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Research Article

IN VITRO ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF *JATROPHA CURCAS* SEED EXTRACT

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ABSTRACTS
The antimicrobial effects of the methanol, ethyl acetate and hexane extracts of *J.curcas* seed at concentration ranging from 50-200mg/ml were tested against some pathogenic organisms using agar diffusion method; the extracts exhibited antimicrobial activities with the zones of inhibition ranging from 10-25, 8-23, 10-20 and 12-21(mg/ml) for *Staphylococcus aureus*, *Escherichia coli, Salmonella typhi and Candida albican,* respectively. The Minimum Inhibitory Concentration (MIC) which ranges from 3.13-12.5mg/ml was determined using the broth dilution method; the Minimum Bactericidal Concentration (MBC) ranges from 25-12.25mg/ml. The phytochemical analysis revealed the presence of alkaloid, glycosides,flavonoid and carbohydrate. The ability of the crude seed extracts of *J.curcas* to inhibit bacteria and fungi is an indication of its broad spectrum antimicrobial potential which may be employed in the management of microbial infections. It is necessary to determine the active dosage level so as to be able to formulate it into a pharmaceutical dosage for use in chemotherapy.
Keyword: Antimicrobial activities, Microbial infections, Inhibition.

INTRODUCTION

Jatropha curcas, Barbados nut, physic or purging nut is a perennial shrub normally up to 5- 6m high. Some of its common names are lapalapa (Yoruba), Bindiazuzu (Hausa), Kashala(Nupe), Majingalu (Kanubari) and Muaayi (Gbagi). It belongs to the family Euphorbiaceae (spurge family).

It is mainly grown, in Asia and Africa where it is known as pourghe re. It is a shrub of tree with spreading branches and stubby twinges with milky or yellowish refescent exudates[1]. 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[2].

The leaves are utilized extensively in West Africa ethno medical practice in different forms to cure various ailments like fever, mouth infections, Jaundice, guinea worm sores and joint rheumatism[3,4].

The sap and crushed leaves have also shown Anti-parasitic activity[5]. The water extract of the branches also strongly inhibited the HIV induced cytopathic effects with low cytotoxicity[6].

The roots are used in decoction as a mouth wash for bleeding gums, toothache, eczema, ring worm, scabies, and gonorrhea[7]. It is also reported that the root methanol extract exhibit anti-diarrhea activity in mice through inhibition of prostaglandin synthesis and reduction of osmotic pressure[8]. The leaf decoction drank for veneral diseases[9]. Colombians and Costa Ricans apply the latex to burns, hemorrhoid, ring worm, and ulcers[9]. Seeds are used also for dropsy, gout. Paralysis and skin ailments[10] the latex has been used in management of skin ulcer, gonorrhea, whitlow, ringworm and eczema[11]. The roots and stem bark and used in treatment of sexually transmitted disease[12].

MATERIALS AND METHODS

The plants used for this study was collected along David mark road Tunga, minna Niger state samples of the seeds was taken to the Department of Medicinal Plant and Traditional Medicine NIPRD, Abuja, where the plant was identified and authenticated. The seed was air dried at room temperature for two weeks in the pilot plant section in NIPRD. The dried seed was grounded into fine powder using mortar and pestle.

Source of test organism

The micro-organisms used for this study (Bacteria: *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, Salmonella typhi.* Fungi: *Candida albicans* were obtained from the stock culture collections of the Department of Microbiology and Biotechnology of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja.

Preparation of the test isolate

The isolates were first subcultured into Trypton Soya broth (TSB) and incubated at 37^{o}C for 18h while fungi isolate were subcultured on Sabouraud Glucose Agar (SGA);the different isolates was subcultured into

different selective media to ascertain purity.the purified isolates were standardized to correspond to 0.5 macfarland turbidity standard.

Extraction procedure

According to soxhlet procedure, solid material is extracted by repeated washing (percolation) with an organic solvent under reflux in a special glass apparatus.

In this method the sample was dried, ground into small particles 100g wasss weighed and placed in a porous cellulose thimble. The thimble was placed in an extraction chamber which was suspended above a flask containing the solvent and below a condenser The flask is heated and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. The extraction chamber was designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask. At the end of the extraction process, which lasts a few hours (about 6-7hours), the flask containing the solvent and lipid is removed.

The process was repeated successively for all the solvent, starting with a non polar solvent (Hexane, 300ml), followed by less polar solvent (ethyl acetate, 350ml), then a polar solvent (methanol, 300ml)[13].

Concentration of extracts

Each of the extracts was concentrated in vacuum using a rotary evaporator which ensures evaporation of bulky solution to small volume without bumping at temperature between 40-60^{o}C and it was further dried to constant weight at the same temperature range in steam bath.

The resultant concentrates was weighed and kept in refrigerator until ready for use.

ANTIMICROBIAL ACTIVITY

The antimicrobial activity of the crude extracts was determined in accordance with the agar well diffusion method described by[14].

Muller Hinton agar (MHA) and SDA were prepared for bacteria and fungi respectively according to the manufacture's instruction.

Immediately after autoclaving, the media was allowed to cool to 45-50^{o}C using the water bath.

The freshly prepared and cooled media was poured into glass flat-bottomed Petri-dishes (90mm in diameter) placed on a horizontal surface (faster$^{(R)}$ laminar flow) to give a uniform depth of approximately 4mm; the agar media was allowed to solidify at room temperature.

About 0.2ml (200*ul*) of the standardized test inoculums was evenly spread on the surface of the solidified media using a sterile swab stick.

Four equidistant wells of 5mm in diameter and 4mm in depth were then made on the seeded agar plates using sterile cork borer; two more wells for positive(+) and negative (-) control were made at the middle. About 0.25ml (25µl) of the plant extracts and control were filled into the wells. Concentration of crude extract ranges from 50-200mg/ml.

Positive controls were Erythromycin and Ciprofloxacin for gram positive and gram negative, Nystatin for fungi; negative controls were DMSO (dimethylsulfoxide) and distilled water.The wells were labelled to correspond with the code numbers of the crude extracts and control. The plates were allowed for at least 30minutes to pre diffuse into the agar before incubation was done at 37^{o}C. Antimicrobial activity was determined by measuring the diameter of zones of inhibition in mm**.**

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the crude extracts was determined using the broth dilution method in test tubes9ml of nutrient broth was dispense into different test tube which was covered with a plug(cotton wool + foil),these tubes were sterilized at 121^{o}Cfor 15mins.using the autoclave. Tubes were allowed to cool at room temperature, it was labelled and arranged serially to correspond to the concentrations. from the reconstituted extracts, 1ml was transferred into the first tube, 1ml was drawn from it into the second tube this continued till the 9th tube where 1ml was taken and discarded, then 0.2ml of the standardized organisms that were active was transferred into each tubes, control tubes were incubated along with other reaction tubes at 37^{o}C for 24h. Tubes were observed for turbidity and the least concentration that show no turbidity was recorded as the MIC.

Determination of Minimum Bactericidial Concentration (MBC)

The tube with no visible turbidity was subcultured into freshly prepared nutrient agar and was incubated at 37^{o}C for 24hrs and the plates were observed for growth. The plates with no visible growth were recorded as the MBC.

Processing and Phytochemical Screening

The seed was air dried in MPR Department at NIPRD, Idu, Abuja. For three weeks, it was then grounded to a powdered using sterile mortal and pestle. Then the screening of the seed extract was carried out according to the method described by Sofowora [15,16] for the detection of active components like saponins, tannin, alkaloid, glycosides, terpene, steroids, anthraquinone, carbohydrate, volatile oil and flavonoids

RESULTS

Table 1 contains secondary metabolites from the three extracts of the plant. The Hexane extract contains abundance of Terpenes, Steroids, Flavonoids and Glycosides but traces of Tannins while the Ethanol and Methanol extracts revealed the presence of Steroids, Tannins, Flavonoids and Glycosides in abundance but traces of Flavonoids and Tannins respectively.

Activities of extracts against test organisms were expressed in form of diameter zone of inhibition around extracts. Table 2 indicates that Staphylococcus aureus, Salmonella typhi, Escherichia coli and Candida albicans were susceptible at the lowest concentration of 50mg/ml while Bacillus subtilis and Pseudomonas aeruginosa were only susceptible at a very high concentration of 200mg/ml. However, all the test organisms were susceptible to Erthromycin and Ciproflaxin.

All the test organisms were resistant to the extracts at lower test concentrations of 50 and 100mg/ml. Only S. aureus, S. typhi and E. coli were susceptible but at a higher concentration of 200mg/ml Table 3. Organisms were susceptible to Ciproflaxin used as standard antibiotic.

Table 4 revealed that only S. aureus was susceptible to the oil at various test concentrations. The remaining organisms were resistant except C. albicans which was susceptible only at the concentration of 0.4µg/ml.

Table 5 indicates the minimum inhibitory concentration (MIC) of methanol extract for the organisms ranging from 3.13-12.50 µg/ml while the minimum bactericidal concentration (MBC) ranged from 6.25-25.00 µg/ml.

DISCUSSION

Table 1 show that Flavonoid, Sterole, Terpene, Volatile oil and Glycoside were very present while carbohydrate, Tannin was slightly present and alkaloid, saponin and anthraquinone are absent.

In this study the result obtained as shown in Table 2,3 and 4 indicates that the methanolic extracts of the plant inhibited the growth of the test isolates but that of ethyl acetate was resistance to Bs, Ps, and Ca.the observed antimicrobial properties corroborates its use in traditional medicine, used in treating skin ailments, dysentery and as hair stimulant.

The large zone of inhibition exhibited by the extracts on *S.aureus* and *E.coli* justified their use by traditional practitioner in the treatment of sores, bores and control of diarrhea and dysentery. The hexane extract (oil) was very active against *Staphylococcus aureu*s this confirmed it use traditionally in the treatment of skin ailment (scabies,trachoma,etc.).

The low MIC exhibited by the methanolic extract on *S.aureus* is of great significance in the health care delivery system since it could be used as an alternative to orthodox antibiotics in the treatment of infections due to the microorganisms especially as they frequently develop resistance to known antibiotics[17].

It was also observed from this work that the higher the concentration the more their activity and as the concentration decreases the lower the antimicrobial effect

Hence an acceptable and effective dosage can be prepared for the control and eradication of this pathogen.

CONCLUSIONS

This study has revealed the presence of many secondary metabolites in the seeds of *J. curcas.* It has further confirmed that the extracts could be use for the treatment of various infections caused by the pathogens. The results lend credence to the folkloric use of this plant in treating microbial infections and shows that *J. curcas* could be exploited for new potent antibiotics.fom the physic chemical analysis the expressed oil may be used in making soap, and hair stimulants.

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Table 1: Phytochemical analysis of *Jatropha curcas* seed extract

Extracts	% yield	Secondary metabolites						
		Alkaloid	Saponin	Terpenes	Steroids	Tannins	Flavonoids	Glycosides
JCH	27	—	—	++	++	+	++	++
JCE	1.5	—	—	+	++	++	+	—
JCM	2.0	—	—	+	++	+	++	++

Key: JCH (*Jatropha curcas* hexane), JCE (*Jatropha curcas* ethyl acetate) JCM (*Jatropha curcas* Methanol)
+ Slightly present
++ Highly present
— Absent

Table 2: Result of Antimicrobial screening of different concentrations of the crude methanolic extract of *J. curcas*

Concentrations of extracts (mg/ml) and controls	Microorganisms/zones of inhibition(mm) methanol					
	Sa	St	Bs	Ec	Pa	Ca
200	**27**	**20**	**15**	**23**	**10**	**21**
100	**15**	**16**	**0**	**12**	**0**	**19**
50	**10**	**10**	**0**	**8**	**0**	**15**
Eryth.(25mg/ml)	**22**	**25**	**15**	**20**	**10**	**NT**
Cipro.(25mg/ml)	**24**	**26**	**12**	**45**	**23**	**24**
Dmso	0	0	**0**	**0**	**0**	**0**
Nystatin	**NT**	**NT**	**NT**	**NT**	**NT**	**25**

KEY: Sa =S.aureus, St= S. typhi, Ec=E.coli, Pa=P.aeruginosa, Ca= C.albican, Bs= B.subtilis, O=no activity NT= not tested

Table 3: Result of Antimicrobial screening of different concentrations of the crude Ethyl acetate extract of *J.curcas*

Concentrations of extracts(mg/ml) and controls	Microorganisms/zones of inhibition(mm) Ethyl Alcohol					
	Sa	St	Pa	Bs	Ec	Ca
200	**12**	**8**	**0**	**0**	**10**	**0**
100	**5**	**0**	**0**	**0**	**0**	**0**
50	**0**	**0**	**0**	**0**	**0**	**0**
Eryth.(25mg/ml)	**NT**	**NT**	**NT**	**NT**	**NT**	**NT**
Cipro.(25mg/ml)	**25**	**20**	**10**	**22**	**20**	**NT**
Dmso		**0**	**0**	**0**	**0**	**0**
Nystatin	**NT**	**NT**	**NT**	**NT**	**NT**	**23**

KEY: *Sa =S.aureus, St = S. typhi, Ec =E.coli, Pa =P.aeruginosa, Ca = C.albican, Bs = B.subtilis,* 0=*no* activity, *NT* =not tested

Table 4 Diameter zones of inhibition in mm of Hexane Extract of *J. curcas* (oil) on the test organisms

Isolates	0.4µl	0.25 µl	0.2 µl	0.1 *µl*
S.aureus	*22*	*12*	*10*	*5*
S.typhi	*0*	*0*	*O*	*0*
E.coli	*0*	*0*	*0*	*0*
C.albican	*20*	*0*	*0*	0

Table 5: The MIC and MBC of the extracts of the seed of *J. curcas*.

Isolates	Methanol(mg/ml)	
	MIC	MBC
S.aureus	6.26	12.5
S.typhi	12.5	25
E.coli	3.13	6.25
C.albicans	12.5	25

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