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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF MORINGA OLEIFERA AGAINST PATHOGENIC BACTERIA IN SUDAN

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ABSTRACT

The antibacterial activity of Moringa oleifera Aqueous and alcoholic leaf extract the highest concentration of extract is (100 mg/m l) and the lowest one is (12.5 mg/ml) against different four pathogenic organisms Bacillus cereus pseudomonad aeruginosa, Escherichia coli and Salmonella typhimurium were carried out by using a disc diffusion technique. In aqueous leaf extract of Moringa the highest antibacterial activity was detected against Escherichia coli in all different concentration of Moringa and the lowest inhibition zone against pseudomonad aeruginosa), In alcoholic leaf extract the highest antibacterial activity was detected against pseudomonad aeruginosa and Escherichia coli and the lowest inhibition zone against Salmonella typhimurium). The antibacterial activity of the synthetic antibiotics, (Ciprofloxacin, Tetracycline Ceftriaxone, Chloramphenicol and Gentamycin, were tested by the disc diffusion method, and by measuring zones of inhibition, shows that the highest activity of antibiotic against bacteria was due to the action of ciprofloxacin,. among all antibiotic and the lowest activity was due to the action of Tetracycline, the highest inhibition zone among the bacteria by antibiotic against Salmonella typhimurium and the lower inhibition zone against Bacillus cereus, all Aqueous and alcoholic leaf extract of Moringa have high activity against Escherichia coli, .so that there was no different between antibiotic and Moringa leaf extract against Escherichia coli and Bacillus cereus in this study.

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INTRODUCTION

Moringa oleifera is a woody tree mainly distributed in the tropical and subtropical regions of Asia, Africa and the Middle East (Leone *et al*., 2005). Family Moringaceae (Olson, 2010). used as a nutritional source such as essential amino acid, vitamins D and A, Minerals such as potassium, calcium, phosphorus, iron, and used as a medicinal plant (Leone *et al*., 2015; Teixeira *et al*., 2014). It has traditionally been used in the treatment of malaria, parasitic diseases, skin diseases, hypertension and diabetes, it exhibits pharmacological properties such as antioxidant, anti-inflammatory, anti-cancer, anti-hyperglycemic and anti-hyperlipidemic properties (Leone *et al*., 2015; AbdullRazis *et al*., 2014; Anwar *et al*., 2007), the aqueous, and alcohol extracts of Moringa oleifera exhibited various biological activities including antioxidant, tissue protective, and analgesic properties (Stohs and Hartman, 2015). Many studies have suggested that different crude extracts from different tissues of Moringa oleifera show antibacterial activities against both Gram-negative and Gram-positive bacteria. Using the zone of inhibition test, Bacillus cereus has been implicated in food-borne intoxication. Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa cause diseases like mastitis, abortions and upper respiratory complications streptococcus faecalis is a pathogenic bacteria commonly found in the intestines of birds (Pal *et al*., 1995; Granum and Lund, 1997). Antifungal activity of Moringa oleifera has been described in multiple studies.

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showed the antifungal effect of Moringa oleifera against the human pathogenic fungi Trichophyton rubrum, Trichophyton mentagrophytes, Epidermophyton floccosum and Microsporum canis. both the seed extracts and essential oils exhibited antifungal activity on the four different fungi with different MIC while the leaf extract and the sub-fractions had little effect on these dermatophytes. Moringa oleifera can be used to treat fungal infections of skin (Chuang *et al*., 2007). Moringa oleifera show antiviral activities in several different studies (Murakami *et al*., 1998) reported that Epstein-Barr virus (EBV) activation in Raji cells could be inhibited by niaziminin, a thiocarbamate identified from the leaves of Moringa oleifera Anti parasitic Activity of Moringa oleifera Leishmania is a genus of trypanosomes that are responsible for Leishmaniasis disease (Ansari *et al*., 2016; Kaur *et al*., 2014). showed that 70% ethanolic extract of Moringa oleifera roots exhibit antileishmanial activity with IC50 values of 83 µg/ml and the methanolic extract of leaves showed antileishmanial activity with IC50 values of 47.5 µg/ml. Niazinin, which was isolated from the methanolic extract of leaves, showed the most antileishmanial activity with IC50 values of 5.25 µM (Singh *et al*., 2015). Objectives: The objective of this study was to assess the antibacterial activity of Moringa oleifera.

MATERIALS AND METHODS

Plant material: The experimental part of this research was carried out using Moringa oleifera purchase in herbiest in the super market and identified in thedepartment of plant Botany Faculty of Agriculture, Khartoum University. The Moringa oleifera leaf extract was then prepared by grounding 50 g of leaves using mortar and pestle, and the yield was successively soaked in distilled water for aqueous extract, for alcoholic extract soaked in 80 % ethanol for about 72 hours, with daily filtration and evaporation. Solvents were evaporated under reduced pressure to dryness using rotary evaporator apparatus. Filtration and extraction were carried out in the Center of Medicinal and Aromatic Plants, Khartoum (Sudan). Extracts were exposed to air till complete dryness.

Microorganism: All the microorganisms used in this work were obtained from the National Centre for Research, Khartoum Sudan Laboratory. Bacterial identification was carried out by conventional biochemical methods according to the standard microbiological techniques these microbes were Bacillus cereus pseudomonad aeruginosa, Escherichia coli and Salmonella typhimurium.

Microbial Sensitivity Test: The antimicrobial sensitivity testing was conducted by the agar disc diffusion method. The sensitivity medium (Muller-Hinton agar) was prepared by adding 3.8g of Muller Hinton agar powder to 100 ml distilled water and autoclaved at 121°C for 15 minutes at 15 lbs., and poured in sterile Petri plates up to a uniform thickness of approximately 4 mm and the agar was allowed to set at ambient temperature before use. The bacterial isolates were suspended in peptone broth and incubated at 37° C for 3-4hours before used as inocula. The turbidity of the broth culture was adjusted to 0.5 McFarland units.

This gives a suspension containing approximately 1-2 x 106 colony forming units (CFU)/ml. A sterile cotton swab was inserted into the bacterial suspension, rotated, and then compressed against the wall of the test tube to express any excess fluid. The swab was then streaked on the surface of the Muller-Hinton agar plate. To ensure a uniform, confluent growth, the swab was streaked three times over the entire plate surface. To test antibacterial activity of Moringa leaf extract, it was first dissolved in a methanol solvent, and then varying concentrations of the extracts (100µg, 50µg, 25µg, 12.5µg, and 6.25µg) were soaked on autoclaved discs of Whatmann filter paper. These filter paper discs were placed on a streaked Muller-Hinton agar plate surface. The plates were incubated overnight at 37° C for 18-24hours. The antimicrobial activity was detected by measuring zones of inhibition.

Microbial Sensitivity Test of the antibiotics: To test antibacterial activity of the synthetic antibiotics, standardized discs of Ciprofloxacin (5µg), Gentamycin (10 µg), Ceftriaxone (30µg), Chloramphenicol (10µg), Tetracycline (25µg), were tested by agar disc diffusion method by placing on a streaked Hinton agar plate surface. The antimicrobial activity was also detected by measuring zones of inhibition.

RESULTS AND DISCUSSION

Table 1. show that the antibacterial activity of Moringa oleifera Aqueous leaf extract against different four pathogenic organisms Bacillus cereus pseudomonad aeruginosa, Escherichia coli and Salmonella typhimurium, (the highest concentration of the aqueous leaf extract is (100 mg/m l) and the lowest one is (12.5 mg/ml), the highest antibacterial activity was detected against Escherichia coli in all different concentration of Moringa and the lowest inhibition zone pseudomonad aeruginosa,), these results conformity with those

Table 1. Inhibition zoon (in mm) for different concentrations of Moringa oleifera aqueous leaf extract

Microorganism	Concentration of the leaf water Extract from the Moringa oleifera)) µg/disc			
	100	50	25	12.5
Salmonella typhimurium	15	14	11	10
Pseudomonas aeruginosa	15	0	0	0
Escherichia coli	15	15	14	13
Bacillus Cereus	15	10	10	0

Table 2. Inhibition zoon (in mm) for different concentrations of Moringa oleifera Alcoholic leaf extract

Microorganism	Concentration of the leaf alcoholic extract from the Moringa oleifera) µg/dis			
	100	50	25	12.5
Salmonella typhimurium	10	10	-	-
Pseudomonas aeruginosa	16	14	12	10
Escherichia coli	15	14	13	10
Bacillus Cereus	15	14	10	10

Table 3. Inhibition zoon (in mm) for different antibiotics Ciprofloxacin (5µg), Gentamycin (10 µg), Chloramphenicol (10µg), Tetracycline (25µg) and Ceftriaxone (30µg)

Acterial test strains (No. tested)	Antibiotics concentration in (µg/disc)m				
	Ciprofloxacin	Gentamycin	Chloramphenicol	Tetracycline	Ceftriaxone
Salmonella typhimurium	27	17	20	11	14
Pseudomonas aeruginosa	28	10	14	14	16
Escherichia coli	16.9	13	15	10	11
Bacillus cereus	23	I5	10	13	6

obtained in previous studies (Pal *et al*., 1995; Sato *et al*., 2004; Cushine and Lamb, 2005). Table 2. show that the antibacterial activity of Moringa oleifera alcoholic leaf extract against different four pathogenic organisms Salmonella typhimurium, pseudomonad aeruginosa, Escherichia coli and Bacillus cereus, (the highest concentration of the alcoholic leaf extract is (100 mg/ml) and the lowest one is (12.5 mg/ml), the highest antibacterial activity was detected against pseudomonad aeruginosa, Escherichia coli and the lowest inhibition zone Salmonella typhimurium,) these results conformity with those obtained in previous studies the antimicrobial activities. of Moringa oleifera leaf extracts against four Gram-positive bacteria (Bacillus cereus, Bacillus subtilis, Staphylococcus aureus and Sarcinalutea) and two Gram-negative bacteria (Escherichia coli and Acid fast Mycobacterium phlei), the results revealed that the average zone of four Gram-positive bacteria and two Gram-negative bacteria was inhibited by the ethanol extract of Moringa oleifera leaves. Using modified disk diffusion to screen antibacterial activity the aqueous and ethanol extracts of Moringa oleifera leaves showed effective antibacterial activity against Staphylococcus aureus, Vibrio parahaemolyticus, Enterococcus faecalis and Aeromonas caviae, (Peixoto *et al*., 2011; Dzotam *et al*., 2016). Table 3. exhibits the zones of inhibition (in mm) for the different synthetic antibiotic shows that the highest activity of antibiotic against bacteria was due to the action of ciprofloxacin,. among all antibiotic and the lowest activity was due to the action of Tetracycline, the highest inhibition zone among the bacteria by antibiotic against Salmonella typhimuriumand the lower inhibition zone against Bacillus cereus, there was no different between antibiotic and Moringa leaf extract in this study.

Conclusion

This work comes to conclude that the Aqueous and alcoholic leaf extract of Moringa oleifera had potent antibacterial activity against pathogen bacteria, and have no high different between it and synthetic antibiotic in the activity against microorganism, and I recommended to isolate and separate the bioactive compound responsible for antibacterial activity.

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