

Full Length Research Paper

Antimicrobial and phytochemical activity of methanolic extract and its fractions of *Jatropha curcas* Linn. (Euphorbiaceae) stem bark

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The stem bark of *Jatropha curcas* Linn (Euphorbiaceae) widely used in ethno medicine was studied. The methanolic extract (ME) and methanolic extract fractions (MEF) namely: chloroform fraction (CF), ethyl acetate fraction (EAF) and methanol fraction (MF) of *J. curcas* were subjected to antimicrobial evaluation and phytochemical screening. Results showed that saponins and steroids were present only in the ME and CF. However, the ME and MEF were containing alkaloids, terpenoids, glycosides, resins, carbohydrates. Furthermore, on comparison with gentamicin sulphate (GS) (10 µgml⁻¹), a known antibiotic, the ME and MEF (at the tested concentrations of between 0.625 and 20 mgml⁻¹) elicited commensurate antimicrobial activity on the tested gram positive bacteria, but exhibited no activity neither on the tested gram negative bacteria, *Plasmodium aeruginosa* nor the fungus, *Candida albicans*. Altogether, the study suggests that methanolic extract and fractions of *J. curcas* stem bark may serve as source for compounds with therapeutic potentials and dietary energy. The study therefore prompts further investigations, including toxicological evaluation, to harness the possible potential use of *J. curcas* stem bark from Nsukka-Nigeria either as drug, food or pharmafood.

Key words: Phytochemicals, antimicrobial, *Jatropha curcas*, stem bark, pharmafood.

INTRODUCTION

Ever before the advent of orthodox medication, plants and plant parts in forms of squeezed, aqueous (cold, warm or hot) and illicit gin extracts have served as dependable sources of medicine in traditional African societies and beyond. Indeed, according to WHO (2002) report, traditional medicine using plant extracts will continue to provide health coverage for over 80% of the world's population, particularly the developing countries. The emergence of hitherto unknown disease causing microbes is of immense public health concern. This requires renewed strategies on treatment and prevention, essentially through the development of new antimicrobials (Iwu et al., 1999). Thus, further drug research and development from any viable source is imperative, and the investigation of indigenous plants

may yield positive results.

Jatropha species (*J. berlandieri*, *J. podagrica*, *J. nudicaulis*) are ornamental plants aptly called 'ogiri oke' in recognition of its common use as boundary mark in Ojoto and other Igbo speaking parts of South East Nigeria. Apart from that, they are used in traditional medication against various ailments in Africa, Asia and even Latin America (Burkill, 1994). Earlier reported works indicated that *Jatropha* species exhibit bioactive activities against fever and rheumatism (Oliver-Bever, 1986), parasites (Fagbenro-Beyioku, 1998), HIV induced cytopathic effects (Matsuse et al., 1999), diarrhoea (Mujumdar et al., 2001), and recently, organisms implicated in sexually transmitted diseases (Aiyelaagbe et al., 2007). However, most of the previous studies on *Jatropha* were not on curcas specie and those on curcas specie were centered on the leaf and root, but not on the stem bark, extracts. The present study therefore, attempts to determine the phytochemical contents of methanolic extract and fractions from stem bark of

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Table 1. The secondary metabolites results of the phytochemical analysis of the ME and MEF (CF, EAF and MF) of *J. curcas* stem bark.

Secondary metabolites	ME	MEF		
		CF	EAF	MF
Alkaloids	++	++	++	++
Flavonoids	-	-	-	-
Glycosides	++	++	+	++
Steroids	++	++	-	-
Saponins	+++	+	-	-
Terpenoids	+++	+	++	+++
Resins	++	++	++	++
Tannins	-	-	-	-

-: Not present; +: Present but low in abundance; ++: Present and moderate in abundance; +++: Present and high in abundance.

Jatropha curcas in relation to their antimicrobial activity on some common pathogens. The aim is to contribute to scientific knowledge on the development of potential human uses for *J. curcas* stem bark, either as drug, food or pharmafood. Therefore, the objective of the study is to ascertain the antimicrobial and phytochemical activity of methanolic extract and its fractions of *J. curcas* stem bark.

EXPERIMENTALS

Solvent and chromatographic materials

The solvents used (ethyl acetate, chloroform and methanol) are Sigma-Aldrich® grade. Distilled water and nutrient agar were used for the column chromatography. Other chemicals and standard grades of silica gel (70 to 230 mesh) and reagents used were of certified grade and quality and were used without further purification.

Test organisms

The test organisms were clinical isolates of patients attending University of Nigeria Medical centre Nsukka donated to the Department of Microbiology, University of Nigeria Nsukka, Nigeria and include *Bacillus subtilis* (*B. subtilis*), *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Plasmodium aeruginosa* (*P. aeruginosa*) and *Candida albicans* (*C. albicans*).

Collection and preparation of plant material

Fresh stem bark of *J. curcas* was collected from Obukpa in Nsukka, Enugu State in the South East of Nigeria during August, 2009. The specimen was then identified by Mr. Ozioko of Bioresources Conservation and Development Programme (BCDP) Nsukka.

A known amount, 1000 g of the resultant powdery specimen, was extracted with 4.0 L of methanol at room temperature (25±2°C) for 7 days by cold maceration method. At the end of the seventh day, the extract was filtered with Whatman filter paper. The filtrate was

concentrated with rotary evaporator and then divided into two parts. One part was preserved in a refrigerator as concentrated extract until subsequently used for the research study. The other part (10 g of the dried ME) was dissolved in chloroform and then introduced into a fractionating column for chromatographic separation (Raghavendra et al., 2008). This was achieved by successive elution with 750 ml of chloroform, 800 ml of ethyl acetate and 700 ml of methanol to obtain the respective solvent fractions. These fractions were then stored in a refrigerator until used for the phytochemical and antimicrobial screening.

Phytochemical screening

The phytochemical analysis of the methanolic extract and fractions of *J. curcas* was by standard methods as described in Evans (2000). Specifically, the extract and fractions were screened for saponins, glycosides, proteins, steroids, reducing sugars, fats and oils, alkaloids, flavonoids, tannins, terpenoids, carbohydrates, amino acid compounds and resins.

Antimicrobial activity screening

The minimum inhibitory concentrations (MIC) agar dilution assay

The minimum inhibitory concentration was determined by agar dilution method, described by Adeniyi et al. (1996) as the preliminary test indicated poor diffusion of the methanol extract in agar. Essentially, two-fold serial dilution of the extract (0.625, 1.25, 2.5, and 5 mg ml⁻¹) were prepared in sterile distilled water and poured into separate sterilized Petri dishes. The concentration was selected based on the preliminary sensitivity tests on the micro-organisms as has been used and reported by many authors (Cleudson et al., 2007; Esimone et al., 2003). 20 ml of molten nutrient agar Sabouraud dextrose agar (SDA) (for fungi) was poured into the Petri dishes, swirled slowly and then allowed to set and dry. Each set of agar plate was streaked with the broth culture of bacteria (*B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*) and a fungus (*C. albicans*).

The agar plates containing no extract (that is the negative control) and the plates containing 1.10, 2.19, 4.38, 8.75 and 17.5 µg ml⁻¹ of gentamicin sulphate (GS) (that is the positive control) were also streaked with the micro-organisms. The agar plates were incubated at 37°C for 24 h (for the bacteria) and at 25°C for 48 h (for the fungus). MIC was determined according to Harbone (1998).

RESULTS

Phytochemical screening

The preliminary phytochemical results indicated the presence of saponins and steroids in the methanol extract and the chloroform fraction only. However, the extract and the different solvent fractions from the stem bark of *J. curcas* showed the presence of alkaloids, terpenoids, glycosides and resins but neither flavonoids nor tannins. Qualitatively, the secondary metabolites appear more abundant in the methanol extract than in the different solvent fractions (Table 1). In addition, carbohydrates and reducing sugars but, no oils, proteins or amino acids were detected in the methanol extract or the different solvent fractions of *J. curcas* (Table 2).

Table 2. Preliminary nutritional results of the phytochemical analysis of the ME and MEF (CF, EAF and MF) of *J. curcas* stem bark.

Secondary metabolites	ME	MEF		
		CF	EAF	MF
Carbohydrates	+++	+	++	+++
Proteins	-	-	-	-
Oils	-	-	-	-
Amino acid compounds	-	-	-	-
Reducing sugars	+	+	+	+

-: Not present; +: Present but low in abundance; ++: Present and moderate in abundance; +++: Present and high in abundance.

Antimicrobial study

The ME and purified methanolic extract fractions (MEF) showed potent antimicrobial activity against the tested organisms at MIC of 20 $\mu\text{g L}^{-1}$ whereas the ME in particular displayed MIC of as low as 0.625 mg ml^{-1} against *B. subtilis*. However, the ME and MEF at MIC range of 0.625 to 20 mg ml^{-1} failed to elicit any inhibition on *P. aeruginosa* (a gram negative bacteria) and *C. albicans* which is a fungus (Table 3).

DISCUSSION

Generally, secondary metabolites in plants have been reported to confer plants with therapeutic activities (Rabe, 2000). In particular, alkaloids were reported as the most efficient, therapeutically significant plant substance (Njoku and Akumefula, 2007) hence, the moderate abundance of alkaloids, glycosides, terpenoids and resins detected in the tested extract and fractions of *J. curcas* stem bark, might explain the basis for its reported efficacy/use in ethnomedicinal therapy. However, the presence of saponin, an antioxidant phytochemical (Gupta, 2002) and steroid in only the methanolic extract and chloroform fraction, highlights the possibility that the therapeutic benefit(s) of *J. curcas* stem bark might be dependent on its solvent medium and mode of extraction.

Tannins and flavonoids have biological activities that are of benefit in the prevention and management of many ailments (James et al., 2007). Earlier, Erah et al. (1996) had associated antimicrobial activity with the presence of tannins and flavonoids. Therefore, the absence of tannins and flavonoids in the methanolic extract and fractions from the stem bark of *J. curcas* (an indication of the absence of these essential secondary metabolites) might limit its therapeutic potentials and uses. This probably explains the apparent narrow spectrum antimicrobial activity of *J. curcas* stem bark extracts observed in the present study.

In particular, flavonoids are known to inhibit platelets

aggregation (Formica and Regelson, 1995) and could exert a membrane stabilizing action that may protect the liver cells from injury (Perrissoud, 1986). These were linked to the possible efficient detoxification and antioxidant activities (Iweala, 2009) resulting, probably, from an enhanced induction of phase I and II enzymes by flavonoids (Ren et al., 2003). Thus, the absence of flavonoids in the screened stem bark methanolic extract and fractions of *J. curcas*, in addition, suggests its possible lack of hepatoprotective potential and therefore, may not be of benefit in any formulation intended for the management or prevention of liver diseases.

Generally, the ME and MEF exhibited varying degree of anti bacterial activities on most of the tested pathogens. The results appear to be in consonance with that of Igbinosa et al. (2009) thereby supporting their conclusion that *J. curcas* could be of benefit in the development of potent drugs against diseases caused by these pathogens. Further to this, ME followed by MF appear to be more potent than the other fractions. A similar observation had been reported by Kowalski and Kedzia (2007) and was attributed to the presence of soluble phenolic and polyphenolic compounds in ME and MF. However, both (ME and MF) failed (along with the other fractions) to inhibit *P. aeruginosa* and *C. albicans*. The observations were not entirely surprising. *P. aeruginosa* is a gram negative bacterium, hence more complex and less susceptible (Gould and Booker, 2000). As a result, it might have resisted the ME and MF of *J. curcas* stem bark, probably due to its cell permeability (James et al., 2007) or inherent point mutation. On the other hand, *C. albicans* is a fungus and the present result is a possible pointer that ME and MEF does not possess antifungal potential, further suggesting that the stem bark extract and fractions of *J. curcas* may not be useful as broad spectrum antibiotic.

The result of the present phytochemical screening of the ME and MEF of *J. curcas* stem bark revealed the presence of carbohydrates but the absence of proteins, amino acids and oils. The presence of carbohydrate in the extract and fractions of *J. curcas* seems to be in agreement with the report of the presence of carbohydrate in *Jatropha cathartica* seed (Oladele and Oshodi, 2007) indicating that, the stem bark of *J. curcas* could also be a good source of dietary energy. However, it might not be of benefit for regeneration, growth and overall biosynthesis owing to the noted absence of amino acids, proteins and oils. Some results of the present study apparently contradicted similar work by Igbinosa et al. (2009) who reported antifungal activity of *Jatropha* stem bark extracts against *C. albicans* and also the presence of tannins and flavonoids in the stem bark extracts of *J. curcas*. The discrepancies could be attributed to the difference in either or both the location (collection site) of the specimen and/or extract concentration used in the two studies. Recently, Ejikeme et al. (2010) had reported location or environment related

Table 3. Inhibition zone diameters (IZD) of *J. curcas* stem bark against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa* and *C. albicans*.

Test	Tested	IZD of ME and MEF of <i>J. curcas</i> stem bark (mm)							
		Gram	Concentrations (mg ml ⁻¹)						
Samples	Pathogens		20	10	5	2.5	1.25	0.625	10
ME	<i>B. subtilis</i>	+	16.00	14.50	14.00	11.00	10.00	9.75	18.34
CF	<i>S. aureus</i>	+	12.00	12.00	11.00	9.00	0	0	20.10
EAF	<i>B. subtilis</i>	+	11.75	10.75	9.25	0	0	0	18.34
EAF	<i>S. aureus</i>	+	9.00	0	0	0	0	0	20.10
MF	<i>E. coli</i>	-	15.75	13.75	12.08	10.00	10.00	0	21.00
ME	<i>P. aeruginosa</i>	-	0	0	0	0	0	0	18.70
CF	<i>P. aeruginosa</i>	-	0	0	0	0	0	0	18.70
EAF	<i>P. aeruginosa</i>	-	0	0	0	0	0	0	18.70
MF	<i>P. aeruginosa</i>	-	0	0	0	0	0	0	18.70
ME	<i>C. albicans</i>		0	0	0	0	0	0	NT
CF	<i>C. albicans</i>		0	0	0	0	0	0	NT
EAF	<i>C. albicans</i>		0	0	0	0	0	0	NT
MF	<i>C. albicans</i>		0	0	0	0	0	0	NT

GS- Gentamicin sulphate; + (positive); - (negative); NT (not tested).

significant variation in some physico-chemical parameters of *Azelia africana* seed and oil. However, the observed discrepancies in the two studies have been noted and the environmental effect on the presence and level of phytochemicals on the specimen studied is receiving detailed attention in our laboratory.

Taken together, the study suggests that methanolic extract and fractions of *J. curcas* stem bark, may serve as source for compounds with therapeutic potentials and also, dietary energy. However, further investigations, including toxicological evaluation, are required to harness the possible potential use of *J. curcas* stem bark either as drug, food or pharmafood.

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