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Preparing New Beverage from *Moringa oleifera* Leaves

Eman M. Abd El-Rahim ; A. I. Abd El-Gawwad ; M. M. Rabie and Rania E. El-Gammal
Food Industries Department, Faculty of Agriculture, Mansoura University, 35516, Mansoura, Egypt

ABSTRACT

Consumption of phenolic-rich beverages were widely spread nowdays, this could be due to their effect against different diseases, medical and nutritional values. So, this work was carried to prepare new beverage from *Moringa Oleifera* leaves. Chemical composition, sensory evaluation and bioactive phenolic compounds were determined. Results of chemical composition showed that *M.Oleifera* leaves considered as a rich source of protein, fibers and minerals which reached to 21.40, 7.46 and 5.33 % respectively. Fractionation and identification of bioactive phenolic compounds using HPLC indicated that *M.Oleifera* leaves powder contained nearly about 24 phenolic compounds, the predominant one was e-vanillic being 2022.38 ppm followed by syringic acid 1011.28 ppm. Sensory evaluation of sixteen formula from *M.Oleifera* leaves and other common beverages, namely (green tea, peppermint, anise and cinnamon) at different ratios were prepared. Results of sensory evaluation indicated that Moringa beverage recorded the highest consumer acceptability followed by the formula containing (1.25 Moringa: 0.75 Green tea) then (1.25 Moringa : 0.75 Anise) respectively. Also results showed formula containing (1.25 Moringa : 0.75 Green tea) had a high contont of total phenolic compound compared with Moringa beverage and formula containing (1.25 Moringa: 0.75 Anise). Results of Antioxidant activity (DPPH%) for prepared beverages showed that Moringa beverage recorded 92.58% followed by formula containing (1.25 Moringa: 0.75 Green tea) which was 89.13% . Obtained results of mineral contents showed a gradual increase in Ca, K and Fe in Moringa beverage in compared with formula containing (1.25 Moringa: 0.75 Green tea) and the formula containing (1.25 Moringa: 0.75 Anise). So, from this study it could be concluded that the possibility of using *M.Oleifera* leaves as a daily beverage alone or combined with other commercial beverages.

Keywords : *Moringa Oleifera*, Green tea, Anise, phenolic compounds and antioxidant activity.

INTRODUCTION

Moringa Oleifera belongs to the family of Moringaceae included 14 species which growing fast in the tropics and subtropics areas is native to India, Africa, Asia Minor and sub-Himalayan tracts, drought tolerant, the height of the tree ranged from 5 to 10 m in three years. It is spread in North and South America, Cambodia, The pacific and Caribbean islands (Morton, 1991; Anwar and Bhanger, 2003; Anwar *et al*., 2005; Crosby, 2007; Oluduro, 2012 and Rockwood *et al*., 2013).

Moringa called as the miracle tree which contain 36 anti-inflammatories, more than 539 bio-chemical activities and 46 antioxidants natural components. Its leaves a rich source of high nutrition value such as various phenolics, essential minerals like calcium and potassium, vitamins like B,C and A amino acids and β-carotene, so it can used in modern science to prevent many diseases and cultivated in Malnutrition areas and remote countries for medical and nutrition values benefits (Debnath and Guha, 2007; Toba *et al*., 2010; Mishra *et al*., 2011; Oluduro, 2012 and Misra and Misra, 2014).

Moringa leaves act as antimicrobial, antidiabetic, antioxidant, anti-atherosclerotic and hypertensive agents and anticancer, which a cure liver diseases, cardiovascular and hypoglycemic actions, asthma, malaria, eye and ear infections, headaches, skin diseases, hyperglycemia, diarrhea, scurvy, heart burn, bronchitis, syphilis, Strengthens the immune and digestion systems, hypocholesterolemic and regulate thyroid hormone, also enhance the shelf life of fat containing food to contain antioxidant compounds like phenolics, ascorbic acid and carotenoids (Dillard and German, 2000; Sreelatha and Padma, 2010; Mbikay, 2012 and Rockwood *et al*., 2013).

Moringa leaves is eaten raw as a salad green or combined with other vegetables and grains or cooked as spinach and other greens, introduced as a side dish with other food or as a nutrition main course, the dried powder from leaves added to soups, sauces or spinkled on other food to raise the nutritional value, and is used the dried powder in supplement form or as a healthful drink like tea or mixed with cold or hot drinks (Fahey, 2005).

So, the objective of this work was to study the possibility of preparing a new beverages formula from *Moringa Oleifera* leaves rich in natural phenolic compounds and antioxidants in compare with other common beverages.

MATERIALS AND METHODS

Raw materials:

Moringa Oleifera (M. Oleifera) trees have been cultivated to ensure the plants were healthy and uninfected, leaves was collected from Faculty of Agriculture farm, Mansoura University, El-Mansoura City, El-Dakahleia Governorate, Egypt.

Commercial beverages:

Commercial Green tea, Peppermint, Anise and Cinnamon were purchased from local Market (Sun Mall), El-Mansoura City, El-Dakahleia Governorate, Egypt.

Chemicals:

All chemicals were purchased from El-Gomhouria pharmaceutical company, El-Mansoura City, El-Dakahleia Governorate, Egypt.

Methods

Preparation of *Moringa Oleifera* leaves:

Leaves of *M.Oleifera* were collected and washed under running tap water to eliminate dust and other foreign particles, then dried in the greenhouse (30- 45ºC) for one week then crushed to fine powder using domestic blender (BRAUN). Powdered was stored in polyethylene plastic bags at 5±1ºC until analysis.

Preparation of *Moringa Oleifera* beverages:

Powder of *M.Oleifera* leaves was added to green tea, peppermint, anise and cinnamon as shown in Table (1), each packet contain 2 gm, then 200 ml hot water was added, finally prepared beverage were panel tested at Food Industries Dept., Fac. of Agric., Mans.University.

Table 1. Formulas of prepared beverages from *M.Oleifera* leaves powder, Green tea, Peppermint, Anise and Cinnamon powders.

Formula	Powder of *M.Oleifera* leaves (gm)	Green tea (gm)	Peppermint (gm)	Anise (gm)	Cinnamon (gm)
M	2	-	-	-	-
G_1	1	1	-	-	-
G_2	1.25	0.75	-	-	-
G_3	1.50	0.50	-	-	-
G_4	1.75	0.25	-	-	-
B_1	1	-	1	-	-
B_2	1.25	-	0.75	-	-
B_3	1.50	-	0.50	-	-
B_4	1.75	-	0.25	-	-
A_1	1	-	-	1	-
A_2	1.25	-	-	0.75	-
A_3	1.50	-	-	0.50	-
A_4	1.75	-	-	0.25	-
C_1	1	-	-	-	1
C_2	1.25	-	-	-	0.75
C_3	1.50	-	-	-	0.50
C_4	1.75	-	-	-	0.25

Proximate chemical analysis:

Moisture content, crude oil, crude protein, crude fiber and ash of *M.Oleifera* leaves was estimated according to (A.O.A.C. 2000) .

Identification and fractionation of phenolic compounds:

Phenolic compounds of dried *M.Oleifera* leaves was determined using HPLC at Food Tech. Res. Institute, Agric. Res. Center, El-Giza, Egypt, according to (Goupy *et al*., 1999) .

Determination of total phenolic compounds (TPC):

The Folin-Ciocalteu method was used for determining of total phenolic compounds of dried *M.Oleifera* leaves and other combined beverages by using standardized spectrophotometric method at Food Tech. Res. Institute, Agric. Res. Center, El-Giza, Egypt according to (Ivanova *et al*., 2010).

Determination of radical scavenging activity (DPPH%):

2,2 diphenyll-picrylhydrazyl (DPPH%) assay of dried *M.Oleifera* leaves and beverages were carried out according to the method of (Brand-Williams *et al*., 1995) at Food Tech. Res. Institute, Agric. Res. Center, El-Giza, Egypt.

Minerals content:

Calcium, potassium and iron were determined using Sens AA ″GBC scientific equipment″ model ″Sens AA Dual″ made in Dandenong, Victoria, Australia at Atomic absorption, Micro-Analysis unit, Faculty of Science, Mansoura University, Egypt.

Statistical analysis:

Data were statistically analyzed according to the technique of analysis variance (ANOVA), the least significant difference (L.S.D) and Duncan's methods was used to compare the differences between the means of treatment values to the methods described by (Gomez and Gomez, 1984). All statistical analyses were performed using analysis of variance technique by means of Co STATE computer software.

RESULTS AND DISCUSSION

Proximate chemical composition of *Moringa Oleifera* leaves powder:

The proximate chemical composition of *M.Oleifera* leaves powder was determined, results in Table (2), revealed that *M.Oleifera* leave powder could be considered as a good source of crude protein, crude ash and fibers .

From data presented in Table (2), it could be noticed that the moisture content of *M.Oleifera* leaves powder (MOLP) was 8.16%, the protein content reached to 21.40 %, which consider *M.Oleifera* leaves as a good and cheap source of protein supplement in countries suffering from malnutrition. These obtained results were lower than those obtained by (Ilyas *et al*., 2015 and Ismael *et al*., 2016) who mentioned that the crude protein of (MOLP) were 28.11 and 38.1 % respectively .

Table 2. Proximate chemical composition of *Moringa Oleifera* leaves powder.

Component (dry wet basis%)	*Moringa Oleifera* leaf powder
Moisture	8.16
Crude protein	21.40
Crude fiber	7.46
Crude oil	10.02
Ash	5.33

Also results presented in Table (2), showed that fiber content of (MOLP) was 7.46 %, this higher fibers content aids indigestion and prevention of many diseases (Saldanha, 1995). These results was similar to those reported by (Ismael *et al*., 2016) who found that the crude fibers of (MOLP) was 7.40% but was lower than those mentioned by (Ilyas et al., 2015) who reported that the crude fibers of (MOLP) was 19.61 ± 0.38 % .

As shown in the same Table (2), it could be observed that the oil content of (MOLP) was 10.02 %. The ash content of (MOLP) was 5.33% which indicated the presence of suitable quantity of minerals in the leaves, these results was lower than those mentioned by (Ilyas *et al*., 2015 and Ismael *et al*., 2016) who found that ash content of (MOLP) was 10.50 and 6.80 % respectively. So the differences in chemical composition would be attributed to the differences in the stage of maturity of the plants as well as the soil fortified with different chemical fertilizers and geographical location of the plants as reported by (Ilyas *et al*., 2015) .

Identification and fractionation of phenolic compounds content (ppm) in *Moringa Oleifera* leaves powder:

Phenolic compounds are known as antioxidants which have long been recognized to have protective function oxidative damage in diet and may provide health benefits with reduced risk of chronic diseases (Karppinen *et al*., 2004). Therefore phenolic compounds were determined and identified in *M.Oleifera* leaves powder and results presented in Table (3), it can be observed that *M.Oleifera* leave powder contained 24 fractionated and identified phenolic compounds. The predominant phenolic compound being e-vanillic was (2022.38 ppm) followed by syringic acid (1011.28 ppm) and benzoic (939.99 ppm), while moderate amounts of pyrogallol, catechol, protocatchuic and caffeine recorded (515.68, 468.86, 289.70 and 111.11 ppm) respectively .

Table 3. Identification and fractionation of phenolic compounds of dried *Moringa Oleifera* leaves powder.

Phenolic compound	content (ppm)
Syringic	1011.28
Gallic	7.88
Pyrogallol	515.68
4- Amino-benzoic	5.56
Protocatchuic	289.70
Catechein	53.99
Chlorogenic	45.67
Catechol	468.86
Epicatechein	84.95
Caffeine	111.11
P-OH-benzoic	63.66
Caffeic	52.17
Vanillic	56.35
Ferulic	22.11
Iso-ferulic	29.07
E-vanillic	2022.38
Ellagic	91.20
Alpha-coumaric	8.85
Benzoic	939.99
Salycilic	46.03
3,4,5-methoxy-cinnamic	5.39
Coumarin	27.41
p-coumaric	8.83
Cinnamic	3.02

Also, an adequate amounts of cinnamic, 3,4,5-methoxy-cinnamic, 4-amino-benzoic, gallic, p-coumaric, alpha-coumaric, ferulic, Coumarin, iso-ferulic, chlorogenic, salycilic, caffeic, catechein, vanillic, p-oH-benzoic, epicatechein and ellagic were registered.

The presence of these compounds in *M.Oleifera* leaves powder can also modulate the lipid peroxidation involved in atherogenesis, coagulation and carcinogenesis in humans (Siddhuraju and Becker, 2003) .

Sensory evaluation of Moringa beverages:

Sensory evaluation considered as an important indicator of potential consumer preferences. In spite of its short comings, it will remain the most serious quality indicator. Results of sensory evaluation include appearance, taste, aroma, colour and overall-acceptability are presented in Table (4) .

Data in Table (4), indicate that control beverage sample contained only *M.Oleifera* have an acceptable level up to 40.400 while the other beverage formula which containing (*M.Oleifera* : green tea) with the ratio of 1.25 : o.75 and the formula containing (*M.Oleifera*: anise) with the ratio of 1.25 : 0.75 nearly showed the same overall-acceptability which recorded 40.025 and 39.700 respectively .

Also, from the same Table it can be observed that addition of (*M.Oleifera* : green tea) at the ratio of 1.75:0.25 enhanced the taste to be more accepted in compared with control one. Also results showed that non significant differences observed in aroma was found in the beverage formula M and G_2. Results in the same Table showed that addition of (*M.Oleifera* : pepperiment) at the ratio of 1:1 and 1.75 : 0.25 could improve the taste in compared to control formula with score 7.550 .

Table 4. Sensory evaluation of beverages *Moringa Oleifera* leaves powder

Samples	Apperance	Taste	Aroma	Colour	Overallacceptability	Total
M	8.600^{a}	7.500^{ab}	7.950^{a}	8.200^{ab}	8.150^{a}	40.400^{a}
G_1	8.300^{abc}	7.550^{ab}	7.750^{abc}	7.825^{abc}	8.100^{a}	39.525^{abc}
G_2	8.300^{abc}	7.700^{ab}	7.950^{a}	8.050^{ab}	8.075^{a}	40.025^{ab}
G_3	8.150^{bcd}	7.500^{ab}	7.700^{abcd}	8.000^{ab}	7.700^{abc}	39.000^{abcd}
G_4	8.300^{abc}	7.850^{a}	7.400^{bdef}	8.100^{ab}	7.775^{abc}	39.225^{abc}
B_1	7.700^{ef}	7.550^{ab}	7.700^{abcd}	7.925^{abc}	7.925^{ab}	38.750^{abcd}
B_2	7.850^{def}	7.425^{ab}	7.775^{abc}	7.975^{ab}	8.000^{ab}	38.925^{abcd}
B_3	8.175^{abcd}	7.450^{ab}	7.525^{abcde}	7.950^{abc}	8.000^{ab}	38.750^{abcd}
B_4	8.000^{bcde}	7.550^{ab}	7.250^{def}	8.050^{ab}	7.525^{abc}	38.375^{abcd}
A_1	8.350^{ab}	7.450^{ab}	7.500^{abcde}	8.100^{ab}	7.825^{abc}	39.075^{abc}
A_2	8.350^{ab}	7.550^{ab}	7.700^{abcd}	8.250^{ab}	7.950^{ab}	39.700^{abc}
A_3	8.300^{abc}	7.450^{ab}	7.325^{bcdef}	8.300^{a}	7.700^{abc}	39.075^{abc}
A_4	8.200^{abcd}	7.200^{ab}	7.050^{ef}	7.850^{abc}	5.475^{d}	37.475^{bcde}
C_1	7.850^{def}	7.300^{ab}	7.300^{cdef}	7.550^{bcd}	7.200^{bc}	37.200^{cde}
C_2	8.300^{abc}	7.375^{ab}	7.800^{ab}	7.650^{abcd}	7.625^{abc}	38.650^{abcd}
C_3	7.900^{cdef}	6.950^{b}	7.150^{ef}	7.300^{cd}	7.200^{bc}	36.450^{de}
C_4	7.500^{f}	6.850^{b}	7.000^{f}	7.150^{d}	7.075^{c}	35.575^{e}
LSD at 5%	0.67	0.79	0.69	0.74	0.99	2.84

Also in the same Table (4), it could be noticed that formula contained (*M.Oleifera* : anise) at ratio of 1.25 : 0.75 enhanced taste to be more accepted in compared with control with score 7.550, and addition of the (*M.Oleifera* : anise) at ratio of 1.25 : 0.75 and 1.50 : 0.50 also improved colour in compare with control one .

Total phenolic compounds, antioxidant activity and some minerals content of prepared beverages with or without *Moringa Oleifera*:

Amount of phenolic compounds could be considered as a good preventative tool against many diseases. From data presented in Table (5), it can be noticed that The highest content of total phenolic compounds (TPC) was observed in G_2 beverage formula which contained 1.25 Moringa : 0.75 green tea which recorded (31.5 mg/g) followed by Moringa beverage formula (17.17 mg/g). Results also in the same Table (5), indicated that prepared extract from Moringa beverage had the highest level of antioxidant activity (92.58%), these results were higher than those obtained by (Ilyas *et al*., 2015) who reported that the antioxidant activity of *Moringa oleifera* leaf powder was up to (87.02%), while the G_2 beverage which contained 1.25 Moringa : 0.75 green tea recorded (89.13%) followed by A_2 beverage which contained 1.25 Moringa : 0.75 anise (87.72%) .

Table 5. Total phenolic compounds, antioxidant activity and some minerals content of prepared beverages with or without *Moringa Oleifera*:

Sample	M	G_2	A_2
Total phenolic compounds (mg /gm)	17.17	31.5	12.87
DPPH %	92.58	89.13	87.72
Calcium (ppm)	61.10	32.26	45.61
Potassium (ppm)	13.33	8.80	12.89
Iron (ppm)	2.455	1.512	1.356

M=*M.Oleifera* beverage G_2=1.25 *M.Oleifera*: 0.75 Green tea A_2=1.25 *M.Oleifera*: 0.75 Anise

Minerals content of Moringa beverages, namely (Ca, K and Fe) is also presented in Table (5). Calcium (Ca) considered as essential element for transport of oxygen and cellular activity, for blood clotting, stabilizes blood pressure, contributes to normal brain function and bone health as reported by (Antia *et al*., 2006). Our obtained results showed that calcium exhibited the highest amount of minerals content in Moringa beverage being (61.10 ppm) in compared with formula G_2 beverage (32.26 ppm) and formula A_2 beverage (45.61 ppm) . Potassium (K) is an essential nutritional element for transmission of nerve impulses and electrolyte balance. From data presented in Table (5), it can be noticed that the potassium (k) of Moringa beverage reached to (13.33 ppm), while in the formula A_2 beverage 1.25 Moringa: 0.75 anise reached to (12.89 ppm) followed by G_2 beverage 1.25 Moringa: 0.75 green tea (8.80 ppm). Finally iron (fe) play an important role in energy metabolism, gene regulation, cell growth, enzyme reaction and treatment and prevention of anemia. Deficiency of iron could resulted in decreased work capacity, depressed immune, increased rates of infection, increased lead and cadmium absorption and fetal growth retardation (Antia *et al*., 2006). Obtained results in Table (5), indicate that the iron was higher in Moringa beverage (2.455 ppm) than G_2 beverage (1.512 ppm) and A_2 beverage (1.356 ppm). So, These obtained results indicated that *Moringa Oleifera* is a good source of some essential minerals for human health and could be recommended as daily used beverage .

So, from above mentioned data it could be observed that addition of *Moringa Oleifera* could particularly enhanced some sensorial properties of prepared beverages and could be accepted alone or combined with other commercial beverages .

CONCLUSION

Results of present study indicated that *Moringa Oleifera* leaves is a rich source of nutritional value, phenolic compounds and antioxidant activity. Beverages prepared from *M.Oleifera* leaves also showed agood minerals content, strong antioxidant properties and a rich source of phenolics. So, the study recommended *M.Oleifera* leaves as a daily beverage alone or combined with some other commercial beverages.

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تحضير مشروب جديد من اوراق نبات المورينجا اوليفيرا

ايمان محمود عبد الجواد ، عبد الحميد ابراهيم عبد الجواد ، ممدوح محمد ربيع و رانيا ابراهيم الجمال
قسم الصناعات الغذائية – كلية الزراعة – جامعة المنصورة – جمهورية مصر العربية

انتشر فى السنوات الاخيره استهلاك المشروبات الغنيه بالمركبات الفينوليه لما لها من تاثير واقى ضد الامراض المختلفه ولمنافعها الطبية وقيمتها الغذائية. ولذا اجريت هذه الدراسة بغرض اعداد مشروب جديد من اوراق نبات المورينجا اوليفيرا. وتمت دراسه التركيب الكيماوى والتقييم الحسى والمركبات الحيويه الطبيعيه لهذا المشروب. اظهرت نتائج التحليل الكيماوى ان اوراق المورينجا مصدر غنى بالبروتين والالياف والمعادن حيث وصلت نسبتها الى ٢١,٤٠ ، ٧,٤٦ و ٥,٣٣ % على التوالى. بينما اظهرت نتائج تحليل المركبات الفعالة لاوراق المورينجا احتوائها على ٢٤ مركب فينولى وكان المركب e-vanillic هو اكثر المركبات السائدة حيث وصلت نسبته الى ٢٠٢٢,٣٨ جزء فى المليون يليه Syringic acid حيث كانت نسبته ١٠١١,٢٨ جزء فى المليون . كما تم تحضير سته عشر توليفه من اوراق المورينجا مع مشروبات اخرى كالشاى الاخضر والنعناع والينسون والقرفه بتركيزات مختلفه. واوضحت نتائج التقييم الحسى ان مشروب المورينجا سجل اعلى نسبه قبول للمستهلك يليه التوليفه المحتويه على مورينجا وشاى اخضر بنسبه (٠,٧٥:١,٢٥) متبوعه بالتوليفه المحتويه على مورينجا وينسون بنسبه (٠,٧٥:١,٢٥). كما اظهرت النتائج ان التوليفه المحتويه على مورينجا وشاى اخضر بنسبه (٠,٧٥:١,٢٥) كان محتواها من المركبات الفينوليه الكليه اعلى بالمقارنه بمشروب المورينجا والتوليفه المحتويه على مورينجا وينسون بنسبه (٠,٧٥:١,٢٥). اظهرت النتائج ايضا ارتفاع التاثير المضاد للاكسده لمشروب المورينجا الى ٩٢,٥٨ % متبوعا بالتوليفه المحتويه على مورينجا وشاى اخضر بنسبه (٠,٧٥:١,٢٥) الى ٨٩,١٣ % وارتفاع نسبه كل من الكالسيوم والبوتاسوم والحديد فى مشروب المورينجا بالمقارنة بالتوليفة المحتوية على مورينجا وشاى اخضر بنسبه (٠,٧٥:١,٢٥) والتوليفه المحتويه على مورينجا وينسون بنسبة (٠,٧٥:١,٢٥). ولذا نخلص من هذه الدراسه الى امكانيه اعداد مشروب يومى غنى بالمركبات الفينولية من اوراق المورينجا فقط او ممزوجا بمشروبات تجاريه اخرى.