

Effects of *Jatropha curcas* leaves on common Dermatophytes and causative agent of Pityriasis versicolor in Rivers State, Nigeria

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ABSTRACT: The antifungal activity of *Jatropha curcas* leaves used by traditional medicine practitioners against the three major Dermatophytes – *Trichophyton*, *Microsporum*, *Epidermophyton* together with *Malassezia furfur* [the causative agent of *Pityriasis versicolor* (Eczema)], was studied by well-in-agar diffusion technique using different concentrations of ethanolic extracts. Isolates from the scalp, skin, toes and feet of forty individuals (mainly children) were obtained in four locations namely Aluu, Choba, Rumuosi and Emohua areas of Rivers State, Nigeria. The study revealed a significant inhibitory effect of *Jatropha curcas* leaves on the fungal isolates at five different concentrations of 250mg/ml, 200mg/ml, 150mg/ml, 100mg/ml and 50mg/ml used. Assessment of the various minimum inhibitory concentrations (MIC) showed that *Jatropha curcas* leaves have the most potential for use as an antidermatophytic agent. The minimum inhibitory concentration (MIC) of the ethanol extract was between 19.95 and 79.43 mg/ml. The zones of inhibition exhibited by the extracts against the test fungal species ranged between 0 and 20mm. The ability of the crude stem extracts of *J. curcas* to inhibit the growth of fungi is an indication of its broad spectrum antifungal potential which may be employed in the management of fungal infections. The implications of these findings in the use of *Jatropha curcas* in traditional medicine are discussed.

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1. INTRODUCTION

Plants are one of the most important sources of medicine. Plant derived compounds (phytochemicals) have been attracting much interest as natural alternatives to synthetic compounds (Kalimuthu et al., 2010). Extracts of plants were used for the treatment of various diseases and this forms the basis for all traditional systems of medicine (Kalimuthu et al., 2010).

The treatment and control of diseases by the use of available medicinal plants in a locality will continue to play significant roles in medical health care implementation in the developing countries (Ekundayo et al., 2011). Various antifungal agents are currently available for the treatment and control of fungal infections and diseases. The use of these medicines as therapeutic agents however is limited. This is due to various challenges such as drug solubility, stability, adsorption and toxicity (Cedric, 2004). In addition, some of these drugs are expensive and generally unavailable to citizens of developing countries, especially those residing in the rural areas (Sule et al., 2011). The shortfalls in the use of chemotherapeutic agents as control agents in fungal diseases, further encourages the use of plants as a form of alternative medicine.

Jatropha species belong to the family Euphorbiaceae and are used in traditional folklore medicine to cure various ailments in Africa, Asia and Latin America (Burkill, 1994; Igbinosa et al., 2009). *Jatropha curcas* Linn is commonly called physic nut, purging nut or pig nut. *Jatropha curcas* Linn is commonly grown as hedges and fences around gardens and households in Northern Nigeria (Oyi et al., 2007). It has been documented to have medicinal uses for human and veterinary purposes (Irvine, 1961). Previous studies have reported that the plant exhibits bioactive activities for fever, mouth infections, jaundice, guinea worm sores and joint heumatism (Irvine, 1961; Oliver-Bever, 1986; Igbinosa et al., 2009). Fagbenro-Beyioku (1998) investigated and reported the anti-parasitic activity of the sap and crushed leaves of *J. curcas*. The water extract of the branches also strongly inhibited HIV induced cytopathic effects with low cytotoxicity (Matsuse et al., 1999; Igbinosa et al., 2009).

The latex combined with the powdered leaves is applied to sluggish wounds while when formulated as enema it is used for the treatment of gonorrhoea (Irvine, 1961). It is also used as an antiseptic against cuts and wounds (Oyi et al., 2007). The healing effect of curcain a proteolytic enzyme from the latex on wound has been demonstrated (Nath and Dutta,

1992). *Jatropha curcas* latex has also been reported to have strong antimicrobial activities (Oyi *et al.* 2002, 2007). Previous works have shown that many *Jatropha* species possess antimicrobial activity (Aiyelaagbe *et al.*, 2000; Aiyelaagbe, 2001; Igbinsola *et al.*, 2009).

Several studies have confirmed the antimicrobial efficacy of different *Jatropha* species; however, there is insufficient information regarding the antimicrobial activities of *J. curcas* Linn. Whatever limited information available on the medicinal properties of *J. curcas* is mostly on the leaf extracts of the plant. The use of antimicrobial agents for the control of pathogenic bacteria is helpful in the treatment of infections and diseases (Ekundayo *et al.*, 2011). Hence, there is need to investigate the antimicrobial properties of plant extracts that have not been done. In this study, the antifungal property of ethanol extracts of the *J. curcas* leaves has been studied as part of the exploration for new and novel bioactive compounds, thus contributing to the list of plants used in the treatment of infections.

2. MATERIALS AND METHOD

2.1. Plant Collection and Identification

Fresh leaves of *Jatropha curcas* were collected from Oredo Local Government Area, Benin City, Edo State, Nigeria. They were identified at the herbarium of the University of Port Harcourt, Rivers State, Nigeria.

2.2. Preparation and Extraction of crude extracts

The leaves of *Jatropha curcas* were separated manually. The materials were cleaned with sterile distilled water, air dried and finely ground using a grinder mill. Twenty grams of the fine powder from *Jatropha curcas* leaves were placed in 250 ml of solvent (95% ethanol), placed in a conical flask and refluxed at 50°C for 60 min (Chen *et al.*, 2007). The extracts were filtered through Whatman filter paper No. 1 and ethanol extracts were evaporated to dryness using a hot air oven at a much reduced temperature (40°C). The residues obtained were dissolved in 1% DMSO (for antimicrobial assay). The weight of the extract was determined and stored below ambient temperature.

2.3. Specimen Collection

The specimens were collected from different parts of the body of various individuals of different age groups, mainly children. Forty (40) individuals were sampled. This procedure was carried out using forty (40) new surgical blades for each individual. Specimen were collected by scraping affected spots into clean sheets of paper which were then transferred into sterile containers that had been properly labelled with respect to each individual's data, these were brought to the laboratory for inoculation.

2.4. Clinical Appearance of Specimen

The lesions on the body of the individuals had various appearances. These ranged from the formation of dense to flat mass of skin which could be black or reddish in colour. Others were a mixture of black and red lesions. The shapes of the lesions were also variable. Some were circinate, these were also dry, irregular, and scaly with thin marginated epidermis, not healing at the centre. Some other lesions were diffused having broken hairs which were grey to white in appearance.

2.5. Specimen Inoculation

The Sabouraud Dextrose Agar (SDA) was prepared and poured into forty (40) sterile Petri dishes and allowed to solidify. The media was then inoculated with each of the specimen after which the culture was incubated at room temperature for growth to occur. Subculturing into fresh SDA agar was carried out after about four days of incubation. The plates were then incubated at room temperature for about four days. This was followed by macroscopic and microscopic examination.

2.6. Evaluation of Antifungal Activity

The ethanolic extract of the *Jatropha curcas* leaves was applied on the fungal isolates *Epidermophyton*, *Trichophyton*, *Malassezia furfur*, *Microsporium* using agar diffusion as described by Igbinsola *et al.* (2009) in order to determine their antifungal activity on these isolates. The fungal isolates were allowed to grow on a Sabouraud dextrose agar (SDA) (Oxoid) at 25°C until they sporulated. The fungal spores were harvested after sporulation by pouring a mixture of sterile glycerol and distilled water to the surface of the plate and later scraped the spores with a sterile glass rod. The harvested fungal spores and bacterial isolates were standardized to an OD_{600nm} of 0.1 before use. One hundred microliter of the standardized fungal spore suspension was evenly spread on the SDA (Oxoid) using a glass spreader. Wells were then bored into the agar media using a sterile 6 mm cork borer and the wells filled with the solution of the extract taking care not to allow spillage of the solution to the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 h to allow for proper diffusion of the extract into the media. Plates were incubated at 25°C for 96 h and later observed for zones of inhibition. The effect of the extract on fungal isolates was not compared with amphotericin B and miconazole at a concentration of 1 mg/ml.

2.7. Minimum inhibitory concentration (MIC)

The MIC estimation of the crude extract was determined using the methods of Sahm and Washington (1999), Akinpelu and Kolawole (2004) and Igbinsola *et al.* (2009). Two-fold dilutions of the crude extract was prepared and 2 ml aliquots of different concentrations of the solution were added to

18 ml of pre-sterilized molten SDA for fungi at 40°C to give final concentration regimes of 0.050 and 10 mg/ml. The medium was then poured into sterile Petri dishes and allowed to set. The surface of the medium was allowed to dry under laminar flow before streaking with 18 h old fungal cultures. The plates were later incubated at 25°C for up to 72 h for fungi, after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented the growth of the test fungus.

3. RESULTS

3.1. Evaluation of the Antifungal Activity of the Different Plant Extracts

At the end of the incubation, the plates (Petri dishes) were collected and the zones of inhibition that developed were measured. The average of the zones of inhibition for each extract was calculated. Results are shown in Table 1. The zones of inhibition exhibited by the extracts against the test fungal species ranged between 0 and 20mm. In general, zones of inhibition decreased with decrease in the concentration of the extract (Table 1).

Table 1: Antimicrobial Activity of Ethanolic Extracts of *Jatropha curcas* on the Fungal Isolates

Concentration of Extract (mg/ml)	Log of Conc.	Zone of Inhibition (mm)			
		<i>Microsporum</i>	<i>Trichophyton</i>	<i>Epidermophyton</i>	<i>Malassezia furfur</i>
250	2.40	19.70	16.11	18.00	20.00
200	2.30	18.50	15.40	16.80	18.80
150	2.20	16.80	14.10	16.80	15.20
100	2.00	15.50	13.00	13.20	0.0
50	1.75	13.10	12.30	10.40	0.0

3.2. Minimum Inhibitory Concentrations of the Ethanolic Extracts of *Jatropha curcas* (mg/ml)

Table 2 shows the minimum inhibitory concentrations of the ethanolic extracts of *Jatropha curcas* (mg/ml). The highest MIC value of 79.43mg/ml was obtained for *Jatropha curcas* against *Microsporum* and least MIC value of 19.95mg/ml was obtained against *Epidermophyton* (Table 2).

Table 2: Minimum Inhibitory Concentrations of the Ethanolic Extracts of *Jatropha curcas* (mg/ml)

Test isolates	Minimum Inhibitory Concentrations (mg/ml)
<i>Epidermophyton</i>	19.95
<i>Trichophyton</i>	30.20
<i>Microsporum</i>	79.43
<i>Malassezia furfur</i>	29.50

DISCUSSION

The intractable problems of antimicrobial resistance has led to the resurgence of interest in herbal products as sources of noble compound to suppress or possibly eradicate the ever increasing problems of emergence of newer diseases though to be brought under control (Wurochekker et al., 2008; Ekundayo et al., 2011). *Jatropha curcas* L. (physic nut, purging nut) is a medicinal plant used for the treatment of various diseases such as malarial fever, arthritis, gout, and jaundice (Sarin et al., 2010). In some regions of Africa, physic nut leaves are used as a purgative (Ajiwe et al., 1996; Shweta et al., 2005; Sarin et al., 2010). Traditionally, different parts of *J. curcas* have been used in treatment of different forms of infection (Namuli et al., 2011).

The aim of this present study was to investigate the antifungal activity of *Jatropha curcas* leaves used by traditional healers in the treatment of *Pityriasis versicolor* and other dermatophytoses. The antifungal activity of the plant extracts were tested on selected clinical fungal isolates. The plant extracts were obtained through ethanolic extraction. Ethanol is believed to be a suitable solvent for the extraction process (Obi et al., 2000). Pyrogallol has also been reported to be an effective antimicrobial agent and its toxicity is attributed to the three hydroxyl groups present in its structure (Cowan, 1999; Kocacaliskan et al., 2006; Namuli et al., 2011). Acetic acid is also a well known antimicrobial agent used in food industry (Namuli et al., 2011). Haesebrouck et al. (2009) observed that acetic acid solution (0.5%) exerted bactericidal effect against *Staphylococcus pseudintermedius*. Similarly, root wood extract which contained high percentage of acetic acid showed strong antibacterial activity against *K. pneumonia* K36 (Namuli et al., 2011). However, Huang et al. (2010) observed that acetic acid exhibited no antimicrobial activity against various oral microorganisms. Generally in a study by Sarin et al. (2010), the methanol extract of *J. curcas* had the highest activity against both bacterial and fungal isolates, followed by the ethanol extract and the least was observed in the water extract.

The extract from *Jatropha curcas* also inhibited the growth of the fungal isolates at various concentrations. Consequently, the minimum inhibitory concentrations (MIC) of the ethanolic extracts of *Jatropha curcas* in relation to the fungal isolates were determined. The highest MIC value of 79.43mg/ml was obtained for *Jatropha curcas* against *Microsporum*. The fungal activities of the plant extracts were not tested against the antifungal activity of any known antifungal drug. However, phytochemicals present in the leaves extracts may have been high as shown by the observed high

antifungal activity, probably due to the fact that different phytochemicals exert their effects differently (Namuli et al., 2011). In concentrations where no antifungal activity were not observed, potent antifungal flavonoids may not diffuse through the paper disc due to their low rate of diffusion (Cushnie and Lamb, 2005; Namuli et al., 2011).

The observation made on the minimum inhibitory concentration (MIC) of the extract seemed to correlate with the report that organisms varied widely in the degree of their susceptibility (Emeruwa, 1982; El-Feraly et al., 1983; and Willey et al., 2008; Arekemase et al., 2011). In a study by Ekundayo et al. (2011), the ethanolic extracts of *Jatropha curcas* inhibited pathogenic bacteria with the minimum inhibitory concentration (MIC) ranging from 2.2 to 10.0 mm. The antibacterial activity of *Jatropha curcas* was also found to be effective at MIC value of 10mg/ml in a study by Sangeetha et al. (2009). Also in a study by Sarin et al. (2010), the extracts of *Jatropha curcas* showed broad antimycotic activity against the tested fungal isolates at a final concentration of 10 mg/ml and the performance of the extracts were similar to the antibacterial activity. Sarin et al. (2010) also reported that crude extracts of *J. curcas* seeds inhibited *E. coli* at 30 mg/ml and 50 mg/ml; *P. aeruginosa* at 50 mg/ml, *S. aureus*, *B. cereus*, and *B. megaterium* at different concentrations except for *Salmonella typhi* which was not inhibited by any of the crude extracts. The ability of the ethanolic extracts of the leaf and bark of *J. curcas* to inhibit growth of the test organisms is an indication of its antimicrobial potency which may be employed in treatment of microbial infections (Ekundayo et al., 2011).

The inhibitory effects of the ethanolic extracts of leaves of *J. curcas* can introduce the plant as a potential candidate for the treatment of ailments caused by these pathogens. The inhibitory activity of plant extract is largely dependent on the concentration, parts of the plant used and the microbes tested (Kalimuthu et al., 2010; Ekundayo et al., 2011). Researchers have extensively studied the biological properties of *Jatropha curcas* and their results showed that this plant is ethno-medically valuable (Singh et al., 2012). The Methanolic extracts of the leaves, roots and the stem have already been shown as to possess the antimicrobial activity; both antibacterial as well as antifungal properties (Thomas, 1989; Singh et al., 2012). Igbiosa et al. (2009) reported that the ethanolic extract of the stem bark of *J. curcas* inhibited *B. subtilis*, *E. coli*, *P. vulgaris* which is in agreement with this present study. Kalimuthu et al. (2010) also reported the inhibitory ability of the methanolic extract of *in vivo* leaves and *in vitro* derived callus (30 days old) of *J.*

curcas against *Pseudomonas*, *E. coli*, *Klebsiella* and *S. aureus*. In a study by Oyi et al. (2007), the latex of *Jatropha curcas* also showed a very good activity against *Candida albicans* and *Trichophyton* sp.

In this study, the zones of inhibition exhibited by the extracts against the test fungal species ranged between 0 and 20mm. This is comparable to what as obtained in other studies by some authors. In a study by Ekundayo et al. (2011), the ethanolic extracts of *Jatropha curcas* inhibited pathogenic bacteria with zones of inhibition ranging from 30.6 to 38.5 mm. In addition, a crude extract of *J. curcas* has been found to inhibit HIV-induced cytopathic effects with low cytotoxicity (Matsuse et al., 1998; Haas and Mittelbach, 2000). In a study by Oyi et al. (2007), the diameter of zones of inhibition ranged between 20 to 26 mm, when the activity of the latex of *Jatropha curcas* was compared with that of established antibiotics, it was shown that the latex seemed to be more active. In a study by Singh et al. (2012), the methanolic extracts of the *Jatropha curcas* Seed Kernel showed the maximum zone of inhibition of 25.5mm.

The inhibitory activity of plant extracts is generally depends upon the concentration, type of parts used and microbes tested (Balandrin et al., 1985), the accumulation and concentration of secondary metabolites which are responsible for inhibitory activity is varied according the plant parts (Essawi and Srouns, 2000; Rekha, 2010). It may be a reason for the variation in the inhibitory activity of extracts of *J. curcas* (Kalimuthu et al., 2010). Results of the study by Kalimuthu et al. (2010) support the folkloric usage of this studied plant and suggested that its methanol extract posses' compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens.

The susceptibility of these fungi to *J. curcas* extracts is significant, as most of these fungi have recently been implicated in cases of immunocompromised patients who frequently develop opportunistic infections (Portillo et al., 2001). Aiyelaagbe et al. (2007) reported that the presence of some secondary metabolites in the root extract of *J. curcas* inhibited some microorganisms isolated from sexually transmitted infections. From the study by Sangeetha et al. (2009), it was reported that leaf and bark ethanol extract showed a significant response against *Bacillus subtilis*, *E. coli* and *Pseudomonas aeruginosa*. The anti – bacterial activity of the extracts was compared with standard rifampicin and was found effective but not as effective as standard drug (Sangeetha et al., 2009). In a study by Kalimuthu et al. (2010), the antifungal activities of the leaf extract *in vivo* were noteworthy. However,

the methanol extract of leaf derived callus of *Jatropha curcas* showed higher antifungal activity with concomitant increase in concentrations in the same study by Kalimuthu et al. (2010). Recently, Oskoueian et al. (2011a) reported that extract of root and latex of *J. curcas* plant which contained phenolics, flavonoid and saponins showed notable antioxidant, anticancer and anti-inflammatory activities. These compounds have been reported to be involved in the biological activities of the plant (Balasundram et al., 2006; Oskoueian et al., 2011b).

Although some antibacterial and antifungal activities have been reported previously against pathogenic bacteria and fungi (Aiyelaagbe et al., 2007; Thomas et al., 2008), such activities have never been studied elaborately. This is the basis for the study of antimicrobial activity of *J. curcas* in Bangladesh (Gupta et al., 2010). Methanolic and hot water extracts of kernel meal of *J. curcas* showed antimicrobial activity against both Gram positive and Gram negative pathogenic bacteria (inhibition range: 0–1.63 cm) at the concentrations of 1 and 1.5 mg/disc in a study by Oskoueian et al. (2011b). In the study by Gupta et al. (2010), the ethyl acetate extract of *Jatropha curcas* showed moderate zone of inhibition against some of the test organisms, except *Aspergillus fumigatus*, *Rhizopus oryzae* and *Candida krusei*. The isolated compound JC-1 exhibited mild inhibitory activities against *Candida albicans* (Gupta et al., 2010). JC-2 demonstrated prominent zone of inhibition against *Rhizopus oryzae* (Gupta et al., 2010). The antimicrobial activity test in a study by Ejelolu et al. (2010) revealed that the oil extracted from seeds of *Jatropha curcas* and *Mucuna solan* showed sensitivity reaction against *E. coli O157:H7* and *Streptococcus pyogenes*. Cytotoxicity assay results carried out by Oskoueian et al. (2011b) indicated the potential of methanolic extract as a source of anticancer therapeutic agents toward breast cancer cells. The ability of methanolic extract to inhibit breast cancer cell growth indicates the potential value of *J. curcas* kernel meal as an alternative source of therapeutic agents which requires further investigation (Oskoueian et al., 2011b).

The study by Namuli et al. (2011) lend support that stem bark, root bark and kernel meal of *J. curcas* contained compounds with antibacterial activities which made them potential source of antibacterial compounds. The ability of the *J. curcas* extracts to inhibit the growth of several bacterial and fungal species is an indication of the broad spectrum antimicrobial potential of *J. curcas*, which makes the plant a candidate for bioprospecting for antibiotic and antifungal drugs (Igbinsosa et al., 2009; Sarin et al., 2010).

CONCLUSION

The results obtained in this study revealed that the significant inhibitory effect of the ethanolic extract of *Jatropha curcas* on the fungal isolates *Trichophyton*, *Microsporum*, *Epidermophyton* and *Malassezia furfur* that causes *Pityriasis versicolor*. The appreciable levels of inhibition recorded for *Jatropha curcas* on the fungi isolates indicates that these plants could be potent sources of novel antifungal drugs. It is concluded that *J. curcas* leaves could be a potential source of active antimicrobial agents, and a detailed assessment of its *in vivo* potencies and toxicological profile is ongoing. The results lend acceptance to the folkloric use of this plant in treating microbial infections and shows that *J. curcas* could be exploited for new potent antibiotics. However, there is need to conduct toxicological assessment of the leaves to ascertain their safety on human. Further studies should be carried out to unravel the identity of the active ingredients as well as its medicinal properties.

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