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Phytochemical Investigation and Characterization on the Root Bark Extract of Prunus Africana

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Abstract
Prunus africana is one of the most popular to treat benign prostate hyperplasia (BHP), and to treat diarrhoea, dysmenorrhoea, infertility, irregular menstruation, kidney disease, disorders, fevers, obesity, pneumonia, hypertension, antigonorrheic, antimalarial, chest pain; other various diseases. The air dried and powdered plant material (200g) was first soaked with 500 mL n-hexane for 72 hours and yielded 1.3 g of n-hexane extract. Marc was soaked with 400 mL of ethyl acetate for 72 hours and afforded 2g of ethyl acetate extract. Finally, Marc was soaked with 400mL of methanol and yielded 12.6 g of methanol extract. The ethyl acetate extract of the root bark of *P. africana* afforded a compound coded as **AYU**. Its Structural determination was accomplished by means of spectroscopic techniques, namely IR, ^{1}H NMR, ^{13}C NMR and DEPT-135. The compound, **AYU** was isolated and characterized from the root bark of *P. african*. It was shown that spot on TLC only up on spraying 1% vanillin sulphuric acid and after heating for a few minutes. Generally, chromatographic techniques like PTLC and HPLC are required to isolate more compounds from leaves of *P. africana*. Also MS and 2D NMR spectroscopic techniques are needed to fully characterize the isolated compound.
Keywords: P.africana; benign prostate hyperplasia; characterization; chromatographic techniques; spectroscopic techniques.
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1. Introduction

Plants have long history of being used for a wide variety of purposes including food, clothing, shelter, tools, *weapons, and therapeutic agents*.[1] phytochemical study of plants is of the great importance in developing drugs. Drugs are strictly defined as chemical substances that are used to prevent or cure diseases in humans, animals and plants. Drugs from natural products are secondary metabolites and their derivatives. Natural products have been a major source of drugs for centuries, with more than 25% of the pharmaceuticals in use today derived from natural products. Natural product chemistry is a part of organic chemistry that covers the chemistry of naturally occurring organic compounds: their biosynthesis, function in their environment, metabolism and more conventional branches of chemistry such as structural elucidation and synthesis. Primary metabolism is the system of biochemical reactions whose products are vital for their life cycles. Secondary metabolism refers to the functions of an organism yielding products that are not necessary for the essential biochemical events. Secondary metabolites are thus compounds which are often species dependent. The most challenge of human civilization is getting the proper drug treatment. According to the World Health Organization (WHO) estimation that around 80% of the world population in developing countries relies on traditional medicines for primary health care needs, of which a major proportion corresponds to plant extracts.[2] The World Health Organization has also recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines.[3] From 70-95% of citizens in a majority of developing countries like in Asia, Africa, Latin America and the Middle East use traditional medicine for the management of primary health care. A survey made by the World Health Organization (WHO) showed that 70-90% populations of some industrialized nations like Canada, France, Germany and Italy, for instance, are using traditional medicines under the title “complementary” or “alternative” medicines. The survey also showed that in some African countries like Ghana, Mali, Nigeria and Zambia, around 60% of children due to malaria are treated at home with herbal medicine.[1] Worldwide, infectious diseases are the leading causes of death accounting for approximately one half of all deaths in tropical countries and a significant problem in developed nations. It is estimated that infectious diseases are the underlying causes for 8% deaths occurring in US. Despite the use of herbal medicines over many centuries of the world, only a relatively small number of plant species has been studied for possible medical applications. [4]

1.1 Prunus africana (P. africana)

P. africana is a geographically widespread tree to forest habitats of the African continent. It is an evergreen hardwood tree; over 30 m-60 m in height and up to 1.5 meter diameter. The leaves are simple and alternately arranged. The flowers are small, androgynous, and insect-pollinated. It is known commonly as African cherry or Pygeum africanum. The Rosaceae family includes many well known and beloved species of economic importance particularly edible temperate zone fruits, ornamentals and some important medicinal plants.[5, 6] The Rosaceae is the

19th largest family of plant and it includes more than 100 genera and 2830-3100 species among which P.africana has well claimed medicinal value. It is widely distributed in Angola, Mozambique, Zambia, Zimbabwe, Burundi, Congo, Kenya, Rwanda, Sudan, Tanzania, Uganda, Cameroon, Nigeria, Sao Tome, and Ethiopia (northwest highlands, southeast Highlands Harerge, Illubabor, Kefa, Arsi and Wolega).[5]

1.2 P. africana Species and Their medicinal uses

The known Prunus species are plums (P. domestica L.), cherries (P. avium L.), peaches (P. persica L.), almonds (P. dulcis), and apricots (P. armeniaca L.). All of them are commercially interesting species.[7] It is most popular to treat benign prostate hyperplasia (BHP) which is a non-cancerous enlargement of the prostate all over the world. Traditional healers across Africa use P. africana as a medicine to treat diarrhoea, dysmenorrhoea, epilepsy, impotency, infertility, irregular menstruation, kidney disease, mental illness, eye disorders, fevers, obesity, pneumonia, arthritis, haemorrhage, hemorrhoids, hypermenorrhea, hypertension, prostate gland enlargement , antigonorrheic, antihelmentic, anti-inflammatory, antimalarial, chest pain, antiparasiticide and anti-rheumatic.[5, 8] The pharmacological efficacy is believed to be due to various compounds of known and unknown. Among the known compounds, three groups are great important: (A) phytosterols especially β-sitosterol, have anti-inflammatory properties that inhibit the swelling of the prostate gland, (B) pentacyclic triterpenoids that provide anti-edematous activity, and (C) ferulic acid esters, which have a powerful hypocholestero-lernic activity in the prostate, as well as anti-tumor activity.[9] Leaves are used as an inhalant for fever or are drunk as an infusion to improve appetite.[10] It is also used as a remedy for stomach pain, and insanity.[11]

1.3 Uses of P. africana with Exploitation

The discovery of the medicinal properties of Prunus bark initiated a decades' long harvest for international market needs, in which Cameroon supplied over half, followed by Madagascar, the Democratic Republic of the Congo (DRC), Kenya, Uganda, and Equatorial Guinea.[7] For the last 35 years, the African cherry (*P. africana*) has been used in the treatment of benign prostatic hyperplasia and other disorders. The bark, from which the treatment is derived, is entirely wild-collected. The major exporters of bark include Cameroon, Madagascar, Equatorial Guinea, and Kenya. To the group of Fournier France, Indian, and Italy which exported 86% of the world bark extracts. Worldwide exports of dried bark in 2000 have been estimated at 1350-1525 metric tons per year, down from its peak of 3225 tons in 1997. Bark extracts (6370-7225 kg per year) are worth an estimated $ 4.36 million per year.[11] While, in Ethiopia, the *African cherry* is basically used for fuel wood, charcoal, ornamental, windbreaks and green manure.[12] Bark infusions were used in the treatment of chest pains and as a purgative for cattle.[13] In Africa, 500 tonnes of bark were harvested for use in traditional medicine annually.[14] The use of the species in pharmaceutical industry commercially began in 1970s. In 1980, only 200 tonnes of *P. africana* bark were harvested.[15] The demand for the bark in pharmaceutical industry increased from then and by 1997 the demand had risen to 3500 tonnes.[16] This involved processing and marketing of bark and bark extracts for the treatment of benign prostate hyperplasia.[17] Benign prostatic hyperplasia (BPH) is a condition common in most men and manifests itself as increased frequency in urination, pain in passing urine, inability to empty the bladder and post urinary dribbling.[18] Phytosterols eliminate vassal congestion and excess blood hence reduces the size of prostate adenomas. The pentacyclic triterpenoids block enzymatic activity consequently inhibits inflammation in the prostate.[19, 20] Thus, the study was aimed at phytochemical investigation and characterization, on the root bark of *P.africana*.

2. Materials and Methods

2.1 Plant Material

The root bark of P. africana was collected from Shero kebele borderline of Abose and Weshiso land, Misha Woreda, Hadiya Administrative Zone, Southern Nations Nationalities and People Regional State.

2.2. Instruments, Apparatus and Chemicals

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance 400 MHz spectrometer in $CDCl_3$ with TMS as internal started. The ultra-violet and visible (UV-Vis) spectra were taken on GENESY'S 2PC UV-Vis scanning spectrometer (200-800 nm). IR spectra were obtained on Perkin-Elmer BX infrared spectrometer (400-4000 cm^{-1}) using KBr. Melting point was recorded using digital melting point apparatus. Analytical thin layer chromatograms were run on 0.2 mm thick layer of silica gel coated on aluminum foil. Column chromatography was performed using silica gel (70-230 mesh). List of solvents were used in this research like: n-hexane, ethyl acetate, methanol and others. Some of the apparatus were used: funnels, round bottom flasks, vials, glass wares, refrigerator, Whatman No.1 filter papers, grinder, drying oven, measuring cylinders, TLC Chamber, capillary tubes, cuvette quartz etc...

2.3. Extraction and Isolation

The air dried and powdered plant material (200g) 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 and yielded 1.3g. The solvent free Marc was then soaked with 400 ml of ethyl acetate for 72 hours and the extract was collected. This filtrate was evaporated under reduced pressure using the Rotavapor and afforded 2g. Finally, the solvent free Marc 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 and it was yielded sample of 12.6g, and its extract was afforded many spots on TLC. Solvent was removed using rotary evaporator. Compound on TLC was detected after spraying 1% vanillin sulphuric acid and after heating for a few minutes. There was no visible spot for n-hexane extract, but ethyl acetate extracts were showed three colored spots by using solvent system of n-hexane: ethyl acetate (6:4), and its yield of 2g crude extract was dissolved in itself with equivalent amount of silica gel, dried using Rotavapor and applied to a silica gel (200g) column chromatography which was packed with n-hexane (100%).

Table1: The column chromatography elution of *P. africana* root bark extract with their respective volumes and masses

No. of fractions	Volume (mL)	Mass (mg)	No. of fractions	Volume (mL)	Mass (mg)	No. of fractions	Volume (mL)	Mass (mg)
1	20	1.700	50	20	3.327	81	20	4.821
2	20	2.400	51	20	7.654	82	20	2.813
3	20	5.100	52	20	9.371	83-94	240	-
4	20	2.700	53	20	13.500	95	20	0.520
5	20	4.100	54	20	5.500	96	20	0.038
6	20	5.100	55	20	7.974	97	20	0.068
7	20	3.700	56	20	3.500	98	10	1.047
8	20	4.900	57	20	4.852	99	10	0.841
9-27	380	-	58	20	7.469	100	10	2.039
28	20	40.490	59	20	5.374	101-164	640	-
29	20	63.470	60	20	4.346	165	10	0.024
30	20	76.214	61	20	3.421	166	10	0.019
31	20	87.090	62	20	0.6784	167	10	1.072
32	20	120.670	63	20	7.204	168	10	2.008
33	20	94.050	64	20	9.063	168A	50	6.005
34	20	56.478	65	20	1.845	169	50	-
35	20	100.290	66	20	4.562	170-195	260	-
36	20	83.940	67	20	1.784			
37	20	68.326	68	20	2.453			
38	20	78.192	69	20	1.963			
39	20	39.156	70	20	7.270			
40	20	41.345	71	20	2.846			
41	20	35.094	72	20	0.248			
42	20	44.220	73	20	0.251			
43	20	54.021	74	20	3.618			
44	20	12.355	75	20	24.380			
45	20	12.114	76	20	9.0531			
46	20	13.980	77	20	4.731			
47	20	27.139	78	20	6.039			
48	20	57.275	79	20	0.371			
49	20	61.723	80	20	3.521			

Key: sign (-) indicates the absence of mass.

The numbers of fractions were collected. Out of these fractions which were collected using the solvent systems increased polarity, some of them were showed the characteristic colored spots on TLC up on spraying 1% vanillin sulphuric acid and after heating for a few minutes. The remaining fractions did not show the characteristic colored spots on TLC up on spraying 1% vanillin sulphuric acid and after heating for a few minutes. Among fractions, fraction 58 showed single spot on TLC using the solvent system n-hexane: ethyl acetate (6:4) upon spraying 1% vanillin sulphuric acid and after heating for a few minutes. Finally, the dried sample of this fraction was afforded 17mg of the compound, **AYU**.

3. Results and Discussion

3.1 Phytochemical Screening of root bark extract of P. africana

The extracts phytochemical analysis for identification of chemical constituents was done using standard procedures from literature.

1. Tannins: About 0.1g of the extract was put in a test tube and 20mL 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. A brownish green color or a blue-black coloration indicated the presence of tannins.
2. Saponins: About 0.1g of the extract was mixed with 5mL of water and vigorously shaken. The formation of stable form indicated the presence of saponins.
3. Flavonoids: About 0.1g of the extract was added in to a test tube. To the test tube 5mL of dilute ammonia and 2mL of concentrated sulphuric acid was added and heated for about 2 minutes. The appearance of a yellow color indicated the presence of flavonoids.
4. Terpenoids: About 0.1g of the extract was taken in a clean test tube; 2mL of chloroform was added and vigorously shaken, then evaporated to dryness. To this, 2mL of concentrated sulphuric acid was added and heated for about 2 minutes. A grayish color indicated the presence of terpenoids.
5. Glycosides: About 0.1g of the extract was mixed with 2mL of chloroform and 2mL of concentrated sulphuric acid was carefully added and shaken gently, then the observations were made. A red brown color indicate the presence of steroidal ring (glycone portion of glycoside)
6. Alkaloids: About 0.1g 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.
7. Steroids: About 0.1g of the extract was put in a test tube and 10mL of chloroform added and filtered. Then 2mL of the filtrate was mixed with 2mL of a mixture of acetic acid and concentrated sulphuric acid. Bluish green ring indicated the presence of steroids.
8**. Phenols**: About 0.1g of the extract was put in a test tube and treated with a few drops of 2% of $FeCl_3$; blue green or black coloration indicated the presence of phenols.[21]

Table 2: Results of phytochemical screening of root bark extract of *P. africana*

Phytochemical Constituents	**Extracts**	
	Ethyl acetate	Hexane
Tannins	+	+
Saponins	+	_
Flavonoids	+	+
Terpenoids	+	_
Glycosides	_	+
Alkaloids	+	+
Steroids	+	_
Phenols	+	+

Key: + indicates presence of chemical Constituents and _ indicates absence of chemical Constituents

The air dried and powdered leaves of the *P. africana* (200g) were extracted with solvents of n-hexane, ethyl acetate and methanol. These extracts when developed on TLC both the n-hexane and ethyl acetate extracts have shown three colored spots, but methanol extract was afforded many spots on TLC. The orange organic extract of ethyl acetate (2g) was subjected to column chromatography on silica gel.

3.2 Characterization of Compound, AYU

The compound, AYU was obtained as a white solid that showed a characteristic color change to violet on TLC plate upon spraying 1% vanillin sulphuric acid and after heating for a few minutes. It has retention factor, RF value 0.8 using hexane: ethyl acetate (6:4) as solvent system. In the IR spectrum of the compound, AYU the absorption band at 3400cm^{-1} showed the O-H stretching that indicated the presence of a hydroxyl group. The strong absorption band at 2922cm^{-1} showed the presence of the C-H stretching of the olefinic group. The weak absorption band at 2850cm^{-1} showed the presence of the C-H stretching for sp^3 groups. Two strong absorption band at1690cm^{-1} and 1609cm^{-1} showed the presence of the olefinic C=C stretching. The absorption band at 1170cm^{-1} showed the presence of the C-O bond stretching.

Table 3: IR spectral peak values and functional groups obtained from the root bark extract of *P. africana* (AYU)

Extract prepared in	peak values in cm^{-1}	functional groups
Ethyl acetate	3400	-OH (hydroxyl group)
	2922	Sp^2 C-H stretching
	2850	SP^3 C-H stretching
	1690 and 1609	Olefinic group of C=C stretching
	1468	Methylene group bending
	1358	Methyl group bending
	1170	CO bond stretching

The ^{1}H NMR spectrum showed ttiplet peaks at δ 5.29, integrating for one proton, corresponded to the methine

proton groups and assigned to C-1. Coupled proton peaks, which is triplet peaks at δ 2.10; 1.99, integrating for two protons, which were corresponded to the methylene proton groups and assigned to C-2. The pentet peaks at δ 3.41, integrating for one proton, corresponded to the methine proton group that assigned at C-3. Coupled proton peaks, which is triplet peaks at δ 1.89; 1.60, which integrating for two protons corresponded to the methylene proton groups and attached to C-4. Triplet peaks at δ 1.90, integrating for one proton corresponded to the methine proton, which were assigned to C-5. The quartet peaks at δ 3.3, integrating for one proton, corresponded to the methine proton and assigned to C-9. Coupled proton peaks, which is doublet peaks at δ 2.30; 2.10, integrating for two protons corresponded to the methylene proton which was attached to C-11. The pentet peaks at δ 2.10 which integrating for one proton corresponded to the methine proton and attached to C-12. Coupled proton peaks, which is doublet peaks at δ 2.30; 2.10, which integrating for two protons corresponded to the methylene proton and attached to C-13. Singlet peak at δ 1.20 which integrating for six protons corresponded to the methyl protons and attached to C-14 and C-15. Doublet peaks at δ 1.10, which integrating for three protons corresponded to the methyl protons and attached to C-16. Doublet peaks at δ 1.23, which integrating for three protons corresponded to the methyl protons and it attached to C-17.

The ^{13}C NMR and DEPT-135 spectrum of compound, AYU showed well resolved resonance of 17C atoms of which 4, 4, 5, and 4 of them were methyl, methylene, methine, and quaternary carbon groups, respectively.

Table 4: ^{1}H NMR spectral data of compound (AYU)

S. No.	Peaks (δ)	Peak multiplicities	Proton No.	Assigned carbon	Remark
1	5.29	Triplet	One	C-1	Methine
2	2.10; 1.99	Triplet	Two	C-2	methylene
3	3.41	Pentet	One	C-3	Methine
4	1.89; 1.60	Triplet	Two	C-4	Methylene
5	1.90	Triplet	One	C-5	methine
6	3.30	Quartet	One	C-9	Methine
7	2.30; 2.10	Doublet	Two	C-11	Methylene
8	2.10	Pentet	One	C-12	Methine
9	2.30; 2.10	Doublet	two	C-13	methylene
10	1.20	Singlet	Six	C-14 and C-15	Methyl
11	1.10	Doublet	Three	C-16	Methyl
12	1.23	Doublet	Three	C-17	Methyl

The ^{13}CNMR and DEPT-135 spectrum of compound (**AYU**) showed well resolved resonance of 17 carbon atoms and from them were: four methyl groups, four methylene groups, five methine groups and four quaternary carbons.

Table 5: ^{13}C NMR and DEPT-135 (400 MHz, $CDCl_3$) spectral data of Compound (AYU)

Carbon No.	^{13}C NMR (in ppm)	DEPT (in ppm)	Remark
1	122.66	122.65	CH
2	35.61	35.62	CH_2
3	68.00	68.00	CH
4	33.00	33.10	CH_2
5	41.55	41.52	CH
6	32.96	-	C (Quaternary carbon)
7	140.87	-	C (Quaternary carbon)
8	129.9	-	C (Quaternary carbon)
9	52.20	52.21	CH
10	139.05	-	C (Quaternary carbon)
11	40.08	40.08	CH_2
12	23.21	23.22	CH
13	39.00	39.10	CH_2
14	25.90	25.86	CH_3
15	25.90	25.86	CH_3
16	24.72	24.71	CH_3
17	20.41	20.40	CH_3

Finally, from the all above data, namely IR spectral data, ^{13}C NMR, DEPT-135 and ^{1}H NMR spectral data the proposed structure of compound (**AYU**) would be shown below:

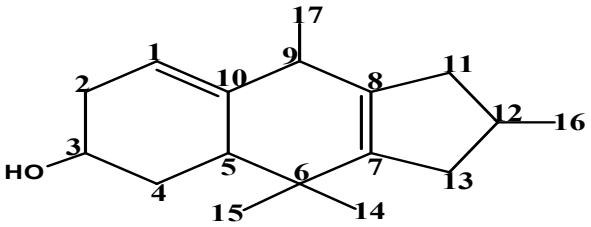

Fig. 1. The proposed structure of the compound, AYU
Key: AYU= code, which was given for proposed structure, there is no other meaning

4. Conclusions and Recommendations

P. africana is a commercial by its stem bark which is most popular to treat benign prostate hyperplasia (BHP). In the this study, root bark of *p.africana* were showed the presence of phytochemical constituents such as alkaloids, flavonoids, terpenoids, saponins, tannins, steroids, and phenols of ethyl acetate extracts of it and also confirms that the absence of saponins, terpenoids, and steroids in the hexane extracts of *P.africana* root bark. The root bark of *P.africana* was extracted with solvents of n-hexane, ethyl acetate, and methanol and their yields 1.3g, 2g, and 12.6g, respectively. The orange organic extract of ethyl acetate (2g) was subjected to column chromatography on silica gel and numbers of fractions were collected. That is, from the IR spectrum, the absorption band at 3400cm^{-1} shows the O-H stretching that confirms the presence of a hydroxyl group. Also the strong absorption band at 2922cm^{-1} shows the presence of the C-H stretching. Two strong absorption band at 1690cm^{-1} and 1609 cm^{-1} shows the presence of the C=C stretching of the olefinic group.

As a final point, from this study, the compound AYU was elucidated and characterised by incorporating by spectroscopic techniques such as IR spectral data, ^{13}C NMR, DEPT-135, and ^{1}H NMR spectral data obtained.

Despite the traditional use of this plant for the treatments of various ailments, in many parts of the world there is no more report on phytochemical analysis on the root bark of *P. africana*. This is provoked tricky to compare and contrasts my work with the relative of other work. Thus, this study may serve as baseline for researchers who are inspired and interested to conduct such type of research in the future.

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Competing Interests
Authors have declared that no competing interests exist.

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