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Antibacterial Activity of Sea Buckthorn (*Hippophae rhamnoides L.*) against Pathogenic Microbes

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Abstract: *Sea buckthorn (Hippophae rhamnoides L.) is a unique and valuable plant and has recently gained worldwide attention mainly for its medicinal and nutritional potential. It is a thorny bush with yellow-orange pearl shaped fruits and has a high content of vitamins, minerals, natural antioxidants and omega-3,6 fatty acids.Doses of 2 mg/ml, 4 mg/ml and 6 mg/mlof aqueous extract of plant berry powder were evaluated against Gram positive and Gram negative microbes by usingdisc diffusion and agar well diffusion method. The zone of inhibition was compared with the standard drugs vancomycin andKanamycin(30 µg/ml). It was concluded that the aqueous extract of berry powder has antibacterial activity, which may be used to preventvarious diseases and can be incorporated in human and animal diet.*

Keywords: Hippophae rhamnoides L., Antibacterial Activity, Minimum Inhibitory Concentration, Gram Positive, Gram Negative

I. INTRODUCTION

Herbal formulations have been in use for many years globally not only as therapeutic but also as prophylactic and health promotive agents. Sea buckthorn (*Hippophae rhamnoides L.*), a unique and valuable plant has recently gained worldwide attention mainly for its medicinal and nutritional potential. It is a thorny bush with yellow – orange, pearl shaped fruits, nitrogen fixing deciduous shrub from the Elaeagnaceae family. It grows widely in various regions of Asia, Europe, and North America. The whole plant and especially its berries are considered to be a good source of a large number of bioactive compounds with medicinal and nutritional properties. The greatest attention has been drawn to its high content of vitamins, minerals, natural antioxidants, omega-3 and omega-6 fatty acids, and proteins. Among them ascorbic acid, tocopherols, carotenoids, and flavonoids exhibit antioxidant activity. Antioxidants protects the body from the detrimental effect of free radicals generated as byproduct of normal metabolism and plays important role in preventing pathogenic processes related to cancer and cardiovascular disease and they can also enhance immune function.The plant has wide application mainly in folk medicine for the treatment of cough, skin diseases, gastric ulcers, asthma, and lung disorders.

According to Jana Krejcaroval *et al*. (2015), at present, sea buckthorn has become popular especially for its positive effects on the human organism. Sea buckthorn is valued for its antioxidant, cardio-protective, anti atherogenic, antidiabetic, hepato-protective, anti-carcinogenic, immune-modulating, antiviral, antibacterial, anti-inflammatory and vasodilating effects. It also reduces occurrence of stomach ulcers, supports wound healing, accelerates treatment of skin disorders and reduces pain. Other important properties of sea buckthorn include its cyto-protective effects and it also positively affects metabolic diseases with the ability to slow down ageing and protect against radiation-induced damage, accelerate the healing of burns and frost bites, and reduce hair loss and this plant has no associated toxicity or side effects.

The antimicrobial activity of sea buckthorn berries, seeds, leaves and oil has been reported. Antimicrobial activity is a collective term for all active principles (agents) that inhibit the growth of bacteria, prevent the formation of microbial

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colonies, and may destroy microorganisms. The aqueous and hydro alcoholic leaf and berry extracts showed a growth-inhibiting effect against Gram positive and Gram negative bacteria i.e. *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli, Listeria monocytogenes*, *Yersinia enterocolitica*, and *Enterococcus faecalis, Salmonella typhi, Klebshiela pneumoneae.*

The increasing resistance of microorganisms towards antibacterial agents has been responsible in recent years for serious health issues. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases. Martina Blaskoval and Eva Ivanisoval et al (2020).

Muhammad Imran Qadir *et al*. (2016) has reported that, Methicillin-resistant *Staphylococcus aureus* (MRSA) is thought to be a serious clinical problem because of its resistance to a number of antibiotics. *S.aureus* is responsible for serious skin infections and also causes life threatening diseases like endocarditis and pneumoniae. MRSA strains was found resistant not only to methicillin but also to other penicillins, macrolides, tetracyclins, β-lactamase inhibitors, carbapenems, cephalosporins, sulphonamides, aminoGlycosides, lincosamides and trimethoprim. Because of its resistance to a wide variety of antibiotics, they are very difficult to treat. The study has proved that sea buckthorn berries and leavesshows antibacterial activity against Methicillin-resistant *Staphylococcus aureus.*

Thus, the author has decided to perform and determine the antibacterial activity of Hippophae rhamnoides berries aqueous extract by using agar well diffusion and disc diffusion method.

II. Material and Methods

2.1 Plant Material Collection:

Sea buckthorn (*Hippophae rhamnoides L.*) berriespowder was purchased from local market of Mahim (W), Mumbai, Maharashtra.

2.2 Soxhlet Aqueous Extraction Method

For soxhlet extraction, a thimble of 10gm dried sea buckthorn berries powder was prepared using whatman filter paper, was kept in 100ml of distilled water and left for 8 hr. at 60^{o}C.

2.3 Test Microorganisms

Gram positive (*Staphylococcusaureus* and *Bacilluscereus*) and Gram negative (*Escherichia coli, Klebshiela pneumoneae, and Pseudomonas aeruginosa*) microorganisms were collected from Himedia laboratory, Thane, Mumbai, Maharashtra. These bacterial cultures were maintained through sub culturing on suitable agar media and preserved at 4^{0}C.

2.4 Bacterial Culture Suspension

Bacterial suspensions were prepared by inoculating 24 hrs old bacterial colonies in saline solution and optical density was adjusted to 0.6 at 600 nm (10^{8} CFU/ml).

2.5. Antibacterial Activity

Vancomycin and kanamycin were used as control drugs for the zone of inhibition comparison. Antibacterial activity of Sea buckthorn berries powder was tested on Gram positive (*Staphylococcus aureus* and *Bacillus cereus*) and Gram negative (*Escherichia coli, and Pseudomonas aeruginosa*) bacteria by using following methods:

2.6 Antibiotic Disc Diffusion Test

Mueller Hinton agar was poured in sterile Petriplates and bacterial cultures were spread using sterile cotton swabs aseptically. 6mm diameter discs were prepared using whatman filter paper no. 1 and were sterilized using autoclave.Filter paper discs were impregnated with 15 µL of the plant material extract and placed on the inoculated plates and incubated at 37^{o}C for 24 hrs. The diameters of the zone of inhibition were measured in millimeters after incubation.

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2.7 Agar Well Diffusion Test

Sterile Mueller Hinton agar along with bacterial culture was poured in sterile Petri plates aseptically. Wells of 6mm diameter was prepared using sterile cork borer. Different concentrations of plant extract were added to the well and were incubated at 37^0C for 24 hrs. After incubation zone of inhibition was measured.

2.8 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) is the lowest concentration of a sample, which prevents visible growth of bacteria. Different concentrations ranging from 2µg/ml to 500µg/ml of plant berry extract were prepared from stock solution(1000 PPM). Mueller Hinton broth was distributed in different sterile test tubes followed by the addition of stock solution and 0.1ml bacterial culture suspension (OD adjusted 0.6 at 600nm) aseptically. These inoculated tubes were incubated at 37^0C for 24 hrs. Afterincubation turbidity was observed.

III. Result and Discussion

Zone of inhibition in mm of aqueous extract of berry powder against *S. aureus, B. cereus, E.coli* and *P. aerugenosa* were measured and are given in table 1 and 2. All four Gram positive and Gram negative microbes showed zone of inhibition in increasing order in both disc diffusion and agar well diffusion method. In disc diffusion method *S. aureus* and *E.coli* were found more sensitive to berry powder aqueous extract as they showed larger zone of inhibition as compare to *B. cereus* and *P. aeruginosa*. The lowest antibacterial activity was measured against *P. aeruginosa* as it showed 10.01mm zone of inhibition for 6mg/ml and highest antibacterial activity was measured against S. aureus as it showed 18.31mm zone of inhibition for 6mg/ml concentration.In agar well diffusion method, *S. aureus* showed 23.4mm zone of inhibition for 6mg/ml concentration and it was found more sensitive as compare to the other microbes. The lowest antibacterial activity was recorded against *P. aeruginosa* as it showed 12.1 mm zone of inhibition for 6mg/ml concentration.

Table 3 shows Minimum Inhibitory Concentration against Gram positive and Gram negative microbes where *S. aureus* showed complete bacterial growth inhibition at 250 µg/ml, *B. cereus* at 125 µg/ml, *E. coli* at 4 µg/ml and *P. aeruginosa at* 300 µg/ml. Aqueous extract of *Hippophae rhamnoides* berries powder inhibits E. coli at very less concentration and was found more susceptible, where *P. aeruginosa* inhibits at high concentration and it was found least susceptible.

All three test results were compared with the previous studies and it was found similar and no significant differences were observed.

Table 1: Antibiotic Disc Diffusion test

Sample	Concentration (mg/ml)	Zone of Inhibition (mm)			
		S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*
Vancomycin (Standard)	30 µg disc	27	-	-	09
Kanamycin (Standard)	30 µg disc	-	34	22	-
Aqueous extract of *Hippophae rhamnoides* berries powder	2 mg/ml	13.12	11.0	13.53	6.71
	4 mg/ml	14.02	12.0	14.67	7.09
	6 mg/ml	18.31	13.1	16.0	10.01

Table 2: Agar well Diffusion test

Sample	Concentration (mg/ml)	Zone of Inhibition (mm)			
		S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*
Vancomycin (Standard)	30 µg disc	27	-	-	13
Kanamycin (Standard)	30 µg disc	-	35	25	-
Aqueous extract of *Hippophae rhamnoides* berries powder	2 mg/ml	18.3	19.2	16.0	9.2
	4 mg/ml	20.2	20.1	17.2	10.5
	6 mg/ml	23.4	20.0	18.0	12.1

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Table 3: Minimum Inhibitory Concentration (MIC)

Sample	Name of organisms	MIC value (µg/ml)
Aqueous extract of *Hippophae rhamnoides* berries powder	*S. aureus*	250 µg/ml
	B. cereus	125 µg/ml
	E. coli	4 µg/ml
	P. aeruginosa	300 µg/ml

IV. CONCLUSION

It was concluded that, the aqueous extract of sea buckthorn (*Hippophae rhamnoides L.*) berry powder have antibacterial activity against Gram positive and Gram negative microbes and it matches with standard antibiotics results. It was confirmed, the sea buckthorn berries are rich source of antioxidants and fatty acids which may be used for the treatment of bacterial infections and can be incorporated in the animal and human nutrition.

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