Evaluation of antibacterial effects of *Melia azedarach* fruit extracts against some isolated pathogenic bacteria

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Abstract

In ancient times plants have been a great source of medicine. Many of traditionally known plants have been extensively studied by advanced scientific techniques and reported for various medicinal properties such as anticancer, antinflammatory, antidiabetic, anthelmintic, antibacterial, antifungal, hepatoprotective, antioxidant, larvicidal. The aim of the present study was to evaluate the antibacterial effects of methanolic and aqueous extracts of *Melia azedarach* fruit against isolated strains of *Pseudomonas aeruginosa*, *E. coli, Staphylococcusaureus, Bacillussubtilis,* Proteus and Klibsiella using well and disc diffusion methods. Both extracts established significant antibacterial activity against tested bacteria. In well and disc methods, *Staphylococcus aureus, Pseudomonas aeruginosa*, Klibsiella and Proteus showed sensitivity at significant level to aqueous extract of Melia azedarach fruit. In comparison between two methods well and disc *E. coli* was significantly different at 50 mg/mL. and *Pseudomonas aeruginosa* at 100 and 200 mg/mL. *Staphylococcus aureus* and Proteus showed a significant difference in sensitivity to alcohol extract of M. azedarach fruit in well method. In disc method *Pseudomonas aeruginosa* and Proteus showed a significantly different sensitivity to alcohol extract of M. azedarach fruit. In comparison between well and disc method no significant difference was observed on alcohol extract of Melia azedarach. In comparison between alcohol and aqueous extract no significant difference was observed in both well and disc methods.

Introduction

All parts of *Melia azedarach* L. are found to possess numerous medicinal properties such as antioxidant,[1,2] antibacterial.[3] The antibacterial potential of M. Azedarach L. was tested using crude leave, flower and fruits seed extracts against pathogenic bacteria strains.[3] Aim of this study was to evaluate antibacterial effects of Melia azedarach fruit extracts against some isolated pathogenic bacteria.

Materials and Methods

Melia azedrach fruits were collected from different sites of Baqubah, Diyala, Iraq. The fruit crushed and grind to a powder. Of powder 25 g of plant fruit seed were filled in the thimble and extracted successively with 300 mL of methanol using a Soxhlet extractor for 72 hours. The extract was concentrated using rotary evaporator, after complete solvent evaporation. The solvent extract was weighed and preserved at 4°C in airtight bottles until use. The powder of plant fruit seed were mixed thoroughly with 300 mL of distilled water in a beaker on magnetic stirrer for 24 h. Then filtered through Whatman No.1 filter paper. The extract was concentrated using rotary evaporator, after complete solvent evaporation. The solvent extract was weighed and preserved at 4°C in airtight bottles until use.

In the study of the antibacterial activity, both extracts were diluted in dimethyl sulfoxide (DMSO). The concentrations used in this experiment were 50, 100, and 200 mg/mL.

Antimicrobial activity was tested using a modified disc diffusion assay (DDA) method originally described by Bauer[4] and Ncube and colleagues.[5] The inoculums for each microorganism were prepared from broth culture (10^5 CFU/mL). A loop of culture from the nutrient agar (NA) slant stock was cultured in Luria-Bertani (LB) medium over night and spread with a sterile swab into Petri-plates. Sterile disc (6 mm) impregnated with the plant extracts (50, 100 and 200 mg/mL) were placed on the cultured plates and incubated for 24 h at 37°C. The solvent loaded disc without extracts in it served as control in the study. Clear inhibition zones around discs indicated the presence of antimicrobial activity. All data on antimicrobial activity were average of triplicate. Antimicrobial activities of both extracts were evaluated by the agar well diffusion method. For agar well diffusion method,[6,7] antimicrobial susceptibility was tested on solid agar – agar media in petri plates. Nutrient agar (NA) plates were swabbed (Sterile cotton swabs) with 8 hours old – broth culture of respective bacteria. Well (10 mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Stock solution of fruit extracts was prepared at a concentration of 50 mg/mL, 100; 200 mg/mL in aqueous and methanolic extracts. About 100 µL of different concentration of plant solvents extracts were added sterile syringe into the wells and allowed to diffuse at room temperature for 2h.

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Key words: Melia azedarach; Well diffusion; Disc diffusion; Methanol extract; Aqueous extract.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 16 June 2015.
Revision received: 30 July 2015.
Accepted for publication: 5 August 2015.

Veterinary Science Development 2016; 6:6080
doi:10.4081/vsd.2016.6080

The plates were incubated at 37°C for 24 h. The diameter of the inhibition zone (mm) was measured triplicate were maintained and the experiment was repeated thrice, for each replicates the reading were taken in three different fixed directions and the average values were recorded.

The following bacterial strains were used as test organisms: Staphylococcus *aureus, Bacillus subtilis, E. coli, Pseudomonas aeruginosa, Klibsiella* and Proteus. All the bacterial strains were obtained from Department of Microbiology, College of Veterinary Medicine, University of Diyala, Iraq.

All values are expressed as the mean ± the standard error of the mean (SEM). The data were analyzed by using one way analysis of variance ANOVA, and then the test of the least significant differences between the means of inhibitory zones.[8] The significant level of test was $P<0.05$.

Results

The results revealed that both alcohol and aqueous extracts are potent antimicrobial against all the microorganisms studied.

The results revealed that aqueous extract of *Melia azedarach* fruit in well and disc methods showed no significance in inhibitory zone against *E. coli*. But there was higher significant difference at 50 mg/mL concentration in disc method comparing with well method. Against *Pseudomonas aeruginosa* in well method there was significant difference in inhibition at 200 mg/mL in comparison with 50

mg/mL. While in disc method the inhibitory zone was significance at 100 and 200 mg/mL comparing with 50 mg/mL. When compared well and disc methods there was significant difference at 100, and 200 mg/mL. In case of *Klebsiella* in well method there was significant difference in inhibition at 200 mg/mL in comparison with 50 mg/mL. While in disc method there was significant difference in inhibition at 100 and 200 mg/mL in comparison with 50 mg/mL. In comparison between well and disc method *Klebsiella* did not show any significant difference. While in case of Proteus in well method there were significant difference at 100, and 200 mg/mL in comparison with 50 mg/mL. In disc method there was significant difference at 200 mg/mL in comparison with 50 mg/mL. In comparison between well with disc method Proteus showed no significant difference. Bacillus in well and disc methods did not show any significant difference. In comparison between disc sand well methods also there was no significant difference. Against *staphylococcus aureus* in well method there was significant difference at 100 and 200 mg/mL in comparison with 50 mg/mL. In disc method the significance was at 100 and 200 mg/mL in comparison with 50 mg/mL. In comparison between well and disc method there was no significant difference (Table 1).

The results revealed that in alcohol extract of Melia azedarach fruits showed no significant inhibitory difference against *E. Coli*; *Bacillus subtilis* and Klebsiella in both well and disc methods. In disc method *Pseudomonas aeruginosa* showed a significant inhibitory difference at 200 mg/mL in comparison with 50, and 100 mg/mL. In comparison between well and disc there was no significant

Table 1. Diameter of inhibitory zone in mm of aqueous extract of *Melia azedarach* fruit against isolated pathogenic bacteria.

Bacteria	Well			Disc		
	50 mg/mL	100 mg/mL	200 mg/mL	50 mg/mL	100 mg/mL	200 mg/mL
Escherichia coli	5.17±1.19^{Aa}	6.67±0.67^{a}	6.33±1.02^{a}	8.67±1.74^{aB}	8.50±0.99^{a}	7.33±1.09^{a}
Staphilococcus aureus	6.83±0.75^{a}	11.67±1.80^{b}	14.33±2.32^{b}	5.6±1.18^{a}	10.67±2.33^{b}	13.17±2.20^{b}
Pseudomonas aeruginosa	4.0±0.68^{aA}	4.67±0.71^{aA}	6.67±0.95^{bA}	4.67±0.67^{aA}	7.5±1.06b^{B}	9.67±0.88^{bB}
Klebsiella	6.6±1.47^{a}	8.83±1.99^{a}	12.17±1.81^{b}	7.33±0.80^{a}	11.83±2.24^{b}	12.67±1.80^{b}
Proteus	4.83±0.65^{a}	10.17±2.74^{b}	9.5±2.17^{b}	6.5±1.18^{a}	9.5±1.65^{a}	11.5±1.89^{b}

M±MSE; ^{a,b},significance between concentration; ^{A,B}, significance between methods. The significance was at P<0.05.

Table 2. Diameter of inhibitory zone in mm of alcohol extract of *Melia azedarach* fruit against isolated pathogenic bacteria.

Bacteria	Well			Disc		
	50 mg/mL	100 mg/mL	200 mg/mL	50 mg/mL	100 mg/mL	200 mg/mL
Bacillus subtilis	5.33±1.77^{a}	6.67±1.34^{a}	8.00±1.00^{a}	6.33±2.34^{a}	7.67±3.18^{a}	9.33±2.91^{a}
Staphylococcus aureus	9.33±1.36^{a}	9.83±0.83^{a}	13.33±1.82^{b}	9.17±2.21^{a}	8.17±1.01^{a}	10.67±2.22^{a}
Pseudomonas aeruginosa	5.83±1.38^{a}	5.67±0.84^{a}	8.50±1.48^{a}	6.67±0.67^{a}	7.00±0.58^{a}	8.67±0.34^{b}
Proteus	5.50±1.41^{a}	8.83±1.76^{a}	10.17±1.66^{b}	6.17±1.51^{a}	8.83±1.38^{a}	11.33±1.93^{b}

M±MSE; ^{a,b},significance between concentration; ^{A,B},significance between methods. The significance was at P<0.05.

Table 3. Diameter of inhibitory zone in mm of aqueous and alcohol extracts of *Melia azedarach* fruit against isolated pathogenic bacteria in disc method.

Bacteria	Well			Disc		
	50 mg/mL	100 mg/mL	200 mg/mL	50 mg/mL	100 mg/mL	200 mg/mL
Staphylococcus aureus	5.6±1.18^{a}	10.67±2.33^{b}	13.17±2.20^{b}	9.17±2.21^{a}	8.17±1.01^{a}	10.67±2.22^{a}
Pseudomonas aeruginosa	4.67±0.67^{aA}	7.5±1.06^{b}	9.67±0.88^{b}	6.67±0.67^{aB}	7.00±0.58^{a}	8.67±0.34^{bc}
Klebsiella	7.33±0.80^{a}	11.83±2.24^{b}	12.67±1.80^{b}	8.67±1.43^{a}	11.00±1.71^{a}	11.83±1.45^{a}
Proteus	6.5±1.18^{a}	9.5±1.65^{a}	11.5±1.89^{b}	6.17±1.51^{a}	8.83±1.38^{a}	11.33±1.93^{b}

M±MSE; ^{a,b},significance between concentration; ^{A,B}, significance between methods. The significance was at P<0.05.

Table 4 diameter of inhibitory zone in mm of aqueous and alcohol extracts of *Melia azedarach* fruit against isolated pathogenic bacteria in well method.

Bacteria	Well			Disc		
	50 mg/mL	100 mg/mL	200 mg/mL	50 mg/mL	100 mg/mL	200 mg/mL
Staphylococcus aureus	5.6±1.18^{a}	10.67±2.33^{b}	13.17±2.20^{b}	9.17±2.21^{a}	8.17±1.01^{a}	10.67±2.22^{a}
Pseudomonas aeruginosa	4.67±0.67^{aA}	7.5±1.06^{b}	9.67±0.88^{b}	6.67±0.67^{aB}	7.00±0.58^{a}	8.67±0.34^{bc}
Klebsiella	7.33±0.80^{a}	11.83±2.24^{b}	12.67±1.80^{b}	8.67±1.43^{a}	11.00±1.71^{a}	11.83±1.45^{a}
Proteus	6.5±1.18^{a}	9.5±1.65^{a}	11.5±1.89^{b}	6.17±1.51^{a}	8.83±1.38^{a}	11.33±1.93^{b}

M±MSE; ^{a,b},significance between concentration; ^{A,B}, significance between methods. The significance was at P<0.05.

difference in sensitivity of *Pseudomonas aeruginosa* against alcohol extract of *Melia azedarach* fruit. Against Proteus in well and disc methods, there was significant inhibitory difference at 200 mg/mL in comparison with 50 mg/mL. In comparison between well with disc method there was no significant difference against *staph aureus* there was significant inhibitory difference at 200 mg/mL in comparison with 50 mg/mL in disc method (Table 2).

In comparison between alcohol and aqueous extract, the results revealed that in case *E. coli*; Proteus; Bacillus and *Staphylococcus aureus* none. In case of *Klibsiella* in well method none. In disc method significant at 50 mg/mL. In case of *Pseudomonas aeruginosa* in disc method at 50 mg/mL (Tables 3 and 4).

Discussion

Among the different solvents extracts studied methanol and ethanol showed high degree of inhibition followed by petroleum ether and aqueous extract.[9] Methanol extract showed maximum inhibition zone in *E. coli* and *B. cereus*, more specifically, aqueous extract represented higher susceptibility to all bacterial strains.[9]

Dichloromethane leaf extract of *M. azedarach* was found to be more effective against gram positive than gram negative bacterial strains, while ethanol extract inhibited the growth of three gram positive and two gram negative strains, ethyl acetate, methanolic fraction and aqueous extract were found to be effective against all tested bacterial strains.[10]

Conclusions

Results of this study confirm that the fruit extracts showed an antibacterial effect. *E. coli* and *Bacillus* were the least sensitive bacteria *Pseudomonas*, *Staphylococcus*, *Proteus* and *Klibsiella* were the sensitive bacterial strains to *M. azedarach* fruit aqueous and alcohol extract. No differences were observed between two methods well and disc; and between extract aqueous and alcohol.

Results of this study strongly confirm that the fruit extracts could be effective antibacterial compounds.

References

1. Ahmed MF, Ibrahim M, Ahmed MA, et al. Antioxidative activity of Melia azedarach Linn leaf extract. Iranian J Pharmacol Ther 2008;7:31-4.
2. Nahak G, Sahu RK. In vitro antioxidative activity of Azedirachtaindica and Melia azedarach leaves by DPPH scavdengingassay. Am J Sci 2010;6:123-8.
3. Khan AV, Khan AA, Shukla I. In vitro antibacterial potential of Melia azedarach crude leaf extracts against some human pathogenic bacterial strains. Ethnobotanical Leaf 2008;12:439-45.
4. Bauer RW, Kirby MDK, Sherris JC Turk M. Antibiotic susceptibility testing by a standardized single disc method. Am J Cl Pathol 1966;45:493-6.
5. Ncube NS, Afolayan AJ, Okoh A. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr J Biotechnol 2008;7:1797-806.
6. Murray PR, Baron EJ, Pfaller MA, et al. Manual of Clinical Microbiology, 6th ed. Washington DC: ASM press, 1995. pp 15-8.
7. Olurinola PF. A laboratory manual of pharmaceutical microbiology. Abuja, Nigeria: National Institute for Pharmaceutical Research and Development, 1996.
8. Steel RG, Torrie JH. Principles and procedures of statistics, a biometrical approach, 2nd ed. Singapore: McGraw – Ho; 1985. pp 183.
9. Sen A, Batra A. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: Melia azedarach L. Int J Curr Pharm Res 2012;4:67-73.
10. Ramya S, Jepachanderamohan PJ, Alaguchamy N, et al. In vitro antibacterial prospective of crude leaf extracts of Melia azedarach Linn. against selected bacterial strains. Ethanobotanical Leaf 2009;13:254-8.