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Research Article

Anti-Bacterial Activity and Phytochemical Screening of *Moringa oleifera* Leaves

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Abstract

Plants have been used as medicine throughout history. Medicinal plants are widely and successfully used all over the world. *Moringa oleifera* is one of these plants which has been found useful in nutrition, agriculture, soil control, water purification, industrial applications, cattle feed and also for treating various types of diseases in humans and livestock. In this study, the antibacterial activity of *Moringa oleifera* leaves anti-bacterial activity was investigated. The bioactive compounds from *Moringa oleifera* leaves were extracted by different solvents (Petroleum Ether, Chloroform, Methanol, Ethanol and water). The Phytochemical screening tests showed that the *Moringa oleifera* leaves contained; tannins, alkaloids, flavonoids, anthraquinones, glycosides, triterpenes, saponin, and cummarins. The extracts were applied to different bacterial cultures (*Bacillus cereus (B. Cereus),*

Staphylococcus aureus (S. Arous), Escherichia coli (E. Coli), and Pseudomonas aeruginosa (P. Aeruginosa). The *Moringa oleifera* extracts revealed efficient anti-bacterial activity which was almost twice the anti-bacterial activity of the standard drug *Gentamycin*. Based on the findings of this study, it can be concluded that *Moringa oleifera* is a promising antibacterial agent. This confirms its efficiency as an antibacterial traditional medicine.

Introduction

Plants have been used as medicines throughout history. Indeed, studies of wild animals showed that they instinctively eat certain plants to treat themselves from certain diseases. Medicinal plants are widely and successfully used on every continent. In Asia, the practice of herbal medicine is extremely well established and documented. As a result, most of the medicinal plants that have international recognition come from Asia, particularly from China and India. In Europe and North America, the use of herbal medicine is increasing fast, especially for correcting imbalances caused by modern diets and lifestyles. Many people now take medicinal plant products on a daily basis, to maintain good health and to treat illness as well. In Africa, attitudes towards traditional, herbal medicines vary strongly, due to the confusion between herbal medicine and witchcraft.

It is proved by research that *Moringa oleifera* leaves extract is effective in regulating hyperthyroidism [1], anti-proliferation and induction of apoptosis on human cancer cell [2]. It works as anti-oxidant [3] Hypocholestrolemic agent in obese patients [4] and Ethno-medicine to treat diabetes mellitus [5]. It works as anti-bacterial [6] and anti fungal besides the use of its essential oil as an anti-skin disease agent [7]. This study aimed to determine the chemical constituent of the leaves of *Moringa oleifera* and to investigate their antibacterial activity, using different solvents.

Materials and Methods

Materials

The *Moringa oleifera* leaves used in this work were collected from Alsamrab, Sudan. Microorganisms were collected from the Faculty of Medical Laboratories Science, Omdurman Islamic University, Khartoum, Sudan. The chemicals used in this research were; Petroleum ether, Chloroform, Methanol, and Ethanol (Sd. fine-cHEm limited Mumbai, India).

Methods

Preparation of Extracts

Preparation of Fresh (MOL) Extract

The fresh *Moringa oleifera* leaves, were extracted using water. The extract was then dried using a freeze drier and kept for further tests. Fresh *Moringa oleifera* leaves also were extracted with ethanol then ethanol was evaporated using rotary evaporator.

Etahnolic Extract

Ten grams of the *Moringa oleifera* leaves powder were refluxed with 100ml of 80% ethanol in a round bottle flask for 4 hours. The solution was cooled, filtered, and 80% ethanol was added to complete the filtrate to 100ml. The *Moringa oleifera* leaves extract was used for phytochemical tests.

Successive Extraction

Petroleum Ether (350ml) was added to 60g of dry powder of *Moringa oleifera* leaves in thermal soxhlet extraction apparatus. The extraction took place for 18 hours. The extract was dried and kept for further tests. The same sample was dried from Petroleum Ether and re-extracted by 540ml of chloroform using the same apparatus. Then, the sample was dried from chloroform and extracted again with 350ml of methanol. The three samples were used for anti-bacterial test.

General Phytochemical Screening

Phytochemical screening was applied to the sample for presence of different constituents such as tannins, alkaloids, flavonoids, anthraquinones, glycosides, sterol, deoxy sugar, triterpenes, and saponin [8]. Cummarin was analysed according to Harborne, (1973) [9].

Anti-Bacterial Tests

Preparation of Bacterial Suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were equally distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100ml of normal saline to produce a suspension containing about (10^8 - 10^9) colony forming units/ml. The suspension was stored in the refrigerator at 4°C for further tests.

Testing for Anti-Bacterial Activity

The cup-plate-agar diffusion method was adopted to assess the anti-bacterial activity of the extracts. Three ml of each of the four standardized bacterial stock suspensions (10^8 - 10^9) Colony Forming Unit/ml (CFU/ml) were thoroughly mixed with 300ml of sterile melted nutrient agar which was maintained at 45°C. Incubated nutrient agar of 20ml was distributed into sterile petri-dishes. Then, agar disks were removed. The cups were filled with 0.1ml samples from the concentration of 1g/10ml of extracts) of each of the extracts using transferable pipette adjustable volume automatic micro titer pipette, and allowed to diffuse at room temperature for two hours. The same amount of 0.1ml sample was taken from the standard drug Gentamycin with 2 concentrations of 8mg/L and 20mg/L. The plates were then incubated in the upright position at 37°C for 18 hours. After incubation the diameters of the resultant growth inhibition zones were measured and mean values were tabulated.

Results and Discussion

Phytochemical Screening

The Phytochemical screening test showed that the *Moringa oleifera* leaves consisted of tannins, alkaloids, flavonoids, anthraquinones, glycosides, triterpenes, saponin, and cummarins.

Result of Anti-Bacterial Activity

The results of anti-bacterial activity using dried *Moringa oleifera* leaves extracts and the standard drug (*Gentamycin*) are shown in Tables (1 and 2), respectively. The anti-bacterial activity for different extracts with different bacteria compared to the standard drug (*Gentamycin*) are shown in Figure (1), while Figures (2, 3, 4 and 5) shows the inhibition zone (mm) for different bacteria.

Table 1: *Anti-bacterial activity of Moringa oleifera leaves extracts*

Tested Bacteria / Extract	Inhibition zone (mm)			
	B. Cereus	*S. arous*	*E. Coli*	*P. Aeruginosa*
Fresh Aqueous	30	32	35	0
Fresh Ethanolic	33	31	31	35
Petroleum Ether	30	0	22	13
Chloroform	40	20	15	24
Methanol	8	8	30	15

Table 2: *Anti-bacterial activity of standard drug (Gentamycin)*

Tested Bacteria / *Gentamycin* Concentration	Inhibition zone (mm)			
	B.Cereus	*S. Arous*	*E. Coli*	*P.Aeruginosa*
10mg/L	22.5	0	20	16
20mg/L	20	21.5	26	23

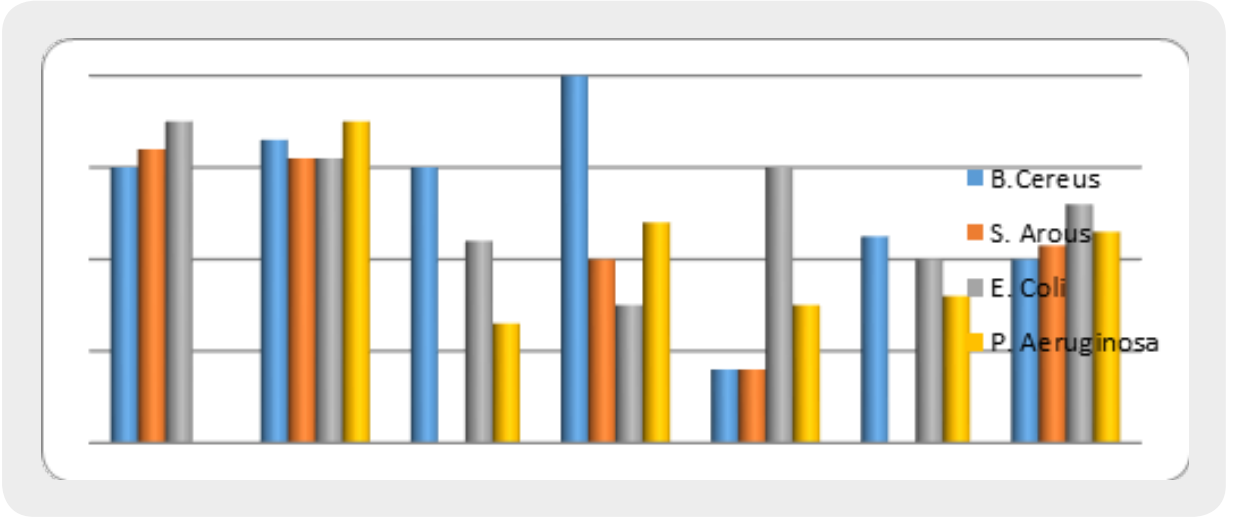

Figure 1: *The anti-bacterial activity for different extracts compared to Gentamycin*

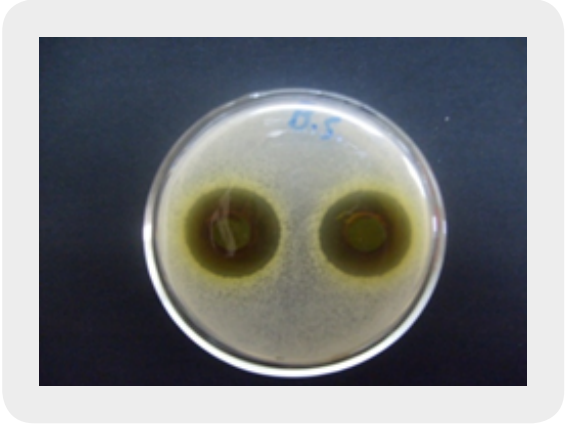

Figure 2: *B. Cereus*

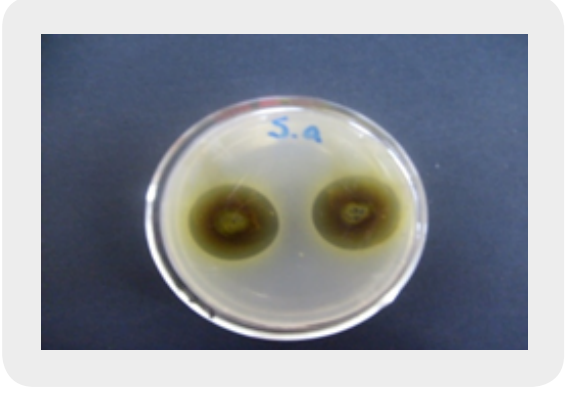

Figure 3: *S. Aureus*

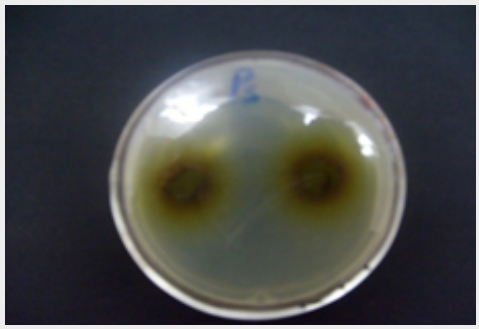

Figure 4: *E. Coli*

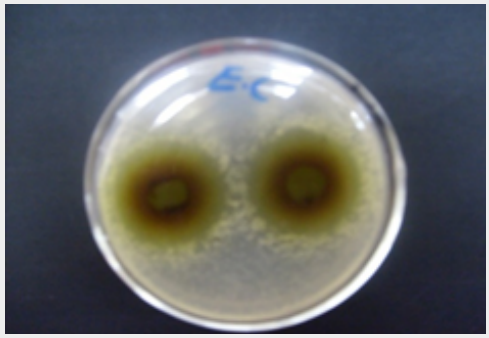

Figure 5: *P. Aeruginosa*

The results of the phytochemical screening of *Moringa oleifera* leaves revealed presence of, tannins, alkaloids, flavonoids, anthraquinones, glycosides, sterol, deoxy sugar, triterpenes, saponin, and cummarins.

Moringa oleifera phytochemical screening for ethanolic extract revealed the presence of sterol in agreement with [10], alkaloid, flavonoids, saponin and tannins which was reported by [11]. In this current study, cummarins, glycosides, and deoxy sugars were found in *Moringa oleifera* leaves but are not yet reported by other studies.

For the anti-bacterial activity, it was found that different extracts of *Moringa oleifera* leaves were active against bacteria such as *E. Coli, S. Arous, P. Aeruginosa*, and *B. Cereus,* as these organisms range from pathogenic and toxigenic organism liable to cause food borne illnesses, and foodspoilage due to bacteria presence. [12] reported that possession of alkaloids, tannins and flavonoids enhanced anti-bacterial properties of plant. In addition, the anti-bacterial activity of *Moringa oleifera* leaves for different species of bacteria was highlighted by many authors [11,13-16].

The experiments were carried out using five extracts of *Moringa oleifera* leaves (Fresh aqueous, fresh ethanolic, petroleum ether, chloroform, and methanolic extract), which was applied on four species of bacteria (*S. Arous, P. Aeruginosa, E. Coli,* and *B. Cereus*), it was found that Fresh ethanolic extract was highly active against all mentioned species. *B. Cereus* and *E. Coli* are highly sensitive to petroleum ether extract while *S. Aureus,* and *P. Aeruginosa* were resistant.

B. Cereus was the most sensitive bacteria to chloroform extract; *P. Aeruginosa* and *S. Aureus* were also sensitive, while *E. Coli* showed lower sensitivity. Methanol extract is highly active against *E. Coli*, but has a lower activity against *P. Aeruginosa* but *S. Arous* and *B. Cereus* are highly resistant.

Conclusions

It can be concluded that *Moringa oleifera* leaves extracts with different solvents are effective and with good anti-bacterial activity against certain bacteria. Ethanolic extract is very effective against the four types of bacteria in this study and other extracts are effective selectively for certain bacteria. Compared to standard drug (*Gentamycin*), ethanolic extract showed double activity against bacteria. Therefore, it is recommended to consider *Moringa oleifera* leaves as anti-bacterial medicine.

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Bioavailability

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