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Determination Of The Effects Ofaqueous Extracts Of Leaves, Seeds And Stem Bark Of *Moringa Oleifera* On Bacterial Isolates From Spoilt Locally Produced Soymilk As A Measure Of Their Preservative Potential.

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Abstract: *The preservative potentials of the aqueous extracts of Moringa oleifera seeds, leaves and stem bark were studied by evaluating their antibacterial activities against some bacteria isolated from spoilt locally produced soymilk at different concentrations of 2g/100ml, 4g/100ml, 6g/100ml, 8g/100ml and 10g/100ml each of the seeds, leaves and stem bark powder. This was aimed at finding an alternative, but safer and cheaper way of preserving soymilk. The bacteria isolates were Escherichia coli,* Enterococcus *faecalis, and Staphylococcus aureus. The result of the study showed that the seeds, leaves, and stem bark of aqueous extracts of Moringa oleifera were active against the isolated bacteria. The isolated organisms were more susceptible to the effects of the extracts at concentration range of 6g/100ml to 10g/100. Thus by fortifying soymilk with Moringa oleifera seed or leaves due to their nutritive value, or by adding a calculated amount of stem bark extract, the activities of these bacteria associated with the spoilage of soymilk can be inhibited, thus preserving the soymilk and extending its shelf- life.*

Keywords: *soymilk, Moringa oleifera, Escherichia coli,* Enterococcus *faecalis, and Staphylococcus aureus, antibacterial, preservative.*

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I. Introduction

The *Moringa oleifera* plant is an inexpensive nutrient rich plant. *Moringa* has rich combination of nutrients, amino acids, antioxidants, anti-aging and anti-inflammatory properties (Fahey, 2005; Hsu *et al*., 2006; Oduro *et al*., 2008; Sanchez-Machado *et al*., 2009;Kasolo *et al*., 2010; Busani *et al*.,2011). *Moringa* species are well documented plant herbs due to their extraordinary nutritional and medicinal properties. It is known in folk medicine as having value in treating a wide variety of ailments. They are known to be anti-helminthic, antibiotic, detoxifiers, immune builders and have been used to treat malaria (Armando *et al*., 1991; Saroj *et al*., 1995; Thilza *et al*., 2010; Jackson *et al*., 2011; Abalaka *et al*., 2012). Soymilk is an aqueous extract of soybeans (*Glycine max*), and it can be used as an alternative for dairy milk (Xi-Lin *et al*., 2014). It is not expensive, free of lactose and cholesterol, nutritious and highly digestible (Liao *et al*., 2007; Soya Be, 2006)). It is rich in polyunsaturated fatty acids of phospholipids (Momoh *et al.,* 2011; Raja *et al*., 2014; Smita *et al*., 2015), and has very low fat content (Jacqueline, 2013; Berry *et al.* 2006).). It's also a good source of calcium and soluble fiber (Bahareh and Peiyman, 2008). Kolapo and Oladimeji, (2008) produced soy-corn milk by fortifying soymilk with maize, the quality evaluation of which they reported to have increased nutrient content and sensorial attributes. Kolawole *et al*., (2015) produced yoghurt from bambara groundnut (*Vigna subterranea*), and soybeans which they reported to have considerable quality. Spoilage of Soymilk has been shown by various researchers to be caused by some microorganisms (Osuntogun and Aboaba, 2004; Mbajiuka *et al*., 2014; Xi-Lin *et al*., 2014), because it contains the nutrients that are also required for the growth of most of these spoilage organisms (Momoh *et al.,* 2011). Some synthetic additives used in preserving milk are also used in preserving soymilk. Xi-Lin *et al*., (2014) reported the used of nipagin, complex esters, nisin, sodium dehydroacetate which are synthetic materials, and heat treatment to control spoilage microorganisms in soybean milk. There is paucity of information on the use of plant parts extracts for the preservation of soymilk. Thus, this study aimed at finding an alternative, but safer and cheaper way of preserving soymilk by evaluating the antimicrobial activities of *Moringa oleifera* seeds, leaves and stem bark aqueous extracts on bacterial isolates from spoilt locally produced soymilk.

II. Materials And Methods

Sample collection

Soybean, *Moringa oleifera* seeds, leaves and stem bark used for this study were purchased at Bodija market in Ibadan, Oyo state, Nigeria.

Sample preparation

Soybeans were sorted and winnowed. *Moringa oleifera* seeds, leaves and stem bark were thoroughly oven dried at 40^{o}C. The leaves were pulverized using a blender while the seeds and the stem bark was grounded using mortar and pestle.

Preparation of Aqueous Plant Extracts

Moringa seeds, leaves and stem bark aqueous extracts were prepared by soaking 2g, 4g, 6g, 8g and 10g each of grounded seeds, leaves and stem bark in 100ml of distilled water for 48 hours, after which each was filtered into an 100ml standard flask and make up to the volume with distilled water.

Soybean Milk Processing

Soymilk was produced locally from 200g of sorted soybeans using the method reported by Lee *et al*., (1990). The milk thus obtained was pasteurized by boiling and then kept in a formerly sterilized bottle for 72 hours to spoil.

Serial Dilution and Inoculation

One ml of the spoilt soymilk was picked using sterile pipette after shaking vigorously and a tenfold serial dilution according to Duawale and Lamaster, (2003) was carried out. The pour plate method as reported by Mbajiuka *et al*., 2014 was used in plating the samples. The plates were transferred into an incubator set at 37^{o}C and incubated for 24 hours.

Sub- Culture and purification

After incubation period, discrete colonies from the bacteria plates were picked with a flamed wire loop and sub-cultured onto a newly prepared nutrient agar plates and were incubated at 37^{o}C for 48 hours Purified colonies were transferred into slants and stored properly for further characterization.

Characterization of Purified Culture (Bacteria)

Each purified bacteria was examined microscopically for colony morphology, and then gram stained to determine the gram status, as well as some standard biochemical tests as reported by Mbajiuka et al., (2014).These include catalase, indole, methyl red, voges-Proskauer, urease and sugar fermentation tests.

Determination of antimicrobial activity

The antimicrobial properties of *Moringa oleifera* seeds, leaves and stem bark aqueous extracts *were* investigated against the bacterial isolates using agar well diffusion method. Wells were made on the nutrient agar plate using a sterile cork borer of 8mm diameter; the different concentrations of the extracts were added into the wells. Duplicates were made to ascertain the results obtained. The plates were left on the bench for 2hrs to allow the extract to diffuse properly into the nutrient agar. The bacterial plates were incubated uprightly at 37°C for 24hrs. The resulting zones of inhibitions were measured in mm, and were compared to a standard interpretation chart by (NCCLS, 1999).

III. Results

The results of the study are as presented in the tables below

Table 1. Antibacterial activity of seed extract of *Moringa oleifera* on bacteria isolated from spoilt soymilk presented as zone of inhibition in mm.

Seed Extract Concentration in g(Powder)/100ml	*E. coli*	*E. faecalis*	*S. aureus*
2	9	11	14
4	12	13	17
6	17	19	20
8	20	23	22
10	22	24	27

Table 2. Antibacterial activity of stem bark extract of *Moringa oleifera* on bacteria isolated from spoilt soymilk presented as zone of inhibition in mm.

Stem Bark Extract Concentration in g(Powder)/100ml	*E. coli*	*E. faecalis*	*S. aureus*
2	16	15	19
4	17	18	21
6	20	19	23
8	21	22	24
10	21	24	25

Table 3. Antibacterial activity of leaves extract of *Moringa oleifera* on bacteria isolated from spoilt soymilk presented as zone of inhibition in mm.

Leaves Extract Concentration in g(Powder)/100ml	E. coli	E. faecalis	S. aureus
2	16	13	19
4	20	16	22
6	22	21	26
8	24	24	29
10	26	27	32

IV. Discussion

Three bacteria were isolated from the spoilt milk. These are *Escherichia coli, E. faecalis and S. aureus*. The result of this study showed that the seeds, leaves, and stem bark aqueous extracts of *Moringa oleifera* were active against the isolated bacteria at all concentrations. The isolated organisms were more susceptible to the effects of the extracts at concentration range of 6g/100ml to 10g/100 as could be seen from the result presented in Tables 1, 2 and 3. The effects of the extracts seemed to be more pronounced on *S. aureus* than others. The leaves aqueous extracts has greater activity against the organisms at these concentrations than the seeds and stem bark aqueous extracts. These extracts effectively inhibited the growth of the isolated organisms from soymilk, thus, by fortifying soymilk with predetermined *Moringa oleifera* seed or leaves due to their nutritive value, or by adding a calculated amount of stem bark extract, the activities of these bacteria associated with the spoilage of soymilk can be inhibited, thereby preserving the soymilk and also extending its shelf- life. The result of this study supports the work of Jackson et al., (2011) who evaluated the antibacterial effect of aqueous and ethanolic *Moringa* leaf extracts in vitro, and found that both the aqueous and ethanolic *Moringa* leaf extracts contain compounds with wide spectrum antibacterial activity capable of inhibiting the growth of gram-positive and negative bacteria. Agboke *et al*., (2011) also evaluated the microbiology quality of some soybean milk products consumed in Nigeria, from which *E. coli, E. faecalis* and *S. aureus* amongst other bacteria were isolated.

V. Conclusion

Moringa oleifera seeds, leaves and stem back could be employed in calculated amount in the preservation of soymilk. It is therefore recommended that the concerned food industries should utilize this great potential of moringa oleifera parts in soymilk preservation

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