Antibacterial Evaluation of Extracts of Seed Kernel and Leaf of *Moringa Oleifera* Against Bacteria from Raw Cow Milk

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Abstract: *Moringa oleifera*has been found very useful in a lot of health related problems owing to its medicinal components and values, these has led to research on its antibacterial activity against food borne pathogens. Aqueous and acetone extracts of *Moringa oleifera* seed kernel and leaf were evaluated for antibacterial activity against *Bacillus cereus, Staphylococcus aureus* and *Escherichia coli* isolated from raw cow milk. The antibacterial assay was carried out at concentrations of 250, 125, 62.5 and 31.25mg/ml using modified agar well diffusion method. Aqueous extract inhibited the growth of test isolates at varying degrees. Aqueous extract of seed kernel was highly inhibitory on *Staphylococcus aureus* with a zone of inhibition (23.33±1.20mm) at concentration of 250mg/ml. Minimum Inhibitory Concentration showed that both extracts inhibited the growth of *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli* at concentrations ranging from 125 to 21.25mg/ml. The antibacterial effect of standard antibiotics was performed and it was observed that Gentamycin inhibited growth of the three test isolates. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, saponin, tannin, phenols, glycosides and carbohydrates compound in the extracts. From the present study, *Moringa oleifera* seed kernel and leaf can be a promising source of phytochemical components and further studies is therefore recommended.

Key words: Food-borne pathogens, Minimum Inhibitory Concentration, *Moringa oleifera*, Phytochemical screening, Raw cow milk

Introduction

Conventional medicines provide health coverage for over 80% of the world's population, mostly in the developing world (Nyenje and Ndip 2011). Some natural substances that have effective antimicrobial properties have been used as seasonings and they can affect the growth and metabolism of bacteria, activating or inhibiting the growth depending on their constitution and concentration ((Shan *et al*., 2007;Nazzaro *et al*., 2009).*Moringa oleifera* is a highly valued plant, distributed in many countries of the tropics and subtropics. It is considered one of the world's most useful trees, as most of the part has enormous range of medicinal uses (Bamishaiye *et al*., 2011; Abalaka*et al*., 2012)..

Production of milk and various milk products usually take place under poor sanitary conditions in the developing nations. Potentially pathogenic microorganisms isolated from milk have capacity to cause infectious diseases in human (Mogessie, 1990; Edward and Inya, 2013).

The presence of these pathogenic microorganisms in milk has emerged as a major public health concern especially for those individuals consuming it.

This work focuses on investigating claims of efficacy of seed kernel and leaf of *Moringa oleifera* and screening of phytochemical components responsible for such antibacterial action.

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Material and Methods

Sample Collection

Moringa oleifera seeds and leaves were plucked from a household in Ilorin, Nigeria and taken to the Herbarium, Department of Plant Biology,University of Ilorin for identification. Voucher number UIH/559 was assigned to the samples.

Test Organisms

Bacillus cereus, Staphylococcus aureus and Escherichia coli that were previously isolated from raw cow milk were obtained from culture collection unit of the department of microbiology, University of Ilorin. The organisms were confirmed using standard methods as described by Cheesebrough, (2000) and Fawole and Oso, (2004).

Preparation of Extracts

Fresh leaves of *Moringa oleifera* were cleaned and the seeds dehulled. Both were dried for one and two weeks respectively at room temperature. The dried leaves and kernels were ground into fine powder using an electrical grinder (EXCELLA MIXER GRINDER). Maceration of the materials was as described by Tiwari *et al.* (2011). Thirty five gram (35g) of the powdered seed kernels were separately macerated in each of 350ml of 70% acetone and water for 24hrs. These were then filtered, and the resulting filtrates were concentrated separately using vacuum dryer (DZF - 6020).

Media Preparation

The following media were used in this research; Nutrient agar (Oxoid),Eosin Methylene Blue agar (Oxoid), Mannitol salt agar (Oxoid), Mueller Hilton (MH) agar and broth (Biolab, Hungary). All media were prepared according to manufacturer's instructions. Sterility of the media was checked by incubating overnight before use.

Antibacterial Susceptibility Testing

Antibacterial activity of aqueous and 70% acetone extract of the seed kernel and leaf were assayed using the agar well diffusion method (Bauer *et al*., 1966). Inocula were prepared from subcultures of bacteria as follows: colonies of each isolate were emulsified in sterile Mueller Hilton (MH) broth to achieve cell concentrations (corresponding to 0.5 McFarland standards) and incubated overnight (Ndip*et al*., 2007). The standardized test isolates were swabbed on separate solidified petri dishes. Wells were made aseptically on the agar surface with a 5mm cork borer and different concentrations of the extracts (250, 125, 62.5 and 31.25mg/ml) were introduced into the wells using micropipette. The plates were left for 1 hour to allow for diffusion of the extracts into the agar before incubated at 37^{o}C for 24 hours. After incubation, diameters of zones of inhibition around the wells were measured to the nearest millimeter. All the result were compared with standard antibiotic Gentamycin (10ug) (Abtek Biological Ltd, England) as a positive control. Acetone (70%) and sterile distilled water were used as a negative control.

Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined using the broth macro-dilution technique as described by Cetin-Karaca, (2011) with slight modifications in which the broth volume for each antimicrobial concentration is ≥ 1.0 ml contained in test tubes by substracting with difference of 10 (e.g. 62.5, 52.5, 42.5 and 32.5). One milliliter (1 ml) of each extract was added in nine milliliter (9ml) of MH broth and then a loopful of the test organism previously diluted to 0.5 McFarland turbidiometric standard was introduced to the tubes. After 18-24hrs incubation, the tubes were examined by checking for absorbance at wavelength of 470 nM with the aid of a spectrophotometer (GENESY 20) model. The lowest absorbance was considered the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined from test tubes in the MIC test that showed no growth were streaked on sterile MH agar. A positive control was done by streaking sterile MH agar plates with the test organisms. The plates without growth were considered as the MBC after incubation at 37^{o}C for 24 (NCCLS, 2000).

Qualitative Phytochemical Analysis

Qualitative phytochemical analysis is the process of detecting the bioactive principle present in the extracts with the use of standard phytochemical methods (Edeoga*et al*., 2005). Preliminary phytochemical studies to determine the presence of phytochemicals was carried out using the method described by Tiwari *et al.* (2011) and Harborne (2005).

Statistical analysis

The results were expressed as mean ± standard error of three replicates. Data were subjected to either student's t-test or one-way analysis of variance (ANOVA) and the differences between samples were determined by Duncan's Multiple Range test.

Results

Effect of Seed Kernel Extracts of *Moringa Oleifera* on Test Bacteria

Table 1 shows the data for the effect of varying concentration of aqueous and acetone extracts of *Moringa* kernel on the test organisms.

The effects of both extracts on inhibiton of *B.cereus* is relatively similar at concentrations of 250mg/ml and 125mg/ml producing zone of inhibition of 13.23±0.92mm and 14.00±0.57mm respectively.

E.coli had significantly different ($P < 0.05$) inhibitory effect at 250mg/ml and 125mg/ml while relatively similar inhibitory effect at concentrations 62.5mg/ml and 31.25mg/ml.

S.aureus had significantly different ($P < 0.05$) inhibitory effect at 250mg/ml – 62.5mg/ml for both extraction solvents and relatively the same effect at 31.25mg/ml.

Table 1: Effect of seed kernel extracts of *Moringa oleifera* on test bacteria

Concentration (mg/ml)	Zone of Inhibition (mm)					
	Water			Acetone		
	Bc	Ec	Sa	Bc	Ec	Sa
250	13.23±0.92^{c}	10.63±1.04^{d}	23.33±1.20^{d}	15.67±0.67^{d}	8.10±0.10^{c}	17.33±2.85^{b}
125	10.10±0.67^{b}	4.83±0.17^{c}	20.00±1.16^{c}	14.00±0.57^{c*}	6.43±0.05^{b*}	15.57±1.79^{ab}
62.5	8.53±0.39^{ab}	2.23±0.15^{b}	13.67±0.67^{b}	7.77±0.12^{b}	6.12±0.27^{b*}	15.00±2.00^{ab}
31.25	7.33±0.33^{a*}	0.00±0.00 ^{a}	7.30±0.17^{a}	0.00±0.00 ^{a}	0.00±0.00^{a}	8.33±1.86 ^{a}

Values are means of triplicates (n=3) ± SD. Values with different superscripts across a column are significantly different from each other. Values with * are significantly different from the corresponding solvents for a particular organism.
KEY: Bc- *Bacillus cereus*; Sa- *Staphylococcus aureus*; Ec- *Escherichia coli.*

Effect of Leaf Extracts of *Moringa Oleifera* on Test Isolates

B. cereus and *S. aureus* showed relatively similar inhibitory effect ($P < 0.05$) at all concentrations for both extracts. *Escherichia coli* showed significantly different inhibitory effect at all concentrations tested for both extraction solvents (Table 2).

Table2: Effect of leaf extracts of *Moringa oleifera* on test bacteria

Concentration(mg/ml)	Zone of Inhibition (mm)					
	Water			Acetone		
	Bc	Ec	Sa	Bc	Ec	Sa
250	8.00±0.17^{d}	12.00±0.00^{d*}	9.00±0.17^{d}	11.33±0.33^{d*}	5.13±0.43^{c}	8.83±0.17^{d}
125	6.33±0.33^{c}	10.33±0.33^{c*}	7.12±0.30^{c*}	9.00±0.58^{c*}	3.33±0.20^{b}	5.53±0.29^{c}
62.5	3.33±0.00^{b}	8.67±0.88^{b*}	5.23±0.12^{b*}	4.67±0.33^{b*}	0±0.00^{a}	3.73±0.22^{b}
31.25	0±0.00^{a}	6.00±0.00^{a*}	0±0.00^{a}	2.90±0.10^{a*}	0±0.00^{a}	0±0.00 ^{a}

Values are means of triplicates (n=3) ± SD. Values with different superscripts across a column are significantly different from each other. Values with * are significantly different from the corresponding solvents for a particular organism

.**KEY:** Bc- *Bacillus cereus;*Sa- *Staphylococcus aureus;*Ec- *Escherichia coli.*

Antibacterial Susceptibility Pattern of Test Organisms to Standard Antibiotic

Table 3 shows antibacterial susceptibility pattern of gentamycin against the test organism. Result shows that *Escherichia coli* was the most susceptible recording the highest zone of inhibition (19.00mm) while the least is *Staphylococcus aureus* (12.00mm).

Table 3: Antibacterial Susceptibility pattern of test organisms to standard antibiotic

Test organisms	**Diameters zones of inhibition (mm) of Gentamycin (positive control)**
Bacillus cereus	16.66
Escherichia coli	19.00
Staphylococcus aureus	12.00

Determination of Minimum Inhibitory Concentration.

The MIC of the seed kernel aqueous extract against *B.cereus* was 125mg/ml while seed kernel acetone, leaf aqueous and acetone extracts had MIC values of 62.5mg/ml against this test organism (Table 3). For *E. coli*, seed kernel aqueous, leaf aqueous and leaf acetone extracts had MIC values of 125mg/ml while kernel acetone extract had an MIC of 62.5mg/ml. The MIC values against *S. aureus* ranged from 125mg/ml to 21.25mg/ml.

Table 4: Minimum Inhibitory concentration of *Moringa oleifera*

Test organisms	Minimum Inhibitory Concentration(MIC)(mg/ml)			
	Seed Kernel aqueous	Seed Kernel acetone	Leaf aqueous	Leaf acetone
B. cereus	125	62.5	62.5	62.5
E. coli	125	62.5	125	125
S. aureus	31.25	21.25	125	21.25

Phytochemical Screening

Results of qualitative screening for the presence of some phytochemical components in the aqueous and acetone extracts of seed kernel and leaf of *M.oleifera* are shown in Table 4. Seed kernel acetone extract was the only extract that possessed carbohydrates. Aqueous and acetone extract of the kernel lacked phenol while leaf aqueous and acetone extract contained phenol. Alkaloids, flavonoids, glycoside and steroids were present in aqueous and acetone extracts of seed kernel.

Table 4: Qualitative phytochemical analysis of *M. oleifera*

Extract*	Alkaloids	Flavonoids	Tannin	Glycosides	Steroids	Phenols	Carbohydrate	Saponin
Kernel acetone	+	+	-	+	+	-	+	-
Kernel aqueous	+	+	+	+	+	-	-	+
Leaf acetone	-	+	+	+	-	+	-	-
Leaf aqueous	+	-	-	-	+	+	-	+

KEY: +, Present; -, Absent; *, 250mg/ml, concentration of extract used.

Discussion

The antibacterial activity of *Moringa oleifera* aqueous and acetone extracts exhibited inhibitory effect at varying degrees with increase in extract concentrations. This observation is in agreement with the work done by Nwaiwu*et al.* (2011); Saadabi and Zaid (2011). However, this is in contrast with the report by Aiyegoro*et al.* (2008); Ashafa*et al.* (2008) and Busani*et al.* (2012). *Moringa oleifera* seed kernel aqueous extractinhibited*Staphylococcus aureus* (23.33±1.20) from this study while Yang *et al.* (2006) found out that *Moringa oleifera* aqueous leaf extract exhibited a superior inhibition on *Staphylococcus aureus* isolated from food and animal intestine which concise with test organism in this study that is also food strain. The reason for this contrasting result could be due to the geographical location or season and difference in the extraction solvents could also be a reason, this agrees with the earlier studies of Tijjani*et al.* (2009) and Ayinde*et al.* (2007) that not all phytochemicals are present in all plant parts. The inhibitory activities of plant extracts against them are attributed to the presence of bioactive principles (phytochemicals) some of which were screened for in this research (Enwa*et al.*, 2013). Kasolo*et al.* (2010) reported that *Moringa oleifera* leaves contain alkaloids, nitrogen containing naturally occurring compound that has the ability to intercalate with DNA of microorganisms that could be responsible for the much acclaimed medicinal values which agrees with the present study that shows the presence of alkaloid in aqueous leaf extract. Walter *et al.* (2011) reported the presence of recombinant protein in *Moringa* seed and in this study tannin present in aqueous *Moringa* seed

kernel extract which could also be a reason for superior activity to *S. aureus*. Generally, mechanism of action by the phytochemical constituents of *M. oleifera* is the reason for antibacterial activity thereby causing bacterial enzyme inhibition such as the sortase inhibitory effect, DNA replication, bacterial toxin action and causing the lysis of bacterial cells. It had been suggested that pterygospermin acts by the inhibition of the transaminase enzyme and through cell membrane perturbations (Onyekaba*et al.*, 2013). The activity of *M. oleifera* seed kernel and leaf extracts were compared to standard antibiotics; gentamycin, itshowed higher antibacterial activity against all the test organisms when compared to the crude extracts. This is in close agreement with the work done by Saadabi and Abu Zaid (2011). High antibacterial activity of the crude extracts of *Moringa oleifera* therefore justifies its uses in folkloric medicine and can be harnessed in the treatment of some food borne infections. Further research work on isolation and characterization of the bioactive metabolites, and should also be tested *in vivo* to determine the toxicity and the optimum dose to be used.

Conclusion

Since aqueous extracts showed relatively high antibacterial activity over acetone extracts against the test organisms used during this study, it is evidence that water is more suitable for extraction of *Moringa* preparations. Also, the presence of phytochemical components encourages the use of *Moringa oleifera* seed kernel as the antibacterial agents for controlling food borne infections.

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