Pak. j. sci. ind. res. Ser. B: biol. sci. 2012 **55** (1) 35-39

Studies on Antimicrobial and Antifungal Activities of *Ziziphus mauritiana* Against Human Clinical Bacterial and Fungal Pathogens

Adnan Amin[a]*, Swahid Shah[a], Saadia Andaleeb[b], Muhammad Mohibullah Khan[c] and Muhammad Ayaz Khan[a]

[a]Gomal Center of Biochemistry and Biotechnology (GCBB), Gomal University, D.I. Khan, KPK, Pakistan
[b]NCVI, NUST, Islamabad, Pakistan
[c]Department of Plant Breeding and Genetics, Faculty of Agriculture, Gomal University, D.I. Khan, KPK, Pakistan

(received April 20, 2011; revised November 3, 2011; accepted March 2, 2012)

Abstract. The antimicrobial and antifungal activities of crude extracts of *Ziziphus mauritiana* leaves were investigated against six selected bacterial (*Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter*, *Klebsiella pneumoniae*) and one fungal pathogen (*Aspergillus niger*). The crude extract was further fractionated in butanol, choloroform, *n*-hexane and methanol. Agar well diffusion and agar dilution assay were employed for determination of zones of inhibition and MICs, respectively, whereas MBC was determined using broth dilution test. The butanol fraction presented encouraging antimicrobial activity (15.0±0.02), while methanol (7.03±0.05) and chloroform (7.0±0.05) fractions emerged with significantly low susceptibility. The *n*-hexane fraction was recorded as almost inactive (0±0) against all bacterial pathogens. Unlike the antibacterial activities, all fractions possessed momentous antifungal activities except the methanol fraction (0±0). The *n*-hexane fraction showed widest zone of inhibition (11±0.05) followed by butanol (8.0±0.02) and chloroform (7.0±0.02).

Keywords: crude extract, antimicrobial activities, traditional medicine, *n*-hexane fraction, *Aspergillus niger*, *Ziziphus mauritiana*

Introduction

Ziziphus mauritiana Lam (Family Rhamnaceae) is a common medicinal plant in the tropical and subtropical parts of the world. Commonly it is referred as Indian plump, Chinese date (Morton, 1987) or Bair in Pakistan and India. The extract of *Z. mauritiana* is well known for its alkaloids, flavonoids, glycosides, saponins and volatile oil (Dahiru *et al*., 2006), vitamin C, mucilage, protein (Adzu *et al*., 2003), caffeic acid, ferulic acid and *p*-coumaric acid (Muchuweti *et al*., 2005). The *Z. mauritiana* whole plant has an array of uses for management of ample human ailments (Dubey *et al*., 2010), including healing of wounds, ulcers (Adzu *et al*., 2001), hepatic disorders (Dahiru *et al*., 2005; Michel, 2002), pregnancy associated abdominal discomforts (Kaaria, 1998), gouty arthritis (Morton, 1987) and skin infections (Adzu *et al*., 2001), sperm immobilization (Dubey *et al*., 2010), antioxidant (Dahiru and Obidoa, 2008) and antitumor activity (Mishra *et al*., 2011). However, insufficient information is available about its antifungal and antimicrobial activities (Abalaka *et al*., 2010; Mahesh and Satish, 2008). This research intended to determine the antimicrobial and antifungal potential of *Z. mauritiana*.

*Author for correspondence; E-mail: adnan_amin@gu.edu.pk, dani_amin79@yahoo.com

Materials and Methods

Plant material. Fresh plant leaves were collected locally in district D.I.Khan, Khyber Pukhtunkhwa (KPK) Pakistan. Plant identification was established in Department of Pharmacognosy, Faculty of Pharmacy, Gomal University, where the plant specimens were deposited.

Preparation of crude extracts. The plant material (leaves) were collected at early morning hours and subjected to sun shade drying (15-20 days). The leaves were subsequently powdered by Wiley Mill (300 mm mesh). About 200 g of dry powdered leaves were extracted with 300 mL of 95% ethanol using rotary shaker (190-200 rpm) overnight, followed by filtration and concentrating it to one-fifth of the total volume. Afterwards the dried crude extract was prepared by subjecting plant material (10 g) to slow heat (oven) for 6-8 h (Vlietinck and Vanden, 1991).

Preparation of fractions. About 100 g of dried plant material was extracted with *n*-hexane, chloroform, butanol and methanol using soxhlet apparatus. The solvent was evaporated under reduced pressure and the semisolid mass obtained was stored in silica gel beads. (Ieven *et al*., 1979).

Microorganisms. Six clinical bacterial species; *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter*, *Staphylococcus aureus* (methicilline resistant), *Micrococcus luteus* and one fungal strain; *Aspergillus niger* were used for antimicrobial and antifungal assay. These clinical strains were obtained from Pakistan Institute of Medical Sciences (PIMS) Islamabad, Pakistan. The strains were further identified and characterized in microbiology research lab (MRL) Microbiology Department, Quaid-i-Azam University-Islamabad, Pakistan. The strains were maintained on agar slants at 4 C° in Gomal Center of Biochemistry and Biotechnology (GCBB) for susceptibility tests. Microorganisms were incubated overnight at 37 °C in Mueller-Hinton broth (Oxoid) at pH 7.4.

Antimicrobial screening. *Agar well diffusion assay.* The antibacterial activity was determined using agar well diffusion method (Boakye *et al*., 1977). All bacterial cultures were first grown in nutrient broth at 37 °C for 24 h and turbidity of inoculum was matched with McFarland's turbidity standard (0.5×108 cfu/mL). The inocula of the respective bacteria were applied on to the surface of sterilized Muller Hinton agar (Oxoid) plates with a sterilized cotton swab to make certain uniform thickness of bacterial growth after incubation and sterilized cork borer (6 mm diameter) was employed to figure well on agar plates. About 100 µL of each plant extract fraction was applied in respective well and plates were subsequently allowed to stay for 1-2 h at room temperature for proper diffusion. The plates were later incubated at 37 °C for 18-24 h and observed for zone of inhibition (mm). The streptomycin (10 µg) (Oxoid) was used as reference antibiotic.

Determination of minimum inhibitory concentration. The minimum inhibitory concentration (MIC) of the crude extract, defined as the lowest concentration of the sample, that produced visible growth (90%) of a microorganism, was determined by agar dilution method (Mukherjee, 2002; EUCAST, 2000). The sterilized Muller Hinton agar (oxoid) was allowed to cool to 50 °C and about 19 mL of this was added to sterilized test tubes containing 1 mL of different concentration of crude extract. This mixture was thoroughly mixed and poured into sterilized petri plates. The concentrations of the extracts used in this test ranged from 30 mg to 0.007 mg/mL. The microbial suspension with density adjusted to 0.5 McFarland turbidity standard were inoculated (0.05 µL) onto the series of agar plates using micropipette. The plates were thereafter incubated at 37 °C for 24 h.

Determination of minimum bactericidal concentration. Minimum inhibitory concentration (MBC) of the selected plant parts was calculated by the viable cell count method (Toda *et al*., 1989), and the results were expressed as number of viable cells as a percentage of the control.

Screening for antifungal activity. The requisite amount of fungal strain (*A. niger*) was suspended in 2 mL of Sabauraud dextrose broth. This suspension was consistently stretched on petri plates containing Sabauraud dextrose agar media using sterilized cotton swabs. Wells were prepared using sterilized cork borer (6 mm). The crude plant extracts (100 µL) were applied into the wells using micropipette and incubated at 25 °C for 3 days. The plates were then examined for zones of inhibition around each well. Itraconazole (30 mg/mL) was used as a positive control.

Results and Discussion

The antimicrobial resistance pattern of all human clinical bacterial and fungal pathogens was determined (Table 1) that are admissible of multidrug resistance (MDR). The results obtained in the present study manifested that the butanol fraction showed a considerable antimicrobial activity against *M. luteus* and possessed very little or no activity against other bacterial strains. Conversely the methanol and chloroform fractions proclaimed proportionately ailing antimicrobial activities against *M. luteus* but little or no activity against other bacterial strains. None of crude fractions were reported as active against *E. coli* and *P. aeruginosa* (Table 2). With the analogous susceptibility pattern, consequent MIC and MBC of all fractions were disseminated as significantly high (15-30 mg/mL) (Table 3-4).

The data pertaining to susceptibility of all four crude extract fractions of *Z. mauritiana* leaves against *A. niger* affirmed potential antifungal activities, with the exception of the methanol fraction (0.00 mm). The *n*-hexane fraction demonstrated aberrant zones of inhibition (11±0.05) at concentration of 30 mg/mL followed by butanol (8.0±0.02) and chloroform (7±0.02) fraction (Table 5).

The bacterial and fungal infections are threatening human health around the globe regardless of the emergence of new therapeutic regimens to address such infections (Youngabi *et al*., 2000). People all over the world are spinning back towards the use of medicinal plants to manage the situation anticipated by development of resistance in microorganism. Like many medicinal plants numerous therapeutic claims are associated with *Ziziphus* species that is an integral part in Ayuveda (Dafni *et al*., 2005).

During present investigation the susceptibility test outcomes established the butanol fraction as most active fraction in comparison with other fractions (hexane, chloroform and methanol) against all clinical strains whose resistance pattern has not been established previously. Nearly all fractions exhibited placid activity against *M. luteus* while very little or no activity was observed against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Enterobacter* and *S. aureus* (methicillin resistant). These provisions are not surprising in analogy with earlier reports (Abalaka *et al*., 2010; Mahesh and Satish, 2008). Nevertheless, the non responsiveness of majority of clinical strains to crude leaf fractions is an apparent manifestation of sprite of resistance among clinical isolates (Bouchillon *et al*., 2004) as apparent from resistance pattern of clinical strains (Table 1). The consequent bactericidal and bacteriostatic concentrations (MIC and MBC) reported were radically higher against and considered as indicators of presence of less potent active constituents in crude leaf fractions (Adamu *et al*., 2000). These observations limit the use of

Table 1. Resistance pattern of human clinical bacterial and fungal pathogens

Microorganism	Resistance pattern
Bacterial pathogen	
Staphylococcus aureus	MET,AML,ATM,AMC,CRO,CIP,CE,
Micrococcus luteus	MET,AML,FEP,STP,ATM,AMC,CRO
Escherichia coli	AML,FEP,STP,AMC,CRO,CE,ATM,MET
Pseudomonas aeroginosa	AML,STP,FEP,ATM,AMC,CRO
Enterobacter	STP,ATM,AMC,CRO,MET
Klebsiella pneumoniae	AML,STP,FEP,ATM,AMC,CRO,CE
Fungal pathogen	
Aspergillus niger	KET, MY

AMC = amoxicillin/clavulanic acid; AML = amoxicillin; ATM = azteronam; CE = cephradine; CIP = ciprofloxacin; CRO = ceftrioxone; FEP = cefepime; KET = ketoconazole; MET = methicillin; MY = myconazole; STP = streptomycin

Table 2. Zone of inhibition of *Z. mauritiana* crude extract fractions against microorganisms

Fraction	Ec	Ps	Kp	Ent	Sta	Ml
	Zone of inhibition (mm)					
Methanol	na	na	na	na	na	7.03±(0.05)
Butanol	na	na	2.03±(0.05)	2.0±(0.05)	2.0±(0.05)	15.0±(0.02)
n-Hexane	na	na	na	2.0±(0.05)	2.03±(0.05)	2.0±(0.11)
Chloroform	na	na	2.06±(0.1)	2.0±(0.05)	na	7.0±(0.05)
Streptomycin	9.0±(0.1)	9±(0.0.5)	11±(0.1)	10±(0.1)	12±(0.1)	12±(0.1)

Ec = *E. coli*; Ps = *P. aeruginosa*; Kp = *K. pneumoniae*; Ent = *Enterobacter*; Sta = *S. aureus*; Ml = *M. luteus* (methicilline resistant); na = not active

Table 3. Minimum inhibitory concentrations (MIC) of *Z. mauritiana* fractions

Fraction	Ec	Ps	Kp	Ent	Sta	Ml
			Microorganism/MIC (mg/mL)			
Methanol	na	na	na	na	na	30±(0)
Butannol	na	na	30±(0)	30±(0)	30±(0)	15±(0)
n-Hexane	na	na	na	>30±(0)	30±(0)	30±(0)
Chloroform	na	na	>30±(0)	>30±(0)	na	30±(0)
Streptomycin	0.93±(0)	0.93±(0)	0.46±(0)	0.46±(0)	0.93±(0)	0.46±(0)

Ec = *E. coli*; Ps = *P. aeruginosa*; Kp = *K. pneumoniae*; Ent = *Enterobacter*; Sta = *S. aureus*; Ml = *M. Luteus* (methicilline resistant); na = not active

Table 4. Minimum bactericidal concentrations (MBC) of *Z. mauritiana* fractions

Fraction	Ec	Ps	Kp	Ent	Sta	Ml
			Microorganism/MBC (mg/mL)			
Methanol	na	na	na	na	na	>30±(0)
Butannol	na	na	>30±(0)	>30±(0)	>30±(0)	15±(0)
n-Hexane	na	na	na	>30±(0)	>30±(0)	>30±(0)
Chloroform	na	na	>30±(0)	>30±(0)	na	>30±(0)
Streptomycin	3.72±(0)	3.72±(0)	1.84±(0)	1.84±(0)	3.72±(0)	1.84±(0)

Ec = *E. coli*; Ps = *P. aeroginosa*; Kp = *K. pneumoniae*; Ent = *Enterobacter*; Sta = *S. aureus*; Ml = *M. luteus* (methicilline resistant)

Table 5. Antifungal activities of *Z. mauritiana* fractions against *A. niger*

Fraction	Zones of inhibition (mm)
Methanol	0.00
Butannol	8.0±(0.02)
n-Hexane	11±(0.05)
Chloroform	7±(0.02)
Itraconazole	12±(0.05)

Z. mauritiana leaf extract confronting multiresistant bacterial pathogens (Bouchillon *et al*., 2004).

A peer literature review reveals little knowledge covering antifungal activities of *Ziziphus* species (Mahesh and Satish, 2008). Conversely unlike inadequate antibacterial activities, nearly all fractions of *Z. mauritiana* L. illuminated comparatively enhanced antifungal activities with exception of methanol fraction. These findings are comparatively better than earlier reports (Abalaka *et al*., 2010; Mahesh and Satish, 2008; Adamu *et al*., 2006).

Conclusion

The findings of present research ascertain the limited medicinal potential of *Z. mauritiana* against human bacterial and fungal pathogens.

References

Abalaka, M.E., Daniyan, S.Y., Mann, A. 2010. Evaluation of the antimicrobial activities of two *Ziziphus* species (*Ziziphus mauritiana* L. and *Ziziphus spinachristi* L.) on some microbial pathogens. *African Journal Pharmacy and Pharmacology*, **4:** 135-139.

Adamu, H.M., Abayeh, O.J., Ibok, N.N.E., Kafu, S.E. 2006. Antifungal activities of extracts of some cassia detarium and *Zizipus* species against dermatophytes. *Natural Products Radiance*, **5:** 357-360.

Adamu, H.M., Bilar, A., Odis, M., Matchave, C. 2000. Antimicrobial activity and phytochemical screening of some selected medicinal plants in Buchi (Nigeria). *Journal of Economics and Taxonomic Botany*, **24:** 123-127.

Adzu, B., Amos, S., Amizan, M.B., Gamaniel, K. 2003. Evaluation of the antidiarrhoeal effects of *Ziziphus* spina-christi stem bark in rats. *Acta Tropica*, **87:** 245-250.

Adzu, B., Amos, S., Wambebe, C., Gamaniel, K. 2001. Anti nociceptive activity of *Ziziphus* spinachristi root bark extract. *Fitoterapia*, **72:** 334-350.

Boakye, Y.K., Fiagbe, N., Ayim, J. 1977. Antimicrobial properties of some west African medicinal plants. *Journal of Natural Product*, **40:** 543-545.

Bouchillon, S.K., Jonson, B.M., Hoban, D.J., Johnson, J.L., Dowzicky, M.J., Wu, D. H., Visalli, M.A., Bradford, P.A. 2004. Determining incidence of extended spectrum β-lactamase producing *Enterobacteriaceae*,vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* in 38 centers from 17 countries: the PEARLS Study 2001-2002. *International Journal of Antmicrobial Agents*, **24:** 119-124.

Dafni, A., Levy, S., Lev, E. 2005. The ethnobotany of Christ's Thorn Jujube (*Ziziphusspina-christi*) in Israel. *Journal of Ethnobiology and Ethnomedicine*, **1:** 8.

Dahiru, D., Obidoa, O. 2008. Evaluation of the antioxidant effects of *Ziziphus mauritiana* Lam. Leaf extracts against chronic ethanol induced hepatotoxicity in rat liver. *African Journal Traditional Complementary and Alternative Medicine*, **5:** 39-45.

Dahiru, D., Sini, J., Jhon, M., John, A.L. 2006.

Anti-diarrhoeal activity of *Ziziphus mauritiana* root extract in rodents. *African Journal of Biotechnology*, **5:** 941-945.

Dahiru, D., William, E.T., Nadro, M.S. 2005. Protective effect of *Ziziphus mauritiana* leaf extract on carbon tetrachloride-induced liver injury. *African Journal of Biotechnology*, **4:** 1177-1179.

Dubey, R., Kushagra, D., Sridhar, C., Jayaveera, K.N. 2010. Sperm immobilization activity of aqueous, methanolic and saponins extract of bark of *Ziziphus mauritiana*. *Der Pharmacia Sinica*, **1:** 151-156.

EUCAST, 2000. Determination of minimum inhibitory concentrations (MICs) of antimicrobial agents by agar dilution. *EUCAST Definitive Document, E*., **6:** 509-515.

Ieven, M., Berghe, V., Mertens, D.A., Vlietinck, F., Lammens, A.E. 1979. Screening of higher plants for biological activities. *Planta Medica*, **36:** 311-321.

Kaaria, I. 1998. Seed production, dispersal and germination in *Cryptostegia grandifolia* and *Ziziphus mauritiana*, two invasive shrubs in tropical woodlands of Northern Australia. *Australian Journal of Ecology*, **21:** 324-331.

Mahesh, B., Satish, S. 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World Journal of Agricultural Science*, **4:** 839-843.

Michel, A. 2002. *Tree, Shrub and Liana of West African Zone*. 440 pp., Margraf Publishers GMBH, Paris, France.

Mishra, T., Madhu, K., Aruna, B. 2011. Anticancer potential of aqueous ethanol seed extract of *Ziziphus mauritiana* against cancer cell lines and ehrlich ascites carcinoma. *Evidance based complementry and Alternative Medicine*, **6:** 1-11.

Morton, J. 1987. Indian Jujube. In: *Fruits of Warm Climates*. J. F. Morton (ed.), Miami, Florida. 272-275. Last updated: 23/4/2004. Accessed on 5/5/2004 at http://www. Indian Jujube. htm.

Muchuweti, M., Gretchen, Z., Ashwell, R.N., Abisha, K. 2005. Sugars, organic acid and phenolic compounds of *Ziziphus mauritiana* Fruit. *European Food Research and Technology*, **221:** 570-574.

Mukherjee, P.K., 2002. *Quality Control of Herbal Drugs, an Approach to Evaluation of Botanicals*. 256 pp., *Business Horizons*, New Delhi, India.

Toda, M., Okubo, S., Hiyoshi, R., Shimamura, T. 1989. The bactericidal activity of tea and coffee. *Letters in Applied Microbiology*, **8:** 123-125.

Vlietinck, A.J., Vanden, B.D.A. 1991. Can ethnopharmacology contribute to the development of antiviral drugs. *Journal of Ethnopharmacology*, **32:** 141-153.

Youngabi, K.A., Dhukku, U.H., Agho, M.O., Chindo, I.H. 2000. Studies on the antifungal properties of *Urtica dioica* urticaceae (stinging nettle). *Journal of Phytomedicine and Therapeutics*, **5:** 39-43.