



Phytochemical, Analgesic And Acute Toxicity Study Of *Jatropha Curcus* Root

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ABSTRACT

Jatropha curcus Linn. is a multipurpose plant in the *Euphorbiaceae* family. The ethnomedical practice in West Africa showed the application of *Jatropha curcus* leaves in different forms to cure various ailments like fever, mouth infections, jaundice, guinea worm sores and joint rheumatism. The roots of *J. curcus* have been used as a decoction for bleeding gums, toothache, eczema, ringworm, and scabies and to cure dysentery. This study therefore assessed the phytochemical and analgesic properties of *Jatropha curcus* root. The acetic acid induced writhing test in mice was used to study the analgesic effect. The analgesic activity on methanol extract of the root (25mg/kg, 50mg/kg and 100 mg/kg) in the writhing test showed significant reduction of pain induced by acetic acid with the 50mg/kg being more potent. Phytochemical results of the three solvent extracts showed presence of tannins, triterpenes, alkaloids, carbohydrate and flavonoids. The quantitative phytochemical analysis showed that alkaloids (94.0 mg/g) was the highest phytochemical detected in the root while the lowest was saponins and phenols (23.0 mg/g). The results of this investigation may be useful for deriving doses that are safe for human consumption medicinally of *Jatropha curcus* root. Collectively, this study provides scientific data for the use of *Jatropha curcus* in the treatment of pains and contribute to the analgesic knowledge of this species. Further study should be carried out to find out the mechanism of its action and also to isolate, identify and characterize the active agent responsible for these effects in this plant.

Key words: Analgesic, *Jatropha curcus*, Acetic acid, Phytochemicals, Writhing test.

1.0 INTRODUCTION

Pain is a principal reason of medically associated job absence because it accompanies nearly every disease condition and is the most common cause for medical actions (Schim and Stang, 2004). Pain causes millions hospital visits yearly costing billions of dollars every year in health care loss due to lack of effective analgesic or wrong treatment (Kivell and Prinszano, 2010; Schim and Stang, 2004). Some consequences of inadequately treated pain include metabolic

disorders manifesting as weight loss, and increase in the use of healthcare resources (Joshi and Ogunnaike, 2005). The conventional analgesics have serious adverse effects such as gastric ulcerations, renal damage, respiratory depression, tolerance and addiction among other side effects coupled with the fact that they are not effective in neuropathic pain, making it necessary to search for more effective remedies with mild side effects but cheap and reliable. Good management of pain with cost-effective analgesics is therefore needed

to overcome these problems resources (Joshi and Ogunnaike, 2005). Some medicinal plants have a long history of use in traditional medicine to manage pain. The validation of traditional claims of these medicinal plants will provide the scientific basis for the conservation of tropical medicinal plants that are vanishing, the deployment of the beneficial ones as phytomedicines in the primary health care and the development of potential bioactive constituents. These could provide novel compounds or precursors in drug development and utilization of isolated compounds as research tools in drug development (Kivell and Prisinzano, 2010).

Jatropha curcus is one of such medicinal plants reputed for its wide ethno-medicinal use including psychosis, diabetes, stomach ulcer and various painful conditions in Nigeria and some West African countries (Adamu *et al.*, 2007; Magaji *et al.*, 2009; Meda *et al.*, 2013; Mohammed *et al.*, 2008; Mothana *et al.*, 2009; Musa *et al.*, 2011; Okatch *et al.*, 2012). However, there is no comprehensive study on the assumed analgesic effects and the safety/toxicity profile of this plant. This study, therefore, investigates the traditional claim of pain relief by *Jatropha curcus* and provides scientific evidence for analgesic effects of the plant using different assays of chemical, mechanical and thermal models of pain in rodents. Further isolation and evaluation of the active chemical constituents of *Jatropha curcus* responsible for the analgesic effects and clarification of the structural characters of responsible components will lead to standardization and quality control of this herbal thereby improving patient acceptability of the product (Okatch *et al.*, 2012).

Materials and Methods

Collection and Identification of Plant Materials

The roots of *Jatropha curcus* were collected from local farm in May, 2019 at Babura Local Government Area, Jigawa state. The plant was identified and authenticated in the Herbarium of the Plant Biology Department of Bayero University, Kano by Malam Bahawuddeen and was compared with a voucher specimen number of BUKSAN60.

Experimental Animals

Mice of both sexes weighing 18-31g were obtained from the Department of Pharmacology

and Therapeutics, Bayero University, Kano. The animals were allowed free access to standard feed and water *ad libitum*. They were kept in clean cages filled with saw dust, which was replaced every three days.

Phytochemical Screening

Phytochemical screening was carried out on the dried extract using simple chemical tests to detect the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, anthraquinones, and cardiac glycosides. The following methods were used:

Tests for carbohydrates

Molish's (General) Test for Carbohydrates:

To 1 ml of the filtrate, 1 ml of Molish's reagent was added in a test tube, followed by 1 ml of concentrated sulphuric acid down the test tube to form a lower layer. A reddish colour at the interfacial ring indicates the presence of carbohydrate (Evans, 2009).

Tests for Saponins

Frothing test

About 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30seconds. The tube was allowed to stand in a vertical position and was observed for 30mins. A honeycomb froth that persists for 10-15mins indicates presence of saponins.

Test for Flavonoids

Shinoda Test

A portion of the extract was dissolved in 1-2ml of 50% methanol in the heat metallic magnesium chips and few drops of concentrated hydrochloric acid were added. Appearance of red color indicates presence of flavonoids (Evan, 1996).

Test for Alkaloid

Wagner's Test

Few drops of Wagner's reagents were added to a portion of the extract, whitish precipitate indicates the presence of alkaloid (Evans, 1996).

Test for Steroid and Triterpenes

Liebermann-Burchard's test

To a portion of the extract, equal volume of acetic acid anhydride was added and mixed gently. 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. A colour change observed immediately and later indicates the presence of steroid and triterpenes. Red, pink or purple colour indicates the presence

of Triterpenes while blue or blue green indicates steroids (Trease and Evans, 1996).

Test for Cardiac Glycoside

Kella-killiani's test

A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Observed carefully at the interphase for purple-brown ring, this indicates the presence of deoxy sugars and pale green colour in the upper acetic acid layer indicates the presence of cardiac glycosides (Evans, 1996).

Test for Tannins

Ferric chloride test

To a portion of the extract, 3-5 drops of ferric chloride was added. A greenish black precipitate indicates presence of condensed tannins while hydrolysable tannins give a blue or brownish blue precipitate (Evans, 1996).

Test for Anthraquinones

Bontrager's test

To a portion of the extract in a dry test tube, 5ml of chloroform was added and shaken for at least 5mins. This was filtered and the filtrate shaken with equal volume of 10% ammonium solution, bright pink colour in the aqueous upper layer indicates the presence of free anthraquinones (Evans, 1996).

Quantitative Phytochemical screening of the root extract of *Jatropha curcus*

Alkaloid Determination using Haborne (1973) Method

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

Flavanoid Determination by the Method of Bohm and Kocipal – Abyazan (1994)

10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room

temperature. The whole solution was filtered through Whatman filter upper No. 42 (125mm). The filtrate was later be transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Saponin Determination

The method of Obadoni and Ochuko (2001) was used. Out of the grinded samples 10g was weighed for each and put into a conical flask and 100ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200ml, 200% ethanol. The combined extracts were reduced to 40ml over water bath at about 90°C. The concentrate was transferred into a 250ml separatory funnel and 20ml 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 60ml of n – butanol was added. The combined n-butanol extracts were washed twice with 10ml 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 by Van-Burden and Robinson (1981) Method

500 mg of each sample was weighed into a 50ml plastic bottle and 50ml of distilled water was added and shaken for 1hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up of the mark. Then 5ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1M FeCl₃ in 0.1M HCl and 0.008M potassium ferrocyanide. The absorbance was measure at 120 mm within 10 min.

Determination of Total Phenols by Spectrophotometric Method

The fat free sample was boiled with 50ml of ether for the extraction of the phenolic component for 15minutes. About 5ml of the extract was pipetted into a 50ml flask, and then 10ml of distilled water was added. About 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were added also. The sample was made up to mark and allowed to react for about 30 minutes for colour development. This was measured at 505nm.

Acute Toxicity Studies (LD50)

The LD50 of the extract was determined using Lorke's method (1983). The study was carried out in two phases and animals (mice) were deprived of food for 16-18h prior to administration of the extract. In phase 1, three groups of three animals per group were used. The extract was administered orally in geometrically increasing doses (10mg/kg, 100mg/kg and 1000mg/kg). The treated animals were observed for four hours post administration for signs of toxicity. After 24 hours, when no death occurred, phase 2 was initiated. In phase 2, four groups of one animal each were given the extract orally in geometrically increasing doses (1500 mg/kg, 2250 mg/kg, 3250 mg/kg and 5000 mg/kg). The animals were then observed for signs of toxicity for the first 4 hours and mortality for 24hours. The arithmetic mean of the lowest dose that killed an animal and the highest dose that did not kill was taken as the mean lethal dose (LD50) of the extract.

Analgesic studies

Acetic acid induced writhing in mice

Acetic acid induced writhing method described by (Koster *et al.*, 1959) was adopted for evaluation of analgesic activity. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, turning of trunk (Mishra *et al.*, 2011). 30 Swiss albino mice of both sexes were divided into five groups, 1 and 5 served as negative control (distilled water 10 ml/kg) and positive control (Piroxicam 20 mg/kg), while groups 2, 3, and 4 received 100 mg/kg, 50 mg/kg, and 25 mg/kg of the extract. Sixty minutes after treatment, the mice received 0.6% acetic acid (10ml/kg) interperitoneally to induce pain. 5minutes after acetic acid injection, the animals were observed and number of writhes by each mouse was counted for 15minutes. Percentage inhibition was calculated using the following formular:

$$\text{Percentage inhibition} = \frac{\text{Average number of writhes (control)} - \text{Average number of writhes (test)}}{\text{Average number of writhes (control)}} \times 100$$

(Koster *et al.*, 1959; Mishra *et al.*, 2011).

RESULTS

Table 1. Mass and Percentage Yield for the Extracts of *Jatropha curcus* root

Extract	Mass (g)	Percentage Yield (%)	Extract Appearance
Hexane	7.2	3.6	Light brown
Ethyl acetate	12.9	6.5	Brown
Methanol	20.1	10.1	Red

Phytochemical screening of hexane, ethyl acetate and methanol extracts revealed the presence of alkaloid, flavonoids, carbohydrates, triterpenes and tannins. Saponins and glycosides were

absent in hexane extract but present in ethyl acetate and methanol extract. Anthraquinones was absent in all the extracts

Table 2. Qualitative Phytochemical screening of Hexane, Ethyl acetate and methanolic root extracts of *Jatropha curcus*

Metabolites	Inference		
	Hexane	Ethyl acetate	Methanol
Alkaloid	+	+	+
Flavonoid	+	+	+
Saponins	-	+	+
Cardiac glycoside	-	+	+
Tannins	+	+	+
Steroid	-	-	+
Triterpenes	+	+	+
Phenol	-	+	+

Anthraquinones	-	-	-
Carbohydrate	+	+	+

Key: + Present, - Absent

The alkaloid (94.0 mg/g) was the highest phytochemical detected in the plant while the lowest was saponins and phenols (23.0 mg/g).

Table 3. Quantitative Phytochemical screening of methanolic root extract of *Jatropha curcus*

Metabolite	Quantity (mg/g)
Alkaloids	94.0±0.0
Flavonoids	76.0±0.0
Saponins	23.0±0.0
Tannins	68.0±0.0
Phenols	23.0±0.0

No death was recorded in the first phase of the study in rats. In the second phase, doses of 1500, 2250, 3250 and 5000mg/kg were used and no death was also recorded. The oral median lethal

dose (LD50) for the methanol root-extract of *Jatropha curcus* was therefore estimated to be greater than 5000mg/kg and no sign of behavioural changes were also observed.

Table 4. Acute toxicity studies of methanolic root bark extract of *Jatropha curcus*

Plant species	Group	Number of Animals	Dose (mg/kg)	Mortality recorded after 24hrs
Phase I	I	3	10	0/3
	II	3	100	0/3
	III	3	1000	0/3
Phase II	I	1	1500	0/1
	II	1	2250	0/1
	III	1	3250	0/1
	IV	1	5000	0/1

The extract significantly decreased the number of writhes caused by acetic acid in a dose independent manner as shown in Table 5. The effects observed at 25 mg/kg and 50 mg/kg were

more than that of 100 mg/kg of the extract. The effect observed in 50 mg/kg group was comparable to that of the standard.

Table 5: Effect of methanol root extract of *Jatropha curcus* on Acetic Acid Induced writhing in mice

Treatment	Dose (mg/kg)	Mean No. of Writhes ± SEM	Inhibition (%)
Distilled water	10ml/kg	20.33±0.49 ^a	-
Piroxicam	20	3.83±0.31 ^e	81.2
Extract	25	8.50±0.43 ^c	55.7
Extract	50	6.00±0.37 ^d	70.5
Extract	100	11.50±0.43 ^b	43.4
LSD	-	0.79	-

DISCUSSION

The preliminary and quantitative phytochemical screening of the root extracts of *Jatropha curcus* revealed the presence of several constituents as shown in Table 2 and 3. These constituents are

known to be responsible for several pharmacological activities. Flavonoids were reported as prostaglandin synthetase inhibitors (Watanebe *et al.*, 2000). Prostaglandins are known to be involved in the pain perception

(Helms and Barone, 2008). This suggests that reduced availability of the prostaglandins by flavonoids might have been responsible for their analgesic activity. The presence of tannins and saponins possibly might have given rise to the observed analgesic property and contributed to the anti-pyretic activity of the plant extract. Saponins possess a wide range of therapeutic actions in the body including anti-inflammatory, expectorant, diuretic, anti-malarial and haemolytic effects on red blood cells, while tannins are used in compress for cuts and wounds, haemorrhoids, varicose veins and in medicine for diarrhoea, catarrh, heavy menstrual flows and inflammatory conditions of the digestive tract (Evans, 1989). Cardiac glycosides increase the force of myocardial contraction and reduce conductivity within the atrioventricular (AV) node. They are used in the treatment of supraventricular tachycardias, especially for controlling ventricular response in persistent atrial fibrillation (Prassas and Diamandis, 2008). The oral median lethal dose value for the methanol root extract of *Jatropha curcus* obtained in rats was found to be above 5000mg/kg. This suggests that the plant extract is non-toxic as no death was recorded. Acute toxicity studies are usually carried out to determine the dose that will cause death or serious toxic manifestations when administered singly or severally at few doses in order to establish doses that should be used in subsequent studies (Wanda *et al.*, 2002). The Organization for Economic Cooperation and Development (OECD), Paris, France, recommended chemical labelling and classification of acute systemic toxicity based on oral median lethal dose values as: very toxic if ≤ 5 mg/kg, toxic if > 5 mg/kg but ≤ 50 mg/kg, harmful if > 50 mg/kg but ≤ 500 mg/kg, and non-toxic or not harmful if > 500 mg/kg or ≤ 2000 mg/kg (Walum, 1998). Based on this classification, the oral median lethal dose obtained for rats found to be above 5000 mg/kg, is relatively safe orally. However, the situation

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may not the same on repeat dose experiment in sub-chronic toxicity studies.

Acetic acid-induced writhing is among the sensitive methods for evaluating potential analgesic drugs or compounds that act peripherally. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, or turning of trunk (Mishra *et al.*, 2011). This model of response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway. The acetic acid induced writhing model has been associated with increased level of prostaglandins (PGE2 and PGF2 α) in peritoneal fluids as well as lipoxygenase products; this enhances inflammatory pain by increasing capillary permeability (Lakshman *et al.*, 2006; Khan *et al.*, 2010). The mechanism, by which any substance that inhibits these writhings causes analgesia, will be preferably by inhibition of prostaglandin synthesis, which is a peripheral mechanism of pain inhibition (Somachit and Shahid, 2003). The data presented suggests that the methanol extract of *Jatropha curcus* root possess peripheral analgesic property. The extract at all doses was shown to have analgesic property as evidenced in the model used. The reduction in the number of writhes caused by the extract, suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of prostaglandins and other endogenous substances

CONCLUSION

The methanol root extract of *Jatropha curcus* was found to possess several bioactive constituents including alkaloid, flavonoids, saponins, tannins, cardiac glycosides among others, associated with potent pharmacological activities. The extract was found to possess considerable analgesic at doses tested. This partly justifies the claim for the traditional use of the plant in the treatment of toothache.

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