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ResearchArticle

TOXICOLOGICAL ACTIVITY OF CRUDE SAPONIN EXTRACT OF FICUS PLATYPHYLLA

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ABSTRACT

Due to problems associated with the use of synthetic drugs and lack of affordability, effort has been made to prepare useful drugs from medicinal plants which would have less significant side effects and can be easily affordable to the poor people. The use of medicinal plants in therapy is becoming significant in Africa with the publishing of African Pharmacopeia which regulates and set standard for the use of various medicinal plants in Africa. It is commonly used for alleviating disease conditions like insomnia, pain, depression and agitation.

The plant was obtained from the forest and three parts namely the leaf, stem bark and root bark were grinded into powder. The powder was triturated with natural feed and cassava was added to make mixed feed cake. After conditioning two albino rats in the metabolic cage for eight weeks, they were fed with the prepared mixed feed cake. The urine obtained was used for heamolysis test. At both 26^{o}C and 27^{o}C room temperature, extraction of saponins from leaf, stem bark and root bark were done for a period of 20 hours and 33 minutes respectively in both water and methanol solvent and the duration of heamolysis on blood cells was obtained.

The result of the research showed that shaking of the grinded samples of the leaf, stem bark and root bark produced lather (foam) that persisted for 40 minutes. The blood cells were also heamolysed leaving a clear solution on top and a dark-brownish cloudy suspension below. At both room temperature measurements, the heamolysis duration for methanol was shorter for methanol than water as solvent. More urine was obtained when the animals were fed with the plant extracts than when they consumed pure animal feed and the color of urine also changed from dark-yellow cloudy urine to reddish-brown cloudy urine. The rats eventually died after ingestion of mixed feed cake.

Methanol was undoubtedly a better solvent for saponins. Though there are many biological activities of saponin which are of importance to man such as reduction of serum cholesterol, antiviral and anti-fungal activities and their effects on the uterus and vaginal infection, the use of saponin is limited due to its toxicity. The surface activity of saponin on erythrocyte and its tendency for accumulation have limited the use of saponin.

Key words: Saponin, Toxicity, Heamolysis, Erythrocytes, Ficus platyphylla, Temperature.

INTRODUCTION

Ficus platiphylla is a large tree with very large broad leaves and small figs with stalks often considerably longer than the fig. The tree is 18m high and 6cm in girth with large widely spreading branches and a very broad crown. Bark rusty red, flaking off in scattered patches and grey beneath, slash pink. Leaves are 7-40cm long by 10-28cm broad, mostly broadly elliptic, rounded blunt at the apex, deeply and narrowly cordate, thick finely velvety or glabrous. Stalk staut up to 12cm long. Fruits emerge October –January and April to May on the shoots just below the new leaves about 12cm smooth (but warted when dry), very finely hairy or glabrous with stander stals 1-3cm long. The plants usually grow in savanna habitat of West Africa and extend from Senega to Somalia, Egypt, Sudan and Zimbabwe. Preliminary phytochemical screening of the methanolic extract of the stem bark showed positive test for flavonoids, tannins and saponins. Phytochemical analysis of the leaf in Egypt revealed the presence of the following compounds, chalcone-3,5-dihydroxy-4,0-Beta flavonoid, D-Glucosideconsmosiin-flavonoid, Gluteolin flavonoid, Luteolin-5- Rutinoside flavonoid, UmbelliferoneCoumarin, XanthotoxinCoumarin and Xanthotoxolcoumarin[1].

The cold water extract, powder or decoction of the roots or bark are used in various conditions as dysphoria, pain, insomnia and when someone is possessed with evil spirit. The extract or decoction is ingested while the powders is mixed with food when eating or placed in a burning charcoal and saturate the environment with its smoke to drive away evil spirit(s). The plant is a useful traditional medicine in Nigeria for the control of mania and its efficacy was acclaimed [1, 2].

Previous pharmacological studies on Ficus platyphylla was illustrated using methanolic extract of the plant stem bark for the investigation of both inflammatory and antinorciceptive activities. The anti-inflammatory activity was tested against egg-albumin induced oedema while the analgestic effects were studied using the acetic acid induced wriggling and formalin test in mice. The result showed that the extract of the plant possessed significant dose-dependent anti-inflammatory effect. The methanolic extract was claimed to inhibit pain caused by acetic acid in mice dose-dependently and reduce pain episodes induced by formalin in both first and second phases confirming antinorciceptive and anti-inflammatory effects in mice [3]. Another study group led by Olusola investigated analgesic, sedative and antipsychotic activities of methanolic extract of the bark of Ficus platyphylla. The effect against amphetamine stimulated stereotyped behavior in adult white albino mice was observed. Acetic acid induced wriggling in mice was used as the pain model while the Benwich Data logger was used to observed the effect on locomotor activity. The extract showed profound inhibition of amphetamine induced stereotyped behavior and suppression of locomotor activity. The conclusion of the study claimed the presence of sedative principles that may be responsible for the observed effects against amphetamine and pain [4].

The behavioural effect of methanolic extract of Ficus platyphylla bark was studied on pentobarbitone sleeping time, spontaneous motor activity, amphetamine induced stereotyped behavior and pentylenetrazole(PTZ) induced seizure in mice and rats. The crude extract prolonged pentobarbitone sleeping time, reduced spontaneous motor activity, significantly antagonized amphetamine induced hyperactivity and reduced the intensity of PTZ induced seizures in mice. The conclusion of the study showed that there was presence of sedative principle in the extract [5]. Another study involving methanolic extract of Ficus platyphylla bark was evaluated for gastrointestinal activity.

As many people consume parts of Ficus platyphylla to treat their disease conditions, it is important to investigate the toxicological effects of this plant on individuals. In-vitro researches have been conducted on isolated tissues, however, this research study aimed at investigating the toxicological activities of the plant on red blood cells.

MATERIALS AND METHODS

The plant materials were collected in June at Shika forest and identified by the keepers of the Herbarium of Department of Biological Science. A voucher specimen AMG1 was kept at the Herbarium. After obtaining approval from Animal Protection Unit, two male albino rats weighing 110g and 120g, aged 13 weeks were obtained from Animal House of Department of Pharmacology and Clinical Pharmacy for the study. The rats were conditioned for a period of eight weeks.

Animal Feed: 403g of Ficus platyphylla stem bark powder was triturated with the 322g pure animal feed until it was well mixed. 81g cassava powder was suspended in a small quantity of cold water and sufficient quantity of boiled water was added into the suspension to make a paste. The paste was incorporated into the mixed feed. The mixed feed was moulded into balls and dried in open air for 48 hours at room temperature.

Test for Saponin: (a) Frothing test was carried out using 1g of the powdered material shaken in water for 30 seconds. (b) Heamolytic test was done by pouring 5ml of a 10% suspension of red blood corpuscles in normal saline into two test tubes. 5ml of normal saline (control) was added to one tube and to the other 5ml of sample extract in which 0.045g of sodium chloride has been previously dissolved was added. The solution sin the tubes were mixed by gently inverting the tubes.

Metabolic Procedure: the rats were fed constantly in the metabolic cage with the pure feed as well as water for four weeks. The urine samples from the rats were collected every day at regular time. All the urine samples were pooled together and exposed to atmospheric air to concentrate. Thereafter, the rats were fed with 50% amended animal feed for three weeks. The urine was collected at the same time each day and pooled together. It was concentrated on exposure to atmospheric air. The urine samples were tested for heamolysis using the procedure.

Extraction of Plant Materials: The stem bark and root bark were cleaned as well as the leaf and exposed to open air for fifteen days. It was completely dried in the oven for 24 hours.

The dried materials were grinded to coarse powders and 1g of it was macerated with 10ml each of water and chloroform for 30 minutes. Another set of extraction was done with maceration time lasting for 24 hours. Each of the extracts was then filtered through a cotton wool. After filtration, the methanol extract was evaporated on water bath and the residue was dissolved in 10ml of water.

Chromatogram: Ten chromatographic plate of size 5×20cm were coated with silica gel with a thickness of 0.5mm. The plates were exposed to open air for 10 minutes and then dried in oven at 115^{o}C for I hour.

Solvent System: Three solvent systems were used. The first is Chloroform: Methanol: Water (5:4:1), the second, Chloroform: Methanol (3:1), the third, Buthanol: Acetic acid: Water (5:1:4).

After the saturation of the tanks with each solvent system, two plates were spotted with the two urine samples collected. The plates were inserted into the tank s and run for 1 hour. The chromatogram obtained from each of the solvent system was viewed under ultraviolent light. Then they were sprayed with Liebermann-Burchard reagents.

Salkowski Test: 2ml each of the urine samples were put in different test tubes. 1ml chloroform was added to the test tubes and 1ml of concentrated sulphuric acid was also added to each of the tubes.

RESULTS

1. Frothing Test: Shaking of the grounded samples of the leaf, stem bark and root bark produced lather (foam) that persisted for 40 minutes.

2. Heamolysis Test: The blood cells were heamolysed leaving a clear solution on top and a dark-brownish cloudy suspension below.

Table 1: Hemolysis Time

S/N	ROOM TEMPERATURE	EXTRACTION DURATION	EXTRACTION SOLVENT	PLANT PART	HEAMOLYSIS DURATION (minutes)
1	26^{o}C	20 hours	Water	Leaf	46
2	26^{o}C	20 hours	Water	Bark	30
3	26^{o}C	20 hours	Water	Root	59
4	26^{o}C	20 hours	Methanol	Leaf	15
5	26^{o}C	20 hours	Methanol	Bark	7
6	26^{o}C	20 hours	Methanol	Root	19
7	27^{o}C	33 minutes	Water	Leaf	17
8	27^{o}C	33 minutes	Water	Bark	9
9	27^{o}C	33 minutes	Water	Root	20
10	27^{o}C	33 minutes	Methanol	Leaf	5
11	27^{o}C	33 minutes	Methanol	Bark	3
12	27^{o}C	33 minutes	Methanol	Root	9
13	26^{o}C	1 hour	Water	Leaf	16
14	26^{o}C	1 hour	Water	Bark	6
15	26^{o}C	1 hour	Water	Root	17
16	26^{o}C	1 hour	Methanol	Leaf	6
17	26^{o}C	1 hour	Methanol	Bark	2
18	26^{o}C	1 hour	Methanol	Root	13

Table 2: **Urine Sample for Heamolysis**

ACTIVITY	URINE BEFORE FEEDING WITH MIXED ANIMAL FEED	URINE AFTER FEEDING WITH MIXED ANIMAL FEED
Average volume of urine collected per day	2.96ml	3.2ml
Colour	Dark-yellow cloudy urine	Reddish-brown cloudy urine
Heamolysis test	Negative	Negative

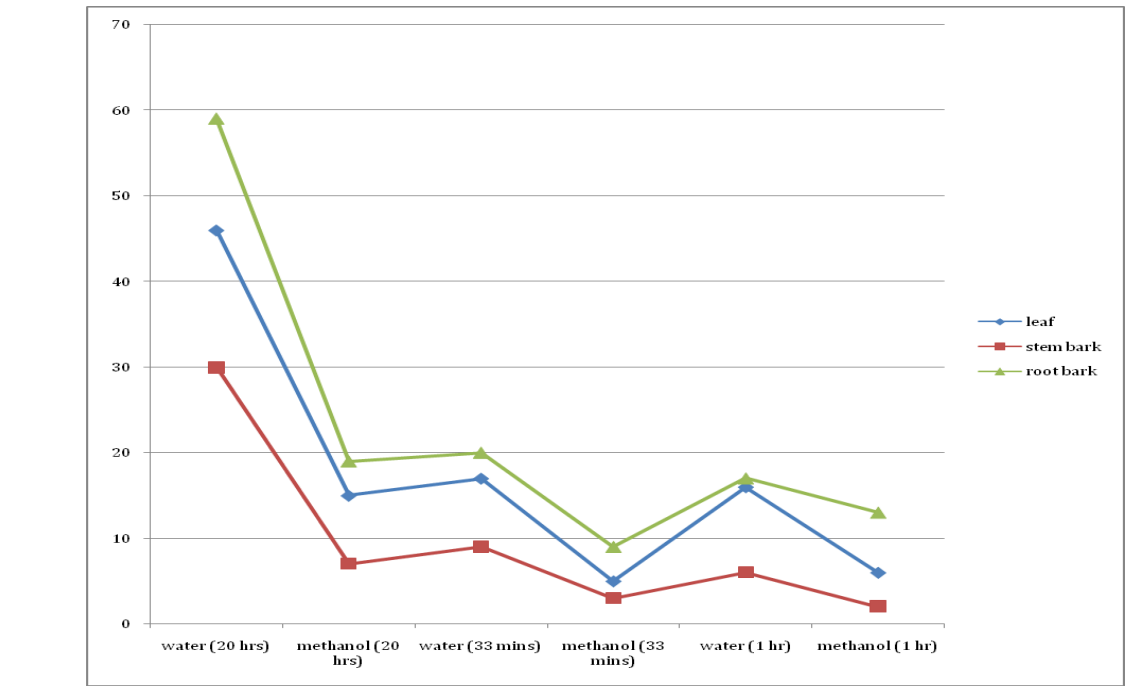
NB: The rats died after three weeks of feeding with crude pant materials (mixed animal feed).

3. Thin Layer Chromatography

After spraying the chromatogram with Lieberman Burchard reagent and heated in the oven, an orange or pale yellow spot was observed from the area of spot of untreated urine sample (before the rats were fed with the crude plant extract). The spot area with treated urine sample (after feeding with plant extract) showed a reddish-Purple line. This indicated that the urine sample collected after feeding the rats with the plant material contained steroidal saponin.

4. The urine sample collected before feeding the rats with crude plant materials did not give positive result while the urine sample collected after the rats were fed with crude plant material indicated the presence of sterol.

Figure 1: Heamolysis Time Graph



DISCUSSION

The extracted saponin was tested on both human and Animal blood for heamolysis and the result was positive in both indicating the activity of saponin as a potent surfactant to alter the membrane permeability of both human and animal red blood cells alike. The comparison of activity of saponin from both water and methanol extract of leaves, root bark and stem bark of the plant showed hemolysis time was shortest for stem bark followed by leaves and root bark respectively (fig. 1). This showed that stem bark has the highest amount and activity of saponin followed by the leaves and the root bark. The heamolysis of both the water and methanol extract of saponin from the plant where also compared showing that methanol has a shorter duration of heamolysis time than the water extracts (fig. 1). This might imply that water aids hydrolysis leading to proportionate hydrolysis of saponin in water extract resulting in reducing activity of saponin hence the heamolysis time of saponin is

prolonged. On hydrolysis, saponin yields sapogenin and corresponding glycosides with loss of activity. Comparing the heamolysis time at the same temperature (26^{o}C) at different duration of extraction, it was observed that the longer the duration of extraction the longer the heamolysis time observe in water extract. This also suggested that saponin hydrolyses with time and non-stability of saponin in solvent especially in water while saponin seems to be stable in methanol. When the temperature was varied, it was observed that the heamolysis time of the water extract increased while that of methanol extract decreased. This suggested that temperature aided hydrolysis of saponin in water while in methanol extract the methanol evaporated leaving concentrated saponin hence, heamolysis time decreased.

The urine collected after feeding the rats with plant materials did not heamolyse blood. This implied that saponin was well absorbed into the system ic circulation and it also possessed high plasma protein binding which prevented its elimination via the kidney. The death of the rats could not be ascertained through autopsy as it was sudden. However, earlier research suggested the presence of ficin in ficus plant responsible for digesting protein inside the animal. Ficin is probably assumed to have had effect on organs of the rats leading to their death. It was also suggested that the surface activity of saponin might prevent the circulating blood to carry sufficient oxygen round the body of rats which could lead to heart failure and consequently death.

The result of thin layer chromatography showed that there was trace of saponin in the urine after feeding the rats with the crude plant feed. This suggested that saponin is slowly eliminated from the body with risk of accumulation leading to toxicity. The result of salkowski test also confirmed the presence of saponin in the urine sample collected after feeding the rats with crude plant feed.

In conclusion, methanol was a better solvent for saponin. Though there are many biological activities of saponin which are of importance to man such as reduction of serum cholesterol, antiviral and anti-fungal activities and their effects on the uterus and vaginal infection, the use of saponin is limited due to its toxicity. The surface activity of saponin on erythrocyte and its tendency for accumulation have limited the use of saponin.

Further research can be done to evaluate the topical effect of saponin on the skin.

REFERENCES

1. Amos S., Chindo B. et al. Anti-inflammatory and Anti-norciceptive Effects of the Methanolic Extract of Ficusplatyphylla Stem bark. Journal of Herbs, Species and Medicinal Plants 2002; 9: 47-53.
2. Merel H., Akpovi A., Jos L. Medicinal Plants Used to Treat Malaria in Southern Beinin. Economy Botany 2004; 58 Suppl S:239-254.
3. Chindo B. Joseph A. et al. Anticonvulsant properties of Saponins from FicusPlatyphylla Stem Bark. Brain Research Bulletin 2009; 78Issues 6: 276-282.
4. Olusola A.,Saidu K., Onah J., Orisadipe A., Wambebe C., Gamaliel K. Antiplasmodial, Analgesic and Anti-inflammatory Activities of Aqueous Extract of Erythrinasenegalensis. J. Ethnopharmacol 2000; 71: 275-280.
5. Gammaniel K., Amos S. et al. Behavioural Effects of the Methanolic Extract of Ficusplatyphylla Bark in Mice and Rats. Journal of Behavioural Pharmacology 1998.
6. Basu N., Rastogi R.P. Triterpenoidsaponins and sapogenins. Journal of Phytochemistry 1967; 1249-1270.

7. Canell, Richard J.P. National Products Isolation, New Jersey: Humana Press; 1998. Pg. 358
8. Davenport H.W.Physiology of the Digestive Tract. Year Book Medical Publishers; 1966. Pg. 111.
9. Kenneth C., Yu W. Regulation of Cholesterol Synthesis and Esterification in Primary Cultures of macrophages Following Uptake of Chylomicron Remnants. Journal of Biochemistry and Molecular Biology International 1997; pg 33-39.
10. Mahato S.B., Ganguly A.N. Steroidal Saponins (Review). Journal of Phytochemistry 1982; pg 959-978.
11. Rui-Chao L., Bernard H. et al. Aferoside A: A Steroidal Saponin from Costusafer. International Journal of Plant Biochemistry 1996; pg 667.
12. SahuN.P.TriterpenoidSaponins of Mimusopselengi. The International Journal of Plant Biochemistry 1996; Pg 883.
13. Trease and Evans Pharmacognosy. 14th Edition. London:W.B. Saunders; 2000 pg .35-36.