International Journal of Natural and Applied Sciences, 2(3): 225-229, 2006
©Tapas Institute of Scientific Research and Development, 2006

PHYTOCHEMICAL, TOXICOLOGICAL AND HISTOPATHOLOGICAL STUDIES OF SOME MEDICINAL PLANTS IN NIGERIA

F. C. Onwuliri[1], J. D. Mawak[2], D. L. Wonang[1] and E. A. Onwuliri[1]
[1]Applied Microbiology Unit, Department of Botany, University of Jos, Nigeria
[2]Department of Microbiology, University of Jos, Nigeria
Corresponding author: Dr F. C. Onwuliri; E-mail: faconwuliri@yahoo.com

ABSTRACT

Phytochemical, toxicological and histo-pathological studies were carried out on the leaves and stem bark extracts of *Grewia mollis, Boswellia dalziellii, Jatropha curcas* and *Pterocarpus erinaceus* claimed to be of medicinal values in Nigeria. The soxhlet apparatus was used for extraction of the crude materials. The result revealed the presence of tannins, saponins, flavonoids, glycosides, balsam, phenols, terpenes, steroids and the absence of alkaloids in *Grewia mollis* (bark), *Boswellia dalziellii* (leaf) and *Pterocarpus erinaceus* (bark) as well as cyanogenic glycosides in all the plant materials. Toxicological result showed that the plant is safe for human consumption but with caution having produce LD_{50} at 1500 mg/kg body weight. The extracts showed no structural effects on the liver and heart. In the kidney however, the convoluted tubules were filled with fluids, which suggested that the extract should be used with caution on patients with renal failure. The plants therefore possess some important biological activities that could be harnessed and employed beneficially in the management of viral and bacterial infections.

Keywords: Phytochemistry, toxicology, histo-pathology, rat, medicinal plants, Nigeria

INTRODUCTION

Plants comparatively have a much greater biosynthetic ability than animals. Not only do plants make all the biomolecules that are universally required in living organisms, they also synthesize a vast array or complex chemicals, which are unique to them. Phytochemicals are products of secondary metabolism defined variously as a metabolic cul de sac or pheripheral metabolic pathways leading off from the universal (or primary) metabolism. Phytochemicals ranges from cell wall substances through photosynthesis pigments, terpenes and terpnoids, the alkaloids, plants phenolics to plant hormones, plant non-proteins, amino acids and cyanogenic glycosides (Harborne, 1973). Many phytochemicals have been shown to be bioactive. that is they exhibit pronounced biological activity in other living organisms (Harborne, 1973). Many of the bioactive phytochemicals have found usefulness as chemotherapeutic agents, pesticides, food additives, and other biologicals.

On the other hand, many human and animal diseases condition, nutritional and otherwise have been traced to exposure to certain bioactive phytochemicals in food and fodder. Many plants used for nutrition and as therapeutic agents have been reported to cause instant death after use. Therefore, toxicity has been one of the major limitations of the use of tropical plants as therapeutic agents. The claims by the traditional medicine practitioners in Nigeria that *Grewia mollis, Boswellia dalziellii, Jatropha curcas* and *Pterocarpus erinaceus* are effective in the management of various infectious diseases needs to be evaluated.

Phytochemical analysis is a routine investigation in biochemical studies of plant extracts and in nutritional studies on foodstuff of plant origin. Some of the phytochemical substances that are usually tested for in plant extracts include alkaloids, terpernoids, tannins, flavonoids and saponins among others (Onwuliri and Umezurumba, 2003).

Jatropha curcas (Bubble bush) is a drought – resistant perennial, growing well in marginal, poor soil. It is easy to establish, grows relatively quickly, producing seeds for 50 years (Gill, 1987). Jatropha produces seeds with oil content of 37%. The oil can be combusted as fuel without being refined. The by-products are press cake, which serves a good organic fertilizer. The plant grows in many parts of Nigeria. It is rugged in nature and can survive with minimum input.

Medicinally, it is used in the treatment of diseases like cancer, snakebites, dropsy and paralysis among others (George and Pamploma-Roger, 1999). The roots, stems, leaves, seeds and fruits of the plant have been widely used in traditional folk medicine in many parts of West Africa. The seeds of *J. curcas* have been used as a purgative, anthelminthic and abortifacient as well as for treating ascites,

paralysis and skin diseases (Gills, 1987). Oil from the seed has been used as an ingredient in the treatment of rheumatic conditions, itches, fever, jaundice, gonorrhea, as a diuretic agent and as a mouthwash. The leaf has been used as a haemostatic agent and also in the boasting of immunity against some infectious diseases. The bark is used as a fish poison (George and Pamploma-Roger, 1999).

Grewia mollis (False button weed) belongs to the family Tiliaceae and is composed of 50 genera and 450 species distributed in the tropical regions. The plant is mostly trees or shrubs and rarely herbs. The stem is tall, erect, simple or branded with mucilage. The leaves are alternate, simple, entire or toothed and stipulate (Gills, 1987). *Grewia mollis* is of great importance as it contains chemical constituents, which have medical properties. The plant is also known to yield timber. The leaves, stems and fruits are used to cure gonorrhea (Gills, 1987).

Boswellia dalzeielii, Hutch (Family: Burseraceae) is a tree of the savanna forest recognizable by its papery bark peeling off in a ragged manner. The bark yields a whitish gum resin, which dries readily and friable. The plant products (such as the gum resin) and different parts of the plant are widely employed in traditional medicine. The gum resin is used along with other materials as stomach disturbance remedy and for the treatment of venereal diseases. The bark is boiled in large quantities and used for fever, rheumatism and gastrointestinal troubles treatment. The root decoction boiled along with *Hibiscus sabdariffa* is used for the treatment of syphilis. The root decoction with *Damiellia oliveri* is used in wounds. In Nigeria (specifically in Adamawa State), the fresh bark is often used as an anti-emetic and relief of giddiness and palpitations.

This study was therefore designed to determine the essential chemical composition of these plant materials that give them their medicinal properties as well as the toxicity of the plant extracts in experimental rats.

MATERIALS AND METHODS

Collection, preparation and extraction of plant materials: The leaves and stem barks of *Grewia mollis, Boswellia dalziellii, Jatropha curcas* and *Pterocarpus erinaceus* claimed to be of medicinal values in Nigeria were collected from Jos North L.G.A. of Plateau State, Nigeria and used for the study. The plants were identified at the Department of Botany, University of Jos, Nigeria.

The leaves and barks were dried in open air under shade to prevent the direct sunlight from inactivating the chemical constituents. The samples were pulverized into powders and stored in polyethylene bags until required for analysis. Altogether, six samples made up of leave and stem backs of *Boswellia dalziellii,* and *Pterocarpus erinaceus,* leaf of *Jatropha curcas* and back of *Grewia mollis.*

Fifteen grams (15 g) each of the six-powdered plant sample was extracted using a soxhlet extractor according to standard procedure. The resultant concentrates of extraction were then evaporated in a water bath at 60^0C to obtain the extract in solid. The extract was then stored in airtight containers and kept in the refrigerator at 4^0C until needed. Percentage yield was then calculated as

$$\% \text{ Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Crude Drug}} \times \frac{100}{1}$$

Phytochemical screening of the plants extracts: The phytochemical screenings of the plant extracts were carried out using standard qualitative procedures as described by Trease and Evans (1984), Sofowora (1982) and Onwuliri (2004). Altogether, the parameters screened were alkaloids (dragendorf reagent), flavonoid (lead acetate), tannins (ferric chloride), saponins (general test), cardiac glycoside (salkowski), cyanogenic glycoside and terpenes/steroids (phenol, resin and balsam)

Histo-pathological Test: A total of 6 adult winster rats were used determine the toxicity of the plant extracts. Each of the rats was administered 500 mg/kg of on of the plant extract intra-peritoneally for two weeks. The rats were maintained on commercial concentrate ration and water *ad-libitum.* Thereafter, the animals were scarified and their kidney, heart and liver harvested for histo-pathological studies. The tissues were prepared histo-pathological examination according to the method described by Brenda and Rack (1989). The sections were cut between 5-10 µm. And thereafter mounted as permanent slides ready for microscopic examination.

LD_{50} determination: A pilot test was first carried out using ten rats, which were grouped into five groups, two in each group. Different doses of the extracts were administered to the rats intra-peritoneally (IP) according to their body weight. The rats were then observed for 24 hours, after which the dose regimen for the LD_{50} determination was drawn up to be 500 mg/kg, 1000 mg/kg, and 2000 mg/kg body weight.

Another group of 20 rats were divided into four groups of 5 rats and each group was administered one of the doses of the drug extract intra-peritoneally based on the body weight of each group. The animals were kept for 24 hours and observations recorded. The LD_{50} was determined by the graphical, probit value on the herb or arithmetic methods (Trease and Evans, 1983).

RESULTS

The results of phytochemical screening of the crude extract of plant materials are represented in Table 1. The extracts contained alkaloids, flavonoids, tannins, saponins, terpenes, steroid, phenol, cardiac glycosides and balsam while cyanogenic glycoside was absent. The result of the percentage mortality of the mice is represented in Table 2. The result revealed that group D rats receiving 2000 mg/kg body weight of the test extract had the highest mortality rate.

The result of the mean death (*Md*), dose differences (*Dd*), and their products are shown in Table 3. The dose difference was similar (50.0 mg/kg) in all groups, while mean death (*Md*) was shown to be 3,250. The lethal median dose (LD_{50}) occurred in group C rats, where 50% of the population was killed by the dose (1,500 mg/kg). The group D rats with the highest log dose had the highest percentage cumulative death (Table 4).

From the gross and histological studies, the kidneys of the unmarked pilot rat had tabular necrosis (Localized death), which was more pronounced, at the cortical region. Its kidneys and liver were dark brown and edematous while the marked rats kidneys and liver were bright red in color. Structural differences were also noted in the kidneys of treated rats against the untreated where the convoluted tubules were seen to be filled with fluids.

Table 1: phytochemical characteristics of the extracts of the leaves and barks of the plant used

Chemical Components	Crude Extracts					
	A	B	C	D	E	F
Alkaloids (dragendorf reagent)	-	-	+	+	+	-
Flavonoid (lead acetate)	+	+	+	+	+	+
Tannins (ferric chloride	+	++	++	+	+	+
Saponin (general test)	+	+	+	+	+	+
Cardiac Glycoside (salkowski)	+	+	+	++	+	+
Cyanogenic glycoside	–	–	–	–	–	–
Terpenes/Steroids	+	+	+	+	+	+
Phenol	+	++	++	+	++	++
Resin	+	+	+	+	+	+
Balsam	+	++	++	+	++	+

Key:

A = *Grewia mollis (The bark)*
B = *Boswellia dalzielii (The leaf)*
C = *Boswellia dalzielii (The bark)*
D = *Jatropha curcas (The leaf)*
E = *Pterocarpus erinaceus (The leaf)*
F = *Pterocarpus erinaceus (The bark)*
+ = *Present.*
++ = Highly Present
- = Not Present

DISCUSSION

The phytochemical constituents of the test plants investigated in this study are alkaloids, balsam, flavonoids, tannins, saponins, terpenes, steroids, phenols and cardiac glycosides at variable concentrations. Sofowora and Olaniyi (1979), Sofowora (1982) and Onwuliri, (2004) have also observed the presence of such constituents as alkaloids, saponins, flavonoids, tannins, glycosides among others in tropical plants growing in Nigeria. Some of which have been shown to exhibit varying biological activities. These include the inhibition of dental plaque formation, analgesic, anti sickling and antiviral activity. The most significant property of these plants was reported to be their anti-microbial and anti-viral activities against common pathogens.

Table 2: Number of death and percentage mortality for mice with plant extracts

Group	No. of Mice	Average weight of mice	Dose (mg/kg)	Number of Death	(%) Mortality
A	5	20	500	0	0
B	5	30	1000	1	20
C	5	18	1500	3	60
D	5	34	2000	5	100

Table 3: Determination of mean death (md), dose difference (dd) and product for mice tested with plant extracts

Group of Mice	Dose for mice (mg/kg)	Dose diff (Dd) mg/kg	Number of Death	Mean Death	Product of Md and Dd (MdDD
A	500		0		
B	1000	500	1	0.5	250
C	1500	500	3	2	1000
D	2000	500	5	4	2000
Total	5000	1500	9	6.8	3250

Table 4: Determination of cumulative (death and survived), percentage cumulative death and log dose for mice tested with plant extracts.

Group of Mice	Dose (mg/kg)	Death	Survived	Cumulative Death	Survived	% Cumulative Death	Log Dose (mg/kg)
A	500	0	5	0	5	0	2.69
B	100	1	4	1	9	11.11	3.00
C	1500	3	2	4	11	44.44	3.17
D	2000	5	0	9	11	100	3.30

The presence of glycosides in the plant materials is expected, as glycosides are known to occur universally in the higher plants (Trease and Evans, 1983). It is of interesting that none of these plant materials showed the presence of cyanogenic glycosides. This makes the plants safer for consumption since cyanogenic glycosides are known to release on hydrolysis, the very poisonous cyanic acid (Harbone, 1973).

The presence of steroids in these plant materials suggests that they could be of some uses other than the ones already known. It is possible that these steroids occur as part of the aglycone moieties of other constituents of the plants like saponins and alkaloids (Harbone, 1973).

The LD_{50} is a measure of how lethal/toxic chemotherapeutic agents can be half of the population under study. (Onwuliri and Umezurumba, 2003). The results showed that for all doses used, only the dose of 1500mg/kg body weight and above produced LD_{50} and doses with values less than 1000mg/kg body weights are of no practical interest (Lorke, 1983). From the gross histological studies, the kidneys of the unmarked pilot rat had tabular necrosis (localized death), which was more pronounced, at the cortical region. Its kidneys and liver were dark brown and edematous while the marked rats kidneys and liver were bright red in color. This agrees with the findings of Aguiyi *et al.* (1996) where hepatic congestion and necrosis of the kidney of experimental rats was precipitated by an overt decomposition of the renal tubules. The edematous nature of the post mortem organs could be due to tissue biodegradation leading to carbon dioxide production and accumulation in the organs. Structural differences were also noted in the kidneys of treated rats against the untreated where the convoluted tubules were seen to be filled with fluids, which suggested that the drugs should be used with caution, most especially for patients with renal failure.

CONCLUSION

The traditional claims of these plants for the treatment of some infectious diseases seem justified. This is because the present of chemical compositions and toxicity studies tend to support the use of these herbs. The claims by the traditional medicine practitioners of herbs that are effective against various diseases may provide new research interests for pharmaceutical industries.

ACKNOWLEDGEMENT

The author wishes to thank the Management of Victory Laboratory for using their laboratory facilities in the course of this project.

REFERENCES

Aguiyi, J. C., Johnson, P. B., Obi, C. I., Onwukeme, K. and Dafur, S. J. (1996). Studies on the rodenticidal activities of indomethacin. *West African Journal of Pharmacology and Drug Research*, 1: 37-40.

George. P. and Pamploma – Roger, M. D. (1999). *Encyclopedia of Medicinal Plants*. Editorial Safeliz S. L. Aranvaco. 10: 57 – 59; 20: 49-52.

Gills, L. S (1987). *Taxonomy of Flowering Plants*. Africana Fep Publishers., Onitsha 281-287pp.

Harborne, J. B. (1973). Phytochemical screening methods: *A guide to modern techniques in plants analysis. Chapman and Hall*. London Pp: 47 – 74.

Lorke, D. (1983). A New approach to practical Acute toxicity testing. *Arch. Toxical.,* 54: 275-289.

Onwuliri F. C. and Umezurumba I. C. (2003). Studies on the investigation of the claimed antimicrobial potency of selected plant parts on *Salmonella typhi. Journal of Medicine in the Tropics*, 5 (1): 30 – 35.

Onwuliri, F. C., (2004). Antimicrobial studies of the extracts of *Acalypha wilkesiana L.* on microorganisms associated with wound and skin infections. *West African Journal of Biological Science*, 15: 15-19.

Trease, G. E. and Evans, W. C. (1983). *Pharmacognosy*. 12th Edition. Publ. Bailere Tindall-Macmillan, London.

Sofowora, E. A., and Olaniyi, A. A. (1979). Phytochemcial examination of the stem, bark of Dracaena manni. *Proceeding of conference*. Ile-Ife: University Press, 70p.

Sofowora, E. A., (1982). *Medicinal Plants and Traditional Practice in Africa*. Publ. John Wiley and Sons, New York.