Journal of Medical Sciences

ISSN 1682-4474

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Research Paper

J. Med. Sci., 8 (2): 143-147
15th February, 2008

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

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The Antimicrobial Activity of *Jatropha multifida* Extracts and Chromatographic Fractions Against Sexually Transmitted Infections

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This study was carried out to investigate the antimicrobial activity of the plant against different microorganisms especially those responsible for sexually transmitted infections which is becoming endemic in Africa. Hexane, ethyl acetate and methanol extracts of the plant and chromatographic fractions were screened against seven pathogenic organisms comprising gram positive and gram negative bacteria and fungi. The extracts and fractions displayed potent antimicrobial activity against these organisms, giving Minimum Inhibitory Concentrations (MIC) ranging from 0.75 to 12.5 μg mL^{-1} for the fractions. The results suggest that extracts of *J. multifida* could be used in treating sexually transmitted bacterial infections. Phytochemical screening of the extracts also showed the presence of steroids, alkaloids, saponins, tannins and glycosides.

Key words: *Jatropha multifida*, Euphorbiaceae, antimicrobial activity, sexually transmitted infections

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INTRODUCTION

Jatropha multifida (Linn) (Euphorbiaceae) is a multipurpose shrub commonly grown as hedge plant around homes in West Africa for aesthetics and its medicinal properties.

Jatropha species are used in traditional folklore medicine to cure various ailments in Africa (Burkill, 1994). *Jatropha multifida* is used in West Africa ethnomedicine for treating various infections. The latex is used to cure thrush on tongues of babies and for treating infected wounds and skin infections. The seed and seed oil are used as purgative. The seed oil is also used along with burnt plantain leaves in making homemade hard soap and also as a lubricant and for making candles (Burkill, 1994). Glucosides and a peptide have been reported from the latex of *J. multifida* (Kosasi *et al*., 1989a, b; Van der Berg *et al*., 1995). Furthermore, the roots of *Jatropha* species including the title plant have demonstrated antimicrobial activities (Dominguez *et al*., 1980; Aiyelaagbe *et al*., 2000; Aiyelaagbe, 2001).

An estimated 200-400 million people worldwide are infected with sexually transmitted diseases which are caused by bacteria, fungi and viruses.

The objective of this study was to further assay the extracts and fractions of *J. multifida* against bacteria and fungi-causing sexually transmitted diseases. This paper reports the results of the antimicrobial assays of the stem and root of this plant.

MATERIALS AND METHODS

Plant materials: *Jatropha multifida* (Linn) (Euphorbiaceae) was collected from different locations in Ibadan and Ekiti in south western Nigeria in June 2001 and June 2003. The plants were authenticated at the Forestry Research Institute Ibadan, where voucher specimens were deposited (Herbarium voucher number FHI 107675).

The plant samples were air-dried, cut into small pieces and ground.

Extraction of plant material: The dried and ground plant materials: leaves (1.37 kg); stemwood (2.1 kg); stembark (1.48 kg); rootwood (1.84 kg) and rootbark (1.28 kg) were extracted in the soxhlet extractor with hexane, ethyl acetate and methanol (10 L each) successively to give the respective extracts which were concentrated in vacuo.

Phytochemical screening: The extracts were tested for the presence of some secondary plant metabolites following standard procedures (Sofowora, 1984; Trease and Evans, 1989).

Microbial strains: The following microorganisms were employed in the assay: *Gardnerella vaginalis* (STI/UCH 2305), *Neisseria gonorrhoea*, (ATCC 19424), *Escherichia coli* (NCTC 9001), *Proteus mirabilis* (laboratory strain), *Pseudomonas aeruginosa* (NCTC 6750), *Staphylococcus aureus* (NCTC 6571) and *Candida albicans (*STI/UCH 0992) (Key: ATCC -American Typed Culture Collection; NCTC-National Culture Typed Center; STI-Sexually Transmitted Infection; UCH-University College Hospital, Ibadan).

The following chemotherapeutic agents were used as control: Gentamycin (10 μg mL^{-1}) and Tioconazole (5 μg mL^{-1}).

Determination of antimicrobial activity: The extracts were subjected to antimicrobial assays using the procedures described in literature (Kavanagh, 1972; Adeniyi *et al*., 1996).

The sensitivity test agar plates and Sabourand and Dextrose agar plates were each seeded with 0.2 mL of a 1:100 dilution of an overnight culture of some bacteria and the fungi, respectively. Mueller-Hinton agar was used for testing the *Neisseria gonorrhoea* and *Gardnerella vaginalis*. The seeded plates were allowed to dry in the incubator at 37°C for 20 min. A standard cork borer of 8 mm diameter was used to make equidistant and uniform wells on the surface of the agar and into different wells were placed 60 μL of the different extracts/fractions re-suspended in 20% DMSO at a final concentration of 20 mg mL^{-1}. The plates were incubated at 37°C for 24 h and at room temperature for 72 h for bacteria and fungi, respectively after which diameter of zones of inhibition were measured. Since each of the extracts were reconstituted in 20% DMSO before being tested, 20% DMSO was included in each plate as a solvent control besides the chemotherapeutic agents included as positive controls. The antimicrobial studies were done in triplicates and diameters of zones of inhibition (mm) are expressed as Means and Standard errors on Means.

Determination of Minimum Inhibitory Concentration (MIC): The MIC of the crude extracts and fractions were determined using broth dilution method in 96 well microtiter plates. The highest concentration of extracts and fractions tested were 20 and 10 mg mL^{-1}, respectively, dissolved in 10% of dimethyl sulphoxide (DMSO) and serial 2-fold dilutions were made with water as diluent.

Ten microlitres of each test dilution (in Mueller-Hinton broth and Tryptic soya broth for bacteria and fungi, respectively) was added to wells of a 96-well plate and each well was inoculated with 190 μL of a logarithmic

phase test culture diluted to 0.5 Macfarland standard which correspond to 10^7 cfu (when plated on Mueller Hinton agar in petri dish) of a logarithmic phase test culture. The petri dish (for control viable count) and the 96-well plates (for monitoring test) were incubated at 37°C for 24 h after which the lowest concentration that showed no visible growth was recorded as the Minimum Inhibitory Concentration (MIC) for each organism (Adeniyi *et al*., 2000).

Fractionation of the extracts: The root extracts were subjected to Vacuum Liquid Chromatography (VLC) to fractionate them following standard procedures (Coll and Bowden, 1986; Pelletier *et al*., 1986). Five gram of each extract was dissolved in chloroform and preadsorbed on TLC-grade silica gel (Merck Kieselgel 60G). A solvent gradient of hexane, ethyl acetate, chloroform and methanol was used. The concentrated fractions were subjected to TLC and similar fractions were pooled together.

The fractions were also subjected to antimicrobial assay.

RESULTS AND DISCUSSION

Phytochemical screening of the extracts revealed the presence of different secondary metabolites, including steroids, saponins and alkaloids (Table 1). All the extracts are rich in steroids. The ethyl acetate and methanol extracts are richer in the metabolites than the hexane extracts, which may be due to the relatively higher polarity of these solvents, hence their ability to extract more components. However, it is noteworthy that the methanol extracts of the stem wood and bark (JMSWM, JMSBM) and the ethyl acetate of the stem wood (JMSWE) showed potent broad-spectrum activity against all the microorganisms. Some of the extracts were even more active than the control drugs, gentamycin and tioconazole. For example, the stem wood and bark ethyl acetate and methanol extracts were more active against *Proteus mirabilis* than gentamycin. It is also significant that five of the extracts (Table 2) were active against *P. aeruginosa* used, which was resistant to gentamycin. The antimicrobial activity of these extracts may account for the many uses of the plant in ethnomedicine.

The MIC results further confirmed the potency of these extracts against the microorganisms. The MIC showed that the hexane extract of the root bark (JMRBH) is the most potent against *Neisseria gonorrhoea* with an MIC value of 0.78 μg mL^{-1}. The JMRWH, JMRWE and JMRBH were also the most active against *Staphylococcus aureus* with an MIC value of 0.78 μg mL^{-1}. The most active extracts against *Candida albicans* with an MIC value of 3.12 μg mL^{-1} were the JMRWH, JMRWE and JMRBM. The stem bark ethyl acetate and methanol extracts (JMSBE, JMSBM) also showed high potency against *Proteus mirabilis* with MIC value of 1.56 μg mL^{-1} (Table 3).

The pooled fractions from the VLC of the root extracts also showed potent broad-spectrum antimicrobial activity against the microorganisms (Table 4) and were more active than the crude extracts. The observed activities were comparable to that of gentamycin and even higher with respect to *E. coli* and *S. aureus*. The MIC of these fractions is also very significant and confirmed the potency of these fractions (Table 5).

J. multifida latex is used for treating thrush on tongues of babies, infected wounds and skin infections

Table 1: Yield and result of phytochemical screening of *J. multifida* extracts

		Secondary metabolites				
Extracts[a]	Percentage yield (w/w)	Steroids	Alkaloids	Saponins	Tannins	Glycosides
JMSWH	0.71	+	-	-	+	+
JMSWE	3.53	+	+	-	+	+
JMSWM	2.35	+	+	-	-	-
JMSBH	2.53	+	-	-	-	-
JMSBE	2.13	+	+	+	+	+
JMSBM	5.87	+	+	+	+	+
JMRWH	0.38	+	+	-	-	+
JMRWE	0.60	+	+	+	+	+
JMRWM	0.87	+	+	+	+	+
JMRBH	0.70	+	+	-	-	+
JMRBE	0.70	+	+	-	-	+
JMRBM	0.90	+	+	+	+	+

Extracts[a], JMSWH = Hexane extract of *J. multifida* stem wood, JMSWE = Ethyl acetate extract of *J. multifida* stem wood, JMSWM = Methanol extract of *J. multifida* stem wood, JMSBH = Hexane extract of *J. multifida* stem bark, JMSBE = Ethyl acetate extract of *J. multifida* stem bark, JMSBM = Methanol extract of *J. multifida* stem bark, JMRWH = Hexane extract of *J. multifida* root wood, JMRWE = Ethyl acetate extract of *J. multifida* root wood, JMRWM = Methanol extract of *J. multifida* root wood, JMRBH = Hexane extract of *J. multifida* root bark, JMRBE = Ethyl acetate extract of *J. multifida* root bark, JMRBM = Methanol extract of *J. multifida* root bark, + = Positive, - = Negative

Table 2: *In vitro* antimicrobial activity of the extracts of *J. multifida* against STD organisms

	Microorganisms[b]/Zone of inhibition[c] (mm)						
Extracts[a]	GV	NG	EC	PM	PA	SA	CA
JMSWH	13±0.4	12±0.3	-	-	-	-	-
JMSWE	14±0.2	12±0.4	11±0.3	17±0.4	13±0.3	15±0.3	13±0.3
JMSWM	17±0.3	13±0.4	12±0.3	18±0.3	14±0.4	16±0.4	14±0.2
JMSBH	14±0.3	12±0.3	-	-	-	15±0.4	12±0.4
JMSBE	15±0.4	-	14±0.4	19±0.4	12±0.3	15±0.3	12±0.2
JMSBM	15±0.4	12±0.4	11±0.3	18±0.3	14±0.3	14±0.4	11±0.3
JMRWH	17±0.5	NT	20±0.5	NT	14±0.5	20±0.4	18±0.5
JMRWE	15±0.4	NT	15±0.5	NT	14±0.4	20±0.4	18±0.4
JMRWM	15±0.3	NT	15±0.4	NT	-	20±0.5	-
JMRBH	12±0.5	20±0.4	15±0.5	-	-	20±0.4	13±0.4
JMRBE	-	20±0.3	20±0.3	-	-	-	17±0.5
JMRBM	16±0.4	18±0.3	15±0.3	11±0.3	-	-	18±0.3
GENT	24±0.5	22±0.5	14±0.3	16±0.3	R	18±0.3	NT
TIOC	NT	NT	NT	NT	NT	NT	19±0.3
DMSO	-	-	-	-	-	-	-

Extracts[a], JMSWH = Hexane extract of *J. multifida* stem wood, JMSWE = Ethyl acetate extract of *J. multifida* stem wood, JMSWM = Methanol extract of *J. multifida* stem wood, JMSBH = Hexane extract of *J. multifida* stem bark, JMSBE = Ethyl acetate extract of *J. multifida* stem bark, JMSBM = Methanol extract of *J. multifida* stem bark, JMRWH = Hexane extract of *J. multifida* root wood, JMRWE = Ethyl acetate extract of *J. multifida* root wood, JMRWM = Methanol extract of *J. multifida* root wood, JMRBH = Hexane extract of *J. multifida* root bark, JMRBE = Ethyl acetate extract of *J. multifida* root bark, JMRBM = Methanol extract of *J. multifida* root bark, Concentration of Extracts = 20 mg mL^{-1}, GENT = Gentamycin (10 μg mL^{-1}), TIOC = Tioconazole (5 μg mL^{-1}), [b]Microorganisms: GV-*Gardnerella vaginalis* (STI/UCH 2305), NG - *Neisseria gonorrhoea* (ATCC 19424), EC = *Escherichia coli* (NCTC 9001), PM = *Proteus mirabilis* (laboratory strain), PA = *Pseudomonas aeruginosa* (NCTC 6750), SA = *Staphylococcus aureus* (NCTC 6571), CA = *Candida albicans* (STI/UCH 0992), [c]Zone of Inhibition: Zone of inhibition of triplicate result expressed as means and standard error on means size of well = 8 mm, - = No zone of inhibition, NT = Not Tested, R = Resistant

Table 3: Minimum Inhibitory Concentration (MIC) of *Jatropha multifida* Extracts

	Microorganisms[b]/MIC[c] (μg mL^{-1})						
Extracts[a]	GV	NG	EC	PM	PA	SA	CA
JMSWH	25.00	100.00	-	-	-	-	-
JMSWE	25.00	100.00	12.5	6.25	50.0	12.5	25.0
JMSWM	3.12	25.00	25.0	3.12	12.5	6.25	12.5
JMSBH	1.56	50.00	-	-	-	6.25	50.0
JMSBE	6.25	-	12.5	1.56	50.0	12.5	50.0
JMSBM	6.25	100.00	100	1.56	12.5	12.5	50.0
JMRWH	6.25	NT	1.56	NT	12.5	1.56	3.12
JMRWE	12.50	NT	6.25	NT	12.5	0.78	3.12
JMRWM	6.25	NT	6.25	NT	>200	0.78	>200
JMRBH	100.00	0.78	100	>200	>200	0.78	50.0
JMRBE	50.00	1.56	1.56	-	-	-	6.25
JMRBM	12.50	3.12	25.0	50	-	-	3.12

Extracts[a], JMSWH = Hexane extract of *J. multifida* stem wood, JMSWE = Ethyl acetate extract of *J. multifida* stem wood, JMSWM = Methanol extract of *J. multifida* stem wood, JMSBH = Hexane extract of *J. multifida* stem bark, JMSBE = Ethyl acetate extract of *J. multifida* stem bark, JMSBM = Methanol extract of *J. multifida* stem bark, JMRWH = Hexane extract of *J. multifida* root wood, JMRWE = Ethyl acetate extract of *J. multifida* root wood, JMRWM = Methanol extract of *J. multifida* root wood, JMRBH = Hexane extract of *J. multifida* root bark, JMRBE = Ethyl acetate extract of *J. multifida* root bark, JMRBM = Methanol extract of *J. multifida* root bark, Concentration of extracts = 20 mg mL^{-1}, GENT = Gentamycin (10 μg mL^{-1}), TIOC = Tioconazole (5 μg mL^{-1}), [b]Microorganisms: GV = *Gardnerella vaginalis* (STI/UCH 2305), NG = *Neisseria gonorrhoea* (ATCC 19424), EC = *Escherichia coli* (NCTC 9001), PM = *Proteus mirabilis* (laboratory strain), PA = *Pseudomonas aeruginosa* (NCTC 6750), SA = *Staphylococcus aureus* (NCTC 6571), CA = *Candida albicans* (STI/UCH 0992), [c]MIC-Minimum Inhibitory Concentration (μg mL^{-1})

Table 4: *In vitro* antimicrobial activity of VLC fractions of *Jatropha multifida*

	Microorganisms[b]/Zone of inhibition[c] (mm)					
Fractions[a]	GV	NG	EC	PA	SA	CA
JMRWM1	14±0.2	-	20±0.2	16±0.4	16±0.4	-
JMRWM2	-	NT	-	16±0.4	14±0.2	-
JMRWM3	-	-	-	-	-	-
JMRWM4	-	-	11±0.4	15±0.3	-	-
JMRBH1	20±0.4	18±0.4	26±0.3	-	20±0.3	12±0.4
JMRBH2	25±0.3	25±0.3	24±0.3	14±0.2	20±0.2	-
JMRBE1	25±0.3	18±0.3	25±0.3	12±0.4	22±0.3	12±0.3
JMRBE2	20±0.4	20±0.4	24±0.2	15±0.3	23±0.4	-
JMRBM1	20±0.4	19±0.4	20±0.4	14±0.2	20±0.4	-
JMRBM2	17±0.4	13±0.2	15±0.3	-	16±0.4	-
DMSO	-	-	-	-	-	-
GENT	25±04	25±0.2	15±0.3	R	18±0.4	NT
TIOC	NT	NT	NT	NT	NT	20±0.4

[a]Fractions: JMRWM (1-4) = Pooled VLC fractions of Methanol extract of *J. multifida* root wood, JMRBH (1-2) = Pooled VLC fractions of Hexane extract of *J. multifida* root bark, JMRBE (1-2) = Pooled VLC fractions of Ethyl acetate extract of *J. multifida* root bark, JMRBM (1-2) = Pooled VLC fractions of Methanol extract of *J. multifida* root bark, Concentration of Fractions = 20 mg mL^{-1} GENT = Gentamycin (10 μg mL^{-1}), TIOC = Tioconazole (5 μg mL^{-1}) [b]Microorganisms: GV = *Gardnerella vaginalis* (STI/UCH 2305), NG = *Neisseria gonorrhoea* (ATCC 19424), EC = *Escherichia coli* (NCTC 9001), PA = *Pseudomonas aeruginosa* (NCTC 6750), SA = *Staphylococcus aureus* (NCTC 6571), CA = *Candida albicans* (STI/UCH 0992), [c]Zone of Inhibition: Zone of inhibition of triplicate result expressed as means and standard error on means, Size of well = 8 mm, - = No zone of inhibition, NT = Not Tested, R = Resistant

(Burkill, 1994), but there was no report on the potency of the other parts in the literature. Hence, these results indicate that the stem and roots of this plant could be exploited medicinally to treat sexually transmitted diseases and other infections transmitted by bacteria and fungi. These results support the folkloric uses of this plant and suggest that the plant could be exploited for more potent antibiotics in the future. This is the first report of antimicrobial activity of the extracts and fractions of *Jatropha multifida* against bacteria and fungi STD microorganisms.

Table 5: Minimum inhibitory concentration of VLC fractions of *Jatropha multifida*

	Microorganisms[b]/MIC[c] (μg mL^{-1})					
Fractions[a]	GV	NG	EC	PA	SA	CA
JMRWM1	6.25	>12.50	1.56	6.25	6.25	>12.5
JMRWM2	>12.50	>12.50	>12.50	6.25	>12.50	>12.5
JMRWM3	>12.50	>12.50	>12.50	>12.50	>12.50	>12.5
JMRWM4	>12.50	>12.50	>12.50	6.25	>12.50	>12.5
JMRBH1	0.75	1.56	1.56	>12.50	1.56	>12.5
JMRBH2	0.75	0.75	0.75	6.25	0.75	>12.5
JMRBE1	0.75	1.56	0.75	>12.50	0.75	>12.5
JMRBE2	0.75	1.56	0.75	6.25	0.75	>12.5
JMRBM1	1.56	3.12	1.56	6.25	3.12	>12.5
JMRBM2	3.12	>12.50	6.25	>12.50	3.12	>12.5

[a]Fractions: JMRWM (1-4) = Pooled VLC fractions of Methanol extract of *J. multifida* root wood, JMRBH (1-2) = Pooled VLC fractions of Hexane extract of *J. multifida* root bark, JMRBE (1-2) = Pooled VLC fractions of Ethyl acetate extract of *J. multifida* root bark, JMRBM (1-2) = Pooled VLC fractions of Methanol extract of *J. multifida* root bark, Concentration of Fractions = 20 mg mL^{-1} GENT = Gentamycin (10 μg mL^{-1}), TIOC = Tioconazole (5 μg mL^{-1}) [b]Microorganisms: GV = *Gardnerella vaginalis* (STI/UCH 2305), NG = *Neisseria gonorrhoea* (ATCC 19424), EC = *Escherichia coli* (NCTC 9001), PA = *Pseudomonas aeruginosa* (NCTC 6750), SA = *Staphylococcus aureus* (NCTC 6571), CA = *Candida albicans* (STI/UCH 0992), [c]MIC = Minimum Inhibitory Concentration (μg mL^{-1})

CONCLUSION

The study has confirmed the presence of many secondary metabolites in this plant. It has further showed that these plant extracts could be exploited for the treatment of sexually transmitted infections. The results support the folkloric use of this plant in treating microbial infections and shows that *J. multifida* could be investigated further for new potent antibiotics especially for sexually transmitted diseases.

ACKNOWLEDGMENTS

This research was supported by the Committee on Scientific and Technological Cooperation of the Organization of Islamic Conference (COMSTECH/OIC), Islamabad, Pakistan and the International Foundation for Science (IFS), Stockholm, Sweden, through a grant to Dr. Olapeju O. Aiyelaagbe. The financial support of MacArthur Foundation through a grant to the University of Ibadan for Staff Development (given to Dr. Olapeju O. Aiyelaagbe) also facilitated the write-up of this study.

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