Study on antibacterial and antioxidant activities of raw and fermented *Moringa Oleifera* lam. leaves

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**ABSTRACT**

The *Moringa* plant (*Moringa oleifera* Lam.) has been the object of much research due to its multiple uses and well-known bactericidal potential with good nutritional and therapeutic values. Raw (non-fermented) and fermented leaves were evaluated for antibacterial and antioxidant properties. The present study revealed that fermented leaves have great antibacterial activities against pathogenic microbes such as *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Alcaligenes* spp., *Micrococcus* spp., *Shigella* spp., as well as two KTCC cultures of *Bacillus cereus*. The fermented leaves showed highest inhibition (16mm) to *Proteus* spp. and lowest inhibition (7 mm) to *Pseudomonas* spp. This result suggests that the fermented leaves are superior to non-fermented dried leaves considering antibacterial activities. Although, in comparison with raw leaves, fermented leaves showed reduced phenol content and DPPH radical scavenging activity.

**Keyword:** *Moringa oleifera* Lam leaf, Sauerkraut process, antibacterial activities, antioxidant properties

**INTRODUCTION**

Medicinal plants are of great importance to the health of individuals and communities. Herbal medicines serve the health needs of about 80% of the world’s population, especially for millions of people in the vast rural areas of developing countries. The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide, especially in developing countries causes of morbidity and mortality in immuno-compromised patients [1]. The increasing prevalence of multi-drug resistant bacterial strains and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of untreatable bacterial infections and require searching for new infection-fighting strategies [2,3]. Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for noble antibiotic sources [4].

*Moringa oleifera* Lam. under the family Moringaceae is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan [5]. A wide variety of nutritional and medicinal virtues have been attributed to its different parts. Phytochemical analyses have shown that its leaves are particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, as well as such known antioxidants such as β-carotene, vitamin C, and flavonoids [6].

Antimicrobial activities of various *M. oleifera* Lam. morphological parts against some pathogenic microorganisms have been reported by Doughari *et al.* [7]. The fresh leaf juice and aqueous extracts from the *Moringa* seeds inhibit
the growth of Pseudomonas aeruginosa and Staphylococcus aureus and no activity was demonstrated against other pathogenic Gram-positive and Gram-negative bacteria and Candida albicans [8]. Dahot [9] investigated an antibacterial action of Moringa leaves against E. coli Kl. aerogenes, Kl. pneumoniae, S. aureus, Aspergillus niger and B. subtilis.

It is also used for treating fungal infection, anti-inflammation, sexually-transmitted diseases, malnutrition and diarrhea. Moringa species have long been recognized by folk medicine practitioners as having value in the treatment of tumors [10]. It has high levels of antioxidant activity. Kumar and Pari [11] investigated antioxidant potential of Moringa oleifera on lipid peroxidation. It is reported that due to presence of kaempferol properties Moringa leaves showed antioxidant potential [12].

Generally, fermented foods are food substrates that are invaded or overgrown by edible microorganisms whose enzymes, principally amylases, proteases and lipases; hydrolyze polysaccharides, proteins and lipids are responsible to produce fermented food. Moreover, these enzymes also improve enjoyable flavors, aromas and textures which are more attractive to the human consumption [13].

Sauerkraut fermentation process has received substantial research in order to commercialize and standardize production. The 'sauerkraut fermentation process' can be applied to any other suitable type of vegetable product. In the present study, to improve of Moringa oleifera Lam leaves nutritionally and medicinally Sauerkraut fermentation process was used by using lactic acid bacteria in anaerobic condition. Leuconostoc mesenteroides, Lactobacillus plantarum, Lactobacillus brevis, and Enterococcus faecalis are main contributing micro-organisms in Sauerkraut process.

There is no sufficient information in Bangladesh regarding antibacterial activities as well as antioxidants properties of fermented Moringa oleifera Lam. leaves. Therefore elaborate study is required. Hence, the present study was undertaken to investigate the effectiveness of dried and fermented M. Oleifera Lam. leaves as a potential antimicrobial agent against some human pathogenic bacteria. The present study also focused on antioxidant activity to demonstrate the impact of fermentation on the qualitative and quantitative polyphenol composition in the plant material.

MATERIALS AND METHODS

2.1 Plant material
The fresh leaves of M. oleifera Lam. leaves were collected from Atomic Energy Research Establishment complex, Savar, Dhaka, Bangladesh. The plant species was identified by a Taxonomist of Bangladesh National Herbarium, Bangladesh Botanical Garden.

2.2 Plant leaves fermentation process (Sauerkraut method)
Sauerkraut is a popular process to improve plant origin food product by using self microbial flora, including Leuconostoc mesenteroides, Lactobacillus plantarum, Lactobacillus brevis, and Enterococcus faecalis fermentation process.

In this experiment M. oleifera Lam. fresh leaves were taken (200 gm) and treated with 3% sodium chloride. The leaves and salt were placed in alternating layer in the two wide-mouthed jars. After that a wooden boards were placed over of the leaves-salt mixture and press gently to squeeze out a layer of juice from the leaves. Then weight has to be placed on the both boards to maintaining the anaerobic condition of the fermentation condition and cover the jars with cheese cloths. The jars were incubated for 21 days at 30°C.

2.3 Determination of Lactic acid
The total percentage of Lactic acid was measured from the fermentation process by the following equation [14].

\[
\% \text{ lactic acid} = \frac{\text{ml of alkali} \times \text{normality of alkali} \times 9}{\text{weight of sample in gm}}
\]

2.4 Methanol (90%) extracts of dried leaves and fermented leaves extraction
100 gm of fresh leaves of M. oleifera were shade dried through drier at 50 to 55°C over a period of 5 days. The dried leaves were ground into powdered using grinder machine and stored into cork tight bottle for further use. 20 gm of dried and fermented leaves powdered were taken into 500 ml conical flasks with 100 ml 90% methanol. The conical flasks were plugged with rubber cork, and then shaken 30 min by using Shaker (GFL-3017) at 120 rpm and
allowed to stand at room temperature for 5 days. The extracts were separately filtered using sterile what’s man no.1 filter paper and filtrates were then concentrated in a vacuum drying oven (VS-1202V5) and subsequently lyophilized to dryness.

2.5 Antibacterial assay
Antibacterial activity of methanol extraction of dried and fermented leaves were tested against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Alcaligenes* spp., *Micrococcus* spp., *Shigella* spp., and *Bacillus cereus* (KTCC 11204 and 40935 cultures). In vitro antibacterial test was carried out by paper disc diffusion method [15,16] using $10^8$ cfu ml of standardized suspension of tested bacteria spread on nutrient agar media. The discs (6 mm in diameter) were impregnated with 10 µl of fresh dried leaf and fermented leaves extracts, and kept them for self air drying and were placed on fresh cultured seeded agar plates. For control identification there used sterile paper disc with soaked with same Dichloromethane (DCM) solvent. The plates were incubated at 37°C for 24h. Antimicrobial activity was evaluated by measuring the zones of inhibition against the tested bacteria. Each assay was carried out in triplicate.

2.6 Determination of antioxidant markers
For the antioxidant determination two gram of dried and fermented leaves were suspended in 20 ml 80% methanol, then kept stirring condition for 1 hr and have to filtered through filter paper (Whatman 11.0 cm). Then the filtrates were used for subsequent analysis.

2.6.1 Total phenol content
According to the Folin-Ciocalteu (FC) method [17] total phenol content was determined. At first aliquot of both samples (0.5 ml) was added to diluted 2N FC reagent (1:10) (2.5 ml). After 3-4 min, 7.5% sodium carbonate solution (2 ml) was added to the mixture in the test tubes and kept those in a dark place for 2 hr at room temperature. Through using UV visible spectrophotometer at wavelength 765 nm the absorbance of the all solutions were measured. Here, Gallic acid was used as a calibration standard and the data were expressed as mg Gallic acid equivalents/100 g samples.

2.6.2 DPPH• radical scavenging activity
According some modification of method of [18] the stable free radical (DPPH•) scavenging activity of the both leaves samples were determined. The stock DPPH• solution was prepared by dissolving in methanol (2.4 mg/mL). Prior to use, stock DPPH• radical solution was diluted through 80% methanol and measured the initial OD 517 using UV visible spectrophotometer which was at 0.89. An aliquot of both samples (150 µL) were added separately to 4.5 ml of diluted DPPH• solution, vortexes them well and kept them in a dark place for 15 min at room temperature.

The both samples absorbance were taken at 517 nm. Total antioxidant capacities were calculated virtually to the reactivity of Trolox, which is act as a standard value and the results were shown as µM Trolox equiv./g dried and fermented leaves. The ability to scavenge DPPH• was determined by following equation.

$$\%\text{ Inhibition} = \frac{Ab - As}{Ab} * 100$$

Here Ab and As were the absorbance of the blank and the sample, respectively.

RESULTS AND DISCUSSION
During fermentation process of *Moringa* leaves, 1.44 % to 1.56 % lactic acid was found from 10 gm leaves. This percentage of the lactic acid level was good. So, commercially higher amount of lactic acid can be produced from *Moringa* leaves by using this easy and cheaper fermentation process.

3.1 Antibacterial activities of raw and fermented *Moringa* leaves
Most pathogenic organisms are becoming resistant to antibiotics [19]. To overcome this alarming problem, the investigation of novel active compounds against new targets is a matter of urgency. By this way, in this study, antibacterial activities of dried and fermented *Moringa* leaves against enteropathogens *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas* spp., *Alcaligenes* spp., *Micrococcus* spp., *Shigella* spp., with two types of KTCC cultures of *Bacillus cereus* (40935, and 11235) were investigated.
The leaves of both extracts showed some antibacterial activities as the growth of inhibition zones were up to 16 mm. The total antibacterial results were showed in the following figure 1. The fermented leaf extract has greater antibacterial activity than dried leaf extract against Staphylococcus aureus, Proteus vulgaris, Pseudomonas spp, Micrococcus spp, and Shigella spp. Whereas, against Alcaligens spp and two types of KTCC cultures of Bacillus cereus dried leaves extract showed better inhibition than fermented leaves.

![Figure (1): Antibacterial activities of Dried and Fermented M. oleifera Lam. Leaves](image)

Dried leaves samples showed lowest amount of growth of inhibition against Staphylococcus aureus, Proteus, and Pseudomonas spp, which were all 7 mm (figure 2), while highest inhibitions were showed Alcaligens spp., which was 10 mm. At the fermented sample highest inhibition was found against Proteus spp. that was 16 mm, whereas lowest inhibition (7 mm) was found against Pseudomonas spp. Against KTCC-40935 strain of Bacillus spp. dried leaves sample showed highest inhibition (14mm) while fermentation samples give lower level of zone of inhibitions (8 mm). But the KTCC - 11204 strain showed two different zone of inhibition (14 mm and 36 mm) in case of dried leaves (figure 3).

![Figure (2): Normal zone of inhibition of Proteus spp. (a) and Bacillus cereus KTCC 40935 strain (b) of against dried & fermented leaves](image)
Among different extract solvent (ethanol, distilled water and methanol), methanolic extract of *Moringa* leaves provide better antibacterial activity [20]. Also Rahman *et al.* [21] found that fresh *Moringa* leaf juice, powder from fresh leaf, cold water extract of fresh leaf, showed a potential antibacterial activity against all the tested organisms such as *Shigella, P. aeruginosa, Pseudomonas* spp., *S. aureus, B. cereus, Vibrio* spp. Khesorn [22] reported the antibacterial activity from the methanolic crude extract and purified dichloromethane (DCM) extract of *Moringa oleifera* capsules by agar-well diffusion. The methanolic crude extract showed no activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The purified dichloromethane extract and isolated parts from column chromatography showed antibacterial activity against these bacteria.

Spiliotis *et al.* [23] reported antimicrobial activity from different types of *Moringa oleifera* seeds against *Bacillus cereus, Candida albicans, Streptococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas aeruginosa, E.coli* and *Aspergillus niger*. Nikkon *et al.* [24] showed that antimicrobial and antifungal activities of *Moringa* seeds against *Shigella boydii, Shigella dysenteriae* and *Staphylococcus aureus*.

The finding of this study is variance with Doughari *et al.*, [7] who reported in their study on the antibacterial activity from the aqueous, acetone and ethanol extracts of the leaves of *Moringa* that ethanol extract of the plant demonstrated the highest activity, while the aqueous extract showed the lowest activity. It has been reported that different solvents have different extraction capabilities and spectrum of solubility for the phytoconstituents [25, 26]. Moreover it was found that fermented leaves was more active against Gram-negative bacteria whereas , dried leaves was more active against Gram Positive bacteria - *Staphylococcus aureus, Micrococcus spp. Bacillus spp. KTCC 11204 and 40935*. The activity of the plant against both Gram-positive and Gram-negative bacteria may be indicative of the presence of broad-spectrum antibiotic compounds in the plant [27].

3.2 Effect of fermentation on antioxidant markers of *M. oleifera* Lam. leaves

Phytochemically, *M. oleifera* Lam. leaf is known as rich source of quercetin glucosides, rutin, sterols, chlorogenic acid, flavanol, kaempferol glycosides [28]. In this study, total phenol content and DPPH-radical scavenging activity were measured as antioxidant markers. The results showed that in non-fermented (raw leaves) and fermented leaves, total phenol content was 308.28mg and 186.99mg (Gallic acid equivalent per 100gm) respectively. After the fermentation period (21 days), the total phenol content was significantly decreased to 60.66 % (figure 4,a) compared to raw leaves. Several investigations reported lower phenolic content in fermented products. The decreased level might due to the diffusion of phenolic compounds in cell liquids and oxidation of diffused phenolic by the action of an enzyme polyphenol oxidase present in food stuff or produce by the micro-organisms during fermentation [29]. Accordingly, free radical scavenging activity has been found to be significantly reduced (figure 4,b) in fermented *Moringa* leaves (85.64 % loss). These phenomena are consistent with other reports Lasekan *et al.*, 2013; Adetuyi *et al.*, [29, 30] who also found the decreased level of phenol content as well as antioxidant activity with the increased duration of fermentation of their experimental plant materials. The decrease of phenolic compound as well as antioxidant activity of fermented leaves can be explained with the fate of tocoferols after fermentation, because
these are the abundant bioactive compound in *M. oleifera* Lam. leaf [31]. Several studies reported a decreased level of tocoferols in fermented products [32].

![Graph showing the effect of fermentation on total phenol content](image)

**Figure (4):** Effect of fermentation on total phenol content (a) and antioxidant activity (b) of dried and fermented *M. oleifera* Lam. leaves

## CONCLUSION

The main principle of the present study was to measure the efficacy of Moringa leaves extract for antibacterial activities and antioxidants levels of raw and fermented type. Although fermentation caused lower antioxidant levels, but showed higher antibacterial potential against Staphylococcus aureus, Proteus spp., Micrococcus spp., and Shigella spp. compared to raw leaves. Thus *M. oleifera* Lam. fermented leaves could become natural antimicrobial agents with potential applications in pharmaceutical industry for controlling the pathogenic bacteria. The fermented leaves can be used as salad, salad dressing, vegetable substitutes, side dishes of rice, meat foods. However, issues of safety and toxicity should be considered.

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