



PHYTOCHEMICAL SCREENING AND TOXICITY STUDIES ON THE LEAVES OF
MANNIOPHYTON FULVUM

^{1*}Agbaire P.O., ²Emudainohwo J.O.T and ³Peretiemo-Clarke, B. O

^{1,3}Department of Chemistry, Faculty of Sciences, Delta State University, Abraka, Nigeria.

²Department of Pharmacology and Therapeutics Delta State University, Abraka, Nigeria.

Corresponding Author: E-mail: patagbaire@gmail.com Tel: +234(0)8135868184

ABSTRACT: With regards to current drug development strategies, natural products have continually provided an essential source for new drugs. In this study, the phytochemical constituents present in the leaf extract of *Manniophyton fulvum* were screened and its toxicological properties *in vitro* were also established. The phytochemical screening of the ethanolic leaf extract of *Manniophyton fulvum* revealed the presence of alkaloids, saponins, sterols/terpenes, reducing sugars, anthraquinones, and cardiac glycosides. The leaf extract produced toxicity at a dose of 1050 mg/kg. The observed effects could not be unconnected to the presence of the phytochemical constituents in the extract. The outcome of this study lends support to the trado-medicinal uses of *Manniophyton fulvum* in the treatment of various medical conditions.

Keywords: *Manniophyton fulvum*, Euphorbiaceae, phytochemical screening, acute toxicity studies

INTRODUCTION

The identification of plants with biochemically active compounds has in recent times, assumed a broad significance in the search for newer and effective chemotherapeutics agents [1]. Long before now, many useful chemotherapeutic agents have been sourced from some of the common medicinal plants used by traditional medicine practitioners in the treatment of several diseases [2]. This is because, plants are known to synthesize very complex molecules which apart from their definite stereochemistry, also possesses biological compounds with unique mode of action [3]. These compounds known collectively as Phytochemicals include the water or ethanol soluble compounds like peptide; unsaturated long chain fatty acids; alkaloids that possesses mood-changing and mode enhancement properties; phenols that possesses antioxidant and venotonic properties; tannins which possesses natural antibiotic properties; saponins that possesses cleaning properties and also as foam producers; flavonoids; and essential oils [4,5]. While the actions of most clinical drugs currently in use today are based on the activities of their constituent Phytochemicals [6], it is equally an established research model, to scientifically identify active biochemical compounds present in medicinal herbs as a way of unearthing the basis for their pharmaco-biological actions [7].

Manniophyton fulvum remains one of the popular herbs amongst local traditional medicine practitioners in the south-south region of Nigeria. It belongs to the family Euphorbiaceae. It is also geographically distributed widely in tropical Africa, from Sierra Leone to Sudan, and South-ward to Angola [8]. In African Traditional medicine the root, stem, bark and leaf are credited with analgesic properties against diarrhea, stomach ache, cough and bronchitis [9]. According to unconfirmed folklore in the south-south region of Nigeria, traditional herbalists have used the root extract of *Manniophyton fulvum* to treat erectile dysfunction. The red stem sap is credited with haemostatic properties, while the leaf sap is used against ear problems, caries [10]. In Congo (Brazzaville), it is considered a cicatrisant on wounds, and also good for treating dysentery, piles, haemophthysis and dysmenorrhea [11,12]. The red stem-sap is used topically in Ivory Coast on herpes and other dermal infections [13].

While the leaf-sap is similarly applied to areas of leprosy in Congo (Brazzaville) [12] in Zaire, the leaf-sap is an ear-instillation for ear-troubles [14]. A decoction of the young shoots, bark and stem, the husk of the nut and the sap are used as a remedy for cough in Congo (Brazzaville), Ivory coast and Sierra Leone [11,12,15]. However, with no research information on the phytochemical analysis of the leaf extracts, as well as of the toxicological profile of *Manniophyton fulvum*, this study will help to discover the chemical constituents of the aqueous and ethanol leaf extracts of *Manniophyton fulvum* as well as determine the oral acute toxicity (LD₅₀) of the two extracts in Wistar rats.

MATERIALS AND METHODS

Plant collection and identification.

The fresh leaves of *Manniophyton fulvum* were collected at the side of the Medical Hostel Site III of the Delta State University, Abraka, Nigeria. They were identified at the Botany Department of the same University.

Preparation of extract.

The leaves of *Manniophyton fulvum* were sun-dried for one week. The dried leaves were blended into powdered form with grinding machine and weighed. The powder was divided into two equal parts of 250g each. Each portion were soaked in 2000ml of distilled water and 2000ml of ethanol respectively for 72 hours. The extract was obtained using an electrical evaporator extraction apparatus (rotory evaporator). The solvent was extracted under heat pressure and a paste like extract was obtained. It was then oven dried to complete solid and grinded to yield powder.

PHYTOCHEMICAL SCREENING

Tests for Alkaloids: In testing for Alkaloids, Safowora, 2008[16] method was employed. About 0.5g of each extract was stirred with 5ml of 1 per cent aqueous hydrochloric acid on a water bath; 1ml of the filtrate was then treated with a few drops of mayer's reagent and another 1ml portion was treated with Dragendorff's reagent. Turbidity or precipitation with either reagents was an indication of the presence of alkaloids.

Terpenoids and Steroids (Liebermann Buchart test): A small quantity of each extract was dissolved in trichloromethane, and a minimum volume of concentrated sulphuric acid was then added to its content. A blue or green color or a mixture of these two shades was taken as positive test for steroidal compounds [17].

Test for tannins: The plant extract (0.2 g) was re-extracted with ethanol. The solution obtained was later treated with 5 % ferric chloride. A blue-black or blue-green appearance was taken as positive test for tannins [17].

Tests for Saponins: A small portion of each extract was added to 2 ml of distilled water and boiled for 3-5 minutes. The resultant mixture was filtered, allowed to cool with the filtrate shaken vigorously. Honey comb froth higher than the aqueous layer was taken as strongly positive for saponins. Froth as high as the aqueous layer was taken as moderate as and lower than this as negative for the presence of saponins [17].

Test for Phlobatannins: An aqueous extract of the plant part was boiled with 1 per cent aqueous hydrochloric acid, a deposition of a red precipitate was taken as evidence for the presence of phlobotannins [18]

Test for Anthraquinones: Borntrager's test was used for the detection of anthraquinones. 5g of each plant extract is to be shaken with 10ml benzene, filtered and 5ml of 10 per cent ammonia solution added to the filtrate. The mixture is to be shaken and the presence of a pink, red or violet colour was an indication of the presence of anthraquinones[19].

Test for Cardiac Glycosides: Lieberman's test. 0.5g of the extract was dissolved in 2ml of acetic anhydride and cooled well in ice; sulphuric acid was then carefully added. A colour change from violet to blue to green was an indication of the presence of a steroidal nucleus (i.e. aglycone part of the cardiac glycoside) [20].

Test for Reducing Sugars: To 2mls of the extract, 5ml of a mixture (1:1) of Fehling's solution 1A and Fehling's solution 11B was added and the mixture boiled in a water bath for five minutes. A brick-red precipitate indicated the presence of free reducing sugars [21]

Test for Resin: 10ml of petroleum ether was put in a test-tube and the same amount of copper acetate solution was added and the mixture was vigorously shaken. It was then allowed to separate, a green colour indicated the presence of resin [19].

ANIMAL

Procurement and Handling.

Healthy adult albino rats of Wistar strain, weighing between 150 - 200 g were obtained from the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The rats were housed in separate well ventilated cages, and kept at room temperature ($24 \pm 2^\circ\text{C}$) with a 12:12 hour light/dark cycle. The animals were fed with standard pellet diet and provided drinking water *ad libitum*. The animals were acclimatized for two weeks and received humane care in compliance with the ethical guide for the care and use of Laboratory Animals approved by the College of Health Sciences, Delta State, University Abraka, Delta State, Nigeria. After the two weeks of acclimatization, the experimental handlings of the groups were varied.

Acute Toxicity Studies.

The LD₅₀ was carried out by adopting the method outlined by Lorke (1983)[22]. A total of thirty-four rats were used for these studies.

In the **initial phase**, eighteen rats were grouped into six groups of three rats each, and were treated with both aqueous and ethanol extracts of *Manniophyton fulvum* at doses of 500, 1000 and 1500mg/kg body weight orally. The animals were observed for 24 hours. In the **second phase** which is deduced from the first phase, sixteen rats were grouped into four groups of four rats each, and were treated with both aqueous and ethanol extracts of *Manniophyton fulvum* at doses of 200, 400, 800 and 1200mg/kg body weight orally. They were also observed for 24 hours as in the first phase, and the final LD₅₀ values were determined.

RESULTS

Some physical properties of the aqueous and ethanol extract of *Manniophyton fulvum* are given in Table 1. Phytochemical constituents observed from the aqueous and ethanolic extract of the leaves of this plant are reducing sugars, flavonoids, tannins, sterols/terpenes, alkaloids and Cardiac glycosides (Table2).

Table 1: Some physical properties of the aqueous and ethanol extract of leaves of *Manniophyton fulvum*

Physical parameters Inference

Groups	pH	Colour	Solvent	Odour	Texture
Aqueous	6.92	Dark-green	Distilled Water	Characteristic	Dry powder
Ethanol	4.98	Brownish-black	Ethanol (90-96%)	Characteristic	Jelly-like

Table 2: Some Secondary Metabolites of the aqueous and ethanolic extract of the leaves of *Manniophyton fulvum*

Constituents Present

Substance Screened for	Aqueous	Substance Screened for	Ethanolic
Reducing sugar	++	Reducing sugar	++
Anthraquinone	-	Anthraquinone	-
Flavonoids	++	Flavonoids	++
Saponins	+	Saponins	-
Tannins	++	Tannins	++
Sterols/ Terpenes	+	Sterols/ Terpenes	++
Alkaloids	++	Alkaloids	+
Resins	-	Resins	-
Cardiac glycosides	++	Cardiac glycosides	++

Key (+) = present, (++) = highly present (in appropriate quantity) (-) = absent

The oral LD₅₀ value of the aqueous and ethanol extract of the leaves of *Manniophyton fulvum* was determined to be 950-1000mg/kg and 850mg/kg body weight in rat respectively. However, some signs of toxicity observed in some animals were hypersensitivity, convulsions, weakness, dizziness, loss of appetite tremor and death as summarized in Table 3 below.

Table 3: Acute toxicity tests of the aqueous and ethanol leaf extracts of *Manniophyton fulvum* Doses (mg/kg) Survival rate (phase 1)

Concentration	Aqueous	Ethanol
500	0/3	0/3
1000	1/3	3/3
1500	3/3	3/3

Doses (mg/kg) Survival rate (phase II)

Concentration	Aqueous	Ethanol
200	0/2	0/2
400	0/2	0/2
800	0/2	2/2
1600	2/2	2/2

(By determining the cube root of the three values, (Lorke, 1983))

DISCUSSION

The result obtained from this study revealed that the aqueous and ethanol plant extract of *Manniophyton fulvum*, a family of Euphorbiaceae, contains bioactive agents. These agents are Alkaloids, Saponin, Sterols/Terpenes, Reducing Sugar, Anthraquinones, Cardiac Glycosides, tannins and flavonoids. These are the main effective compounds of medicinal plants and these active medicinal constituents have some medicinal values as shown in various studies [22, 23, 24].

The Saponins are produced by plant to stop bacterial and fungal attacks. They have been reported as natural antibiotics helping the body to fight infections and capable of knocking out some tumor cells particularly lung and blood cancers [22,23,25]. The most important properties of the saponins were reported to include expectorant, diuretic, analgesic and promotion of wound healing [26].

Tannins have been reported to possess astringent properties; hasten the healing of wounds and inflamed mucus membranes [25].

Some forms of glycosides are known to possess analgesic and anti-inflammatory properties. Cardiac glycosides have a strong and direct action on the heart, helping to support its strength and rate of contraction when it is failing. Cardiac glycosides are also significantly diuretic. They also help to transfer fluids from the tissues and circulatory system to the urinary tract, thereby lowering blood pressure [27].

Anthraquinones have an irritant laxative effect on the large intestine, causing contractions of the intestinal walls which stimulate bowel movement and make the stool more liquid thereby easing bowel movements [28]

The discovery of saponins, tannins, flavonoids, alkaloids, anthraquinones and glycosides as phytochemical constituents in the extract provides some justification for the many medicinal uses of the parts of this plant.

The aqueous and ethanol extract of the leaf of this plant possess an LD₅₀ value of 1050mg/kg body weight in rat, orally. However, some signs of toxicity observed in some animals were loss of appetite, stoolings, weakness, dizziness, convulsions, tremor and death as summarized in Table 3. As compared to earlier studies done on the aqueous and ethanol root extract of *Manniophyton fulvum*, the result shown in table 3, the leaf extract of *Manniophyton fulvum* is less toxic than that of the root extract, being that the dosage required to cause death in 50% of the animals using the root extract is about 850 mg/kg [29], whereas the leaf extract produced toxicity at a dose of 1050 mg/kg.

CONCLUSION

This study provides for record purposes, information on the LD₅₀ of the aqueous and ethanol extract of the leaves of *Manniophyton fulvum* determined by the oral route as well as the results of a phytochemical screening. The confirmation of the presence of Alkaloids, Saponins, Sterols/Terpenes, Reducing Sugars, Anthraquinones, and Cardiac Glycosides as constituents of the extract also possibly justifies some of the medicinal uses of this plant, as these constituent are known to possess pharmacological properties related to such uses. This shows that the plant is a good medicinal plant and it has a moderate toxicological value.

RECOMMENDATION

With the results obtained from this study, an extensive Phytochemical screening accompanied with toxicological analysis on the aqueous and ethanol on the bark and stem of *Manniophyton fulvum* will be recommended to establish whether such products are suitable for human consumption. Further studies should also be carried out on decoction, concoction extraction of the bark, root, stem and leaf. There is equally a need for research that will describe the anatomical structure and physical properties of the fibres. The antioxidant properties of the leaves may offer an opportunity to obtain natural anti-oxidant compounds useful in food conservation.

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