



ANTIBACTERIAL ACTIVITY OF *MORINGA OLEIFERA* LEAF EXTRACTS AGAINST BACTERIAL ISOLATES FROM PATIENTS WITH BACTERIAL KERATITIS

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ABSTRACT

The aim of the study was to determine the antimicrobial activity of *Moringa oleifera* leaf extracts against bacterial isolates from patients with bacterial keratitis attending National Eye Centre, Kaduna. Sixty four (64) patients with bacterial keratitis were sampled at National Eye Center between the period of four months. Corneal scrapings were examined, Gram stained and then cultured using standard methods. The cultured bacterial organisms were further identified using standard biochemical tests. Agar disc diffusion technique was used for the antibacterial bioassay. Five concentrations were prepared from the stock solution (4000µg, 2000µg, 1000µg, 500µg, 250µg/mL). Of the 64 patients examined, 41(64%) were males while 23(36%) were females. The age range was 18 to 73 with a mean age of 37. Forty seven 47 (73.4%) had bacterial growth while 17(26.6%) had no growth. The organisms isolated were *Staphylococcus aureus* 34 (72.3%), *Pseudomonas aeruginosa* 6 (12.8%) and *Streptococcus pneumoniae* 7 (14.9%). Phytochemical analysis of the extract revealed the presence of tannins, saponins, reducing sugars, flavonoids, alkaloids and glycosides. The result of the antibacterial activity revealed that the chloroform extract has the highest activity on *Pseudomonas aeruginosa* with 16mm zone of inhibition diameter, while the least activity (7mm) was shown by the hexane extract against *S. aureus*. The result has shown the Antibacterial potential of the *M. oleifera* extracts against bacteria associated with bacterial keratitis, which could be exploited for drug development.

Keywords: Keratitis, extracts, *Moringa*, Leaf, Antibacterial

INTRODUCTION

Moringa oleifera is native to western and sub-Himalayan region, India, Pakistan, Asia, Africa and Arabia. The *Moringa* tree is cultivated and used as a vegetable (leaves, pods flowers, roasted seeds), for spice (mainly roots), cooking and cosmetic oil (seeds) and as medicinal plant (all plant organ) (Farooq *et al.*, 2012). Important medicinal properties of the plant includes antipyretic, antiepileptic, anti-inflammatory, anti-ulcerative, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities (Rafael *et al.*, 2011). In addition, *Moringa oleifera* seeds possess water purifying powers. They are known to be anti-helminthic, antibiotic, detoxifiers, immune builders and have been used to treat malaria and it can also be used as a less expensive bio-absorbent for removal of heavy metals (Thilza *et al.*, 2010). *Moringa oleifera* is a highly valued plant, distributed in many countries of the tropics and subtropics. It has impressive range of medicinal uses with high nutritional value. Different part of this plant contain a profile of important minerals, and a good source of proteins, vitamins, carotene, amino acids and various phenolics (Ashikin *et al.*, 2016).

Furthermore, Bukar *et al.*, (2010) reported that *M. oleifera* leaf ethanol extract exhibited broad spectrum activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterobacter aerogenes* with MIC values of between 2.0 and >4.0mg/ml. However, *M. oleifera* seed chloroform extract was only active against *E. coli* and

Salmonella Typhimurium with MIC values of 1.0 to >4.0mg/ml (Bukar *et al.*, 2010).

Most of the studies on antimicrobial activity of *Moringa oleifera* leaf extracts were conducted on non-ocular samples. Therefore, there is paucity of data in the literature on the antimicrobial activity of *Moringa* against isolates from eye infection. The present research was conducted to determine the antibacterial activity of *Moringa* against the isolates obtained from ocular samples in patient with bacterial keratitis.

MATERIALS AND METHODS

Collection and Identification of *Moringa oleifera* Leaf

The fresh leaves of *Moringa oleifera* were obtained from Birnin Kudu local government area of Jigawa State. It was transported in a clean container to Department of Plant Biology, Bayero University, Kano where it was identified by a botanist in comparison with information in the herbarium database of the Department with catalogue number BUKHAN 0011.

Processing of Plant Material

The leaves of *Moringa oleifera* were handpicked and washed thoroughly with distilled water and then air dried under shed at ambient temperature for two weeks. This was later pounded using a clean mortar and pestle into the powder form and then stored for future use (Bukar *et al.*, 2010).

Extraction Procedure

Hundred Grams (100g) of air dried and powdered *Moringa* leaves were extracted by Soxhlet extraction method using 500 ml of chloroform and ethanol. The extracts were concentrated using a hot oven. The concentrated extracts were labelled CE (*Moringa* leaf chloroform extract) and EE (*Moringa* leaf ethanol extract), ME (*Moringa* leaf methanol extract) and HE (*Moringa* leaf hexane extract). The extracts were then stored in the refrigerator for future use.

Isolation and Identification of bacterial isolates

Specimens were obtained by corneal scrapings with a kimura spatula after the instillation of a topical anaesthesia in the margin of the lesion, usually an ulcer were gently scraped under slit lamp visualization by an Ophthalmologist at the National Eye Centre, Kaduna.. The material was inoculated into thioglycolate agar, blood agar, chocolate agar and Mackonkey agar. The thioglycolate, blood and Mackonkey agar were kept in an incubator at 37°C for 24hrs while the chocolate agar was kept in an anaerobic jar (candle jar) at 37°C for 24hrs. After incubation the resulting colonies were Gram stained and identified using standard biochemical procedures (Sharma, 2012).

Identification of *Staphylococcus aureus*

Catalase Test

Two millitre of the hydrogen peroxide solution into was poured into a test tube. Using a sterile a glass rod several colonies of the test organism were removed and immersed in the hydrogen peroxide solution. Immediate bubbling was observed. Presence of bubbles indicated positive for *Staphylococcus* species (Sharma, 2012).

Coagulase Test

Two separate drops of distilled water were placed on a glass slide. A colony of the test organism (previously checked by gram staining) was emulsified in each of the drops to make two thick suspensions. A loopful of plasma was added to one of the suspensions, and was mixed gently, clumping of the organism was observed within 10 seconds. No plasma was added to the second suspension, this was used to differentiate any granular appearance of the organism from true coagulase clumping. Positive coagulase reaction indicated *Staphylococcus aureus* (Sharma, 2012).

Identification of *Streptococcus pneumoniae*

Optochin Sensitivity.

Optochin (p) disk (6mm, 5microgramme) SIGMA ALDRICH 74042 was obtained from commercial vendors. Using an inoculating loop, two suspect colonies of a pure culture to be tested were streaked on blood agar. An optochin disk was placed within the streaked area of the plate. The blood agar plate was incubated at 37°C with carbondioxide (candle jar) for 24hrs. The growth on the blood agar plate was observed near the optochin disk. Absence of growth indicated sensitivity to optochin which confirmed *Streptococcus pneumoniae* (Sharma, 2012).

Identification *Pseudomonas aeruginosa*

Kligler Iron Agar (KIA)

The media was inoculated with the test organism using a straight wire by first stabbing the butt and then streaking the slope in a zig – zag pattern. The media was incubated at 37°C for 24hrs. On Kligler Iron Agar *Pseudomonas aeruginosa* produced a characteristic red slope and red butt is produced. No gas was formed and no hydrogen sulphide was produced.

Oxidase Test

A piece of filter paper was placed in a clean Petri dish and 2 drops of freshly prepared oxidase reagent was added. Using a piece of glass rod a colony of the test organism was removed and smeared on the filter paper. The development of a blue – purple colour within a few seconds was observed (Sharma, 2012). All media and biochemical test where selected according to standard methods as described above.

Standardization of the Inoculum of Test Bacteria

Few colonies of an overnight culture of each of the isolates were taken and emulsified in a tube containing 2ml of sterile physiological saline such that its turbidity matched with that of 0.5 McFarland standard (Sharma, 2012).

Antimicrobial Susceptibility Testing

Disc agar diffusion technique was used for antibacterial bioassay. Plates of Mueller Hinton agar were dried in an oven to remove excess moisture from the surface of the agar plates. The plates were inoculated by streaking using swab stick dipped in a standardized inoculum. With a sterile pair of forceps, the Ciprofloxacin antibiotic disc (10µg) which served as the control and paper discs impregnated with the plant extract at different concentrations (4000µg/mL, 2000µg/mL, 1000µg/mL and 500µg/mL) were placed on the surface of the inoculated plates. The plates were incubated for 24hrs at 37°C. After incubation, zone diameter of inhibition formed in the medium was measured.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was carried out using broth dilution technique. Stock solution of 64000µg in 8ml DMSO of each of the extracts was prepared. Four millilitre (4mL) from the stock solution of the extract was taken and 2ml was added to each of the two test tubes containing 2ml of Mueller Hinton broth to arrive at 4000µg/mL. From the stock solution serial dilution was carried out to arrive at concentrations of 2000µg/ml, 1000µg/ml and 500µg/mL respectively. Another test tube containing 2mL of Mueller Hinton broth and 0.1mL of the standardized test organism served as the control. Four test tubes containing different concentrations of the extracts and Mueller Hinton broth were inoculated with 0.1 ml of the standardized test organism while the remaining 4 test tubes that were not inoculated served as the extract control. Nine test tubes for each batch of extract were used. All the test tubes were incubated at 37°C for 24hrs. The lowest concentration of the extract at which no turbidity was observed was recorded as the minimum inhibitory concentration (Oluduro, 2012).

Minimum Bactericidal Concentration

To determine the minimum bactericidal concentration, Mueller Hinton agar plates were inoculated with samples from each of the test tubes that showed no turbidity from MIC and were incubated 37°C for 24hrs. The plates were observed for bacterial growth. The lowest concentration with no bacterial growth was recorded as the minimum bactericidal concentration (Oluduro, 2012).

Statistical analysis

Mean values of zone of inhibition diameters from the activity of the highest concentrations of the extracts were compared

using one way ANOVA to determine whether significant difference or otherwise exist at 5% probability level.

RESULTS

Percentage occurrence of isolates recovered from samples

The organisms isolated were *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* as shown in (Table 1). It also shows that *Staphylococcus aureus* was the commonest organism with 72.3% occurrence, while *S. pneumoniae* the least with 12.8% occurrence

Table 1: Bacterial Isolates from Patients with Bacterial Keratitis Attending National Eye Centre Kaduna.

ISOLATES	No. (%)
<i>Staphylococcus aureus</i>	34 (72.3)
<i>Streptococcus pneumoniae</i>	7 (12.8)
<i>Pseudomonas aeruginosa</i>	6 (14.9)
Total	47 (100)

Physical Characteristics of the Extract

The details of physical properties of the extracts were shown in Table 2. The extract with the highest percentage recovery is *M. oleifera* ethanol extract with 16.5%, while the least is *M.*

oleifera hexane extract with 10.3% recovery. All the extracts were dark green in colour. Chloroform and methanol extracts were gummy, while ethanol and hexane extracts were oily in texture.

Table 2: Physical Properties of the Extracts

Physical parameters	CE	EE	ME	HE
Weight used for extraction(g)	100.0	100.0	100.0	100.0
Weight of extract (g)	14.4	16.5	15.9	10.3
% extract recovered	14.4	16.5	15.9	10.3
Colour of extract	Dark green	Dark green	Dark green	Dark green
Texture	Gummy	Oily	Gummy	Oily

KEY: CE = chloroform extract, EE = ethanol extract, ME = methanol extract, HE = hexane extract.

Phytochemicals in *Moringa oleifera* Extracts

The result of phytochemical analysis of *Moringa* extracts is presented in Table 3. Alkaloids were present in all the extracts except hexane extract. Saponins were present in all the extracts except chloroform extract while tannins were

present in the chloroform extract but absent in the rest of the extracts. Flavonoids were present in all the extracts except hexane extract.

Table 3: Phytochemicals in *Moringa oleifera* Extracts

Phytochemicals	CE	EE	HE	ME
Alkaloids	+	+	-	+
Saponins	-	+	+	+
Tannins	+	-	-	-
Flavonoids	+	+	-	+
Reducing Sugars	+	+	-	-
Glycosides	+	+	+	+

Key: + Present, - Absent

Antibacterial Activity of *Moringa oleifera* Extracts

Chloroform extract CE showed activity against *Streptococcus pneumoniae* at 4000µg/mL and 2000µg/mL, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were both sensitive to the extract at 4000µg/ml (Table 4). Ethanol extract EE showed higher activity on *Pseudomonas aeruginosa* than against *Staphylococcus aureus* and *Streptococcus pneumoniae* at 4000µg/mL. *Pseudomonas aeruginosa* showed highest sensitivity of 15mm at 4000µg/mL than *Streptococcus pneumoniae* (13mm) and *Staphylococcus aureus* (11mm) (Table 4). *Pseudomonas aeruginosa* (8mm) and *Staphylococcus aureus* (7mm) were sensitive at highest concentration while *Streptococcus pneumoniae* showed no activity at all.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Moringa oleifera* Leaf Extracts

Chloroform extract has MIC of 1000µg/ml against *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* (Table 5). *Staphylococcus aureus* has MBC 1000µg/ml with CE and EE and 4000µg/ml with HE and ME. *Streptococcus pneumoniae* has MBC of 1000µg/ml with EE, CE and 4000µg/ml with HE and 2000µg/ml with ME. *Pseudomonas aeruginosa* has MBC of 1000µg/ml CE and EE and 2000µg/ml with HE and ME (Table 5).

Table 4: Antibacterial Activity of *Moringa oleifera* Extracts (CE)

Conc (µg/mL)	CE			EE			ME			HE			
	PA	SA	ST	PA	SA	ST	PA	SA	ST	PA	SA	ST	
4000	16	12	11	11	10	10	15	11	13	8	7	0	
2000	11	11	8	9	9	8	8	7	8	7	0	0	
1000	7	0	0	7	0	0	7	0	0	0	0	0	
500	0	0	0	0	0	0	0	0	0	0	0	0	
CPX	10	20	17	19	20	17	19	20	17	19	20	17	19

CE = Anova showed significant difference between mean zone of inhibition of control and extracts highest concentration (df 3, f 9.2 p 0.001)

EE - Anova showed significant difference between mean zone of inhibition of control and extracts highest concentration (df, 3, f, 9.2, p 0.002)

ME - Anova showed significant difference between mean zone of inhibition of control and extracts highest concentration (df, 3, f, 9.2, p 0.002)

HE - Anova showed significant difference between mean zone of inhibition of control and extracts highest concentration (df 4 f 0.9 p 0.04).

Key: SA: *Staphylococcus aureus*. ST: *Streptococcus pneumoniae*. PA: *Pseudomonas aeruginosa* Control: ciprofloxacin.

Table 5: Minimum Inhibitory Concentration (MIC) of *Moringa oleifera* Leaf Extracts

	MIC (µg/ml)				MBC			
	CE	EE	HE	ME	CE	EE	HE	ME
<i>Staphylococcus aureus</i>	1000	1000	2000	2000	1000	1000	4000	4000
<i>Streptococcus pneumonia</i>	1000	1000	2000	1000	1000	1000	4000	2000
<i>Pseudomonas aeruginosa</i>	1000	1000	1000	1000	1000	1000	2000	2000

Key: CE = chloroform extract, EE = ethanol extract, HE = Hexane extract, ME = methanol extract

DISCUSSION

The result of this study showed that *S. aureus*, *P. aeruginosa* and *S. pneumonia* were the common organisms responsible for bacterial keratitis with *S. aureus* being the commonest organism isolated. Similar result was reported in Nigeria where *S. aureus* was the commonest organism (Oladigbolu *et al.* 2006). This is contrary to what was reported in Ghana and Thailand where *P. aeruginosa* was found to be the commonest organism. High percentage of bacterial corneal ulcers are caused by *S. aureus*, *S. pneumonia* and *P. aeruginosa* (Oladigbolu *et al.*, 2014). The probable reason for the high prevalence of *S. aureus* might be related to the use of traditional eye medication and poor personal hygiene among the patients with bacterial keratitis (Sharma, 2012).

The result of phytochemical screening from this research shows absence of flavonoids in HE, flavonoids have been shown to enhance antimicrobial property of a plant extract (Vergara *et al.*, 2017).

Compounds like pterygospermin, benzyl glucosinolate and benzyl isothiocyanate have however being isolated from *M. oleifera* leaves and these compounds have been reported to have antimicrobial properties against a wide range of bacteria (Rafael *et al.*, 2011). The leaves of *M. oleifera* have also been known to contain a number of phytochemicals such as flavonoids, tannins, saponins and other phenolic compounds that have antimicrobial activities (Farooq *et al.*, 2012). This would suggest that the antimicrobial activities observed in this study could be attributed to such compounds. The antibacterial activity of *M. oleifera* extracts validates some medicinal uses of *M. oleifera* (Rafael *et al.*, 2011).

The extracts have varying degree of antibacterial activities against the isolates. The antimicrobial activity increased with the increasing concentration 4000µg/mL being the highest concentration. All the different extracts of *Moringa oleifera* leaf have shown activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumoniae*. This implies that the *Moringa* leaf extracts have broad spectrum antibiotic activities against both the gram positive and gram negative organisms. Devendra *et al.*, (2011) reported similar result with chloroform extract zone of inhibition of 9.5mm, 6.2mm and 7.0mm against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes* respectively. However, Moyo *et al.*, (2012) reported no activity of the chloroform extract against *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

It can be deduced that *M. oleifera* chloroform extract CE has the highest spectrum of activity against all the isolates at the highest concentration of 4000µg/mL. The organism were also sensitive at 2000µg/mL where *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* were more sensitive at this concentration. Auwal *et al.* (2013) also reported that CE showed activity on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Devendra *et al.*, (2011) also reported that chloroform extract of *M. oleifera* was active against *Pseudomonas aeruginosa*, *Staphylococcus aureus*. Abakala *et al.*, (2012) reported the antibacterial activity of CE against *Pseudomonas aeruginosa*. It can be deduced that *Moringa*

oleifera leaf ethanol extract EE was active against the test bacteria. All the organisms show high sensitivity at three different concentrations with the largest zone of inhibition at the highest concentration. This correlates with the work of Bukar (2011) who reported broadest spectrum of activity with EE against some food spoilers including *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Mensah *et al.*, (2012) reported the activity of alcoholic extract of *Moringa oleifera* against *Staphylococcus aureus*.

The methanolic extract of *M. oleifera* also showed activity against all the bacterial isolates with *Pseudomonas aeruginosa* showing the largest zone of inhibition followed by *Streptococcus pneumoniae* and *Staphylococcus aureus*. The antimicrobial effect of the methanol extract against these organisms may be due to the ability of methanol to extract some of the active components of these plants like phenols compounds such as saponins, bryophyllin and others secondary metabolites which are reported to be antimicrobial in nature (Bichi *et al.*, 2012).

Moringa oleifera leaf hexane extract was active only against two of the bacterial isolates. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were sensitive to the extract at 4000µg/mL. *Streptococcus pneumoniae* was not sensitive to any of the concentrations tested. The result shows that HE is the least extract with activity on the tested organisms. Its low antibacterial activity might be associated with its phytochemical contents.

CONCLUSION

The study has demonstrated that chloroform, ethanol and methanol extracts of *M. oleifera* have antibacterial activity against *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* followed and *Staphylococcus aureus* isolated from patients with bacterial keratitis.

REFERENCES

- Abalaka., M.E., Daniyan, S.Y., Oyeleke, S.B. and Adeyemo S.O. (2010). Antibacterial Evaluation of *Moringaoleifera* leaf extracts on selected bacterial pathogens. *Journal of Microbiology research*. 2(2): 1-4.
- Ashikin, N., Karim, A., Ibrahim, M. D., Kntayya, S. B., Rukayadi, Y., Hamid, H. A., and Razis, A. (2016). *Moringa oleifera* Lam : Targeting Chemoprevention, 17, 3675–3686.
- Auwal, M. S., Tijjani, A N., Sadiq, M. A, Saka, S., Mairiga, I. A, Shuaibu, A, and Gulani, I. A. (2013). Antibacterial and haematological activity of *Moringa oleifera* aqueous seed extract in Wistar albino rats, *Sokoto of Vetenary Sciences Journal*.11(1), 28–37.
- Bichi, M. H., Agunwamba, J. C., Muyibi, S. A., and Abdulkarim, M. I. (2012). Effect of Extraction Method on the Antimicrobial Activity of *Moringa oleifera* seed extract. *Journal of American Science*, 8(9), 450–458.
- Bukar, A., Uba, A. and Oyeyi, T. (2010). Antimicrobial profile of *Moringa oleifera* Lam. Extracts against some food –

- borne microorganisms. *Bayero Journal of Pure and Applied Sciences*. 3(1):43 - 48
- Devendra, B. N., Srinivas, N., Talluri, V. S. S. L. P., and Latha, P. S. (2011). Antimicrobial activity of *Moringa oleifera* lam leaf extract against selected bacterial and fungal strains. *International Journal of Pharm. and Biosciences*.2(3):13–18.
- Farooq, F., Rai, M., Tiwari, A., Khan, A. A., and Farooq, S. (2012). Medicinal properties of *Moringa oleifera*: An overview of promising healer. *Journal of Medicinal Plants Research*.11(1) 28-37
- Mensah, J. K., Ikhajiagbe, B., Edema, N. E., and Emokhor, J. (2012). Phytochemical , nutritional and antibacterial properties of dried leaf powder of *Moringa oleifera* (Lam) from Edo Central Province , Nigeria. *J. Nat. Prod. Plant Resour.*, 2(1), 107–112.
- Moyo, Busani, Masika, Patrick Juluis and Muchenje, Voster (2012): Antimicrobial activities of *Moringa olieifera* lam leaf extracts. *African Journal of Biotechnology* 11 (11): 2797 – 2602..
- Oladigbolu, K., Rafindadi, A., Abah, E., and Samaila, E. (2014). Corneal ulcers in a tertiary hospital in Northern Nigeria. *Annals of African Medicine*, 12(3), 165–70.
- Oluduro, A. O. (2012). Evaluation of Antimicrobial properties and nutritional potentials of *Moringa oleifera*. *Malaysian Journal of Microbiology*, 8(2), 59–67.
- Rafael, J., Peixoto, O., Silva, G. C., Costa, R. A., Fontenelle, L. D. S., Hitzschky, G., and Vieira, F. (2011). *In vitro* antibacterial effect of aqueous and ethanolic *Moringa leaf* extracts, *Asian Pacific Journal of Tropical Medicine*20(11): 201–204.
- Sharma, S. (2012). Diagnosis of infectious diseases of the eye. *Eye*, 26(2), 177–184.
- Vergara-jimenez, M., Almatrafi, M. M., and Fernandez, M. L. (2017). Bioactive Components in *Moringa leifera* Leaves Protect against Chronic Disease, *Journal of Antioxidants*2(1): 1–13.