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Antimicrobial Activity of *Moringa oleifera* Leaf Extract in *Pangasianodon hypophthalmus* Minced Meat

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Abstract Fish being highly perishable and readily susceptible to chemical and microbial deterioration leads to reduction in the quality, wastage and economic loss. Maintenance of high-quality fish, therefore calls for adequate, effective and affordable preservative techniques of these protein resources. *Moringa oleifera* commonly known as drumstick is native to India, Africa and South East Asia is of special interest in food preservation, as it contains a variety of bioactive substances and contributes towards the taste and aroma. These bioactive subtances have a considerable role in extending the shelf life. Anti microbial activity of *Moringa oleifera* leaves extract on the minced *Pangasianodon hypophthalmus* meat was studied and found that the fresh leaves extract have a wide range of antimicrobial activities against both gram-positive and gram-negative bacteria in disc diffusion assay. The extract displayed the largest average halo against *Escherichia coli* (19.6 mm) and the smallest average halo against *Aeromonas hydrophila* (8.35 mm), indicating that the phenolic compounds of leaves extract resulted in microbial inhibition, protecting muscle against the internal protease and finally inhibits protein breakdown and amine production.

Keywords Antimicrobial, *Moringa oleifera*, Natural preservative, *Pangasianodon hypophthalmus*.

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Introduction

Fish is an important source of protein, supplying all essential amino acids in good proportion needed for tissue building and repair (Dholakia 2010). On a global scale, more and more consumers are turning towards fishery products, unlike red meat, fishery products are a good source of easily digestible proteins, several micronutrients and therapeutically important polyunsaturated fatty acids (Abdullahi et al. 2001). In modern days, there is an ever-increasing awareness about healthy foods and fish is finding

more acceptances because of its special nutritional qualities. Fish protein is rich in lysine which lacks in vegetable protein. Moreover, the nature of fatty acids present in fish lipid is unique making fish a healthy food. Fish lipid contains large amount of ω-3 fatty acids, like Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), which are helpful in reducing coronary diseases and autoimmune diseases (Bender 1997). Interestingly, fish is also a good source of various vitamins (A,D, B6, B12) and minerals (iron, zinc, iodine, selenium, potassium, sodium, calcium).

Moringa oleifera a terrestrial plant which exhibits antibacterial, antifungal as well as antioxidant properties leading to its wide application in food preservation. *M. oleifera* Lam. is native to the Indian subcontinent and became naturalized in the tropical and subtropical areas around the world, belonging to one of the 14 species of the family Moringaceae (Iqbal and Bhanger 2006). All parts of this tree such as leaves, roots, bark, fruits, flowers, immature pods and seeds are edible and have long been consumed by humans. Due to the presence of several important substances like ascorbic acid, estrogenic substances, beta-sitosterol, iron, calcium, phosphorus, copper, vitamin A, B and C, alpha tocopherol, beta carotene, protein and essential amino acids like methionine, cysteine, tryptophan and lysine, drumstick leaves can be considered as a dietary supplement.

The antimicrobial properties of this plant were investigated worldwide and most of the studies revealed to have a promising anti-inflammatory, anti-helminthic, anti-fungal, pro-coagulant and flocculating as well as anti-microbial properties. Ethanolic extract of *M. oleifera* showed a broad spectrum antimicrobial property against many pathogens including *Staphylococcus aureus, Escherichia coli, Bacillus* sp., *Pseudomonas aeruginosa, Corynebacterium* sp., *Klebsiella pneumonia* and *Acinetobacter* sp. (Rajamanickam and Sudha 2013).

The crude extract of *M.oleifera* leaves can actively scavenge free radicals, thus preventing cellular damage (Sreelatha and Padma 2009). The leaves of *M. oleifera* have also been known to contain a number of phyto-chemicals such as flavonoids, saponins, tannins and other phenolic compounds exhibiting the antimicrobial activities (Sato et al. 2004). These compounds might be responsible for a significant decrease in the number and variety of microorganisms. The mechanisms of actions of the bioactive compounds are mainly via cell membranes perturbations. This is coupled with the action of β-lactams on the transpeptidation of the cell wall leading to enhanced antimicrobial effect of the combinations (Esimone et al. 2006) inducing membrane destabilization and thus the bacteria are thought to be killed due to leakage of cytoplasmic contents, loss of membrane potential, change of membrane permeability, lipid degradation, the entry of the peptide and blocking of anionic cell components or the triggering of autolytic enzymes (Zasloff 2002). The proteins or peptides are also involved in a defense mechanism against pathogenic fungi by inhibiting the growth through diverse molecular modes, such as binding to chitin or increasing the permeability of the fungal membranes or cell wall (Chuang et al. 2007).

Commercial use of different synthetic preservatives like chemical antioxidants (BHT, propyl gallate, nitrate, phosphate), nutritive compounds (alpha-tocopherol, beta-carotene, vitamin C) and chemical antibiotics is very common but in recent year's interest in the application of naturally occurring preservatives in muscle, foods have amplified. The demand for natural extracts is gaining importance due to the negative effect and health problems associated with chemical preservatives. *M.oleifera* being herbal preservative exhibits potent pharmacological activities, low toxicity and economic viability compared with other synthetic drugs (Paliwal et al. 2011). This plant is of special interest in food preservation beacuse in addition of contributing taste and aroma to foods, it also contains a variety of bioactive substances, which are of considerable use in extending shelf life and making it a promising natural antimicrobial agent with potential application both in the pharmaceutical and food processing industry. Based on this, the objective of the present study was to determine the antimicrobial activity of leaf extract of *Moringa oleifera.*

Classification of *Moringa oleifera*

M. oleifera Lam. belongs to one of the 14 species of

the family Moringaceae is native to the Indian subcontinent and now became naturalized in the tropical and subtropical areas around the world (Iqbal and Bhanger 2006). The tree is known by various regional names, such as Benzolive, drumstick tree, Horseradish tree, Kelor, Marango, Mlonge, Mulangay, Saijihan, Sajna, ben oil tree, miracle tree (Fahey 2005). The leaves, seeds, flowers, pods (fruit), bark and roots are all seen as a vegetable and consumed by human in diverse culinaary ways (Iqbal and Bhanger 2006) for its nutritional value, purported medicinal properties and industrial purposes (Khalafalla et al. 2010). The classification of *M. oleifera* is provided below :
Kingdom : Plantea
Sub kingdom : Tracheobionta
Super Division : Spermatophyta
Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Dilleniidae
Order : Capparales
Family : Moringaceae
Genus : *Moringa*
Species : *Oleifera*

Materials and Methods

Preparation of *Moringa oleifera* leaf extract

Fresh *M. oleifera* leaves were collected and washed well with water to remove the adhering dust. Then the leaves were air dried properly. 20g of fresh leaves were homogenized with 180 ml of an ethanol-water solution containing 50% sterile distilled water and 50% ethanol as described by Peixoto et al. (2011). The liquid portion was separated from the residue by filtration using Whatman no. 1 filter paper and this solution was used as *Moringa oleifera* leaf extract for the experiment.

Spectrum of antimicrobial activity of *Moringa oleifera* leaf extract

Preparation of media for test organisms

Selective media for test organisms like Tergitol-7 agar media (*Escherichia coli*), Baird Parker media (*Staphylococcus aureus*), Bismuth Sulfate Agar(BSA) media (*Salmonella typhimurium*) and Nutrient agar media (*Aeromonas hydrophila*) were prepared separately and autoclaved at 121^{o}C for 15 minutes at 15 lbs. After cooling, the media was poured into sterile petri plates and allowed to set at ambient temperature and then were used for the antimicrobial study.

Antimicrobial activity by agar disc diffusion method

Determination of the antimicrobial activity of *M. oleifera* leaf extract was carried out by agar disc diffusion method. Firstly, the inoculums from the pure culture of the test bacteria were applied on the specific media by swabbing under laboratory condition. Then sterile Whatman no. 1 filter paper discs of 5 mm diameter were soaked into test solutions and were placed on the agar surface separately under aseptic conditions as described by Kalpana et al. (2013). Acetic acid was taken as positive control whereas distilled water was taken as negative control. All the plates were maintained at room temperature for 2 h to allow the diffusion of the solutions into the medium. The plates were then incubated at 37^{o}C for 48 h and the zones of inhibition were measured (Pal et al. 1995).

Results and Discussion

The antimicrobial properties in terms of zone of inhibitions or halos (mm) exhibited by four different strains of bacteria when treated with the leaf extract. The extract displayed the largest average halo against *Escherichia coli (*19.6 mm) and the smallest average halo against *Aeromonas hydrophila* (8.35 mm) presented in Fig. 1. The order of the diameters of inhibition zones obtained as *Escherichia coli* (19.6 mm) >*Salmonella typhimurium* (18.55 mm) > *Staphylococcus aureus* (13.8 mm) >*Aeromonas hydrophila* (8.35 mm).

Both gram-positive and gram-negative bacteria are susceptible to the leaf extract of *M. oleifera*. The result is supported by the findings of Siddhuraju and Becker (2003) and vaghasiya and Chanda (2007) who stated that the plant has antimicrobial activity against both gram-positive and gram-negative bacteria due to the presence of broad-spectrum antibiotic com-

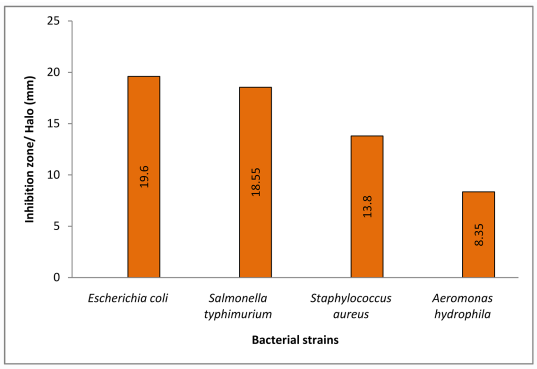

Fig. 1. Antimicrobial activity of *Moringa oleifera* leaf extract against some bacteria.

pounds. The similar result were reported by Rahman and Sheikh (2009) in the fresh leaf juice, powder from fresh leaves and cold water extract of fresh leaves from drumstick displaying a potential antimicrobial activity against both gram-positive and gram-negative bacteria determined by disc diffusion and minimum inhibitory concentration method.

The present study reveals the susceptibility of *E. Coli* against *M. oleifera* leaf extract which is supported by the findings of Falowo et al. (2016) reporting the drumstick leaf extract to have the best inhibitory effect against *E. coli* where the diameter of the zone of inhibition obtained was 19 mm. Similarly Adeyemi et al. (2013) reported that the treatment of fish with *M. oleifera* extract was useful in the elimination of *E. coli.* But , in contradiction, Rajendrhan et al. (1998) reported *E. coli* to be resistant to *M. oleifera* extracts. Bhawasar et al. (1965) also showed *M. oleifera* extracts to be ineffective against *E. coli.* The difference could be attributed to variation in the environment from where the plant was collected, the season and the physiological stage of the plant when leaves were harvested (Taylor and Van Staden 2001) as it effects the chemical composition and the number of compounds in the plant.

The study also shows that drumstick leaf extract is effective against another important pathogen *Salmonella typhimurium*. This is in concurrence with the findings of Doughari et al. (2007) who observed the inhibition halos up to 8 mm when challenging *Salmonella* with aqueous and ethanolic *M. oleifera* leaf extract. Adeyemi et al. (2013) also reported that *Salmonella* was not found in any *M. oleifera* treated fish. This observation is in in line with the report of Nwaiwu et al. (2011) who established the activity of *M. oleifera* hexane extract against *Salmonella, Shigella* and *Enterobacter*. Cáceres et al. (1991) reporting that *M. oleifera* leaf extract can inhibit the growth of *Staphylococcus aureus* which is similar with the findings of the present study. This result is also supported by Peixoto et al. (2011) who observed the antimicrobial effect of aqueous and ethanolic *M. oleifera* leaf extract on *Staphylococcus aureus* and reported that the extracts produced bacteriostatic and bactericidal effects on *S. aureus.* The results also coincides with the findings of Bukar et al. (2010) who reported the bioactivity of *M.oleifera* ethanol extract on the pathogenic bacteria such as *E. coli, S. aureus, P.aeruginosa, S. typhimurium* and *Enterobacter aerogenes.*

Conclusion

The antimicrobial activity of *M. oleifera* leaf extract was determined by disc diffusion method which proved *M. oleifera* a promising and potential natural preservative due to its wide range of inhibition against both gram-positive and gram-negative bacteria. The antimicrobial properties were established in terms of zone of inhibitions or halos (mm) exhibited by four different bacterial strains against this extract, the largest inhibition zone was displayed by *Escherichia coli* (19.6 mm) followed by *Salmonella typhimurium* (18.55 mm), *Staphylococcus aureus* (13.8 mm) and *Aeromonas hydrophila* (8.35 mm). Thus, *M. oleifera* leaves being easily available and cost-effective, can be used as a potent natural antimicrobial agent in food industry.

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