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PHYTOCHEMICAL ANALYSIS OF CRUDE AQUEOUS EXTRACT OF FICUS PLATYPHYLLA STEM-BARK AND ITS TOXICITY STUDY ON WISTAR ALBINO RATS

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ABSTRACT

This study was carried out to determine the phytochemical composition of crude aqueous extract of *Ficus platyphylla* stem-bark and its potential toxicity on Wistar albino rats. The following phytochemicals were identified: terpenoids, tannins, flavonoids, anthraquinones, free reducing sugars, combined reducing sugars, ketoses, pentoses, cardiac glycosides, and saponins. Terpenoids were in highest concentration followed by anthraquinones, combined reducing sugars, flavonoids, free reducing sugars, ketoses and tannins that were moderate in concentration, while cardiac glycosides, saponins and pentose sugars were present in the least concentration. The study revealed that aqueous extract of *Ficus platyphylla* stem-bark possesses fairly high oral LD₅₀ value (> 5000 mg/kg in rats), suggesting its low toxicity, orally. When administered intraperitoneally however, the LD₅₀ was calculated to be 113.1mg/kg body weight. This LD₅₀ (113.1mg/kg) value suggests its potential toxicity. The aqueous stem-bark extract of *Ficus platyphylla* should therefore be used with some degree of caution when parenteral route is the choice.

Keywords: Stem-bark, *Ficus platyphylla*, toxicity, lethal dose (LD₅₀), aqueous extract, phytochemicals.

1. INTRODUCTION

Plants are widely used for medicinal purposes in many countries and are a source of many potent and powerful drugs (Ahmad *et al.*, 2007) and (Srivastava *et al.*, 1996). *Ficus platyphylla* has different names in Nigeria: *Gamji* in Hausa, *Barwada* in Kanuri, *Epo-Obo* in Yoruba and *Ogbagba* in Nupe. The tree is about 18m high, 6m in girth, with large and long branches and broad leaves and widely distributed in many African countries especially in sub-Saharan region (Kubmarawa *et al.*, 2009; Mudi *et al.*, 2011).

Ficus platyphylla possesses medicinal properties that are effective in the management of convulsive disorders (Wakeel *et al.*, 2004). The plant also constitutes important sources of raw material for industrial processing and preparation of various chemical compounds (Penzo, 1980).

Preparations of the plant have been used in the Nigerian traditional medicine for management of insomnia, epilepsy, psychosis, depression, pain and inflammation especially in northern Nigeria (Audu, 1989).

Past studies showed that the plant contains active substances with potential central nervous system, anti-inflammatory, and gastrointestinal activities in rodents (Gamaniel *et al.*, 2000; Amos *et al.*, 2001, 2002; Chindo *et al.*, 2003, 2008, 2009). Plants of medicinal importance are still relevant in both developing and developed countries of (Farombi, 2003). In many parts of the world, herbal drugs continue to gain popularity because of their efficacy and cost effectiveness with minimal side effects (Subramoniam and Pushpangadan, 1999) and (Shinwari and Khan, 2000).

Various plant species are widely in use in both human and veterinary medicine today (Amponsah *et al.*, 2002; Amresh *et al.*, 2004; Dahiru *et al.*, 2006; Gupta, 1994; Kar *et al.*, 2003).

2. METHODOLOGY

Stem bark of *Ficus platyphylla* was collected at Molai ward, Maiduguri, Borno State. The plant was authenticated at the Department of Biological Sciences, University of Maiduguri, where a voucher specimen was deposited in the herbarium for future reference.

2.1 Extraction of Plant Material For Phytochemical Analysis

The stem bark was first air-dried at ambient temperature and pulverized to powder using a clean grinding machine. Reflux method was used for the extraction. 250g of the powdered sample was put into a 5 liter round bottom flask, and enough distilled water was added up to above the sample level. The mixture was heated for about 3 hours, after which it was removed and decanted. The decanted solution was filtered using Whatman filter paper. Some fresh distilled water was added to the residue and heated again for about 2 hours, removed, decanted and filtered. The solution was transferred onto an open tray, and dried in an oven at 40°C, for 24hours. Greenish residue was obtained, weighed and kept for further analysis.

2.2 Phytochemical Screening Test For Free Reducing Sugars (Fehling's Test)

0.2g of the extract was dissolved in distilled water and filtered. The filtrate was heated with 5ml of equal volumes of Fehling's solutions A and B. Red precipitate of cuprous oxide (Cu₂O) was formed, indicating the presence of reducing sugars.

2.3 Test For Combined Reducing Sugars

0.2 g of the extract was hydrolyzed by boiling 5ml of dilute hydrochloric acid and the resulting solution neutralized with sodium hydroxide solution. Few drops of Fehling's solution were added and heated on a water bath for two minutes. Reddish-brown precipitate of cuprous oxide (Cu₂O) was formed, indicating the presence of combined reducing sugars.

2.4 Test For Soluble Starch

Small quantity of the extract was boiled with 5% potassium hydroxide (KOH), cooled and acidified with H₂SO₄. A yellow colouration was formed, indicating the presence of soluble starch.

2.5 Test For Tanins Using Ferric Chloride Test

0.5g of the extract was stirred with about 10ml of distilled water and then filtered. Few drops of ferric chloride solution were added to 2ml of the filtrate. A blue- black precipitate was formed, indicating the presence of tanins.

2.6 Test For Cardiac Glycoside Using Salwoski Test

0.5g of the extract was dissolved in 2ml of chloroform. Tetraoxosulphate (IV) acid was carefully added by the side of the test tube to form a lower layer. A reddish-brown colouration was formed at the interphase, indicating the presence cardiac glycoside.

2.7 Test For Flavonoids Using Ferric Chloride Test

The extract was boiled with distilled water and then filtered. 2 drops of 10% ferric chloride were added to 2ml of the filtrate. A green-blue colouration was formed, indicating the presence of flavonoids.

2.8 Experimental Animals

Twenty-six (26) (13 for oral and 13 for intraperitoneal routes) Wistar-Lewis albino rats of both sexes were used. They were obtained from the Department of Animal Science, Faculty of Agriculture, University of Maiduguri. They were kept in the Department of Veterinary Physiology, Pharmacology and Biochemistry for two (2) weeks for acclimatization, where feed and water were given *ad libitum*.

2.9 Acute Toxicity Study

The acute toxicity (LD_{50}) was estimated through intraperitoneal and par os in rats ($n = 13$ in each case) following Lorke's method (1983). In the first phase, nine (9) rats were randomly divided into three groups of three rats per group and were given 10, 100 and 1000 mg extract/kg body weight orally (via a cannula), respectively. The rats were observed for signs of adverse effects and death for 24 hours. In second phase the procedure was repeated using another set of three rats randomly divided into three groups of one rat each, given 1600, 2900 and 5000 mg extract/kg body weight, respectively. The rats were observed for 24 hours for signs of toxicity which included paw-licking, salivation, stretching, rubbing of nose on the floor and wall of cage, change in body weight and death. The number of deaths in each group within 24 hours was recorded and the final LD_{50} values were calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred)

3. RESULT

Table 1. Phytochemical analysis of crude aqueous extract of *Ficus platyphylla* stem-bark.

S/No.	Phytochemical content	Result
1.	Alkaloids	-
2.	Anthraquinones	++
3.	Cardiac glycosides	+
4.	Combined reducing sugars	++
5.	Flavonoids	++
6.	Free reducing sugars	++
7.	Ketoses	++
8.	Pentoses	+
9.	Phlobatannins	-
10.	Saponins	+
11.	Soluble starch	-
12.	Tannins	++
13.	Terpenoids	+++

Keys

(+) = Present in low concentration

(++) = Present in moderate concentration

(+++)= Present in high concentration

(-) =Absent

The phytochemicals obtained from the extract include, terpenoids, tannins, flavonoids, anthraquinones, free reducing sugars, combined reducing sugars, ketoses, pentoses, cardiac glycosides, and saponins. Alkaloids, Phlobatannins and soluble starch were absent. Terpenoids were in highest concentration followed by anthraquinones, combined reducing sugars, flavonoids, free reducing sugars, ketoses and tanins appeared in moderate concentration, while cardiac glycosides, saponins and pentose sugars were the least in concentration (table. 1).

Table 2. LD_{50} value of aqueous stem-bark extract of *Ficus platyphylla* in Wistar albino rats by oral route.

Dosage mg/kg	Mortality
Phase I	
10	0/3
100	0/3
1000	0/3
Phase II	
1600	0/1

2900	0/1
5000	0/1

Table 3. LD₅₀ value of aqueous stem-bark extract of *Ficus platyphylla* in Wistar albino rats by intraperitoneal route.

Dosage mg/kg	Mortality
Phase I	
10	0/3
100	2/3
1000	3/3
Phase II	
20	0/1
40	0/1
80	0/1
160	1/1

At different dose levels tested in both first and second phases of the study, no mortality was recorded in the different groups of rats that received *Ficus platyphylla* orally after 24 hours (Table. 2). No untoward clinical signs were observed either. There were no changes in the nature of stool, urine and eye color of all the rats.

However, in the first phase of the intraperitoneal route study, there were two mortalities out of three, in the rat group that received 100 mg/kg body weight; and 100% mortality in rat group that were given 1000 mg/kg. In the second phase of the study, the mortality occurred in the rat that received 160 mg/kg body weight after 24hours, with no mortalities in those groups that were given 20 mg/kg, 40 mg/kg and 80 mg/kg (table. 3).

The LD₅₀ was calculated using the formula $LD_{50} = \sqrt{a \times b}$

Where a = highest dose that does not kill the rats

b = least dose that kills the rats

In table 3 phase II, 80 mg/kg is the highest dose that did not kill the rats, and 160 mg/kg is the least dose that killed the rats.

$$\begin{aligned} \text{Thus } LD_{50} &= \sqrt{80 \times 160} \\ &= \sqrt{12800} \\ &= 113.1\text{mg/kg.} \end{aligned}$$

The LD₅₀ of the aqueous extract of *Ficus platyphylla* stem-bark is therefore 113.1mg/kg intraperitoneally and greater than 5000mg/kg orally in rats, in this study.

4. DISCUSSION

Clarke and Clarke (1977) stated that, “In classifying toxicity of substances according to LD₅₀ values, substances with oral LD₅₀ values of 1000 mg/kg body weight and intraperitoneal LD₅₀ values of 500 mg/kg body weight can be said to be of low toxicity and therefore safe”. This study showed that the aqueous stem-bark extract of *Ficus platyphylla* possesses high (> 5000 mg/kg in rats) oral LD₅₀ value indicating its low toxicity when administered orally. When given intraperitoneally however, the LD₅₀ was determined to be 113.1mg/kg body weight. This LD₅₀ (113.1mg/kg) value indicates its potential toxicity compared to when given orally. The aqueous stem-bark extract of *Ficus platyphylla* should therefore be used with caution especially when it is to be given intraperitoneally.

5. CONCLUSION

This study revealed that, the aqueous extract of *Ficus platyphylla* stem-bark contained active agents and could be a promissory candidate for drug development and further validates the tribal / folkloric claim, as a cure for some ailments. This claim is also confirmed, as the extract indicated a relatively moderate number of phytochemicals. It is suggested that more research be conducted that will further elucidate and characterize the active components and possible mechanism(s) of action of these agents in the ethno medical practices.

The result of this study revealed that aqueous stem bark extract of *Ficus platyphylla* is of low toxicity, and therefore safe when used orally in Wistar albino rats. However, by acute intraperitoneal route, the extract is relatively toxic in Wistar albino rats. Due to its potential toxicity in Wistar albino rats when used intraperitoneally, it is recommended that sub-acute toxicity studies be

conducted (for both oral and parenteral routes) on the aqueous stem-bark extract of *Ficus platyphylla*

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