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Original Research Article

Antibacterial Activities of *Moringa oleifera* Leaf Extract on Some Human Pathogenic Bacteria

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Abstract

The aim of the present study was to evaluate the antibacterial activity of *Moringa oleifera* leaf extracts, Escherichia coli and Klebsiella were used. The bioactive compounds Extracted from *M. oleifera* leaves by using many solvents, ethanol, ethyl acetate, water and methanol. The qualitative phytochemical analysis of *M. oleifera* leaves were carried out using standard procedures to identify the constituents of bioactive compounds (Alkaloids, Flavonoids, Tannins and Phenols). The methanol crude leaves extract were used to determine the Minimum Inhibitory Concentration on E.coli and Klebsiella by using the method of Greenwood as well as the inhibitory zone. Results; this study shown that the extract is active against bacterial isolates, whereas the inhibitory effect of the isolate is dose depending, where higher activity was clear by dose 200 mg/L. Also, the sensitivity of the bacterial isolate to the extract differs whereas Klebsiella is more sensitive to the extract with average zone 3.73 mm while E. coli less sensitive by average zone of inhibition 3.47 mm at a maximum concentration 200mg/Lin comparison with a control.

Keywords: Antibacterial activity; *Moringa oleifera* leave extract, Phytochemical screening, Chromatographic Purification.

INTRODUCTION

To prevent and cure different human diseases recently considerable attention has been paid to eco-friendly and bio-friendly medicinal plants (Dubey NK *et al*., 2004 and Shahid M *et al.,* 2013). The medicinal plants are the main source of pharmaceuticals and healthcare products in many countries (Ivanova D *et al*., 2005 and Jamshidi-Kia F *et al*., 2018), as well as, they are widely used in different traditions all over the world where they are becoming more popular in modern scientific communities as natural alternatives to synthetic chemicals (VaN Wyk Be *et al.,* 2004 and Vishwakarma A.P *et al*., 2013). As we know, the infections are a serious health problem that affects millions of people such as urinary tract infection and respiratory tract infections. These infections are treated with classical antibacterial drugs which becomes less effective nowadays, because of emergency of many resistant or multi resistant causative pathogenic microorganisms, in addition to this, the high cost and adverse side effects are commonly associated with popular synthetic antibiotics (Jasmine *et al*., 2013). So, there are need to search for alternative therapeutic options. Plants are the oldest source of pharmacologically active compounds and have provided human kind with various medically useful compounds for centuries (Mbwambo *et al.,* 2007), also nowadays the plants gets more attention in this field, because of they are cheap and safe, and they are considered as an alternative sources of antimicrobials (Pretorius CJ *et al*., 2001; Sharif MDM *et al*., 2006; Doughari JH *et al*., 2007; Jasmine R *et al*., 2013 Mbwambo ZH *et al*., 2007 and Belguith H *et al*., 2010). The medicinal plants are consists of many secondary metabolites such as alkaloids, Flavonoids, phenolic compounds, which possesses an antimicrobial activities (Silver LL *et al*., 1993 and Liu Y *et al*., 2018).

Moringa oleifera is one of these medicinal plants that tested nowadays for its medical properties. Its mainly distributed in the tropical and subtropical regions of Asia, Africa and the Middle East (Leone A *et al,* .2015). All parts of this plant possess medicinal properties, whereas the flowers, leaves, seeds, pods, stem and bark of the tree have high nutritional value (Anwar F *et al*., 2003, Anwar F *et al*., 2005 and Anwar F *et al*., 2007).

The leaves of *M. oleifera* are rich source of calcium, potassium, protein, β-carotene, antioxidants (ascorbic acid, flavonoids, phenolic, carotenoids), and they are exhibit many pharmacological properties such as antioxidant, anti-inflammatory, anti-hyperglycemic and anti-cancer properties (Abdull Razis AF *et al*., 2014), in addition, have been reported that, the various *M. oleifera* parts possess antimicrobial activities against some pathogenic microorganisms (Caceres A *et al*., 1999; Doughari JH *et al*., 2007; Kekuda N *et al*., 2010 and Jamil A *et al*., 2007).

where the studies showed that, the Leaf extracts of *Moringa oleifera* contain protein that has varying antibacterial activities on many bacterial species such as E. coli, K. aerogenes, S. aureus and Bacillus subtilis (ISITUA CC *et al*., 2016 and Dahot MU *et al*., 1998**)**, whereas kiran singh, showed in 2014 that this extract was more effective than traditional antibiotics to combat this pathogenic bacteria (kiran singh GM *et al*., 2014). However, there is no extensive work on its antimicrobial properties has been done. Hence, the present study was an attempt to examine the role of leaf extracts of *M. Oleifera* as a potential antimicrobial agent against some human pathogenic by using the Libyan ecotype of *Moringa oleifera.*

MATERIAL AND METHODS

Extraction of *M. oleifera* leaves

The experimental plant *M. oleifera* was collected from a farm at Tripoli Libya *Moriga Oleifera* leaves was healthy and uninfected. The leaves were air dried at room temperature and grinded in powder form (Roise, Viller 1987). 150 gm of the powdered leaves were extracted with 600 ml of methanol. The extract was separately filtered using Whitman's no1 filter paper. Then the extract was concentrated in vacuum using a rotatory evaporator at 40 C the methanol remaining in the extract removed by placing it at room temperature overnight to give a residue weighing 8 g.

Preliminary Phytochemical Screening of SuccessiveExtracts of M. oleifera Pods

Qualitative phytochemical analysis of M. oleifera leaves was carried out using standard procedures to identify the constituents, Alkaloids, Flavonoids, Tannins and Phenols as described by (Patel *et al*., 2014).

Test for alkaloids

1% of HCL prepared and added to the *Meringa Oleifera* extract in test tube and heat it for 20 min with gently shake then leave it to cool a bit. Take 1 ml of the extract and added few drops of Wagner's reagent notice a creamy brown indicate the presence of Alkaloids.

Test for flavonoids

3 ml of *Meringa Oleifera* extract added to 10ml of distilled water and mix it well notice a yellow color indicate the presence of Flavonoid.

Test for tannins:

2ml of *Maringa Oleifera* extract and put it in test tube and gently heat it for 2min add 3 drops of Ferric chloride notice orange color indicate the presence of Tannin.

Test for phenols

3ml of *Maringa Oleifera* extract added to 5ml distilled water then add few drops of 5% Ferric chloride notice dark green indicate the presence of phenols.

METHODS

Chromatographic purification TLC was carried out to isolate the principle components that were presents in most effective extracts of plants the TLC was used by different solvent systems Solvent phase: Chloroform:methanol: ethanol (1:1:1), Chloroform:methanol: ethanol (2:2:0.5) Chloroform: Glacial acetic acid: methanol (4:5:1) (Sharma, Paliwal 2013).

The plant extract was with 4 different solvents each extract applied on pre coated TLC plate by using capillary tubes. Drawing a light line on the plate and dots to know the place of each extract applied on the plate. After using each mobile phase, the TLC plate were air dried and observed under ultra violet light. They were later sprayed with iodine vapors for the development of the separated bands the movement was expressed by its retention factor (Rf) values were calculated for different sample.

$$RF = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solute front TLC plate}}$$

Preparation of Bacterial Isolate

Two different types of bacterial strains were obtained from the medical laboratories which are Escherichia coli and Klebsiella.

Screening of antimicrobial activity

Media for test organisms

36g of Muller Hinton Agar was added to 1000 ml of sterile distilled water and autoclaved at 121oC for 30 minutes at 1.5 lbs. After cooling both the agar was poured into sterile Petri plates approximately 4mm and allowed to set at ambient temperature and used. Sterile Mueller Hinton agar plates were inoculated with the test culture by surface spreading using sterile wire loops and each bacterium evenly spread on the entire surface of the plate to obtain uniformity of the inoculum. The culture plate then had at most 4 holes of 7 mm diameter and 5 mm depth made into it using a sterile agar glass

borer. The density of suspension inoculated onto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of Barium sulphate solution (Cheesbrough, 2002).

Inhibition Activity of Different Concentration of Meringa oleifera Extracts

This was carried out using agar well diffusion method. 200 µl of different concentration of the ethanoic extracts (25mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml) of *Maringa oleifera* were dispensed separately in wells already seeded with the test isolates and incubated at 37°C for 24 h. After incubation, the inhibitory activity of the minimum concentration of the extracts against the test organisms was determined by measuring the clear zones around the wells in diameter. Standard antibiotic discs were used as a positive control to compare the antibacterial activity. The discs loaded with test extracts, and the standard antibiotic were placed with help of sterile forceps carefully with adequate spacing between each other. After incubation, the antibacterial activity of the extracts against the test organisms was determined by measuring the clear zones around the wells in diameter.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration of the crude leave extract of *Meringa oleifera* was determined using the method of Greenwood (1989) as described by Geidam *et al*., (2007). Serial dilution of the extract at the concentrations of 25, 30,35, 40, 45,50,100 and 200 mg/ml. Where 18 mg of the Muller Hinton Agar media was prepared in 500 ml of distilled water and autoclaved at 121 C and 5Ib for 30 minutes then cooled, the media filled in tubes, each tube contains 17 ml. Astandarazed inoculum for each bacterial strain was prepared to give an inoculum size approximately 10^{-5} in 5 tubes each tubes contain 10 ml of distilled water. Each extract concentration poured in tube containing 3ml of distilled water and mixed properly then taken off by a sterile syringe and filtered by filter paper and poured to the prepared M.H.A broth and mixed properly then add 100µlof bacterial isolate and mixed again then put them in autoclaved petri dishes and move the dishes in different directions to homogenize the plant extract. The control sample containing only the bacteria without extract. Then all dishes kept at 37 C for 24 hrs. in incubator. Then determine minimum inhibitory concentration and recorded as the least concentration of the extract that completely inhibited the growth of the organisms.

RESULT AND DISCUSSION

RESULTS

Analytic detection

After drying the plate and exposed with iodine vapor all plates were visualized with the help of UV and all different spots that were observed was calculated.

Phytochemical Screening of Sequential Extracts of *M. oleifera* leaves

Phytochemical screening of the sequential extract of M. oleifera leaves showe the presence of various bioactive components which are phenol, alkaloids, flavonoids and tannin are the most prominent components and the result of phytochemical test is presented in Table 1. Among these phytochemical tests, ethanol and ethyl acetate extract were found to contain maximum of alkaloids, flavonoids, tannin and phenol in comparison with other solvents. All these phytochemicals possess good antioxidant activities and has been reported to exhibit multiple biological effects including anti‑ inflammatory and antitumor activities.

Table 1: Qualitative phytochemical screening of sequential extracts *of Moringa oleifera* leaves

Solvent used	Alkaloids	Flavonoids	Tannin	Phenol
Methanol	++	+++	++	+++
Ethanol	+++	+++	+++	+++
Ethyl acetate	+++	+++	+++	+++
Water	++	+++	+++	+++

Chromatographic Purification: TLC

Chloroform: Methanol: Ethanol (1:1:1) TLC of pet methanol extract of *M. oleifera* leaves revealed the presence of 4 compounds having Rf values of 0.01, 0.89, 0.92 and 0.93 respectively when a solvent phase of was used. With ethanol extract by same solvent showed 5 bands having Rf values of 0.046 ,0.66,0.83, 0. 90 0.93 respectively. With ethyl acetate extract shows 4 bands having Rf of0.046,0.50,0.56, 0.89. However, with water extract shows no bands chloroform: methanol: ethanol (2:2:0.5) Methanol extract shows 3 bands having Rf values of 0.090,0.090, 0.81. with ethanol extract shows 3 bands having Rf values of 0.090,0.8, 0.81. with ethyl acetate shows nobands with water extract show 2 bands having Rf values of 0.63, 0.76 Chloroform: Glacial acetic acid: methanol (4:5 :1) with methanol extract shows 2 bands having Rf values of 0.45, 0.78. while, Ethanol extract show no bands and with ethyl acetate extract shows 3 bands having Rf values of 0.4,0.76, 0.83. however, with water extract show no band.

The present study was concentrated on the determining antibacterial activity by using agar well diffusion method by measuring the inhibition zone in mm against two bacterial strains E. coli and Kleibsiella

species and determine the phytochemical screening in Leaves of Moringa oleifera with different solvents like water, 70% ethanol, 80% methanol and petroleum ether. The extract used in this study was the methanol extract.

Antibacterial activity of the leaves extracts:

By using a different concentration of the leaves extract on the bacterial isolate, the results shown in Table2, where the extract is active against the isolates. However, the inhibitory effect of the isolate is depending on the dose, where higher activity was clear by dose 200 mg/L. Also, the sensitivity of the bacterial isolate to the extract differs. Klebsiella is more sensitive to the extract with average zone 3.73mm while E. coliisless sensitive by average zone of inhibition 3.47mm at a maximum concentration 200mg/Lin comparison with a control.

Table 2: Antibacterial Activity of *Moringa oleifera* leaves Extract against bacteria (*E.coli* and *Klebsiela)* concentration (mg/mL)/ Zone of inhibition (mm)

Organism	**25**	**50**	**100**	**200**	**Control**
E.coli	2.40 ± 0.40	2.68 ± 0.29	3.13 ± 0.06	3.47 ± 0.06	4.40 ± 0.00
P-value	0.001	0.000	0.000	0.000	
Organism	**25**	**50**	**100**	**200**	**Control**
Klebsiella	2.33 ± 0.29	2.40 ± 0. 69	3.10 ± 0.17	3.47 ± 0.06	4.40 ± 0.00
P-value	0.000	0.007	0.000	0.038	

The minimum inhibitory concentration (MIC) value of methanol extract of *Moringa oleifera* against Kleibsilla.According to Table 3 the MIC value of methanol extract treated on Kleisilla *was* found to be 45 mg%.

Table 3: MIC of methanolic moringa leaves extract for Kleibsilla

Concentration of Extract	**No of colonies**
200	**no**
100	**no**
50	**no**
45	**no**
40	**187**
35	**177**
30	**304**
25	**121**

DISCUSSION

The rising prevalence of pathogenic microorganisms' resistant to the newer antibiotics has been expressed in the last three decades (Valarmathy *et al*., 2010). The current study exhibits that *Moringa oleifera* leave extract shows phytochemical bioactive compounds like flavonoids, tannins, alkaloids, saponins and phenols in methanol extract shows in Table 1. That they exhibit antibacterial activity.

Alkaloids are natural bioactive compounds contain basic nitrogen atoms. They also have pharmacological effect and are used as herbal medications (Rhoades, 1979). Flavonoids promote the effect of vitamin C and act as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes (Korkina, 1997). Tannins have revealed potential Antiviral, Antibacterial and Anti-parasitic effects. Saponins cause hemolysis of red blood cells (Winter WP, 1993).

Antibacterial activity of *Moringa oleifera* was seen against several bacteria namely Escherichia Coli, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vulgaris, Streptococcus mutants, Bacillus subtilus and Staphylococcus epidermidis (Naplean P, 2009). The methanol extract showed antibacterial effect against both E. coli and Klebseilla Table 3. The results showed that increasing the concentration of the methanol extracts increased the zone of inhibition. The medicinal plant *Moringa oleifera* exhibits good antibacterial activity against, *Klebsiella*, and *E. coli* in this study. However, many previous results revealed the strains E. coli, P. aeruginosa and S. enterritidis (IH) were resistant to many treatments (Jackson *et al*., 2011). This resistance observed were matched from a study on the antibacterial properties of Indian plants showing Moringa extracts to be ineffective against E. coli. Thus, the results in current study revealed significant inhibitory effect of methanol leaves extract of *Moringa oleifera* on the E. coli and Kleibsella by using 200 mg % concentration with inhibitory zone about 3.47 ± 0.06 and 3.47 ± 0.06 respectively in comparison with the control where the zone of inhibition was 4.40 ± 0.00.

CONCLUSION

This study has revealed that the methanol extracts of *Moriga oleifera* leaf possess some degree of antibacterial effects. Were the leaf extracts shown to contain bioactive compounds with a clear antibacterial activity, capable of inhibiting the growth of gram negative bacteria, E. coli and Kleibsilla. More and further studies can achieve that *Moringa oleifera* can be used to discover which bioactive compounds responsible on the antibacterial activity.

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