

Phytochemical Evaluation and Characterization of Methanol Extracts from the Stem-back of *Jatropha curcas* Plant

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ABSTRACT

The phytochemical screening and characterization of the alkaloid and glycosides fractions of methanol extracts of stem-back of *Jatropha Curcas* plant were carried out. The phytochemical screening showed that the stem-back had $0.62 \pm 0.18\%$ flavonoids, $0.52 \pm 0.42\%$ tannin, $6.99 \pm 0.37\%$ Saponin, $28.78 \pm 0.39\text{mg}/100\text{g}$ alkaloid and $51.92 \pm 0.35\text{mg}/100\text{g}$ glycosides. Furthermore, two fractions of these phytochemicals (*i.e.* the alkaloid and glycoside) were characterized using standard methods. The alkaloid fraction was found to contain curcine ($0.44 \pm 0.20\text{mg}/100\text{g}$), tetramethylpyrazine ($1.22 \pm 0.12\text{mg}/100\text{g}$), atropine ($2.10 \pm 0.32\text{mg}/100\text{g}$), diphenoxylate ($0.81 \pm 0.32\text{mg}/100\text{g}$), curcacycline A ($0.49 \pm 0.17\text{mg}/100\text{g}$) and curcine ($0.80 \pm 0.20\text{mg}/100\text{g}$). The glycoside fraction contained linamarin ($0.25 \pm 0.09\text{mg}/100\text{g}$), lotaustralin ($2.14 \pm 0.09\text{mg}/100\text{g}$), prunasin ($0.40 \pm 0.12\text{mg}/100\text{g}$), dhurin ($2.49 \pm 0.32\text{mg}/100\text{g}$), amygdalin ($15.90 \pm 0.55\text{mg}/100\text{g}$), quabain ($3.98 \pm 0.45\text{mg}/100\text{g}$), digitoxin ($13.69 \pm 0.40\text{mg}/100\text{g}$), digitalis ($4.14 \pm 0.25\text{mg}/100\text{g}$) and dioxin ($8.89 \pm 0.51\text{mg}/100\text{g}$). These active ingredients found in this herbal plant are of great importance in the pharmaceutical industry and can be harnessed to better the lot of humanity more especially in this Covid-19 era when all hands are on deck to find a possible cure for the pandemic that has ravaged the entire world.

Keywords: Alkaloids, glycosides, *Jatropha curcas*, screening, phytochemicals, stem-back

INTRODUCTION

Nature has endowed mankind with all that is required to take care of it on planet earth. The earth is filled with so many natural resources at man's disposal. These enormous resources at man's disposal includes the vegetation (*i.e.* abundant plant resources) that serves not only for food but also to take care of the health need of man. Several plants exist with very high nutritive and medicinal values and yet remain unexploited for human and animal benefits. Plants are very vital because they are a fundamental part of life on earth, because they generate oxygen,

food and medicine that allows human and other higher life forms to exist (Izevbige *et al.*, 2004). In addition, to using green leaves as food, specific green leaves make excellent natural medicines. They work as Hippocrates, the father of medicine said “let food be your medicine and medicine be your food” (Izevbige *et al.*, 2004). Plants form the main ingredients of medicine in traditional system of healing and have been the source of inspiration for several major pharmaceutical drugs (Obute, 2005). Africa happens to be one of the continent that is greatly blessed with different varieties of this nature's endowment known as plants, the majority of which are of medicinal value. But her short coming has been how to fully harness this nature's gift and get the best out of it. The use of medicinal plants is increasing worldwide, in view of the tremendous expansion of traditional medicine and growing interest in herbal treatments. Plants are used in medicine to maintain and augment health-physically, mentally and spiritually as well as to treat specific condition and ailments (Igoli *et al.*, 2005). The plant kingdom contributed immensely to human health when no synthetic medicines were available, and when no concept of surgery existed. There is therefore need to conserve these plants associated with indigenous knowledge for our development and good health (Esimone *et al.*, 2009). One of the smallest countries in Africa (Madagascar) came to global limelight and popularity this Covid-19 period for their alleged claim of having been able to come up with herbal remedy that could cure covid-19 ailment. This claim drew the attention of some Western Countries like Russia and others who placed order for the claimed herbal remedy. Unfortunately, for them, the clinical trials conducted on this herbal remedy by these Western nations could not prove its efficacy, which is an indication of work not properly done. This fact does not therefore rule out the efficacy of herbal remedies. It is in the light of these, that this study, Phytochemical evaluation of extract of stem-bark of this medicinal plant, *Jatropha curcas* is carried to ascertain the active ingredients (Phyto-chemicals) in its stem-bark that could be of great benefits to the pharmaceutical industries in formulating or compounding remedies (drugs) that could be of great use in treatment of ailments, Covid-19 not excluded.

MATERIALS AND METHODS

The *Jatropha curcas* stem-bark used for the work was obtained in one of the local communities (Egwi) in Etche Local Government Area of Rivers State, Nigeria. It is a life tree that is used for medicinal purpose. The stem-bark was sun-dried and pulverized and stored in an air-light container for further use (Nwala *et al.*, 2013).

Preparation of Extract

About 250g of the pulverized stem-bark sample was extracted with methanol. The extract was filtered using Whatman's No 1 filter paper and the filtrate was concentrated to dryness in a vacuo using a rotary evaporator to remove the methanol (Nwala, *et al.*, 2013).

Phytochemical Screening Test for the Extract

A small portion of the dry extract was subjected to the phytochemical test using methods to test for tannins, saponins, flavonoids, alkaloids and glycosides (Makkor & Becker, 1999; Hodek, *et al.*, 2002; Li *et al.*, 2003; Akinpelu *et al.*, 2009; Kaur & Arora, 2009; Nwala *et al.*, 2013; Igbinosa, *et al.*, 2009).

Test for Tannins

About 1g of the sample was put into 250ml conical flask containing 100ml of distilled water and the flask was boiled for 1 hour and then filtered through a Whatman (No 541) filter paper (While

Warm) into a volumetric flask. The sample solution was hence made up to 100ml after cooling at room temperature.

Tannic acids standard solution was freshly prepared by dissolving 0.01g of tannic acid in distilled water diluting to 100ml (1.0mg/ml). Zero to 10ml (0-10ml) tannic acids standard solution was pipette into 100ml volumetric flask to give standard solution of concentration varying from 0.00-10mg tannic acid. Ten milliliters (10ml) of the sample were then pipetted into 100ml volumetric flask. Then 5ml of folin-Denis reagent and 20ml of 17% (W/V) sodium carbonate solution were added to both the standard and sample solutions. The solutions were then diluted to the 100ml mark of the volumetric flask with distilled water and mixed. They were kept in a water bath at 37°C for 20 minutes. Their absorbances were measured spectrophotometrically at 760nm. The value of the tannin content of the sample was extrapolated from the standard graph as mg tannin/100g sample.

Saponin Content Determination

One green (1g) of the sample was folded in a filter paper (in a thimble) and put in a socket extractor and a reflux condenser filtered on top. Extraction was done with acetone in 250ml capacity round bottomed flask for 3 hours. The apparatus was dismantled and a 15ml capacity round bottom flask containing 100cm³ of methanol was filtered to the extractor and another extraction was carried out for 3 hours with methanol. The methanol was recovered by distillation and the flask oven dried and weighted. The second extraction was done in order to maintain the change in weight at the end of the second extraction and the saponin content was then calculated.

Determination of Flavonoids

Ten grams of the sample was extracted in a clinical flask with a rubber lip placed in an ultrasonic bath with 100ml Ethanol - water (80;20, v/v) solution at 25°C for 15 minutes repeated 3 times. The extract was isolated by membrane filtration and then transferred to a rotatory evaporator devices and concentrated under vacuum until the residual volume was 5ml; the residual was washed by Ethyl acetate successively followed by filtering and drying, the flavonoid was then calculated.

Determination of Alkaloids

Two hundred (200ml) of 20% acetic acid in ethanol was added to 5g powdered sample, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to 1/4 of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle; collected precipitation was washed with diluted ammonium hydroxide and then filtered. The residue was, weighed and expressed as the Alkaloids.

Determination of Glycosides

One gram (1g) of the sample was weighed into 800ml round bottom flask and 200ml of distilled water added to the flask and allowed to stand for 24hrs. The solution was then distilled to 150ml of distillate, were added 8ml of 6M ammonium hydroxide (NH₄OH) and 2ml of 5% potassium iodide (KI). The resultant solution was titrated against 0.02N silver nitrate (AgNO₃). The titre value was multiplied by 1.08 mg HCN. One (1) ml of 0.02N AgNO₃ being equivalent to 1.08mg HCN was used and the glycoside content was then calculated.

Determination of Types of Alkaloids in *Jatropha curcas* Stem Bark Sample

This determination was carried out by the method modified by Suau *et al.* (2002) in similar research studies. 5.0g of the pulverized sample was macerated in 25ml of hexane for about 72 hours. The extract was filtered and the residue was air-dried, later treated with 10% aqueous NH_3 and macerated in CHCl_3 for 24hrs. After the filtration and evaporation at reduced pressure, the resultant crude extract was extracted three times with CHCl_3 . The CHCl_3 fraction was washed with water. The extract was poured into the round bottom flask of the rotator evaporator arrangement. It was separated by driving the solvent off the extract. Then the concentration extract was dried of water by using the anhydrous sodium sulphate before gas chromatography analysis (Gc-Ms).

The analysis was carried out using a Hewlett packed (HP; Palo ALTO, CA, USA) model 5988A Mscouple to an Hp 5980 GC equipped with an HP-1 Fisedsilicallary (300mx 0.25mm 1.d; 0.25 mm film thickness). The increase at 10°C/min to 250°C and quantitative determination was based on the total ion current corrected for the detector response of each individual alkaloid. The mass range from 125 to 450 450 atomic mass unit (amu) was scanned at a rate of 2.6 scan s/s. For analysis, alkaloid fractions (10mg) were dissolved in dichloromethane (10ml) and 0.5-1.5ml aliquots were injected directly. Alkaloids were identified by direct comparison of their MS and retention time (Rt) with those of authentic sample and with data from the Ms library.

Determination of various Types of Glycoside in *Jatropha curcas* Stem-bark Extract

The glycosides extraction was also carried out by the methods modified by Strinvas *et al.* (2003) and Nwala *et al.* (2013) earlier. 5.0g of the pulverized sample was macerated in 25ml of hexane for about 72 hrs. The extract was filtered and the residue air-derived, later treated with 10% aqueous NH_3 and macerated in CHCl_3 for 24 hours. After the filtration and evaporation at reduced pressure, the resultant crude extract was treated with 7.5ml of 5% aqueous HCl. The aqueous phase was made alkaline with aqueous NH_3 and extracted three times with CHCl_3 . The CHCl_3 fraction was then washed with water. The extract was poured into the round bottom flask of the rotator evaporator arrangement. The extract was separated by driving the solvent off the extract. Then the concentrated extract was dried by using the anhydrous sodium sulphate before gas chromatographic analysis.

The chromatographic analysis was carried out using Hewlett Packard (HP; Palo Alto, CA USA) HP 6890GC powered with HP-Chem-station Rev. A. 09.01 (1206) software with AC-5 capillary Colum (30m x 0.25mm x 0.2mm). The Colum temperature was initially held at 200°C for 0.8min, then increased at 10°C to 250°C and then held at 250°C for 24mins; the nitrogen flow rate was 10ml/min for analysis. The glycoside fraction (10mg) were dissolved in dichloromethane (10ml) and 0.5-1.5mm aliquots were injected directly. The chromatographic peaks of the analysis were confirmed by comparing their retention times and UV spectra with those of the integration of the peak using external standard method.

RESULTS

The results are presented in Tables 1,2 and 3 below. The phytochemical screening or test showed that the methanol extract of *Jatropha curcas* stem bark contains tannins, Saponins, flavonoids, alkaloids and glycosides. The values of these phytochemicals in the J. curcas stem-bark extract are tannins ($0.52 \pm 0.35\text{mg}/100\text{g}$), flavonoids ($0.62 \pm 0.18\%$), saponins ($6.99 \pm 0.57\%$), alkaloids ($28.78 \pm 0.39\text{mg}/100\text{g}$), and glycosides ($51.92 \pm 0.35\text{mg}/100\text{g}$). The

glycosides had the highest value, while the tannins had the least value. The value of glycoside was significantly different from that of the others apart from the alkaloid.

The characterization of the alkaloid fraction of the phytochemicals showed that it contained curcumin, tetramethylpyrazine, atropine, diphenoxylate, curcacycline A, Jatrophine, Jatropharm and Curcain. The values of these compounds are 0.44 ± 0.20 mg/100g (curcumin), 1.22 ± 0.13 mg/100g (tetramethylpyrazine), 2.10 ± 0.32 mg/100g (Atropine), 0.81 ± 0.32 mg/100g (Diphenoxylate), 0.49 ± 0.17 mg/100g (curcacycline A), 6.24 ± 0.42 mg/100g (Jatrophine), 7.12 ± 0.35 mg/100g (Jatropharm) and 0.80 ± 0.20 mg/100g (curcain). The value of Jatropharm (7.12 ± 0.55 mg/100g) was significantly higher than that of the others except that of Jatrophine.

The other phytochemical that was characterized is the glycoside. The glycoside fraction contained linamarin, lotaustralin, prunasin, dhurrin, amygdalin, quabain, digitoxin, digitalis and digoxin. The concentrations of these compounds are 0.25 ± 0.09 mg/100g (Linamarin), 2.14 ± 0.09 mg/100g (Dhurrin), 15.90 ± 0.55 mg/g (Amygdalin), 3.98 ± 0.45 mg/100g (Quabain), 13.69 ± 0.40 mg/100g (Digitoxin), 4.14 ± 0.25 mg/100g (Digitalis) and 8.89 ± 0.51 mg/100g (Digoxin).

Amygdalin (15.90 ± 0.55 mg/100g) had the highest concentration, followed by Digitoxin (13.69 ± 0.40 mg/100g), while the least concentration was that of Linamarin (0.25 ± 0.09 mg/100g). The concentration of Amygdalin is only significantly different ($P < 0.05$) to that of Linamarin, Lotaustralin, Prunasin and Digoxin and not to the rest of the compounds.

Table 1: Phytochemical constituents of methanol extract of *Jatropha curcas* stem-bark.

Composition	Stem-bark Extract
Tannins (%)	0.52 ± 0.13^{abc}
Saponins (%)	6.99 ± 0.57^{ac}
Flavonoid (%)	0.62 ± 0.18^a
Alkaloids (Mg/100g)	28.78 ± 0.39^c
Glycosides (Mg/100g)	51.92 ± 0.35^c

Values are mean $\pm$ standard deviation of triplicate determination and means in the same row with different superscript letters were significantly different at the 0.05 level.

Table 2: Composition of types of Alkaloids in *Jatropha curcas* Stem-bark Extract

Parameters (Mg/100g)	Stem-bark Extract
Curcumin	0.44 ± 0.20^{bc}
Tetramethylpyrazine	1.22 ± 0.13^a
Atropine	2.10 ± 0.32^{abc}
Diphenoxylate	0.81 ± 0.32^a
Curcacycline A	0.49 ± 0.17^{bc}
Jatrophine	7.12 ± 0.55^c
Jatropharm	7.12 ± 0.55^c
Curcain	0.80 ± 0.20^{ac}

Values are means $\pm$ standard deviation of triplicate determinations and means in the same row with different superscript letters were significantly different at the 0.05 level.

Table 3: Composition of types of Glycosides in Jatropha Carcass Stem-Bark Extract

Parameters (mg/100g)	Stem-Bark Extract
Linamorin	0.25±0.09 ^{bc}
Lotaustralin	2.14±0.09 ^a
Prunasin	0.40±0.12 ^{bc}
Dhurrin	2.49±0.32 ^c
Amygdalin	15.90±0.55 ^c
Quabain	3.98±0.45 ^c
Digitoxin	13.69±0.40 ^c
Digitalis	4.14±0.25 ^c
Digoxin	8.89±0.51 ^{bc}

Values are means ± standard deviation of triplicate determinate and means in the same row with different superscript letter were significantly different at the 0.05 level.

DISCUSSION

The phytochemical evaluation showed that the methanol extract of stem-bark of *J. carcass* contained tannins, saponins, flavonoids, alkaloids and glycosides. According to Li *et al.* (2003), Akinpelu *et al.* (2009) and Nwala *et al.* (2013), these phytochemical compounds are known to support bioactive activities in medicinal plants and hence their use as local remedies in the treatment of some diseases.

Tannins had the lowest value (0.52±0.13) in the stem-bark extract of *J. curcas*. Akinpelu *et al.* (2009) reported the presence of tannins in the leaf of *J. curcas*. Igbinosa *et al.* (2009) reported the presence of tannins in the stem-bark, while Adeoye and Oyedapo (2004) also reported the presence of tannins in the extract of stem-bark of *Erythrophleum guineense*. Tannins are perceived to be useful in the treatment of inflamed or ulcerated tissues and they are known to be active in cancer prevention (Li *et al.*, 2003). Hence, the possession of this compound by *J. curcas* may be useful as a source of bio-active compound in the treatment of cancer if the extracts from the plant is improved upon by the chemist. The saponin level or value of the stem-bark was 6.99±0.57%. This level was higher than the ones reported by Makkar and Becker (1999) and Martinez-Herrera *et al.* (2006) in similar studies. Saponins are known to produce inhibitory effect on inflammation and are key ingredients in traditional Chinese medicine (Liu & Henkel, 2002).

The flavonoid content of the stem-bark of *J. curcas* was significantly lower (0.62±0.18%) than that of the other phytochemicals except the tannins. Igbinosa *et al.* (2009) acknowledged the presence of flavonoids in stem-bark of *J. curcas*, while Akinpelu *et al.* (2009), extracted flavonoids from the leaf sample of *J. curcas*. Flavonoids exhibited a wide range of biological activities like anti-microbial, anti-inflammatory and anti-angionic properties (Hodek *et al.*, 2002).

The alkaloid content of the stem-bark of *J. Curcas* was second to the largest phytochemical as shown by the results of the evaluation. Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effect on humans and this has led to the development of powerful pain killer medications (Nobori *et al.*, 1994). The glycoside level of the stem-bark extract of *J. curcas* was significantly ($P<0.5$) higher than the other phytochemical except that of

the alkaloid level. Egbe and Akinyele (1990) found 420mgkg⁻¹ of cynogenic glycoside in lima beans. It is therefore evident that cynogenic glycoside is widely distributed in nature and that some amounts are consumed as part of our daily diets. The characterization of the alkaloid fraction of the phytochemicals showed that it contained curcin, tetramethylpyrazine, Atropine, diphenoxylate, curcacycline A, Jatrophine, jatropharm and curcain.

Curcin is the least in value of all the compounds in the alkaloid fraction of the phytochemicals. Aregheore *et al.*, (2003), reported that curcin was one of the irritants or toxic compounds isolated from *J. curcas* seeds. Tetramethylprazine also was found in appreciable level (1.22 ± 0.13 mg/100g) in the alkaloid fraction of the *J. Curcas* extract phytochemicals. Atropine which is one of the components of lomotil used for the pharmacological evaluation of anti-diarrhoeal activities, was another alkaloid found in the extracts of *J. Curcas*. The value of atropine in the alkaloid is significantly ($P < 0.05$) different from the rest of the alkaloid fractions. Diphenoxylate is another component of lomotil and the alkaloid fraction of *J. Curcas* stem-bark extract. These compounds are also very important pharmacologically especially in anti-diarrhoeal activities (Srinivas *et al.*, 2003). Curcacycline A is another compound in the alkaloid fraction of *J. Curcas*. Its value (0.49 ± 0.17 mg/100g) was significantly ($P < 0.05$) different from all the other fraction except in curcin, jatrophine and jatropharm which have the highest value of the alkaloid fraction of *J. Curcas* extract. These values, 6.24 ± 0.42 mg/100g and 7.12 ± 0.55 mg/100g respectively were significantly higher ($P < 0.05$) than those of the others in the fraction. Curcas was also Characterized as a component of the alkaloid fraction of *J. Curcas* Stem-bark. Its value, 0.80 ± 0.55 mg/100g was significantly ($P < 0.05$) different from others. The kind of alkaloid reported by Suaur *et al.* (2002), for fumaria species were different from the types observed in *J. curcas* stem-bark extract.

The glycoside fraction of the phytochemicals was also characterized, and the result showed that it contained linamarin, Lotaustralin, Prunasin, Dhurrin, Amygdalin, Quabain, digitoxin, digitalis and digoxi. Linamarin is a Cynogenic glycoside found in the leaves and roots of plant such as cassava, lima beans and flax. Upon exposure to gut enzymes in humans, linamarin and its methylated relative, lotaustralin can decompose to toxic chemical compound, hydrogen cyanide. Therefore, uses of plants that contain significant quantities of linamorphin as food, require extensive preparation and detoxification (Soto-blanco *et al.*, 2002). Lotuaustralin is a methylated relative of linamarin and both may be hydrolyzed by the enzyme, linamarase to form glucose and a precursor to the toxic compound, hydrogen cyanide (Akgul *et al.*, 2004). Quabain is also one of the important compounds found in glycoside. In France and Germany intravenous quabain has a long history in the treatment of heart failure and some continue to advocate its use intravenously and orally in angina pectoris and myocardial infarction (Murrel, 2005; Balzan, 2007). Digitoxin was second to the highest compound in value. Digitoxin (cardiac glycoside) has similar structure and effects like digoxin (though the effects are longer lasting). Unlike digoxin (which is eliminated from the body via the kidney) it is eliminated via the liver, so could be used in patients with poor or erratic kidney function. However, it is now rarely used in current western medical practice (Haux *et al.*, 2001)

CONCLUSION

J. Curcas stem-bark extract contains phytochemicals which could be used as herbal remedies in the treatment of some diseases. The fractions that were characterized also contained some important compounds that were already formulated into drugs. Now that the search for the

cure for Covid-19 is in top gear more work should be done on this wonder plant perhaps the expected cure for the pandemic will be derived or obtain there.

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