



# ANTIMICROBIAL ACTIVITY, SHELF LIFE AND STANDARDIZATION OF RECIPES WITH RAW AND PROCESSED *MORINGA* *OLEIFERA* SEEDS

Shyama Reji

University of Madras

**Abstract:** *Moringa Oleifera* commonly called drumstick seeds is usually used as a vegetable and for medicinal purposes. *M. oleifera* is found to have numerous benefits and the present study was carried out to determine the antimicrobial potential and shelf life of raw, germinated and fermented *M. oleifera* seeds. The study also discusses the benefits and methods of incorporating the samples into food products. The results were found to be positive thus concluding that Moringa seeds exhibit high antimicrobial value when processed. Processed seeds have an expected shelf life and are accepted when incorporated into food items. Its high nutritive value is suitable for either nutritional or medicinal purposes. Studies have also proved that fortifying staple foods with processed moringa seeds could enhance the flavour and texture, increasing the nutrition quality. Thus, incorporating the foods with moringa seed powder can prevent malnutrition

**Index Terms** – Moringa oleifera, Germinated, Fermented, Sensory Evaluation, antimicrobial, Shelf life

## 1. INTRODUCTION

Foods which aid in the prevention or treatment of diseases or disorders are called Nutraceuticals.<sup>1</sup> Moringaceae is a family of shrubs and trees which comprises 13 species distributed in the Indian Subcontinent, Kenya, Northern and South Western Africa, Arabia and Madagascar.<sup>2</sup> Processing the *Moringa oleifera* seeds can enhance their nutritional value which is already proven.<sup>3</sup> Moreover, the antibacterial potential of Moringa seed was almost equal compared to that of commonly used antibiotics. The need for new natural antimicrobial agents is important, as there are many complications linked to most synthetic antibiotics. Several studies have proved the antifungal activity of moringa seed extracts.<sup>4</sup> There are increasing pieces of evidence to prove that functional plants can alter the treatment for non-severe cases of infectious diseases. They could act as a source of new and low-cost antibiotics to which pathogenic strains are resistant.<sup>5</sup>

Non-communicable diseases have created a need for intensive approaches to prevention and treatment. With the concept of food as medicine, gaining importance in the multipronged approach to disease management, there is a growing interest in functional foods. Hence, there is a need to formulate novel, yet, readily available foods and food products, which help in the prevention and management of diseases. Moringa seeds are likely used in different parts of the globe and are a regular part of the South Indian diet. This study seeks to determine the synergistic effect of moringa seeds concerning the antioxidant and antimicrobial properties of raw (RMO), germinated (GMO) and fermented (FMO) *M. oleifera* seeds. It is found to have essential phytochemicals, micronutrients and macronutrients<sup>5</sup> which can be used as natural remedies to prevent various diseases and deficiencies.

## 2. MATERIALS AND METHODS

An experimental study design was adopted for the study. The study protocol was approved by the institutional ethics committee of Women's Christian College, Chennai. Seeds of *M. oleifera* were collected at Madhavaram, Chennai, Tamil Nadu. The Family, genus and Species of *M. oleifera* were identified in the Plant Biotechnology Department as PKM1 Variety.

### 2.1 Processing of raw, germinated, and fermented *moringa oleifera* flour samples

*M. oleifera* seeds were sorted, rinsed, sundried, dehulled and milled using a mechanical blender. The sample is sieved to obtain RMO seed flour. Seeds were sorted and soaked in potable water for 2 hours. Excess water was removed and the seeds were tied in a wet cloth for 72 hours (3 days) at room temperature. Germinated seeds were washed, sundried, dehulled, and ground to powder using a mechanical blender and sieve to obtain GMO seed flour. Finally, for fermentation, seeds were sorted, dehulled,<sup>6</sup> and soaked in water for 72 hours (3 days) to ferment. The fermented seeds were sundried and milled using a mechanical blender and sieved to obtain FMO seed flour. The flour was packed in a plastic container sealed and stored at room temperature before analyses.

To prepare the extract, about 20 g of each sample was soaked in 100 ml of ethanol for 72 h. The pale-yellow supernatant liquid was filtered by Whatman filter paper.

### 2.2 Experimental procedure

The agar well diffusion method is the most widely used technique for assessing antimicrobial activity.<sup>7</sup> In this technique, a well or reservoir containing the test compound at a known concentration is brought into contact with an inoculated medium and the diameter of the clear zone around the reservoir (zone of inhibition diameter) is measured at the end of the incubated period. Different types of reservoirs can be used, such as filter paper discs or holes punched in the medium.<sup>8</sup>

#### 2.2.1 Assessment of antimicrobial activity of RMO, GMO and FMO

The antimicrobial property of RMO, GMO and FMO seeds was detected using agar disk diffusion methods using Muller Hinton agar. The results were obtained after incubating the plates at 37°C for 24 hours. The antimicrobial property was determined against three foodborne disease-causing pathogens which are *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*. Diameter with a zone of inhibition of 10 or less indicates the test product has low antimicrobial activity against the test pathogen.<sup>9</sup> The diameter zone of inhibition of 11 to 15 indicates the test product has an intermediate microbial activity against the pathogen. A diameter with a zone of inhibition of 16 or more indicates a high antimicrobial activity against the test agar.

#### 2.2.2 Preparation of the Muller Hinton Agar (MHA agar)

3.8 grams of MH agar were weighed and dissolved in 100 ml of deionized water in a 250 ml conical flask. The mouth was plugged with a sterile cotton plug. The flask was microwaved for 3 minutes and then autoclaved at 15 lbs pressure for 15 minutes at 121 degrees Celsius. The autoclaved agar was poured into the sterile petri plates within a laminar flow chamber. The agar in the Petri plates was allowed to cool and solidify within the chamber. The agar was streaked with the prepared bacteria culture with a sterile cotton swab.

#### 2.2.3 Preparation of the bacteria subculture

A sterile loop was used to transfer the organisms (the inoculum) from the pure culture to the sterile growth medium. The sterile growth medium would contain the inoculum with the required quantity of nutrient broth. The Inoculating loop was heated until it was red hot before and after the sub-culturing procedure to prevent any contamination the neck of the bottle should be passed through a hot Bunsen flame before and after the inoculation. The inoculation procedure mentioned above was followed for the preparation of all three subcultures *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli* and labelled separately.

## 2.2.4 Anti-bacterial test

After 24 hours, the three sterile petri plates with solidified agar were streaked with subcultures of *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli* using sterile swabs within the laminar flow chamber. Three wells were made in each Petri plate using the cork borer and a concentration of 20µl of each raw, germinated and fermented *M. oleifera* ethanol extract was loaded in each of the wells using a micropipette to compare the antimicrobial activity of the test sample and the standard samples. The setup was incubated at room temperature for 24 hours.

The zone of inhibition was observed for all the petri plates. The zone of inhibition was a clear region around the wells where the sample was loaded. The clear region is an indication of the absence of bacterial growth and it reveals the potency of the sample as an antibacterial agent. The diameter of the zone of inhibition was measured using a scale; the result is expressed in millimeters.

## 2.3 Evaluation of the shelf-life of the samples

The shelf life of the samples was evaluated at room temperature on the 1<sup>st</sup>, 15<sup>th</sup> and 30<sup>th</sup> day of storage. The shelf life was analysed for physical signs of deterioration and microbial quality. The samples were examined for changes in appearance, texture changes, moisture and odour. The standard plate count method was used to evaluate the microbial quality.

## 2.4 Development of Standardized Recipes

A comparative study on the sensory evaluation of three standardized recipes incorporating RMO, GMO and FMO seed powder was conducted. The appearance, colour, taste, texture, flavour and overall acceptability of the foods were marked by the participants using a hedonic scorecard.

Twenty subjects (male and female), aged 20 to 30 years willing to participate in the study will be chosen as members of the taste panel for sensory evaluation of the standardized recipes. Participants with common ailments like colds that could affect taste sensitivity or undergoing any treatment and those who are allergic to any specific foods were excluded from the study.

## 3. RESULTS AND DISCUSSION

The current study deals with the antimicrobial activity and shelf-life of raw and processed moringa seeds. The data collected was processed, tabulated and subjected to descriptive and inferential statistical analysis.

### 3.1 The antimicrobial activity of samples against *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*

It is evident from Table 1 that, GMO followed by FMO exhibits a high antimicrobial activity towards all three pathogens compared to RMO.

Table 1. Antimicrobial activity of samples against *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*

| Bacteria                     | Zone of inhibition (mm) samples at a concentration of 20µl |      |      |
|------------------------------|------------------------------------------------------------|------|------|
|                              | RMO                                                        | GMO  | FMO  |
| <i>Salmonella typhi</i>      | 16.0                                                       | 20.0 | 18.0 |
| <i>Staphylococcus aureus</i> | 15.0                                                       | 19.0 | 17.0 |
| <i>Escherichia coli</i>      | 16.0                                                       | 18.0 | 17.0 |

### 3.2 Evaluation of shelf life of samples

The shelf life of the RMO was evaluated by the physical quality and the standard plate count technique for the evaluation of microbial quality. Samples were stored in the same conditions, in a closed container at room temperature for 1 month revealing that there was a significant difference between the samples. The results obtained are listed in Table 2.

Table 2: Microbial quality of the samples

| Day of evaluation    | Number of microbial colonies formed (n) |               |               |
|----------------------|-----------------------------------------|---------------|---------------|
|                      | RMO (n)                                 | GMO           | FMO           |
| 1 <sup>st</sup> day  | 0                                       | 0             | 0             |
| 15 <sup>th</sup> day | 63                                      | 81            | More than 100 |
| 30 <sup>rd</sup> day | 68                                      | More than 100 | More than 100 |

As observed in Table 2, the number of colonies formed on the first day was zero for all three samples, and on the 15th day, it was 63 colonies FMO had more than 100 colonies and RMO had the least number. On the 30<sup>th</sup> day of storage, the number of colonies increased steadily in GMO and FMO but in RMO, only a slight increase was observed. Gram-positive bacteria were present in all three samples.

The physical quality of the RMO was found to be good on the 15th and 30th day of storage and free from any unpleasant odour. On the 30<sup>th</sup> day, the texture also appeared to be the same as on the 1st day but few lumps were identified. On the other hand, GMO was found to be average whereas FMO had a great difference on the 15<sup>th</sup> and 30<sup>th</sup> day of storage. A lump formed on the 15<sup>th</sup>, day of storage and the number of lumps increased on the 30<sup>th</sup> day of storage and a foul smell was observed on the 30<sup>th</sup> day of storage. The texture changed on the 30<sup>th</sup> day. There was more moisture content in the GMO on the 30<sup>th</sup> day. Whereas, there was a change in the colour of the FMO. On the 15<sup>th</sup> day, the FMO became darker than the 1<sup>st</sup> day of storage and on the 30<sup>th</sup> day it became dark brown. There was a lump formed on the 15<sup>th</sup> day of storage and the number of lumps increased on the 30<sup>th</sup> day of storage. There was a slight texture change on the 30<sup>th</sup> day. The physical quality and standard plate count of the samples on the 1<sup>st</sup>, 15<sup>th</sup> and 30<sup>th</sup> day are shown in Figure 1.

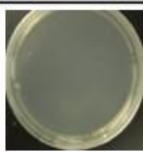
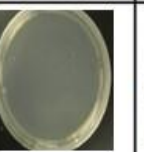
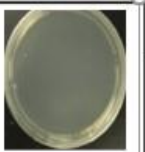
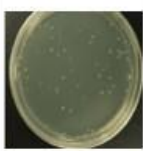
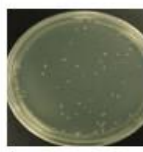
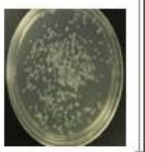
| Day    | FMO                                                                                 |                                                                                     | GMO                                                                                 |                                                                                      | FMO                                                                                   |                                                                                       |
|--------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
|        | Physical quality                                                                    | Standard Plate count                                                                | Physical quality                                                                    | Standard Plate count                                                                 | Physical quality                                                                      | Standard Plate count                                                                  |
| Day 1  |  |  |  |  |  |  |
| Day 15 |  |  |  |  |  |  |
| Day 30 |  |  |  |  |  |  |

Figure 1: Physical Quality and standard plate count of the sample on the 1<sup>st</sup>, 15<sup>th</sup> and 30<sup>th</sup> day of storage.  
Standardization of recipes using samples

Three recipes were standardized by incorporating 5g – 10g of RMO, GMO and FMO seed powder. The selected recipes were popular South Indian main dishes, accompaniments and snacks. The three recipes standardized incorporating samples were wheat semolina upma, drumstick flower stir fry and pumpkin rice balls



### 3.3 Sensory evaluation of recipes incorporating samples

The recipes standardized were tested for their acceptability using the five-point hedonic scale for five sensory attributes; appearance, colour, taste, Texture and flavour as well as the overall acceptability of the product. The standardized recipes were assessed by twenty panelists of the age group 18 – 30 years. The panel members analysed the sensory quality of the standardized recipes incorporating raw, germinated and fermented *M. oleifera* seed powder. The overall acceptability of the standardized recipes is given in Table 3.

Table 3. Comparison of mean overall acceptance of sensory score of wheat semolina upma, drumstick flower stir fry and pumpkin rice balls incorporating RMO, GMO and FMO seed powder

| Food Items                | RMO<br>(Mean $\pm$ SD) | GMO<br>(Mean $\pm$ SD) | FMO<br>(Mean $\pm$ SD) |
|---------------------------|------------------------|------------------------|------------------------|
| Wheat semolina Upma       | 4.5 $\pm$ 0.51         | 3.4 $\pm$ 0.51         | 2.6 $\pm$ 0.74         |
| Drumstick Flower Stir fry | 3.8 $\pm$ 0.48         | 3.4 $\pm$ 0.51         | 2.7 $\pm$ 0.55         |
| Pumpkin Rice Balls        | 3.1 $\pm$ 0.55         | 3.3 $\pm$ 0.48         | 2.5 $\pm$ 0.60         |

From Table 3 it can be inferred that the overall acceptability of all three food products ranged from 2.5 and 3.5 except for wheat semolina incorporated with RMO (4.5  $\pm$  0.51). The colour of RMO incorporated wheat semolina upma had a score of 4.5  $\pm$  0.51. GMO and FMO had the same scores of 4.4  $\pm$  0.60. Wheat semolina upma incorporated with RMO had the highest score of 3.7  $\pm$  0.44 and FMO had the lowest score of 2.5  $\pm$  0.68. The scores for texture and flavour were 4.6  $\pm$  0.50 which was the same for all three samples. The overall acceptability of the RMO-incorporated wheat semolina upma was higher at 4.5  $\pm$  0.51 and GMO had an average score of 3.4  $\pm$  0.51 but FMO had the least scores of 2.6  $\pm$  0.74.

The wheat semolina upma, drumstick flower stir fry and pumpkin rice balls incorporated with RMO and GMO received a mean score > 3 for overall acceptability indicating that the taste panel considered the recipes to be good or very good whereas food products standardized by adding FMO got a mean score > 2 for the overall acceptability indicating that the taste panel considered the recipes to be acceptable.

About drumstick flower stir fry, the score for appearance (3.6  $\pm$  0.50) was the same for all three samples. The score for the colour of the dish incorporating RMO, GMO and FMO was 3.6  $\pm$  0.48. In the third dish, pumpkin rice balls, the score for appearance (2.8  $\pm$  0.41) was the same for all three samples and the score for colour was 3.4  $\pm$  0.50 for all three samples.

The most accepted sample was RMO and the least accepted was FMO. The product added RMO and GMO seed powder received a mean score of > 3 whereas the product added with FMO got a mean score of > 2 for the overall acceptability indicating that the taste panel considered the recipes to be acceptable

## 4. DISCUSSION

The study was conducted to analyse the antimicrobial activity and shelf life of raw and processed moringa seeds. The research was found to quote relevant information regarding processed moringa seeds. *Salmonella typhi* causes typhoid fever in humans.<sup>10</sup> A study conducted by Enan<sup>11</sup> reported that moringa seed extract has a higher antimicrobial efficiency against disease-borne pathogens. Also, another study conducted with Moringa leaves reveals that the following isolated bacteria *Corynebacterium pseudotuberculosis* (30.4%), *Staphylococcus aureus* (25.8%), *Escherichia coli* (17.8%), *Corynebacterium ulcerans* (10.5%), *Klebsiella pneumoniae* (8.5%), *Pseudomonas aeruginosa* (8.5%), *Micrococcus spp.* (6.7%), *Proteus vulgaris* (5.2%), *Citrobacter spp.* (4.2%), and *Staphylococcus epidermidis* (1.7%) found to have a superior antibacterial effect with *M. oleifera* ethanol extracts.<sup>12</sup>

*Escherichia coli* is a common colonizer of the human gastrointestinal tract, often appearing soon after birth and persisting for decades.<sup>13</sup> They may be limited to the colonization of a mucosal surface or can spread throughout the body and can cause urinary tract infection, sepsis meningitis and gastrointestinal infection. The human gastrointestinal tract is susceptible to diarrhoea genic *E. coli* infections.<sup>14</sup> Studies done on the antimicrobial activity of moringa seed extract have already proved that the aqueous extract of Moringa seeds

at 40 % concentrations was found to have an inhibition zone of 48 mm.<sup>15</sup> Here all three samples were found to have a good antimicrobial potential towards the pathogens. Thus, fortification with processed *M. Oleifera* is highly recommended.

Even though moringa pods are easily available, it is time-consuming to process the seeds for more benefits. Thus, investigating the shelf life of the processed seeds is done in the study. The shelf life was evaluated using physical examination for a sign of deterioration and spoilage and the standard plate count technique for evaluation of microbial quality. The shelf life of any food product can be easily detected using the standard plate count method, based on the number of colonies as a regular period of checking. Any sterile product would have no colonies of any particular microorganism. The most sensitive technique of detecting the presence of viable bacterium is to allow simplifying itself to form a viable colony.<sup>16</sup> Many studies<sup>17,18</sup> have found that *M. oleifera* seeds lose their viability and vigour within 6–12 months depending on the conditions in which they are stored which agrees with the results of the current research. The sun-dried seeds can be stored without dehulling for 2 – 3 months. RMO seeds have an extended shelf-life than GMO and FMO seeds. GMO and FMO can be refrigerated for extended shelf life. But at room temperature, the shelf life is only 10 – 15 days.<sup>18</sup>

The findings of the present study led to the conclusion that *M. oleifera* can be treated to enhance their nutrient quality and thus can be incorporated into food to prevent malnutrition and other deficiencies.<sup>19</sup> *M. oleifera* seeds can be consumed as raw or in a processed form, and incorporated in food products. The present study proves that processed *M. oleifera* seed has excellent antibacterial activity. As the moringa seeds are rich in bioactive components, it is used to treat various diseases.

## 5. LIMITATIONS OF THE STUDY

There are various methods to assess the antimicrobial and antioxidant potential of the plant samples. However, in the present study, only preferred methods were used to analyse each of the activities. These assessments can also be carried out by various other methods which can provide more accuracy to the result.

## 6. ACKNOWLEDGEMENTS

I would like to thank the ARMATS Biotek Training and Research Institute (ABTRI) for giving consent to conduct the research.

**CONFLICT OF INTEREST:** No conflict of Interest

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