International Journal of Academic Health and Medical Research (IJAHMR)
ISSN: 2643-9824
Vol. 4 Issue 1, January – 2020, Pages: 1-5

Antibacterial Activity of Ethanol and Aqueous Extracts of *Moringa Oleifera* Lam. against Some Human Pathogenic Bacteria (*In Vitro* Study)

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Abstract: *Medical plants are considered one of the most important sources of food and treatment worldwide and especially in Sudan. Sudanese were using medical and aromatic plants - including Moringa - for the treatment of many diseases. On the other hand, scientists and health care providers are facing the continuous problem of bacterial antibiotic resistance, which has become a great challenge in eradication of infectious diseases. This study was conducted in Wad Medani College of medical sciences and technology (MST) in the period from Augusts to November 2014, to evaluate the antibacterial activity of Moringa oleifera leaves extract against Gram positive and Gram negative bacteria. The organisms under study include: staphylococcus aureus, streptococcus pyogen, Salmonella typhii, Escherichia coli and pseudomonas aeroginosa. Extraction of active substances from moringa leaves was done by using two extraction methods, aqueous extract and ethanol extract. The antimicrobial susceptibility tests were performed according to the standard procedures. The results of this study revealed that the aqueous moringa extract mean zone of inhibition was 29 mm for staphylococcus aureus, 25 mm for streptococcus pyogen, 30 mm for pseudomonas aeroginosa. 22 mm for Escherichia coli and 32 mm for Salmonella typhii, And the Ethanolic moringa extract mean zone of inhibition was 31 mm, 30 mm, 29 mm, 25 mm and 31 mm for the previous bacteria respectively. This study verifies that Moringa oleifera leaves extract has an (in vitro) antibacterial activity against the organisms under studding. Clinical trials (in-vivo) are needed to test the activity of Moringa oleifera against bacterial infections.*

Keywords (Moringa oleifera, folk-medicine, Well diffusion, McFarland stander, Sudan)

Introduction:

Plants have been an important source of natural materials that used in protecting of human health. (Jackson Rafael *et al., 2011*). The antimicrobial action of plants has been studied through hundreds of researches that demonstrate the high degree of usefulness and effectiveness of substance which obtained from plants in treatment of pathogenic microorganisms, According to world health organization, more than 80% of the world's population depends on traditional medicine for their primary health care needs. (M. Mashiar Rahman. *et al.,* 2009). The frequency of infections caused by pathogenic microorganisms has increased worldwide and become an important cause of mortality and morbidity especially in developing countries (Jawetz *et al.,* 2007). The uncontrolled administration of antimicrobial agents lead to the appearance of resistant strains of microorganism and reduce the susceptibility to antibiotic, which require from the scientist and researches centers to develop a new methods for infection enucleating (Rogas *et al.,* 2006). Antimicrobial resistance has become a global issue, thus the importance of complementary medicine was increased in the last years due to the continuous emersion of resistant strains of pathogens and side effect of chemical antimicrobial agents. (Vikash Kumar *et al.,* 2012). *Moringa oliefera* is a medicinal species belonging to the monogeneric family moringaceae , (order Brassicales) .It has 33 species of trees and distributed in sub-Himalayan ranges of India, Sri Lanka , North Eastern and South Western Africa and Madagascar (Shaficur Rahman *et al,*. 2008). Today, it has become cultivated in many regions of the tropics and is widely used in Africa, Ceylon, Thailand, Burma, Singapore, West Indies, Srilanka, India, Mexico Malabar, Malaysia, the Philippine and Sudan. Different parts of this plant contain a group of precious, substance which is considered a good source of proteins and minerals (Mahajan SG *et al.,* 2007*)* The plant principally contains alkaloids, protein, vitamin, mineral, fixed oil, fatty acid and many carbohydrates, etc. (Santosh Kumar 2013). Root is used as stimulant in

intermittent fever, epilepsy and as carminative, diuretic, cardiac and circulatory tonic (Jackson Rafael *et al.,* 2011).

Study location

The study was done at Wad Medani College of medical sciences and technology (MST). Wad Medani, Sudan. Wad Medani is the capital of Gezira state, lies on the western bank of Blue Nile River about 85 Km from Khartoum, the capital of Sudan.

Study design

The prospective, laboratory-based observational study ran for 3 months from August to November 2014 aim to detect the antibacterial activity of *Moringa oliefera* against many pathogenic bacteria by using different extraction methods.

Specimen collection

Five samples were collected from Department of Microbiology in Faculty of Medical Laboratories Sciences, Gazira University Sudan.

Methods:

Preparation of culture media:

All culture media was prepared as instructed by the manufacturer. The pH of the medium was adjusted at **7.2- 7.4** and Sterilize by autoclave at **121°C** for **15** minutes, after cooling culture media was dispensed aseptically in 25 ml amounts in sterile Petri dishes. The media was tested by putting one agar plate in the incubator overnight and looking for growth.

The medium was given a patch number and the date of preparation was written for each patch. The plates were stored at 2–8°C, in sealed plastic bags to prevent loss of moisture. **(Cheesbrough M. 2006)**

Collection of plant material:

The plant was obtained from the local market and was identified in the Faculty of Pharmacy, University of Gazira.

3.7.3 Preparation of moringa extract:

Plant material (100 g) was crushed in sterile water (250 ml) for preparation of aqueous extract. The extract was separated using sterile muslin cloth and filters through sterile filter paper. And another (100 g) was ground into fine powder using a stainless-steel grinder, and dipped in100% ethanol (200 mL) for overnight for preparation of ethanol extract. The ethanol fraction was separated using sterile muslin cloth and filters through sterile filter paper. The filtered extract was concentrated and stored in the optimum conditions (Himal Pauel *et al.,* 2008).

Preparation of turbidity standard equivalent to (McFarland 0.5):

Barium sulfate was used to adjust the turbidity of the inoculums. It was prepared by adding 1% v/v solution of sulfuric acid (which was prepared by adding 1 ml of concentrated sulphuric acid to 99 ml of water) to 1.17% w/v solution of barium chloride (which was prepared by adding 2.35g of dehydrate barium chloride in 200 ml of DW). To prepare the turbidity standard, 0.5ml of barium chloride solution was added to 99.4ml of the sulfuric acid solution **(Mackie & McCartney, 1996).**

Sensitivity test: (Well diffusion method)

Colonies from bacterial was suspended in sterile normal saline (0.9%) and was adjusted to produce a suspension containing about 10^8 Colony Forming units (CFu) (compared with McFarland standard).

After preparation of Muller Hinton agar medium and before it be solid (45°C) 25 ml of medium was added to each plate, 200 µl of bacterial suspension then was filled in the agar plate (90 mm) and mixed gently. After complete solidification of media, five circular wells (10 mm in diameter) was cuts in the medium using sterile test tube and the agar discs was removed. By using an adjustable pipette, two wells was filled with 100 µl of aqueous , and ethanol extracts of *Moringa oleifera* , the third and fourth was filled with the control antibiotics (Cefuroxime and Chloramphenicol) and the last well was filled with DW (negative control). The plates was left in the room temperature for 40 minutes and then incubated in the upright position over night at 37°C in the incubator. (Emeruwa, 1982).

Data analysis

Data obtained from this study was analyzed by simple tables frequency using Excel software program

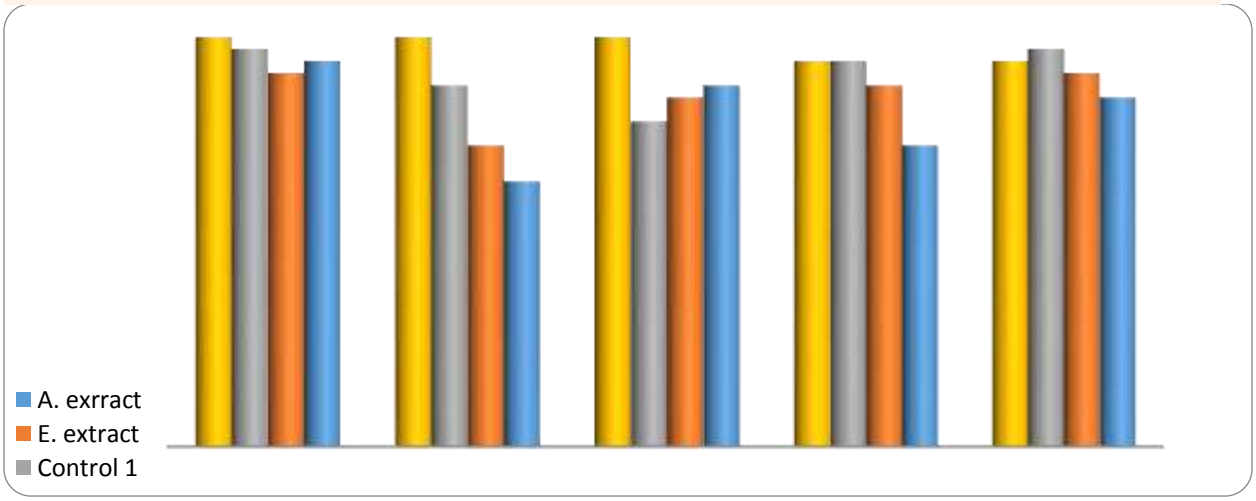
Results:

The antibacterial activity of ethanol and aqueous extracts of *Moringa oleifera* Lam was investigated using agar well diffusion method, against the selected human pathogens such as *Escherichia Coli, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhii and streptococcus pyogen*. All the examined extract showed varying degrees of antibacterial activities against the pathogens. The susceptibility tests were run **3** times to insure the accuracy and reliability of the results.

(Table 1) describe the mean of inhibition zones of (moringa aqueous and Ethanol extract Cefuroxime and Chloramphenicol) against the selected organisms.

(Figure 1) describe the mean of inhibition zones of (moringa aqueous and Ethanol extract Cefuroxime and Chloramphenicol) against the selected organisms.

Bacteria	Aqueous extract zone of inhibition (mm)	Ethanolic extract zone of inhibition (mm)	Cefuroxime zone of inhibition (mm)	Chloramphenicol zone of inhibition (mm)
Staphylococcus aureus	**29**	**31**	**33**	**32**
streptococcus pyogen	**25**	**30**	**32**	**32**
Pseudomonas aeroginosa	**30**	**29**	**27**	**34**
Escherichia Coli	**22**	**25**	**30**	**34**
Salmonella typhii	**32**	**31**	**33**	**34**



Discussions:

The present study was conducted to obtain prefatory information on the antibacterial activity of water and ethanol extracts of Moringa oleifera lam leaves in Sudan. The antimicrobial activity of the fruit extract of Moringa oleifera was investigated against five potentially pathogenic microorganisms Escherichia Coli, Pseudomonas aeroginosa, Staphylococcus aureus, Salmonella typhii and streptococcus pyogen using two methods of extraction. The agar well diffusion method was used in this study. The ethanol extract has greater antibacterial activity than water extracts. This result are agree with Nair et al., (2005) and it is interesting because in the traditional method of bacterial infection therapy, decoction of parts or boiling the plant in water is used wh

ereas, according to present study, preparing an extract with an organic solvent was shown to provide a better antibacterial activity. The aqueous extract of Moringa oleifera showed a broad-spectrum antibacterial activity with a zone of inhibition of 20 to 32 mm and maximum zone of inhibition was obtained for Staphylococcus aureus and S. typhii. The Ethanol extract of Moringa oleifera also showed a broad-spectrum antibacterial activity with a zone of inhibition ranging from 26 to 33 mm and-maximum zone of inhibition was obtained also for Staphylococcus aureus and S. typhii. These results are similar to that obtained by (Vikash Kumar et al., 2011) and (Onsare JG et al., 2013). The results showed that there was no significant difference between the antibacterial activity of aqueous and Ethanol extracts of moringa, with P value of (0.472) But there was significant difference between the moringa extracts and the control antibiotic Chloramphenicol with P value of (0.011) Gram-negative bacteria have been found less susceptible to plant extracts in earlier studies done by other researchers (Kuhnt et al., 1994; Afolayan and Meyer, 1995). In this study we observed that aqueous and Ethanol extracts of moringa were more active against all—Gram-negative bacteria tested along with employed Gram-positive bacteria It was interesting to observe that extracts were effective against pseudomonas aeroginosa which is one of the most resistant bacteria to many antimicrobial agents, In accordance with the results obtained by (Santosh Kumar Singh 2013) The study also revealed that all tested organisms were sensitive to the control antibiotics Cefuroxime and Chloramphenicol.

Conclusion:

These findings offer a new track in production of a potent antimicrobial agent from Moringa oleifera. Present study indicates that the plant contains antimicrobial compound that can be further developed as phytomedicine for therapy of bacterial infections. This in vitro study demonstrated that folk medicine can be as effective as modern medicine to struggle pathogenic Microorganisms. The use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. Extracts were found to be effective in inhibiting the growth of Gram-positive bacteria as well as Gram-negative bacteria. Further studies are needed to verify the active ingredients of Moringa oleifera and in which part (target) of bacterial cell they work. And Clinical trials (in-vivo) are needed to test the activity of Moringa oleifera against bacterial infections.

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