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The phytochemical components and acute toxicity of methanolic stem bark extract of *Prunus africana*

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Abstract: currently there is a growing interest in use of medicinal plants. This has led to amplified need of scientific analysis of their safety and extractive phytochemical component, thus providing health care workers with adequate knowledge regarding the plants, and this in turn will assist patients make informed choice on their utilization. The bark extract of Prunus africana (P.africana) has been used traditionally for decades in the treatment of various conditions such as abdominal upset, decreased appetite, fever, malaria, prostate cancer, and benign prostatic hyperplasia. The stem bark of P.africana was evaluated for its phytochemical constituents and acute toxicity effect on fifteen female wistar rats. The bark extract was collected in Mukurweini in Nyeri County, Kenya.

The bark extract of P.africana was soaked in methanol. The mixture was filtered and the organic solvent was evaporated to near dryness by vacuum evaporation using rotary evaporator. The bark extract was subjected to a phytochemical screening where extractive protocols were applied to detect majority of molecules present. The evaluation of acute toxicity of methanolic extract of the bark followed the modifiedLorke's model.

The phytochemical screening of the methanol bark extract revealed carbohydrates, flavonoids, tannins and saponins. The methanolic bark extract of P.africana at dose less than or equal to 5000 mg/kg body weight was found to be safe, therefore it isrelatively harmless based on Loomis and Hayes classification of acute toxicity.

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I. INTRODUCTION

Prunus africana, also known as *Pygeum africana*is ever green canopy tree native in Africa countries[1]. It is mainly wellestablished on tallhighlands forests across the Africa continent[2].Pygeum belongs to a member of Rosacea family[3].In Africa the tree are found in the rainforests of equatorial region, in Angola, Congo, Cameroon, Ghana, Kenya, Ethiopia, Madagascar, Mozambique Malawi, South Africa, Uganda, Tanzania, Zimbabwe and Zambia. The trees are usually 10 to 25 meters long but can grow up to 45 meters, their trunk is straight cylindrical with a dense round crown. The leaves have a deep green and glossy appearance. The flowers are small and the colour ranges from white to whitish cream. The fruits resembles cherry and their colour ranges from red to purplish-brown. The wood is pale red in color and has a strong cyanide smell when freshly cut. The bark, bruised leaves, and fruits have a strong and a bitter-almond smell[2].The *P.africana*bark extract has been used for several medicinal purposes; leaves has been used as inhalant for fever or drunk as appetizer, water extract from powdered bark has been used as a remedy for stomach ache or as a purgative for cattle[5]. *P.africana*bark, bruised leaves, and fruits have a strong and a bitter-almond smell. *P.africana*,it been crucial in the clinical management of BPH for numerous decades[1].The concentration of most compounds used in treatment of BPH in these trees either from the wild or domesticated habitats do not vary significantly, but some phytochemicals concentration vary hence the need of a phytochemical analysis[6].The extensive usages of *P.africana* to-date these trees are at the verge of extinction [3,4]

II. MATERIAL AND METHODS

2.1 Experimental procedures

2.1.1 Identification and harvesting of stem bark of *P.africana*

P.africana bark was sourced from Mukurweini in Nyeri County, a taxonomist from the Department of Botany in Jomo Kenyatta University of Agriculture and Technology (JKUAT) was involved during plant identification and harvesting. A voucher specimen of *P.africana* plant was deposited in Jomo Kenyatta university of Agriculture and Technology botanical Herbarium voucher number Rosacea0001.

Sustainable harvesting was done by cutting the old branches and prunes i.e. "renewable plant sourcing" this was to preserve the source and keep it getting renewed[1]. The *P.africana* branches and stem were be debarked using a sharp-edged knifes to obtain 10 kilograms of the wet bark.

2.1.2 Preparation of stem bark of *P.africana*

The stem bark was air dried in mesh bags until the moisture content is about 10-15%.The dried bark was be weighed and chopped into small pieces and grounded into fine powder using a mill. The powder was then packed in air tight plastic containers.

2.1.3 Extraction of bark extract of *P.africana*

One kilogram of powdered material was soaked in 1000ml methanol for 72 hours .The mixture was filtered through Whatman filter paper No.1 and the organic solvent was evaporated to near dryness by vacuum evaporation using rotary evaporatoras per J.B. Harborne, in 1984[7].The obtained bark extract was a greenish mass with bitter almond smell containing beta-sitosterol ,which concentration is was about 15-18% by weight of the pygeum[1].

The bark extracts was then weighed, labelled and stored in sterile air tight bijou bottles at 4 °C prior to use.

2.2 Qualitative Phytochemical analysis of the methanolic bark extract

The following standard protocols were used for qualitative analysis of samples to check for the presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, saponins, tannins, terpenoids, quinones and proteins[8,9]

Test for Flavonoids

To 2 ml of the extract few drops of 20% sodium hydroxide was added, formation of intense yellow colour is observed. To this, few drops of 70% dilute hydrochloric acid were added and yellow colour disappeared.

Formation and disappearance of yellow colour indicates the presence of flavonoids in the sample extract.

Test for Alkaloids: To 1 ml of the extract, 1 ml of marquis reagent, 2ml of concentrated sulphuric acid and few drops of 40% formaldehyde were added and mixed, appearance of dark orange or purple colour indicates the presence of alkaloids.

Test for Saponins

To 2 ml of the extract, 6 ml of distilled water were added and shaken vigorously; formation of bubbles or persistent foam indicates the presence of saponins.

Test for Tannins

To 2 ml of the extract, 10% of alcoholic ferric chloride was added; formation of brownish blue or black colour indicates the presence of tannins.

Test for Phenols

To 2 ml of the extract, 2 ml of 5% aqueous ferric chloride were added; formation of blue colour indicates the presence of phenols in the sample extract.

Test for Proteins

To 2 ml of the extract, 1 ml of 40% sodium hydroxide and few drops of 1% copper sulphate were added; formation of violet colour indicates the presence of peptide linkage molecules in the sample extract.

Test for Cardiac Glycosides

To 1 ml of the extract, 0.5ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution were added, formation of brown ring at the interface indicates the presence of cardiac glycosides in the sample extract.

Test for Terpenoids

To 1 ml of the extract, add 0.5 ml of chloroform followed by a few drops of concentrated sulphuric acid, formation of reddish brown precipitate indicates the presence of terpenoids in the extract.

Test for Carbohydrates

To 1 ml of the extract, add few drops of Molisch's reagent and then add 1 ml of concentrated sulphuric acid at the side of the tubes. The mixture was then allowed to stand for 2 to 3 minutes. Formation of red or dull violet colour indicates the presence of carbohydrates in the sample extract.

2.3 Experimental animals

Fifteen female nulliparous non-pregnant wistar rats were obtained from SAFARI animal biomedical department in Jomo Kenyatta University of Agriculture and Technology (JKUAT). Female wistar rats were used because they are more sensitive to toxicity in drug under investigation as compared to male[10].The rats were weighing approximately 110g. They were housed in standard rat cages (one for each group) and exposed to 12 hour light/dark cycles under humid tropical conditions. Each was be cage was labelled with a cage card showing experiment number, date of starting the experiment, dosage level, Age, Number of animals, Species and sex of

the animal. The rats were allowed unrestricted access to standard feed Rodent pellets obtained from UNGA Mills and water ad libitum throughout the experimental period. The rats were handled in accordance with the guidelines for the care and use of laboratory animals.

2.4 Acute toxicity (LD50) study of the bark extract

The acute toxicity was determined by use of modified Lorke's method[11].The experiment was conducted in two phases using a total of fifteen animals. The female nulliparous non-pregnant wistar rats were fasted overnight prior administration of the Bark extract of *P.africana*. Phase I; four groups each with 3 rats, group 1, 2 & 3 animals were administered with single oral dose of 10, 100 & 1000 mg/kg of the bark extract in 5% dimethyl sulfoxide (DMSO), respectively. Group 4; was the control group with three rats, which were given 5% DMSO in distilled water (5 ml/kg body weight). Phase II; it had three animals, and each received a single oral dose of 1600, 2900 and 5000 mg/kg of the bark extract in 5% DMSO respectively. The bark extract was administered orally using sterile gavage needles. All the animals were monitored closely for signs of toxicity which are mortality, changes in gross appearance of the skin and fur, mucous membrane of the eye, respiratory distress, somatomotor activity, behavior, and special attention was given to observation of tremors,salivation, diarrhea, coma and convulsions, changes during the first 48 hours post dosing. The observation schedule was as follows; immediately, ½ an hour, 1 hour, 4 hour, 24 and 48 hours, the monitoring for signs of toxicity continued daily for 14 days. The body weight was monitored as follows; day 0 (initial weight), day 7 and day14 (terminal weight)

Terminal sacrifice of all surviving animals done on day 15th by fasting them overnight and euthanizing them with carbon dioxide and gross necropsies were performed .All Organs and tissue were harvested, examined and weighed.

Then the LD50 is calculated by the formula;

$LD50 = \sqrt{D0 * D100}$

D0 = Highest dose that gave no mortality

D100 = Lowest dose that produce mortality

2.5 Ethical clearance

The ethical clearance was sought from JKUAT Animal Ethical Committee (AEC) before initiation of the study.

2.6 Statistical analysis

The results were expressed as mean ± standard error of the mean (SEM) for all values. The data were statistically analyzed using one-way ANOVA (SPSS version 24.0) followed by Tukey's post hoc multiple comparison tests. The results were considered to be significant at $P<0.05$.

III. RESULTS

3.1 Yields of Methanol bark extract extracts

Percentage yield = weight of plant after extraction/weight of plant before extraction x 100%

The total solid of *P.africana* crude methanolic extract recovered was 68grams. The extract was a greenish mass with bitter almond Smell.

Table 1. Phytochemical constituents of *P.africana* bark extract

Sample Extract	**Phytochemical screened**							
	carbohydrate	steroids	Triterpenes	Glycosides	Tannins	Flavonoids	alkaloids	saponins
Methanolic B.E *P.africa*	++	-	+++	+	++	+++	++	+++

+++ = Highly present, ++ = moderately present, - =absent

3.2 Acute oral toxicity study of *P.africana*

The following parameter were observed after single dose administration of *P.africana* methanolic bark extract, death, respiratory distress, injury, pain distress, allergic reactions, changes in fur appearance,moribund condition, paralysis on hind limbs, ataxia,increased or reduced activity and sedation.

Table 2. Showing observation and mortality rate

Experiment	Doses (Mg)	Observation in hours						Mortality	Mortality rate (%)
		Immediate	½ hour	1 hour	4 hours	24 hour	48 hours		
Phase I (B.E in 5% DMSO)	10	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0/3	0
	100	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0/3	0
	1000	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0/3	0
Control [Distilled water + 5%DMSO]	0	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0/3	0
Phase II (B.E in 5% DMSO)	1600	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0/1	0
	2900	Respiratory distress	Respiratory distress	Respiratory distress	Respiratory distress	Respiratory distress	Normal activity	0/1	0
	5000	Respiratory distress	Respiratory distress	Respiratory distress	Respiratory distress	Normal activity	Normal activity	0/1	0

DMSO-dimethyl sulphoxide
B.E- bark extract of *P.africana*

Table 3. Post mortem results of gross pathology findings in acute toxicity of rats administered methanolic extract of *P.africana*.

	Gross pathology results					
Organ	**Dose (mg/ kg body weight)**					
	10	**100**	**1000**	**1600**	**2900**	**5000**
kidney	None	None	None	None	None	None
Lungs	None	None	hyperaemia	None	Lung abscess	Hyperaemia
Liver	None	None	None	None	Liver congestion	Liver congestion
Spleen	None	None	None	None	None	None
Brain	None	None	None	None	None	None
Prostate	None	None	None	None	None	None
Testis	None	None	None	None	None	None

Table 4. Effect of administering different doses of *P.africana* bark extract on body weight of rats over a period of 14 days.

Phases of experiment	**Dose in (mg/kg bwt)**	**Initial Weight (g) Day 0**	**Weight(g) Day 7**	**Terminal weight(g) Day 14**
Phase I	10	179.67±1.76	213.67±3.93	229.00±1.73
	100	167.67±1.53	204.00±3.06	228.00±1.00
	1000	157.33±2.03	185.00±2.52	201.67±2.91[a]
Control	0	157.00±2.08	212.33±4.91	230.67±3.92[a]
Phase II	1600*	169.00	180.00	209.00
	2900*	155.00	172.00	189.00
	5000*	156.00	178.00	181.00

Significantly different from the control ($p < 0.05$).
*Dose groups with single rat per group ($n<3$) were not compared due to absence of measure of variability

Table 5.Effect of administering varying doses of *P.africana* bark extract on the absolute organ weight of rats

	Dosage in (Mg/kg body weight)						
	10	**100**	**1000**	**Control**	**1600 ***	**2900***	**5000 ***
Brain	1.42^{a} ±0.281	1.14^{a}±0.1048	1.08^{a}±0.1827	1.32^{a}±0.2987	0.99	1.25	1.22
Kidney	2.29^{b}±0.2554	2.11^{b}±0.1474	2.33^{b}±0.1375	2.21^{b}±0.2186	2.84	1.88	2.29
Heart	1.28^{c}±0.212	1.12^{c}±0.2119	1.16^{c}±0.3214	1.19^{c}±0.2347	1.21	1.02	0.96
Testis	4.82^{d}±0.3781	4.35^{d}±0.2364	4.54^{d}±0.3601	5.58^{d}±0.2222	5.25	4.41	4.82
Prostate	0.54^{e}±0.3732	0.53^{e}±0.2178	0.56^{e}±0.2985	0.57^{e}±0.3511	0.54	0.53	0.56
Liver	10.06^{f}±0.327	12.17^{f}±0.2167	10.39^{f}±0.2081	11.36^{f}±0.3008	12.09	10.17	10.06
Spleen	1.4^{g}±0.6216	1.71^{g}±0.3580	1.92^{g}±0.4022	1.07^{h}±0.3072	1.95	1.93	1.89
Lungs	2.78^{i}±0.0987	2.6^{i}±0.2879	2.01^{i}±0.1173	3^{i}±0.1189	2.62	2.9	2.78

The test of significance was performed in rows. Different superscripts indicate significantly different from the control ($p < 0.05$)

*Weight values of organs where n<3 were not compared due to absence of measure of variability.

Table 6. Effect of oral treatment with methanolic bark extract of *P.africana* on percent organ-body weight ratios (OBR) of rats after the acute toxicity study

Dosage in (Mg/kg bwt)							
	10	**100**	**1000**	**Control**	**1600***	**2900***	**5000***
Brain	0.5796^{a}±0.1842	0.4597^{a}±0.0414	0.5023^{a}±0.0423	0.507^{a}±0.2082	0.4736	0.6613	0.674
Kidney	1.0837^{b}±0.0222	0.9525^{b}±0.0201	0.9793^{b}±0.1015	0.9883^{b}±0.0281	1.3589	0.9947	1.2652
Heart	0.3294^{c}±0.2062	0.4154^{c}±0.0732	0.3902^{c}±0.1781	0.3922^{c}±0.1194	0.5789	0.5397	0.5303
Testis	2.1552^{d}±0.051	2.0323^{d}±0.2251	2.3295^{d}±0.1807	2.2077^{d}±0.1995	2.512	2.3333	2.663
Prostate	0.1957^{e}±0.0607	0.2171^{e}±0.1251	0.1661^{e}±0.1147	0.152^{e}±0.1087	0.2584	0.2804	0.3094
Liver	4.153^{f}±0.2478	5.2153^{f}±0.37541	4.9955^{f}±0.1269	4.4593^{f}±0.397	5.7847	5.381	5.5580
Spleen	0.5714^{g}±0.1582	0.7273^{g}±0.0427	0.7268^{g}±0.1471	0.431^{h}±0.1597	0.9330	1.0212	1.0442
Lungs	1.18^{i}±0.0987	1.0291^{i}±0.1579	1.121^{i}±0.1759	1.2078^{i}±0.1109	1.2536	1.5344	1.5359

The test of significance was performed in rows. Different superscripts indicate significantly different from the control ($p < 0.05$)

*Weight values of organs where n<3 were not compared due to absence of measure of variability.

IV. DISCUSSION

4.1 Phytochemical analysis

Qualitative phytochemical screening gives a brief clue about the nature of active phytochemical constituents found in herb extract. The phytochemical constituents of the extract will either have beneficial or harmful effects on the animals. The tannins and anthraquinones are believed to have both proxidant and antioxidant effects on the body. While the antioxidant is crucial in protection of the organs and body tissues, the proxidant are injurious to the organs and tissues [12,13] The result of the qualitative phytochemical screening of the methanolic bark extract *P.africana* indicated the presence of carbohydrates, triterpenes, glycosides, tannins, flavonoids, alkaloids and saponnins (Table 1).These results conformed to a previous studies which reported the presence of tannins, triterpenes, glycosides, alkaloids, saponins, flavonoids and carbohydrates in *P. africana* bark[14].

Changes in the animals weight during observation period is more visible at higher doses, and in the presence of tannins and other phenolics which are believed to hinder the absorption of nutrients in intestines making them inaccessible and thereby reducing the voluntary feed intake even though the animals were provided with free access to feeds and water [12]. The bark extract at higher doses might have caused an interference since phyto-analysis indicated the existence of tannins and other compounds which inhibits absorption of nutrient in small intestine leading to wasting in the animals

4.2 Acute toxicity

The acute toxicity study provides evidences on the safety range of drugs in the animal; it is also vital in estimation the therapeutic index (LD50/ED50) of chemotherapeutics and xenobiotics[15–17].

The acute toxic effect of *P.africana* on (Table 2) shows that no mortality within 48 hours post dosage. The phase II animals (2900 &5000mg/kg) animals respiratory distress was noted in first 24 hour, this signs was not seen in other dose group. The LD50, being greater than 5000 mg/kg body weight is thought to be safe as proposed by Lorkes in 1983[11]. Again, lack of mortality among animals in all the dose groups during the entire 2 weeks of experimental period seems to back-up this claim.

In Phase II animal the lungs hyperemia, liver congestion, lung abscess appeared to be the main gross pathology associated administration of *P.africana* bark extract the animals. (Table 3).Liver congestion and hyperemic lungs was attributed to saponins in bark extract (Table 1), it is possible that with increase in dose the quantity of saponin increased to toxic level in the lungs and liver. Saponins are recognized to have harmful haemolysing effect on the erythrocytes in circulation[18,19]. Moreover, the liver congestion can also be attributed, to one of its roles which is biotransformation of xenobiotic[20,21]. The lung abscess observed in the right lung in one of Phase II animal (2600mg/kg body weight) could be as result of accidental aspiration of bark extract in the lung.

Dose-dependent weight loss that was recorded, were found not to be statistically significant ($p>0.05$) when compared with the control group (Table 4).

On the absolute organ weights and OBR values only spleen that showed a dose-dependent enlargement, with increased dose of the bark extract, the absolute weight and OBR value of spleen increased (Table 5 and 6 respectively) and were statistically significant ($p>0.05$) from the control, this could be attributed to hemolytic effects of saponin to red blood cell In all the other organs observed there was no statistically significant ($p>0.05$). This suggests that the bark extracts did not interfere with the other organs.

V. CONCLUSION AND RECOMMENDATIONS

From the results oral intake of the methanolic bark extract *P.africana* at dose less than or equal to 5000 mg/kg body weight appears to be safe, since there is no mortality that occurred even after administration of the highest dose. Therefore this study hypothesized that bark extracts is relative harmless based on Loomis and Hayes classification of toxicity,hence it's usage as a traditional remedy is safe. Higher doses should, however, be avoided and users should not rule out entirely the likelihood of a splenomegaly emerging with the continued use of *P.africana* bark extract.

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Ethical Approval

Author hereby affirms that the experimental protocol was approved by the Jomo Kenyatta University of Agriculture and Technology Animal ethical Committee (JKUAT AEC). The animals were only used once. They were all sacrificed using humane end points at the end of the study[22].The protocol followed to the letter theGuidelines for Care and Use of Laboratory Animals in Biomedical Research[23]

CONFLICT OF INTEREST: None

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