Journal of Applied and Advanced Research, 2018: 3(1) 18–21
http://dx.doi.org/10.21839/jaar.2018.v3i1.129
ISSN 2519-9412 / © 2018 Phoenix Research Publishers

Research Article – Pharmacology

Preliminary toxicity and thin layer chromatographic studies of *Pteleopsis habeensis* leaves

Falmata M. Aliyu*[1], Fave Tata[2], Fatimah H. Ashemi[2]
[1]*Department of Pharmaceutical Chemistry, University of Maiduguri, P.M.B 1069, Maiduguri, Nigeria.*
[2]*Department of Pharmacology and Toxicology, University of Maiduguri, P.M.B 1069, Maiduguri, Nigeria*

(Received: 16-02-2018; Accepted 27-02-2018; Published Online 28-02-2018)
*Corresponding author

Abstract

Oral acute toxicity test in which a single dose is used in an animal on one occasion is to identify the gross behaviour and the dose that will cause the animal to die. To investigate the phytoconstituents and toxicity of different extracts of *P. habeensis* in wistar strain Albino rats. The acute toxicity was determined through oral administration of different doses of *P. haeensis* leaf extracts to Wistar albino rats in increasing concentrations to ascertain its safety. The animals were monitored daily for 10 days for signs of toxicity such changes in their general behaviour and death as end point. The lethal dose (LD_{50}) of *P. habeensis* leaves extract were found to be ≥ 4000 mg/kgand there was no any sign of toxicity or changes in the gross behaviour when observed. The extracts were found to contain some important phytochemical constituent such as alkaloid, flavonoids, Tannin and Triterpenoids. TLC studies illustrated the spots of different phytoconstituent presents. The findings revealed that with the different extracts of *P. habeensis* no sign of toxicity on short term exposure of a single dose and contains different active constituent which are associated with some pharmacological effects.

Keywords: *Pteloepsis habeensis*, Phytoconstituents, TLC, Acute toxicity

Introduction

A large number of the world population relies mainly on plants and plant extracts for health care and more than 30% of the entire plant species, at one time or the other was used for medicinal purposes [1]. Africa has been a rich source of medical plants and compounds which have proved to be useful in a number of diseases. The World Health Organization (WHO) has estimated the level of populace that use traditional medicine in developing countries at about 80%. These medicinal products have been used for a variety of ailments. Extraction and characterization of several active phytochemicals from these green factories have given birth to some high activity profile drugs [2]. The use of traditional medicine is an important part in Nigeria and was practiced by ancients long before the introduction of modern medicine. Nigeria is blessed with a wide variety of medicinal plant that are use in the management of many disease conditions. Many Nigerians resorts to the use of folk remedies due to their affordability, accessibility, less side effects as well as potency. The ability of such plants to treat ailments might be related to the phytochemicals present in such plants. Even though, people use traditional medicine for treating diseases, toxicity profile of most plants are yet to be explored. *Pteolopsis habeensis* (Aubrev ex Keay) belongs to the family Combretaceae, is known as Lallen giwa in Hausa language in northern part of Nigeria of Sub Saharan Africa [3]. It is used traditionally in treating Malaria, Stomach ache, Cancer and as an aphrodisiac [3]. It is found in Mali, Ghana, some parts of Nigeriaand Benin [4]. As no work has been done on ingestion of *P. habeensis* at high doses, the systemic approach in evaluating their efficacy and safety profile is needed. Therefore, the present study was aimed to evaluate the safety of *P. habeensis* leaves extract in acute toxicity tests in Wistar strains albino rats.

Materials and Methods

Dragendorff reagents, distilled water, Methanol, conc Sulphuric acid, Mayers reagent, Wagners reagent, Fehlings solution A and B, 10% Ferric chloride. All the chemicals and reagents used in this study were of analytical grade.

Collection and Preparation of Sample Materials

P. habeensis leaves were collected at the outskirt of Maiduguri, Borno state, Nigeria. The samples collected were packed separately in clean sterilized polythene bags and brought to the herbarium of the department of Biological Sciences, University of Maiduguri for identification and authentication. The fresh samples were washed with tap water and air dried under shade. Dried samples of plant material collected were milled into fine powder using high capacity grinding machine and subsequently stored separately in sterilized polythene bags until required for further use.

Extraction of plant material

500g of the powdered material was extracted successively with 95% hexane followed by Chloroform then ethanol and Methanol respectively each for 48h. Different fractions of the extract were filtered separately and evaporated to dryness. Extracts were evaluated for phytochemicals and toxicological study.

Phytochemical screening

The phytochemical analysis of the different leaves extracts of P. habeensis was done in accordance with standard methods [5,6].

TLC Chromatography

Three grams (3g) of each hexane, Chlroform, Methanol and Ethano extracts was dissolved in their respective solvent,

Table I: The Phytochemical constituent of different extracts of *P. Habeensis*

S.No	Phytoconstituents	Test	Hexane	Chloroform	Ethanol	Methanol
1	Carbohydrates	Molisch Test	+	+	+	+
		Barfoed	-	+	+	+
		Fehlings	-	+	-	+
2	Tannins		+	+	+	+
3	Anthraquinones		-	-	-	-
4	Cardiac glycosides steroid		+	+	+	+
	Triterpenoids		+	+	+	+
5	Saponins		-	-	-	+
6	Alkaloids	Dragendorff	+	+	+	+
		Mayer	+	+	+	+
		Wagner	+	+	+	+
7	Flavonoids	NaOH Test	-	+	+	+

i.e. n-hexane, Chloroform, Methanol and Ethanol respectively to form a sample solution. The first solvent system was made from n- hexane and ethyl acetate 4:1 (v/v) and the second was made from n- hexane 100%. Capillary tube was used to spot a sample solution on the silica gel TLC plate at 1cm from the edged of the prepared commercial plates and the drop is allowed to dry. The plate was placed in TLC (Chromo tank) and allows ascend the TLC plate by capillary action. The plate was removed and the solvent front was marked then allowed to dry. 10% Sulphuric acid was used as the visualizing agent to detect the spots. A meter rule was used to measure the distance moved by the solvent and distance moved by spot, from which the retention factor (Rf values) of the various spots was calculated.

Rf = Distance move by spot front/ Distance move by solvent front

Experimental Animal

A total of 20 wistar strain albino rats of both sexes were used for the toxicity study. Average weight of 95-120g were used. The animal were allowed to acclimatize for 7 days and allow accesses to free food and water. The animal were kept and handled under standard conditions as described by ICLAS and CIOMS guidelines of 2012.

Acute toxicity

LD_{50} Determination Using the OECD method

The acute toxicity (LD_{50}) of ethanol, methanol, hexane and chloroform extracts of *P. habeensis* were determined using organization for economic cooperation and development (OECD) guideline in rats (OECD Test Guideline 425) [7]. The limit dose test, up and down procedure as modified in this study was adopted to evaluate the acute toxicity of the extracts of *P. habeensis* following oral administration in Wistar strain albino rats (95–120 g) which were maintained under the standard conditions and allow free access to food and water.

The animals (n = 2 per dose) were fasted 4 h prior to the experiment. Animals were administered with single dose of extract of *P. habeensis* at a dose of 2000 mg/kg and observed for their mortality and signs of toxicity during the first 4 h, 24 h, 48 h for the short-term toxicity outcome and finally monitored the surviving animals for the next 10 days study period for any delayed toxic effects or death and the dose was increased up to 4000 mg/kg and were observed up to 10 days. The animals were observed for 24 h to48 h before dosing the next animals. The toxicity observed include increased motor activities, rolling, writhing, depression, behavior pattern, diarrhea, sleep, coma, strength of grip, tremors, convulsions, stimulation, respiratory frequency and any death present [8].

Results

Phytochemical screening

Determination of Acute Toxicity

OECD method was used to determine the acute toxicity (LD_{50}) of the leave extracts (ethanol, methanol, hexane and chloroform) of *P. habeensis* via oral route and showed that the LD_{50} were greater than or equal to ≥ 4000mg/kg mg /kg. There were no death or any sign of toxicity recorded as all the animals were stable for the period of study (10 days).

Thin Layer Chromatography

Figure 1: TLC screening of bioactive compounds present in the fractionated fractions of *P. habeensis* with 100% Chloroform as developing system

Figure 2: TLC screening of bioactive compounds present in the fractionated fractions of *P. habeensis* with 75% n-Hexane and 25% Ethyl acetate as developing system

The result of thin layer chromatography of *P. habeensis* extracts showed different spot on the TLC plates as illustrated in Fig1-3

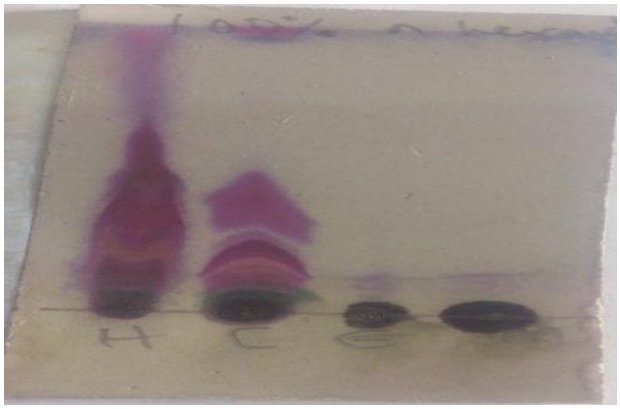

Figure 3: TLC screening of bioactive compounds present in the fractionated fractions of *P. habeensis* with 100% n-Hexane as developing system

Discussion

The present study demonstrated the presence of different secondary metabolites like tannins, saponins alkaloids, glycosides, flavonoids, and others from qualitative phytochemical analysis of *P. habeensis*, thus, indicating that the plant contains bioactive compounds.

The study is in conformity with that of [9] that's howed the presence of Alkaloids and Saponin in Methanol extracts. However, the study is dissimilar with the study of [10] that reveal the presence of Anthraquinone in Methanol extract which is absent here. From Table I, the methanol and ethanol extract of the extracts contains more active phytochemicals compared to the nonpolar extracts. This indicates that polar solvents (Methanol and ethanol) have more extractive effects than the nonpolar solvents (Hexane and Chloroform). This buttress the report of [11], thus the extracting solvents significantly affect the measured total phytochemical content as a result of solvent polarity which is an important parameter that affects the yield of a plant material and that organic solvents such as alcohols have been found to provide more abundant elements.[11]From the finding, it could also inferred that the extracts obtained from organic solvent will have more efficacy and to certain extent toxicity as compared to the inorganic extracts because the safety and therapeutic efficiency of medicinal plants are attributed to their constituents active component [12].

Table II: Acute toxicity study of *P. habeesis* leaves extract in Wistar strain albino rats

Extracts	Route of administration	Lethal Dose (LD_{50})
Ethanol extract	Oral	≥ 4000 mg/kg
Methanol extract	Oral	≥ 4000 mg/kg
Hexane extract	Oral	≥ 4000 mg/kg
Chloroform extract	Oral	≥ 4000 mg/kg

Toxicity is a manifestation of harmfulness of a chemical entity on a biological system, indicating the state of adverse effects led by the interaction between toxicants and cells [13].

The oral acute toxicity (LD_{50}) of *P. habeensis* extracts obtained from the different extracting solvents did not produce any lethality or toxic effects on the experimental rats after single dose exposure of different doses (Table II) Thus, the LD_{50} were found to be greater than or equal to 4000mg/kg. It is the first study to determine the toxicity profile of *P. habeensis*.

Conclusion

Conclusively, the results obtained in the present study indicates that the extracts have the potential to act as a source of useful drugs because of presence of various phytochemical components. The plant is nontoxic on short term exposure as no signs of toxicity and mortality were recorded at different doses of extracts used. However, further research need to be carried out to determine their effect on biochemical and haematological parameter.

Acknowledgement

The authors wish to express their sincere gratitude to Department of Pharmaceutical Chemistry ad Department of Pharmacology, University of Maiduguri, P.M.B 1069, Maiduguri, Nigeria for providing necessary facilities to carry out this research work.

Conflict of interest

We declare that we have no conflict of interest.

References

[1] S. Akroum, D. Satta, K. and Lalaoui, K. N Antibacterial Activity And Acute Toxicity Effect of Flavonoids Extracted From Mentha longifolia.*Eur. J. Sci. Res*, **2009,** 2:289-295

[2] O.Y.Sato, H. Singyouchi, K. Ohtsubo,T. Kihara, H.M. Shibata, *International Journal of Pharmaceutical Sciences and Research*, **1997,** 20**:**401-403

[3] Abdullahi, M., Muhammad, G., and Abdulkadir, N. U. Medicinal and economic plant of Nupe land 1st edition , Jube Evans Books and publication, Bida. (2003), P 71.

[4] Oyen, L.P.A., (2010). *Pteleopsis habeensis* Aubrév. Ex Keay. In: Brink, M. & Achigan-Dako, E.G. (Editors). Prota 16: Fibres/Plantes à fibres. [CD-Rom]. PROTA, Wageningen, Netherlands

[5] Trease GE, Evans WC. Trease and Evans' pharmacognosy: A physician's Guide to Herbal Medicine. 13th Edition, Bailliere Tindall London; 1989

[6] Soforowora LA. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan. Harbone.1993; 55-71.

[7] Organisation for Economic Coorperation and Development Guidance document on acute oral toxicity testingOrganisation for Economic Coorperation and Development,Paris (2001)[outline]

[8] Gaya I, Mohammad O, Suleiman A, Maje M, Adekunle A (2008). Toxicological and Lactogenic Studies on the Seeds of Hibiscus sabdariffa Linn (Malvaceae) Extract on Serum Prolactin Levels of Albino Wistar Rats. The Internet J. Endocrinol.5:2

[9] Mohammed, S. L. Phytochemical Screening and Antimicrobial Activities of Crude Methanolic extract of Pteleopsis Habeensis (Aubrev Ex Keay) Stem Bark against drug Resistant Bacteria And Fungi" *International Journal of Technical Research and*

Applications e-ISSN: 2320-8163, www.ijtra.com Volume-2. (2014), PP. 26-30

[10]F. M. Aliyu, A. Bababe, M. Kachallah, H. B. Yesufu, F. A. Goje and E. A. Gwa. Phytochemical screening, TLC profile and antioxidant activities of crude Methanol extract of *Pteleopsis habeensis* (Aubrev ex keay) *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*. 5(4), 2017, 150-155.

[11]Bimakr, M (2010) Comparison different extraction method for the extraction of major bioactive flavonoid compounds from Spearmint (Mentha Spicata L.) leaves. Food Bioprod Process;1-6.

[12]Tabasu, S. M and Khare,S (2016) Safety of Medicinal Plants: An important concern.Int *J.Pharm Bio Sci*7(1):237-243.