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ANTIMICROBIAL ACTIVITIES OF DIFFERENT PARTS OF TWO GEOGRAPHICALLY DISTINCT VARIETIES OF *JATROPHA CURCAS* LINN. FRUIT IN PAKISTAN

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Abstract

Antimicrobial activities of deoiled seed kernel (mechanically pressed), fruit coat and seed coat of *Jatropha curcas* Linn. collected from two regions (Bannu and Peshawar) of Pakistan were investigated. The antimicrobial activities were carried out against *Klebsiella pneumoniae (*ATCC 43816*), Escherichia coli (*ATCC 10536*), Staphylococcus aureus (*ATCC 6538*), Bacillus subtilis (*ATCC 6633*)* and two clinical fungal isolates *Aspergillus fumigatus* and *Candida albicans* using agar well diffusion method. The antibacterial activities of Peshawar sample were found to be higher than Bannu, against selected strains. While antifungal activities of both samples were similar. Highest zone of inhibition 31.5 ± 0.7 mm was exhibited by *n*-hexane extract of deoiled seed kernel of Peshawar sample against *Bacillus subtilis* (ATCC 6633). The minimum inhibitory concentration of ethanolic extracts of deoiled seed kernel and seed coat of Peshawar sample was 31.25 - 25 mg/ml. Whereas, minimum inhibitory concentration of ethanolic and *n*-hexane extracts of Bannu sample was 62.5 - 125 mg/ml. The results suggested that antimicrobial potential of *J. curcas* Linn. varied with geographical distribution. The investigation of different varieties of medicinal plants belonging to the same species will greatly enhance the chances of best pharmaceuticals discovery.

Introduction

Traditionally, medicinal plants have been used to cure various infectious diseases and approximately 20,000 species of medicinal plants have been reported to date (Compean and Ynalvez 2015). Antibiotic resistance has been increasing at an alarming rate around the globe. The rise of bacterial resistance has diverted researchers' interest toward herbal antimicrobial agents (Dastan *et al.* 2016). Medicinal plants' extracts have high antimicrobial potential and are effective against infectious agents that causing diseases and are difficult to cure with conventional/standard drugs (Madduluri *et al.* 2013). The extracts of various *Jatropha* species have shown cytotoxic, anti-tumor and antimicrobial activities (Magda *et al.* 2015). *Jatropha curcas* Linn. belonging to Euphorbiaceae family is a multipurpose, drought resistant plant. *J. curcas* Linn. can be grown easily in desert and marginal areas, which makes it ideal to be grown on uncultivated areas of Pakistan (Kumar and Sharma 2008, Kamel *et al.* 2018). *J. curcas* Linn. has capability to grow in acidic, alkaline and sandy environment (Dada *et al.* 2014). Being a medicinal and oil rich plant, it could be used for the production of biodiesel and value-added products in biorefinery context to increase its economic viability for biofuel production.

A limited number of studies have been conducted on antimicrobial activities of various parts of *J. curcas* such as seeds, leaves, bark and roots. The seeds have different components and each component may have certain antimicrobial activities. Therefore, there is a need to investigate anti-

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microbial potential of its different parts. No study has been conducted to date on fruit coat, seed kernel and seed coat separately of two distinct species of *J. curcas.* However, the quantity and variety of nutritional and antimicrobial compounds varies from species to species depending upon the climatic conditions and geographical distribution (Paul *et al.* 2016). Therefore, searching and investigating medicinal plants species on the basis of geographical distribution and climatic condition will lead to selection and extraction of high quantity and potent antimicrobial agents. Such studies are ideal for boosting economic value of natural compounds as well as to overcome limited production problems. To the best of knowledge of the authors, there is no data available that offer comparison of antimicrobial activities of geographically distinct *J. curcas* fruits. The plants grown in two different locations, having different climatic and fertility conditions, are expected to vary in their antimicrobial activities. In view of this, the present study was designed to evaluate and compare antimicrobial activities of two geographically different *J. curcas* fruit.

Materials and Methods

Jatropha curcas Linn. fruits were collected from districts of Peshawar and Bannu, Khyber Pakhtunkhwa, Pakistan with latitude and altitude 34.0151° N, 71.5249° E and 32.986111° N, 70.604164°, respectively and confirmed in herbarium of Plant Sciences Department, Quaid-i-Azam University, Islamabad, Pakistan. Both samples were air-dried and the three parts of seeds i.e. seed kernel, seed coat and fruit coat were separated. Oil was extracted from seed kernel using a clean mechanical expeller leaving behind deoiled seed kernel. Extracted oil can be used for biodiesel production and deoiled seed kernel was used for antimicrobial purpose in the present study. All three parts were ground into fine powder using mortar and pestle and stored at 4 °C till further use. The three parts i.e. deoiled seed kernel, seed coat, and fruit coat of *J. curcas* fruit collected from Peshawar and Bannu were termed in this article as deoiled seed kernel^{P}, seed coat^{P}, fruit coat^{P} and deoiled seed kernel^{B}, seed coat^{B}, fruit coat^{B}, respectively.

This study targeted four American Type Culture Collection bacterial strains; *Klebsiella pneumoniae (*ATCC 43816*), Escherichia coli (*ATCC 10536*), Staphylococcus aureus (*ATCC 6538*), Bacillus subtilis (*ATCC 6633*)* and two clinical fungal isolates i.e. *Aspergillus fumigatus* and *Candida albicans*. The bacterial and fungal strains were preserved at 4°C on nutrient agar and potato dextrose agar (Sigma Aldrich) media, respectively.

The extract was prepared according to Basri and Fan (2005) using aqueous, 99.7% ethanol, and *n*-hexane solvents. After drying the extract was re-dissolved in dimethyl sulfoxide (Sigma Aldrich) to concentration of 250 mg/ml. About 100 µl filtered extract was spread on nutrient agar and potato dextrose agar plate and incubated at 37°C for 48 hrs to check sterility. Amoxicillin sodium powder (100 µg/ml) and Nystatin (10,000 units/ml of sterile distilled water) were used as positive controls for antibacterial and antifungal activities, respectively.

The antibacterial activities were determined by using agar well diffusion method Boakye-Yiadom (1979). A 0.5 McFarland standard solution (10^6 CFU/ml) was used to achieve equal concentration of bacterial strains. Then, each strain was swabbed using sterile cotton swabs, on plates containing equal volume of Mueller Hinton Agar (Sigma Aldrich). The 8 mm wells punched were then filled with 100 µl of respective extracts of deoiled seed kernel, seed coat and fruit coat. All of the plates were left at room temperature for an hour in order to allow diffusion of treatments before proper growth of microorganisms commenced. Afterwards incubated at 37°C and diameters of zones of inhibition (ZI) in mm was measured after 24 hrs. The results presented are averages of duplicate assays.

The fungi (yeast cells and molds) after proper growth on PDA, were harvested and a mixture of sterilized distilled water was poured on the surface of the plate followed by scrapping them with

sterilized glass rod. The harvested yeast and mold were standardized to an OD_{600nm} of 0.1 before use (Igbinosa *et al*. 2009). The standardized yeast and mold suspension (100 µl) were then evenly spread on PDA using sterile glass spreader. Same procedure was used for antifungal assay as that for antibacterial. The plates were incubated at 30°C for 72 hrs and observed for ZI.

The minimum inhibitory concentration (MIC) of the extracts that exhibited antimicrobial activities against selected microorganisms was determined. All of the extracts were diluted four times by two-folds dilution in DMSO. The suspensions of the microorganisms were prepared. A 100 µl of each dilution was added into their respective well, left for one hour to diffuse properly and were incubated at their respective temperature at 37°C for 24 hrs in case of bacterial and at 30°C for 72 hrs in case of fungal strains. The MIC was considered as the lowest concentration of a given extract added in the plate at which no growth of microorganisms occurred and was evident from the zone of inhibition.

The antibacterial and antifungal tests for each treatment were carried out in duplicate. Mean value for ZI and standard deviation was calculated. One-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparison were applied to compare the antimicrobial activities of samples collected from Peshawar and Bannu districts (using GraphPad Prism Software).

Results and Discussion

Antimicrobial activities of aqueous, ethanolic and *n*-hexane extracts of two geographically different samples of *Jatropha curcas* fruit against selected strains were evaluated. *J. curcas* has ethno medicinal importance and its biological properties have been extensively investigated. Alkaloids, anthraquinones, flavonoids, saponins and tannins have antimicrobial potential and most of them are present in different parts of *J. curcas* which traditionally suggest their use in curing various illnesses that include coated tongue, dysentery, infertility, gonorrhoea, haemorrhoids, skin infections, inflammation etc. (Hassan *et al*. 2004). Table 1 shows antibacterial activities of deoiled seed kernel, seed coat and fruit coat extracts, from both samples of *J. curcas.* The extracts of both samples i.e. *J. curcas* fruits collected from districts of Peshawar and Bannu, had shown the antimicrobial activities. The antimicrobial activities exhibited by Peshawar sample was higher than that of Bannu. Although the antimicrobial activity varies from strain to strain, the antimicrobial activities of deoiled seed kernel of both samples were highest compared to other parts evaluated. Among these, ethanolic extracts showed a broader range of activity, while aqueous and *n*-hexane extracts showed relatively narrow range of activities against selected test microorganisms. Ethanol as a solvent has capability to dissolve a broad range of organic compounds such as saponins, tannins, flavonoids and steroids; therefore, showing higher potency compared to aqueous and *n*-hexane extracts which is in agreement with results of Al-Bayati and Al-Mola (2008), Penecilla and Magno (2011), Kusuma *et al*. (2017). Highest zone of inhibition 31.5 ± 0.42 mm was observed by *n*-hexane extract of deoiled seed kernal^{P} against *B. subtilis* (ATCC 6633) followed by *n*-hexane extract of deoiled seed kernal^{P} against *K. pneumoniae (*ATCC 43816*)* and ethanolic extract of seed coat^{P} against *E. coli* (ATCC 10536) both with ZI of 28 ± 0.28 mm and aqueous extract of fruit coat^{P} against *S. aureus* (ATCC 6538) with ZI of 23.5 ± 0.7 mm. On the contrary, samples from Bannu exhibited maximum activity at ZI of 20 ± 0 mm for ethanolic extract of fruit coat^{B} against *S. aureus* (ATCC 6538) followed by 19 ± 1.41 mm of *n*-hexane extract against *K. pneumoniae* (ATCC 43816) and aqueous extract of seed kernel^{B} with ZI of 18 ± 0.28 mm against *B. subtilis* (ATCC 6633). On the whole, ethanolic extracts have shown a broad range of potency compared to others (Table 1). These results are in agreement with the

previous studies by Suo and Wang (2010). The values of respective negative controls have been subtracted from the values of extracts.

Table 1. Comparison of antibacterial activities of *J. curcas* fruits collected from the districts of Peshawar and Bannu.

Plant extract	Extracts and standard	Zone of inhibition in mm ± standard deviation			
		Escherichia coli (ATCC 10536)	*Staphylococcus aureus* (ATCC 6538)	*Klebsiella pneumoniae* (ATCC 43816)	*Bacillus subtilis* (ATCC 6633)
Seed kernel^{P}	Aqueous	-	27.7 ± 0.56	19.1 ± 1.27	-
	Ethanolic	16.25 ± 0.07	21.65 ± 1.9	25.6 ± 0.56	15.5 ± 0.4
	n-hexane	13.65 ± 0.77	25.15 ± 1.2	28 ± 0.28	31.5 ± 0.42
Seed coat^{P}	Aqueous	-	21 ± 1.13	19 ± 1.41	-
	Ethanolic	28 ± 0.28	27.5 ± 0.7	20 ± 0	16 ± 0.28
	n-hexane	-	13 ± 1.41	-	-
Fruit coat^{P}	Aqueous	-	23.5 ± 0.7	22 ± 0.7	-
	Ethanolic	16 ± 0.28	17 ± 1.41	16 ± 1.41	12 ± 1.41
	n-hexane	-	19.5 ± 0.7	20 ± 1.41	17.5 ± 0.7
Deoiled seed kernel^{B}	Aqueous	10 ± 0	13.6 ± 0.56	-	18 ± 0.28
	Ethanolic	13.6 ± 0.5	11.55 ± 0.63	12 ± 0.14	12 ± 0
	n-hexane	15.05 ± 0.07	13 ± 1.13	15.5 ± 0.28	12 ± 0.28
Seed coat^{B}	Aqueous	10 ± 0	14 ± 0.42	11.5 ± 0.7	13.5 ± 0.7
	Ethanolic	14 ± 0	14.5 ± 0.7	13 ± 0	14.5 ± 0.7
	n-hexane	11.5 ± 0.7	14 ± 0.14	10.5 ± 0.7	10.15 ± 0.07
Fruit coat^{B}	Aqueous	-	19 ± 1.4	17 ± 1.41	-
	Ethanolic	11 ± 0.28	20 ± 0	15.5 ± 0.7	10 ± 0.28
	n-hexane	11.5 ± 0.7	18.5 ± 0.7	19 ± 1.41	14 ± 0.28
Standard	Amoxicillin Sodium	43.15 ± 0.07	42.2 ± 0.14	41.15 ± 0.07	42 ± 0.14

^{P}Shows samples collected from Peshawar, ^{B}Shows samples collected from Bannu district, - Indicates no inhibition; values are mean ± Sd (standard deviation) of two replicates.

The antifungal activities of both samples slightly varied. All the extracts of the fruit coat collected from districts of Peshawar and Bannu had antifungal activity against the selected strains. The ethanolic extract of seed kernel^{P} showed highest zone of inhibition 16 ± 0 mm against *A. fumigatus* followed by 15.5 ± 0.7 mm of ethanolic extract of fruit coat^{B} against *A. fumigatus* and 14.5 ± 0.7 mm by ethanolic extracts of seed kernel^{B} against *C. albicans* as shown in Table 2. These results are in conformity with previous studies (Al-Bayati and Al-Mola 2008 and Penecilla and Magno 2011). Different classes of secondary metabolites present in medicinal plants are responsible for antimicrobial activities (Salem *et al.* 2011 and Agarwal *et al.* 2012). These variations in antimicrobial activities of *J. curcas* species may be attributed to environmental factors or nutritional conditions of plants such as average annual temperature of area, amount of sunlight in different hours of the day, height from sea level, quality of soil, per cent precipitation, amount of humidity and various physico-chemical parameters; these conditions greatly influence secondary metabolite production, this observation is in close agreement with previous findings (Jamshidi *et al.* 2009, Akula and Ravishankar 2011, Abdollahi *et al.* 2012). Similar study was also

conducted by Paul *et al*. (2016) stating that concentration of tannins, alkaloids, total phenols, flavonoids, combined and free anthraquinone varies in *J. curcas* plants with climatic conditions resulting in varied antimicrobial activities.

Table 2. Comparison of antifungal activities of *J. curcas* fruits collected from the districts of Peshawar and Bannu.

Plant extract	Extracts and standard	Zone of inhibition in mm ± standard deviation		Plant extract	Extracts and standard	Zone of inhibition in mm ± standard deviation	
		Candida albicans	*Aspergillus fumigatus*			*Candida albicans*	*Aspergillus fumigatus*
Deoiled seed kernel^{P}	Aqueous	-	10 ± 0	Deoiled Seed kernel^{B}	Aqueous	-	-
	Ethanolic	13 ± 0.7	16 ± 0		Ethanolic	14.5 ± 0.7	13 ± 0
	n-hexane	-	-		*n*-hexane	-	-
Seed coat^{P}	Aqueous	-	-	Seed coat^{B}	Aqueous	-	10 ± 1.41
	Ethanolic	10.1 ± 0.07	12 ± 1.41		Ethanolic	13.5 ± 0.7	13 ± 0.28
	n-hexane	-	12.5 ± 0.7		*n*-hexane	-	12.5 ± 0.7
Fruit coat^{P}	Aqueous	10 ± 0.28	12.5 ± 0.7	Fruit coat^{B}	Aqueous	12.5 ± 0.7	14.5 ± 0.7
	Ethanolic	11.5 ± 0.7	13 ± 1.41		Ethanolic	14 ± 0.41	15.5 ± 0.7
	n-hexane	13 ± 0.42	11.5 ± 0.7		*n*-hexane	12.5 ± 0.7	13.5 ± 0.7
Standard	Nystatin	19 ± 0.28	17.2 ± 0.28	Standard	Nystatin	19 ± 0.28	17.2 ± 0.28

^{P}Indicates samples collected from Peshawar district, ^{B}Indicates samples collected from Bannu district, - Indicates no inhibition; values are mean ± Sd (standard deviation) of two replicates.

Table 3. Comparison of minimum inhibitory concentration of deoiled seed kernel collected from the districts of Peshawar and Bannu.

Extracts	MIC (mg/ml)					
	Staphylococcus aureus (ATCC 6538)	*Klebsiella pneumoniae* (ATCC 43816)	*Escherichia coli* (ATCC10536)	*Bacillus subtilis* (ATCC 6633)	*Aspergillus fumigatus*	*Candida albicans*
Aqeous^{P}	125	125	NA	NA	125	NA
Aqeous^{B}	NA	125	NA	62.5	NA	NA
n-hexane^{P}	125	125	125	125	NA	NA
n-hexane^{B}	125	125	125	62.5	NA	NA
Ethanolic^{P}	31.25	62.5	NA	62.5	NA	62.5
Ethanolic^{B}	125	62.5	125	NA	NA	125

^{P}Shows sample collected from Peshawar, ^{B}Shows sample collected from Bannu, NA: No activity.

The minimum inhibitory concentration (MIC) of all extracts varied against selected strains. In case of MIC of deoiled seed kernel, ethanolic extracts of deoiled seed kernel^{P} showed lowest MIC (31.25 mg/ml) against *S. aureus* (ATCC 6538). On the contrary, the MIC of deoiled seed kernel^{B} ethanolic extracts was 62.5 mg/ml against *K. pneumoniae* (ATCC 43816) and *B. subtilis* (ATCC 6633). The MIC of aqueous and *n*-hexane extracts of deoiled seed kernel is presented in Table 3. Similarly, for seed coat, ethanolic extracts of seed coat^{P} showed lowest MIC of 31.25 mg/ml

against *S. aureus* (ATCC 6538) and *K. pneumoniae* (ATCC 43816) followed by *n*-hexane extract of seed coat^{B} that was 62.5 mg/ml against *Bacillus subtilis* (ATCC 6633) and *A. fumigatus* (Table 4). Furthermore, the MIC of ethanolic extracts of fruit coat^{P} was found to be 62.5 mg/ml against *S. aureus* (ATCC 6538) followed by *K. pneumoniae* (ATCC 43816) and *C albicans*. In case of fruit coat^{B}, 62.5 mg/ml was the lowest MIC value recorded for ethanolic and *n*-hexane extracts against *K. pneumoniae* (ATCC 43816) and *C. albicans* (Table 5). Higher MIC was observed for bacteria, which were resistant to high concentration of extracts. The ethanolic extract of seed kernel^{P} as well as seed coat^{P} showed lowest MICs. For Bannu sample, the lowest MIC was represented by the ethanolic as well as *n*-hexane extract. The MIC results indicate that overall, ethanolic extracts were most potent followed by n-hexane and aqueous extracts. The antimicrobial activities of all three parts of *J. curcas* fruit indicate high potential of broad-spectrum antimicrobial compounds.

Table 4. Comparison of minimum inhibitory concentration of seed coat collected from the districts of Peshawar and Bannu.

Extracts	MIC (mg/ml)					
	Staphylococcus aureus (ATCC 6538)	*Klebsiella pneumoniae* (ATCC 43816)	*Escherichia coli* (ATCC10536)	*Bacillus subtilis* (ATCC 6633)	*Aspergillus fumigatus*	*Candida albicans*
Aqeous^{P}	NA	NA	NA	NA	NA	NA
Aqeous^{B}	NA	NA	NA	125	NA	NA
n-hexane^{P}	NA	NA	NA	NA	62.5	NA
n-hexane^{B}	NA	125	125	62.5	62.5	NA
Ethanoli^{P}	31.25	31.25	NA	125	125	NA
Ethanolic^{B}	125	125	NA	62.5	NA	125

^{P}Shows sample collected from Peshawar, ^{B}Shows sample collected from Bannu, NA: No activity.

Table 5. Comparison of minimum inhibitory concentration of fruit coat collected from the districts of Peshawar and Bannu.

Extracts	MIC (mg/ml)					
	Staphylococcus aureus (ATCC 6538)	*Klebsiella pneumoniae* (ATCC 43816)	*Escherichia coli* (ATCC 10536)	*Bacillus subtilis* (ATCC 6633)	*Aspergillus fumigatus*	*Candida albicans*
Aqeous^{P}	125	62.5	NA	NA	62.5	NA
Aqeous^{B}	125	125	NA	NA	NA	NA
n-hexane^{P}	NA	62.5	NA	NA	62.5	NA
n-hexane^{B}	125	62.5	NA	NA	NA	NA
Ethanolic^{P}	62.5	125	NA	NA	NA	62.5
Ethanolic^{B}	125	62.5	125	NA	NA	62.5

^{P}Shows sample collected from Peshawar, ^{B}Shows sample collected from Bannu, NA: No activity.

Based on above findings, it may be clearly stated that geographical divisions, environmental factors and nutritional conditions have effects on the antimicrobial activities of medicinal plants. Therefore, both samples of *Jatropha curcas* fruit showed variations in their activities against selected microorganisms. Also these finding indicate that further research is necessary to resolve the impact of climate on the production of biological active compounds.

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