
ANTINUTRITIONAL FACTORS, TOXIC ELEMENTS AND PHYTOCHEMICALS PRESENT IN JATROPHA CURCAS FRUITS, KERNELS, SEEDS AND ETHANOL SEED EXTRACT

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ABSTRACT

Jatropha curcas (*J. curcas*) seeds have been exploited as a source of biodiesel and for its ethno medicinal uses and agro feed potential, however, toxic properties have been associated with the seeds. The study was aimed at determining the toxic constituents present in *J. curcas* fruits, seeds and kernels and ethanol seed extract. Standard methods were employed in the investigations, including those of the Association of Analytical Chemists and Trease and Evans. *J. curcas* fruits, seeds and kernels from Sierra Leone contained cyanogenic glycosides (in mg/100g) of 7.10, 5.10 and 16.96 while the corresponding values for the Nigeria samples were 11.60, 10.15 and 15.92; all greatly above the maximum permissible limits of 0.05-0.35. Similarly, the tannin contents of the fruits, seeds and kernels from Sierra Leone (1.66, 1.46, and 1.80) and of the seeds and kernels from Nigeria (1.40, 1.48) approximated the maximum permissible limits of 1.5 mg/100g or were higher. In contrast, the phytates and saponins which ranged from 1.78-2.14 and 1.64-2.42 for both sources of *J. curcas* were significantly below the maximum permissible levels of 500 mg/100g (for phytates) and 100 mg/100g (for saponins). Heavy metals like cadmium, copper, chromium and lead were also detected, but their concentrations were below the maximum permissible limits. These constituents were mostly similar regardless of the country source of *Jatropha*. Ethanol extract of *J. curcas* seeds was found to contain toxic phytochemicals and heavy metals. *J. curcas* as food cannot be ruled out especially for animals since cooking, fermentation and heat treatment can significantly reduce some of these antinutritional factors.

Key words: Antinutritional factor, Heavy metals, *Jatropha curcas*, Phytochemicals

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INTRODUCTION

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga *et al.*, 2005).

Jatropha curcas (physic nut or purging nut), a drought resistant shrub or tree belonging to the family Euphorbiaceae is regarded as a wonder plant because of its numerous attributes. For instance, the seeds contain up to 60% oil with a fatty acid pattern similar to that of edible oil, and the percentage of essential amino acids and mineral content can be compared to those of other seeds (Makkar *et al.*, 1999). Apart from the seed oil, *Jatropha curcas* (*J. curcas*) is a significant source of many phytochemicals with varying biological activities. The plant contains alkaloids, lignans, cyclic peptides and terpenes. Among the terpenes, diterpenes from different species of *Jatropha* have been documented to have a wide range of biological activities such as tumor-promoting, irritant, cytotoxic, anti-inflammatory, antitumor, molluscicidal, insecticidal and fungicidal activities (Rakshit *et al.*, 2010). The nut of the plant has also been used as folk medicine traditionally for the treatment of many ailments, including burns, convulsions, fever and inflammation (Osoniyi and Onajobi, 2003).

During the past two decades, the *Jatropha* plant has gained interest in particular for its oil, which can be used for biodiesel production (Kumar and Sharma, 2008). In spite of the myriad of ethno medical uses and agro-feed potential of *J. curcas* seeds and the potential for production of biodiesel, it is important to note that toxic properties have also been adduced to parts of the plant, especially the seeds (Watt *et al.*, 1962, El Badawi *et al.*, 1995).

In addition, several cases of *J. curcas* nut poisoning in humans have been reported following accidental consumption of the seeds with symptoms of giddiness, vomiting, diarrhoea and in extreme cases death (Abdu-Aguye, 1986, Becker and Makkar, 1998). *J. curcas* seeds toxicity has also

been reported in rodents and livestock (Heller, 1996). Toxicity of *J. curcas* seeds could be caused by several components, including saponins, lectins (curcin), phytates, hydrogen cyanide, protease inhibitors, phorbol esters and curcalonic acid which is a stronger purgative than ricinolic acid (Neuwinger, 1994).

Being a widely used plant with many medicinal uses and its potential to be used as livestock feed, biodiesel and fences around farms and residential houses, the objective of the study was to determine the toxic constituents present in *J. curcas* fruits, seed and kernels and ethanol seed extract.

MATERIALS AND METHODS

J. curcas Fruits Collection and Identification and Extraction of seeds

J. curcas plant and fruits from Sierra Leone were identified, collected and authenticated by Mr. B.M.S.Turay, Taxonomist, Faculty of Pharmaceutical Sciences, College of Medicine and Allied Health Sciences, University of Sierra Leone.

J. curcas fruits from Nigeria were harvested from the *Jatropha* plantation of the Institute for Agricultural Research (IAR), Ahmadu Bello University (ABU), Zaria, Kaduna State, and authenticated by Professor Joshua Ogunwole, Seeds and Legumes Programme Leader, IAR, ABU, Zaria, Kaduna state, Nigeria. The seeds were extracted as recently described by Abiri and colleagues in 2015 (Abiri *et al.*, 2015).

Antinutritional factor determination Determination of Hydrogen Cyanide (HCN) Content

HCN content was determined by the method previously described by the Association of Analytical Chemists (AOAC) in 1980 (AOAC, 1980). Briefly, ten grams (10 g) of grind fruit, seed and kernel samples from Sierra Leone and Nigeria were each soaked in a mixture of 200 ml of distilled water and 10 ml of orthophosphoric acid. The mixture was left for twelve (12) hours to release all bounded hydrocyanic acid. A drop of an antifoaming agent (paraffin) and antibumping

agent was added and the solutions distilled until 150 ml of the distillate was collected. Twenty millilitres (20 ml) of the distillate was diluted with 40 ml of distilled water in a conical flask. Eight millilitres (8 ml) of 6.0M ammonium hydroxide (NH₄OH) and 2 ml of 5% potassium iodide (KI) solutions were added. The mixture was titrated against 0.02M silver nitrate solution using a micro-burette until a faint, but permanent turbidity was obtained. Using a simple proportion (1 ml 0.02M AgNO₃ = 1.08 mg HCN), the amount of HCN was estimated in relation to the volume of silver nitrate that produced permanent turbidity.

Determination of Phytate Levels

Phytate levels were determined by the method previously described by Reddy and colleagues in 1982 (Reddy *et al.*, 1982). Briefly, 4g each of grind samples of *J. curcas* fruits, seeds and kernels from Sierra Leone and Nigeria were soaked in 100 ml of 2% hydrochloric acid for 5 hours and filtered. Twenty-five millilitres of the filtrate was taken into a conical flask and 5 ml of 0.3% ammonium thiocyanate solution was added. The mixture was titrated against a standard solution of iron (III) chloride until a brownish-yellow colour persisted for 5 minutes. Using a simple proportion (1 ml of 0.025M FeCl₃ = 6.601 mg phytate), the amount of phytate was estimated in relation to the volume of iron (III) chloride that produced a persistent brownish-yellow colour.

Determination of Saponins Content

The method previously described by Hudson and El-Difrawi in 1979 was used to determine the saponin content (Hudson and El-Difrawi, 1979). Briefly, 10 g of the ground sample of *J. curcas* seeds from Sierra Leone and Nigeria was taken into 100 ml of 20% aqueous ethanol in water and agitated with a magnetic stirrer for 12 hours at 55°C. The solution was filtered using Whatman No.1 filter paper and the residue was re-extracted with 300 ml of 20% aqueous ethanol. The extract was combined and reduced to about 40 ml under vacuum using a rotary evaporator. The extract and 20 ml diethyl ether were transferred into a 250 ml separator funnel and shaken vigorously. The

aqueous layer was discarded. The process of purification was continued until a colourless aqueous extract was obtained. The pH of the remaining aqueous solution was adjusted to about 4.5 by adding 4 g of sodium chloride and the solution was shaken successively with butanol. The butanolic extract was washed twice with 10 ml of 5% sodium chloride and evaporated to dryness in a fume cupboard, to give the saponin, which was weighed and expressed as a percentage.

Determination of Tannin Levels

The tannin content was determined by the method previously described by Allen and colleagues in 1974 (Allen *et al.*, 1974). Briefly, 0.5 g oven-dried sample of *J. curcas* fruits, seeds and kernels from Sierra Leone and Nigeria were each weighed into 100 ml conical flasks. Fifty millilitres of distilled water was added and allowed to boil gently for 1 hour on a hot plate. The solution was filtered while warm into a 50 ml volumetric flask. 0.3 ml aliquot of tannic acid was taken into a range of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml (corresponding to 0.05 mg, 0.1, 0.15, 0.2, 0.25, and 0.3 mg). Three millilitres of the sample was pipetted into a 50 ml volumetric flask. To both sample and standard, water was added until half full. 2.5 ml of Folin-Denis reagent was added to each flask followed by the addition of 10 ml 17% sodium carbonate solution and diluted to 50 ml mark and mixed. These were allowed to stand in water bath at 25°C for 5 minutes. The optical density (absorbance) readings were taken at 760nm wavelength using water as reference. Soluble tannin (%) was calculated as:

$\frac{\text{Concentration} \times \text{extract volume}}{10 \times \text{aliquot} \times \text{weight sample}}$

Determination of Heavy Metal Contents

The method previously described by the AOAC in 2003, was used for the determination of heavy metals such as Cr, Cu, Co, Cd and Pb (AOAC, 2003). Briefly, 0.3 g of the ground samples were weighed and put into digestion glass tubes and 30 ml of the reagent was added. The tubes were placed into the digestion block pre-heated to 150°C for wet digestion; indicated by the appearance of a blue-green coloured liquid. The digestion was completed in about 80 minutes and the mixture

was left to cool. The contents of the tubes were transferred to 100 ml volumetric flasks and distilled water was added to the 100 ml mark. The digest was used for heavy metal determination by atomic absorption spectrophotometer (ASS). The concentration of each heavy metal was recorded in parts per million (ppm).

Determination of Toxic Phytochemicals

Qualitative phytochemical screening for saponin glycosides, tannins and alkaloids were done using the methods previously described by Evans and Trease in 1996 (Evans and Trease, 1996). The presence of saponin glycoside was determined by the frothing and haemolysis tests. For the frothing test, the formation of honeycomb froth in a mixture of a small portion of the extract dissolved in 10 ml distilled water which persisted for 15 minutes indicated the presence of saponin glycosides. In addition, haemolysis in the test tubes containing the extract and 2ml of 1.8% aqueous sodium chloride to which 5 drops of human blood was added was used to confirm the presence of saponin glycoside.

Ferric chloride and Lead sub-acetate tests were done for the detection of tannins. The appearance of a greenish-black precipitate following the addition of 3 drops of ferric chloride solution to the extract indicated the presence of condensed tannins. In addition, the appearance of a colored precipitate following the addition of 3 drops of lead sub-acetate confirmed the presence of tannins.

The presence of alkaloids in the extract was confirmed by the Mayer's, Dragendoff's, Wagner's and Picric acid tests; which were confirmed separately by the appearance of a creamy, reddish brown, whitish, or yellowish precipitate following the addition of 2 drops of Mayer's, Dragendoff's, Wagner's and 1% picric acid to a portion of the extract respectively.

Statistical analysis

The data were expressed as means \pm standard errors (SEM) where appropriate. The statistical analysis of the data was done using Statistical Package for the Social Scientist (SPSS) software,

version 14. Test of significance was done using Student's t-test with $p < 0.05$ considered significant.

RESULTS

Antinutritional factors, heavy metals and toxic phytochemical constituents of *J. curcas* from Sierra Leone and Nigeria

The dried fruits, seeds and kernels of *J. curcas* from Sierra Leone and Nigeria were analysed for the following antinutritional factors: cyanogenic glycoside, hydrogen cyanide, phytates, saponins and tannins.

Antinutritional factors such as cyanogenic glycoside, hydrogen cyanide, phytates, saponins and tannins were found in both extracts from Sierra Leone and Nigeria. The cyanogenic glycoside in the fruits and seeds of the Sierra Leone extract was significantly lower than those in the Nigeria extract ($p < 0.05$) and all the other factors were statistically similar (Table 1)

Table 1: Antinutritional factors present in fruits, seeds and kernels of *Jatropha curcas* from Sierra Leone and Nigeria

Country source	Plant part	Amount (mg/100g)				
		Cyanogenic glycosides	Cyanide	Phytate	Saponin	Tannin
Sierra Leone	Fruit	7.10 \pm 0.10	0.68	2.14	1.82	1.66
	Seed	5.10 \pm 0.12	0.46	1.82	1.64	1.46
	Kernel	16.96 \pm 0.03	0.48	2.10	2.42	1.80
Nigeria	Fruit	11.60 \pm 0.12*	ND	ND	ND	ND
	Seed	10.15 \pm 2.41*	0.24	2.06	1.66	1.40
	Kernel	15.92 \pm 3.49	0.44	1.78	1.84	1.48

Furthermore, analyses of both extracts for the presence of heavy metals such as Chromium (Cr), Copper (Cu), Cobalt (Co), Cadmium (Cd) and Lead (Pb) revealed the presence of cadmium,

chromium, copper and lead. Cobalt was not detected in the samples (Table 2).

Table 2: Heavy metals present in fruits, seeds and kernels of *Jatropha curcas* from Sierra Leone and Nigeria

Country source	Plant part	Cadmium	Cobalt	Chromium	Copper	Lead
Sierra Leone	Fruit	0.013	0.000	0.147	0.170	0.082
	Seed	0.020	0.000	0.099	0.375	0.060
	Kernel	0.013	0.000	0.123	0.328	0.061
Nigeria	Fruit	ND	ND	ND	ND	ND
	Seed	0.014	0.000	0.145	0.760	0.044
	Kernel	0.016	0.000	0.234	1.483	0.073

Ethanol extracts of *J. curcas* seeds from Sierra Leone and Nigeria were subjected to qualitative phytochemical screening for saponin glycosides, tannins and alkaloids, all of which were found to be present, but with some variations in their relative distribution between the country sources (Table 3).

Table 3: Toxic phytochemical constituents of ethanol extracts of *Jatropha curcas* seeds from Sierra Leone and Nigeria

Constituents	Test	Sierra Leone	Nigeria
Alkaloids	Dragendoff	-	+
Alkaloids	Mayer	+	-
Alkaloids	Picric acid	+	+
Alkaloids	Wagner	-	++
Saponins	Frothing	-	-
Saponins	Haemolysis	+	+
Tannins	Ferric chloride	+	+
Tannins	Lead sub-acetate	+	+

DISCUSSION

In the current study, *J. curcas* fruits, seeds and kernels from Sierra Leone and Nigeria were shown to have some antinutritional factors (cyanogenic glycosides, cyanide, phytates, saponins and tannins), heavy metals (cadmium, copper, chromium and lead) and toxic phytochemical constituents (alkaloids, saponins and tannins). There was no significant difference in the amount of phytates, saponins and tannins in *J. curcas* fruits, seeds and kernels from Sierra Leone when compared to those from Nigeria.

With respect to saponins, while the frothing test was negative for both extracts, the haemolytic test showed the presence of saponins - underscoring differential sensitivity in tests employed and the need to utilize multiple tests for analyzing the same constituents, whenever possible. Saponin was found in *J. curcas* kernels and seeds from Sierra Leone and Nigeria, although the values were significantly lower than the maximum permissible limits (Ndidi *et al.*, 2014). Since saponins are often bitter in taste, their presence may potentially reduce plant palatability in livestock (Sen *et al.*, 1998). Although *J. curcas* saponins from Mexico have been shown to be non-haemolytic (Becker and Makkar, 1998), the haemolysis test in this study was positive indicating these saponins could be harmful. These findings are in line with earlier reports by Sivastra and colleagues in 2010, and Oseni and colleagues in 2011 (Sivastra *et al.*, 2010; Oseni *et al.*, 2011). These authors independently reported that ethanol plant extract of *J. curcas* seeds from Nigeria (Oseni *et al.*, 2011) and India (Sivastra *et al.*, 2010) contained saponins, tannins and alkaloids. The presence of these antinutritional factors and toxic alkaloids such as saponins, lectins (curcin), phytates, hydrogen cyanide, protease inhibitors, curcalonic acid (which is a stronger purgative than ricinolic acid) have earlier been documented to be responsible for some of the toxic effects of *J. curcas* seeds (Neuwinger, 1994).

In the current study, the highest concentration of cyanogenic glycoside was found in the *J. curcas*

kernels, compared to the fruits and seeds, irrespective of the country source; with a significantly higher concentration of the cyanogenic glycosides from the *J. curcas* fruits and seeds from Nigeria compared to Sierra Leone. The difference in the amount of cyanogenic glycoside may be attributed in part to the difference in cultivars and environmental factors such as soil and climate, as reported by Cardoso and colleagues in 2005 (Cardoso *et al.*, 2005).

Regardless of the aforementioned country differences, the values recorded in this study for cyanogenic glycosides were significantly higher than the maximum permissible limits in edible plants, being 0.05-0.35 mg/100g (Bradbury, 1991) or 0.09 mg/100g (FAO /WHO, 2011). Consumption of cyanogenic glycoside above the permissible limits will result in the development of neurological manifestations in human (Montgomery, 1980), acute and chronic poisoning, adverse effects on the central nervous system (CNS), kidneys, liver, increase in respiration rate, vascular and immune system (Jabeen *et al.*, 2010). The relatively high levels of cyanide and cyanogenic glycosides documented in the current study may account in part for the signs observed during the acute toxicity studies carried out in the course of this investigation (Abiri *et al.*, 2015). Death in rats, mice and chicks was akin in some respects to cyanide poisoning and reduced following appropriate antidotal therapy with combination antidotes specific against cyanide, namely: sodium nitrite and sodium thiosulphate (Abiri *et al.*, 2015).

Although phytate was shown to be present in *J. curcas* fruits, seeds and kernels from Sierra Leone and Nigeria, the values were significantly lower than the maximum permissible limit in edible plants (Ndidi *et al.*, 2014). When these molecules are consumed along with diet, the phytates chelate with divalent and/or trivalent mineral ions such as Ca^{2+} , Mg^{2+} , Zn^{2+} , Cu^{3+} , and Fe^{3+} , resulting in these ions becoming unavailable for absorption (Duffus and Duffus, 1991). Phytates also form sparingly

digestible phytate-protein complexes, thus reducing the availability of dietary protein (Richardson *et al.*, 1985). Presence of phytate in edible plants can also lead to drug and food interaction with antacids and milk (Duffus and Duffus, 1991). Since other sources of phytate may be present in diets, even the low amounts observed here could be of toxicological significance.

All parts of *J. curcas* plant (fruit, seeds and kernel) from both countries were shown to have high levels of tannins. The tannin levels from Sierra Leone (1.66 mg/100g fruit and 1.80 mg/100g kernel) were higher than the maximum permissible limits whereas as those from Nigeria (1.40-1.48 mg/100g) were close to the maximum permissible limits as earlier documented by Ndidi and colleagues (Ndidi *et al.*, 2014). Tannins are phenolic substances associated with toxic and antinutritional effects including reduced food intake, impaired nutrient absorption and growth retardation (Butler *et al.*, 1986). The presence of tannins may be responsible in part for the reduced food intake observed during the behavioural studies in which there was a reduction in food intake by chicks, mice and rats which might lead to growth retardation (Abiri *et al.*, 2015).

In the current study, the kernels were found to contain more of the antinutritional factors than the seeds, and were generally, above permissible levels. Although cyanogenic glycosides have been reported to be absent in *J. curcas* kernel meal and seeds from Mexico (Makkar *et al.*, 1997), the presence of antinutritional factors such as saponins, tannins, phytates and cyanide have been detected in variable amount in the ethanol plant extract of *J. curcas* seeds from Nigeria (Annongu *et al.*, 2010, Oseni *et al.*, 2011, Ameen *et al.*, 2011). The variability in the levels so reported may be due to differences in the provenances of *J. curcas* studied.

Heavy metals such as cadmium, cobalt, chromium, copper and lead were also quantified in the current study. The quantity of cadmium, chromium, copper and lead detected in the ethanol extract of *J. curcas*

fruits, seeds and kernels from both countries were below the maximum permissible values in an edible plant as jointly documented by FAO and WHO in 1984 (FAO/WHO, 1984). However, no cobalt was detected in the samples from both countries. On the other hand, Azza and Colleagues in 2009 reported the absence of lead, nickel and copper in ethanolic *J. curcas* seed extract from Egypt (Azza *et al.*, 2009).

These heavy metals have been documented to cause several disease conditions. For instance, high levels of cadmium above permissible limits (FAO/WHO, 1984) may cause acute and chronic poisoning, adverse effects on the kidneys, liver, vascular and immune system (Jabeen *et al.*, 2010). Chronic exposure to chromium may result in liver, kidney and lung damage, skin rashes, nose irritations, bleeding and stomach upset (Khan *et al.*, 2008). In addition, toxic ingestion of copper may cause skin and hair discoloration, dermatitis, anemia, acne, adrenal hyperactivity, allergies, hair loss, arthritis, autism, cancer, depression and elevated cholesterol (Ullah *et al.*, 2012). Chronic exposure to lead frequently results in accumulation that causes typical symptoms in humans such as colic, anaemia, headache, convulsions, chronic nephritis of the kidneys, brain damage and other central nervous system disorders (Khan *et al.*, 2008).

CONCLUSION

The fruits, seeds, kernels and ethanol seed extract of *Jatropha curcas* seeds were found to contain antinutritional factors, toxic phytochemicals, negligible heavy metals and absence of cobalt. Although some of these factors were found in excess of the permissible limits, the potential use of *J. curcas* as food cannot be ruled out especially for animals as cooking, fermentation and heat treatment can significantly reduce some of these antinutritional factors.

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