REPORT

Antibacterial activity of sea buckthorn (*Hippophae rhamnoides* L.) against methicillin resistant *Staphylococcus aureus* (MRSA)

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Abstract: Objective of the present study was to investigate the antibacterial activity of Sea buckthorn (*Hippophae rhamnoides* L.) berries and leaves against methicillin resistant *Staphylococcus aureus* (MRSA) by using the standard disc diffusion method. Chloroform, n-hexane and aqueous extract of the plant parts were used. Doses of 2mg/ml, 4 mg/ml and 6mg/ml were tested against the microorganism, and the zone of inhibition was compared against the standard drug vancomycin. Results indicated that n-hexane and chloroform extracts of berries and n-hexane extract leaves showed significant ($p<0.05$) antibacterial activity comparable with vancomycin. It was concluded from the study that extracts berries and leaves of *Hippophae rhamnoides* have antibacterial activity against MRSA.

Keywords: Methicillin-resistant *Staphylococcus aureus* (MRSA), *Hippophae rhamnoides.*

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is thought to be a serious clinical problem because of its resistance to a number of antibiotics. It is responsible not only for serious skin and skin structure infections but also causes life-threatening diseases like endocarditis and pneumonia (Lee *et al.*, 2010). MRSA strains are resistant not only to methicillin but also to other penicillins, macrolides, tetracyclins, β-lactamase inhibitors, carbapenems, cephalosporins, sulphonamides, amino glycosides, lincosamides and trimethoprim (Kuroda *et al.*, 2001). Because of its resistance to a wide variety of antibiotics, they are very difficult to treat. MRSA was once susceptible to quinolones but it resisted so quickly that today more than 80% of the MRSA strains are resistant to quinolones. FDA approved medicine for the treatment of MRSA is vancomycin (Welte and Pletz, 2010). The increasing prevalence of MRSA infections (Bukhari *et al.*, 2011) has created a tremendous demand for effective and safe antimicrobial agents other than the historic anti-MRSA agent vancomycin.

The emergence of resistance and tolerance to the existing drugs has created a decreased efficacy of these drugs. This problem has been tried to be overcome by increasing the drug delivery by the use of polymers (Khalid *et al.*, 2009; Hussain *et al.*, 2011) or through nanotechnology (Naz *et al.*, 2012; Ehsan *et al.*, 2012), synthesis of new drugs, either by the use of proteomics (Qadir, 2011; Qadir and Malik, 2011), or synthesis from lactic acid bacteria (Masood *et al.*, 2011) or marine microorganisms (Javed *et al.*, 2011). However, now a day, the trend is being changed from synthetic drugs to the natural drugs to control the diseases. The natural products are constantly being screened for their possible pharmacological value particularly for their anti-inflammatory (Qadir, 2009), hypotensive (Qadir, 2010), hepatoprotective (Ahmad *et al.*, 2012; Ali *et al.*, 2013), hypoglycaemic (Nisa *et al.*, 2009; Qadir and Malik, 2010), amoebicidal (Asif and Qadir, 2011), anti-fertility, cytotoxic, spasmolytic, bronchodilator (Janbaz *et al.*, 2013a), antioxidant (Janbaz *et al.*, 2012), anti-diarrheal (Janbaz *et al.*, 2013b) and antimicrobial (Amin *et al.*, 2012) properties.

Hippophae rhamnoides L. (family Elaeagnaceae) commonly known as Sea buckthorn is native to Eurasia. It is widely distributed in Pakistan, India, Nepal, China, Myanmar, France, Finland, Romania, Germany, Russia and Britain. Phytochemical analysis of the plant confirmed the presence of mineral acids, carotenes, flavonoids, vitamins, carbohydrates and amino acids (Chaman and Syed, 2011). For last 50 years, preparations of Sea buckthorn have been used clinically to treat burns, inflammations and ulcers in China as an alternative medicine (Negi *et al.*, 2005). The plant has recently proved to be antibacterial against *Staphylococcus aureus* (Upadhyay *et al.*, 2010). Therefore objective of this research was to investigate the antibacterial activity of *Hippophae rhamnoides* berries and leaves against methicillin resistant *Staphylococcus aureus* (MRSA).

MATERIAL AND METHODS

Berries and leaves of *Hippophae rhamnoides* were collected from Gilgit, Pakistan and identified from Botany

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Table 1: Zone of inhibition in mm (Mean ± SE) by different extracts of leaves and berries of *Hippophae rhamnoides* against Methicillin-resistant *Staphylococcus aureus*

	Strength	n-Hexane extract	Chloroform extract	Aqueous extract
Vancomycin (Standard)	30 µg disc	29.36 ±0.4[a]	28.71±0.34 [a]	27.76±1.87 [a]
Hippophae rhamnoides berries	2 mg/ml	15.46±2.55[c]	14.69±2.65[cd]	13.53±3.17[d]
	4 mg/ml	18.35±2.06[b]	17.99±1.88[b]	14.02±2.98[cd]
	6 mg/ml	22.93±1.27[a]	23.37±1.22[a]	18.01±2.87[b]
Hippophae rhamnoides leaves	2 mg/ml	14.70±2.49[e]	14.63±2.48[e]	12.76±3.08[f]
	4 mg/ml	17.54±2.06[cd]	18.05±1.92[c]	14.79±3.12[e]
	6 mg/ml	24.93±1.39[a]	20.95±1.74[b]	17.23±3.25[d]

Similar letter in a row or in a column are statistically non-significant (p>0.05)

Department, University of Agriculture, Faisalabad, Pakistan. The plant parts were kept in herbarium for future reference. They were shade-dried and powdered. The material was extracted by cold maceration using n-Hexane, Chloroform and Distilled water. Rotary evaporator was used for concentrating n-Hexane and Chloroform extracts. Aqueous extract was dried by using lyophilizer (Nandagopal and Kumari 2007).

Whatman filter paper No. 1 was used to prepare extract discs (6 mm). Discs were sterilized using autoclave. All the six extracts (n-hexane, chloroform and aqueous extract of berries and leaves of *Hippophae rhamnoides*) were diluted in their respective solvents to give final concentrations of 2 mg/ml, 4 mg/ml and 6 mg/ml. Each sterilized disc was immersed for 24 hours in test tubes of respective concentration. After 24 hours, the discs were taken out and kept for drying (Hannan *et al.*, 2008).

Mueller-hinton agar medium was prepared and poured in a flask. After autoclaving the flasks, they were kept in a water bath maintained at 50°C to avoid any solidification. Medium was poured in plates and was allowed to solidify. Inoculum was prepared using the process of direct colony suspension, a method, which is recommended for Staphylococci. The strains of MRSA were taken from Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan. The cultures were transferred using three to five well-isolated colonies by sterile cotton swab and making a direct saline suspension. It was then adjusted to match 0.5McFarland turbidity standards using a vortex mixer. A sterile cotton swab was dipped in the suspension within 15minutes after adjusting suspension. The cotton swab was pressed firmly and rotated many times inside the tube above fluid level. Mueller-hinton agar medium was inoculated by streaking the swab over the surface. The process was repeated two times after rotating the plate at approximately 60° so that the inoculum is evenly distributed. Drug impregnated discs were placed in the plates. Vancomycin (30µg) discs were used as a standard drug. Plates were incubated for 24 hours at 30-35°C. Diameter of inhibition around each disc was measured in mm using digital caliper.

RESULTS

Zone of inhibition in mm (Mean ± SE) by different extracts of leaves and berries of *Hippophae rhamnoides* against methicillin-resistant *Staphylococcus aureus* (MRSA) are given in table 1. Zone of inhibition for 6 mg/ml of n-hexane and chloroform extract of *Hippophae rhamnoides* berries were significant ($p<0.05$) compared to all other doses and extracts of *Hippophae rhamnoides* berries and non-significant ($p>0.05$) as compared to standard (vancomycin). Zone of inhibition for 6 mg/ml of n-hexane extract of *Hippophae rhamnoides* leaves were significant ($p<0.05$) compared to all other doses and extracts of *Hippophae rhamnoides* leaves and non-significant ($p>0.05$) as compared to standard (vancomycin).

DISCUSSION

Mickel *et al.* (2012) reported antibacterial activity of *Hippophae rhamnoides* leaves extract against *Staphylococcus aureus.* But the literature lacks the antibacterial activity against methicillin-resistant *Staphylococcus aureus*. Our study showed that 6mg/ml of both the n-hexane and chloroform extracts of *Hippophae rhamnoides* berries and 6mg/ml of n-hexane extract of leaves of the plant significantly ($p<0.05$) inhibited the growth of methicillin-resistant *Staphylococcus aureus*. However, antibacterial activity against methicillin-resistant *Staphylococcus aureus* was shown by all the other extracts that was non-significant ($p>0.05$). This antibacterial activity may be significant at higher doses, that estimation is only possible by isolating the active constituents.

CONCLUSION

It was concluded from the present study that n-hexane and chloroform extracts of *Hippophae rhamnoides* berries and n-hexane extract of leaves of the plant have antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA).

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