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Impact of *Moringa* aqueous extract on pathogenic bacteria and fungi *in vitro*

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ABSTRACT

Moringa peregrine have many benefits. In this study aqueous extract of *Moringa* plant inhibited the activity of these bacteria which include *Bacillus cereus*; *Staphylococcus aureus*; *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*; *Enterococcus cloacae*; *Salmonella typhi* and; *Proteus vulgaris*. *Moringa* extracts has shown an impact on the growth of bacteria on the Blood with inhibition zone variable (23.5 ± 0.45 to 12.5 ± 0.50 mm) according to the type of bacteria. The mean growth inhibition percentages were 85.9 ± 0.42 to 65.3 ± 0.34 nm against all tested bacteria. As regards to fungi, high potency extract displayed zones of inhibition of ⩾10 mm, moderate potent extracts gave zones of inhibition between <10 and 9 mm. The results indicated that, *Moringa* aqueous extract played variable antifungal activity ranged from high (18 ± 0.54 mm), moderate (13.2 ± 0.58 mm) and low (6.6 ± 0.47 mm). The inhibition zones diameter in millimeters against *A. niger*, *A. flavus*, *P. italicum*, *F. oxysporum*, *R. stolonifer*, *Alternaria* sp., *C. albicans*, *C. parapsilosis* were 15.2 ± 0.52, 12.4 ± 0.55, 10.5 ± 0.26, 9.4 ± 0.71, 13.2 ± 0.58, 6.6 ± 0.47, 12 ± 0.44 and 18 ± 0.54, respectively. On the other hand, the mean inhibition of growth (as percentages) were 75.2 ± 0.55, 59.4 ± 0.75, 58.2 ± 0.63, 46.5 ± 0.63, 62.5 ± 0.77, 24.5 ± 0.65, 20.3 ± 0.75 and 80.00 ± 0.70% respectively. Thus, the aqueous extract of *Moringa leaves* showed antimicrobial activity against tested bacteria, fungi and yeasts at different concentrations.

Introduction

Moringa oleifera (Moringaceae), also known as "the tree of life", is mainly native to India and Africa. It is considered one of the most useful trees in the world because every part of the *Moringa* tree can be used for food, medication and industrial purposes (Moyo et al., 2011). In particular, the leaves can be eaten fresh in salad, cooked, or stored as dried powder for many months without loss of nutritional value. Apart from treating malnutrition, in rural areas of Uganda, its leaves are used to treat a wide range of medical conditions such as HIV/AIDS-related symptoms, bronchitis, ulcers, malaria and fever (Kasolo et al., 2010). In this regard, leaf extracts of *M. oleifera* have been reported to exhibit antioxidant activity both *in vitro* and *in vivo* due to their abundance of phenolic acids and flavonoids (Vongsak et al., 2013; Al Khateeb et al., 2013). Previous phytochemical analysis of *M. oleifera* from different countries have shown that the leaves are particularly rich in potassium, calcium, phosphorus, iron, vitamin D, essential amino acids, as well as known antioxidants such as carotene, vitamin C, and flavonoids (Mbikay, 2012). *M. peregrina* (Forssk Fiori) is also widely grown, but to a much lesser extent than *M. oleifera* in Saudi Arabia, India and south of Iran (Ghodsi et al., 2014). The specific components of *Moringa* preparations that have been reported to have hypotensive, anticancer, and antibacterial activity include 4-(4′-O-acetyl-α-L-rhamnopyranosyloxy)benzylisothiocyanate, (-α-L-rhamnopyranosyloxy) benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-(α-L rhamnopyranosyloxy) benzyl glucosinolate. It is also rich in a number of vitamins and minerals as well as other more commonly recognized phytochemicals such as the carotenoids (including β-carotene or provitamin A) (Nepolean et al., 2009). Bacteria and fungi are of human and veterinary importance as outlined later. *Bacillus cereus* has been implicated in food-borne intoxication (Granum and Lund, 1997). *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* cause diseases like mastitis, abortions and upper respiratory complications (Fraser, 1986). *Streptococcus faecalis* is a pathogenic bacteria commonly found in the intestines of birds (Granum and Lund, 1997). *Penicillium notatum* induces hypersensitivity, pneumonitis

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in animals. *Candida albicans* is reported to cause vaginitis and yeast mastitis. These necessitate searching for antibiotics that could be used against microbes. *Moringa oleifera* Lam is one of the best known, widely distributed (Anwar et al., 2007). For this reason, the purposes of this study was to treat pathogenic bacteria, fungi and yeasts using aqueous *Moringa* plant extract as antimicrobial and impact on biofilm formation. As well as determination of Minimum inhibition concentration (MIC) for Moringa aqueous extract.

Material and methods

Moringa oleifera (*Moringaceae*) leaves were collected from Virological greenhouse of the Microbiology Dept., Faculty of Agriculture, Ain Shams University, Egypt.

Preparation of Moringa leaves extract

Leaves extract were prepared according to Oludura. The fresh leaves were air dried at room temperature to constant weight. The dried leaves were ground into powder. A weight of 25 g of the powdered leaves was extracted in 100 ml of demonized distilled water in conical flask (aqueous extract). The conical flask was shaken at 120 rpm for 30 min. The extract was filtered by filtration system using membrane filter (pore size 0.45 μm) before use.

Microorganisms

For this study, eight isolates of bacteria and ten Fungi and yeast isolates (Tables 1 and 2) were obtained from Microbiology Lab. of the Microbiology Dept., Faculty of Agriculture, Ain-Shams University, Egypt.

Antibacterial activity

Disc diffusion assay

The susceptibility of the bacteria to *Moringa* aqueous extract was estimated by measuring the diameter of zone inhibition and values as average of three replicates according to Narms (2002).

Antifungal activity

Agar well diffusion assay

The susceptibility of the fungi to *Moringa* aqueous extract was estimated on Sabouraud dextrose Agar (SDA) by measuring the diameter of zone inhibition and values as average of three replicates according to Albuquerque et al. (2006).

Mean growth inhibition percentage by ELISA reader

The bacterial suspension was diluted to (10^6 CFU/ml) using Mueller Hinton Broth (MHB). As well as the fungi suspension was diluted to (10^6 cfu/ml) in Sabouraud dextrose broth (SDB). The adjusted microbial inoculums (100 μl) were added to each well of sterilized 96 well flat – bottomed micro titer plate containing the tested concentration (100 mg/ml) of *Moringa* aqueous extract. As a result, last inoculums concentration of (5×10^5 CFU/ml) was obtained in each well. Two wells containing bacterial suspension without *Moringa* aqueous extract (Growth control) and two wells containing media only as background control were included in this plate. Optical density was measured at 620 nm after 24 h at 37 °C incubation using ELISA micro plate reader (Sun Rise-TECAN, Inc., USA). The percentage of bacterial growth reduction (GR) was calculated using as reference the control treatment (without extract) as:

$$GR\% = C - T/C \times 100$$

where C = cell concentration (control) and T = cell concentration (extract treatment). Three replicates were considered. The results were recorded as means ± SE of the triplicate experiment (NCCLS/CLSI, 2007).

Table 2
Screening of antifungal activity of (100 mg/ml) aqueous extract of *Moringa* leaves.

Tested fungi/yeasts	Clear zones diameter[a] (mm)	Mean inhibition %[b]
Aspergillus niger	15.2 ± 0.52	75.2 ± 0.55
Aspergillus flavus	12.4 ± 0.55	59.4 ± 0.75
Penicillium italicum	10.5 ± 0.26	58.2 ± 0.63
Fusarium oxysporum	9.4 ± 0.71	46.5 ± 0.63
Rhizopus stolonifer	13.2 ± 0.58	62.5 ± 0.77
Alternaria sp.	6.6 ± 0.47	24.5 ± 0.65
Candida albicans	12 ± 0.44	20.3 ± 0.75
Candida parapsilosis	18 ± 0.54	80.00 ± 0.70

[a] The test was done using the diffusion agar technique, well diameter: (6.0 mm).
[b] Broth micro-dilution method, the experiments were performed in triplicate and the data are expressed in the form of mean ± SE.

Determination of minimum inhibition concentration (MIC)

MIC of *Moringa* aqueous extract was determined by the broth micro dilution method as approved by the guidelines of Clinical and Laboratory Standards Institute (NCCLS/CLSI, 2007).

Antivirulence activity

Biofilm formation assay of bacterial isolates

Tissue culture plate method as a quantitative test as described by Christensen et al. were implemented in this study. The inoculated trypticase soy broths with tested bacteria were incubated at 37 °C for 24 h. The cultures were diluted 1:100 with broth medium. Individual well of sterilized 96 wells of flat bottom polystyrene Tissue culture plates (Sigma-Aldrich, USA) were filled with 200 μl of diluted cultures. Negative control wells were filled with 200 μl of diluted broth medium. After incubation, the wells were washed with 0.2 ml of phosphate buffer (pH, 7.2) three times for removing free floating bacteria by gentle tapping. Biofilm formed by bacteria adherent to the wells were fixed by 2% sodium acetate and stained by crystal violet (1%). Excess stain was removed by deionized water and dried. Optical density of stained bacteria adherent recorded by using ELISA micro-titer - plate reader (Sun Rise – TECAN, Inc., USA).

Table 1
The growth inhibition of *Moringa* aqueous extract on bacterial isolates using micro-dilution method and measured by ELISA plate reader.

Bacterial isolates	Growth inhibition (O.D.620)		Bacterial isolates	Growth inhibition	
	ELISA reader[a]	Inhibition zone[a]		ELISA reader	Inhibition zone (mm)
B. cereus	85.9 ± 0.42	21.8 ± 0.55	*P. aeruginosa*	69.9 ± 0.20	12.5 ± 0.50
K. pneumonia	75.5 ± 0.78	15.2 ± 0.48	*S. aureus*	75.6 ± 0.70	15.5 ± 0.60
E. cloacae	74.63 ± 1.2	23.4 ± 0.27	*S. typhi*	79.7 ± 0.36	23.5 ± 0.45
E. coli	85.0 ± 0.27	20.8 ± 0.55	*P. vulgaris*	65.3 ± 0.34	15.5 ± 0.56

[a] Mean of 3 replicates ± standard error ((100 mg/ml)/well).