CHEMICAL AND BIOLOGICAL EVALUATION OF MORINGA SEEDS

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Abstract

The chemical composition of two cultivars of *Moringa peregrina* and *Moringa oleifera* seeds were studied and compared with that for soybean seeds for moisture content, crude protein, crude oil, total ash and total carbohydrates.

The highest content of oil and protein were found in *M. peregrina* which were 53.35 % and 20.66 %, respectively in comparison with that found in *M. oleifera* which were found to be as 45.05 % and 19.01 %, respectively.

Minerals content of the three samples were studied and the obtained results indicated that the three samples contained 16 elements, and potassium was the major one meanwhile cadmium (Cd), Lead (Pb) were the lowest. The Amino acids were determined by Amino acid analyzer, for the defatted meal of *M. peregrina* and *M. oleifera* where 17 amino acid were identified. It contains the major essential amino acids, also it contains arginin and glutamic in high percentages, meanwhile tryptophan was found in low percentage when compared with the amino acids of soybean meal.

As for the biological evaluation, The oil , water extract and ethanol extract of the meal and hulls were tested against some organisms for the two cultivars .

The obtained results indicated that the water extract and ethanolic extract of the two cultivars meal were effective against both *E-coli* (negative-gram) and *Saccharomyces cerevisia*.

Meanwhile the water extract and ethanolic extract of *M. oleifera* and only ethanolic extract of the *M. peregrina* were effective against *Staphillococcus aureus.* It also found that water extract of the two cultivars tested were effective against *Candida* (yeast).

The results also revealed that the water extract and ethanolic extract of each from the oil or hulls of the two cultivars had no effect against all microorganisms.

INTRODUCTION

The Moringa family consists of ca. 10 xerophytic species distributed from tropical Africa to the east India (Sengupta and Gupta, 1970). Four main species exist, namely *Moringa aptera, Moringa concanensis, Moringa olifera* and *Moringa pterygosperma*. All the species expect *Moringa peregrina* grow wild and are rapidly growing trees of 25-30 ft high which bear long seed pods, each pod containing ca. 20 seeds. *Moringa peregrina*, locally called "yassar" contains seeds which have long been used as a source of oil (Morton, 1991, Somali *et al.* 1984 and FAO, 1988). The *Moringa peregrina* kernel contains 1.8% moisture, 54.3% oil, 22.1% protein, 3.6% fiber, 15.3% carbohydrates and 2.5% ash (Somali *et al.,* 1984). AL-Kahatani and A.Abou-Arab (1993) studied the minerals in *Moringa peregrina* and soybean flour. They found that potassium and sodium were the predominant minerals in both *M. peregrina* and soybean flour. Amino acid contents were determined for Al-Ban seed products and protein fractions and amino acid scores were calculated for Al-Ban seed products. The defatted flour from Al-Ban seed contains all the essential amino acids in varying amounts. These products also contained high amounts of histidine, isoleucin, valine and leucine and contained low levels of lysine and tryptophan Al-Husssain and Al-Othman (2003) and Young and Pellet (1991) .

The antimicrobial activities of the leaves, roots, bark and seeds of *Moringa oleifera* were investigated *in vitro* against bacteria, yeast, dermatophytes and helminthes pathogenic to man. The aqueous extract from the seeds inhibted the growth of *staphillococcus aureus* while extractions temperatures above 56°C inhibited this activity. No activity was demonstrated against 4 other pathogenic gram-positive and gram- negative bacteria and *candida utilis* (Caceres *et al.* 1991).

Therefore, the present work aims to study the chemical and biological evaluation of two Moringa seeds.

2- MATERIALS AND METHODS

2.1 Materials

2.1.1 *Moringa peregrina* seeds

Moringa peregrina seeds were obtained from Marsa Alm, Alkosir, city

2.1.2 *Moringa oleifera* seeds

Moringa oleifera seeds were obtained from North Sinai Desert Station for Research and Extension.

2.1.3 Soybean seeds

Soybean seeds were obtained from soy processing plant, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt.

2.2 Methods

2.2.1 Extraction of the oil

The kernels of *Moringa peregrina* and *Moringa oleifera* seeds were pressed with laboratory – type of Carver hydraulic press under 10.000 Ib/in^2 (psi) pressure for 1h at room temperature according to the methods of Ustun *et al.* (1990). The produced oil was filtrated and kept in dark glass bottles in the refrigerator until analysis.

2.2.2 Chemical analysis

Determination of moisture, total lipids, crude protein, crude fiber and ash were determined according to the methods outlined in A.O.A.C. (2000).

2.2.3 Minerals content

Minerals content were determined in the diluted solution of ash samples by using the Plasma Optical Emission – mass Spectrometer Thermo elemental described in the AOAC (1990).

2.2.4 Determination of amino acid composition

Amino acids were determined according to Becker, *et al.* (1981) as follows: Samples were hydrolyzed using 5 ml. HCI (6N) sealed tubes at 110° C for 24hr. The digested samples were filtered and the volume was completed to 50 ml with distilled water, 5 ml were transferred to a glass dish and left to dry at room temperature, the

residue was dissolved in 5 ml of 0.2 N sodium citrate buffer PH 2.20. The solution was filtered through 0.22 in membrane.

Twenty incroliter of the final filtrate were loaded in the instrument capsule for the determination of the amino acids This volume contains 20 microgram protein.

Amino Acid Analyzer LC 3000 eppendorf was used in determination of the amino acids in all samples, under investigation

2.2.4.1 Determination of biological value

Under the term *in-vitro* biological value, chemical indexing was used here, that is biological value is calculated from amino acid profiles of the samples investigated. The chemical score of the protein was calculated by the determination of the limiting essential amino acid according to the standard FAO/WHO (1973) procedure., as follows: the contents of each of the essential amino acids in the protein samples were divided by those of the reference pattern. The concentration of the essential amino acids were expressed in gram of the individual amino acid per 16 grams of nitrogen. The amino acid with the smallest value of the above- mentioned ratios was the limiting essential amino acid. The reference pattern is presented in Table (1). Chemical scores (CS) were calculated as reported in (FAO/WHO, 1973) using the following formula.

$$\text{Amino acid score} = \frac{\text{Concentration of amino acid in the sample}}{\text{Concentration of amino acid in the reference}} \times 100$$

Table 1. FAO/WHO (1973) reference pattern.

Essential amino acid	G/16 gN
Isoleucine	4.00
Leucine	7.04
Lysine	5.44
Phenylalanine + Tyrosine	6.08
Methionine + Cystine	3.52
Threonine	4.00
Tryptophan	0.96
Valine	4.96

2.2.4.2 Determination of tryptophan

Tryptophan was determined colorimetrically in the alkaline hydrolysate according to the method of Blauth, *et al.* (1963). In this method, approximately 0.25gm of sample was accurately weighed and heated in a sealed tube with saturated barium hydroxide solution (14%) for 24 hours in an oven at 120 C. After hydrolysis, the excess alkali was neutralized to PH 7.0 with carbon dioxide and the excess of which was removed by moderate heating. The hydrolysate was diluted to 100 ml with distilled water in a volumetric flask. One ml of the neutral hydrolysate was quantitatively transferred to a test tube. Two ml of acetic acid containing iron [Ferric chloride 0.27 mg ($Fe\ Cl_3.6H_2O$) was dissolved in 0.5 ml. of water and glacial acetic acid was added to the one liter mark] was added, followed by 2 ml of concentrated sulphuric acid. The tube was then shaked vigorously and left to stand for 15 min. The absorbance of the developed color was measured in a Unicam spectrophotometer at 460 nm. and standard calibrated curve was prepared using known concentrations of tryptophane solutions and developed in the same manner as the unknown samples

2.2.5 Determination of the antimicrobial activity of *Moringa peregrina* and *Moringa oleifera* oil and aqueous, ethanolic alcohol extract of kernel seed meal and husk.

2.2.5.1 preparation of aqueous extracts

100 ml distilled water were added to 10 gm grinding seed meal or grinding seed husk then shacked using orbital shaker at 250 r.p.m. for 8 hours. The mixture was filtered using cheese cloth and the extract was taken to determine its antimicrobial activity.

2.2.5.2 Ethanolic alcohol of *Moringa peregrina* and *Moringa oleifera* grinding kernel seed meal and grinding seed husk

10 gm. Of grinded seed meal or grinded seed husk were added to 100 ml. ethanolic alcohol 95%, then shacked using orbital shaker at 250 r.p.m. for 8 hours. The mixture was filtered using cheese cloth and the extract was taken to determine its antimicrobial activity.

2.2.5.3 Microorganisms

All microorganisms used for this study were kindly obtained from Faculty of Agriculture, Ain Shams University. The bacterial strains used were *Bacillus cereus* ATCC 33018, *Staphillo coccus aureus* ATCC 25923 (Gram positive bacteria), *Esherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 20231 (Gram negative bacteria). The yeast strains were *Saccharomyces cerevis ceae, Candida utilis*, and the fungi strain was *Aspergillus niger*.

2.2.*5.4* Propagation media

Bacterial strains were propagated and enumerated in nutrient agar media according to Oxoid Manual (1979). Culture were incubated at 30°C (except *E.coli* at 37°C) for 24 hours. Mold and yeast strains were maintained and propagated on potato dextrose agar (PDA).

2.2.5.5 Testing media

Nutrient agar media was used in testing the antimicrobial activity of the studied *Moringa peregrina* and *Moringa oleifera* oil and aqueous, ethanolic alcohol extract of kernel meal and seed husks against the growth of bacteria. The antimicrobial activity of these extracts against fungi and yeast growth were determined using (PDA).

2.2.5.6 Determination of antimicrobial activity

The in-vitro antimicrobial properties of *Moringa peregrina* and *Moringa oleifera* oil and aqueous, ethanolic alcohol extract of kernel meal and husks were evaluated separately towards various species of bacteria, fungi and yeast employing the filter-paper-disk-diffusion plate method according to Hassan (1994).

RESULTS AND DISCUSSION

Chemical composition of *Moringa peregrina* ,*Moringa oleifera* and soy bean seeds and defatted meals

The chemical composition of two cultivar, namely *Moringa peregrina* and *Moringa oleifera* seeds were studied and compared with soybean seed for its moisture content, crude protein, crude oil ,total ash and total carbohydrates as shown in table (2). The result found in table (2) reveal that *M. oleifera* and *M. peregrina* can be considered as rich source for oil since it contained 45.05 % and 53.35%, respectively, meanwhile

soybean seeds contained 19.96% oil, in addition it could be noticed that moisture content was 5.55% ,4.19% and 7.65% for the three samples respectively .

The crude protein content of the two Moringa samples were 19.01% and 20.66% respectively, it was 28.83% in soybean seeds, the results in table (2) also show that the total carbohydrates was higher in *M. oleifera* (25.11%) and soybean seeds (32.44%) than in *M. peregrina* (12.99%) while the fiber content was 2.54%, 6.63% and 6.51% in the three samples respectively. In addition the ash content was 2.17%, 2.74% and 4.61% respectively.

It could be noticed that the defatted meal of the three samples contained a higher content of crude protein, total carbohydrates, fiber and ash than the seeds. These results are in accordance with those obtained by Somali *et al.* (1984).

Amino acid composition of defatted *Moringa peregrina*, *Moringa oleifera* and soybean meals

Amino acid content and types are very important parameters to evaluate the protein. The amino acid composition of samples under investigation was quantitatively determined by Amino acid analyzer LC 3000, and the result is calculated on the base gm Amino acid /100gm protein.

Results of the Amino acid composition of the samples found in table (3) indicated that, argenine and glutamic acid in *Moringa peregrina*, *Moringa oleifera* and soybean protein are the most abundant amino acid which recorded 11.9 and 7.9, 14.4 and 9.8, 8.6 and 7.8 mg/100gm protein respectively, On the other hand, tryptophan in *Moringa peregrina* and *Moringa oleifera* protein as well as methionine, tryptophan and cystine in soybean protein are present in lesser quantities of amino acid.

The concentration of the previous amino acids were 0.78, 0.91 mg/100gm protein respectively as well as 0.53, 1.12 and 1.98 mg/100gm protein respectively. Therionine and cystine are nearly the same in *Moringa peregrina*, *Moringa oleifera* and soybean proteins which were as 2.1, 2.3 and 2.9 mg/100gm protein for thrionine and as 1.1, 1.2 nd 1.9 mg/100gm protein for cystine respectively. In addition, tyrosine has the same concentration in *Moringa peregrina*, *Moringa oleifera* proteins 1.6 mg/100 gm protein.

These results are in agreement with Al. Hussain and Al-Othman (2003) and lopez *et al.* (1991)

IN vitro Biological Value of _Moringa peregrine, Moringa oleifera_ and soybean proteins

The data presented in table (4) show the essential amino acid composition and amino acid scores of *Moringa peregrine, Moringa oleifera* and soybean meals according to (FAO/WHO 1973) pattern. The results of chemical score indicated that 1/sine in soybean were the Limiting essential amino acids, their chemical scores recorded 37%, 44.4% and 58%.

On the other hand, the aromatic amino acids (phenylalanine and tyrosine) in *Moringa peregrine, Moringa oleifera* and soybean were the most abundant amino acids which recorded 133.8%, 100.6% and 133.3% respectively.

These results are in agreement with that reported by Al Hussain et al., 2003 and pellet et al., 1980)

Minerals content of Moringa peregrina ,Moringa oleifera and soybean meals

Data of mineral content are presente in table (4). it shows that, calcium is the predominant mineral in *Moringa peregrina* meal which recorded 406.33 mg/100gm sample and potassium is the most abundant mineral in *Moringa oleifera* and soybean meals which recorded 416.66 , 1116.6 mg / 100 gm sample respectively

On the other hand, Pb (lead) of *Moringa peregrina*, soybean meal and Pb (lead), cadmium of *Moringa oleifera* meal are present in low quantities.

The concentrations of previous minerals were 0.0033, 0.0016 and 0.0033 mg/100gm sample respectively. Moreover, Chromium is nearly the same in *Moringa peregrina, Moringa oleifera* and soybean meal which recorded 0.45, 0.52 and 0.57 mg/100gm) sample respectively. In addition, pb (lead) has the same concentration in both *Moringa peregrine* and *Moringa oleifera* meals which was as 0.0033 mg/100gm sample while cd (cadmium) of both *Moringa peregrina* and soybean meals was as 0.005 mg/100gm sample for both meals. These results are nearly agreement with Al-Kahatani and Abou-Arab (1993) .

Table 2. Chemical Composition of *Moringa peregrine, Moringa oleifera* and Soybean seeds and defatted meals:

Component% (dry wt basis)	Moringa peregrina		Moringa oleifera		Soybean	
	Seed	Defatted meal	Seed	Defatted meal	Seed	Defatted meal
Moisture	4.19	-	5.55	-	7.65	-
Crude oil	53.35	-	45.05	-	19.96	-
Crude protein	20.66	48.67	19.01	38.49	28.83	39.83
Total carbohydrate	12.99	31.26	25.11	50.82	32.44	44.82
Crude fiber	6.63	14.95	2.54	5.14	6.51	9.00
Ash	2.17	5.12	2.74	5.55	4.61	6.35

Table 3. Amino acid composition of defatted *Moringa peregrine, Moringa oleifera* and soybean meals (mg/100gm protein)

Amino acids	Moringa peregrina	Moringa oleifera	Soybean
Asparitic	3.40	3.81	5.42
Therionine	2.12	2.37	2.92
Serine	2.11	2.59	3.15
Glutamic	7.93	9.85	7.80
Glycine	3.41	3.96	3.04
Alanine	3.95	4.63	3.85
Valine	2.69	3.04	2.86
Methionine	1.09	1.62	0.53
Cystine	1.19	1.21	1.98
Isoleucire	2.85	4.03	3.73
Leucine	4.35	4.70	5.06
Tyrosine	1.64	1.62	2.66
Phenylalanine	4.21	4.80	5.39
Histidine	3.80	3.97	4.50
Lysine	2.05	2.40	5.93
Argenine	11.97	14.48	8.64
Tryptophain	0.78	0.91	1.12

Table 4. Essential amino acid composition and amino acid score of *Moringa peregrina*, *Moringa oleifera* and soybean meals according to FAO/WHO 1973 pattern.

Amino acids	FAO/WHO (1973) pattern (g/100g protein)	Amino acid content (g/100g protein)			Amino acid scores		
		Moringa peregrina	Moringa oleifera	soybean	Moringa peregrine	Moringa oleifera	Soybean
Isoleucine	4	2.8	4.00	3.7	70	100	92.5
Leucine	7	4.3	4.7	5.00	61.4	67.1	71
Lysine	5.4	2.00	2.4	5.9	37.00*	44.4*	109.2
Total sulphur amino acid (cystine+methionine)	3.5	2.28	2.83	2.5	65.1	80.8	71.4
Total aromatic amino acid							
(phenylalanine+tyrosine)	6	8.00	6.4	8.03	133.3	106.6	133.8
Threonine	4	2.1	2.4	2.9	52.5	60	72.5
Tryptophan	1	0.78	0.91	1.12	78	91	112
Valine	5	2.7	3.00	2.9	54	60	58*

* First limiting

Table 5. Mineral contents of *Moringa peregrina, Moringa oleifera* and soybean meals (mg/100gm sample)

Mineral	Moringa peregrina	Moringa oleifera	Soybean
Al	5.14	4.43	4.70
B	3.73	1.57	5.81
Ca	406.33	192.17	309.83
Cd	0.005	0.0033	0.005
Co	0.025	0.10	0.06
Cr	0.45	0.52	0.57
Cu	1.27	1.00	1.89
Fe	39.30	42.70	48.05
K	42.50	416.66	1116.66
Mg	254.66	328.66	231.50
Mn	1.89	2.21	4.189
Na	108.33	133.30	150.00
Ni	0.56	0.33	0.45
Pb	0.0033	0.0033	0.0016
Sr	1.55	4.16	1.80
Zn	25.18	5.05	4.80

Antimicrobial activity of *Moringa peregrina* and *Moringa oleifera* oil, meal and husk extracts

The antimicrobial activity of tested extracts of cake, husks and oil of each of *M. peregrine* and *M. oleifera* against selected microorganisms associated with food spoilage and human diseases are listed in Table (6). It is obvious from Table (6) that each of water, ethanolic extracts of *M. peregrina* and *M. oleifera* husks and oil had no effect against all tested microorganisms. Concerning *M. peregrina* extracts the maximum antimicrobial activity was shown with aqueous extracts against *E. coli, Saccharomyces cerevisiae* and *Candida sp.* The diameters of inhibition zones were 25, 25 and 1.0 mm, respectively. While the diameter of inhibition zones formed by the ethanolic extract were 1, 2, 15 mm against growth of each of *E. coli, Staphylococcus aureus* and *Saccharomyces cerevisiae,* respectively. Table (6) indicated also that aqueous extract of *Moringa oleifera* had the same effect as aqueous extract of *M. peregrina* against *E. coli* and *Saccharomyces cerevisiae.* The inhibition zones diameter were 25 and 25 mm, respectively. Also, aqueous extract had an effect on *Staphillococcus aureus* and *Candida sp.* growth. The diameter of inhibition zones were 2 and 0.7 mm respectively. It could be concluded from Table (6) that aqueous extracts of each *M. peregrina* and *M. olifera* had strong antimicrobial effect

than ethanolic extracts. An unexpectedly results was that water of the meal remained after oil extraction of *M. peregrina* and *M. olifera* by pressing was found to have strong effect on the tested microorganisms comparatively with ethanolic extract of such cake. This strong polar compound in aqueous extracts. These results agreed with finding of Caceres *et al.* (1992) who found that aqueous extract of *M. olifera* seed had antimicrobial activity against each of *Seudomonas aeruginoa* and *Staphillococcus aureus*

Table 6. Antimicrobial activity of *Moringa peregrina* and *Moringa oleifera* oil, meal and husk extracts.

Inhibition zone Diameter in mm.											
	Moringa peregrina					Moringa oleifera					control
Extract / Organism	Aqueous Extract		Ethanolic Alcohole Extract		Oil	Aqueous Extract of		Ethanolic Alcohole Extract		Oil	
	meal	husk	meal	husk		meal	husk	meal	husk		
Bacillus cereus ATTCC 33018	—	—	—	—	—	—	—	—	—		—
Escherichia coli ATCC 25922	25	—	1	—	—	25	—	12	—	—	—
Salmonella typhimuriun ATCC 20231	—	—	—	—	—	—	—	—	—	—	—
Staphillococcus aureus ATCC 25923	—	—	2	—	—	2	—	0.8	—	—	—
Saccharonyces cerevisceae	25	—	15	—	—	25	—	18	—	—	—
Candida utilis	1	—	—	—	—	0.7	—	—	—	—	—
Aspergillus niger	—	—	—	—	—	—	—	—	—	—	—

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التقييم الكيميائي والبيولوجي لبذور المورينجا

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تم دراسة التركيب الكيماوي لصنفين من بذور المورينجا وهما :- *Moringa peregrina* ,*Moringa oleifera* ومقارنة ذلك ببذور فول الصويا وذلك من حيث نسبة الرطوبة والبروتين و الزيت و الرماد الكلي والكربوهيدرات الكلية. ولقد وجد أن صنف *M. peregrina* يتميز بارتفاع نسبة الدهن والبروتين حيث بلغت ٥٣,٣٥٪و ٢٠,٦٦٪ على التوالي وهى تعتبرأعلى من تلك الموجودة في صنف *M. oleifera* حيث كانت٤٥,٥ ٪ و ١٩,٠١٪على التوالي.

كما تم تحليل العناصر المعدنية وذلك للعينات الثلاثة تحت الدراسة حيث تبين أنها تحتوي على حوالي ١٦ عنصر ووجد أن عنصر البوتاسيوم يمثل أكبر نسبة بينما عنصر الكادميوم والرصاص هما أقل نسبة.

كذلك تم تفريد الأحماض الأمينية بواسطة جهاز Amino Acid Analyzer في كسب بذور المورينجا (الصنفين) حيث أمكن التعرف على ١٦ حامض أميني وتشمل معظم الأحماض الأمينية الأساسية وكذلك تحتوي على نسبة عالية من حامض الأرجنين والجلوتاميك بينما تواجد الحامض الأميني التيروزين والميثيونين بنسبة قليلة وذلك مقارنة بتلك الأحماض الأمينية في كسب بذور فول الصويا.

تم دراسة التأثير المضاد لبعض الميكروبات لكلاً من الزيت والمستخلص المائي والإيثانولي لكسب وقشور صنفي المورينجا حيث تبين أن كلاً من المستخلص المائي وكذلك الإيثانولي لكسب صنفي المورينجا له تأثيرمضاد للبكتريا *E.Coli*(السالبة لجرام) وكذلك مضاد للخميرة *Sacharomyces cerevisia*

كما أن المستخلص المائي والإيثانولي لكسب صنف *M. oleifera* وكذا المستخلص الإيثانولي فقط لكسب صنف *M. peregrina* كان له تأثير مضاد لبكتريا *Staphillococcus Aureus* بينما كان هناك تأثير مضاد لـ*Candida* وذلك بواسطة المستخلص المائي لكسب صنفي بذور المورينجا.

كما أظهرت النتائج أن المستخلص المائي والإيثانولي لزيت وقشور بذور صنفي المورينجا ليس لها أي تأثير مضاد للميكروبات تحت الدراسة .