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Short Communication

THE TOXIC AND NON-APHRODISIAC POTENTIALS OF OILS FROM *JATROPHA CURCAS* SEEDS ON MICE

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ABSTRACT

Jatropha curcas is a multipurpose, drought resistant, perennial plant belonging to the Euphorbiceae family which is gaining lots of importance for the production of biodiesel from its oil. This study revealed the other potentials of the oil. Qualitative analysis was carried out on oil extract from the seed of *J. curcas* which revealed the presence of phytochemicals. The oil extract was also tested for aphrodisiac potentials and toxicity on mice. Results indicated the presence of saponins, terpenoids, cardiac glycosides, flavonoids and steroids which have been reported to have pharmacological attributes. The oil extract did not exhibit aphrodisiac potentials on the male mice at different doses administered (0.5, 1.0 and 1.5 ml). Mortalities were recorded for the oil extract tested on both the male and female mice. Hence, the studies indicated that the oil extract from the seed of *J. curcas* does not possess aphrodisiac potentials but contained toxins which can be removed to make the oil edible.

Keywords: n-hexane, extraction, seed-oil, *Jatropha curcas*, toxic phytochemicals, non-aphrodiasiac potentials and mice.

INTRODUCTION

Jatropha curcas is a species of flowering plant in the spurge family, Euphorbiceae that is native to the American tropics especially Mexico and Central America. It is cultivated in tropical and sub-tropical regions around the world. The plant can grow in wastelands and almost on every terrain even on gravelly, sandy and saline soil. The plant grows best on well-drained soil with a preferable pH of 6 to 9 with good aeration but it is well adapted to marginal soil with low nutritional content. It grows well with more than 600mm rainfall per year and it can withstand long periods of drought. The plant sheds leaves during prolonged drought. The preferred temperature for the cultivation of *J. curcas* is the average of 20°C to 28°C. However, it withstands a very light frost which causes it to lose all its leaves and may produce a sharp decline in seed yield.

The plant grows to a height of about 6m (20ft). The color of the leaves ranges from green to pale-green with both the male and female flowers being produced in the same inflorescence having an average of twenty male flowers to each female flower. The fresh fruits also have green color with a yellow color indicating maturity (en.wikipedia. org/wiki/*Jatropha curcas*).

Jatropha curcas is a non-edible oil crop mostly used to produce biodiesel. In addition to this the tranesterifcation process of *J. curcas* ends up with by-products that can be used to make great deal of products such as high quality paper, energy pellets, soap, cosmetics, tooth paste, embalming fluid, pipe joint cement, cough medicine, and as a moisturizing agent in tobacco. Moreover, *Jatropha* seed cake which is a waste by-product of the biodiesel transesterification can be used as organic manure.

The seeds of *J. curcas* contain viscous oil, which can be used for the manufacture of candles and soap, in cosmetics industry, as a diesel/paraffin wax or extender. This latter use has important implication for meeting the demand of rural energy services and also exploring practical substitutes of fossil fuels to counter greenhouse gas accumulation in the atmosphere. These characteristics along with its versatility make it of vital importance to developing countries (Foidl and Kashyap, 1999).

Various parts of the plant are of medicinal value. Its stem-bark contains tannin, the flowers attract bees and thus the plant has a honey production potential. Its wood and fruit can be used for numerous purposes including fuel (Kumar and Sharma, 2008).

Jatropha curcas seed-oil can be used as fuel in diesel engines directly and by blending it with methanol (Gubitz *et al*., 1999). The seed oil of *Jatropha* plant was used as a fuel substitute during the World War II. Engines tested with *Jatropha curcas* seed-oil in Thailand, demonstrated satisfactory engine performance (Takeda, 1982).The feasibility of the production of fatty acid ethyl esters from *Jatropha* plant seed-oil has been studied (Eisa, 1997). In addition to being a source of oil, *Jatropha* also provides a meal that serves as a highly nutritious and economic protein supplement in animal feed, if the toxins are

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removed (Berker and Makkar, 1998). Although there is an increased international recognition of the plant, all attention has been drifted to the use of its oil to produce biodiesel due to the increasing demand for energy across the globe. As a result all other potentials of the plant have been relegated to the background.

Most works carried out on the plant presented in journals, articles, books, and other forms of media mostly talk about the production of oil from its seeds which can be used to produce appreciable quantities of biodiesel with little or no consideration to other benefits that could be derived from the plant. The objectives of this research project are to test for the presence of phytochemicals and non aphrodisiac potentials in the oil of *J. curcas* seeds. The work is also aimed at testing for toxicity of the oil on mice and creating awareness of these potentials of the plant.

MATERIALS AND METHODS

The materials used for this research included seeds of *J. curcas* from which oils were extracted, and mice. The seeds were obtained from Tokuroano in the Krachi East district of the Volta region in Ghana. The mice were procured from Kwame Nkrumah University of Science and Technology (KNUST), Ghana.

Justification of seeds

The seed being of *Jatropha curcas* was authenticated by Dr. Isaac Sackey, a botanist at the Applied Biology Department of the Faculty of Applied Sciences, University for Development Studies (UDS), Ghana.

Methods

Sample collection and preparation

Seeds together with its outer coats were randomly collected from *Jatropha curcas* plants. The outer coats were then removed, while the inner coats containing the seeds were dried for about a week. The inner coats were also removed to obtain the actual seeds which were dried for one week to remove moisture, and later ground to fine powder.

Extraction of oil

Soxhlet extractor was used in the extraction of oil from the powdered seeds of *J. curcas* using petroleum ether (40-60°C). Ten grams of the seeds was extracted with 90 ml of petroleum ether for a period of 3 hrs. The solvent was then evaporated in vacuo and the residual oil was dried in an oven at 40°C to a constant weight. The recovered oil was stored in a vial and kept at room temperature.

Tests for phytochemicals

Alkaloids: To 2 ml of the oil, 10 ml of methanol was added and then filtered. 1% HCl was added to the filtrate and steamed. To 1 ml of the steamed filtrate, 6 drops of Mayer's reagent were added which gave a creamish/brown/red/orange precipitate that indicated the presence of alkaloids.

Saponins: To 0.5 ml of the oil, 5 ml of distilled water was added. Frothing persistence indicated the presence of saponins.

Terpenoids: To 5 ml of the oil in a test tube, 2 ml of chloroform was added. 3 ml of conc. H_2SO_4 was then carefully added to form a layer. An interface with a reddish brown coloration indicated the presence of terpenoids.

Cardiac glycosides (Keller killiani test): To 2 ml of the oil, 1 ml of glacial acetic acid which contained Iron (III) Chloride and conc. H_2SO_4 was added. The formation of a green or blue precipitate indicated the presence of cardiac glycosides.

Steroids: To 2 ml of the oil in a test tube, 2 ml of chloroform was added. This was followed by pouring equal amount of concentrated H_2SO_4 by sides into the test tube. The upper layer turned red, and the sulphuric acid layer appeared yellow with green fluorescence. This indicated the presence of steroids.

Flavonoids: To 2 ml of the oil, few drops of conc. H_2SO_4 were added. This was followed by the addition of magnesium ribbon into the mixture. A pink or tomato red color indicated the presence of flavonoids or glycosides.

Tannins: To 2 ml of the oil, 2 ml of $FeCl_3$ solution was added. A blue and black precipitate indicated the presence of tannins and phenols respectively.

Anthraquinones: To 5 ml of the oil, 3 drops of diluted conc. H_2SO_4 were added. The mixture was further extracted with benzene, and 1 ml dilute ammonia was added to it. A rose pink coloration indicated the presence of anthraquinones.

Amino acids: Aqueous mixture of the oil was treated with drops of ninhydrin (n-butanol). The appearance of a purple color indicated the presence of amino acids.

Coumarins: A test tube was filled with 2 ml of the oil. The test tube was covered with a piece of filter paper moistened with dilute NaOH solution, and placed in a hot water bath. After several minutes, the paper was removed and exposed to UV light for few minutes. Coumarins gave a yellow fluorescence under UV.

Test for toxicity

To determine toxicity of the oil extracts, doses of 0, 0.5, 1.0 and 1.5 ml were given to 4 groups of 3 mice each. The mice were then observed continuously for 1 hr for any gross behavioral changes and deaths, if any, for the next 6 hrs and at 24 hrs after dosing. The changes in behavior, if any were observed and recorded (Suresh *et al*., 2000).

RESULTS AND DISCUSSION

The results obtained (Table 1) showed the presence of saponins, flavonoids, cardiac glycosides, steroids and terpenoids in the oil extracts from the seeds of *Jatropha curcas* extracted by soxhlet extractor using n-hexane as solvent.

Table 1. Qualitative analysis results for phytochemicals in oil extracts from the seeds of *Jatropha curcas.*

Phytochemicals	Observation
Saponins	+
Tannins	−
Flavonoids	+++
Cardiac glycosides	+++
Steroids	+++
Terpenoids	+++
Alkaloids	−
Coumarins	−
Anthraquinones	−
Amino acids	−

Key: +++ = strongly present; + = weakly present; − = absent.

A number of herbal extracts contain different phytochemicals that display lots of biological activity with valuable therapeutic index. The protective effects fruits and vegetables have are largely attributed to the composition of their phytochemicals which are non-nutritive plant compounds. Different phytochemicals have been identified possessing wide range of activities capable of protection against chronic diseases. For instance saponins, terpenoids, flavonoids, tannins and steroids have anti-inflammatory effects (Liu, 2003; Manach *et al*., 1996; Latha *et al*., 1998; Akindele and Adeyemi, 2007; Ilkay *et al*., 2007). A report by Rupasinghe *et al*. (2003) indicated that saponins have antidiabetic properties. Also, Luo *et al*. (1999) have reported that terpenoids are capable of decreasing blood sugar levels in animals. Steroids and saponins are responsible for activities of the central nervous system (Argal and Parthak, 2006). Cardiac glycosides are therapeutically used for the treatment of cardiac failure. This effect is caused by the ability to increase cardiac output by increasing intracellular calcium, increasing calcium-induced calcium release and thus contraction (en.wikipedia.org/wiki/Cardiac glycosides).Therefore, the oil extracts from the seeds f *Jatropha curcas* can be said to have medicinal properties such as anti-inflammatory and anti-diabetic activities. It can also enhance the activities of the central nervous system and decrease blood sugar levels. The presence of cardiac glycosides also makes the oil a potential for the prevention of heart failure.

The phytochemicals in the oil extracts showed no aphrodisiac properties. Administration of the oil in doses of 0.5 ml, 1.0 ml and 1.5 ml to the mice in groups of two males for each dose did not display any mounting and mating behavior in the animals. The mounting behavior relatively diminished as the doses increased. In the case of 0.5 ml dose, the male made advances towards the female but no mounting was observed. No such advances were observed for doses at 1.0 and 1.5 ml.

With the oil extracts not displaying any aphrodisiac property, toxicity test was carried out. The oil extracts were again given to the animals in dosages of 0.5, 1.0 and 1.5 ml. There were three mice for each dosage in two different feeding conditions. In the first case, the mice were fed immediately after the dosage, and in the second case 2 hours after the dosage (Tables 2a and 2b).

Gross changes in behavior were observed in the dosages as there were increased respiration, loss of body weight and decline in feeding (food and water). The general behavior of the mice also changed as they become less active, and finally there were mortalities in all cases.

For the first condition where the mice were fed immediately after dosage, the mortality rate increased from 0.5 to 1.0 ml. The time at which deaths occurred at the dose of 1.5 ml was more than at 0.5 and 1.0 ml. This anomaly could be attributed to the prevalence of strong immune systems of the mice at that dosage. Also, no mortality was recorded for one of the mice at the dose of 0.5 ml in the first condition even though there were changes in the other behaviors. This therefore indicated that the toxicity of the oil extracts mostly depended on the dosage (Tables 2a and 2b).

For the second condition where the mice were fed 2 hours after dosage, the mortality rate increased as the dosage increased. Comparing this condition with the first, mortality rate for the second condition was greater at doses of 0.5, 1.0, and 1.5 ml.

The experiment conducted revealed that the phytochemicals present in the oils from the seeds of *Jatropha curcas* lack aphrodisiac potentials as it resulted in sharp decline in the sexual behavior of the mice with increasing dosage. Rather, it could be said that the phytochemicals present in the oil extracts possessed potent pharmacological properties.

The oil extracts upon administration induced changes in the general behavior of the mice which included loss of body weights, poor feeding ability, respiration increase and mortality. These results do not counter the fact that the oil possesses pharmacological activities and other

Table 2a. Toxicity test results for oil extracts from the seeds of *Jatropha curcas* on mice.

Condition	Dosage(ml)	Time elapsed before mortality and after dosage (Hrs min)	Average Duration Before mortality(min)	Weight after Death(g)	Percentage Mortality (%)
Fed immediately after dosages	0.5	42 06	3,127 ± 849.94	23.92	66.67
		62 08		29.80	
		-		-	
	1.0	54 37	2, 447 ± 803.5	22.03	100
		39 51		22.97	
		27 53		21.17	
	1.5	30 50	4, 202 ± 4, 373.3	30.49	100
		154 08		13.16	
		25 08		27.53	
Fed 2 hours after dosages	0.5	83 50	5, 225 ± 1, 949.8	17.90	100
		121 05		15.86	
		56 20		25.87	
	1.0	26 32	1,954.7 ± 462.1	16.80	100
		29 57		26.70	
		41 15		19.50	
	1.5	28 32	1, 847.7 ± 243.7	18.20	100
		35 29		22.20	
		28 22		23.64	

Table 2b. Toxicity test results for oil extracts from the seeds of *Jatropha curcas.*

Condition	Dosage (ml)	Number of mice used	Number of deaths recorded	Percentage Mortality (%)
Fed immediately after dosages	0.5	3	2	66.67
	1.0	3	3	100
	1.5	3	3	100
Fed 2 hours after dosages.	0.5	3	3	100
	1.0	3	3	100
	1.5	3	3	100

beneficial qualities, because it could be treated to remove its toxins, and administered in different modes.

CONCLUSION

This study revealed that the oil extracts from the seeds of *Jatropha curcas* do not possess aphrodisiac potentials. Besides, the oils contained toxins which resulted to the death of mice with the death rate depending mostly on the dosages administered. Also, the phytochemicals present in the oil extracts from the seeds of this plant indicated that the seeds possessed potent pharmacological compounds.

RECOMMENDATION

Further studies should be carried out on the seed-oil of *Jatropha curcas* to ascertain the cause of it being toxic to mice and to eliminate its toxins. This could create room for its aphrodisiac potentials to be examined once again, and could also pave way for the oil to be edible. With the oil possessing phytochemicals which have pharmacological properties, more studies are required to determine the main active components to enhance its full exploitation.

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