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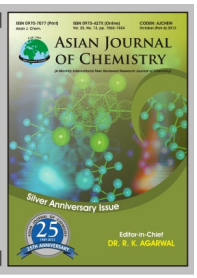

Antimicrobial Potential of Methanolic Extract and Various Fractions of *Jatropha curcas* Roots

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The present study was conducted to explore the antimicrobial effectiveness and phytoconstituents of *Jatropha curcas* roots. Some of phytoconstituents as alkaloids, tannins, steroids, saponins, flavonoids and terpenoids were present in *J. curcas* roots. The antimicrobial activity was determined by the disc diffusion method and minimum inhibitory concentration (MIC) against a panel of microorganisms. Results indicated that all extracts and fractions possessed significant antibacterial and antifungal activities. While the methanolic extract showed magnificent antibacterial effect against *P. multocida*, *E. coli* and *S. aureus* with highest inhibition zones (IZ) and lowest MIC values (IZ = 35.1, 30.0 and 29.6 mm; MIC = 12.5, 28.5, 30.8 mg/mL) than standard drug rifampicin (IZ = 33.0, 27.0, 26.5 mm; MIC = 15.5, 39.1; 41.2 mg/mL), respectively. Comparative results were carried out using rifampicin for bacteria and fluconazole for fungi as standard antibiotics. It is concluded that *J. curcas* roots possessed considerable antimicrobial activity.

Key Words: *J. curcas*, Organic fractions, Rifampicin, Fluconazole, Roots.

INTRODUCTION

Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of side effects that are often associated with synthetic antimicrobials[1]. So there is a need to explore antimicrobials from natural sources. *Jatropha curcas* belongs to the family Euphorbiaceae. *Jatropha*, a crop native to North American region is now distributed in several regions (Africa, India, South East Asia and China) across the World[2]. Euphorbiaceae is a larger family of flowering plants with 300 genera and around 7,500 species. *Jatrophas curcas* is commonly called physic nut, purging nut or pig nut. Previous studies have reported that the plant exhibits bioactive activities for fever, mouth infections, jaundice, guinea worm, sores and joint rheumatism[3]. Crushed leaves of *J. curcas* have been reported to exhibit the anti-parasitic activity[4]. The water extract of the branches also strongly inhibited HIV induced cytopathic effects with low cytotoxicity. Previous works have shown that many *Jatropha* species possessd antimicrobial activities[4]. Fasola *et al.*[5] reported the presence of various phytochemicals *i.e.*, saponin, tannin, glycoside, steroid, alkaloid and flavonoid in stem and leaves of *J. curcas*. These compounds are known to be biologically active and therefore aid the antimicrobial activities of *J. curcas*. These secondary metabolites exert antimicrobial activity through different mechanisms. Herbs that have tannin as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery[6].

Extracts of the stems have been suggested to possess various biological activities including antiinsect, antimicrobial, cytotoxic, antiinflammatory and molluscicidal activities have been reported from the stem of *J. curcas*[7-14]. The seeds and the seed oil are used as purgative and as a remedy for syphilis[15], dropsy, gout, paralysis and skin ailments. In addition, the oil is used as a substitute for diesel oil and as fuel[16,17]. Latex of the plant *J. curcas* is used to dress sores, ulcers and inflamed tongues[18].

The roots of *J. curcas* are used after decoction as a mouth-wash for bleeding gums, toothache, eczema, ringworm, and scabies, and to cure dysentery and venereal diseases, like gonorrhea[19,20]. In this context as part of our studies on indigenous flora of Pakistan[21-25] the antimicrobial potency of different organic fractions of *J. curcas* roots was investigated.

EXPERIMENTAL

Roots of *Jatropha curcas* were purchased from the local market of Faisalabad and further identified by Dr. Mansoor

Hameed, Department of Botany University of Agriculture Faisalabad, Pakistan where a voucher specimen has been deposited.

Preparation of extract and fractions: The shade-dried ground roots (2 Kg) of the plant were extracted with absolute methanol (2 × 5 L) and kept it for 4-5 days at room temperature. After filtering the extract was concentrated through rotary vacuum evaporator. Methanolic extract (180 g) became viscous which was stored at - 4 ºC. The process was repeated three times with intervals of 4 days to obtain a maximum quantity of extract. The residue was further partitioned successively with *n*-hexane (90 g), chloroform (25 g) ethyl acetate (20 g), and acetone (22 g) fractions. All samples (dry residue) were dissolved in 10 % sterile dimethyl sulfoxide for further antimicrobial activity.

Phytochemical analysis: Phytochemical screening of the dry powdered plant was carried out according to the previously reported method[26].

Antimicrobial assay of *J. curcas* roots

Microorganisms: Methanolic extract and its different organic fractions of the plant were individually tested against the following microorganisms including four bacteria, *Escherichia coli* ATCC 25922, *Bacillus subtilis* JS 2004, *Staphylococcus aureus* API Staph tac 6736153 and *Pasturella multocida* locally isolated and three pathogenic fungi, *Aspergillus niger* ATCC 10595, *Aspergillus flavus* ATCC 32612 and *Rhizopus solani* locally isolated. The pure bacterial and fungal strains were obtained from the Department of Veterinary Microbiology, University of Agriculture, Faisalabad, Pakistan. Bacterial strains were cultured overnight at 37 ºC in nutrient agar (Merck), while fungal strains were cultured overnight at 28 ºC using potato dextrose agar (Merck).

Disc diffusion method: The antimicrobial activity of the *J. curcas* roots extract and its different fractions was determined by the disc diffusion method[27]. The discs (6 mm in diameter) were impregnated with 20 mg/mL extract/fractions (100 µL/disc) placed on the inoculated agar. Rifampicin (100 µL/disc) (Oxoid) and fluconazole (100 µL/disc) (Oxoid) were used as positive control for bacteria and fungi, respectively. Disc without samples was used as a negative control. Antimicrobial activity was evaluated by measuring the inhibition zone.

Resazurin microtitre-plate assay: The minimum inhibitory concentration (MIC) of the methanolic extract and different organic fractions was evaluated by a modified resazurin microtitre-plate assay as reported by Sarker and coworkers[28] with modification. Briefly, a volume of 100 µL of extracts/fractions solutions in 10 % dimethyl sulfoxide (DMSO, v/v) was transferred into the first row of the 96 well plates. To all other wells, 50 µL of nutrient broth and muller hinton broth for bacteria and fungi, respectively were added. Two-fold serial dilutions were performed using a multichannel pipette such that each well had 50 µL of the test material in serially descending concentrations. To each well 10 µL of resazurin indicator solution (prepared by dissolving 270 mg resazurin tablet in 40 mL of sterile distilled water) were added. Finally, 10 µL of bacterial/fungal suspension were added to each well.

Each plate had a set of controls: a column with a broad spectrum antibiotics as positive control, a column with all solutions with the exception of the test samples, a column with all solutions with the exception of the bacterial/fungal solution adding 10 µL of broths instead and a column with 10 % DMSO (v/v) solution as a negative control. The plates were prepared in triplicate, and incubated at 37 ºC for 24 h and 28 ºC for 48 h for bacteria and fungi, respectively. The absorbance was measured at 620 nm by micro quant for fungus and at 500 nm for bacteria. The colour change was then assessed visually. The growth was indicated by colour changes from purple to pink or colourless. The lowest concentration at which colour change appeared was taken as the MIC value.

Statistical analysis: All the experiments were conducted in triplicate unless stated otherwise and statistical analysis of the data was performed by analysis of variance (ANOVA), using STATISTICA 5.5 (Stat Soft Inc, Tulsa, Oklahoma, USA) software. A probability value of difference $p \leq 0.01$ was considered to denote a statistically significance. All data were presented as mean values ± standard deviation (SD).

RESULTS AND DISCUSSION

Phytochemical constituents were analyzed in dry powdered plant roots (Table-1). Alkaloids, tannins, saponins, steroids, flavonoids and terpenoids were found to be present in *J. curcas* roots, while anthraquinones were absent.

TABLE-1
PHYTOCHEMICALS IN DRY POWDERED *J. curcas* ROOTS

Phytochemicals	Dry powdered roots
Alkaloids	Present
Tannins	Present
Steroids	Present
Saponins	Present
Flavonoids	Present
Terpenoides	Present
Anthraquinones	Absent

This study also reported the antimicrobial activity of different organic fractions of *J. curcas* roots against various bacteria and pathogenic fungi. The results from disc diffusion method (Table-2) followed by measurement of minimum inhibitory concentration (MIC) (Table-3) indicated that methanolic extract showed significant antibacterial effect against *P. multocida*, *E. coli* and *S. aureus* with highest inhibition zones (35.1, 30.0 and 29.6 mm) than standard drug rifampicin (33.0, 27.0, 26.5 mm) followed by lowest MIC values (12.5, 28.5, 30.8 mg/mL), respectively. Ethyl acetate fraction showed strong activity against *P. multocida* and *E. coli* with inhibition zones (26.8, 25.5 mm) and lowest MIC values (41.2, 46.6 mg/mL), respectively. Least activity was exhibited against *R. solani* and *B. subtilis* with small inhibition zones (19.5, 19.0 mm) and highest MIC values (79.7, 83.2 mg/mL). *A. flavus* and *P. multocida* growth was inhibited by chloroform fraction with highest inhibition zones (30.1, 29.0 mm) and followed by lowest MIC values (27.2, 31.2 mg/mL), respectively. While *R. solani* was resistant against chloroform fraction with small inhibition zone (16.8 mm) and high MIC value (98.5 mg/mL). Acetone fraction was highly potent against *R. solani* and *P. multocida* with good inhibition zones (30.9, 30.5 mm) followed by least MIC values (26.4, 25.4

TABLE-2
ANTIMICROBIAL POTENTIAL OF METHANOLIC EXTRACT AND DIFFERENT ORGANIC FRACTIONS OF *J. curcas* ROOTS BY DISC DIFFUSION METHOD[a]

Microbial strain	Inhibition zone (mm)[b]. Methanolic extract and different organic fractions					
Bacterial strains	Antibiotics rifampicin	Absolute methanol	Ethyl acetate	Chloroform	Acetone	*n*-Hexane
B. subtilis	25.0 ± 0.81[a]	24.0 ± 0.81[ab]	19.5 ± 0.48[c]	24.3 ± 1.24[ab]	22.6 ± 1.24[b]	17.5 ± 1.08[c]
P. multocida	33.0 ± 1.63[b]	35.1 ± 0.98[a]	26.8 ± 0.23[d]	29.0 ± 0.70[c]	30.5 ± 0.48[c]	16.1 ± 0.62[e]
S. aureus	26.5 ± 1.22[b]	29.6 ± 0.23[a]	22.0 ± 1.63[c]	19.5 ± 0.78[d]	25.8 ± 0.84[b]	18.0 ± 0.81[d]
E. coli	27 ± 0.81[b]	30.0 ± 0.47[a]	25.5 ± 0.48[b]	25.0 ± 0.81[b]	26.8 ± 0.62[b]	15.3 ± 0.94[c]
Fungi			Fluconazole			
A. niger	27.0 ± 1.22[a]	25.2 ± 1.08[ab]	23.2 ± 1.08[bc]	24.5 ± 0.52[ab]	22.1 ± 0.61[c]	18.25 ± 0.75[d]
A. flavus	33.8 ± 0.86[a]	26.0 ± 0.81[c]	24.5 ± 0.45[cd]	30.1 ± 0.84[b]	25.0 ± 1.24[d]	16.0 ± 0.47[e]
R. solani	32.0 ± 0.62[a]	28.0 ± 0.81[b]	19.0 ± 0.41[c]	16.8 ± 0.62[c]	30.9 ± 0.23[a]	14.0 ± 0.86[d]

[a]Values are mean ± SD of three separate experiments. [b]Diameter of inhibition zone (mm) including disc diameter of 6 mm. Letters in superscript show the significance of the results against single strain.

TABLE-3
MINIMUM INHIBITORY CONCENTRATION (MIC) OF METHANOLIC EXTRACT AND DIFFERENT ORGANIC FRACTIONS OF *J. curcas* ROOTS AGAINST SOME MICROBIAL STRAINS

Microbial strain	Minimum inhibitory concentration (mg/mL). Methanolic extract and different organic fractions					
Bacterial strains	Antibiotics rifampicin	Absolute methanol	Ethyl acetate	Chloroform	Acetone	*n*-Hexane
B. subtilis	48.5 ± 2.68	50.2 ± 1.32	79.7 ± 3.34	50.4 ± 3.30	61.4 ± 2.52	95.2 ± 5.11
P. multocida	15.5 ± 2.77	12.5 ± 1.36	41.2 ± 4.10	31.2 ± 3.26	25.4 ± 3.71	107 ± 8.81
S. aureus	41.2 ± 3.83	30.8 ± 2.50	61.4 ± 3.65	79.8 ± 2.74	46.6 ± 2.82	88.5 ± 6.30
E. coli	39.1 ± 1.0	28.5 ± 1.26	46.6 ± 2.14	48.5 ± 7.70	41.2 ± 1.31	130 ± 8.16
Fungi			Fluconazole			
A. niger	35.4 ± 1.71	48.5 ± 1.38	55.7 ± 2.42	49.4 ± 2.90	62.4 ± 3.75	84.2 ± 1.29
A. flavus	13.1 ± 0.98	43.4 ± 1.80	50.1 ± 7.42	27.2 ± 0.90	46.4 ± 4.75	105 ± 1.28
R. solani	18.5 ± 1.74	35.4 ± 4.08	83.2 ± 3.83	98.5 ± 3.81	26.4 ± 2.52	135 ± 8.52

Values are mean ± SD of three separate experiments.

mg/mL). While it exhibited least activity against *A. niger* with small inhibition zone (22.1 mm) and followed by high MIC value (62.4 mg/mL). The *n*-hexane fraction showed poor results as compared to other fractions against all pathogens it showed good results against *A. niger* and *S. aureus* with high value of inhibition zones (18.25, 18.0 mm) and least MIC values (84.2, 88.5 mg/mL), respectively. Whilst *n*-hexane fraction showed poor results against *P. multocida*, *E. coli* and *R. solani* with smallest inhibition zones (16.1, 15.3, 14.0 mm) and highest MIC values (107, 130, 135 mg/mL), respectively. In general, the antimicrobial activity of tested extract and fractions was comparable with standard drugs rifampicin and fluconazole. All fractions showed less activity as compared to standard drugs except methanolic extract which showed magnificent antibacterial activity against some bacteria than standard drug rifampicin.

The greater inhibitory effects of the methanolic extract of roots of *J. curcas* can introduce the plant as a potential candidate for the treatment of ailments caused by these pathogens. The inhibitory abilities of *J. curcas* fractions may be due to the presence of photochemical constituents as alkaloids, tannins, saponins, steroids, flavonoids and terpenoids as reported in Table-1. Ayelaagbe and coworkers[9] reported that the presence of some secondary metabolites in the root extract of *J. curcas* inhibited some microorganisms isolated with sexually transmitted infections. Phytochemicals are known to be biologically active and therefore aid the antimicrobial property of *J. curcas*. These secondary metabolites exert antimicrobial property through different mechanisms. The inhibitory activity of plant extract is largely dependent on the concentration, parts of the plant used and the microbes tested[29]. This might be the reasons for the variation in the results obtained. Antimicrobial activity of stem bark and leaves extracts from *J. curcas* has already been reported[30-32]. The differences in the antimicrobial effects may be due to the difference in phytochemical properties and differences among species. It is also possible that the active chemical constituents may not be soluble in a particular solvent. Overall the extract and fractions of the plant roots showed the significant antibacterial and antifungal activity on studied organisms.

Conclusion

This present study revealed that methanolic extract and different organic fractions of *J. curcas* roots have significant antimicrobial activity. Overall the methanolic extract of the roots has shown significant antibacterial effects. Therefore by these results it is assumed that constitutes of roots extract/ fractions of *J. curcas* may serve as a potential source of antimicrobial drugs especially constituents of methanolic extract may be useful in chemotherapy of some bacterial infections. However, there is a need to conduct toxicological assessment of the roots to ascertain their safety on human beings.

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