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RESEARCH ARTICLE

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SEED EXTRACT FROM *Jatropha curcas* Linn.

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ABSTRACT

The present study was under taken to investigate the antibacterial and antifungal activity of seeds of *Jatropha curcas* Linn. The powdered seed materials were extracted using chloroform and hexane solvent. The antimicrobial activity against *Staphylococcus aureus*, (gram positive bacteria) *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhimurium* (gram negative bacteria) and fungi *Candida albicans*, *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Rhizopus* sp., and *Mucor* sp. The antibacterial and antifungal activity *in vitro* using the disc diffusion method, Minimum Inhibitory Concentration (MIC), Minimum Bacterial Concentration (MBC) and Minimum Fungicidal Concentration (MFC) is discussed.

INTRODUCTION

Jatropha curcas is a medicinal crop that belongs to the family Euphorbiaceae and has a long history of cultivation in tropical America, Africa, and Asia (Ravindranath *et al.,* 2004). The seed kernels contain a high amount of oil [58-60% (w/w)] (Aderibigbe *et al.,* 1997), and serve as a potential source of biodiesel currently being used in India, Thailand, and other South East Asian countries. The seeds also contain high protein, antinutritional factors including trypsin inhibitor, lectin, saponin, and phytic acid, and toxic compounds called phorbol esters (Martinez-Herrera *et al.,* 2006). It has been known that all parts of *Jatropha curcas* can be used for a wide range of purposes. Extracts from various parts of *Jatropha curcas*, such as seeds and leaves, have shown molluscicidal, insecticidal, and fungicidal properties (Liu *et al.,* 1997; Meshram *et al.,* 1996; Nwosu and Okafor, 1995; Rug and Ruppel, 2000; Solsoloy and Solsoloy, 1997). *Jatropha curcas* seed extracts were found to inhibit the mycelial growth of *Colletotrichum musae* that causes anthracnose disease in bananas (Thangavelu *et al.,* 2004). Its leaf extract was effective in controlling the fungal pathogen *Sclerotium* sp., which causes *Azolla* disease (Garcia and Lawas, 1990). The chemicals responsible for those effects were suggested to be phorbol esters in the extract. Gübitz *et al.,* 1999; Goel *et al.,* 2007 also state that some derivatives of phorbol esters are known to have antimicrobial and antitumor properties, as well as molluscicidal and insecticidal effects.

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The seeds of *Jatropha curcas* or the expressed oil have been used medicinally as a purgative and as a remedy against syphilis. The oil has been used as a source of fuel, for stimulating hair growth and making candles and soap. The oil burns without smoke and has been employed for street lighting near Rio-de-Janeiro in Brazil. The viscid sap (latex) is employed for cleaning teeth, to cure sores on the tongues of babies and for reducing toothache (Burkill, 1994; Langdon, 1997). Phorbol esters are diterpenes that contain 20 carbon atoms made up of four isoprene units (Ito *et al.,* 1983). They are generally found in plant species of the families *Euphorbiaceae* and *Thymelaeaceae*. Recently, various forms of phorbol esters have been isolated from the aerial parts and seed oil of *Jatropha curcas* (Hass *et al.,* 2002; Naengchomnong *et al.,* 1986; Ravindranath *et al.,* 2004). Phorbol esters become toxic since they have biological effects that include skin inflammation, tumor promotion, tissue damage, activation of blood platelets, lymphocyte mitogenesis, prostaglandin production, and stimulation of degranulation in neutrophils in living cells (Aitken, 1986; Goel *et al.,* 2007).

Jatropha curcas seed cake is generated in considerable quantities as a by-product of *Jatropha curcas* seed oil extraction. This byproduct cannot be utilized owing to the presence of antinutritional factors and toxic compounds. The toxic compounds could be, applied to agricultural applications. The compounds, especially phorbol esters, can be extracted from *Jatropha curcas* seed by using methanol and dichloromethane as an extractant (Makkar *et al.,* 1998; Martinez-Herrera *et al.,* 2006). Those solvents, however, are

both harmful and relatively expensive. Ethanol is an organic solvent that is normally used for extractions from various plant parts, such as *Funtumia elastica* bark extract, *Mallotus oppositifolius* leaves extract (Adekunle and Ikumapayi, 2006), *Casearia sylvestris* leaves extract (Silva *et al.,* 2008) and *Opuntia ficus-indica* stem extract (Lee *et al.,* 2002). In addition, to our knowledge, the extract from *Jatropha curcas* seed cake has not been studied for antibacterial and antifungal activities.

MATERIALS AND METHODS

Plant material

The mature seeds of *Jatropha curcas* were collected from Karur district, Tamilnadu, India in the month of July, 2009. The seeds of *Jatropha curcas* were collected, air dried, and stored.

Preparation of plant extract

The seeds were dried at room temperature and then powdered using a grinder and stored in an airtight container. A sample (50 g) of each powdered plant materials (seeds) were taken and extracted using soxhlet apparatus with 250 ml of different solvents separately, such as acetone, chloroform, ethanol, methanol and hexane and each extract was evaporated to dryness under reduced pressure using rotary evaporator and to obtain the concentrated crude extracts. Then the crude extract was made into suitable concentrations using Dimethyl sulfoxide (DMSO) for present study (antimicrobial activity).

Test microorganisms

The test microorganisms used in this study are the gram positive bacteria, *Staphylococcus aureus* (MTCC 098), gram negative bacteria *Klebsiella pneumonia* (ATCC 25955), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (MTCC 098), *Vibrio cholerae* and (fungi) *Candida albicans, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Rhizopus* and *Mucor* obtained from the department of Clinical Microbiology, Rajah Muthiah Medical College (RMMC) & Hospital Annamalai University, Tamil nadu, India. The bacterial isolates were first sub cultured in a Muller Hinton Agar (MHA) and incubated at 37 °C for 24 hours while the fungal isolates were sub cultured on a Sabouraud Dextrose Agar (SDA) for 72 hours at 25 °C.

Antimicrobial activity screening

The antimicrobial activity of the crude extract was screening against *Staphylococcus aureus*, (gram positive bacteria) *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhimurium* (gram negative bacteria) and five fungi *Candida albicans*, *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus Rhizopus* sp., and *Mucor* sp., (standard laboratory clinical isolated) obtained from the microbiology laboratory, RMMC & Hospital Annamalai university Annamalai nagar. The antimicrobial activity was determined by the disc diffusion method using Mueller Hinton Agar plates (MHA) for all the bacteria and Sabouraud Dextrose Agar (SDA) for all fungi previously inoculated with 18 hours old Nutrient broth (NB) culture for the bacteria (or) spores (10^6 spores / ml for the bacteria), of the test organisms respectively. Sterilized paper discs (6mm), soaked in a known concentration of the crude extracts of *Jatropha curcas* (L.) (5000 µg/ml per disc) in DMSO were applied over each of the culture plates previously seeded with 0.5 McFarland (for bacteria) and 10^6 spores/ ml (for fungi). Antibiotic disc of ciprofloxacin (30 µg/ml) was used as positive control for bacteria, Amphotroxicin (30 µg/ml) was used for fungi and sterilized paper discs without extracts the bacteria and fungi incubations were at 37 °C for 24 hours for bacteria and 25 °C for 72 hours for fungi.

Table 1. Antibacterial activity of *Jatropha curcas* seeds

Bacterial pathogens	Zone of inhibition (mm) (Mean ± SD)[a]					MIC (µg/ml)		MFC (µg/ml)	
	Chloroform		Hexane		*CIP*				
	250 (µg/ml)	500 (µg/ml)	250 (µg/ml)	500 (µg/ml)	10 µg per disc	Chloroform	Hexane	Chloroform	Hexane
S. aureus	7±0.25	13±0.35	4±0.43	8±0.36	14±0.36	250	125	250	250
E. coli	4±0.20	9±0.37	3±0.55	7±0.20	12±0.45	125	125	250	500
K. pnemoniae	8±0.30	12±0.26	6±0.55	10±0.49	9±0.5	62.5	62.5	125	250
P. aeruginosa	7±0.45	10±0.45	5±0.45	9±0.35	15±0.26	500	250	500	500
S. typhimurium	6±0.4	11±0.40	2±0.45	4±0.36	16±0.45	500	250	500	500
V. cholarae	5±0.41	8±0.32	3±0.49	6±0.35	10±0.65	250	62.5	500	500

±: Standard deviation, CIP: *Ciprofloxacin* antibacterial control, [a] Mean of three assays.

Table 2. Antifungal activity of *Jatropha curcas* seeds

Fungal pathogens	Zone of inhibition (mm) (Mean ± SD)[a]					MIC (µg/ml)		MBC (µg/ml)	
	Chloroform		Hexane		*AMP*				
	250 (µg/ml)	500 (µg/ml)	250 (µg/ml)	500 (µg/ml)	10 µg per disc	Chloroform	Hexane	Chloroform	Hexane
C. albicans	8±0.20	15±0.36	7±0.36	11±0.51	13±0.4	250	62.5	125	250
A. niger	6±0.25	14±0.45	5±0.43	12±0.20	15±0.60	250	125	125	250
A. flavus	5±0.45	16±0.47	4±0.36	10±0.60	14±0.41	125	31.25	62.5	500
A. fumigatus	10±0.5	17±0.5	8±0.20	15±0.6	18±0.32	500	250	500	500
Rhizopus sp.,	9±0.30	11±0.36	6±0.36	14±0.36	15±0.36	250	62.25	500	500
Mucor sp.,	10±0.35	10±0.51	9±0.60	13±0.25	16±0.47	250	125	500	500

±: Standard deviation, *AMP*: *Amphotroxicin* antifungal control; [a] Mean of three assays.

RESULTS AND DISCUSSION

Results obtained from this investigation indicate that chloroform and hexane extracts of *Jatropha curcas* seed possessed antibacterial and antifungal activities against the microorganisms tested. A total of 12 microorganisms consisting of six bacteria, one yeast and five fungi were tested. When the chloroform and hexane extracts were assayed against the test organisms by disc diffusion assays (Table 1&2), the mean zones of inhibition was obtained. Minimum Inhibitory Concentration (MIC) values of 62.5-500 µg/ml were obtained for the chloroform extract in the tests with the bacterial agents while the range of 31.25-500 µg/ml was recorded against the fungal isolates. On the other hand the MIC values obtained in antibacterial assays using hexane extract were 62.5-250 µg/ml while the values recorded in the antifungal assays were of the 31.25-250 µg/ml.

The results of the Minimum Bacterial Concentration (MBC) of the extracts (Table 1) showed that with the exception of the antibacterial assays against *Staphylococcus aureus* and *Escherichia coli*, the chloroform extracts exhibited a MBC at a concentration of 500 µg/ml, while the hexane extracts had a MBC values ranged from 250-500 µg/ml. Minimum Fungicidal Concentration (MFC) of the extracts (Table 2) showed that with the exception of the antifungal assays against *Candida albicans*, *A. niger, A. flavus, A. fumigatus, Rhizopus* sp., and *Mucor* sp., the chloroform extracts exhibited a MFC at a concentration of 500 µg/ml while hexane extracts had a MFC values ranged between 250-500 µg/ml. Our results (Table 1 and 2) showed that chloroform and hexane seed extract of *Jatropha curcas* gave favourable results against all the tested microorganisms with MIC values between 31.25 and 500 µg/ml. The present study reveals that the seed extracts of *Jatropha curcas* was very effective against *A. fumigatus, A. flavus, C. albicans, A. niger S. aureus* and *K. pneumoniae* than the other strains tested. In the present study *S. aureus* was more susceptible than gram negative bacteria such as *S. typhimurium, P. aeruginosa, E. Coli* and *V. cholarae.* Methanol extract of *Azadirachta indica* (neem) oil at 1,000 mg/l reduced the growth of *Curvularia lunata* by 26% (Govindachari *et al.,*1998).

The extracts of *Ocimum sanctum* and *Azadirachta indica* inhibited the growth of *C. capsici* by 43% and 64%, respectively (Sinha *et al.,* 2004). Shukla and Tripathi (1987) reported that the oil of *Pimpinella anisum* at 1,000 mg/l exhibited total lethality on C. *capsici*, *Curvularia lunata*, *F. oxysporum*, and *F. semitectum.* Antifungal and antimicrobial activities of extracts from parts of *Jatropha* species have been reported. Aiyelaagbe *et al.,* 2000 found moderate antifungal activity against *Candida albicans* by hexane, chloroform, and methanol extracts from roots of *Jatropha podagrica* at a concentration of 20,000 mg/l. Kumar *et al.,* 2006 reported that 500 mg/l crude extract from leaves of *Jatropha gossypifolia* L. completely inhibited eight microorganisms: *Bacillus cereus* var. *mycoides*, *B. pumilus*, *B. subtilis*, *Bordetella bronchiseptica*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Streptococcus faecalis*, and *Candida albicans*. The antibacterial potential of the seed extract of *P. corylifolia* has been demonstrated. However none of the earlier reports have demonstrated the antifungal potency of *P. corylifolia* against phytopathogenic fungi in general and biodeterioration causing fungi in particular (Chistawar *et al.,* 1992; Haraguchi *et al.,* 2000; Newton *et al.,* 2002). Thus in the present investigation, for the first time the antifungal potency of the plant against phytopathogenic fungi in general and biodeterioration causing fungi of maize in particular has been demonstrated.

The seeds of *P. corylifolia* are used in indigenous medicine as laxative, aphrodisiac, anthelmintic, diuretic and diaphoretic in febrile conditions. They have been specially recommended in the treatment of leucoderma, leprosy, psoriasis and inflammatory diseases of the skin and are prescribed both for oral administration and for local external application in the form of a paste or ointment (Latha and Panikkar, 1999). A number of preparations made from the seeds have been tried in numerous cases of leucoderma and other skin diseases. Oral administration of the powdered seeds to the patients has generally in curing nausea, vomiting, malaise, headache and sometimes purging. The seed extracts inhibit the growth of *Staphylococcus citreus, S. aureus* and *S. albus* including strains resistant to Penicillin. The seeds possesses anthelmintic activity against earthworms, Psoralen being the active principle (Wang *et al.,* 1999).

Conclusions

The extract of *Jatropha curcas* seed would serve as a natural phytochemicals against bacterial and fungal phytopathogens for agricultural applications at a low cost and safe practice. *Jatropha curcas* seed, a by - product generated in large quantities by the biodiesel fuel industry, could thus be utilized as a source of the antibacterial and antifungal agents.

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