic acids) also have anti-inflammatory effects on the prostate [17].

In ATM, *P.africana* is also used to treat myriad of diseases including but not limited to diarrhea, epilepsy, arthritis, hemorrhage, and hypertension [15, 16, 19–21]. 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 [20, 21].

Many well-known and beloved species of Rosaceaefamily have great economic importance. Hence, they are known as "edible temperate zone fruits".The Rosaceae is the 19th largest family of plant which includes more than 100 genera and 2830-3100 species among which *P.africana* has well claimed medicinal value.*P.africana*is a geographically widespread 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)[19,22].The genus name "*Prunus*" is derived from a Latin wordwhich refers to the plum family, and the scientific name"*Prunusafricana*" refers to the species of African origin[19, 23, 24].The genus *Prunus*comprises over 400 species, of which only98 are of great importance [25].

The African cherry is a species of the genus *Prunus*, with a mature stem diameter ofup to 1 m and a height of more than 40 m with open branche sand a blackish-brown bark. Leaves are simple, alternate, oval-shaped, shiny-deep green on thetop side and lighter on the underside, with a conspicuousprominent midrib on the underside. Flowersare greenish or white, and fruits are spherical, 7mm long, 1.3cm wide, pinkish-brown, and bilobed, withthin, dark red to reddish brown pulp when ripe [20].

II. PLANT MATERIALS AND METHODS

The stem bark of *P. africana* was collected from unique place name haqoro, Misha Woreda, Hadiya Administrative Zone, Southern Nations Nationalities and People Regional State (SNNPR). A voucher specimen was stored at the National Herbarium of Addis Ababa University (Voucher no.TA003), Addis Ababa, Ethiopia.

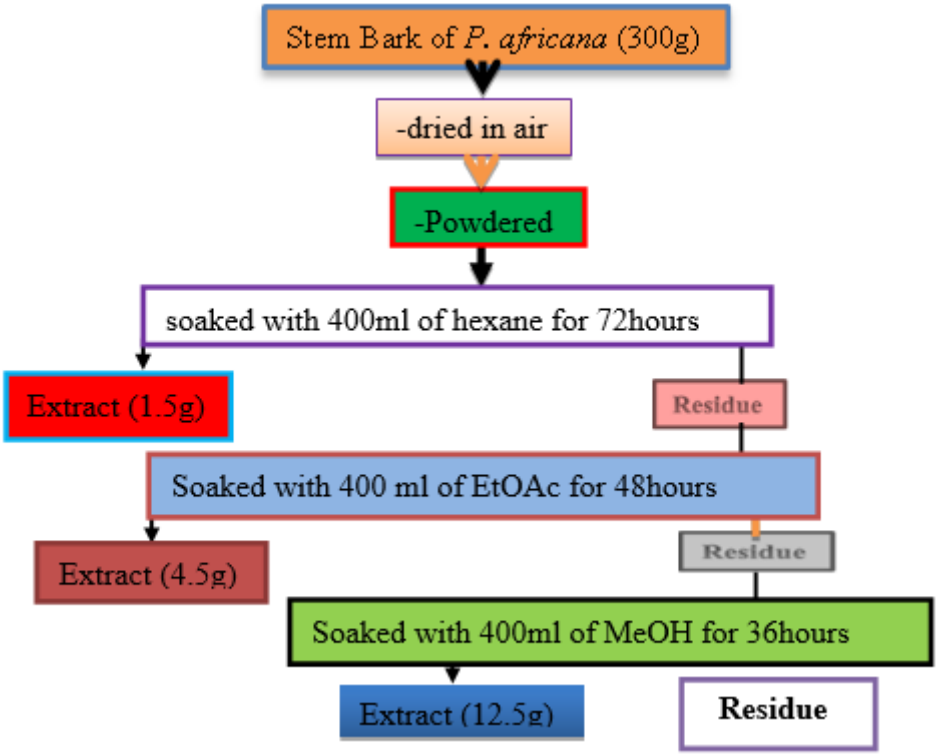
2.1 Apparatus and Chemicals

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 (300g) was first soaked with 400mL 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 400mL of ethyl acetate for 48hours and the extract was collected. This filtrate was evaporated under reduced pressure using the Rotavapor. Finally, the solvent free residue was soaked with 400mLof 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:

https://dx.doi.org/10.22161/ijmpd.4.3.2 *ISSN: 2456-8015*



Scheme 1: Method used to extract stem bark of P. africana

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 121^0C 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 6cm each were drilled in each agar plate using a cork borer. Three of the wells were filled with 0.1ml of the 500mg/mL of the extract. The other wells were filled with 0.1ml of 500mg/mL of penicillin and 0.1ml of 100% DMSO positive and negative controls respectively. The plates were labelled on the underside and incubated at 37^0C for between 24-48 hours and the zones of inhibition measured in mm with the aid of a ruler [26].

The crude extracts of *P. africana* have been claimed to be active against some microbes like *Pseudomonas aeruginosa(P. aeruginosa), Staphylococcus aureus(S.aureus), Streptococcus pneumoniae(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, etc...).It is typically an opportunistic pathogen that seldom causes disease in healthy subjects. Normally, for an infection to occur, some disruption of the physical barriers(skin, mucous membranes) or by passing of them(e.g. urinary catheters, endotracheal tubes or other invasive devices) or underlying dysfunction of the immune defence. Infections are often difficult to eradicate due to *P. aeruginosa* being resistant to many antimicrobials[27].

Escherichia coli species are Gram-negative bacilli that exist singly or in pairs and are commonly present in the intestines of humans and animals. They are motile by peritrichous flagella. *E. coli* are found as a commensal in the intestinal tract of humans and other warm blooded animals. The commensal strains of *E. coli* are non-pathogenic and do not cause an infection in the host;however, some of its strains are pathogenic and can cause several diseases in humans like neonatal meningitis, intestinal infections and urinary tract infections. Almost 80-85% of the uncomplicated urinary tract infections are caused by *UropathogenicE.coli*[28].Pathogenic strains(varieties) of *E. coli* that resutindiarrhoea accompanied by vomiting and fever but the type of diarrhoea differ in each case include *EnterpathogenicE. coli (EPEC), EnteroinvasiveE. coli (EIEC),*

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Enterotoxigenic E.coli (ETEC), and *Enteroaggregative E. coli (EAEC).Uropathogenic E. coli* causes 90% of urinary tract infections in anatomically normal and unobstructed urinary tracts exclusively [29, 30]. The microbial used as test strain were *S. aureus* ATCC26452 for Gram positives, and *P. aeruginosa* ATCC37974, *E. coli* ATCC24534 and *Proteus mirabilis (P. mirabilis)* ATCC23478, for Gram negatives; Chloramphenicalwas used as the standard drug for Gram positives while Fluconazole for Gram negative.

Anti-oxidant Assay

The percentage of antioxidant activity (AA %) of each substance 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*. [31]. 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 absorbance at 517nm after 100 min of reaction using a UVVIS spectrophotometer (DU 800; Beckman Coulter, Fullerton, CA, USA). The mixture of ethanol (3.3mL) and sample (0.5mL) serve as blank. The control solution was prepared by mixing ethanol (3.5mL) and DPPH radical solution (0.3mL) [32].

This research was conducted by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical solution was prepared in methanol, which means 6mg of DPPH mixed with 100mL of methanol, and the sample solutions were also prepared in 3mL of methanol; from lowest to highest 12.5mg/mL, 25mg/mL, 50mg/mL, 100mg/mLand 200mg/mL, then 5mL of DPPH solution was mixed with 2ml of the sample solutions. After incubation for 30 min in the oven at temperature of 40ºC; finally, at the absorbance of 517nm 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 (3mL) and sample (2mL) serve as blank. The control solution was prepared by mixing methanol (4.5mL) and DPPH radical solution (0.5mL).

III. RESULTS AND DISCUSSIONS

3.1 Phytochemical Screening of *P. africana*stem barkExtract

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 0.1g of the extract was added in to a test tube. To the test tube 5mLof 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 0.1 g of the extract was taken in a clean test tube; 2 mLof 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 2 mL 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 2 ml 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.

https://dx.doi.org/10.22161/ijmpd.4.3.2 *ISSN: 2456-8015*

Table 1. Results of phytochemical screening stem bark extracts of P.africana

Phytochemical constituents	**stem bark extracts**	
	Hexane	**Ethyl acetate**
Tannins	+	+
Saponins	+	+
Flavonoids	+	+
Terpenoids	+	+
Glycosides	_	+
Alkaloids	+	+
Steroids	+	_
Phenols	+	+

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

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

Test of strains	**Extract ethyl acetate (mg/m)**	**Inhibition zone in diameter (mm)**	**Standards (mg/ml)**		**Inhibition zone in diameter (mm)**	
			Chloramphenical=X	Fluconazole= Y	X	Y
***Proteus mirabilis* ATCC23478**	2	5	0.05	0.05	15	20
	1	4	0.05	0.05	13	17
***Staphylococcu s. aureus* ATCC26452**	2	11	0.05	0.05	14	16
	1	7	0.05	0.05	17	19
***Escherichia coli* ATCC24534**	2	16	0.05	0.05	17	18
	1	7	0.05	0.05	15	17
***Pseudomonas aeruginosa* ATCC27974**	2	7	0.05	0.05	16	19
	1	6	0.05	0.05	15	18

https://dx.doi.org/10.22161/ijmpd.4.3.2 *ISSN: 2456-8015*

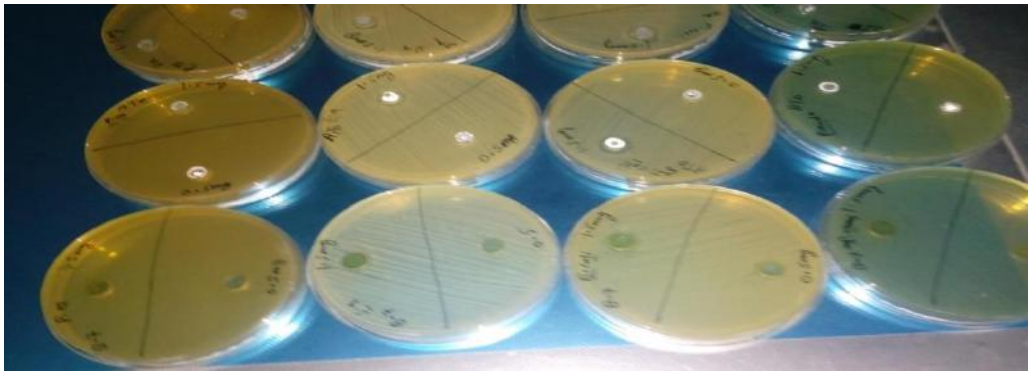

Fig.1: Anti-bacterial activity screening results of stem bark extract of P. Africana

From this conducting, the antimicrobial activities of *P.africana* extracts from stem bark against some microbial strains, namely *Escherichia coli* and *Staphylococcus aureus* were inhibited 15mm and 10mm respectively (Table 2). Thus, the effective anti-bacterial activity was observed with ethyl acetate extract of stem bark against*Escherichia coli*, which inhibited 15mm. A significant anti-bacterial activity was also observed against *Staphylococcus aureus* with 10mm of inhibition zone. The ethyl acetate extract of *P. africana* of stem bark showed promising activity against *E. coli*, ATCC24534 and *S.aureus*, ATCC26452. Hence, *P.africana*that proves plant usefulness as therapeutic agent and it is used for the treatment of various diseases.

Table 3: Anti-oxidant activity results of P. africanastem barkextract

S. No.	Concentrations(mg/mL)	Absorbance	% Scavenging Activity
1	12.5	0.208	77.6
2	25	0.220	76.3
3	50	0.276	70.2
4	100	0.282	69.6
5	200	0.288	68.9

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 0.208 to 0.288, but scavenging activity declined from 77.6% to 68.9%. In spite of the fact, anti-oxidant activities of this study were observed promote results (Table 3) for all concentrations of ethyl acetate extract of *P. africana*of stem bark. Moreover, it has shown prospective result, selectively at lowest concentration and lowest absorbance. That means at the lowest concentration of 12.5 mg/mL and absorbance of 0.208the scavenging activity was 77.6%, while at the highest concentration of 200mg/mL and absorbance of 0.288the scavenging activity was 68.9%. Therefore, the anti-oxidant activities of stem barkof *P. africana* were proved that the plant was held valuable medicinal constituents.

IV. CONCLUSION

Different solvents namely, hexane and ethyl acetate were used for phytochemical screening of plant materials of stem bark of *P.africana*, which was clearly validated in this study the presence of different phytochemical constituents. For instance, the results shows that ethyl acetate extracts were confirmed presence of all phytochemical constituents exceptsteroidsnamely, tannins, saponins, flavonoids, terpenoids, glycosides, alkaloids, and phenols, and also confirms that in hexane extracts of stem bark of *p.africana*absence of glycosides.

The results indicate that majority of the secondary metabolites are contained in the extracts of stem bark of *p.africana*. So this medicinal plant holds promises as source of pharmaceutically important phytochemical

https://dx.doi.org/10.22161/ijmpd.4.3.2 *ISSN: 2456-8015*

constituents. The effective anti-bacterial activity was observed in the ethyl acetate extract of stem bark 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 ethyl acetateextract of *P. africana*leave. The ethyl acetate extract of *P. africana*leave showed promising activity against *E. coli* ATCC24534 and *S.aureus*ATCC26452. Anti-oxidant activities were observed encourage outcomefor all concentrations of ethyl acetate extract of *P. africana*ofstem bark. Moreover, it has shown prospective result, selectively at lowest concentration and lowest absorbance. These findings suggested that *p. africana*stem bark 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 stem barkof*p.africana*. Thus, *p.africana*that proves plant usefulness as therapeutic agent and it is used for the treatment of different ailments.

ACKNOWLEDGEMENT

I would like to give up warmest thanks for one and almighty God! And next I would like to thanks all those who financial supports for this study.

COMPETING INTERESTS

Authors have stated that no competing interests exist.

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