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Research Paper

Effects of *Moringa oleifera*Extracts on Selected Bacteria Associated with Urinary Tract and Enteric Infections in Kaduna Metropolis, Nigeria

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Abstracts:*Moringa oleifera belongs to the Moringaceae family and is characterized by its rapid growth, deciduous nature, and tropical habitat. This genus is distinguished by its thick tuberous roots, light green leaves, and prolific flowering, which results in the development of elongated, pendulous fruits and seeds. Moringa oleifera has been widely employed in traditional medicine to address various health conditions, including but not limited to skin disorders and gastrointestinal disturbances. A urinary tract infection (UTI) refers to an infectious condition resulting from the colonization and proliferation of microorganisms within the urinary tract. The primary objective of this research was to study was to the effects of Moringa oleifera extracts on selected bacteria associated with urinary tract infections (UTIs) and enteric infections. The leaves of Moringa oleifera were gathered and transported to the Department of Biological Sciences at Kaduna State University in Nigeria for the purpose of identification by a professional botanist. The process of preparing the plant extract and conducting a screening of phytochemicals was undertaken. The antimicrobial activity of the aqueous, ethanolic, and methanolic extracts of the leaves was evaluated using the paper disc diffusion method. The values of MIC (minimum inhibitory concentration) and MCB (minimum bactericidal concentration) were determined. The phyto-compound Anthraquinone exhibited the highest concentration in both the aqueous and ethanolic extracts, with values of 9.83 ± 0.04 and 7.6 ± 0.05, respectively. Salmonella typhi exhibited the highest minimum inhibitory concentration (MIC) value of 10.5 mg/ml, along with a minimum bactericidal concentration (MBC) value of 40 mg/ml. Similarly, S. dysenteriae also demonstrated the same MIC and MBC values.*

Keywords:*Moringa oleifera, urinary tract infection, enteric disease, Kaduna, Nigeria*

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I. INTRODUCTION

The cultivation and utilization of the *Moringa* tree encompass various aspects, including the consumption of its vegetables such as leaves, green pods, and flowers, as well as the utilization of its roasted seeds for culinary purposes and as a source of cosmetic oil. Additionally, the *Moringa* tree holds significance as a medicinal plant, with its various plant organs possessing therapeutic properties [1]. *Moringa oleifera* has been widely employed in traditional medicinal practices to address various health conditions, including skin disorders, gastrointestinal disturbances, and as a stimulant for individuals affected by paralysis, epilepsy, and hysteria. Additionally, it has been found to enhance the process of digestion [2]

A urinary tract infection (UTI) is characterized by the colonization and proliferation of microorganisms within the urinary tract [3]. Urinary tract infection (UTI) is a prevalent bacterial infection in humans, manifesting in both community and healthcare settings. It is considered a prominent factor driving individuals to seek medical assistance [4]. Urinary tract infection (UTI) encompasses a range of clinical entities that vary in severity, including asymptomatic infection, acute cystitis, prostatitis, pyelonephritis, and urethritis [5].

Urinary tract infections (UTIs) are commonly observed bacterial infections in the outpatient environment. It has been reported that approximately one in three women will develop a UTI that necessitates antibiotic treatment by the age of 24, and about 50% of women will experience at least one UTI throughout their lifetime [6].

According to Fihn*et al.*[7], the occurrence of hospital acquired infections typically arises subsequent to the colonization by several bacterial species such as *Klebsiella, Enterobacter, Citrobacter, Serratia, Pseudomonas aeruginosa, Providencia, E. faecalis*, or *S. epidermidis*. It is worth mentioning that the age of the patient can have an impact on the specific infective organism that is present. For instance, *Staphylococcus saprophiticus* is currently responsible for 10% of urinary tract infections (UTIs) in young females, whereas its occurrence in elderly female patients is less than 1%.

Urinary tract infection (UTI) is a prevalent condition observed in various populations, including the elderly, individuals with compromised immune systems, those with neurologic dysfunction or renal disease, and females. The higher susceptibility of females to UTIs can be attributed to the anatomical proximity of their genital region to the anus, as noted by Ebie*et al.*[3]. Enteric diseases are prevalent as well. This can be attributed to inadequate personal and sanitary hygiene practices. It is imperative to prevent the escalation of these occurrences. This study aimed at comparing the effects of *Moringa oleifera*extracts on bacteria associated with urinary tract infections and enteric diseases in Kaduna metropolis, Nigeria.

II. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The leaves of *Moringa oleifera* were gathered from a house in Rigasa community and transported immediately to the Department of Biological Sciences at Kaduna State University in Kaduna, where they were subjected to identification by a qualified botanist.The leaves were subjected to a drying process in a shaded environment, followed by grinding into a powdered consistency using a sterile mortar and pestle. The resulting powder was then carefully stored in an airtight plastic container until it was ready for utilization.

2.2 Extractionof Plant Material

A quantity of one gram (1 g) of powdered *Moringa* leaves was introduced into a beaker containing 100 ml of distilled water, where it remained for duration of approximately 48 h. Subsequently, the sample was subjected to a water bath maintained at a temperature of 60 °C prior to undergoing the filtration process. The residue was subjected to a continuous addition of hot water, followed by filtration. The experiment was conducted in triplicate, and the resulting liquid was subjected to evaporation until complete desiccation using a water bath set at a temperature of 60 ^{o}C. A volume of approximately 100 millilitres of ethanol per gramme of plant leaf powder was employed. The ethanol extract was subjected to vacuum drying at a temperature of 40 °C. The desiccated extract was preserved in aseptic containers for subsequent utilisation. Prior to conducting the experiment, a quantity of 10 mg of dry extract was solubilized in 1 ml of ethanol, the same technique was employed for methanol extraction respectively[8].

2.3 Phytochemical Analysis

The extracts were subjected to phytochemical analysis to qualitatively detect alkaloids, flavonoids, tannins, saponins, anthrocyanin, anthraquinone, cardiac glycoside, carotenoids, steroids, and terpenoids[9].

2.4 Collection of Isolates

The bacterial isolates that were obtained from hospitals, namely BarauDikko Teaching Hospital (BDTH), Sabo General Hospital (SGH), and Gwamna Awan General Hospital in Kaduna metropolitan city, Kaduna State, were subjected to bacteriological and biochemical characterization. These isolates were associated with urinary tract infections and enteric diseases. The organisms encompassed in this study are *Escherichia coli, Pseudomonas aeruginosa,Staphylococcus aureus, Salmonella typhi,* and *Shigelladysenteriae.*

2.4.1 Determination of Minimum Inhibitory Concentration

The broth dilution method was employed to determine the lowest inhibitory concentrations of the aqueous and ethanolic extracts derived from the seeds. A total of eighteen (18) tubes were utilised for each extract. The initial sample consisted of 5 millilitres of nutrient broth at double concentration, whereas the subsequent samples consisted of 5 millilitres of nutrient broth at single concentration. Tube one was filled with a volume of five millilitres (5 ml) of the crude extract at the required concentration, which was afterwards stirred extensively. A volume of 5 millilitres (5 ml) from tube one was transferred to tube two and properly mixed. Additionally, a volume of 5 ml from tube two was transferred to test tube three. The aforementioned technique was replicated for the subsequent test tubes, up until tube 8. Tube 9, however, was devoid of any pharmaceutical substance. 0.1 ml of broth cultures of the test organisms was introduced to each of the test tubes numbered 1-9. The tubes were subjected to incubation at a temperature of 35 °C ± 2 °C for duration of 18 - 20 hours. Subsequently, an assessment was conducted to determine the presence or absence of bacterial growth [10].

2.4.2 Determination of Minimum Bactericidal Concentration (MBC)

The determination of the Minimum Bactericidal Concentration (MBC) involved the first selection of tubes that exhibited no growth throughout the process of Minimum Inhibitory Concentration (MIC) determination. A single loopful of each tube's contents was inoculated onto the surface of extract-free nutrient agar and afterwards incubated for a period of 24 hours at a temperature of 35 ^{o}C ± 2 ^{o}C[10]

2.5 Statistical Analysis

Data obtained from quantitative screening of the aqueous, ethanolic and methanolic extracts of *Moringa oleifera* were subjected to statistical analysis using one-way ANOVA of (SPSS) and their statistical significance at P <0.05 were determined.

2.6 Ethical Approval

Ethical approval for the collection of clinical isolates in Kaduna metropolis was sought from the Kaduna State Ministry of Health.

III. RESULTS

The following bioactive compounds of *Moringa* leaf extracts were screened and found to be tannins, flavonoids, terpenoids, steroids, saponins, carotenoids, alkaloids, anthocyanin, anthraquinone, and cardiac glycoside as shown in (Table 1).

Table 1: Phytochemical Screening of Aqueous, Ethanolic and Methanolic Moringa oleifera Leaf Extracts

Phytochemical compound	Aqueous extract	Ethanolic extract	Methanolic extract
Alkaloids	+	+ +	+
Anthocyanin	+	+ +	+
Anthraquinone	+	+	+
Cardiac glycoside	+	+	+
Carotenoids	+	+ +	+
Flavonoid	+	+ + +	++
Saponins	+	+ +	+
Steroids	+	+ +	+
Tannins	+	+ + +	++
Terpenoids	+	+	+

Key:
+ = slightly present
+ + = moderately present
+ + + = highly present

The quantitative screening of the aqueous, ethanolic and methanolic extracts of *Moringa oleifera* revealed that, the phyto-compound anthraquinone had the highest value for aqueous, ethanolic, and methanolic extract with 9.83 ± 0.04, 7.6 ± 0.05, and 5.8 ± 0.02 respectively. Similarly, cardiac glycoside recorded the lowest value for aqueous, ethanolic and methanolic extracts with values of 0.52 ± 0.04, 0.31 ± 0.03, and 0.26 ± 0.04 respectively. It was equally analyzed that both the aqueous extracts of anthraquinone and cardiac glycoside were statistically significant at P <0.05. Other compounds that were statistically significant using aqueous extraction are alkaloids (2.96 ± 0.00), anthocyanin (0.09 ± 0.01), steroids (4.13 ± 0.00), tannins (8.71 ± 0.03) and terpenoids (3.95 ± 0.04). However, the ethanolic extracts of the following compounds were equally statistically significant at P <0.05: flavonoids (2.86 ± 0.01), and saponins (1.84 ± 0.05) respectively. Methanolic extracts of these compounds showed statistical significance as well: alkaloids, flavonoids, steroids and tannins (Table 2).

Table 2 Quantitative Screening of Aqueous, Ethanolic and Methanolic Leaf Extracts of *Moringa oleifera*

Phytochemical compound	Aqueous extract	Ethanolic extract	Methanolic extract
Alkaloids	2.96± 0.00*	1.42±0.02	1.21 ± 0.05*
Anthocyanin	0.09±0.01*	0.08±0.01	0.069 ± 0.03
Anthraquinone	9.83± 0.04*	7.6±0.05	5.8 ± 0.02
Cardiac glycoside	0.52± 0.04*	0.31±0.03	0.26 ± 0.04
Carotenoids	1.18± 0.05*	0.12±0.03	0.09 ± 0.07
Flavonoid	2.63± 0.02	2.86±0.01*	2.91 ± 0.02*
Saponins	1.6± 0.03	1.84±0.05*	1.87 ± 0.04
Steroids	4.13± 0.00*	4.08±0.02	3.92 ± 0.01*
Tannins	8.71±0.03*	8.25± 0.01	8.03 ± 0.03*
Terpenoids	3.95±0.04*	3.60±0.05	3.38 ± 0.02

Values with asterisk are statistically significant at P < 0.0

The aqueous extract of the plant was inhibitory against *Escherichia coli* at concentrations of 40 % and 60 % respectively (14 mm and 15 mm). For *Staphylococcus aureus*, an inhibitory activity of the plant's aqueous extract at 20 %, 40 % and 60 % concentrations were recorded at (12 mm, 15 mm, and 17 mm) respectively. Similarly, the plant recorded an inhibitory activity against *Shigelladysenteriae* at 40 % and 60 % concentrations to be (18mm and 20mm) respectively. However, the aqueous extract of the plant did not show any inhibitory activities against *Pseudomonas aeruginosa* and *Salmonella typhi*. *Escherichia coli* was susceptible to all the concentrations of the ethanol extract of the plant at (12 mm, 16 mm, and 19 mm) respectively. *Salmonella typhi, Staphylococcus aureus, and Shigelladysenteriae*were all susceptible to varying concentrations of the ethanol leaf extract of the plant except for *Pseudomonas aeruginosa* that showed no susceptibility towards the extract. However, a very good inhibitory activity of methanol extract of the plant against all the tested organisms was observed each at different concentrations of the plant extract (Table 3).

Table 3 Antibacterial Susceptibility Pattern of *Moringa oleifera* Extracts against UTI/Enteric Pathogens

		Diameter of inhibition zones of test organisms/mm				
Solvent Extract	Conc. (%)	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. typhi*	*S. dysenteriae*
Aqueous	20	-	-	12	16	-
	40	14	-	15	19	18
	60	15	14	17	21	20
Ethanol	20	-	-	-	16	-
	40	-	-	12	18	-
	60	15	-	15	20	14
Methanol	20	-	-	17	-	-
	40	15	16	-	18	15
	60	17	18	-	20	17
C	20	-	-	-	-	-
	40	-	-	-	-	-
	60	-	-	-	-	-

Key

*Less than 14mm: Resistant

*More than 14mm: Sensitive

C = Control

On the minimum inhibitory and minimum bactericidal concentrations of the plant extracts against the tested bacteria, it was seen that *E. coli* had 12.5 mg/ml for the MIC, while50 mg/mlwas recorded for the MBC. Similarly, *P. aeruginosa* recorded 6.5 mg/mland18mg/ml**.** *Staphylococcus aureus* recorded the highest value for MIC to be 23 mg/mland that of MBC at 12.5 mg/ml**.** *Salmonella typhi* recorded the highest MIC value at 10.5mg/ml and MBC value at 40 mg/ml; the samevalue was equally obtained for *S. dysenteriae*.

Table 4: Minimum Inhibitory and Bactericidal (MIC and MBC) Concentrations of *M.oleifera*Leaf Extract against the Tested Organisms

	Concentration of Extracts (mg/ml)		
Isolates	**MIC (mg/ml)**	**MBC (mg/ml)**	**Extracts**
E. coli	12.5	50	AE
	5.25	20	EE
	10	25	ME
P. aeruginosa	0.00	0.00	AE
	0.00	0.00	EE
	6.5	18	ME
S. aureus	23	23	AE
	12	12	EE
	12.5	12.5	ME
S. typhi	6.25	25	AE
	10.5	20	EE
	25	40	ME
S. dysenteriae	5.25	15	AE
	10	25	EE
	12	40	ME

Key:
AE = Aqueous Extract
EE = Ethanolic Extract
ME= Methanolic Extract

IV. DISCUSSION

Within the field of ethnomedicine, *Moringa oleifera* leaves have been utilised by conventional healthcare professionals to address several health conditions, such as gastric pain, stomach ulcers, diarrhoea, dysentery, and skin infections [11] [12] According to a study conducted by Bukar*et al.*[11], the leaves of *Moringa oleifera* have demonstrated several therapeutic qualities, including anti-tumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, and antioxidant effects.

The extract underwent a phytochemical analysis, which revealed that the plant contains a diverse array of compounds. These compounds contribute to the therapeutic value of the plant and exhibit inhibitory activities against a broad spectrum of microbial pathogens. This includes pathogens associated with urinary tract disorders and enteric diseases. The findings of this current study indicate that the qualitative analysis of leaf extracts from *Moringa oleifera* revealed the presence of many phytochemical components, including flavonoids, anthraquinone, alkaloids, saponins, steroids, terpenoids, cardiac glycosides, anthocyanin, tannins, and carotenoids. This aligns with the findings presented in the study conducted by Kasolo[12]. In contrast to the findings of Oluduro[13], Bamishaiye*et al.* [14] observed the absence of steroids, terpenoids, and cardiac glycosides in the leaf extract. The variability in the composition of *M. oleifera* compounds can be related to regional disparities and the choice of solvent employed during phyto-compound extraction. The findings of this study indicate that the aqueous extract ingredients contained a higher quantity of phytochemicals compared to the ethanol extract.

According to a recent report by Bamishaiye*et al.*[14], the distribution of phytochemicals in *M. oleifera* leaves may vary due to climatic factors and stages of maturity. Additionally, the choice of solvent used for extraction can also influence the extraction capabilities and solubility spectrum of phyto-constituents, as different solvents exhibit different properties in this regard.

In the current study, the antibacterial efficacy of *Moringa oleifera* leaf extract was evaluated using various solvents (aqueous, ethanol, and methanol). The findings revealed that the aqueous extraction of the constituents exhibited significant inhibitory activity against urinary tract pathogens, namely Staphylococcus aureus and *Escherichia coli*. Nevertheless, a greater degree of inhibition was observed in relation to the intestinal pathogens *Salmonella typhi* and *Shigelladysenteriae.* The inhibitory effect of the ethanolic and methanolic extracts was found to be less pronounced against all tested pathogens, except for *Salmonella typhi,* which exhibited a moderate level of susceptibility. The obtained finding provides additional elucidation into the phenomenon that bacteria possess diverse resistance components (genes) that allow them to endure and surmount specific therapies that may pose a threat to their survival. This aligns with the findings of

Oluduro's[13] study on the assessment of the antimicrobial properties of *Moringa oleifera* leaves. The study reported that the ethanolic extract did not demonstrate antimicrobial activity against the orthopaedics' wound organisms tested. However, it did show a negligible effect on the enteropathogens. On the other hand, the aqueous extract exhibited inhibition against nearly all of the organisms tested, suggesting that water is an effective solvent for extracting the leaves. According to Oluduro[13], it was found that the aqueous extract derived from the leaves of *Moringa oleifera* exhibited noteworthy antimicrobial properties against both Gram-negative and Gram-positive bacterial organisms commonly found in wounds. The study suggests that the antimicrobial activity observed in the aqueous extract can be attributed to the higher capacity of water to extract a greater quantity of antimicrobial phytochemicals compared to the ethanolic extract. The antimicrobial phyto-compounds, namely alkaloids, tannins, anthraquinone, and anthocyanin, exhibit their action by adhering to the cell walls of bacteria and subsequently deactivating their enzymes. This mechanism effectively hinders the growth and multiplication of bacteria within the human body [15]. According to Fahey and Akaneme[16], it has been noted that the leaves of *M. oleifera* possess a significant amount of tannins, which may explain their traditional application in the management of urinary tract infections, diarrhoea, wound healing, and dysentery.

V. CONCLUSION

The leaves extract of *Moringa oleifera* plant screened in this study was proven to contained phyto-compounds which are of medicinal importance particularly in the treatment of urinary tract infections and enteric diseases caused by bacterial pathogens with Anthraquinone being the highest abundant phyto-compound while Cardiac glycoside being the least. All the tested bacterial isolates were more susceptible to the aqueous extract of the plant than other solvents (ethanol and methanol). There was equally high inhibitory activity of the extract against the enteric pathogens as compared to that of urinary tract pathogens. It is recommended that a collaborative sensitization awareness/program between medical/community health workers and herbal health practitioners should be encouraged in order to better understand the applications, mechanisms of actions and side effects of using medicinal plants as a remedy for curing some of these infections and diseases such as UTI.

REFERENCES

[1]. Rebecca, H.S.U., Sharon, M., Arbainsyah, A.andLucienne, D. Moringa oleifera medicinal and socio-economic uses. International Course on Economic Botany. National Herbarium Leiden, Netherlands. 2016: Pp 2-6.

[2]. Farooq, F, Rai, M., Tiwari, V., Khan, A. A. and Farooq, S. Medicinal properties of Moringa oleifera: An overview of promising healer. Journal of Medicinal Plants Research, 2020.6 (27), 4368-4374.

[3]. Ebie, M., Kandki-Olukemi, Y.T., Ayanbadejo, J. and Tanyigna, K.B. Urinary tract infections in a Nigerian Military Hospital. Nigerian Journal of Microbiology, 2020.15(1), 31-37.

[4]. Kolawale, A.S., Kolawale, O.M., Kandaki-Olukemi, Y.T., Babatunde, S.K., Durowade, K.A. and Kolawale, C.F. Prevalence of urinary tract infections among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. International Journal of Medical Sciences.2019. (5), 163-167

[5]. Fowler, J.E., and Stamey, T.A. Studies of introital colonization in women with recurrent urinary infections. VII. The role of bacterial adherence, Journal of Urology, 2016.117 (4), 472-6.

[6]. Dielubanza, E.J. and Schaeffer, A.J. Urinary tract infections in women. The Medical clinics of North America, 2021.**95**, 27–41.

[7]. Fihn, S. D., Boyko, E. J., Chen, C. L., Normand, E. H., Yarbro, P. and Scholes, D.. Use of spermicide-coated condoms and other risk factors for urinary tract infection caused by Staphylococcus saprophyticus, Archives of internal medicine, 2018. 58 (3), 281-7.

[8]. Idris, A., Idris, S., Bawa, H., Magaji, R.A. and Ahmad, A. Evaluation of effects of Hexane and methanol extracts of Trigonellafoenum-graecum L. in isolated rabbits heart. Nigerian Journal of Scientific Research, 2018. 17(4): 508-516

[9]. Senguttuvan, J., Paulsamy, S., &Karthika, K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaerisradicata L. for in vitro antioxidant activities. Asian Pacific journal of tropical biomedicine, 2014. 4(Suppl 1), S359–S367.

[10]. Lar, P.M., Ojile, E.E., Dashe, E. and Oluoma J.N. Antibacterial activity of Moringa oleifera seed extracts on some gram negative bacterial isolates. African Journal of Natural Sciences, 2021. 14, 57 - 62

[11]. Bukar, A., Uba, A. and Oyeyi, T.I. Antimicrobial profile of Moringa oleiferalam extracts against some food-borne microorganisms.Bayero Journal of Pure Applied Sciences, 2020. 3(1), 43-48.

[12]. Kirisattayakul, W., Wattanathorn, J., Tong-Un, T., Muchimapura, S., Wan-nanon, P. and JittiwatJ.Cerebroprotective effect of Moringa oleiferaagainstfocalischemic stroke induced by middle cerebral artery occlusion, Oxidative Medicine and Cellular Longevity. 2013. 13, 10–13.

[13]. Oluduro, A.O. Evaluation of antimicrobial properties and nutritional potentials of Moringa oleifera Lam. leaf in South-Western Nigeria. Malaysian journal of microbiology, 2012.8, 59– 67.

[14]. Coker, C., Poore, C. A., Li, X. and Mobley, H. L. Pathogenesis of Proteus mirabilis urinary tract infection. Microbes Infect,2020. 2(12), 1497-505

[15]. Tiwari, P., Kumar, B., Kaur, M., Kaur, G. and Kaur, H. Phytochemical screening and extraction: A Review. InternationalePharmaceuticaSciencia,2016. 1 (1), 98-106

[16]. Fahey, J.Moringaoleifera: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Part 1, Trees for Life Journal, 2015. 1, 15.