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Determination of the Minimum Inhibition Concentration of Moringa *oleifera* Leaf Powder against Some Common Diarrhoea Causing Pathogens

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Abstract The antimicrobial properties and nutrient content of Moringa *oleifera* leaves have made them of great interest in current scientific research. This study determined the Minimum Inhibition Concentration of Moringa *oleifera* leaf powders against some common diarrhoea causing bacteria. Four bacterial strains of Escherichia *coli*, Salmonella *typhi*, Shigella *shiga* and Staphylococcus *aureaus* were used in the study. Moringa *oleifera* leaves powders were thoroughly mixed with sterile Brain Heart Infusion (BHI) broth and centrifuged for 15minutes before being strained using a filter paper. Ten millilitres of sterilized BHI with different levels of moringa leaf powders were each inoculated with 0.05 millilitres of standardised suspension of tested bacteria. Sterilized BHI alone and BHI with varying levels of moringa leaf powder without the pathogens were also prepared to be used in standardising the spectrophotometer. Readings were taken before and after incubating the different samples and bacterial growth was tested by measuring optical density in the broth at 600 nm after incubation for 24 hours at 35°C. The difference in spectrophotometer readings before and after incubation of the samples was used to indicate growth of bacterial pathogens. Results indicated that Moringa oleifera leaf powders inhibited growth in all the four bacterial strains due to its antimicrobial properties. The minimum inhibition concentration of Moringa *oleifera* leaf powder against 0.05 millilitres of standardised Escherichia *coli* varied between 8.4g to 9.8g of moringa *oleifera* leaf powder per 100 millilitres of BHI broth while for Staphylococcus *aureaus* it ranged between 9.8g to 10.2g Moringa *oleifera* leaf powder per 100mls of BHI broth. The results of this investigation can be used to guide low income households and pharmacists on the quantities of dried M. *oleifera* leaf powder to use in an effort to eliminate diarrhoea due to E. *coli* and S. *aureaus* pathogens.

Keywords: *Minimum inhibition concentration, Antimicrobial properties, Moringa oleifera leaves, Diarrhoea causing pathogens*

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1. Introduction

Diarrhoea is known to be a major cause of morbidity and mortality among infants and children worldwide especially in the African and South East Asian regions [1]. Diarrhoea is associated with a decrease in dietary intake, weight faltering and a risk of vitamin A and zinc deficiency [2], contributing greatly to malnutrition among children [3]. The use of plants as therapies in traditional medicine is as old as mankind. In developing countries, World Health Organisation estimates indicate 80% of the African population to utilise medicinal plants for the treatment of infectious diseases [4] . Currently there is a great interest on natural antimicrobial molecule in the hope that it may provide useful solutions to anti-infective drug candidates [5]. Moringa *oleifera* trees are rich in phytochemicals (compounds produced by plants that have health benefits) and are highly demanded for their medicinal value [6]. Moringa *Oleifera* leaves have been reported to have antibacterial activity against Salmonella *typhi*, [5] Escherichia *coli*, Shigella *shiga*, [7] and Staphyloccoccus *aureaus* [8]. Though plants are reported as the cheapest and safest source of antimicrobials [5], often times, the quantities used for eliminating the microorganisms are either so low, resulting in failure to destroy the pathogens or too much and therefore creating undesirable tastes. In our previous studies of optimising millet porridges with Moringa *oleifera* leaf powders, it was noted that the more the quantities of Moringa *oleifera* leaf powders were increased in millet porridges, the more the porridges became undesirable in both taste and colour [9]. Minimum Inhibition Concentration (MIC) is the lowest level of an antimicrobial agent that is required to inhibit growth of microorganisms after an overnight incubation [10]. Strong recommendations for consumption of phytochemicals rich plants such as Moringa *oleifera* exists, but not enough information exists to make specific recommendations of the amounts necessary for their intake [11]. The aim of

this study was to establish the minimum inhibition concentration of Moringa *oleifera* leaves powders that would effectively eliminate the common infections that are responsible for diarrhoea, one of the leading causes of poor nutritional status and health among children.

2. Materials and Methods

2.1. Determination of Antimicrobial Properties of Moringa *oleifera* Leaves

Moringa *oleifera* leaves were obtained from Bujenje County found in the Western part of Uganda. They were dried inside the house and ground with a local miller to a fine powder using a mortar and pestle. The antimicrobial properties for moringa powders were determined by Minimum inhibitory concentration (MIC). MIC is the lowest concentration required to inhibit growth of selective test pathogens in a broth over a 24 hour period [10]. Bacterial growth was determined by measuring optical density in the broth at 600 nm after incubation for 24 hours at 35°C.

2.2. Securing of Microorganisms

Bacterial Cultures of *E. coli*, *S. aureus*, *S. typhi* and *S. shiga* were got from the Department of Public Health, Bacteriology and Toxicology, University of Nairobi.

2.3. Standardisation of Different Pathogen Cells

Cultures of *E. coli*, *S. aureus*, *S. typhi* and *S. shiga* were prepared in nutrient BHI. The different pathogen cells were each inoculated into 10 ml sterile Brain Heart Infusion (BHI) broth, and incubated at 35°C for 24 hours. Their turbidity was standardised to 0.39 optical densities before being inoculated. This was done using sterile BHI broth and a CE 4400/UV DOUBLE BEAM SCANNING SPECTROPHOTOMETER at wave length of 600nm.

2.4. Testing for Minimum Inhibition Concentration (MIC)

Brain Heart Infusion (BHI) broth was mixed with Moringa *oleifera* leaf powder in four different levels beginning with 7.1g and increasing by 20% thereafter up to the least acceptable amounts of Moringa *oleifera* leaf powders in 100mls of millet porridge formulations. The amounts of moringa *oleifera* leaves used were based on our previous studies where the nutrient content of millet flour was improved with moringa leaves without affecting the organoleptic properties of the formulated product [9]. Brain Heart Infusion (BHI) broth was mixed with each of the four quantities of moringa leaf powder. They were thoroughly mixed, centrifuged for 15minutes using Beckman Model T-J-B Centrifuge and strained using filter paper. The same treatment was done to BHI media without moringa. Ten millilitres of each of the prepared media were put in test tubes covered securely with cotton and aluminium foil and sterilised in an autoclave at 210°C. After cooling, 10mls of the BHI alone and 10 mls of BHI and different levels of Moringa *oleifera* leaf powder were each inoculated with 0.05 millilitres of standardised concentrations of the diarrhoea causing pathogens. The controls of BHI alone and BHI with the different levels of moringa *oleifera* leaf powders without the pathogens were also prepared to be used in standardising the spectrophotometer. Bacterial growth was tested by measuring optical density in the broth at 600 nm after incubation for 24 hours at 35°C. The difference in spectrophotometer readings before and after incubation of the samples was used to indicate growth of bacterial pathogens. Each essay was carried out in triplicate and the average obtained. The least concentration of the samples with no visible growth was taken as the MIC [12].

3. Results

3.1. Inhibition of Moringa *oleifera* Leaf Powder against Escherichia *coli*

Figure 1 shows that when 0.05millilitres of standardised tests of Escherichia *coli* were inoculated in sterilised 100millilitres of BHI broth with 7g of Moringa *oleifera* leaf powder, the spectrophotometer reading was 0.067. The reading reduced to 0.03 as the amount of moringa leaf powder was increased by 20% to 8.4g. When the amount of moringa was increased to 9.8g, the spectrophotometer reading was reduced to zero, meaning the growth of E. *coli* was inhibited since there was no indication of E. *coli* growth shown.

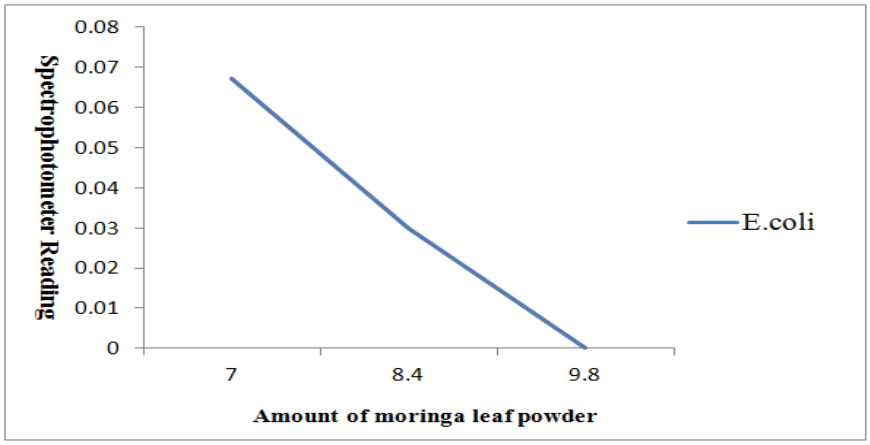

Figure 1. Minimum inhibition Concentration of Moringa *oleifera* leaf powder against Escherichia *coli*

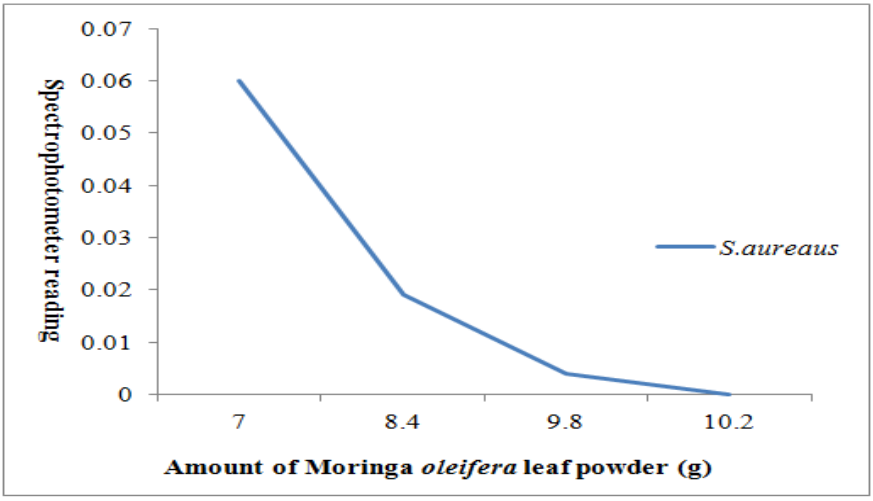

Figure 2. Minimum inhibition Concentration of Moringa *oleifera* leaf powder against Staphyloccocus *aureaus*

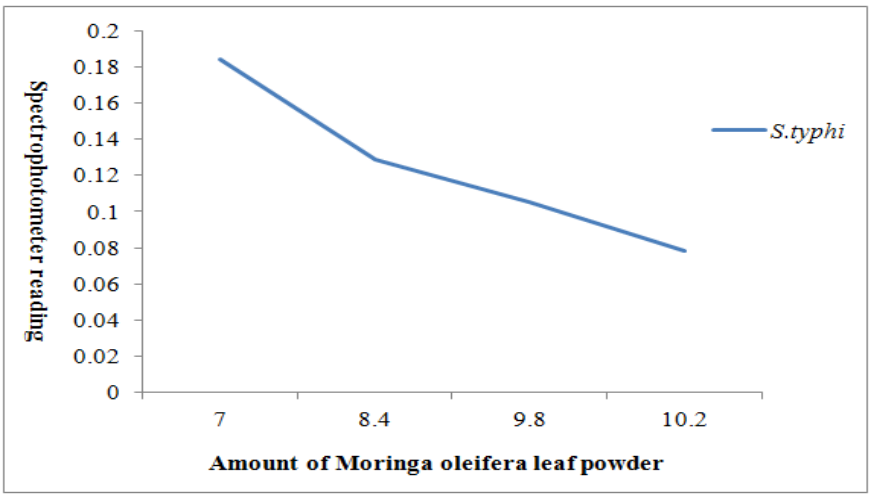

Figure 3. S. *typhi* Inhibition by Moringa *oleifera* leaf powder

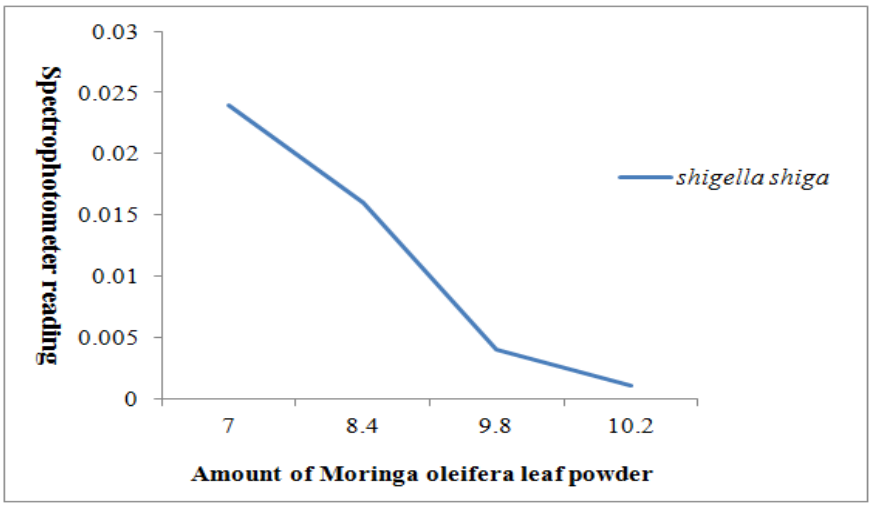

Figure 4. Shigella *shiga* inhibition by Moringa *oleifera* leaf powder

3.2. Inhibition of Moringa *oleifera* Leaf Powder against *Staphyloccocus aureaus*

The average spectrophotometer reading obtained when 0.05 millilitres of standardised tests of *S. aureaus* were inoculated in sterilised 100millilitres of BHI broth with 7g of Moringa *oleifera* leaf powder, was 0.06. The reading reduced to 0.019 as the amount of moringa used was increased by 20% to 8.4g. The spectrophotometer reading was 0.004 as the amount of moringa used was increased to 9.8g, until the point when 10.2 g moringa leaf was used, when the reading became zero meaning the growth of *S. aureaus* had been inhibited since there was no indication of bacteria growth as shown Figure 2.

3.3. Inhibition of Moringa *oleifera* Leaf Powder against Salmonella *typhi*

Figure 3 shows that the amount of Moringa *oleifera* leaf powder used ranged from 7g to 10.2g. When 7g of sterilised Moringa *oleifera* leaf powder in 100 millilitres of BHI was used, the reading on the spectrophotometer was 0.184. It reduced to 0.129 and 0.105 respectively as the amounts of Moringa *oleifera* leaf powder were increased to 8.4g and 9.8 g respectively. The highest amount of moringa powders that could be used was 10.2g and the spectrophotometer reading was 0.078 when it was used. Though moringa has antimicrobial properties against S. *typhi* as indicated by the spectrophotometer reading, total inhibition of S. *typhi* could not be achieved at the desired level of acceptance.

3.4. Inhibition of Moringa *oleifera* Leaf Powder against Shigella *shiga*

The amount of Moringa *oleifera* leaf powder used ranged from 7g to 10.2g. When 7g of sterilised Moringa *oleifera* leaf powder in 100 millilitres of BHI was used, the spectrophotometer reading was 0.024. It reduced to 0.016 and 0.004 respectively as the amounts of Moringa *oleifera* leaf powders were increased to 8.4g and 9.8 g respectively. The highest amount of moringa powders that could be used was 10.2g and the spectrophotometer reading was 0.001 when it was used. Though moringa has antimicrobial properties against S. *shiga* as indicated by the spectrophotometer reading, its total inhibition could not be achieved at the desired level of intake by children.

4. Discussion

Moringa *oleifera* leaf powder was able to inhibit growth of E. *coli*, S. *shiga*, S. *typhi* and S. *aureaus*. This has been reported by other researchers [5,6,8]. Moringa *oleifera* leaf ethanol extract has been reported to exhibit broad spectrum activity against the test organisms of Escherichia *coli* and Staphylococcus *aureus [8]*. Moringa leaves are also widely used in the treatment of bacterial infection, fungal infection and diarrhea [13]. This is attributed to the chemical compounds such as kaempferol and rutin that Moringa *oleifera* leaves contain [14]. Such compounds are reported to have antioxidant and antibiotic properties that are linked to the inhibition of microorganisms [14]. Moringa leaves exhibited better inhibition properties against E. *coli* compared to S. aureaus and S. *typhi*. These findings concur with our previous studies where S. *typhi* had the least inhibition properties, but differ as regards S. *aureaus* which was better inhibited in fermented Moringa millet porridge compared to E. *coli* [9]. This difference could be attributed to fermentation. Using the Minimum Inhibition Concentration (MIC) and disc diffusion method, powder from moringa fresh leaf juice is reported to have exhibited highest zones of inhibition against *Shigella shiga, Shigella sonnei and Staphylococcus aureus* compared with fresh leaf juice [7]. The results can also be compared to an interventional study in Burkina Faso using 10g Moringa *oleifera* leaves powder as a supplement in porridges that resulted in low diarrhoea episodes of 7.8% compared to the control of 80.3% without moringa supplement [15]. Minimum inhibition concentration values of Moringa *oleifera* leaf powder against 0.05 millilitres of standardised Escherichia *coli* varied between 8.4g to 9.8g of Moringa *oleifera* leaf powder per 100 millilitres of BHI broth while for Staphylococcus *aureaus* it ranged between 9.8g to 10.2g Moringa *oleifera* leaf powder per 100mls of BHI broth. This is comparable to study findings where the MIC values for fresh leaf juice, powder from fresh leaf juice, ethanol extract of fresh leaves and cold water extract of fresh leaves were recorded as 1.25 to 2.5 µl disc-1, 229 to 458 µg ml-1, 458 to 916 µg ml-1 and 29.87 to 58.75 mg ml-1, respectively [11]. The MIC of S. *typhi* and S. *shiga* could not be reliably established because of the high concentrations of moringa powders that were required per 100 millilitres of porridges. These high quantities could not be used since when they were put in porridges, they could not be accepted among mothers/caretakers of children below 5 years. There was however evidence of antimicrobial properties against S. *typhi* and S. *shiga.* Since plants such as Moringa ole*ifera* leaf extracts have been reported to form part of traditional medicine in some societies for a long time in history, the established quantities can be used to form part of daily menus to avoid undesirable intakes especially among children. Documentation of such information would also help improve on acceptability of designed formulations utilising Moringa *oleifera* leaves. This is a key factor in not only assessing the palatability of a developed food but also the effectiveness of a designed feeding intervention. These findings can also be utilised by pharmacists to come out with a lasting solution for the current overwhelming challenge of drug resistance for some individuals who have over used antibiotics.

5. Conclusion

The results of this investigation confirm the fact that Moringa *oleifera* leaves have antimicrobial properties against E. *coli, S. aureus, S. typhi* and *S. shiga*. The study also establishes the Minimum Inhibition Concentration of Moringa *oleifera* leaf powder against 0.05 standardised pathogens of Escherichia *coli* and Staphylococcus *aureaus* to vary between 8.4-9.8g of moringa *oleifera* leaf powder per 100 millilitres of BHI broth while for Staphylococcus *aureaus* it varies between 9.8-10.2g of moringa *oleifera* leaf powder per 100 millilitres of BHI broth.

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Statement of Competing Interests

The authors have no competing interests.

List of Abbreviations

BHI	Brain Heart Infusion
E. *coli*	Escherichia Coli
MIC	Minimum Inhibition Concentration
M. *oleifera*	Moringa Oleifera
S. *aureaus*	Staphylococcus Aureaus
S. *shiga*	Shigella Shiga
S. *typhi*	Salmonella Typhi

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