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ANTILISTERIAL ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACTS OF THE LEAVES AND SEEDS OF *MORINGA OLEIFERA*

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ABSTRACT

The in vitro antilisterial activity of the ethanolic and aqueous extracts of the leaves and seeds of Moringa oleifera were evaluated using the agar diffusion method on Mueller Hinton agar. Listeria monocytogenes PCM 2191 serovar 01/2 and the four other L. monocytogenes strains were inhibited by the aqueous extracts at the concentrations of 200 and 250mg/ml of the leaves and 150, 200 and 250mg/ml of the seeds whereas, no inhibitory effects were observed with the ethanolic extracts of both the leaves and seeds. The result of this study has demonstrated the potential application of moringa aqueous extract in the control of L. monocytogenes.

Keywords: Antilisterial, Aqueous, Ethanolic, Listeria monocytogenes, Moringa oleifera.

Contribution/ Originality

This study contributes in the existing literature, a further confirmation of moringa's bio-activity. The study is one of very few studies which have investigated the antilisterial effect of moringa.The papers primary contribution is finding that moringa has a potential application in the control of Listeria monocytogenes.

1. INTRODUCTION

Listeriosis is a severe infection caused by *Listeria monocytogenes* particularly among the elderly, very young and immunocompromised individuals and has also been associated with late-term miscarriages in pregnant women [1-3]. Because of its clinical severity and the high incidence of fatalities, listeriosis is an infection of considerable public health concern [3, 4].

With bacteria having a remarkable ability to develop resistance to every antibiotic, it can be anticipated that even bacterial species such as *Listeria*, which were considered to be susceptible to ampicillin, aminoglycosides, tetracycline, macolides, vancomycin, carbenicillin, cephaloridine, chloramphenicol, erythromycin, furazolidone, methicillin, neomycin, novobiocin, oleandomycin, ticarcillin, azlocillin and less susceptible to chlortetracycline, oxytetracycline, tetracycline, gentamicin, kanamycin, nitrofurantoin, pencillin G, streptomycin, will evolve towards multi-

resistance [5-8]. *Listeria* infections are almost always treated with antibiotics, but antibiotic resistance in *L. monocytogenes* is being reported with increasing frequency for both food and clinical isolates [9-11].

The World Health Organization reported that the use of traditional medicine in developed countries is on the rise due to failure of conventional medicine that can cure chronic diseases, emergence of multi-drug resistant pathogens and parasites, adverse effects of chemical drugs, increasing cost and information of herbal medicine [12].

The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties [13].

The "Moringa" tree, belonging to monogeneric family Moringaceae (order Brassicales) is considered one of the world's most useful trees, as almost every part of the Moringa tree (root, bark, gum, leaf, pods, flowers, seeds and seeds oil) can be used for food, with high nutritional value,impressive range of medicinal uses or some other beneficial properties [12, 14-18]. To reduce health hazards and economic losses due to foodborne microorganisms, the use of natural products as antibacterial compounds [19, 20] seem to be an interesting way to control the presence of pathogenic bacteria and to extend the shelf life of processed food.

This study is therefore aimed to evaluate the activity of ethanolic and aqueous extracts *Moringa oleifera* leaves and seeds against *Listeria monocytogenes*, a highly fatal foodborne pathogen.

2. MATERIALS AND METHODS

2.1. Plant Samples and Microbial Cultures

The leaves and seeds of the *Moringa oleifera* were purchased from spice vendors in Choba market and identified in the Herbarium, University of Port Harcourt, Nigeria. *Listeria monocytogenes* PCM 2191 serovar 01/2 was obtained from the Polish Culture of Microorganisms, Poland while the other for *L. monocytogenes* were previously isolated and characterized [21].

2.2. Moringa Preparation and Extraction Procedure

The moringa leaves and seeds (100g) were air dried for about 1 to 2 weeks and ground into fine powder using electric blender. The extraction was carried out by Soxhlet method for both the ethanolic (absolute) and aqueous extracts. The fine powder (25g) was packed tightly in the Soxhlet extractor and 250ml of ethanol or water used separately as solvent for extraction. The process was carried out for 6hours. The extract was then filtered and re-extracted under the same conditions to ensure complete extraction. The obtained extract were evaporated to dryness under reduced pressure at 60^0 C to get a dried or near dried solid product and stored in dried plastic bags for further analysis at room temperature.

2.3. Preparation of Crude Extract

The method employed for the ethanolic extract was that previously described by Akujobi, et al. [22]. The spices extracts were diluted with 30% dimethylsulphoxide (DMSO) to obtain 250mg/ml (1g in 4ml), 200mg/ml (1g in 5ml), 150mg/ml (1.2g in8ml), 100mg/ml (0.5g in 5ml) and 50mg/ml (0.25g in 5ml). The aqueous extract was used without DMSO. These extracts were stored at 15^0C until required.

2.4. Evaluation of Antilisterial Activity

Agar diffusion method was employed. From an overnight broth culture of the various *L. monocytogenes*, a 1×10^8 cell/ml McFarland standard was prepared (by first centrifuging the overnight broth at 4,000 rpm for 10min and supernatant decanted). Two mills sterile deionized water was then added, vortexed and centrifuged again at 4,000rpm for 10min. The resulting pellets were transferred to a physiological saline while comparing with McFarland standards and 0.1ml aseptically transferred to sterile Petri dishes before adding 20ml molten Mueller Hinton agar cooled to 45-50^0C. The content was thoroughly mixed and then allowed to solidify. Five holes (5.0mm) were made in each plate using a cup borer and 0.2ml of the various moringa concentrations of both the ethanolic and aqueous extracts transferred into each hole aseptically using a pipette. Plates were allowed to stand for prediffusion for 1h before incubation at 37^0C for 24h. Zones of inhibition were measured and the average calculated.

3. RESULTS AND DISCUSSION

The preliminary screening for antilisterial activity of various concentrations (50, 100, 150, 200 and 250mg/ml) of aqueous and ethanolic extracts of the leaves and seeds of *Moringa oleifera* against *L. monocytogenes* PCM 2191 serovar 01/2 (LM1) and four other *L. monocytogenes* revealed the inability of the ethanolic extracts of both the leaves and seeds of moringa to inhibit the tested strains. The aqueous extract of both the leaves and seeds however, showed an inhibitory activity against *L. monocytogenes*, especially at the concentrations of 200 and 250mg/ml for the leaf extract (Table 1) and 150,200 and 250mg/ml for the seed extract (Table 2).

Table-1. The effect of aqueous extract of moringa leaf on *L. monocytogenes*

Concentration(mg/ml)	Average zones of inhibition (mm)				
	LM 1	LM2	LM3	LM4	LM5
250	11	12	10	13	11
200	10	10	7	11	10
150	NI	NI	NI	9	8
100	NI	NI	NI	8	7
50	NI	NI	NI	NI	NI

LM= *L. monocytogenes*; NI= no inhibition.

Table-2. The effect of aqueous extract of moringa seed on *L. monocytogenes*

Concentration(mg/ml)	Average zones of inhibition (mm)				
	LM 1	LM2	LM3	LM4	LM5
250	13	10	12	13	12
200	11	9	10	11	10
150	10	8	9	10	9
100	NI	7	8	9	7
50	NI	6	7	8	7

LM= *L. monocytogenes*; NI= no inhibition

Although, there are no reported cases of antilisterial activity of *M. oleifera* in available peer reviewed journals, the results of this study is in agreement with that of Oluduro [17] who reported the absence of zone of growth inhibition on wound bacteria by ethanolic leaf extract of moringa, whereas, the aqueous leaf extract showed appreciable inhibitory effects at 30mg/ml. The antibacterial activity of various extracts of *Moringa oleifera* have been widely reported against *Staphylococcus aureus*, *Eschrichia coli*, *Vibrio cholera*, *V. parahaemolyticus*, *Salmonella typhi*, *Salmonella gallinarium*, *Salmonella* Enteritidis, *Bacillus cerues*, *B subtilis*, *B. megaterium*, *Psuedomonas aeruginosa*, *Klebsiella pneumonia*, *Streptocuccus pyogenes*, *Shigella shinga*, *Enterobacter aerogenes and Sarcina lutea* [15, 16, 23-31].

The aqueous extracts show promise and form a primary platform for further phytochemical and pharmacological studies for control of *L. monocytogenes*.

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REFERENCES

[1] J. M. Farber and P. I. Peterkin, "Listeria monocytogenes, a food-borne pathogen," *Microbiol. Rev.*, vol. 55, pp. 476–511, 1991.

[2] C. Mena, G. Almeida, L. Carneiro, P. Teixeira, T. Hogg, and P. A. Gibbs, "Incidence of Listeria monocytogenes in different food products commercialized in portugal," *Food Microbiol.*, vol. 21, pp. 213-216, 2004.

[3] M. V. Gianfranceschi, M. C. D'Ottavio, A. Gattuso, A. Bella, and P. Aureli, "Distribution of serotypes and pulsotypes of Listeria monocytogenes from human, food and environmental isolates (Italy 2002–2005)," *Food Microbiol.*, vol. 26, pp. 520-526, 2009.

[4] J. A. Vázquez-Boland, M. Kuhn, P. Berche, T. Chakraborty, G. Domínguez-Bernal, W. Goebel, B. González-Zorn, J. Wehland, and J. Kreft, "Listeria pathogenesis and molecular virulence determinants," *Clin. Microbiol. Rev.*, vol. 14, pp. 584–640, 2001.

[5] H. P. R. Seeliger and D. Jones, *Genus Listeria. In Bergey's manual of systematic bacteriology*. Baltimore, U.S.A.: Williams and Wilkins, 1984.

[6] H. Hof, T. Nichterlein, and M. Kretschmar, "Management of listeriosis," *Clin. Microbiol. Rev.*, vol. 10, pp. 345–357, 1997.

[7] E. Charpentier and P. Courvalin, "Antibiotic resistance in Listeria spp," *Antimicrob. Agents Chemother*, vol. 43, pp. 2103–2108, 1999.

[8] B. Swaminathan and P. Gerner-Smidt, "The epidemiology of human listeriosis," *Microbes Infect.*, vol. 9, pp. 1236-1243, 2007.

[9] D. Walsh, G. Duffy, J. J. Sheridan, I. S. Blair, and D. A. McDowell, "Antibiotic resistance among listeria, including listeria monocytogenes, in retail foods," *J. Appl. Microbiol.*, vol. 90, pp. 517–522, 2001.

[10] P. Aureli, A. M. Ferrini, V. Mannoni, S. Hodzic, C. Wedell-Weergaard, and B. Oliva, "Susceptibility of listeria monocytogenes isolated from food in Italy to antibiotics," *Int. J. Food Microbiol.*, vol. 83, pp. 325–330, 2003.

[11] A. Safdar and D. Armstrong, "Antimicrobial activities against 84 Listeria monocytogenes isolated from patients with systemic listeriosis at a comprehensive cancer center (1955–1997)," *J. Clin. Microbiol.*, vol. 41, pp. 483–485, 2003.

[12] J. G. Onsare, H. Kaur, and D. S. Arora, "Antimicrobial activity of moringa oleifera from different locations against some human pathogens," *Academia J. Med. Plants.*, vol. 1, pp. 080-091, 2013.

[13] B. Adriana, A. N. M. Almodóvar1, C. T. Pereira1, and T. A. Mariângela, "Antimicrobial efficacy of curcuma zedoaria extract as assessed by linear regression compared with commercial mouthrinses," *Braz. J. Microbiol.*, vol. 38, pp. 440-445, 2007.

[14] A. Odebiyi and E. A. Sofowora, "Pytochemical screenings of Nigerian medicinal plants part 11," *Lyodia*, vol. 44, pp. 234-246, 1999.

[15] B. N. Devendra, N. Shrinivas, T. V. S. S. L. Prasad, and P. Swarna Latha, "Antimicrobial activity of moringa oleifera lam., leaf extract, against selected bacterial and fungal strains," *Int. J.Pharma Biosci.*, vol. 2, pp. 13-18, 2011.

[16] M. E. Abalaka, S. Y. Daniyan, S. B. Oyeleke, and S. O. Adeyemo, "The antibacterial evaluation of moringa oleifera leaf extracts on selected bacterial pathogens," *J. Microbiol. Res.*, vol. 2, pp. 1-4, 2012.

[17] A. O. Oluduro, "Evaluation of antimicrobial properties and nutritional potentials of moringa oleifera lam. Leaf in South-Western Nigeria," *Malaysian J. Microbiol.*, vol. 8, pp. 59-67, 2012.

[18] S. Wadhwa, M. S. Panwar, N. Saini, S. Rawat, and S. Singhal, "A review on commercial, traditional uses, phytoconstituents and pharmacological activity of moringa oleifera," *Global J. Traditional Med. Syst.*, vol. 2, pp. 1-13, 2013.

[19] D. E. Conner, *Naturally occurring compounds. Antimicrobials in foods*. New York: Marcel Dekker, Inc, 1993.

[20] H. G. D. Dorman and S. G. Deans, "Antimicrobial agents from plants: Antibacterial activity of plant volatile oils," *J Appl Microbiol*., vol. 88, pp. 308–316, 2000.

[21] O. C. Eruteya, S. A. Odunfa, and J. Lahor, "Listeria spp. in raw cow and goat meat in port harcourt, Nigeria," *British Biotechnol. J*., vol. 4, pp. 204-214, 2014.

[22] C. Akujobi, B. N. Anyanwu, C. Onyeze, and V. I. Ibekwe, "Antibacterial and preliminary phytochemical screening of four medicinal plants," *J. Appl. Sci*., vol. 7, pp. 4328 – 4338, 2004.

[23] S. K. Pal, P. K. Mukherjee, and B. P. Saha, "Studies on the antiulcer activity of moringa oleifera leaf extract on gastric ulcer models in rats," *Phytother. Res*., vol. 9, pp. 463-465, 1995.

[24] M. M. Rahman, M. M. Islam, S. A. Sheikh, A. Sharmin, M. S. Islam, A. Rahman, M. Mizanur Rahman, and M. F. Alam, "Antibacterial activity of leaf juice and extracts of moringa oleifera lam. Against some human pathogenic bacteria," *CMU Journal*, vol. 8, pp. 219-228, 2009.

[25] G. Dewangan, K. M. Koley, V. P. Vadlamudi, A. Mishra, A. Poddar, and S. D. Hirpurkar, "Antibacterial activity of moringa oleifera (Drumstick) root bark," *J. Chem. Pharm. Res*., vol. 2, pp. 424-428, 2010.

[26] G. H. F. Vieira, J. A. Mourão, A. M. Ângelo, R. A. Costa, and R. H. S. F. Vieira, "Antibacterial effect (In Vitro) of Moringa oleifera and Annona muricata against gram positive and gram negative bacteria," *Rev Inst. Med. Trop*., vol. 52, pp. 129-132, 2010.

[27] J. R. O. Peixoto, G. C. Silva, R. A. Costa, J. L. S. Fontenelle, G. H. F. Vieira, A. A. F. Filho, and R. H. S. F. Vieira, "In vitro antibacterial effect of aqueous and ethanolic Moringa leaf extracts," *Asian Pacific J. Trop. Med*., pp. 201-204, 2011.

[28] A. M. Saadabi and Z. A. I. Abu, "An in vitro antimicrobial activity of Moringa oleifera L. seed extracts against different groups of microorganisms," *Asian J. Basic Appl. Sci*., vol. 5, pp. 129-134, 2011.

[29] M. S. Auwal, A. N. Tijjani, M. A. Sadiq, S. Saka, I. A. Mariga, A. Shuibu, E. Adawaren, and I. A. Gulani, "Antibacterial and haematological activity of moringa oleifera seed extracts in wister albino rats," *Sokoto J. Vet Sci*., vol. 11, pp. 28-37, 2013.

[30] S. Kalpana, S. Moorthi, and S. Kumari, "Antimicrobial activity of different extracts of leaf of moringa oleifera (Lam) against gram positive and gram negative bacteria," *Int. J. Curr. Microbiol. Appl. Sci*., vol. 2, pp. 514-518, 2013.

[31] S. D. Patil and J. Rasika, "Antimicrobial activity of moringa oleifera and synergism with cleome viscose," *Int. J. Life Sci*., vol. 1, pp. 182-189, 2013.