Evaluation of Antibacterial Activity of *Euryale ferox* Salisb., a Threatened Aquatic Plant of Kashmir Himalaya

Javid Ahmad Parray1* • Azra N. Kamilli1 • Raies Qadri2 • Rehana Hamid3 • Jaime A. Teixeira da Silva4

1 Department of Environmental Science, University of Kashmir, Srinagar-190006, J and K, India
2 Department of Biotechnology, University of Kashmir, Srinagar-190006, J and K, India
3 Department of Botany, Jamia Hamdard, New Delhi, 1100621 India
4 Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Miki-cho, Ikenobe 2393, Kagawa-ken, 761-0795, Japan

**Corresponding author:** javid06@gmail.com

**Keywords:** inhibition zones, methanol, MIC, Nymphaceae, seed extract

**Abbreviations:** CFU, colony forming unit; GNB, Gram-negative bacilli; GPC, Gram-positive cocci; IZD, inhibition zone diameter; MIC, minimum inhibitory concentration

**INTRODUCTION**

*Euryale ferox*, commonly known as makhana, is an annual aquatic medicinal plant that belongs to the family Nymphaceae. It is widely distributed throughout Russia, Korea, China, Japan, Bangladesh and India (Wu and Raven 1994). It grows wild in Kashmir and is a non-endemic threatened aquatic species (Khan 2000; Dar et al. 2002; Khan et al. 2004). The plant produces starchy seeds and is edible. Traditionally the plant has been used throughout the world to cure many diseases including chronic diarrhoea, kidney problem, leucorrhea and spleen hypofunction (Brown 1995). The plant as a whole is used as an analgesic, aphrodisiac, astringent, deobstruent, oxytonic and tonic in China (Duke 1985). Leaves are used in difficult parturition (Shankar 2010). The plant is internally taken to treat vaginal discharge, impotency, premature and involuntary ejaculation and nocturnal emission (Brown 1995). In China this plant is cultivated for its stem, rhizomes and seeds (Sturtevant 1972). In the Indian traditional system of medicines the dry seeds of the plant are being used as an immunostimulant for mothers after childbirth and invalids with a relatively poor immune status (Puri et al. 2000). Various aspects of significant antioxidant activity have been evaluated in total extracts and fractions derived from *E. ferox*. The plant has also been shown to enhance the activities of Superoxide dismutase, Catalase and Glutathione peroxidase in V79-4 cells (Lee et al. 2002). Recent studies on antioxidant activities of *E. ferox* and its glycoside composition *in vitro* reveal that the plant extracts have potent scavenging activity against reactive oxygen species suggesting that seed of this plant have cardioprotective properties which may link with the ability of makhana to induce TRP32 and TrX-1 protein and to scavenge ROS (Samarajit et al. 2006; Verma et al. 2010).

In view of its traditional use, we decided to determine the antibacterial activity of *E. ferox* extracts for the development of possible new antimicrobial compounds.

**MATERIALS AND METHODS**

**Plant material**

*E. ferox* was collected as a whole plant from Manasbal Lake of Kashmir Himalaya, J&K, India in September 2008. Sampling was carried out immediately after seed formation and plants were collected manually at the age of 7 months in bulk from the Lake. After that four parts i.e., leaves, petioles, rhizomes and seeds were separated and dried separately. The plant was identified by the HOD, Kashmir University Herbarium (KASH), Centre of Plant Taxonomy, Department of Botany, University of Kashmir, Srinagar. A sample was deposited in the herbarium as voucher no. 1015.

**Preparation of plant extracts (hot process)**

Dried powder (50 g) from the seeds, leaves, petioles and rhizomes of *E. ferox* were extracted successively with petroleum ether, chloroform and methanol in a Soxhlet extractor. The solvents from all 12 extracts were concentrated under vacuum at 40–50°C using a rotary flash evaporator (Heidolph, Germany) and crude extracts were air dried at room temperature in a steady air current. The weight of the solid residue was recorded and assumed as the yield of crude extract. The dried extracts were then stored in air-tight jars at 4°C for microbial analysis.

**Microbial strains**

All bacterial strains used in this study were clinical strains, namely *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Proteus vulgaris* and were obtained from the Bacteriology Laboratory, Department of Microbiology, SKIMS, Soura, Srinagar, J&K.
Antimicrobial susceptibility tests

The disc diffusion method (Bauer et al. 1966) was used to determine the antimicrobial activity of all 12 extracts which was determined in a mixed culture of Gram-positive cocci (GPC) and Gram-negative bacilli (GNB). The extracts which exhibited inhibitory activity were then tested against clinically isolated strains using Muller Hinton agar (MHA; Hi-Media). The MHA plates were prepared by pouring 15 ml of MHA into sterile Petri dishes. The plates were allowed to solidify for 15 min and 0.1 ml (0.5 MacFarland standard) inoculum suspension was swabbed uniformly and the plates were allowed to dry for 5-10 min. The concentrations of extracts (160 and 320 μg/ml/disc) were loaded onto 6 mm sterile discs (Hi Media). The loaded discs were placed on the surface of the medium and the compound was allowed to diffuse for 5 min and the plates were incubated. Inhibition zones formed around the discs were measured with a transparent ruler (in mm). Antibiotic discs and solvents were taken as positive and negative controls, respectively.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) of the extracts was performed by using a broth micro dilution method on 96 micro-well plates based on recommendations of the National Committee for Clinical Laboratory Standards (NCCLS 2000). Stored bacteria were restituted, identified and incubated in 5 ml of Muller Hinton broth (HMB) in an incubator at 37°C for 24 hrs to a density equal to that of No.1 McFarland standard. The bacterial suspension was further diluted with a broth to give a final inoculum of 10^6 CFU/ml. To evaluate bacterial sensitivity, serial two-fold dilutions of 500, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 mg/l were prepared with HMB (Phillips et al. 1990). Each micro-well of the 96 micro-well plates contained 50 μl diluted agents and 50 μl bacterial strain suspension. A final test volume of 100 μl was dispensed into each well. Both methanol and gentamicin (Hi Media) were kept in one set of wells to act as negative and positive control, respectively. After 24 hrs incubation of the micro-well plate in an incubator at 37°C, bacterial growth was observed by visual inspection. At the same time, from every micro-well, 50 μl of treated suspension was removed and was inoculated on new MHA plates; bacterial colonies were count after incubation for 48 hrs. The initial concentration of no bacterial growth on the plates was considered as the individual agent minimum inhibitory concentration (MIC) in the micro dilution corresponding to individual strains.

RESULTS AND DISCUSSION

The present investigation of antimicrobial activity of E. ferox against a mixed culture of GPC and GNB reveals that the methanolic extracts of seeds and leaves possessed antibacterial activity (Table 1, Fig. 1A). The inhibition diameter zones (IZD) obtained through the disc diffusion assay of methanolic extracts of seeds and leaves of E. ferox against clinically isolated bacterial strains is shown in Table 2. The MIC values of the methanolic extract of seed and leaf extracts of E. ferox with gentamicin and methanol as positive and negative control, respectively against six standard strains are shown in Table 2. Among the strains, K. pneumoniae and C. freundii were resistant towards the methanolic extract of both seeds and leaves (Figs. 1B, 1C). P. aeruginosa showed the maximum IZD (Fig. 1D) followed by S. typhi (Fig. 1E), E. coli (Fig. 1F) and S. flexnerii (Fig. 1G). S. aureus and S. typhimurium (Figs. 1H, 1I) were sensitive towards only the methanolic extract of seeds whereas P. vulgaris (Fig. 1J) was sensitive to higher concentrations of seed and leaf extracts. The antimicrobial activity of crude extracts from numerous plants has been evaluated by the agar disc dif-fusion assay (Guven et al. 2005) and MIC was determined, based on which anti-infective lead molecules were isolated or used as a therapeutic agent (Sawer et al. 2005). The IZD of some Gram-negative strains like P. aeruginosa was greater than Gram-positive strains (Venkata and Venkata 2008), as determined for fruit extracts of two Syzygium spp. The higher the extract concentration, the greater the inhibitory effect, supporting similar studies such as that by Boakye-Yiadom (1977), who discovered antimicrobial properties of some West African medicinal
The methanolic seed extract exhibited more inhibitory activity against the leaf extract against all tested bacterial strains and hence showed bactericidal activity against standard strains with S. aureus ATCC 25923 having a low MIC value (64 mg/l) and S. flexneri the highest (500 mg/l). The methanolic leaf extract of E. ferox showed bactericidal activity against a standard strain of Methicillin-resistant Staphylococcus aureus (MRSA) and the methanolic seed extracts of both plant parts of E. ferox showed bactericidal activity against Standard bacterial strains of E. coli, Staphylococcus aureus, and Pseudomonas aeruginosa with minimum bactericidal concentration (MBC) ranging from 256-500 mg/l. This may be due to the higher polarity and maximum number of compounds extracted in methanol, which has also proved to be the most effective solvent for extracting a broad spectrum of antimicrobial compounds from plants (Vlachos et al. 1996).

From our study, the methanolic seed extract exhibited more inhibitory activity than the leaf extract against all tested bacterial strains and hence showed bactericidal activity against standard strains with S. aureus ATCC 25923 having a low MIC value (64 mg/l) and S. flexneri the highest (500 mg/l). The methanolic leaf extract of E. ferox showed bactericidal activity against some standard strains i.e., with an MIC value of 128 mg/l against S. aureus ATCC 25923, 256 mg/l against Pseudomonas aeruginosa ATCC 27853 and no activity against a standard P. vulgaris strain. Among the bacterial strains tested S. aureus and P. aeruginosa showed higher activity towards both seed and leaf extracts and some tested strains like Acinetobacter sp., K. pneumoniae were least effective since they are naturally resistant to antibacterial agents (Walker and Edward 1999). The results of MIC revealed a decreasing trend in activity as the concentrations of extracts decreased, which implies that the extracts were more active at higher concentrations. The active results of most of strains tested were in the range of 256-500 mg/l and our findings are in agreement with the results of Idia et al. (2015) for the crude methanolic extracts of Senna allata flowers. The methanolic seed extract exhibited more inhibitory activity than the leaf extract against all tested bacterial strains and hence showed bactericidal activity against standard strains with S. aureus ATCC 25923 having a low MIC value of 64 mg/l and S. flexneri having the highest MIC value of 500 mg/l, while S. aureus and E. coli were inhibited by both seed and leaf extracts. Similarly, Pongpaichit et al. (2005), for the crude methanolic extract of Acorus calamus seed and leaf extracts and Panda et al. (2009), for the methanolic leaf and bark extracts of Vitex negundo, reported significant antibacterial activity against E. coli and S. aureus, respectively. However, results against P. aeruginosa were contradictory to that observed by Pongpaichit et al. (2005). The antibacterial activity of E. ferox may be due to the presence of some antimicrobial compounds like cerebrosides and glycoconjugates (Zhao et al. 1989, 1994; Lin et al. 2003; Li and Xu 2007; Row et al. 2007).

The seed and leaf extracts exhibited marked inhibitory action against S. typhi, a virulent strain that causes typhoid (Prescott 2005). With the increase in resistance to antimicrobial agents, medicinal plants have gained popularity among both urban and rural dwellers in the treatment of the ailment. Similarly, Seena siamae leaf extracts have been shown to have antibacterial activity against S. typhi and S. typhimurium (Doughari and Okafor 2008). The antibacterial activity may be due to the fact that it produces a capsule (Sule and Aghabiaka 2008). Methanolic extracts exhibited higher activity, as also concluded by Babayi et al. (2004) for Eucalyptus camaldulensis and Terminalia catappa. The crude methanolic extracts of E. ferox extract exhibited highest activity against P. aeruginosa and E. coli and similar activity has been reported from other medicinal plants, namely Grewia erythraea, Hymenocrater sessilifolius, Vin setoxicum stockii, Zygo phyllum fabago and Arctothamnium oxycedri (Zaidi et al. 2005; Mudassir et al. 2006).

Among the strains, P. aeruginosa, S. aureus and E. coli exhibited higher zones of inhibition against both methanolic extracts. The antibacterial activity of seed extracts was also reported by Murtaza et al. (1994) while the antibacterial activity of the leaf extract may be due to the presence of alkaloids and flavonoids as reported by Ahmad et al. (2001) in the leaves of Adhatoda vasica Nees. However, the antibacterial activity may be due to the presence of one or more phytochemicals present in plants as most of them are known to have antimicrobial activity (Bruenton 1995). The MIC results depicted that gentamicin exhibited higher activity than the methanolic extracts of both plant parts of E. ferox (Table 3). This could be attributed to the fact that, unlike conventional antibiotics and other pharmaceutical products which are usually prepared from synthetic materials by means of reproducible manufacturing techniques and proce-
deaux, herbal medical products are prepared from materials of plant origin which may be subjected to contamination and deterioration (Babu et al. 2002). The literature reveals that no prior work has been done on evaluating the antimicrobial activity of E. ferox and it is now expected that screening of this plant for antibacterial activity against a wide variety of test organisms will be helpful in obtaining a broad spectrum herbal formulation as well as new antimicrobial substances for the treatment of diseases like kidney problems and urinary tract infections.

ACKNOWLEDGEMENTS

The Authors are highly grateful to Central Research Institute of Unani Medicine Hazratbal, Srinagar and Department of Microbiology, SKIMS, Soura, Srinagar for providing necessary laboratory facilities to carry out these studies.

REFERENCES


Sule IO, Agabiaika TO (2008) Antibacterial effect of some plant extracts on selected Enterobacteriaceae. Ethnobotanical Leaflets 12, 1035-1042


