

Percentage Yield And Acute Oral Toxicity Test Of *Anogeissus Leiocarpus* Crude Extracts From The Wild In Gulum Area Of Jalingo, Taraba State, Nigeria

Ambair I. K.

PhD Student: School of Pure and Applied Science, Department of Microbiology, Modibo Adama University, Yola, Adamawa State, Nigeria.

Prof. Doughari J. H.

Lecturer: School of Pure and Applied Science, Department of Microbiology, Modibo Adama University, Yola, Adamawa State, Nigeria.

Dr. Pukuma M. S.

Lecturer: School of Pure and Applied Science, Department of Microbiology, Modibo Adama University, Yola, Adamawa State Nigeria

Dr. Ewansiha J. U.

Lecturer: School of Pure and Applied Science, Department of Microbiology, Modibo Adama University Yola, Adamawa State, Nigeria.

Corresponding author: Ambair K.I.

Abstract

Background: *Anogeissus leiocarpus* is a deciduous tree plant species of combretaceae family and a typical element of Woodland and Savannahs. Its ethno botanical preparations are of great importance serving as remedy against many diseases in African traditional medicine. Hence, this study was aimed at evaluating percentage yields, color, texture and acute oral toxicity of extracts obtained from this plant,

Materials and methods: Eight (8) crude extracts from root bark and stem bark samples of *Anogeissus leiocarpus* plant were obtained using serial exhaustive extraction by maceration for 72 hours with 8 hourly agitations at room temperature in solvents of increasing polarity vice hexane, chloroform, acetone and water. The color and consistency of extracts were also evaluated visually whereas acute oral toxicity of the extract with the highest percentage yield was similarly determined using a single bolus dose exposure of 0, 10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight of rat.

Results: Acetone root bark crude extract showed the highest percentage yield while chloroform stem bark crude extract the lowest. Polar solvents' crude extracts showed brown to dark brown color with solid powdery texture while the non polar showed light yellow to yellow color with oily to pastry texture. Determination of the acute oral toxicity test of acetone root bark crude extract with the highest yield showed to be practically non-toxic and safe with a lethal dose (LD₅₀) of more than 5,000 mg/kg body weight of albino rat.

Conclusion: Polar solvents can be used as suitable extractants for crude extracts from this plant while its acetone root bark extract as potential source of oral preparations and or medicines in Traditional and orthodox medicine practice because of its safe and non toxic nature.

Keywords: Yield, extracts, toxicity, dosage, oral, LD₅₀, *Anogeissus leiocarpus*

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I. Introduction

Plants are solar-powered biosynthetic laboratories for the synthesis of natural products which are functional end products of organic compounds known as primary and secondary metabolites. Plants use them for survival, growth, reproduction (intrinsic functions) and adaptation to environmental factors (extrinsic function) while human beings use them as indispensable sources of natural products for relief and treatment of different health problems (1) and therefore good and rich sources of phytochemicals useful in pharmaceutical, agrochemical and cosmetic industries. The plant *Anogeissus leiocarpus* has important ethno-botanical uses that vary from country to country such that in Sudan the stem bark decoction is use as cough remedy (2) while in

Nigeria its sticks are use for orodental hygiene. Similarly, in Ivory Cost its use as remedy against malaria, trypanosomes, helminthes as well as dysentery and in Togo its use as remedy for fungal diseases, diabetic ulcers, body pain, blood clot, asthma, cough including tuberculosis (3). Also Mann *et. al.* (4) reported on the excellent activity of leaves extract of this plant against *Mycobacterium tuberculosis*. Hence, based on all these, there is need to determine suitable extraction solvent and toxicity potential of extracts of this plant for safe and effective ethno-botanical therapy.

II. Materials And Methods

Materials: Fresh leaves, flowers, fruits and seeds were collected from the plant *Anogeissus leiocarpus* in the wild in Gulum area of Jalingo Taraba State Nigeria and taken to Taraba State University where they were used to identify and authenticate the plant as *Anogeissus leiocarpus* plant. Thereafter, root and stem barks of the plant were also harvested in November and September, washed in the case of roots, sun-dried, grinded, sieved and parked as powdered plant samples.

Fifteen white albino nulliparous and non-pregnant adult rats of ages 3 weeks to 2 months with weights between 65 to 188.4g were used.

Methods: Powdered plant samples were each weighed and subjected to serial exhaustive extraction by maceration in four different solvents in a ratio of 1:10 starting with the non-polar to most polar solvent vice hexane, chloroform, acetone and water for 72 hours with 8 hourly agitations. The macerate from each plant sample was strained and pressed through a nylon cloth then filtered first through whatman No. one filter paper and centrifuge at 4,000 rpm for 30 minutes and finally filtered through a 0.45µm bacteriological filter before evaporation of the filtrate in a rotary evaporator and drying to constant weight in a laminar flow cabinet at 45°C thereby producing the crude extracts that were kept at 4 °C until required. However and in each case, the obtained deposit from each processed solvent sample solution was weighed and subjected through the same processes as described above starting with the first solvent hexane to the last solvent water.

The acute oral toxicity test of extract was carried out using modified (5) method in two phases 1 and 2. Phase 1 involved the use of twelve healthy, nulliparous and non pregnant adult female white albino rats which were divided into four groups of three rats each vise group 1, 2, 3, and 4 with each rat marked in accordance to the group it belongs as 1a, 1b, 1c; 2d, 2e, 2f; 3g, 3h, 3i and the control 4j, 4k and 4l respectively. All rats were starved for 12 hours overnight and each weighed thereafter before dosing with the test extract. Rats in groups 1, 2, 3 and 4 were administered with doses of 10, 100, 1000 and 0 mg/kg body weight of test extract to each rat and all dosed rats kept in their cage then observed for abnormal behaviors/toxicity signs and mortality over a period of 24 - 48 hours.

Phase 2 was carried out after phase 1 when there were no toxicity signs (abnormal behaviors) and mortality observed. It required the use of three healthy, nulliparous and non pregnant female white albino rats which were divided into three groups of one rat each vise group 5m, 6n and 7o. Rats were then subjected to the same procedure as in phase 1 before dosing them with their respective group doses of 1,600, 2,900 and 5,000 mg/kg body weight of rat of the test extract. All dosed rats were kept in their cage and observed for 24 – 48 hours for abnormal behaviors and mortality as in phase 1.

Calculation of LD₅₀ of Test Extract on Rats in Phase 1 and 2: Results obtained in phase 1 and 2 were used to calculate the LD₅₀ of the test extract on rats by the formula below:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where D₀ = highest dose of extract that gave no mortality

D₁₀₀ = lowest dose of extract that produced mortality

III.RESULTS

Table no. 1: Percentage yield and nature of *Anogeissus leiocarpus* crude extracts

Samples' name & weight (g)	Crude extract	Recovery (g)	Percentage Yield (%)	Color	Texture
Stem bark 70.00	Hexane	1.31	1.87	Light yellow	Oily
Stem bark 63.92	Chloroform	0.23	0.35	Yellow	Pasty
Stem bark 62.90	Acetone	3.83	6.09	Brown	Powdery
Stem bark 45.43	Water	2.33	5.13	Dark brown	Powdery
Root bark 35.00	Hexane	1.36	3.89	Yellow	oily
Root bark 31.50	Chloroform	0.23	0.73	Light yellow	Pasty
Root bark 31.00	Acetone	5.23	16.90	Brown	Powdery
Root bark 17.00	Water	1.66	9.760	Dark brown	Powdery

Table no.2: Animal toxicity sign (abnormality) and mortality (death) rate caused by acetone root bark extract of *Anogeissus leiocarpus* plant in acute oral toxicity test on white albino rats

Phase	Lot No.	Lot dose/rat (mg/kg bwt)	No. of rats/lot	No. of abnormal rats after 24 hrs	No. of abnormal rats after 48 hrs	No. of death rats after 24 hrs	No. of death rats after 48 hrs	Total No. of abnormal rats after 24 hrs	Total No. of abnormal rats after 48 hrs	Total No. of death rats after 24 hrs	Total No. of death rats after 48 hrs	Percentage abnormality after 24 hrs	Percentage abnormality after 48 hrs	Percentage death after 24 hrs	Percentage death after 48 hrs
1	1	10	3	0	0	0	0	0	0	0	0	0	0	0	0
1	2	100	3	0	0	0	0	0	0	0	0	0	0	0	0
1	3	1000	3	0	0	0	0	0	0	0	0	0	0	0	0
1	4(contr ol)	0	3	0	0	0	0	0	0	0	0	0	0	0	0
2	5	1600	1	0	0	0	0	0	0	0	0	0	0	0	0
2	6	2900	1	0	0	0	0	0	0	0	0	0	0	0	0
2	7	5000	3	0	0	0	0	0	0	0	0	0	0	0	0

$LD_{50} = \sqrt{(D_0 \times D_{100})}$ where $D_0 = >5000$ mg/kg bwt & $D_{100} = >5000$ mg/kg bwt from the table thus $LD_{50} = \sqrt{(>5000 \times >5000)} = > 5000$ mg/kg bwt

Keys: bwt = body weight, Abnor = abnormality (toxicity signs), hrs = hours, No. = number, LD_{50} = Amount of extract given at once that causes the death of 50% of group of test rats, > = more than

IV. Discussion

Percentage yields of extracts

Extract yield is the measure of solvent's efficiency to extract specific component from the original sample by a particular method while extract is the sum of the extractable components. Thus results from percentage yield of plant samples' crude extracts studied revealed that all the solvents used for the extraction extracted significant amounts of components in varied amounts showing effects of the various solvent polarities on the solubility of the different components in the plants samples. The components in plants' samples are not the same but vary and so is their solubility in a given solvent (6; 7) as different plant's parts contains different level and type of compounds with a specific polarity requiring a solvent with an equivalent polarity index for extraction (8; 9). Acetone solvent showed the highest percentage yields of 6.69% and 16.90% in both stem and root barks crude extracts and this was followed by water solvent with 5.13% and 9.76% then hexane with 1.89% and 3.89% and finally chloroform the least with 0.35% and 0.73% in both stem and root barks crude extracts (Table 1). This also implies that polar solvents possess higher yields than the non-polar solvents in consonant with the findings of (10; 11; 12). The high yield shown by acetone over water as both are polar solvents can be best ascribed to the plant's samples constituents having the same polarity index with the solvent acetone alongside its ability to extract both polar and non-polar compounds (13). Furthermore, high percentage yield shown in this study depict that the plant understudy can serve as good potential source of phytochemical

compounds with pharmacological importance since the high percentage yield of extracts were based on polarity of the solvents.

Color and texture (nature) of extracts

Crude extracts obtained showed variation in texture and color with the solvents used in their extraction indicating variation in extracts composition and quantities with each extraction solvent. The non-polar solvents showed oily to pasty texture with light yellow to yellow color while polar solvents revealed solid-powdery textures with brown to dark brown colors accordingly.

Animal abnormality and mortality rate caused by acetone root bark extract of *Anogeissus leiocarpus* plant in acute oral toxicity test on white albino rats

The analysis of animal abnormality (toxic signs) and death (mortality) rate caused by acetone root bark extract of *Anogeissus leiocarpus* plant in acute oral toxicity test (Table 2) revealed that there were no abnormalities and deaths of animals (white albino rats) at single bolus doses exposures of 0, 10, 100, 1000, 1,600, 2,900 and 5,000 mg/kg body weight of animal (rat) indicating that the extract is safe at these doses. This is further confirmed by the LD₅₀ value of more than 5,000 mg/kg body weight of rat obtained from the calculated values of death/ mortality in Table 2. Thus, in accordance with Loomis & Hayes (14) classification of LD₅₀ based on dosage, the acetone root bark extract of *Anogeissus leiocarpus* is practically non-toxic and safe as oral phytotherapy. The acute oral toxicity test results in this study agree completely with those of Nuhu and Maigari (15) and Kouangbe *et. al.* (16).

V. Conclusion

Root and stem barks' extracts of the plant *Anogeissus leiocarpus* are highly extractable in polar solvents and so they are recommended for their extraction. They are excellent source of phytochemicals as the high percentage yield of the obtained extracts were based on solvent polarity and so they are also useful raw materials for pharmaceutical, agrochemical, cosmetics, food and other industries especially now that they are found to be practically non-toxic and safe as oral phytotherapy.

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