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Phytochemical Screening of *Terminalia avicennioides* (Guill and Perr). A Potential Pharmaceutical Ingredient

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Abstract
The study was conducted to determine phytochemical properties of Terminalia avicennioides. The stem bark of the plant was obtained and dried under shade, grounded into powder using mortar and pestle, 100g of the dried powder was soaked in 1000ml of distilled water and left for 48hrs and sieved with muslin cloth and then with filter paper to obtain the filtrate. The results obtained showed the presence of flavonoids, tannins, saponins, glycoside, alkaloid, cardiac glycoside, steroids, saponin glycoside, volatile oil and balsam. It was also shown among the results that glycoside had the highest percentage mean of 0.837±0.0060, followed by alkaloid 0.126±00.0830, while saponin had the least with 0.011±0.0081.

Keywords: Phytochemical, Screening, Quantitative, Qualitative, Terminalia avicennioides.

INTRODUCTION
Terminalia avicennioides (Guill and perr) is a tree plant widely distributed and commonly growing in the Savannah region of West Africa (Burkill, 1985). The genus *Terminalia* belongs to family *Combretaceae* consisting of about 514 species of which only 54 are accepted and recognized (Burkill, 1985), is a yellowish brown, hard and durable wood, commonly found in the Savannah region in West Africa (Mann *et al*., 2011). *Terminalia avicennioides* is a tropical herb common in North central vegetation of Nigeria, is a tropical plant with extensive medicinal applications (Adewunmi and Sofowora, 1980; Abdullahi *et al*., 2001). It is locally called Baushe in Hausa dialect (Azeez *et al*., 2015). The plant is known to be active against trypanosomes (Bulus *et al*., 2008) and against conditions like diarrhea (Abdullahi *et al*., 2001), *Candida albicans* (Baba-Mousa *et al*., 1999) and malaria parasite (Sanon *et al*., 2013). Some studies also showed that, the stem bark extract of *T. avicennioides* exhibited both vibrocidal and typhoidal activities (Akinyemi *et al*., 2005) and antimicrobial activity (Mann *et al*., 2008). Its efficacy on the healing of ulcer and wound has been also reported (Akinyemi *et al*., 2005). *T. avicennioides* is among the medicinal plants commonly used traditionally in Nigeria for the treatment of infection.

There is very little information on toxicity studies carried out on the plant. The one conducted so far was on *in vitro* cytotoxicity using brine shrimps and it was observed that, only petroleum ether extract of the plant root bark exhibited remarkable toxicity on brine shrimps larvae at ED_{50} of 63.2µg/ml (Mann *et al*., 2011). Ethylacetate extract produced moderate toxicity at ED_{50} of 297µg/ml while ethanolic extract was nontoxic (ED_{50} >1000µ).

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Bulus *et al*., (2011) investigated acute toxicity effect of aqueous extract of stem bark of *Terminalia avicennioides* on white albino rats and reported LD_{50} >5000mg/kg body weight. There was no significant weight decrease among groups up to 1000mg/kg body weight, however liver congestion was observed with 100mg/kg body weight dose group. Nevertheless, the organ-body weight ratio for kidney, liver and heart were not significantly different from the control group. Liver congestion was the only major pathology associated with treatment of rat with aqueous extract of *Terminalia avicennioides*, which necessitate the need to determine the extent at which ingestion of extracts from the plant is toxic thereby determining the therapeutic dosage for clinical applications.

In addition, a particular study conducted on anticancer activity of the aqueous extracts of *Terminalia avicennioides* revealed that it significantly decreased *in vitro* cancer cell viability with increasing dose and time, indicating cytotoxicity against EAC cell lines (Atawodi *et al*., 2011). This lends support to the possibility of the plant extract to serve as potent anticancer agent. Toxicity study carried out with the ethanolic extract of a member of the same genera (*Terminalia paniculata*) revealed very healthy and protective results (Mann *et al*., 2004). Furthermore, other members of the genera such as *Terminalia belerica, Terminalia mollis, Terminalia chebula*, and *Terminalia arjuna* were reported to show related activities (Bulus *et al*., 2008). However, extract of *T. avicennioides* appeared to be safer in traditional medicine compared with aqueous extract of *T. mollis* ((Bulus *et al*., 2011).

Figure 1: the picture of *Terminalia avicennioides*

Medicinal plants have been used for centuries as remedies for human and animal diseases because they contain certain components of therapeutic value, there are more than 35,000 plant species being used in various human cultures around the world for medicinal purposes of which *Terminalia avicennioides* is inclusive (Fidock *et al*., 2004). According to World Health Organization (2008), medicinal plants will be the best choice to obtain a variety of drugs. About 80% of individuals in the world used traditional medicine (Awulu *et al*., 2013**).** Therefore, such plants should be investigated to better understand their properties, safety and efficiency. The study was aimed at determining qualitatively and quantitatively the bio compounds present in the stem bark of *Terminalia avicennioides*.

MATERIALS AND METHODS

Collection of Plant Sample

The leaves and stem bark of *Terminalia avicennioides* which is popularly known as Baushe in Hausa dialect was collected from Dajin Daraye of Wammakko Local Government Area, Sokoto State, The plant's identity was confirmed at the Herbarium of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto.

Preparation of Aqueous Extract
The stem bark of *Terminalia avicennioides* collected was dried under shade for two weeks, and then grounded into powder using mortar and pestle. About 100g, of the dried powder of the stem bark was weighed and soaked in 1000ml of distilled water, and were left for 48hrs and sieved, first with muslin cloth and then with whattmann size 15cm filter paper. The filtrate was dried at 25^0C using dry cabinet to obtain the concentrate. One gram (1g) of the concentrate was then dissolved in 100ml distilled water, this corresponds to 1000mg in 100ml or 10mg in 1ml as described by Bala (2005).

Phytochemical Screening
The stem bark of *Terminalia avicennioides* collected was taken to Biochemistry Department of Usmanu Danfodiyo University Sokoto, for phytochemical analysis (both Qualitative and Quantitative screening). Standard methods of Sofowora (1991), El-Olemyl *et al*., (1994) and Harbone (1998) were used. The extract was evaluated qualitatively for the presence of flavonoids, tannin, saponins, alkaloids, glycosides, cardiac glycosides volatile oils and steroids while quantitatively, alkaloid, flavanoids, tannins, saponins and glycosides were determined.
The qualitative screening include:

Test for Flavonoids
Two grams (2g) of powdered stem bark of *Terminalia avicennioides* was boiled for 7-10 minutes in 20ml of distilled water and filtered. The filtrate was acidified with 2-3 drops of diluted HCl. The filtrate was then used for the following test:

Five millimeter of aliquots at (PH 10) mixed with NaOH. A yellow colour developed which indicated the possible presence of flavanoids compounds.

Five millimeter aliquot of the filtrate was separately shaken with 5ml amyl alcohol. The alcoholic layer faintly colour yellow indicated the presence of flavanoids glycosides (Sofowora, 1991).

Ten millimeter aliquot of the filtrate was separately shaken with 5ml of amyl alcohol and boiled with 10ml concentrated HCl for 2 minutes. The acidic solution was cooled and divided into portions and will be tested as follows;

The first portion was shaken with amyl alcohol. A yellow colouration produced indicates the presence of flavanoids glycosides.

The second portion, few pieces of magnesium metal was added, red colouration produced indicates the presence of flavanoids (Sofowora, 1991).

Test for Tannins

One ml of freshly prepared 10% KOH was added to 1ml of the extract. Appearance of the dirty white precipitate indicated the presence of tannins.

Two drops of 5% FeCl3 was added to 1ml of the extract. A greenish precipitate indicated the presence of tannins (Sofowora, 1991).

Test for Alkaloids
The extract (0.5g) was stirred with 5ml of 10% aqueous hydrochloric acid in a steam bath for 20 minutes. It was then cooled and filtered. The filtrate was the used for the following test:

One ml of the filtrate was treated with few drops of Meyer's reagent. Appearance of creamy precipitate indicates the presence of alkaloids.

The filtrate was also treated with few drops of Wagner's reagent. A reddish brown precipitate indicates the presence of alkaloids in the extract (Sofowora, 1991).

Test for Saponins

Two grams of the powdered extract was placed into beaker, 20ml of water was then added and heated to boil for 3 minutes. The extract was allowed to cool. The filtrate was subjected to the following test:

One millimetre of the extract was placed in the test and was shaken thoroughly; the whole test tube was filled with froth that would last for several minutes which indicates the presence of saponin.

Three (3ml) of the extract was diluted to 10ml of water. 5-7ml of the above mixture was placed in a test tube, 5ml of castor oil was added and shaken, emulsion were form and that was remain stable for 30 minutes, this indicates the presence of saponin (Harbone, 1998).

Test for Glycosides

Ten cm^3 of 50% H_2SO_4 were added to $1cm^3$ of the extracts in a test tube. The mixture was then heated in boiling water for 15 minutes. $10cm^3$ of Fehling's solution was added and the mixture was boiled. A brick red precipitation was observed, which indicates the presence of glycoside (Harbone, 1998).

Test for Cardiac Glycoside

One ml of the extract and 2 ml of 3.5% ferric chloride were added and allowed to stand for 1 minute. One ml of concentrated H_2SO_4 was poured on the wall of the test tube so as not to interface with upper layer turning green blue indicates the presence of 2 deoxy sugar containing cardiac glycosides (Harbone, 1998).

Test for Anthraquinone Glycoside

Five grams of plant extract was boiled with 10ml aqueous H_2SO_4 and filtered while hot. The filtrate was shaken with 5ml of benzene layer was separated and half of its own volume 10% ammonia solution was added. Pink red or violet colour indicates the presence of anthraquinone glycosides (Harbone, 1998).

Test for Saponin Glycosides

Fehling's solution A and B were added to the extract, Bluish green precipitate indicates the presence of saponin glycosides.

Test for Steroids

One ml of the plant extract in a test tube was dissolve in 2ml of chloroform and 2ml of sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface indicates the presence of steroids (Sofowora, 1991).

Test for Volatile Oil

A small quantity of the plant extract was shaken with dilute HCl. The presence of a white precipitate indicates the presence of volatile oil (Sofowora, 1991).

The quantitative screening includes:

Determination of Alkaloids

Alkaloids were determined using method as reported by Trease and Evans (1978). Five grams of powdered plant sample was extracted with 100ml of methanolic: mixture and solvent evaporated. The residue was mixed with 20ml of 0.0025M H_2SO_4 and portioned with ether to remove unwanted materials. The aqueous fraction was basified with strong NH_3 solution and then extracted with excess chloroform to obtain the alkaloid fraction or separated by filtration. The chloroform extraction was repeated several times and extract was concentrated to dryness. The alkaloid weighed and percentage was calculated with reference to initial weight of powder.

$$\%\text{Alkaloid} = \frac{weight\ of\ alkaloid\ residue}{weight\ of\ sample} \times 100$$

Determination of Flavonoid

Precipitation method of (Bohm and Kocipai, 1994) was used 5 g of plant samples was hydrolyzed by boiling in 100ml of hydrochloric acid solution for about 35minutes. The hydrolysate was filtered to recover the extract. The filtrate was treated with ethyl acetate drop wisely until in excess. The precipitated flavanoids was recovered by filtration using a weighed filter paper after drying in oven at 100^{0C} for 30 minutes: it was cooled in desiccator and reweighed. The difference in weight gave the weight of flavonoids which was express as percentage of the weight of the sample analyzed.

$$\%\ \text{Flavanoids} = \frac{W2-W1}{5g} \times 100$$

Where:
5g = weight of sample.
W1 =weight of empty filter paper.
W2 = weight of paper + Flavonoids precipitate.

Determination of Tannin

Tannin was determined using method of (Trease and Evans, 1978). Powder plant sample of 0.1g was put in to $100cm^3$ conical flask and $50cm^3$ volumetric flasks. The residue was washed several times and the combined solution made up with distilled water to 0, 1, 2, 3, 4 and $5cm^3$ Folin-deni's reagent and $10cm^3$ of Na_2CO_3 solution were added and made to volume with distilled water. The flask was allowed to stand for 20 minutes after which optical density was measured at 760nm. The calibration curve was plotted from which the concentration of tannic acid in the sample was extrapolated.

Determination of Saponins

Saponins were determined using method of El-Olemyl *et al.,* (1994). From powdered plant extract 5g was placed in a 250ml flask containing 30ml of 50% alcohol. The mixture was boiled under reflux for 30 minutes and was immediately filtered while hot through a coarse filter paper. Two gram (2g) of charcoal was added the content was boiled and filtered while hot. The extract was cooled (some saponins may be separated) and an equal volume of acetone was added to complete the precipitation of saponin. The separated saponins were collected by decantation and dissolve in the least amount of boiling 95% alcohol and filtered while hot to remove any insoluble matter.

The filtrate was allowed to cool at room temperature thereby resulting in the precipitation of saponins. The separated saponins were collected by decantation and suspended in about 2ml of alcohol and filtered. The filter paper was immediately transferred to a desiccator containing anhydrous calcium chloride and saponins were left to dry. They were weighed with reference to extract used.

$$\% \text{ Saponins} = \frac{W2-W1}{5g} \times 100$$

Where:
5g = weight of sample.
W1 =weight of empty filter paper.
W2 = weight of paper + Flavanoids precipitate.

Determination of Glycoside

Glycoside was determined using spectrometric method (El-Olemyl *et al*., 1994). One gram of the extract was extracted in 10ml of 70% alcohol and mixture was filtered. From the filtered 8ml of the mixture was added to 8ml of 12.5% lead acetate (to precipitate resins, tannins and pigments). The mixture was shaken well, completed to volume (100ml) with distilled water and filtered. The filtered 50ml was pipette in to another 100ml volumetric flask and 8ml 4.7% disodium hydrogen phosphate (Na_2HPO_4) solution (to precipitate excess lead) was added. The mixture was made up to the volume with distilled water and mixed. The mixture was filtered twice using Whatman filter paper. Baljet reagent (10ml) was added to 10ml of purified filtered. A blank sample of 10ml of distilled water was also added to 10ml Baljet reagent the two were allowed to stand for 1hour (time maximum for colour development). The intensity of the colour was read at 495nm using spectrophotometer against a blank (20ml distilled water). The colour was stable for several hours.

The percentage of total glycoside was calculated digitoxins by using $E^{1cm}_{1\%}$ of given digitoxins (=170)

$$\text{The percentage of glycosides} = \frac{A \times 100}{17} \times g\%$$

Where:
A = Absorbance of the colour at 495nm.
All the reagents were accessed and the screenings were conducted at Biochemistry laboratory of the Usmanu Danfodiyo University Sokoto.

RESULTS

The result of qualitative phytochemical screening showed that flavonoids, tannin, saponin, glycoside, alkaloid, cardiac glycoside, saponin glycoside and volatile oil were present in the stem bark of *Terminalia avicennioides* at moderate amount, steroid and balsam were present at low amount and anthraquinone were not detected (Table 1).

Result of quantitative phytochemical analysis showed that Glycoside had the highest percentage mean of 0.837±0.0060, followed by alkaloid 0.126±00.0830 while saponin had the least with 0.011±0.0081 (Table 2).

Table 1. Qualitative Phytochemical Analysis of *Terminalia avicennioides*

Fraction	Concentration
Flavanoid	+++
Tannin	+++
Saponin	+++
Glycoside	+++
Alkaloid	+++
Cardiac Glycoside	+++
Steroid	++
Saponin Glycoside	+++
Volatile Oil	+++
Anthraquinone	ND
Balsams	++

Note: +++ Moderate Amount, ++ Low Amount. ND Not Detected

Table 2. Quantitative Phytochemical Analysis of *Terminalia avicennioides*

S/No	Fraction	Concentration g (%)
1.	Saponin	0.011±0.0081
2.	Flavanoid	0.018±0.0027
3.	Alkaloid	0.126±0.0830
4.	Glycoside	0.837±0.0060
5.	Tannin	0.035±0.03

Mean concentration (g%w/v) of the fractions ±SEM

DISCUSSION

Phytochemical screening of aqueous extracts of *T. avicennioides* shows the presence of glycosides, saponins, tannins, alkaloids, steroids, balsam and volatile oil, this agreed with the findings of Mann *et al.* (2008) except for the presence of anthraquinone. *Terminalia avicennioides* has been previously shown to be rich in saponin as described by Soforowo (1991), Atawodi (2005), Bulus *et al.* (2008), and Azeez *et al.* (2015), a phytochemical known to have a strong surface action on erythrocyte membrane (Godwin and Theodore, 2001). According to Sofowora, (1986) the presence of secondary metabolites in plants, produce some biological activity in man and animals and it is responsible for their use as herbs. Hence, the presence of the secondary metabolites in *T .avicennioides* may be responsible for its potential use as drug.

CONCLUSION

Phytochemical screening of *Terminalia avicennioides* revealed the presence of flavonoids, tannins, saponins, glycoside, alkaloids, cardiac glycoside, saponins glycoside and volatile oil in the stem bark of *Terminalia avicennioides* at moderate amount, steroid and balsam were present at low amount and anthraquinone was not detected, therefore, the stem bark of the plant constitutes some bio compounds that are of medicinal value. Toxicity of the plant need to be carryout and the phytochemical screening of the other parts of the plant such as the leaves and the rinds should be carryout.

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