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ANTIPLASMODIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF EXTRACTS FROM THE ROOTS AND STEM BARK OF *JATROPHA CURCAS*

Abubakar Sani* and Aisha Kabir

Department of Pure and industrial Chemistry Umaru Musa Yar'adua University, P.M.B,2218, Katsina, Nigeria

Abstract: The powdered roots and stem bark of *Jatropha curcas* were extracted using 95% ethanol. The ethanol extracts obtained from parts of plants were partitioned into fractions using chloroform, distilled water, ethyl acetate, methanol and *n*-hexane solvents of varying polarity. The crude ethanol extract and five soluble fractions of both the roots and stem bark were tested against *plasmodium falciparum*. The results shown a good anti-plasmodial potency at high concentration of the fraction of the roots and stem bark of *J. curcas*. Chloroform and *n*-hexane soluble fractions recorded good activity on the test organisms with 94.3% and 97.1% inhibition. The results indicated that the roots and stem bark extracts contained active compounds which have anti-malaria property. Test for the presence of secondary metabolites in the plant extracts revealed the presence of saponins, flavonoids, steroids, tannins and alkaloids.

Keywords: antimalarial, *Jatropha curcas*, phytochemistry, *plasmodium falciparum*.

Introduction

Plant materials that possess therapeutic properties or exert beneficial pharmacological effects on the human body are generally designated as

* Abubakar, Sani, *email*: abubakar.sani@umyu.edu.ng

medicinal plant.[1] Medicinal plants have been used as a means of curing or preventing diseases known as "phytotherapy" in all regions of the world with regional variations due to the influence of cultural characteristics of the populations as well as its flora, terrain, and climate.[2] Medicinal plants are considerably important and economically essential if they contain active secondary metabolites that are used in the treatment of many human diseases.[3] Current trends in drug development process are focused on natural products of plant origin due to lower cost, availability, fewer adverse effect and perceived effectiveness.[4] Many experimental researches reported the use of plants for medicinal purposes and as such plant materials has become a soft target for the search of bioactive compounds for management of human diseases.[5]

Jatropha curcas (Hausa; Bini da Zugu) is a large shrub 3-4 m high, the leaves are alternately arranged 10-15 cm x 7.5- 12.5 cm. They are broadly oval and connate in shape, usually the leaf have 3 or 5 lobes and glabrous flowers in loose panicles of the cymes. It has a yellowish green fruits about 2.5 cm long, and contains black seed. The seed resembles castor seed in shape but are smaller in size and dark brown in colour.[6] Phytochemical constituents of *J. curcas* stem bark were reported to contains amyrin **1** sitosterol **2** and taraxerol. The leaves of *J. curcas* contains cyclic triterpenes such as stigmasterol **3,** stigmasterol-5-en-3,7-diol, cholest-5-en-3,7-diol, sitosterol and campesterol **4**.[7] The roots part of *J. curcas* contains sitosterol and its d-glucoside, marmesin **5**, propacin **6**, the curculathyranes A and B and the curcusone A- D, diterpenoids jatrophol and jatropholone A and B the caumarin tomentin, the coumarin-lignan jatrophin and taraxerol.[7]

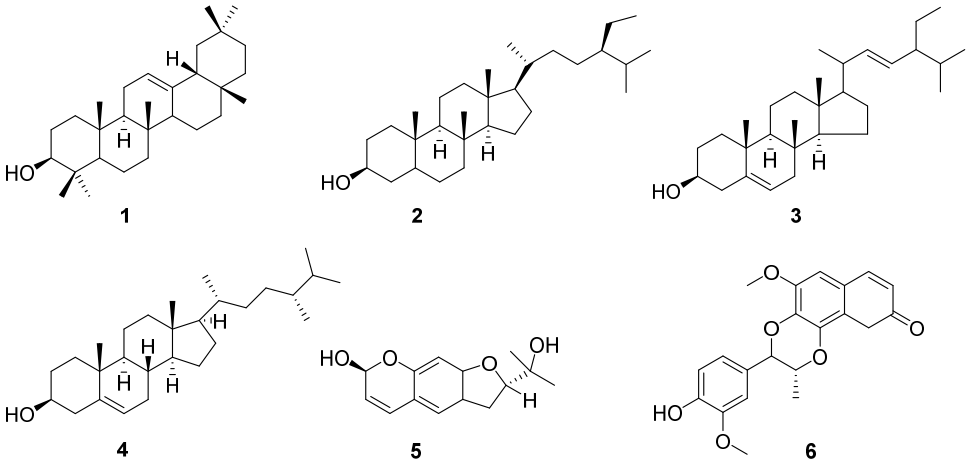


Figure 1. Some phytochemical compounds isolated from the root and stem bark part of *Jatropha curca*.

Results and Discussion

The roots and stem bark of *Jatropha curcus* were extracted using six solvents of different polarity. Physical characteristics of the extracts indicated the variation in the colour, odour and texture as depicted in Table 1. The results of phytochemical analysis (Table 2) revealed the presence of some secondary metabolites in both the roots and stem bark extracts including alkaloid, glycoside and steroids in all the fractions. Flavonoid was found presence only in the ethanol extracts of the root of *J. curcas*. Saponins were observed only in ethanol extract of the stem bark but found to be absent in the ethanol extract of the root and present in all other fractions. Tannins were present only in the ethyl acetate soluble fraction of the root. The results in Table 3 and 4 show a good anti-plasmodial potency at high concentration of the fraction of the roots and stem bark of *J. curcas*. Among the six fractions in both Table 3 and 4, the chloroform and n-hexane fractions recorded 94.3% and 97.1% elimination

of the parasites respectively. It was observed that activity of the extracts increases with increase in concentration.

Table 1. Physical characteristics of extracts obtained from the roots and stem bark of *Jatropha curcas*.

Plant parts	Fractions	Code	Weight(g)	Texture	Appearance
Roots	Ethanol	JC1-01	4.08	Gummy	Reddish brown
	Chloroform	JC1-02	2.45	Sticky	Reddish brown
	water	JC1-03	1.08	Sticky	Reddish brown
	Ethyl acetate	JC1-04	0.59	Sticky	Reddish brown
	Methanol	JC1-05	1.06	Sticky	Reddish brown
	n-hexane	JC1-06	0.95	Sticky	Reddish brown
Stem bark	Ethanol	JC2-01	7.90	Gummy	Greenish yellow
	Chloroform	JC2-02	3.42	Sticky	Greenish yellow
	water	JC2-03	2.54	Sticky	Greenish yellow
	Ethyl acetate	JC2-04	1.12	Sticky	Greenish yellow
	Methanol	JC2-05	1.83	Sticky	Greenish yellow
	n-hexane	JC2-06	1.40	Gummy	Greenish yellow

Key: JC1 = (root part); JC2 = stem bark part; JC-01= ethanol extract; JC-02 = chloroform soluble fraction; JC-02 = water soluble fraction; JC-03 = ethyl acetate soluble fraction; JC-04 = methanol soluble fraction; JC-06 = n-hexane soluble fraction.

Table 2. Phytochemical constituents detected from the roots and stem bark extracts of *Jatropha curca.*

Plant (part)	**Fraction**	**Phytochemicals Constituents**							
		Ter	**Alk**	**Fla**	**Gly**	**Phl**	**Sap**	**Ste**	**Tan**
Roots	JC1-01	+	+	+	+	+	+	-	+
	JC1-02	+	+	+	-	-	+	-	+
	JC1-03	+	+	+	-	-	+	-	+
	JC1-04	+	+	+	-	-	+	-	+
	JC1-05	+	+	+	+	-	+	-	+
	JC1-06	+	+	+	+	-	+	-	+
Stem bark	JC2-01	+	+	+	-	-	+	-	+
	JC2-02	+	-	+	+	+	+	-	+
	JC2-03	+	-	+	+	+	+	-	+
	JC2-04	+	-	+	+	+	+	+	+
	JC2-05	+	-	+	+	+	+	-	+
	JC2-06	+	-	+	+	+	+	-	+

Key: Alk = alkaloid; Fla = Flavonoids; Gly = Glycoside; Phl = Phlobat tannis; Sap = Saponins; Ste = steroids; Tan = Tannis; Ter = Terpenoids.
+ = positive, - = negative

Table 3. Anti-plasmodial activity results of the fractions from the root of *Jatropha curcus.*

Fractions	Conc. mg/mL	Average no. of parasite per field before incubation	Overall average no. of parasite after 48 hours	Total cleared RBC	Percentage of elimination at the end incubation (%)
JC1-01	500	35	08	27	77.1
	1000	35	06	29	82.9
	2000	35	04	31	88.5
	5000	35	03	32	91.4
JC1-02	500	35	11	24	68.6
	1000	35	08	27	77.1
	2000	35	03	32	91.4
	5000	35	02	33	94.3
JC1-03	500	35	10	25	71.4
	1000	35	08	27	77.1
	2000	35	06	29	82.9
	5000	35	04	31	88.6
JC1-04	500	30	16	14	46.7
	1000	30	13	17	56.7
	2000	30	12	18	60.0
	5000	30	08	22	73.3
JC1-05	500	30	12	18	60.0
	1000	30	11	19	63.3
	2000	30	08	22	73.3
	5000	30	02	28	93.3
JC1-06	500	30	19	11	36.7
	1000	30	17	13	43.3
	2000	30	14	16	53.3
	5000	30	12	18	60.0

Table 4. Anti-plasmodial activity results of all the fractions from the Stem bark of *Jatropha curcus.*

Fractions	Conc. mg/mL	Average no. of parasite per field before incubation	Overall average no. of parasite after 48 hours	Total cleared RBC	Percentage of elimination at the end incubation (%)
JC2-01	500	35	10	23	65.7
	1000	35	07	28	80.0
	2000	35	05	30	85.7
	5000	35	02	33	94.2
JC2-02	500	35	03	32	68.6
	1000	35	11	24	86.6
	2000	35	05	30	85.7
	5000	35	03	32	91.4
JC2-03	500	35	15	20	57.1
	1000	35	13	22	62.9
	2000	35	07	26	74.3
	5000	35	03	32	91.4
JC2-04	500	35	12	23	65.7
	1000	35	11	24	68.6
	2000	35	08	27	77.1
	5000	35	05	30	85.7
JC2-05	500	35	16	19	54.3
	1000	35	13	22	62.9
	2000	35	10	25	71.4
	5000	35	06	29	82.9
JC2-06	500	35	09	26	74.3
	1000	35	07	28	80.0
	2000	35	04	31	88.6
	5000	35	01	34	97.1

Experimental

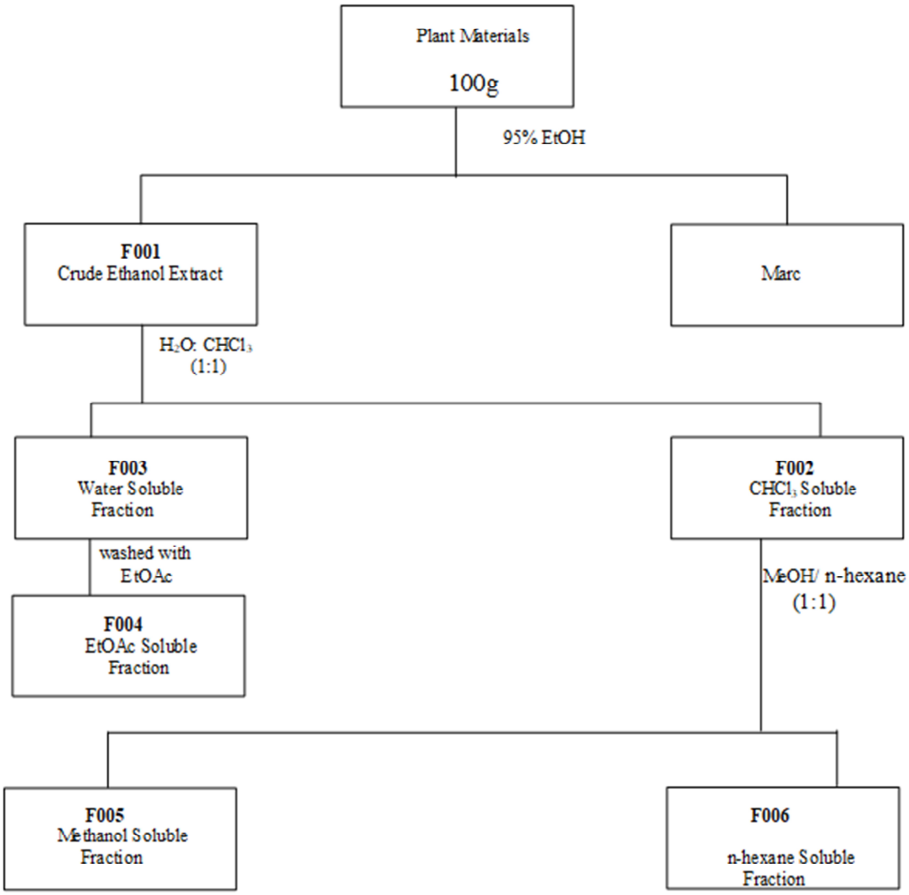
Plant Materials

The roots and stem bark of *Jatropha curcas* were collected on 27th June, 2014 from Tudun katsira in Katsina Local Government Area of Katsina State Nigeria. The plant was identified at Biology Department Umaru Musa Yar'adua University Katsina.

Extraction procedure

The air dried and grounded parts (roots and stem bark) of plant materials (100 g) were extracted by percolation with 95% ethanol (400 mL) at room temperature for two weeks. The crude ethanol extracts were concentrated under reduced pressure using rotary evaporator (R110) at 40°C and ethanol extracts obtained were weighed and labelled F001.[8] Portion of EtOH extracts F001 prepared as mentioned above were transferred into a vial and kept for antibacterial tests while residues were solvent partitioned between $CHCl_3$ and distilled H_2O solvent (200 mL, 1:1), with the aid of separating funnels. $CHCl_3$ soluble fractions, F002 and interface fractions between $CHCl_3$ and distilled H_2O solvents, F001* (where applicable), were separately concentrated to dryness on a rotary evaporator at 40°C whereas distilled H_2O soluble fractions, F003 were washed several times with EtOAc (100 mL), which yielded distilled H_2O soluble fractions, (F003), interface fractions between distilled H_2O and EtOAc solvents, F002* (where applicable), and EtOAc soluble fraction, F004. These soluble solvent fractions were separated and concentrated as $CHCl_3$ soluble fractions and the between $CHCl_3$ and distilled H_2O solvents earlier discussed. Portions of $CHCl_3$ residues obtained as mentioned earlier were transferred into vial and

kept for antibacterial tests while the remainders, were further partitioned between MeOH and n-hexane solvent (200, 1:1), to solvent fractions which were separated and concentrated to give the MeOH soluble fraction (F005) interface fractions between MeOH and n-hexane, F003* (where applicable), and n-hexane soluble fraction (F006) respectively (Scheme 1). All fractions obtained in the process were transferred into distinct vials and preserved.



Scheme 1. Schematic summary of crude fractionation process.

Phytochemical Analysis of the fractions

Phytochemical Analysis for quantitative detection of secondary metabolites were performed on the afforded fractions as was described by.[9,10]

Test for alkaloids

0.5 g of each fraction was dissolved in 5 mL of 5% ethyl ether and stands for 15 minutes. The sample was extracted for 2.0 minutes with 5 mL of aqueous HCl on a boiling water bath. The resulting mixture was centrifuge for 10 minutes at 3000 rpm. 1 mL of the filtrate was treated with few drops of Mayer's reagent and a second 1 mL with Dragendroff's reagent and turbidity was observed.[9,10,11]

Test for anthraquinones

0.5 g of each fraction was shaken with 10 mL of benzene and was filtered. 0.5 mL of 10% ammonia was added to the filtered and the mixture was shaken well and the presence of the violet colour in the upper layer phase indicates the presence of the anthraquinones.[9,10]

Test for flavonoids

A portion of each fraction was heated with 10 mL of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution when yellow colouration was observed.[12]

Test for glycosides

0.5 g of each fraction was dissolved in 5 mL of methanol. 10 mL of 50% HCl was added to 2 mL of methanol extract on a test tube. The mixture was heated in a boiling water bath for 30 minutes. 5 mL of Fehling solution

was added and the mixture was boiled for 5 minutes to observe a brick red precipitate as an indicator for the presence of glycosides.[10]

Test for phlobatannins

A portion of each fraction was boiled with 1% aqueous HCl acid to observed the deposition of red precipitate is an indication for the presence of phlobatannins.[12]

Test for saponins

0.5 g of each fraction was shaken with water in a test tube and it was warmed in a water bath and the persistent of froth indicates the presence of saponins.

Test for steroids

0.5 g of each fraction was dissolved in 5 mL of methanol. 1 mL of the extract was treated with 0.5 mL of acetic acid anhydride and was cooled in ice. This was mixed with 0.5 mL of chloroform and 1 mL of concentrated sulphuric acid was then added carefully by means of a pipette.

Test for tannins

0.5 g of each fraction was stirred with 10 mL of distilled water. This was filtered and ferric chloride reagent was added to the filtrate, a blue black precipitate was taken as evidence for the presence of tannins.[10]

Test for terpenoids

0.5 g of each fraction was dissolved in 5 mL of methanol. 2 mL of the fraction was treated with 1 mL of 2,4-dinitrophenyl hydrazine dissolved in 100 mL of 2M HCl. A yellow orange colouration was observed as an indication of terpenoids.

MALARIA PARASITE BIOASSAY

Preparation of test solution

A stock solution of 10,000 mg/mL was prepared by dissolving 20 mg of each test fractions 2 mL of Dimethyl sulphoxide (DMSO). The sample solutions of 500, 1000, 2,000, 5,000 mg/mL were prepared from the stock solution by serial dilution.

Sourcing of Malaria parasite for Assay

Parasites of infected blood samples containing a *Plasmodium falciparum* were obtained from the Department of Haematology, Umaru Musa Yar'adua University Clinic. The samples were received in K3-EDTA coated disposable plastic sample bottles with tightly fitted plastic corks, and transported to the Biology Laboratory of Umaru Musa Yar'adua University.

Separation of the Erythrocytes (5% parasitaemia) from Serum of Blood samples

50% dextrose solution (0.5 mL) was added to each blood sample (5 mL) which was defibrinated and centrifuged at 2500rpm for 15 minutes in a spectra merlin centrifugation machine.[17] Blood samples with higher parasitaemia (>5%) were diluted with fresh malaria parasite negative erythrocytes.[13]

Preparation of Plasmodium falciparum culture medium

The venus blood (2 mL) from the main vein of white healthy rabbit pinnae was collected using syringe (BD 205WG). This was defibrinated and allowed to settle for at least 45 minutes. The blood was then centrifuged. The sediments were discharged and serum collected was supplemented with RPMI 1640 salt medium (KCl 5.73 mM, NaCl 10.27 mM, $MgSO_4$ 2.56 mM,

$NaHPO_4$ 17.73 mM, $Ca(NO_3)_2$ 0.42 mM, $NaHCO_3$ 2.5 mM and Glucose 11.0 mM (BDH Ltd, UK).[14] The medium was sterilized by 50 gentamicin sulphate.[15]

In vitro Assay of the Activity of the extract on Plasmodium falciparum culture

The assay was performed using RPMI 1640 as the culture medium used for cultivation of *P. falciparum*.[14] Controls were prepared without the plant extracts. Each test solution (0.1 mL) and the culture medium (0.2 mL) were added into test tubes containing 5% parasitaemia erythrocytes and mixed thoroughly. The sensitivity of the parasites to each tested fractions at concentration of 500, 1000. 2000 and 5000 mg/mL was determined microscopically at 37°C after 24 and 48 hours of incubation. The incubation was undertaken in glass bell jar containing a lighted candle to ensure the supply of required quantity of CO_2 (about 5%) O_2 gas 2% and about 93% nitrogen gas.[16]

Determination of the activity

After 24 and 48 hours of incubation, an aliquot of the culture medium was dropped on microscopic slide and strained using Giemsa's staining techniques. The average percent elimination of the erythrocytes that appeared as blue discoid cells was determined using the formula.

$$\%A = N/N_x \times 100$$

where %A = Percentage activity of the extracts;

N = Total number of cleared Red Blood Cells (RBC);

N_x = Total number of parasitized RBC.

Conclusion

The present research work shows that, the extracts of the roots and stem bark extracts of *Jatropha curcas* have good activity against plasmodium parasite particularly the chloroform soluble fraction JC1-02 and n-hexane soluble fractions JC2-06. Thus, the results obtained in this work established the efficacy of the plant extracts used in traditional medicine for treatment of malaria fever in Hausa land.

Recommendation

We recommend further research to be carried out on the bioactivity guided isolation and characterisation of these chemical constituents with a view to supplementing conventional drugs development especially in the developing African countries.

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