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Antibacterial and Antifungal Activity of Different Extract of *Moringa oleifera* Leaves – An In-Vitro Study

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Abstract

Moringa oleifera is a very useful tree in tropical countries; use to treat different human health problems. It had wide range of antimicrobial properties which have been investigated by a number of studies.

water, ethanolic, methanolic and petroleum ether of *Moringa oleifera* leaf extract were screened for in vitro antibacterial and antifungal activity against selected common human pathogens in Sudan by disc diffusion method.

The extracts showed significant effect on the tested organisms. Water extract showed maximum zone of inhibition against *Staphylococcus albus*, methanolic extract was only extract showed activity against *Pseudomonas aeruginosa* , ethanolic extract showed maximum inhibition against *Salmonella spp* and *Yersinia enterocolitica* . Petroleum ether extract was the lowest extract showed activity against tested.

Key words: *Moringa oleifera*, Antibacterial activity, Antifungal activity, leaves extracts, Disc diffusion method.

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Introduction

Traditional medicines become a main source of primary health to majority of population in most developing country, especially in Africa as result of cost effectiveness and viability of antibiotic in addition of antibiotic resistance and their side effect. (Diallo et al. 1999)

Effort for looking to plant as source of facing antimicrobial resistance had done and about 20% of the plants in the world test to their pharmacological or biological effect, result lead to new natural or semi-synthetic antimicrobial drug. (Mothana and Lindequist, 2005)

Moringa oleifera, miracle tree, drumstick tree, horseradish tree and other names all refer to one species of 14 from family of Moringaceae. Sudan, tropics and subtropics Africa, India, Pakistan, Bangladesh, Afghanistan, South America, and different other place are native place of it. (Fahey, 2005)

Various parts of this Moringa oleifera use to treat different human health problems, Scientists worldwide investigates medically important of Moringa oleifera, they reported different benefits of it include: antifertility property, (Shukla et al. 1998) cyanobacteriacidal activity, (Lurling and Beekman, 2010) hypolipidaemic and antiathersclerotic activities. (Pilaiprk et al. 2008) Cardiac and circulatory stimulants, possess anti tumor, (Guevara et al. 1999) antipyretic, antiepileptic, anti inflammatory, antiulcer, (Pal et al. 1995) antispasmodic, diuretic, (Caceres et al. 1992; Morton, 1991) antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities, (Nikkon et al. 2003) and are being employed for the treatment of different ailments in the indigenous system of medicine. (Mughal et al. 1999)

Plants represent the cheapest and safer alternative sources of antimicrobials, Moringa oleifera have wide range of antimicrobial properties which have been investigated by a number of studies, using different part and different way of extraction. (Adriana et al. 2007; Pretorius and Watt 2007)

The present study was an attempt to evaluate in vitro antimicrobial activity of Moringa oleifera against common pathogenic bacterial and fungal infection in Sudan.

Materials & Methods

Plant Material

Plants were collected between the month of January and March 2013 in the Khartoum state, Sudan.

Leaves extract

Extraction was carried out according to method described by (Harborne, 1984): 75 g *Moringa olifera* leaves was successively extracted with petroleum ether and methanol using soxhelt extractor apparatus. Extraction carried out for about four hours for petroleum ether and eight hours for methanol. Solvents were evaporated under reduced pressure using rotary evaporator equipments. Finally extracts allowed to air in Petri dishes till complete dryness and the yield percentages were calculated as: Weight of extract obtained / weight of plant sample * 100.

Preparation of the aqueous extract

obtained from the above extraction was sacked in 100 ml hot distilled water, and left till cooled down with continuous stirring at room temperature; Extract was then filtered through cotton and stored in a refrigerator till used.

Preparation of the ethanol extract

Here, also the same procedure was followed as in cold water treatment.

Test organisms

Tested bacterial and fungal were isolated from different clinical specimens, samples were isolated and identify according to standard laboratory methods. (Cheesbrough, 2000) Isolated bacteria include: Gram negative bacteria (*Providencia spp, Pseudomonas aeruginosa, Yersinia enterocolitica, Salmonella spp, Escherichia coli, Shigella spp, and Klebsiella pneumoniae*) Gram positive bacteria (*Enterococcus faecalis, Staphylococcus auras, Staphylococcus albus*). Isolated fungi include: *Candida Albican, Aspergillus flavus* and *Aspergillus Niger*

Antibacterial sensitivity testing

Antibacterial susceptibility testing of antibiotics was performed by disc diffusion method. (Cheesbrough, 2000) For susceptibility testing, a suspension from one-day-old bacterial cells of each isolate was prepared agar broth (2 ml) equivalent to the McFarland turbidity standard; the suspensions were spread into the surface of the Mueller Hinton agar with sterile cotton swabs. The plates were briefly dried and then the antibiotic disks of Moringa were added to each plate and incubate over night at 37 ºC. The inhibition zone diameters measured in millimeters, with a ruler. Resistance determined by a zone of growth inhibition diameters. Greater zones of complete growth inhibition indicated the presence of susceptible strains. The procedure repeated for cultures that were defined as resistant.

Antifungal sensitivity testing

For this purpose, disk diffusion method was used. Stored isolates of fungi were regrowing on Sabouraud agar. A suspension from 3-day-old fungi cells of each isolate was prepared agar broth (2 ml) equivalent to the McFarland turbidity standard. The suspensions were spread into the surface of the Mueller Hinton agar with sterile cotton swabs. The plates were briefly dried and then the antibiotic disks of Moringa distributed to each plate and incubate microaerobically at 37 ºC for 3-5 days. One plate without Moringa was used as a control.

The inhibition zone diameters measured by millimeters, with a ruler. Resistance determined by a zone of growth inhibition diameters. Greater zones of complete growth inhibition indicated the presence of susceptible strains. The procedure repeated for cultures that were defined as resistant.

Result

Moringa oleifera leaves, water extract exhibit variable activity against bacteria *Staphylococcus albus, Escherichia coli and Shigella spp* show highest zone of inhibition (3, 2, and 1.5 mm) respectively. Providencia spp, *Klebsiella pneumoniae* and *Staphylococcus auras* show same zone of inhibition (1 mm). Other bacteria show no zone of inhibition. (figure. 1)

Methanol leave extraction showed remarkable result against *Enterococcus faecalis, Yersinia enterocolitica, Providencia spp* with (3.5mm, 2.5mm, 2.25mm) respectively, gram positive cocci *Staphylococcus auras, Staphylococcus albus,* showed almost same result with (1.25mm, 1mm). Methanol extraction considered the only extraction give result with *Pseudomonas aeruginosa* (1mm) (Figure. 2)

Susceptible bacteria to ethanol extraction showed almost same result, *Salmonella spp, Yersinia enterocolitica, Escherichia coli, Enterococcus faecalis, Providencia spp* with (1.5mm, 1.5mm, 1.25mm, 1.25mm, 1mm) respectively (figure. 3)

Only four bacteria showed result with petroleum ether which include, *Providencia spp, Yersinia enterocolitica, Enterococcus faecalis, Escherichia coli* (2.5mm, 1.25mm, 1.125mm, 1mm) respectively. (figure. 4)

Our study showed that methanol extraction was the only effective type to *Candida Albican* with (4mm) zone of inhibition. Ethanol extraction was the only extract showed activity to *A. flavus* (4mm) while *A. Niger* resisted to all Moringa oleifera leaves extracts (Figure. 5).

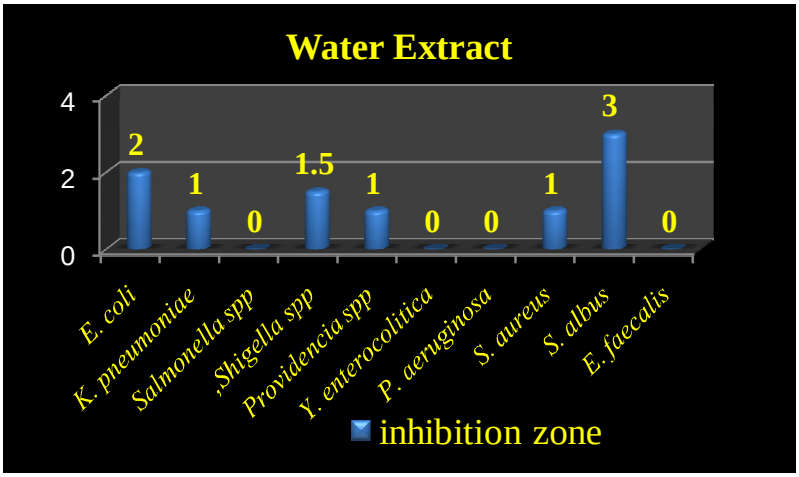

Figure. 1: Antimicrobial activity of Moringa oleifera leaves water extract against human pathogens.

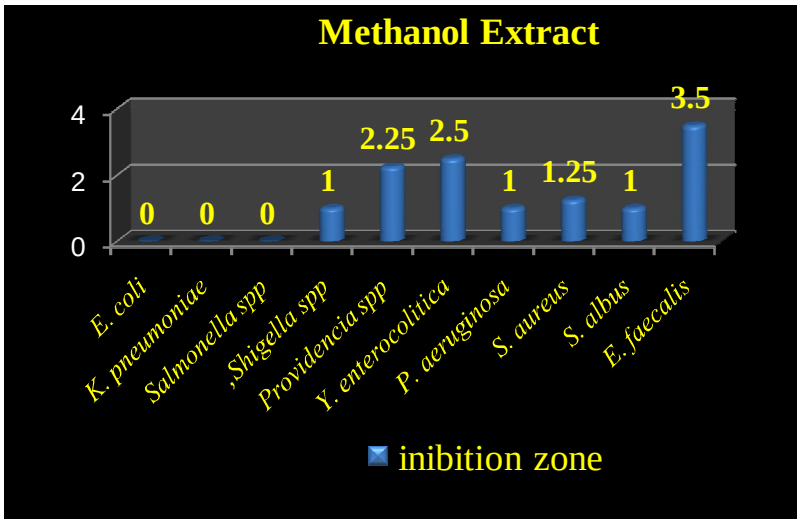

Figure. 2: Antimicrobial activity of Moringa oleifera leaves methanol extract against human pathogens.

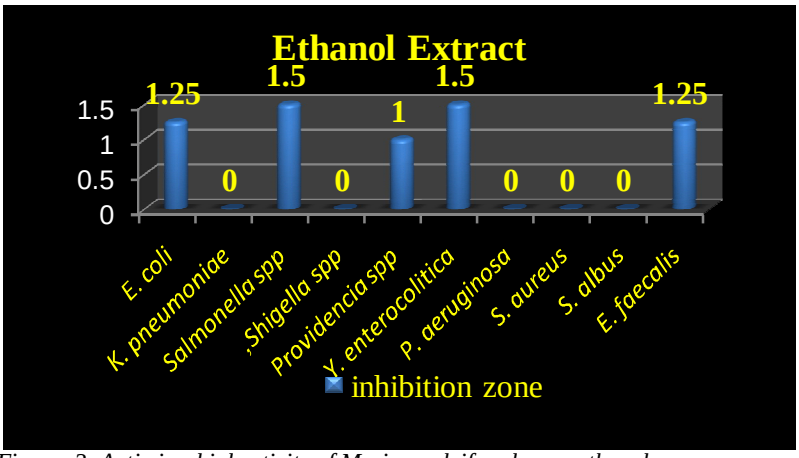

Figure. 3: Antimicrobial activity of Moringa oleifera leaves ethanol

Extract against human pathogens.

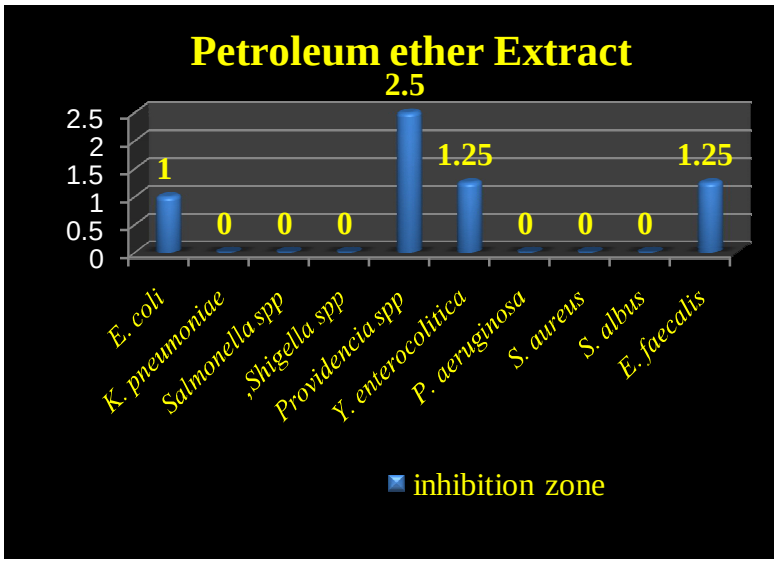

Figure. 4: Antimicrobial activity of Moringa oleifera leaves petroleum ether extract against human pathogens.

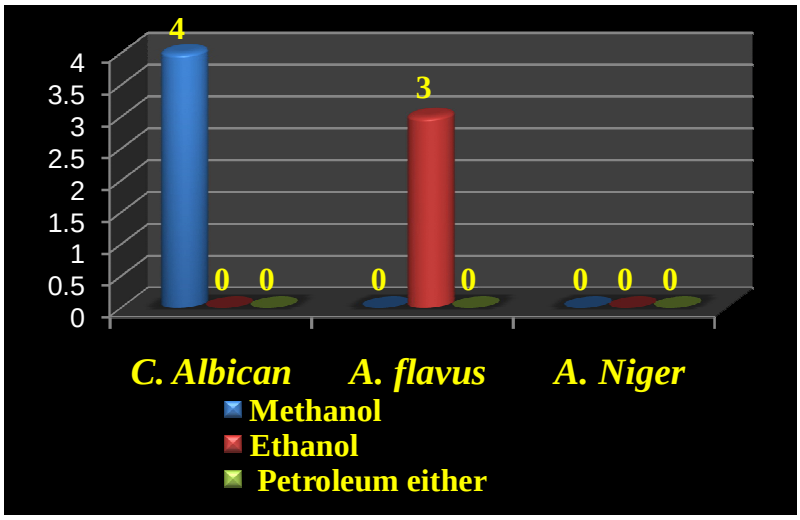

Figure. 5: Antifungal activity of Moringa oleifera leaves extracts against tested fungal

Discussion

The present study was conducted to obtain preliminary information on the antibacterial activity of, water, ethanol, methanol and petroleum ether leave extracts of Moringa oleifera Lam in Sudan. In our investigation, different zones of inhibition were found in extracts from Leaf against all the tested bacteria. Water extracts exhibit variable activity against bacteria; some bacteria like Staphylococcus albus and *Escherichia coli showed* high zone of inhibition and some tested bacteria showed resistance to the Moringa leave water extract.

Various researchers reported antimicrobial activity of Moringa oleifera leave water extract against variety of pathogens, some of them in agree with our result and some had little different due to a variety of bacterial gene that lead bacteria to be resistance to antimicrobial. Similarly to (Priya et al., 2011) which evaluated the antibacterial activity in the aqueous leaf extracts of Moringa against pathogenic bacteria like *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus and Shigella spp.*

(Thilza et al, 2010) evaluated the in vitro antimicrobial activity of *Moringa oleifera* leave extracts against Escherichia coli, *Staphylococcus aureus, Staphylococcus albus, Pseudomonas aeruginosa* and they found that only *Escherichia coli* among tested bacteria showed inhibition zone, their result in correlation with the finding.

(Anthonia, 2010) performed antibacterial activity of Moringa oleifera leaf in South-Western Nigeria and they found that aqueous extract had inhabitation zone different pathogen include *Escherichia coli, Klebsiella pneumoniae, Providencia stuartii , Yesinia enterocolitica*. Locally isolated organism like *Salmonella, Staphylococcus aureus, Enterococcus faecalis showed inhibition zone less than one mm while Pseudomonas aerogenosa* resist to *Moringa oleifera* leaf aqueous extract.

(Vinoth et al, 2012) screened Moringa leave water extract for antibacterial activity, *Staphylococcus aureus* only tested bacteria showed sensitivity while Pseudomonas aerogenosa, *Escherichia coli and Salmonella typhii* no activity was detected.

Maximum zone of inhibition of methanol extract was detected in *Enterococcus faecalis, Yersinia enterocolitica, Providencia spp,* while no activity was founded for *Escherichia coli, Klebsiella pneumonia and salmonella spp*. In addition, it was observed that methanol was the only extracts of Moringa oleifera showed activity against Pseudomonas aerogenosa. These results corroborate by (Patil and Jane, 2013). Further, our results do not match with Priya and his colleagues, which they observed that methanol extracts of Moringa oleifera leaves was founded to had antibacterial activity against *Escherichia coli and Klebsiella pneumoniae*. (Priya et al, 2011)

Petroleum ether was the lowest leave extracts activity against tested bacteria; it had activity to only five bacteria, *Providencia spp, Yesinia enterocolitica, Enterococcus faecalis, and Escherichia coli.* The inactivity of petroleum ether extract may be due to active compound which posses the antimicrobial properties are polar in nature and not possibly extracted by petroleum ether. (Saadabi et al, 2011)

(Priya et al, 2011) also reported that Petroleum ether leave extracts showed moderate inhibition against *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus and Shigella dysentriae.Salmonella spp and Yersinia enterocolitica* showed highest zone of inhibition with Ethanol extract, *Escherichia coli, Enterococcus faecalis, Providencia spp* showed considerable activity, rest of tested bacteria showed no result. (Mashiar et al,, 2009) pointed out that, ethanol extracts of fresh leaves were noticed to be more susceptible to *S. shinga, P. aeruginosa, S. sonnei, Pseudomonas spp*. Compare to this result, our founding shows a stronger activity to wide range of tested bacteria. Similarly to our result, (Vinoth et al, 2012) Investigated the antibacterial activity in the ethanolic leaf extracts of Moringa against pathogenic bacteria, *Salmonella typhii* showed maximum zone of inhibition against while less inhabitation zone measured with *Escherichia Coli*.

The increase in the incidence of fungal infections and the frequent report of resistance and therapeutic failure has promoted the performance of herbal screening for compounds with antifungal properties. *Candida Albican, Aspergillus flavus and Aspergillus Niger* were the most common fungal problems among different age group now a day, responsible for various non life-threatening infections, such as oral thrush, vaginitis, serious lung disease, aspergillosis, common pathogen of cereal grains and legumes.(Jonathan et al., 2012) assessed the antifungal activity of methanol and ethanol extract of Moringa oleifera leave, reported that, *Aspergillus flavus* had highest inhibition zone (30mm) to methanol, *Candida Albican* (5mm) while *Aspergillus Niger* had no zone of inhibition. Ethanol extract showed variable result with (25, 10 and 15mm) to *Candida Albican, Aspergillus Niger and Aspergillus flavus* respectively and this is in correlation with our finding.

Conclusion

Moringa oleifera Lam., an important medicinal plant, is one of the most widely cultivated species of the family Moringaceae. Pharmacologically reported that Different parts of it have been used for different human ailments, extracts showed varying degrees of antimicrobial and antifungal activity on the microorganism tested. Further work is needed to carry out more pharmacological from the extracts in order to support antimicrobial activity of the M. oleifera. Our study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms.

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Reference

- Adriana, B., A.N.M. Almodóvar1, C.T. Pereira1, and T.A. Mariângela. (2007).Antimicrobial efficacy of Curcuma zedoaria extract as assessed by linear regression compared with commercial mouthrinses. Braz. J. Microbiol. 38:440-445.
- Anthonia Olufunke Oluduro. (2011). Evaluation of Antimicrobial properties and nutritional potentials of Moringa oleifera Lam. leaf in South-Western Nigeria. Malaysian Journal of Microbiology, Vol 8(2) 2012, pp. 59-67
- Caceres A, Saravia A, Rizzo S, Zabala L, Leon ED, Nave F. (1992). Pharmacologic properties of Moringa oleifera: 2: Screening for antispasmodic, anti-inflammatory and diuretic activity. J Ethnopharmacol; 36:233–237.
- Cheesbrough, M. (2000). District Laboratory Practice Manual in Tropical Countries Part 2. Cambridge University Press, Cambridge, 136-137. 158,165, 180.
- Diallo D, Hveem B, Mahmoud MA, Betge G, Paulsen BS, Maiga A. (1999). An ethnobotanical survey of herbal drugs of Gourma district, Mali. Pharmaceutical Biol.. 37:80–91.
- Fahey, J.W. (2005). Moringa oleifera: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Trees for Life Journal.1:5.
- Guevara AP, Vargas C, Sakurai H. (1999). An antitumor promoter from Moringa oleifera Lam. Mutat Res; 440: 181–188.
- Harborne JB, (1984). Phytochemical methods, 2nd edition, Chapman and Hall publications, London, NewYork, pp. 288.
- Jonathan, S. G. Olawuyi., O. J. Aina, D. A. Odeniyi, S. O. Adediji, I. O. and Ikhedia, A. (2012). Comparative studies on antifungal, anti-oxidant and phytochemical potential of Momordica charantia and Moringa oleifera. New York Science Journal; 5(12)
- Lurling M and Beekman W. (2010). Anti-cyanobacterial activity of Moringa oleifera seeds. Journal Appl Phycol; 22(4):503-510.
- Mashiar Rahman. M, M. Mominu Islam Sheikh, Shamima Akhtar Sharmin, M. Soriful Islam, M. Atikur Rahman, M. Mizanur Rahmanand M. F. Alam. (2009). Antibacterial Activity of Leaf Juice and Extracts of Moringa oleifera Lam. against Some Human Pathogenic Bacteria. CMU. J. Nat. Sci. Vol. 8(2)
- Morton JF. (1991).The horseradish tree, Moringa pterygosperma [Moringaceae], A boon to arid lands. Econ Bo; 45:318–333.

- Mothana RA and Lindequist U. (2005). Antimicrobial activity of some medicinal plants of the island Soqotra. J. Ethnopharmacol, 96(1-2): 177-181.
- Mughal MH, Ali G, Srivastava PS, Iqbal M. (1999). Improvement of drumstick [Moringa pterygosperma Gaertn.] – a unique source of food and medicine through tissue culture. Hamdard Med; 42:37–42.
- Nikkon F, Saud ZA, Rehman MH, Haque ME. (2003). In vitro antimicrobial activity of the compound isolated from chloroform extract of Moringa oleifera Lam. Pak J Biol Sci; 22:1888–1890.
- Pal SK, Mukherjee PK, Saha BP. (1995). Studies on the antiulcer activity of Moringa oleifera leaf extract on gastric ulcer models in rats. Phytother Res; 9:463–465.
- Patil SD and Jane Rasika. (2013). Antimicrobial activity of Moringa oleifera and its synergism with Cleome viscose. Int. J. of Life Sciences, 2013, Vol. 1(3): 182-189
- Pilaiprk C, Panya K, Yupin S, Srichan P, Morales NP, Laddawal P et al. (2008). The In vitro and ex vivo antioxidant properties, hypolipidemic and antiatheroscleortic activities of water extract of Moringa oleifera (Lam.) Leaves. Journal of Ethanopharmacology; 116:439-446.
- Pretorius, C.J., and E. Watt. (2001). Purification and identification of active components of Carpobrotus edulis L. J. Ethnopharmarcol. 76:87-91.
- • Priya. V, P. Abiramasundari, S. Gayathri Devi and G.P. Jeyanthi. (2011). Antibacterial Activity of the Leaves, Bark, Seed and Flesh of Moringa Oleifera. Vol. 2(8): 2045-2049, ISSN: 0975-8232
- Saadabi Abdulmoneim M and Zaid IE Abu. (2011). An In vitro antimicrobial activity of Moringa oleifera L. seed extracts against different groups of microorganisms. Aust. J. Basic & Appl. Sci., 5(5): 129-134,
- Shukla S, Matur R, Prakash AO. (1998) Antifertility profile of the aqueous extract of Moringa oleifera roots. Journal of Ethnopharmacology; 22(1): 51-62.
- Thilza I.B, Sanni S, Zakari Adamu Isah, F.S. Sanni , Muhammed Talle and Musa Bamaiyi Joseph. (2010). In vitro Antimicrobial activity of water extract of Moringa oleifera leaf stalk on bacteria normally implicated in eye diseases Nigeria Academia Arena, 2010;2(6):80-82] (ISSN 1553-992X).
- Vinoth. B, R.Manivasagaperumal and S.Balamurugan. (2012). phytochemical analysis and antibacterial activity of Moringa oleifera lam, India. International journal or research in biological sciences 2012;2(3):98-102.