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PHYTOCHEMICAL SCREENING, PHYSICOCHEMICAL PROPERTIES AND ACUTE TOXICITY STUDY OF THE LEAVES OF *ZIZIPHUS ABYSSINICA* HOCHST. EX A. RICH. (RHAMNACEAE)

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ABSTRACT

Ziziphus abyssinica belongs to the family Rhamnaceae and is traditionally used in the treatment of pneumonia, tonsillitis, Newcastle disease, snake bite, burns, wounds, tachycardia, pectoral pain, migraines and as pain-killers. The objective of this study is to evaluate the phytochemical, physicochemical analysis and safety margin of *Z. abyssinica* leaf with the hope of assisting in its standardization for quality, purity and safety. The powdered sample of the leaves was extracted with aqueous and methanol and evaluated for physicochemical parameters of the plant. The extracts were subjected to qualitative and quantitative phytochemical analysis and acute toxicity study. Phytochemical screening revealed the presences of alkaloids, flavonoids, saponins, phenols, tannins, glycosides, carbohydrates, anthraquinones and triterpenes in both aqueous and methanol extract. However, steroids were absent; the quantitative phytochemical analysis revealed that flavonoids (134.0 mg/g) were the highest phytochemicals detected in the leaf while the lowest were saponins (6.0 mg/g). The physicochemical parameters evaluated include: moisture content (7.70%), total ash (10.10%), acid insoluble (2.10%), water soluble (7.34%), water extractive value (16.33%) and ethanol extractive value (13.0%). LD_{50} of both extracts was above 5000 mg/kg and did not cause mortality in all the tested rats. The results of this finding may be useful in laying down standards and for the compilation of a suitable monograph of *Z. abyssinica*.

Keywords: Phytochemicals, *Ziziphus abyssinica*, Physicochemical, Acute Toxicity.

INTRODUCTION

Phytomedicine is an important part of therapy throughout the world. It has been widely utilized as effective remedy for the prevention and treatment of variety of disease conditions, for ages, by almost every known nation (Rivera *et al.*, 2013). It is becoming more main stream as improvements in analysis and quality control, along with advances in clinical research, show the value of herbal medicine in treating and preventing diseases (Usman *et al.*, 2018).

The advantages of herbal medicines are their ready availability, inexpensiveness and less or no side effects, however they are easily adulterated (Sumitra, 2014). The major drawback to the use of herbal medicine however is the lack of standardization. This paves way for wrong identification, unintentional substitution of closely related species and intentional adulteration of genuine herbs with low grade ones, in order to meet the growing demands of the market (Chanda, 2014). It is therefore important that standardization parameters be laid down for the correct identifcation, extraction and purifiation of medicinal plants to ensure reproducible quality and efficacy (Aslam and Afridi, 2018).

Ziziphus abyssinica belongs to the family Rhamnaceae and known as Catch thorn in English, *Jujube sauvage* in French and also '*Magaryaa'* in Hausa, is found growing in arid or dry tropical and subtropical regions, with severe heat and slight frost. It occurs at medium to low altitudes in open woodland, open grassland and along riverbanks (Ward *et al.*, 2006). The water extract of the bark of *Z. abyssinica* is used to manage stomach disorders in Northern Kenya. The leaves and other parts of the plant have been used traditionally to treat pneumonia, tonsillitis, Newcastle disease, snake bite, burns, wounds, tachycardia, pectoral pain, migraines and as pain-killers (Burkill, 1985; Okello *et al.*, 2010). The aqueous extract of *Z. abyssinica* has been shown to possess significant activity against *Staphylococcus aureus* and *Candida albicans* (Beentje, 1994). Despite the medicinal applications of *Z. abyssinica*, there is dearth of information on the standardization parameter. Therefore, this study was carried

out to determine the phytochemical, physicochemical parameters and safety margin of *Z. abyssinica* leaf.

MATERIALS AND METHODS

Collection, Identification and Preparation of the Plant Material

The plant material was collected at Babura Local Government Area of Jigawa State. The plant was taxonomically authenticated at the Herbarium unit, Department of Plant biology, Bayero University Kano, Nigeria. The leaves was washed, cleaned and all foreign matter removed, it was then air-dried and comminuted to powder form and stored in an air-tight container for subsequent use.

Extraction method

50 g of the powder sample of *Z. abyssinica* leaves was macerated with 500 ml of aqueous and methanol successively. The extracts was evaporated to dryness on water bath.

Qualitative Phytochemical screening of the aqueous and methanol extracts of *Ziziphus abyssinica* leaves

The aqueous and methanol extracts was subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the standard phytochemical reagents and procedures (Sofowora, 2006; Evans, 2009).

Quantitative Phytochemical screening of the aqueous extract of *Ziziphus abyssinica* leaves

Preparation of Fat free Sample

2 g of the sample was weighed and defatted with 100ml of diethyl ether using a soxhlet apparatus for 2 hours.

Alkaloid Determination

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol were added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until precipitation was completed. The whole solution was allowed to settle and the precipitates were collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried and weighed (Harborne, 2005).

Flavonoid Determination

10 g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Bohm and Kocipal – Abyazan, 1994).

Saponin Determination

The method of Obadoni and Ochuko (2001) was used. Out of the grinded samples 10 g was weighed for each and put into a conical flask and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55ºC. The mixture was filtered and the residue re-extracted with another 200 ml, 200% ethanol. The combined extracts were reduced to 40ml over water bath at about 90ºC. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n – butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Tannin Determination

About 500 mg of each sample was weighed into a 50 ml plastic bottle and 50 ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1M $FeCl_3$ in 0.1M HCl and 0.008M potassium ferrocyanide. The absorbance was measure at 120 mm within 10 min (Oyedeni *et al.,* 2010).

Determination of physicochemical constants of *Ziziphus abyssinica* powdered leaves

Physicochemical parameters such as moisture content, alcohol and water soluble extractive values were studied using the method described by WHO (2011).

Determination of moisture content

The moisture content was determined by "Loss on drying" method (gravimetric determination). Air-dried leaves (3 g) was weighed using KERN EW Electronic Balanced and in a dried and weighed crucible. The crucible was transferred into a hot air sterilizing oven, which was set at 105^0C. After an hour, the crucible was removed, placed in a desiccator over phosphorous pentoxide under atmospheric pressure at room temperature. After 30 minutes in the desiccator, the weight of the powder and crucible were quickly determined and the crucible returned to oven. The heating and weighing were repeated until a constant weight was obtained and noted. Three determinations were conducted and the average of these was taken as the moisture content of the drugs. The moisture content (loss of weight) was calculated using the following formula (WHO, 2011):

$$\% \text{ Moisture content} = \frac{\text{Initial Weight of Powder} - \text{Final Weight of Powder}}{\text{Initial Weight of Powder}} \text{ X } 100$$

Determination of Total Ash Value

A platinum crucible was heated red hot, cooled in a desiccator and quickly weighed. Exactly (2 g) of the air-dried leaves powder was weighed into the previously heated crucible. It was ignited by gradually increasing the heat, until it became white, indicating absence of carbon. It was cooled in a dessicator and weighed. The procedure was repeated three times to obtain average value. The total ash content of the air-dried powder was calculated in percentage, using the following formula (WHO, 2011):

$$\% \text{ Ash Value} = \frac{\text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \text{ X } 100$$

Determination of acid-insoluble ash

To the crucible containing the total ash, 25 ml of dilute hydrochloric acid was added and covered with a watch glass and boiled gently for 5 minutes. About 5 ml of hot water was used to rinse the cover glass. The insoluble matter was

collected on an ash less filter paper and washed with hot water until the filtrate was neutral. This was then transferred back to the crucible and dried on a hot plate and ignited to a constant weight (WHO, 2011). The residue was allowed to cool in a dessicator for 30 minutes and quickly weighed. The acid insoluble ash was calculated as follows:

$$\% \text{ Acid insoluble Ash} = \frac{\text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \text{ X } 100$$

Determination of water soluble ash

To the crucible containing the total ash, 25 ml of water was added and boiled for 5 minutes. The insoluble matter was collected in a sintered glass crucible. It was then washed with hot water and ignited in a crucible for 15 minutes at 105^{o}C. The weight of the residue was subtracted from the weight of the total ash. The water soluble ash of air dried powder was calculated using the following formula:

$$\% \text{ Water Soluble Ash} = \frac{\text{Weight of Total Ash} - \text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \text{ X } 100$$

Water extractives values

Exactly 4 g of air-dried stem-bark powder was weighed into a 250 ml glass stoppered conical flask and 100 ml of water was added to macerate the powder for 6 hours with frequent shaking by using mechanical shaker and was allowed to stand for 18 hours (WHO, 2011). It was then filtered rapidly and 25 ml of filtrate was transferred into a previously dried and weighed evaporating dish and evaporated to dryness on a hot water bath. This was further dried in the oven at 105^{0}C for 6 hours, cooled in a desiccator for 30 minutes and then weighed without delay. The percentage water extractive value was calculated using the following formula:

$$\% \text{ Water Extractive Value} = \frac{\text{Weight of Extract in 25ml X 4}}{\text{Original Weight of Powder}} \text{ X } 100$$

Ethanol extractives value

The procedure above was repeated with 90% ethanol in place of water and the percentage ethanol extractive value was calculated using the following formula:

$$\% \text{ Ethanol Extractive Value} = \frac{\text{Weight of Extract in 25ml X 4}}{\text{Original Weight of Powder}} \text{ X } 100$$

Determination of elemental analysis

The elemental composition of the leaves was determined using Atomic Absorption Spectrophotometry (AAS) (Rajurkar and Damame, 1997).

Acute toxicity studies of aqueous and methanol extracts of *Ziziphus abyssinica* leaves

The lethal dose was determined by Lorke's method (1983). Phase I: Nine wistar rats were used. They were divided into three groups of three animals each. Each groups of animals were administered different doses (10, 100, and 1000 mg/kg) of the extracts and then observed for 24 hours to monitor their behavior as well as mortality. Phase II: Three animals were used. They were divided into three groups of one animal each. The animals were administered higher doses (1600, 2900 and 5000 mg/kg of the extracts and observed for behaviour as well as mortality (Lorke, 1983).

Results and Discussion

The information on the presence or absence and the type of phytochemical constituents especially the secondary metabolites are useful taxonomic keys in identifying a particular species and distinguishing it from a related species, thus helping in the delimitation of taxa (Jonathan and Tom, 2008). The results of phytochemical screening are presented in Table 1, and revealed the presences of alkaloids, flavonoids, saponins, phenols, tannins, glycosides, carbohydrates, anthraquinones and triterpenes detected in both aqueous and methanolic extracts while steroid is absent; this result is in agreement with the finding of Boakye-Gyasi *et al.,* (2017). Phytochemical screening gives a general overview of the classes of active+ constituents present in the leaf which are also responsible for the therapeutic effects.

Table 2 shows the results for the quantitative phytochemical content of the leaf of *Z. abyssinica*. The flavonoids (134.0 mg/g) were the highest phytochemicals detected in the plant and the lowest was saponins (6.0 mg/g). Alkaloids and Tannins were also seen in reasonable quantity. Flavonoids generally serve as flavoring ingredients in plants. Besides their role as flavoring agents they are also expressed in plants in response to microbial infection suggesting their antimicrobial activity (Kittakoop *et al.*, 2014), Flavonoids have also been implicated as antioxidants both in physiological and diseased states. For instance, tea flavonoids have been reported to reduce the oxidation of low-density lipoprotein, lower the blood level of cholesterol and triglycerides (Erdman, 2007). Alkaloids have a wide range of pharmacological activities including antimalarial (e.g., quinine), anticancer (e.g., homoharringtonine) (Kittakoop *et al.*, 2014), antibacterial (e.g., chelerythrine) (Cushnie *et al.*, 2014), and antihyperglycemic activities (e.g., piperine) (Qiu *et al.*, 1997). Tannin is one of the major active ingredients found in plant based medicines (Cushnie *et al.*, 2014), they are used in the dyestuff industry as caustics for cationic dyes (tannin dyes), and also in the production of inks (iron gallate ink), textile dyes, antioxidants in beverages, and coagulants in rubber production as well as possessing antiviral, antibacterial, and antitumor activity (Haslam, 1996; Khanbabaee & Van Ree, 2001). Tannin has been reported to selectively inhibit HIV replication (Kashiwada *et al.*, 1992).

Table 1: Aqueous and Methanolic leaf extracts of *Ziziphus abyssinica*

Metabolite	Inference	
	Aqueous Extract	Methanol Extract
Alkaloid	+	+
Flavonoid	+	+
Saponins	+	+
Cardiac glycoside	+	+
Tannins	+	+
Steroid	-	-
Triterpenes	+	+
Anthraquinones	+	+
Carbohydrate	+	+

Table 2: Quantity of Metabolites in methanol extract of *Ziziphus abyssinica* leaf

Metabolite	Quantity (mg/g)
Alkaloids	110.00 ± 0.33
Flavonoids	134.00 ± 0.29
Saponins	6.00 ± 0.12
Tannins	104.00 ± 0.50

Furthermore, the physico-chemical parameters of the *Z. abyssinica* leaves were assessed for moisture content, total ash, acid insoluble ash, water soluble ash, ethanol and water extractives (Table 3) which may serve as a reference standard in assessing quality and purity of *Z. abyssinica* as a crude drug. The moisture content of 7.70% may discourage the growth of bacteria, yeast, mould and fungi and could be stored for long period of time without spoilage. However, the average percentage of moisture content in crude drug should be within 12-14 % (BHP, 1990; WHO, 2011) and the value obtained was within the permissible limits.

The total ash value (10.10%) obtained could be attributed to organic material like carbonate, oxalate and silicate and presence of other impurities in *Z. abyssinica* leaves. The high value of total ash percentage could be used as criteria to judge the purity of drug (Prasad *et al*., 2012) and load of contaminations that could be directly or indirectly deposited on the aerial part. The acid insoluble ash of 2.10% indicated some levels of contamination with earthy material, sand and other impurities in the crude drug, however, the result is within the permissible standard in evaluation of crude drug (WHO, 2011). The water soluble ash of 7.34% could be used to estimate the amount of inorganic compound present in the crude drugs. These results are in agreement with BHP (1990); WHO (2011); Sumitra (2014) and Ayeni *et al.* (2017) that reported the permissible limits and the necessities for physicochemical evaluation of crude drugs. This study found water extractive value (16.33%) to be the highest and alcohol extractive value to be (13.0%). The high water extractive values probably revealed that water extract have the ability to extract more phytoconstituents than alcohol extract based on their polarity scale. Ajazuddin and Shailendra (2010) reported that having high water extractive value is a better solvent of extraction than ethanol. Nevertheless, water is a universal solvent and it is used mostly as solvent by traditional healers and individual involved in crude drug preparation.

Table 3: Physicochemical Constants of *Ziziphus abyssinica* powdered leaves

Parameters	Values (%w/w) ± SEM*
Moisture content	7.70±0.58
Ash content	10.10±0.21
Acid insoluble ash	2.10±0.88
Water soluble ash	7.34±0.44
Water extractive value	16.33±0.33
Ethanol extractive vale	13.00±0.00

*****Average values of three determinations

Throughout the world, there is increasing interest in the importance of dietary minerals in the prevention of several diseases (Saraf and Samant, 2013). Minerals are of critical importance in diets, even though they comprised only 4-6 % of the human body. However, lack of full understanding of the amount and type of elements found in medicinal plants can cause a lot of danger to consumers as some of these plants may contain toxic elements in high quantities (Burkill, 2000). The elemental composition of *Z. abyssinica* was screened in the present study (Table 4). The mineral element concentrations in milligram per kilogram of the leaf powder of *Z. abyssinica* revealed that it contains Fe, Cu, Pb, Zn, Ni Mn, among others. Iron content (20.09 ppm) was observed to be the element with the highest concentration and this makes *Z. abyssinica* as potential iron containing plant species. Iron is an essential component of respiratory pigments haemoglobin and myoglobin and also of various enzyme systems including the cytochromes, catalases, peroxidases,

and the enzymes xanthine and aldehyde oxidase, and succinic dehydrogenase. As a component of the respiratory pigments and enzymes concerned in tissue oxidation, iron is essential for oxygen and electron transport within the body (Hughes, 1977). Iron deficiency is the most prevalent nutritional deficiency in humans (Lokhande *et al.,* 2010). The recommended daily in-take for iron is 13.7 - 6.5 mg/day for children and 19.3 - 20.5 day for adults (Lokhande *et al.,* 2010; Jeremiah *et al.,* 2019).

Table 4: Elemental analysis of *Ziziphus abyssinica* powdered leaves

Elements	Concentration (ppm)	FAO/WHO (1984) limit* (ppm)
Iron(Fe)	20.085	20.00
Copper (Cu)	0.102	3.00
Lead (Pb)	0.823	0.43
Zinc (Zn)	0.450	27.40
Nickel (Ni)	0.130	1.63
Manganese (Mn)	2.310	2.00
Aluminum (Al)	12.152	-
Cadmium (Cd)	-0.019	0.21
Selenium (Se)	0.608	-
Chromium (Cr)	0.031	-
Arsenic (As)	0.743	-

In this study, median lethal dose (LD_{50}) of the extracts (aqueous and methanol) of the *Z. abyssinica* leaves was carried out orally in rats. The LD_{50} was found to be greater than 5000 mg/kg when administered orally in rats (Table 5) and all the animals remain alive and did not manifest any significant visible signs of toxicity at these doses. These studies showed the extracts of *Z. abyssinica* leaves are practically non-toxic when administered using the oral route. This result is in agreement with Olorunisola *et al.* (2012); Parasuraman *et al*. (2014) and Adesegun *et al.* (2016) that reported acute toxicity of plants could be considered practically nontoxic and safe above oral administration of 5000 mg/kg. However, Maikai *et al.* (2008); Obidike and Salawu (2013) concluded that, further toxicity assay should be carried out in order to reveal possible long term toxicity effects on the physiology and organs for proper recommendation on its utilization.

Table 5: Acute toxicity studies of aqueous and methanol extracts of *Ziziphus abyssinica* leaves administered orally to Wistar Rats

Experiment	Dose (mg/kg)	Number of dead rat after 24 hours	
		Aqueous Extract	Methanol Extract
Phase 1	10	0/3	0/3
	100	0/3	0/3
	1000	0/3	0/3
Phase 2	1600	0/1	0/1
	2900	0/1	0/1
	5000	0/1	0/1

CONCLUSION

The phytochemical and physico-chemical evaluation of the *Z. abyssinica* leaves will serve as baseline in ascertaining its identity, quality and purity and also guide its further pharmaceutical utilization. The Acute toxicity (LD_{50}) of the leaf extracts of *Z. abyssinica* was found to be greater than 5000 mg /kg and is considered relative safe for use. Nevertheless, further studies are encouraged to evaluate toxicity at much higher doses.

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