Original Research Article

PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *Moringa oleifera* LEAVES EXTRACTS AGAINST *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*.

ABSTRACT

This study tests the antibacterial activities of *Moringa oleifera* leaf extract against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*, obtained from Microbiology laboratory, Al-hikmah University Ilorin. Phytochemical analysis reveals the presence of tannins in all the three extracts (Aqueous, ethanolic and N-hexane), while. flavonoids, alkaloids and Saponins were only present in the Aqueous and ethanolic extracts. The leaves extracts were screened for antibacterial activity by agar well diffusion method, employing five different extracts concentrations (100mg/ml, 80mg/ml, 60mg/ml, 40mg/ml and 20mg/ml). The aqueous extracts had a mean activity of 11.50±0.70mm, 7.50±0.70mm and 8.5±0.70mm for *S. aureus*, *E. coli* and *P. aeruginosa* respectively. The ethanol extracts had a mean activity of 12.00±1.41mm, 10.00±1.41mm and 8.00±1.21mm for *S. aureus*, *E. coli* and *P. aeruginosa* respectively, while the N-hexane extracts exert no any activity. The antibiotics discs exert more inhibitory effect compared to the extract. The MIC for the aqueous extracts are at a concentrations of 60mg/ml for *S. aureus*, 80mg/ml for *P. aeruginosa* and 80mg/ml for *E. coli*. While that of the ethanol extract of the leaves are at a concentration of 60mg/ml for *S. aureus,* 80mg/ml for *E. coli* and 100mg/ml for *P. aeruginosa*. The MBC for the aqueous Extract of the leaves are at a concentration of 80mg/ml for *S. aureus,* 100mg/ml for *P. aeruginosa* and 100mg/ml for *E. coli*, while that of the ethanol extract are at a concentration of 80mg/ml for *S. aureus* and 100mg/ml for *E. coli*), while no any MBC was recorded for *P. aeruginosa*. The antibacterial activity tests indicate that the ethanol extract was more active. Among the three isolates, *S. aureus* is more sensitive to the aqueous and ethanol extracts. The activity exhibited by the extracts may be related to the presence of a number of Phytoconstituents.

Keywords*: Moringa oleifera, antibacterial, Ilorin, S. aureus, E. coli and P. aeruginosa.*

1.0 INTRODUCTION

Plants are reported to possess various biological activities and have been an important source of natural products for human health. [1] Many of the existing synthetic drugs are known to cause

various side effects, such as intoxication, nauseas, and other allergies. [2] By implication, herbal medicine is now forming an alternative therapy that has become the mainstream throughout the world due to the growing resistance of pathogens to conventional antibiotics. [3]

Moringa plant provides a rich source of zeatin, quercetin, kaempferom and many other phytochemicals [4] The leaves are outstanding as source of vitamins like beta-carotene of Vitamin A, vitamin B such as folic acid, pyridoxine and nicotinic acid, vitamin C, D and E. [5] The leaves are also rich in minerals like iron, calcium, copper, potassium, zinc and magnesium. [6] The *Moringa oleifera* has been used extensively in traditional medicine for the treatment of several ailments; promote digestion, skin disease, diarrhoea, as stimulant in paralytic afflictions, epilepsy and hysteria. [2] The roots, stem bark, seeds and leaves of the plant have been reported to possess some antibacterial, anti-cancer and anti-inflammatory activities. [7, 8]

This study aimed to investigate the antibacterial activities of aqueous and ethanol extract of *Moringa oleifera* leaves acting separately on *S. s aureus, E. coli* and *P. aeruginosa* along with their minimum inhibitory and bactericidal concentration.

2.0 MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials.

Fresh *Moringa oleifera* leaves were obtained at the biological garden, University of Ilorin, and authenticated at Herbarium Section, Department of Plant Biology, University of Ilorin where a reference voucher specimen number (UILH/001/559) was obtained. The leaves were washed under running tap water and shade dried at a room temperature. The dried leaves were ground to powder for further analysis. [3]

2.2 Collection and Maintenance of Bacterial Isolates.

Clinical isolates of *S. aureus*, *P. aeruginosa* and *E. coli*, were obtained from Microbiology laboratory at Al-Hikmah University Ilorin, Kwara State, and were maintained as stock cultures at $4^{o}C$ in the refrigerator.

2.3 Preparation of Leaves Extracts.

Three solvents were used, Two polar (Aqueous, Ethanol) and one non-polar (N-Hexane) solvent. Seventy (70g) grams of the powdered leaves of *Moringa oleifera* was weighed in triplicate into 3 different bottles containing 300ml of distilled water, 95% ethanol and N-hexane, for aqueous, ethanol and N-hexane extracts respectively. The solutions were shaked, covered and left on the mechanical shaker to extract at 130RPM for 48hrs. The extract was filtered and evaporated to dryness using water bath at $45^{o}C$ [9]

2.4 Phytochemical Screening

Phytochemical screening was carried out for the aqueous, ethanolic and N-hexane extracts of the leaves using the methods as described by Abdulfatai et al. and Sofowara. [3, 10]

2.4.1 Test for Tarpenes

Half (0.5) g of each extract was dissolved in 5ml of water and 2-3 drops of 10% of ferric chloride solution was added. Violet precipitate indicates the presence of Tarpenes.

2.4.2 Test for Phenols and Tannins

Half (0.5) g of each extract was mixed with 2ml of 2% solution of ferric chloride ($Fecl_3^-$). Blue green or black colouration indicates the presence of Phenols and Tannins.

2.4.3 Test for Flavonoids (Alkali reagent Test)

Half (0.5) g of each extract was mixed with 2ml of 2% solution of NaOH. The formation of an intense yellow colour which turned colourless upon the addition of few drops of dilute acid indicates the presence of flavonoids. [11]

2.4.4 Test for Alkaloids

Half (0.5) g of each extract was shaken with 1% hydrochloric acid for two minutes. The mixture was filtered and drops of Mayers reagent was added. Formation of a yellow cream precipitate indicates the presence of alkaloids. [12].

2.4.5 Test for Saponins

Half (0.5) g of each extract was mixed with 5ml of distilled water in a test tube and it was vigorously shaken. The formation of stable foam is an indication of the presence of Saponins. [13]

2.4.6 Test for Tarpenoids

Half (0.5) g of each extract was mixed with 2ml of chloroform followed by the addition of 3ml concentrated H_2SO_4 to form a layer. The formation of reddish brown colour in the interphase indicates the presence of Tarpenoids. [14]

2.5 Concentration Procedure.

Different concentration of the extracts (aqueous, ethanol and N-hexane) were obtained in the following projection;

Solution A: 1g of the extract + 10ml of diluent (distilled water) = 100mg/ml.
Solution B: 4mls of solution A + 1ml of diluent i.e. 4/5 x 100 = 80mg/ml.
Solution C: 3msl of solution A + 2ml of diluent i.e. 3/5 x 100 = 60mg/ml.
Solution D: 2mls of solution A + 3ml of diluent i.e. 2/5 x 100 = 40mg/ml.
Solution E: 1ml of solvent + 4ml of diluent i.e. 1/5 x 100 = 20mg/ml.

2.6 Sterility Test of Leave Extract.

A drop of each of the extracts was place on sterile Muller Hinton agar plate and incubated at 37^{o}C for 24hours. Absence of growth on the plates confirm the sterility of the extracts. [15]

2.7 Determination of Antibacterial Activity of *Moringa oleifera*

The aqueous, ethanolic and N-hexane extracts of leaves of *M. oleifera* were screened for antibacterial activity by agar well diffusion method. The turbidity was adjusted to 0.5% McFarland standard to give a suspension containing approximately 10^6 Cfu/ml. [3, 16]

The standardized inocula were inoculated in an already prepared Mueller Hinton agar. Using a sterile cock borer, five wells of a diameter of 5mm were bored on the agar surface, and 0.1ml of the various extracts concentration (100mg/ml, 80mg/ml, 60mg/ml, 40mg/ml and 20mg/ml) were dispensed into each well. The plates were allowed to stand for one hour for the pre-diffusion of the extracts to occur before incubating for 24 hours at 37^{o}C. The plates were observed for the presence of zone of inhibition and measured accordingly. [9]

2.8 Determination of Antibacterial Activity for Commercially Prepared Antibiotics.

Each of the bacterial inoculum was streaked on an already prepared Mueller Hinton agar surface and the plates were left to stand for 15 minutes, after which the antibiotics discs (Ofloxacin 'OFX 5µg' and Ciprofloxacin 'CIP 5µg') were placed on the surface of the inoculated plate. The discs were pressed down firmly with the aid of sterile forceps to ensure proper contact and the plates were incubated at 37ºC for 24hours. This serves as the positive control.

2.9 Determination of Minimum Inhibitory Concentration (MIC)

Various extracts (Aqueous, Ethanol and N-hexane) concentrations were prepared by dilution using distilled water to obtain different concentrations of 100mg/ml, 80mg/ml, 60mg/ml, 40mg/ml and 20mg/ml. one (1) ml of each extract concentrations and that of Mueller Hinton broth was mixed, and 0.1ml of standardized inoculum (1.5×10^6 CFU/ml) was added to each of the test tubes above. The tubes were incubated at 37^oC for 24 hours. Tubes containing broth and leaf extracts were used as positive control while tubes containing broth and inocula were used as negative controls. The tubes were observed after 24 hours of incubation [10, 17].

2.10 Determination of Minimum Bactericidal Concentration (MBC)

Sterile Mueller Hinton agar plates were separately inoculated with culture from each of the MIC tubes that showed no evidence of turbidity. The plates were incubated at 37^0C for 24 hours. The MBC was determined as the highest dilution that yielded no single bacterial colony on the agar surface. [10, 17]

3.0 RESULTS AND DISCUSSION

The sterility test carried out on all the different extracts (aqueous, ethanol and N-hexane), revealed the absence of any contaminant (Table 1). The antibacterial activity of all the extracts against *S. aureus*, *E. coli* and *P. aeruginosa* indicates that all the extracts except N-hexane showed significant activity against all the isolates.

Table 1: Extract Sterility Test.

S/N	Extract Used	Result of Sterility
1.	Aqueous	-
2.	Ethanol	-
3.	N-hexane	-

Keys: (-) absence of contaminant, an indication for sterility

The phytochemical screening of the various extracts reveals the presence of various bioactive compounds (Table 2). The presence of bioactive compounds in plants is an indication of the presence of compounds which can be inhibitory against clinical isolates. The results of these findings can be compared with the work of Farooq et al., [2] Amabye et al. [19] and Amal and Nashwa [24] with some minor variations in the presence of Tannins and Saponins.

Table 2: Phytochemical Constituents of *Moringa oleifera* Aqueous, Ethanolic and N-hexane Leaf Extracts.

Phytoconstituents	Aqueous Extracts	Ethanolic Extracts	N-hexane Extracts
Tarpenes	-	+	**+**
Phenols	-	-	**-**
Tannins	+	+	**+**
Flavonoids	+	+	**-**
Alkaloids	+	+	**-**
Saponins	+	+	**-**
Tarpenoids	-	-	**+**

Keys: (+) = Positive, (-) = Negative

In the aqueous extract, *S. aureus* has the highest zone of inhibition with a mean activity of 11.50±0.70mm followed by *P. aeruginosa* and *E. coli* having (8.50±0.70mm) and (7.50±0.70mm), all at 100mg/ml concentration respectively, as presented in (Table 3).

Table 3: Zones of inhibition in (mm) for different concentrations of aqueous extracts of *Moringa oleifera* leaves.

		Extracts Concentration in mg/ml				
S/N	**Test isolates**	**20**	**40**	**60**	**80**	**100**
1.	*E. coli*	5.00±0.00	5.00±0.00	5.50±0.70	6.00±0.00	7.50±0.70
2.	*P. aeruginosa*	5.00±0.00	5.500.70	5.50±0.70	6.50±0.70	8.50±0.70
3.	*S. aureus*	5.00±0.00	5.50±0.70	6.00±1.41	7.00±1.73	11.50±0.70

Values represented in the table are means of the duplicate readings and standard error of the zone of inhibition measured in millimetre as analysed using SPSS software version 16.

In the ethanol extract *S. aureus* has the highest inhibition with a mean activity of (12.00±1.41mm), followed by *E. coli* (10.00±1.4mm) and *P. aeruginosa* (8-00±1.21mm) at 100g/ml (Table 4). While the N-hexane extract, exerts no any activity against all the isolates in all concentrations.

All the extract showed highest zones of inhibition at 100mg/ml concentration. The results of these findings is similar with the findings of Kiran and Tafida, [18] who reported a significant activity of the leaves extracts against *P. aeruginosa* and *E. coli.* However, a slight variation was observed when compared with the findings of Amabye and Tadesse, [19] who reported a lower inhibitory activity of the leaves extract on *E. coli, S. aureus* and *P. aeruginosa.*

However, *S. aureus* is more sensitive to both the aqueous and ethanol extract of *Moringa oleifera* leaves than *E. coli and P. aeruginosa*. The commercial antibiotics (Ofloxacin '5µg' and Ciprofloxacin '5µg') used on the test isolate were more effective than the *Moringa oleifera* plant extract, with Ofloxacin having the highest activity on all the test isolates. Oflatoxicin is reported to be effective against *P. aeruginosa* and other Gram negative bacteria and it has low level of bacterial resistance recorded. [20, 21]

The antibacterial activity tests results indicate that ethanol extracts of the plant were more active than the aqueous extracts, while the N-hexane extract exert no any effect on the isolates (table 5). The activity exhibited by the extracts may be related to the presence of a number of Phytoconstituents, especially tannins in addition to flavonoids which were all present in the plant. In particular, flavonoids were reported to be responsible for antibacterial activity associated with some ethno medicinal plants. [7] Phytoconstituents are reported to be responsible for antimicrobial properties of some ethno-medicinal plants and these Phytoconstituents varies with the solvent of extraction and method used. [3, 22]

Table 4: Zones of inhibition in (mm) for different concentrations of ethanol extracts of *Moringa oleifera* leaves.

		Extracts Concentration in mg/ml				
S/N	**Test isolates**	**20**	**40**	**60**	**80**	**100**

1.	*E. coli*	5.00±0.00	5.00±00	5.50±00	8.00±1.41	10.00±1.41
2.	*P. aeruginosa*	5.00±0.00	5.25±0.50	5.75±0.95	6.00±1.73	8.00±1.21
3.	*S. aureus*	5.00±0.00	5.55±1.00	6.00±1.73	10.33±1.15	12.00±1.41

Values represented in the table are means of the duplicate readings and standard error of the zone of inhibition measured in millimetre, as analysed using SPSS software version 16.

Table 5: Zones of inhibition in (mm) for different concentrations of N-hexane extracts of *Moringa oleifera* leaves.

	Extracts Concentration in mg/ml					
S/N	**Test isolates**	**100**	**80**	**60**	**40**	**20**
1.	*E. coli*	0.00	0.00	0.00	0.00	0.00
2.	*P. aeruginosa*	0.00	0.00	0.00	0.00	0.00
3.	*S. aureus*	0.00	0.00	0.00	0.00	0.00

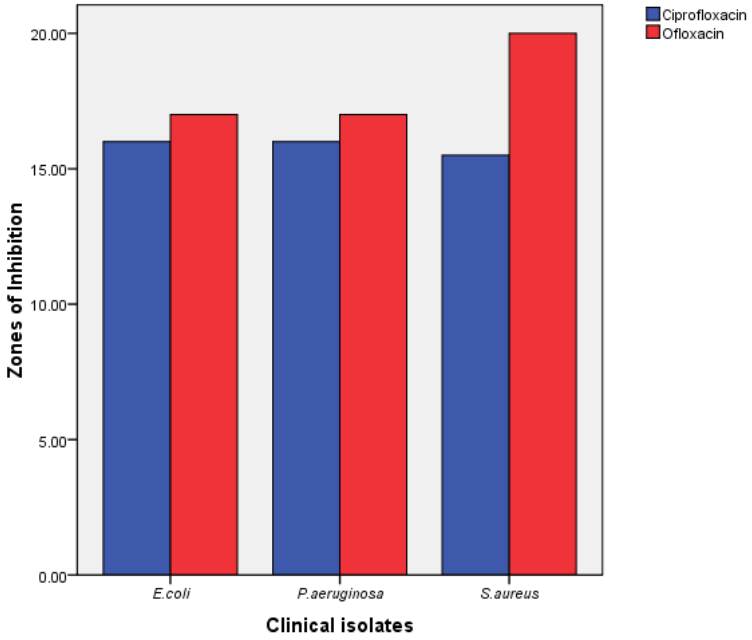

Figure 1: Ranges of the Zones of inhibition of standard commercially sold antibiotics used against the test isolates.

The MIC for the aqueous extract are at a concentrations of 60mg/ml for *S. aureus*, 80mg/ml for *P. aeruginosa* and 80mg/ml for *E. coli*. While that of the ethanol extract are at a concentration of 60mg/ml for *S. aureus,* 80mg/ml for *E. coli* and 100mg/ml for *P. aeruginosa*.

Table 6 present the results of these findings. This is similar with the findings of Kiran and Tafida.[18] who reported a higher activity among the ethanol and aqueous Moringa leaves extracts.

The MBC for the aqueous Extract is at a concentration of 80mg/ml for *S. aureus,* 100mg/ml for *P. aeruginosa* and 100mg/ml for *E. coli*, while that of the ethanol extract are at a concentration of 80mg/ml for *S. aureus* and 100mg/ml for *E. coli*), while no any MBC was recorded for *P. aeruginosa*. Table 7 presents these findings.

The MIC and MBC results obtained in this study, varies with the findings of Gustavo *et al.,* [23] and Pal *et al.* [22] who reported a lower value. However, the disparity may be due to the variation in the plant Phytoconstituents recorded in this study. Phytoconstituents of a plant, varies with the geographical location in which the plant is been collected. [3] The MIC results recorded in this study, varies with the findings of Amal and Nashwa. [24] who reported a lower value of 10mg/L^{-1} with an MBC value of 30 mg/L^{-1} mg.

Table 6: Minimum Inhibitory concentration (MIC) of *Moringa oleifera* Aqueous and Ethanolic Extract Against the Test isolates.

	Extracts Concentration in mg/ml					
Extracts	**Test isolates**	**100**	**80**	**60**	**40**	**20**
	E.coli	_	_	+	+	+
Aqueous	*P. aeruginosa*	_	_	+	+	+
	S. aureus	_	_	_	+	+
Ethanolic	*E. coli*	_	_	+	+	+
	P. aeruginosa	_	+	+	+	+
	S. aureus	_	_	_	+	+

Keys: (+) = Indicate Turbid (growth). (-) = Indicate not Turbid (no growth).

Table 7: Minimum Bactericidal Concentration (MBC) of *Moringa oleifera* Aqueous and Ethanolic Extract Against the Test isolates.

	Extracts Concentrations in mg/ml						
Extracts	**Test isolates**	**100**	**80**	**60**	**40**	**20**	**MBC**
	E.coli	_	+	+	+	+	100
Aqueous	*P.aeruginosa*	_	+	+	+	+	100
	S.aureus	_	_	+	+	+	80
Ethanolic	*E.coli*	_	+	+	+	+	100
	P.aeruginosa	+	+	+	+	+	Nil
	S.aureus	_	_	+	+	+	80

Keys: (+) = Indicate growth. (-) = no growth

4.0 CONCLUSION

The results of this study indicated that, *S. aureus* is more sensitive to both the aqueous and ethanol extract of *Moringa oleifera* leave than the other isolates while N-hexane extract exerts no antibacterial property against the tested isolates. Of all the extracts, the ethanol extracts possess more inhibitory effect on the test isolates. The commercial antibiotics (Ofloxacin '5µg' and Ciprofloxacin '5µg') used on the test isolate were more effective than the *Moringa oleifera* leaves

extract, with Ofloxacin having the highest activity on all the test isolates. The inactivity of the N-hexane extracts could probably be due to their difference in polarity, which results in poor extraction of the Phytoconstituents, which is directly responsible for the plant bioactivity. However, these extract could be a promising reservoir for antibacterial agents with potential application in treating bacterial infections.

REFERENCE

1. Adriana, B., Almodovarl, A.N.M., Pereiral,C.T and Mariangela, T. (2007). Antimicrobial efficacy of curcuma zedoaria extract as assessed by linear regression compared with commercial mouth rinses. *Braz. J. microbiol*. 38:440-445'
2. Farooq, F., Rai, M., Tiwari, V., Khan, A.A and Farooq, S (2012). Medicinal properties of *Moringa oleifera* : An overview of promising healer. *Journal of medicinal plants research*, 6(27):4368-4374.
3. Abdulfatai, K., Abdullahi, B., Jaafaru, I.A. and Rabiu, I. (2018). Antibacterial activity of pigeon pea (*Cajanus cajan)* leaf extracts on *Salmonella* and *Shigella* Species Isolated from stool sample in patients attending barau dikko peadiatric unit kaduna. *European Journal of Biotechnology and Bioscience*, 6, (3); 01-08.
4. Guevara, A.P., Vargas, C., Sakurai, H., Fujiwara, Y., Hashimoto, K., Maoka, T., Kozuka, M., Ito, Y., Tokuda, H and Nishino, H. (2007). An anti-tumor promoter from *Moringa oleifera* lam. *Mutation Research*, 440: 181-188.
5. Lakshmipriya, G., and Kruthi, D. and Devarai, S. K. (2016). *Moringa oleifera*: A review on nutritive importance and its medicinal application. *Food science and Human Wellness,* 5:49-56
6. Talhaliani, P. and Kar, A (2000). Antimicrobial activity of *Moringa oleifera* leaves. *Pharmacological Research*, 41(3):319-323
7. Anwar, F and Rashid, U. (2007). Physio-chemical characteristics of *Moringa oleifera* seeds and seed oil from a wild provenance of Pakistan. *Journal of Botany.* 39(5): 1443-1453.
8. Jamil, A. M., Shahid, M. M., Khan, M and Ashraf, M (2007). Screening of some medicinal plants for isolation of antifungal proteins and peptides. *Pakistan Journal of Botany*. 39(1)211-221.
9. Yusha'u, M., Olonitola, S. O., and Aliyu, B. S. (2007). Prevalence of Extended – Spectrum Beta lactamases (ESBLs) Among members of the Enterobacteriaceae isolates obtained from Mohammed Abdullahi Wase Specialist Hospital, Kano, Nigeria. *International Journal of Pure and Applied Sciences* 1 (3): 42 – 48
10. Sofowara, A., (1986). The state of medicinal plants and research in Nigeria, pp 243-247.
11. Mohammed, G. R. (2018). Extraction, Isolation and Characterization of Natural Products from Medicinal Plants. *International Journal of Basic Sciences and Applied Computing*, 2 (6), 245-251
12. Krishnapriya, T. V. and Suganthi, A. (2017). Biochemical and Phytochemical Analysis of *Colocasia esculenta* (L.) Schott tubers. *International Journal of Research in Pharmacy and Pharmaceutical Sciences,* 2 (3), 21-25
13. Mohammed, A. H., Na'inna, S. Z., Yusha'u, M., Salisu, B., Adamu, U. and Kabeer, Z. M. (2017). Antibacterial, Cytotoxicity and GC-MS Analysis of Extracts. *Bayero Journal of Pure and Applied Sciences,* 10(1): 163 - 169

14. Magashi, A. M. and Abdulmalik, U. (2018). Antibacterial Activity and Phytochemical Screening of Stem Bark Extracts of *Adansonia digitata* on Some Clinical Isolates. *UMYUK Journal of Medical Research*, 3(1).
15. Cheesbrough (2006a). Directions in the study of antimicrobial therapeutics challenges for the development of new antibiotics rethinking the approaches. Natural academics press. NBK19843.
16. National Committee for Clinical Laboratory Standards (NCCLS) (1999): Performance standard for antimicrobial susceptibility testing. NCCL approved standard M100-59.
17. Kawo, A. H., Suleiman, Z. A. and Yusha'u, M. (2011). Studies on the Antibacterial Activity and Chemical Constituents of *Khaya senegalensis* and *Ximenia americana* Leaf Extracts. *African Journal of Microbiology Research*, 5 (26), 4562-4568
18. Kiran, S. and Tafida, G. M. (2013). Antibacterial activity of Moringa oleifera (Lam) leaves extracts against some selected bacteria. *International Journal of Pharmaceutical Science*, 6,52-54
19. Amabye, T. G. and Tadesse, F. M. (2016). Phytochemical and Antibacterial activity of *Moringa oleifera* available in the market of Makelle. *J. Anal Pharm Res,* 2(1):00011
20. National Committee for Clinical Laboratory Standards (NCCLS) (2003). Antimicrobial susceptibility Standards (ATS), ed. 2003, for M7 (CMI)si M100 standard for antimicrobial susceptibility testing. NCCL approved standard M100-59.
21. Pintilie, L. (2012). Quinolones: Synthesis and antibacterial activity. *Antimicrobial agents*,12;219
22. Pal, S.K., Mukherjee, P.K., Saha, K., Pal, M and Saha, B.P (1995). Antimicrobial action of the leaf extract of *Moringa oleifera* lam. *Anc Sci Life*. 14:197-199.
23. Gustavo, H. F., Vieira, J.A., Mourao, Angelo, A.M., Costa, R.A and Vieira, R.H.S. (2010). Antibacterial effect (in-vitro) of *Moringa oleifera* and *Annona muricata* Against Gram positive and Gram negative bacteria. *Revista Institude De Medical Tropicale De Sao Paulo*, 52(3):129-132.
24. Amal, S. O. and Nashwa, A. A. (2017). Antibacterial Effect of the Ethanol Leaves Extract of *Moringa oleifera* and *Camellia sinensis* Against Multi Drug Resistant Bacteria. *International Journal of Pharmacology*, 13(2),156-165