

ANTIMICROBIAL PROPERTIES OF PEPTIDES ISOLATED FROM MORINGA OLEIFERA SEEDS

M. UMAR DAHOT,[#] Z.H. SOOMRO AND M. ASHIQ⁺

^{*}Enzyme and Fermentation Biotechnology Research Laboratory

Department of Biochemistry, Institute of Chemistry

University of Sindh, Jamshoro, Pakistan

⁺HEJ Research Institute of Chemistry

University of Karachi, Karachi, Pakistan

ABSTRACT

Seven and Fourteen peptides from *Moringa oleifera* seeds were separated on Sephadex G-25 from acetone and ethanol prepared sample respectively. An antibacterial action of peptides were tested against *E. coli*, *Kl. aerogenes*, *Kl. pneumoniae*, *S. aureus* and *B. subtilis*. Peptide 1, 3 and 6 in acetone prepared sample and peptide 1, 4, 6, 7, 12, 13 and 14 in ethanol prepared sample of *Moringa oleifera* seeds showed good inhibitory activity against Gram +ve and Gram -ve bacteria. However Peptide 1 in acetone prepared sample and peptides 1, 2, 3, 5, 6, 7, 9, 10 and 14 had a notable inhibitory effect against fungi such as *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Penicillium expansum*.

INTRODUCTION

A large number of shrub,s plants and seeds are extensively used by traditional healers (Hakeems) and herbal specialists to cure various diseases. A small protein, peptide and natural products (such as oils, steroids, alkaloids etc.) have been extracted which have been found to possess antimicrobial activity. Numerous studies have been carried out to extract natural product for screening antimicrobial activity (Rizki *et al.*, 1987; Sheikh *et al.*, 1985; Islam *et al.*, 1989; Rasheed *et al.*, 1990; Farzana & Khan, 1993) but there has been no report on small protein peptide isolation for biological activity from Pakistani shrubs, plants and seeds but during recent years greater attention has been focused to study small protein and peptide having antimicrobial activity have been isolated from plants and seeds (Robert & Selitrenninkoff, 1990; Nair & Bruke, 1990; Robert *et al.*, 1988; Robert & Selitrenninkoff, 1986).

[#]Correspondence

In this communication, we are reporting for the first time the isolation of peptides from *Moringa oleifera* seeds possessing antibacterial and antifungal activity against gram positive, gram negative bacterial and fungal species. *Moringa oleifera* belongs to Moringaceae family which consist three species and differing mainly in colour of flowers. Locally *Moringa oleifera* is known as Sohanjna. *Moringa oleifera* can grow on all types of soil except still clays. The plant can be propagated by seeds or from cuttings. *Moringa oleifera* is widely distributed in Pakistan, India, Arabia, Asia minor, Africa, central and south America and West Indies (Jamieson, 1943). The flowers and fruits appears twice a year and are commonly used as a vegetable. Different parts of *Moringa oleifera* are used in the treatment of skin, respiratory and some diseases by the traditional healers (Hakeems) (Chopra, 1958).

MATERIAL AND METHODS

The seeds of *Moringa oleifera* were collected during July - August in dry state from the plants in local areas from University of Sindh employees colony.

Preparation of soluble extract:

10.0 grams of defatted residue of *Moringa oleifera* seeds were crushed in 33 ml cold distilled water and centrifuged at 4000 rpm. The supernatant was transferred to a 100 ml volumetric flask and this procedure was repeated twice and volume was made upto the mark with distilled water. Protein/peptides were precipitated by the addition of two fold ethanol or acetone. These isolated precipitates were dissolved in sterilized Distilled water and dialyzed overnight in the same.

Separation on Sephadex G-25:

The dialyzed ethanol or acetone precipitated fraction containing protein/peptide was subject to a column of Sephadex G-25 (1.5 x 137 cm) and eluted with 0.2 M acetic acid. Fraction of 4.5 ml each was collected on fraction collector at a flow rate of 48 ml/hr.

Determination of Protein:

The absorbance at 280 nm was measured to monitor the protein during chromatography separation. The protein content of water extract was measured by the method of Lowry *et al.* (1951), using bovine serum albumin as a standard.

Antibacterial activity:

The cultures of bacteria (*E. coli*, *Kl. aerogenes*, *Kl. pneumoniae*, *S. aureus* and *B. subtilis*) grown overnight at 37°C were used for testing the antibacterial activity of different fractions separated on Sephadex G-25. The antibacterial activity was checked

by seed plate method as reported by Rasheed *et al.* (1990). In this technique meat extract nutrient medium containing 1.5% agar was adjusted to pH 7.0, distributed in 40 ml quantity in screw capped bottles and sterilized. The bacterial culture was then added aseptically to the agar medium at 45°C, mixed well and poured immediately in sterilized petri-plates. After hardening, well were cut into agar and the *Moringa oleifera* seeds fractions were placed in these wells. The plates were incubated at 37°C and observations were made after 24 to 72 hours.

Antifungal activity:

Antifungal activity was tested against *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Penicillium expansum*. The diffusion plate method was used to test *Moringa oleifera* seeds fractions as reported by Reddish (1950) and Leven *et al.* (1979). In this technique, 0.1 ml of the fungal spore suspension (grown for 3 days in 10 ml of nutrient Dextrose agar) was thoroughly mixed with 20 ml of melted Sabouraud dextrose agar and poured into sterilized petri plates. When the agar was set, 5 holes of 6 mm diameter bore were made on each of the seeded plate. These holes were filled with 0.1 ml of the testing sample. All these experiments were performed in duplicate. The petri plates were incubated at 28°C for 7-8 days. All the culture plates were examined after 24-94 hours and the results are tabulated. The zone inhibition produced by the plant fractions were compared with zone produced by the standard.

RESULTS AND DISCUSSION

Table 1 illustrates the results of protein content, antibacterial and antifungal activity from the water extract of *Moringa oleifera* seeds. It is clearly noted that aqueous extract of *Moringa oleifera* seeds posses sufficient antimicrobial activity against gram +ve, gram -ve and fungal species.

The initial steps of antibacterial/antifungal protein purification was done as described in materials and methods. Protein/peptide was isolated from the extract of ground *Moringa oleifera* seeds by precipitation with ethanol and acetone. The ethanol/acetone fractions were then separated by sephadex G-25 column chromatography. Fourteen and seven optical density peaks were obtained as shown in Figure 1 and 2. The peptides associated with these optical density peaks from ethanol fraction were termed as EP1, EP2, EP3, EP4, EP5, EP6, EP7, EP8, EP9, EP10, EP11, EP12, EP13 and EP14 but peptide density pecks obtained from acetone fraction were termed as AP1, AP2, AP3, AP4, AP5, AP6 and AP7 in the order, in which they were eluted from the column (Figure 1 and 2).

Antibacterial/antifungal activities were assayed in EP1-EP14 but EP14 display strong antibacterial and antifungal activities by producing clear zones of inhibition

against a number of gram +ve and gram -ve bacteria such as *E. coli*, *Kl. aerogenes*, *Kl. pneumoniae*, *S. aureus* and *B. subtilis* and fungi including *Aspergillus niger*, *A. fumigatus*, *A. flavus* and *Penicillium expansum* as shown in Table 2. Peptide AP1 separated from acetone precipitated sample was found more active against fungi such as *A. niger*, *A. fumigatus*, and *A. flavus* but other fractions such as AP2 to AP7 did not show antifungal activity. However fractions (AP2 to AP7) were found slightly active against *E. coli*, *S. aureus* and *B. subtilis*. This observation provide strong circumstantial evidence that antifungal peptide/protein play an important role in plants antimicrobial defence system (Robert *et al.*, 1988).

The peptides isolated by Sephadex G-25 from ethanol an acetone precipitated fraction were found homogenous (results are not shown). The result of present study confirm that the popular utilization of *Moringa oleifera* seeds as an antimicrobial in skin and respiratory infection has some scientific justification. To our knowledge, this study is the first report on purification and characterization of antimicrobial peptide from plants (*Moringa oleifera* seeds) in this country. This study provides considerable scope in exploiting the local indigenous resources for isolation of antibacterial/antifungal peptides or smaller proteins. Further work is under progress in this Laboratory for isolation, purification and characterization of peptides/smaller proteins for antibacterial/antifungal activity from plants and results will be reported in near future.

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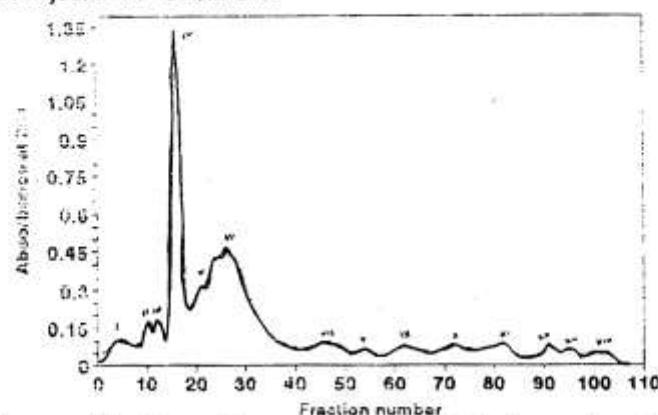


Fig. 1: Elution profile of peptides on Sephadex G-25 from ethanol extracted *M. oleifera*

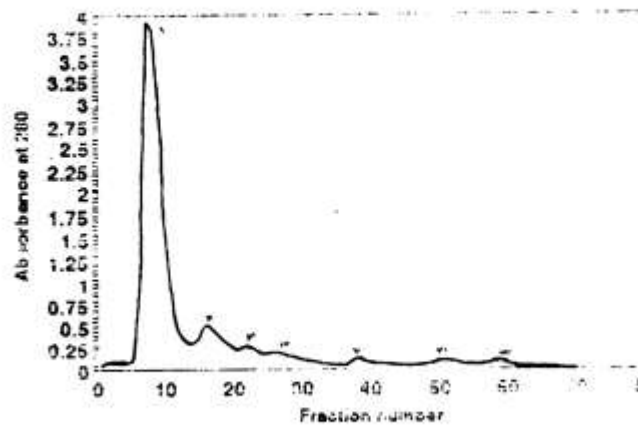


Fig. 2: Elution profile of peptides on Sephadex G-25 from acetone extracted *M. oleifera*

Table 1
Protein content and antimicrobial activity of crude aqueous extract of *Moringa oleifera* seeds

Protein content	3.4 mg/ml				
Antibacterial activity	E. coli	Kl. aerogenes	Kl. pneumoniae	S. aureus	B. subtilis
Inhibition zone	+ ve	Trace	+ ve	+ ve	+ ve
Antifungal activity	Aspergillus niger	Aspergillus fumigatus	Aspergillus flavus	Penicillium expansum	
Inhibition zone	+ ve	+ ve	+ ve	+ + ve	

Table 2
Antimicrobial activity of peptides isolated and purified
from *Moringa oleifera* seeds against bacteria and fungi.

Peptides isolated with ethanol	Bacteria					Fungi			
	<i>E. coli</i>	<i>Kl. aerro- genes</i>	<i>Kl. pneu- moniae</i>	<i>S. aureus</i>	<i>Bacillus sub- tilis</i>	<i>Asp. Funi- gatus</i>	<i>A. nigar</i>	<i>A. flavus</i>	<i>Penicilline expansum</i>
P-1	-ve	-ve	-ve	-ve	+	+	-ve	-ve	-ve
P-2	Trace	-ve	-ve	-ve	-ve	+	-ve	-ve	-ve
P-3	-ve	-ve	-ve	-ve	-ve	+	-ve	-ve	-ve
P-4	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
P-5	-ve	-ve	Trace	-ve	-ve	Trace	-ve	-ve	++
P-6	-ve	Trace	-ve	-ve	+	-ve	-ve	-ve	+++
P-7	Trace	-ve	Trace	+	-ve	-ve	-ve	-ve	++++
P-8	Trace	-ve	-ve	-ve	-ve	-ve	-ve	-ve	Trace
P-9	-ve	-ve	-ve	-ve	Trace	-ve	-ve	-ve	++
P-10	-ve	-ve	-ve	-ve	-ve	Trace	-ve	-ve	+
P-11	-ve	-ve	Trace	-ve	-ve	-ve	-ve	-ve	-ve
P-12	+	-ve	-ve	-ve	-ve -ve	-ve	-ve	-ve	-ve
P-13	+	-ve	-ve	+	++	Trace	-ve	-ve	-ve
P-14	+	+	++	+	++	+	++	++	++
Peptides isolated with acetone									
P-1	Trace	-ve	-ve	+	Trace	+	Trace	+	-ve
P-2	Trace	-ve	-ve	Trace	Trace	-ve	-ve	-ve	-ve
P-3	Trace	-ve	-ve	Trace	Trace	-ve	-ve	-ve	-ve
P-4	-ve	-ve	-ve	Trace	Trace	-ve	-ve	-ve	-ve
P-5	Trace	-ve	-ve	Trace	Trace	-ve	-ve	-ve	-ve
P-6	Trace	-ve	-ve	Trace	Trace	-ve	-ve	-ve	-ve
P-7	Trace	-ve	-ve	-ve	Trace	Trace	-ve	-ve	-ve -ve

Negative = -ve;

Positive = Trace = 2mm

+ = 5 mm inhibitory zone

++ = 10 mm inhibitory zone

+++ = 15 mm inhibitory zone,

++++ = 20 mm inhibitory zone

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