DETECTION OF ANTILISTERIAL ACTION OF AQUEOUS EXTRACT OF *MORINGA OLEIFERA* SEEDS

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Abstract

Listeria monocytogenes is one of the major human as well as animal food borne pathogens. It has been indicated that clinical strains of L. monocytogenes are resistant to a wide range of antibiotics. Use of natural products as anti bacterial compounds seems to be an important way to control the presence of pathogenic bacteria. Moringa is one of the safe and alternative source of antimicrobial agent. Antilisterial action of aqueous extracts of Moringa oleifera seeds (50, 100, 150, 200, 250 mg/ml) was tested against L. monocytogenes and L. innocua. Inhibitory effect was noticed at 200, 250 mg /ml concentrations. The minimum inhibitory concentration was found to be 200 mg/ml.

Keywords- *Moringa oleifera seeds, antilisterial action*, *minimum inhibitory concentration*

Listeriosis is of great public health concern because of its high mortality (20 to 30 %) and its common source epidemic potential. Listeriosis is usually manifested as sudden onset of fever, chills, severe headache, gastrointestinal symptoms, septicaemia, meningitis and encephalitis. Infection during pregnancy can lead to premature labour, miscarriage, infection of the newborn, or even stillbirth (Janakiraman, 2008). In case of cattle, there is high incidence of intestinal carriers.

Antimicrobial resistance of pathogenic microorganisms is a worldwide public health concern because of increasing global trade and travelling. It has been indicated that clinical strains of *L. monocytogenes* are resistant to a wide range of antibiotics. To reduce the health hazards and economic losses due to food borne microorganisms, the use of natural products as antibacterial compounds is an important alternative. *Moringa oleifera* , a widely cultivated plant in tropical countries, is commonly known as moringa, drumstick tree, horseradish tree and ben oil tree. Moringa is one of the safe and alternative source of antimicrobial agent (Doughari *et al*., 2007). All parts of moringa plant have various medicinal properties. It is also used for water purification, hand washing as well as in herbal medicine as cardiac and circulatory stimulants (Kumar et al., 2010).

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Materials and methods

Preparation of aqueous extracts

Three kilogram of drumstick was dried so as to get 150 g of seeds. Seeds were finely powdered in a blender and 50 g was wrapped in a double layered muslin cloth which was then immersed in 500 ml of distilled water and boiled until the volume of water was reduced to half. The extract was removed and the process was repeated twice. The extract obtained was again heated till it was reduced to a semisolid consistency. The extract was then freeze dried at – 37^{0}C for eight hours in freeze dryer (Freeze Operon 7707, Korea) to remove water and reduce it to a powdered form (Vrinda Menon, 2015). The yield of the extract on dry matter basis was three grams. The concentration of seed extract used in the study were 50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml, which were prepared by dissolving measured amount of powder in one millilitre of distilled water (Eruteya and Badon, 2014).

Preparation of Mac Farland standard

The turbidity standard solution was prepared by adding 0.5 ml of 0.048 M $BaCl_2$ to 99.5 ml of 0.36 N H_2SO_4 (1 % w/v). This solution is equal to half the density of No.1 Mac Farland standard solution. This solution was stored in a glass tube and kept in the dark at room temperature for further use. The tube was vigorously agitated just before each use.

Preparation and standardization of inoculum

Standard cultures of *L. monocytogenes* (MTCC 1143) and *L. innocua* (ATCC 33090) were used to analyse antilisterial action of *M. oleifera* seeds. Two isolates of *L. monocytogenes* isolates obtained from beef and dung samples and five isolates of *L. innocua* from beef were also used for the study. The colonies of pure culture from the PALCAM agar was inoculated into sterile nutrient broth and incubated at 37^{0}C for 24 h. *Listeria monocytogenes and L. innocua*, 0.5 McFarland standards were prepared. The turbidity of culture was adjusted using solution having half the density of Mac Farland standard No.1. When the broth culture was found to be more turbid, it was diluted with nutrient broth and when the turbidity was found to be less, culture was incubated for more time to achieve the required turbidity

Detection of minimum inhibitory concentration of *Moringa oleifera* seed against *Listeria* spp.

The agar well diffusion method was followed to determine the minimum inhibitory concentration for antimicrobial activity of the seed extract. The Muellar Hinton agar plates (Himedia, Mumbai) were swabbed with the prepared culture of *L monocytogenes* and *L.innocua*. Wells (8mm diameter and about 2cm apart) were made using sterile cork borer

Table 1. The antilisterial action of of aqueous extract of *M. oleifera* seeds against *L. monocytogenes* isolates

Sl. No.	Concentration (mg/ml)	Average zone of inhibition (mm)		
		L. monocytogenes (MTCC 1143)	LM-1	LM-2
1	50	00	00	00
2	100	00	00	00
3	150	00	00	00
4	200	10	12	12
5	250	12	12	11

LM 1- Isolate from beef, LM 2 – Isolate from dung

Table 2. The antilisterial action of of aqueous extract of *M. oleifera* seeds against *L. innocua***.**

Sl. No	Concentration (mg/ml)	Average zone of Inhibition (mm)					
		L. innocua (ATCC 33090)	LI- 1	LI- 2	LI- 3	LI- 4	LI-5
1	50	00	00	00	00	00	00
2	100	00	00	00	00	00	00
3	150	00	00	00	00	00	00
4	200	11	9	12	10	11	9
5	250	12	10	11	8	10	11

LI 1 to 5 - Isolates from beef

(Vrinda Menon, 2015). Various concentrations of aqueous extracts were prepared in sterile distilled water *viz*., 50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml. Thirty microlitre each of different concentrations of these extracts were added in the wells and allowed to diffuse at room temperature for 2 h. The plates were incubated at 37^{0}C for 18 to 24 h and zone of inhibition was measured. The procedure was repeated six times for each organism. The minimum concentration required to inhibit the organism was also detected and recorded as minimum inhibitory concentration (MIC) of the organism.

Results and Discussion

For both *L. monocytogenes* and *L. innocua* inhibitory effect was noticed at 200, 250 mg /ml concentrations. The minimum inhibitory concentration was found to be 200 mg/ml (Table1 and 2).

The minimum inhibitory concentration found in the present study was higher than the observations of Eruteya and Badon (2014) from Nigeria. They reported the antilisterial action of aqueous extracts of the seeds of *M. oleifera* action against *L. monocytogenes* strains at the concentrations of 50, 100, 150, 200 and 250 mg/ ml. Philip *et al*. (2013) from South Africa also analysed antibacterial activity of seeds of ethanol extract of *M. oleifera* against *Pseudomonas aerogenosa, Escherichia. coli, Klebsiella spp., Salmonella typhi* and *Proteus mirabilis.* Highest inhibitory effect was observed against *P. mirabilis* and *S. typhi.* Kheir *et al*.,(2014) found out that alcoholic and aqueous extract of seeds were active against Gram-positive organisms. The lowest recorded MIC was 31.25 mg/ml against *Staphylococcus aureus.* Vieira *et al*. (2010) documented that aqueous and ethanolic extracts of moringa seeds have antibacterial activity against *S. aureus*, *Vibrio cholerae* and *E. coli*. Eilert *et al*. (1981) identified that 4 (α-L-Rhamnosyloxy) benzyl isothiocyanate was the active antimicrobial agent in seeds of *M oleifera* , which could also be responsible for the antilisterial action. The findings of the present study suggests that *M. oleifera* seeds can be used as antimicrobial agent as well as sanitizing agent in food industry due to its good antilisterial action.

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