Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2021, 13(10): 16-19
(http://www.derpharmachemica.com/archive.html)

Phytochemical Composition and Acute Oral Toxicity Study of Ethanolic Extract of Root of *Plumbago Zeylanica*

Nto Johnson Nto[1,2], Chike I Patrick Anibeze[2], Elizabeth Finbarrs-Bello[2], Emmanuel Anayochukwu Esom[1*], Uloaku Akubueze Nto-Ezimah[3]

[1]*Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Nigeria Enugu Campus*
[2]*Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, Enugu State University of Science and Technology*
[3]*Department of Chemical Pathology, Faculty of Medical Sciences, College of Medicine, University of Nigeria Enugu Campus*

***Corresponding author:** Dr Emmanuel Anayochukwu Esom, Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Nigeria Enugu Campus, E-mail: emmanuel.esom@unn.edu.ng*

ABSTRACT

The phytochemical composition of a plant gives an impression of its medicinal property. Plumbago Zeylanica (Pz) is one of the medicinal plants in sub-Saharan Africa consumed as a supplement for maintaining good health and as an aphrodisiac for enhancing sexual vigor. There is paucity of data on the phytochemical screening and the acute Oral toxicity of root of Plumbago zeylanica harvested in Africa. This study evaluated the bioactive compounds and the acute Oral toxicity of ethanolic root extract of Pz. Phytochemical screening was done using standard and the acute Oral toxicity was conducted and computed using the improved Up-and-Down (UPD) method of Organization for Economic corporation and Development test guideline 425 statistical program which provides a point estimate of LD50. Phytochemical analysis revealed high phenolic and steroid content, a moderate content of flavonoid, terpenoid and alkaloids. Saponin was not detected. The phytochemical composition of ethanolic root extract of Pz suggest a high nutritional and medicinal value, the LD_{50} was >5000mg/kg body and the smallest usable dose recommended is 100mg/kg body weight.

Keywords: *Plumbago Zeylanica*; Acute oral toxicity; Phytochemical; Phenolic; Medicinal Plant

INTRODUCTION

Naturally occurring compounds found in plants and plant materials are known as phytochemicals. Phytochemicals also known as phytonutrients or bioactive agents consist of a variety of chemical compounds which are classified based on their chemical structure or characteristics [1]. These bioactive agents include alkaloids, steroids, phenols, tannins, saponins, flavonoids and glycosides [2,3]. They are found in plant materials such as herbs, seeds, nuts, leaves, fruits, vegetables and grains and are largely responsible for the color, flavor and odor of the plant [4,5]. The immense health benefits derived from medicinal plants are as a result of their phytochemical content [6].

The consumption of plant materials for nutritional and medicinal purposes is on the rise and the phytochemical constituents of these plants may have chronic or acute toxic effects on humans [7]. The adverse effect of a substance that occurs immediately or at a short time interval (usually less than 24 hours) following a single or multiple administrations is called acute toxicity. Acute toxicity assessment gives fact about the lethal dose (LD_{50}), therapeutic index and degree of safety [8]. *Plumbago zeylanica* (*Pz*) is an herbaceous plant that thrives in Africa and Asia. *Pz* is also called chitrak. The Hausa/Fulani's of northern Nigeria calls it 'Gagay' [9]. The plant is cultivated for medicinal purposes and it is locally consumed in northern Nigeria as a remedy for many illnesses, as a supplement for maintaining good health and as an aphrodisiac for enhancing sexual vigor. There is dearth of data on the phytochemical constituents and the LD_{50} of ethanolic extract of root of *Pz* despite the known use of this plant; hence, the need to evaluate the phytochemical constituents and the LD_{50} of ethanolic extract of root of *Pz*.

MATERIALS AND METHODS

Collection of Plant Material

The roots were harvested from the wild by a herbalist from whom the roots were purchased at Pike cattle market in Mubi, Mubi South Local

Government Area, Adamawa State, North-East Nigeria.

Identification of Plant Material

The plant was identified and authenticated as *Plumbago zeylanica* at the National Institute of Pharmaceutical Research and Development, Abuja Nigeria and a specimen was deposited at the herbarium of the institute with voucher no NIPRD/H/7107.

Sample Extraction (Cold Maceration)

One (1) kg of powdered sample was put into a bottle and macerated with 1200 mL of ethanol. The extraction cycle was carried out for three days with occasional shaking after which it was filtered and the filtrate evaporated at room temperature to obtain extracts. Evaporation was maintained at room to prevent denaturation of bioactive constituents inherent in the crude extract. A dark brown gel-like solid was obtained from the root. It was stored in clean capped bottles in a refrigerator for further use.

Phytochemical Screening

Test for Flavonoids

Ten gram (10g) of the sample was weighed and extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The solution is filtered through Whatman filter paper No 42 (125mm). The filtrate is transferred into a weighed crucible. And evaporated into dryness. It was then weighed.

The percentage flavonoid was calculated as follows

$$\%\text{flavonoid} = \frac{\text{weight of flavonoid}}{\text{total weight used}} \times \frac{100}{1}$$

Test for Alkaloids

The gravimetric method was used. Five (5) gram of the sample was weighed out and disperses into 50ml of 10% acetic acid solution in ethanol. The mixture was shook very well and then allowed to stand for 4hours before filtering. The solution was concentrated and then the alkaloids were precipitated with concentrated NH_4OH by adding in drop wise fashion. It was allowed to cool and the precipitate was filtered off with a weighed filter paper. The precipitate in filter paper was dried in an oven at 60°C and reweighed to get the weight of the filter paper and the alkaloid.

Cycloocta[b]pyridine derivatives have many applications. The 4-arylcycloocta [b]pyridine is the main skeleton of antipsychotic drug blonanserin which is used in the treatment of schizophrenia [2].

$$\text{Alkanoid}(\%) = \frac{\text{weight}(\text{weight of filter paper} + \text{alkaloid}) - (\text{weight of filter paper}) \text{of flavonoid}}{\text{weight used}(5\text{g})} \times \frac{100}{1}$$

Test for Tannins

One (1.0) gram of the test sample was weighed and 10.0ml of distilled water was added. It was shook at 5min interval for 30 minutess and then Centrifuged/filtered to get the extract. 2.5ml of the supernatant was transferred into a test-tube. 2.5ml of standard tannic acid solution was also transfered into a 50 ml flask. 1.0ml Folin-Denis reagent was added into the flask, followed by 2.5 ml of saturated Na_2CO_3 solution. The solution was made up to the mark. The absorbance was read after 90mins incubation at room temperature.

$$\%\,\text{Tannin} = \frac{\text{An}}{\text{As}} \times \text{C} \times \frac{100}{\text{W}} \times \frac{V_f}{V_a}$$

Where,

An = absorbance of test sample
As = absorbance of standard solution
C = Conc of standard solution
W = weight of sample used
Vf = total volume of extract
Va = volume of extract analyzed

Test for Saponin

Twenty (20) gram of the sample was weighed into a conical flask. 100ml of 20% aqueous ethanol was added. The samples were heated over hot water bath for 4hrs with continuous stirring at about 55°C. It was then filtered and the residue was re-extracted with another 200ml of 20% ethanol. The extract was concentrated in a water bath to 40ml at 90°C. 20ml of diethyl ether was added and shooken vigorously to separate. The aqueous layer was recovered. 60ml of n-butanol was added and then washed twice with 10ml 5% aqueous sodium chloride. The extract was evaporated in the oven and the weight of saponin was then quantified in percentage.

Test for Steroids

The test was performed based on method described by Solihah et al., [10]. Two ml concentrated sulphuric acid were added to two ml of the extract. Formation of red precipitate indicated presence of steroids.

Quantitative Determination of Steroids

Method described by Fahal et al. [11] was used to get the gravimetric weight. 100ml of the extract was dried at 400C. The residue was re-suspended in 20 ml of chloroform and the volume adjusted to 50 ml with the same solvent, and then cooled to room temperature 25°C, and then filtered. The residue re-extracted twice, using 30 ml of chloroform for 15 min. The two filtrates were collected and were dried at 80°C until constant weight.

Test for Phenolic Compounds

About 2.0 ml of the extract was measured in a test tube and 0.01 moldm-3 Ferric chloride solution was added drop by drop. Appearance of bluish black precipitate indicated the presence of phenolic compounds and tannins.

Determination of Total Phenolics

The total phenolics of the extracts were determined using the Folin and Ciocalteu reagent, following the method described by Singleton and Rossi with slight modifications. Sample and standard readings were made using a spectrophotometer at 765 nm against the reagent blank.

Acute Oral Toxicity Study

The acute Oral toxicity was conducted and computed using the improved Up-and-Down (UPD) method of Organization for Economic Corporation and Development (OECD) test guideline 425 statistical programs which provides a point estimate of $LD_{50,}$ approximate confidence intervals and observed toxic signs for the substance tested while achieving significant reduction in animal use [10]. The dose progression from the AOT425statpgm (version 1.0) was 175mg/kg, 550mg/kg, 1750mg/kg and 5000mg/kg of extracts orally.

RESULTS

Table 1 reveals that the root of *Pz* has high phenolic and steroid content, a moderate content of flavonoid, terpenoid and alkaloids. Saponin was not detected. (Table 2)

Table 1: Result of phytochemical composition of ethanolic root extract of *Pz*

Parameter	Percentage
Percentage yield	5.8%
Alkaloids	3.5%
Flavonoids	9.83%
Tapernoid	6.17%
Steroid	48.03%
Saponin	Not detected
Total Phenolic content	33.64% {GAE}

Table 2: Acute oral toxicity (LD_{50}) of ethanolic root extract of *Pz*

Test Sequence	Animal ID	Dose mg/kg b.wt	Short-term result	Long-term result
1	1	175	0	0
2	2	550	0	0
3	3	1750	0	0
4	4	5000	0	0
5	5	5000	0	0
6	6	5000	0	0

(LD_{50} = Median lethal dose. *Pz* = *Plumbago zeylanica.* X= Died 0=Survived

Statistical estimate based on long term outcome: the LD_{50} is greater than 5000 mg/kg b.wt. The smallest usable dose of *Plumbago zeylanica* determined from the OECD test guideline 425 statistical program was 100mg/kg.

Acute Oral Toxicity Study

Our results show that the acute oral toxicity (LD_{50}) of *Plumbago zeylanica* was greater than 5000mg/kg. The smallest usable dose of *Plumbago zeylanica* determined from the OECD test guideline 425 statistical program was 100mg/kg.

DISCUSSION

The phytochemical composition of a plant gives an impression of its medicinal property. Phytochemicals also known as phytonutrients or bioactive agents consist of a variety of chemical compounds which are classified based on their chemical structure or characteristics [11]. These include alkaloids, steroids, phenols, tannins, saponins, terpenoids, flavonoids and glycosides [12,13].

These bioactive agents are found in plant materials such as herbs, seeds, nuts, leaves, fruits, vegetables and grains and are largely responsible for the colour, flavour and odour of the plant. Our results revealed a high phenolic and steroid content and moderate presence of flavonoid, terpenoids and alkaloid content in our sample. Saponins were not detected.

Plant sourced phenolic compounds have been reported to have antioxidant property [14]. The high phenolic content of root of *Pz* in the result is in agreement with the report of Tilak et al and Vijayakumar *et al*. Pharmaceutical Industries show particular attention to plant sourced steroids as result of their association with gonadal hormones. Flavonoids possess antioxidant and anti-inflammatory properties [9]. Plant sourced Alkaloids has been used in phytoterapy for their bacterial, antispasmodic and analgesic properties [9]. Terpene compounds are used in phytotherapy because of their aromatic properties. Hence, the high presence of polyphenolic compounds suggests high antioxidant property.

The amount of a substance administered all at once that can cause the death of 50% (one half) of a group of test animals is known as LD_{50}. It assesses the short-term poisoning potential (acute toxicity) of a substance. Our results reveal that the LD_{50} of ethanolic root extract of *Pz* is greater than 5000 mg/kg body weight. This is similar to the reports of Sandeep *et al*. The smallest usable dose of ethanolic root extract of Plumbago zeylanica determined from the OECD test guideline 425 statistical program was 100mg/kg.

CONCLUSION

The phytochemical composition of root of *Pz* suggests a high nutritional and medicinal value and the smallest usable dose of ethanolic root extract of *Pz* is 100mg/kg body weight.

REFERENCES

[1] Ajayi IA, Ajibade O, Oderinde RA. Res J Chem Sci. **2011**, 1(3): p. 58-62.
[2] Blench R. Fulfulde Names for Plants and Trees in Nigeria, Cameroun, Chad and Niger. Macdonald Institute for Archaeological Research, University of Cambridge. **2020**, p.55.
[3] Chinedu E, Arome D and Ameh FS. Toxicol Int. **2013**, 20(3): p. 224-226.
[4] Elizabeth FB, Obikili EN, Esom AE et al., J. Phytol. **2016**, 8: 22-25.
[5] Finbarrs-Bello E, Nto NJ, Ikele IT et al., Eur J Med Plants. **2016**, 17(3): 1-7.
[6] Johari MA and Khong HY. Adv Pharmacol Sci. **2019**, 4: p. 1-4.
[7] Igoli JO, Gray AI, Clements CJ et al., INTECH publishers. **2011**, p. 375-388.
[8] Rispin A, Ferar D, Margosches E et al., ILAR Journal.**2002**, 43(4): p. 233-243.
[9] Olorode OO, Idowu MA, Bamgbose A et al., Int J Food Sci Nutr. **2014**, 3(6): p. 572-578.
[10] Sandeep G, Dheeraj A, Sharma NK et al., Asian Pac J Trop Med. **2011**, 4(12): p. 978-84.
[11] Sasidharan S, Chen Y, Saravanan D et al., Afr J Tradit Complement Altern Med. **2011**, 8: p. 1-11.
[12] Tilak JC, Adhikari S and Devasagayam TPA. Redox Report. **2004**, 9(4): p. 219-227.
[13] Vijayakumar R, Latha S, Sasikala C et al., J Pharm Res. **2010**, 3(10): p. 2442-2443.
[14] Yadav R and Munin A. J Phytol. **2011**, 3: p. 10-4.