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Phytoantibiotics Activities Enhancement of *Moringa oleifera* Leaves by gamma irradiation and thermal processing

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Abstract

The present work was conducted aiming to evaluate the effect of different treatments (drying methods and γ-irradiation) on the antioxidant (AA), antibacterial activities (AB), microbiological and chemical characters of Moringa leaves (MLS). ML dried by two methods (the first one at room temperature 20 ^{0}C, the second was carried out at 40^{o}C in the electrical lab. oven, besides, treat another group after room drying completely by γ-irradiation at 5.0 kGy and 10.0 kGy respectively. The obtained results showed a slight reduction effect by thermal or low dose (5.0 kGy) on microorganisms load, i.e. mean counts of total viable cells, fungi, yeasts, and bacteria. Whereas, high dose (10.0 kGy) eliminated all microbe cells completely. AB of ML extract was assayed against seven fungi as *Aspergillus fumigates*, *A. flavus*, *A. niger*,*Penicillium aurantiogriseum*, *P. italicum*, and, *Candida tropicals* as well as seven bacterial species namely, Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilus* and *Streptococcus mutants.* In the same time, Gram-negative bacteria were *Enterobacter cloacae*, *Salmonella typhimurium*, *Escherichia coli*, and *Pseudomonas aeruinosa*. Weak inhibition was resulted by MLS after thermal treatments and low irradiation dose (5.0 kGy), but high dose inhibited most of the tested microorganisms. AA determination by DPPH- scavenging proved increasing the capacity of treated samples either with thermal or irradiation process. The GC-MS analysis of MLS extract showed presence near 23 phenolsicand flavonoids compounds in untreated –control samples –which decreased after thermal treatment to 19number compounds. No changes were observed at 5.0 kGy, but γ-irradiation increased these compounds to 30 after irradiation with 10.0 kGy. Besides, the presence of new phenols and flavonoids have resulted as Papaveroline, Tropine, Elemicine, α-Conidendrin, and Asaron. These new phenols resulted in MLS due to irradiation only at 10.0kGy, but not present in the other treatments. It is maybe resulted from radio- stimulation, induction, or degradation of macro compounds to simple phenols by irradiation processing. These results in parallel with the inhibition data against microorganisms. Therefore it could be recommended that irradiation (10.0kGy) can be used for enhancing the protection of human health by increasing the phytoantibiotic for inhibition of microorganisms.

Keywords: Gamma irradiation, *Moringa oleifera*, Antioxidant activity, DPPH.

1.0 Introduction

Recently, the phytoantibiotics term or plant antibiotics can be regarded as a suitable alternative for therapeutic purposes. The quest for natural food additives has become an increasing concern. Antioxidant (AA)has been widely used as food additives to provide protection against oxidative degradation of foods by free radicals. The Synthetic AA is used for industrial processing, in order to prolong the storage stability of foods. Moringa leaves (MLS)has levels of natural antioxidants to protect the human body from free radicals that may cause some chronic diseases(Kinsella *et al*., 1993 and Lai *et al*., 2001).MLS can be eaten fresh, cooked, or stored as dried powder for later use as a food flavoring or additive (Abdukarima *et al*., 2007). ML are considered a rich source of polyphenols (Bennett *et al.,* 2003), rich in chlorogenic acid, gallic acid, kaempferol and quercetin glycosides (Brahma *et al.*, 2009).

Spores of both *Clostridium perfringens* and *Bacillus cereus* have been found in spices and aromatic herbs (Kneifel and Berger, 1994). Heavy load of fungi may cause a high risk of aflatoxins (Farag *et al.,* 2009). High levels of pathogenic microbes were recorded in foodstuff mainly as being 'ready-to-use'. (Farkas, 1988). Whereas, no additional further food processing which can cause outbreak as recorded before in the USA, due to pathogenic bacteria. According to EU requirements of herbal medicines must meet several requirements. Besides, some countries must prefer the low levels of the total number (Anonymous, 2009).

Conventional decontamination methods are not suitable for spices treatment. Heat cannot be used due to the thermolability of many essential oil components, while ultraviolet radiation, is not effective in decontaminating great volumes due to its short penetration, (Fine & Gervais, 2003). γ-irradiation appeared to be the best way for microbial decontamination of spices and herbs without causing alterations in the condiment quality (Lee *et al.,* 2005). The utilization of γ-irradiation for microbial decontamination of whole spices and spice powders is legally permitted in more than 50 countries worldwide and 40 countries are using this technology commercially (Farag *et al.,* 2013). Food irradiation with recommended doses has been used on a commercial scale now in more than 40 countries as in USA, France, Holland, Belgium, China and now in Egypt (IAEA, 1981; WHO, 1994 and Farag *et al*., 2010).

Therefore, the present work focuson comparison using thermal treatment withγ-irradiation for decontamination of MLS. Besides, evaluation of the quality of treated samples through the microbiological and physical, biochemical characteristic after treating.

2.0 Materials and Methods

2.1 Sampling and Irradiation process

Fresh leaves were harvested from *Moringa oleifera* **(***M. oleifera*) trees cultivated at experimental station of National Center for Radiation Research and Technology-NCRRT (Nasr City, Cairo). Fresh~4.0 kgm. Of MLS were dried naturally at room temperature (20°C) under shadow area then the dried part packaged in big polyethylene bags. One kilogram of naturally dried MLS was γ –irradiated halve kilo per dose (5.0 and 10.0 kGy), besides unirradiated samples which left as control, then all samples were packaged in plastic bags. Another one-kilo fresh MLS were dried at electrical oven at (40 °C) even dry completely. The four treatments were as following, untreated as control(20°C) , drying at oven 40 ^{0}C, natural dried + irradiation at 5.0 kGy and 10,0 kGy were packaged in plastic bags. (250 gm x4 replicates per each treatment).The irradiation processing was done by γ - Cobalt -60, the irradiation was performed at NCRRT. The source had been calibrated by the National Physical Laboratory (NPL, Teddington, UK) using the dichromate dosimetry system. The irradiated samples dosimetry traceable to NPL was performed with the alanine/EPR system. (Farag *et al*, 2009).

2.1.1 Microbiological analysis

2.1.2.1 Microbial count

A 25-g portion of each sample was blended under sterile conditions with 225 ml of sterile saline solution (0.85% NaCl) for 1 min. Then the mixture was diluted ten-fold with the saline solution. Total bacteria count (TBC) were counted in a plate count agar medium using the pour plate technique (American Public Health Association, 1985). Total Fungi Counts (CFU/ml) of samples were conducted according to the method of Ichinoe-Zirnstein *et al* (1983) as follows: samples were ground in a Buhler mill and 10 grams of each ground sample was transferred to sterilize flask containing 90 ml of sterilized saline solution. Serial dilutions i.e. 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were prepared, then 1 ml was transferred.

2.1.2 .2. Antimicrobial activity of extracts (AA)

AA was determined using the agar disc diffusion assay method at Antimicrobial Activity Unit, the Regional Centre for Mycology and Biotechnology(RCMB), Al-Azhar University. The testing was performed according to Hindler *et al* (1994). The Well diameter was 6.0 mm,(100 µl was tested). *In –vitro* antimicrobial screening of treated MLS alcohol extract samples which prepared (10 gm /100 mlethanol90%) and agitated using a rotary shaker at 250°C for 8 h. Extracts were filtered in a Buchner funnel through filter paper (Whatman no. 41) for removing plant particles. The extracts were left by standing overnight, filtration then pure extracted keep even the test in closed black small bottles at cold conditions refrigerator. The used cultures of strain fungi were as *Aspergillus fumigates (RCMB* 002008) (AF), *A. flavus* (002002) (AFL), *A. niger* (002005) (AN), *Penicillium aurantiogriseum* (IMI89372) (PA), *P. italicum RCMB* 001018(1) IMI193019(PI) and *Candida tropicalis* RCMB 005004(1)(CT). As well as seven bacteria species namely, Gram-positive bacteria *Staphylococcus aureus* (RCMB010010) (SA), *Bacillus subtilus RCMB 015(1) NRRL*.) B-543(BS) and *Streptococcus mutants* RCMB017 (1) ATCC 25175(SM).Whereas, Gram-negative bacteria were *Enterobacter cloacae* RCMB0100ATCC 23355 (EC), *Salmonella typhimurium* RCMB006 (1) ATCC 14028(ST), *Escherichia coli* (RCMB010052) ATCC25955 (EC) and *Pseudomonas aeruinosa* ATCC 27853(2) (PA). Gentamycin antibacterial agent was used as references to evaluate the potency of the tested compounds under the same conditions whereas, Ketoconazole was used as antifungal medicine. The plates were done in duplicate. Bacterial cultures were incubated at 37°C for 24h while the other fungal cultures were incubated at (25-30°C) for 3-5 days.

2.1.1 Chemical analysis

2.1.1.1. Determination of DPPH radical scavenging antioxidants-activity

Preparation of plant extracts

Ethyl extracted as 100 ml with 10 g dry sample, In all cases, the extracts were centrifuged (3000 rpm) for 15 min and the supernatants were harvested and filtered using Whatman paper No. 1. The used solvents were finally evaporated through incubation at room temperature. The extraction yields were then calculated as a percent of the used powder**.** The ability of extracts to scavenge DPPH radicals was determined according to the method of Shimada (1992). The absorbance was measured at 517 nm and activity was expressed as percentage DPPH scavenging relative to control using the following equation: DPPH scavenging activity (%) = [Absorbance of control - Absorbance of sample]/ Absorbance of control.

2.1.1.2.. GC/MS Analysis of Phenol and flavonoids Compounds.

One gram of sample powder was extracted using anhydrous ethyl alcohol for three times (15 minutes each time) with the assistance of ultrasonic bath. The obtained turbid solution was filtrated and the solvent of the filtrate was removed by rotary evaporation under reduced pressure. Then the extracted was filtered through a 0.45 μm membrane filter. 1 μL of the subsequent filtrate was injected to GC/MS for analysis at. Regional Centre for Food and Feed (RCFF), Agricultural Research Center (ARC). The analysis was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with a polar Agilent DB-5ms (5%-phenyl methyl polysiloxane) capillary column (30 m × 0.25 mm i. d. and 0.25 µm film thickness). The carrier gas was helium with the linear velocity of 1ml/min. The injector and detector temperatures were 200º C and 250º C, respectively. The volume injected 1µl of the sample. The MS operating parameters were as follows: ionization potential 70 eV, interface temperature 250º C, and acquisition mass range 50–800. The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature.

2.1.1.3. ESR-spectroscopy measurements:

Powdered of MLS were used for ESR spectra measuring with an X-band ESR spectrometer (Bruker, EMX) at room temperature using a standard rectangular cavity (4102 ST) operating at 9.75 GHz. with a 100 kHz modulation frequency. The ESR parameters were chosen to provide the maximum signal-to-noise ratio for a non-distorted signal. The microwave power and modulation amplitudes were 6.3 mW and 1 G, respectively. The response time constant was 40 ms with the field-sweeping rate of 100 G/164s.

The intensity of each sample was measured three times as the peak-to-peak height and average values of these measurements were plotted. The standard deviation was about 0.5% of the mean value. Standard samples of MgO doped with Mn^{2+}, weak pitch and DPPH (a; a-diphenyl-b-picrylhydrazyl) were used to calibrate the ESR intensity and the g-factor of the signal. ESR measurements and analysis were carried out at the National Institute for Standards (NIS). The measurements were conducted on irradiated or thermal treated samples at zero time and 2 weeks later.

3.0 Results

There is a growing interest in the development and evaluation of natural antioxidants from herbs which call phytoantibiotics. Therefore, the hygienic view to find the method for decontamination without affecting the quality of spices by using natural sources.

3.1. Total load of microorganisms:

As shown in Table (1**)** the obtained results showed presence higher numbers of microbes and fungi in untreated samples. Natural levels of bacteria near $90x10^5$ besides $8x10^4$of fungi. This logic due to many observations that MLS consider are contaminated herbs with microorganisms from harvesting to processing, due to fine dust of soils and spreading on the ground surface for drying. Thermal treatment at 40^0C decreased slightly the load microbial one log, whereas, irradiation was more effective to reduce 3 logs, at low irradiation dose (5.0 kGy) for bacteria and 2 log for fungi respectively. But, high dose (10.0kGy) dramatically eliminated completely all microbes.

Table (1) the microbial load of tested MLP.

Treatment	Total bacteria count (TBC/g).	Total Fungi count (CUF/g).
Control(20^0C)	$90x10^5$	$8x10^4$
Thermal process(40^0C)	$80x10^4$	$6.0x10^4$
γ- irradiation kGy - 5.0 -10.0	 $30x10^2$ **ND**	 $3.0x10^2$ **ND**

3.2. Antimicrobial activity of extracts (AA)

In the present work, MLS Ethyl alcohol extract was chosen than other solvents due to its potential as showed by many workers (Kumar and Jain, 2010). MLS extract was evaluated investigation by diffusion agar technique, well diameter 6.0 mm., 100 µm, against six fungal and yeast spp. strains and seven bacteria (Tables 2 and 3).The antifungal and antibacterial activity of MLS extract was tested against 6 strain of fungi and 7 bacteria. The results revealed that untreated MLS extract is not capable of inhibiting all the growth of microbes. Whereas, the irradiated MLS especially at 10.0kGy has the priority against most fungi strains, Gram-positive and Gram-negative bacteria that are used in this study (Tables 2 and 3)**.** MLS Ethanol extract showed weak antifungal activity against *A. flavus, P. italicum, Candida tropicalis*.

The same trend occurred by thermal treatment and low irradiation dose. Whereas high dose (10 kGy), showed strongly high activity against most strains for fungi as *A. flavus*, *P. italicum*, *Candida tropicalis*, *Aspergillus fumigates*, *A. niger* with inhibition area diameter near the antifungal agent ketoconazole Table (2).

Same trends were observed against bacteria, as in Table (3), the results of an untreated sample of MLS extract showed the moderate activity of inhibition zones against (+Gm group) as *Staphylococcus aureus, Bacillus subtilis*, and (-Gm) group as *Streptococcus, Enterobacter cloacae*, *Salmonella typhimurium*. Near values were observed by thermal treatment and low dose. Whereas strongly inhibition was clear at high dose (10.0kGy), its values were near antibiotic (Gentamycin).

Table (2) Antifungal activity of MLP

Name of fungi	control	Thermal treatment	γ-irradiation kGy		Ketoconazole
		40^0C	5	10	
Aspergillus fumigates (RCMB 002008)	NA*	NA	NA	15	17
A. flavus (002002)	12	11	NA	14	16
A. niger (002005)	NA	NA	8	10	15
Penicillium aurantiogriseum (IMI89372)	NA	NA	NA	NA	25
P. italicum RCMB 001018(1)IMI193019	18	NA	12	17	18
Candida tropicalis RCMB 005004(1)	15	12	NA	18	21

NA*=no activity

Table (3) Antibacterial activity of treated Moringa leaves.

Name of bacteria	control	Thermal treatment	γ-irradaion kGy		Gentamycin
Gram(+)		40^0C	5	10	
Bacillus subtilis RCMB 015(1) NRRL.) B-543	9	19	10	16	24
Staph. aureus (RCMB010010)	17	18	14	15	24
Streptococcus mutants RCMB017(1) ATCC 25175	11	14	NA*	15	20
Gram(-)					
Enterobacter cloacae RCMB0100(1) ATCC 23355	10	15	NA	11	27
Salmonella typhimurium RCMB006(1) ATCC 14028	8	12	NA	12	17
Escherichia coli (RCMB010052) ATCC 25955	NA	NA	NA	NA	30
Pseudomonas aeruinosa ATCC 27853(2)	NA	NA	NA	10	17

NA*=no activity.

Also, our results proved that γ-irradiation activated the complicated compounds phenols, flavonoids through degradation, then new content resulted to inhibit the microorganisms by generating free radicals to remove the oxygenated form which caused by microorganisms. As will mention in the next lines.

3.3. Chemical analysis

3.3.1 Radical scavenging activity (RS)

The results of antioxidant capacity DPPH as in Table (4), showed that thermal treatment increased these values. Also, γ-irradiation caused an increment of DPPH free radical scavenging activity as antioxidant capacity Fig. (1).DPPH radical of ethanol extract of treated MLS with values as DPPH inhibition % 44.24, 49.54 ,47.54 and 46.61 / 100mg/L for control, thermal,5.0 kGy and 10.0 kGy respectively.The treating MLS either by mild thermal or low dose of γ-irradiation increased the efficiency of antioxidants as phenolic and flavonoids. These results become more details in the next part of GC-MS analysis.

Table (4) Effect of food processing on DPPH inhibition % content of treated MLP

Treatments	DPPH inhibition % (Conc. 100mg/l)
Thermal -Room temp. 20 0C -Oven Temp.40.0C	44.24 ± 1.1 49.54±1.4 + (12.0%)
γ- irradiation -5.0 kGy- -10.0kGy	47.54 ±1.3 + (7.5%) 46.61 ± 1.2 + (5.4%)

* increase value (%)=value after treatment- value treatment at room temperature before/ value treatment at room temperature before x100

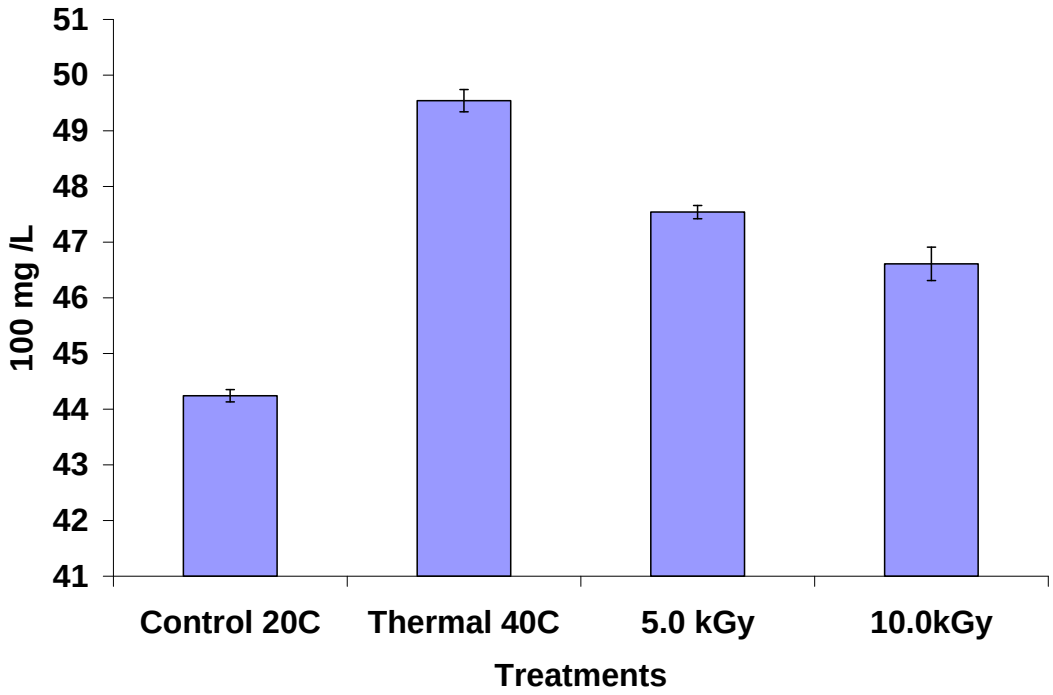


Figure 1. DPPH assays antioxidant (Conc. 100mg/l) of treated Moringa leaves.

3.4. GC/MS Analysis of Phenols and flavonoids Compounds

GC-MS analysis as showed in Table (5) presence of different phenolic and flavonoid compounds in MLS samples. These compounds were near 23 compounds in untreated –control-but decreased by thermal treatment to 19. The detection by GC-MS proved that most of these compounds such as glycosides, flavonoids, tannins, mono- and sesquiterpenoids, diterpenoids and furocoumarins.

Whereas, γ-irradiation caused increase these compounds to 23 and 32 after irradiation with 5.0 and 10.0 kGy in respectively. Irradiation caused degradation of high complicated phenolic compounds or flavonoids to small as mono- and sesquiterpenoids, diterpenoids phenols as Asron, benzoic, Caffeic acid, Elemicin, Papaveroline, Tropine, and α-Conidendrin. .Therefore, γ-irradiation increased the number of last compounds as shown in fig. (2). as shown in Table (5) and Figs. (4) GC-MS analysis resulted in occupied different concentration as (%), these compounds can classify to the minor (less than 5%) and macro compounds (more than 5% concentration). The obtained results showed that irradiation only caused an increase of minor which occupied 55% for minor compounds in irradiated MLS by using 10 .0 kGy. Some of these compounds as arson, benzoic, caffeic acid, Elemicin,Papaveroline,Tropine, and α-Conidendrin.

Table (5) GC-MS of phenols and flavonoids content of MLP

No.	Rt	Name of structure	Thermal treatments(°C)		Gamma irradiation (kGy)	
			(Control)	40	5.0	10.0
1	4.75	2-Coumaranone	1.00	------	2.20	2.70
2	5.49	trans-2,3-Dimethoxycinnamic acid	1.0	2.4	2.0	4.20
3	6.17	Phenol, 4-(dimethylamino)-3,5-dimethyl-	0.44	-------	-------	2.3
4	6.69	Isocoumarin, 3,4-dihydro-8-hydroxy-3-methyl-	0.40	--------	-------	2.8
5	7.13	Sinapic acid	2.13	--------	-------	-------
6	7.45	Benzoic acid	0.7	-------	1.82	2.5
7	7.69	5-Hydroxy-7-methoxy-2-(3-methoxyphenyl)-4H-chromen-4-one	-------	-------	1.8	2.0
8	9.57	3',4',5',5,7,8-examethoxyflavone	----------	--------	-----	5.2
9	10.40	6R,9R,3-oxo-α-ionol	------	2.86		
10	11.54	Papaveroline			7.3	7.5
11	11.66	Cerdren 13 –ol-8-	--------	3.20	------	
12	11.87	2-Methoxy-5-[(Z)-2-nitroethenyl]phenol	--------	--------	2.6	7.3
13	11.89	-αyalangene	3.62	3.59	2.07	2.64
14	12.54	Oleic acid	1.77	1.75	-------	3.9
15	12.59	Flavone, 3-hydroxy-4',5,7-trimethoxy	0.52	------	5.17	5.26
16	12.88	Phytol,acetat	6.15	6.1	3.43	6.3
17	13.03	Phytol	2.32	2.3	0.96	1.56
18	13.16	Pinane	3.89	3.85	1.45	2.75
19	13.44	Dimethyl caffeic acid	-------	-----	-------	1.82
20	13.45	Asaron	------	------	-------	2.5
21	13.58	Conidendrin,α	0.3		5.7	6.5
22	13.73	Folic Acid	1.07		1.34	4.3
23	13.74	Ecgonine	1.04	-------	------	--------
24	13.74	n-Hexadecanoic acid	9.57	9.48	9.63	7.9
25	14.78	Isolongifolol	14.56	14.43	9.22	9.87
26	14.87	Patchoulane	9.58	8.5	9.95	10.32
27	15.09	Elemicin	--------	-------	1.54	3.0
28	15.19	Tropine	--------	-------	1.53	4.0
29	15.31	Coumarin-6-ol, 3,4-dihydro-4,4-dimethyl-5,7-dinitro-	0.32	0.1	------	-------
30	15.70	Geranylisovalerate	0.80	0.79	1.13	1.27
31	15.9	Isomyristic acid	-------	10.01	-----	1.31
32	16.33	Phytanic acid	3.02	3.99	2.82	3.77
33	17.83	Vitamin E	0.91	3.05	-------	3.62
34	19.0	Octacosane	9.11	9.30	12.81	14.81
35	20.0	3,6,3',4'-Tetramethoxyflavone	------	0.62	1.83	1.34
36	22.0	2-Decanol	-------	18.61	31.86	30.62

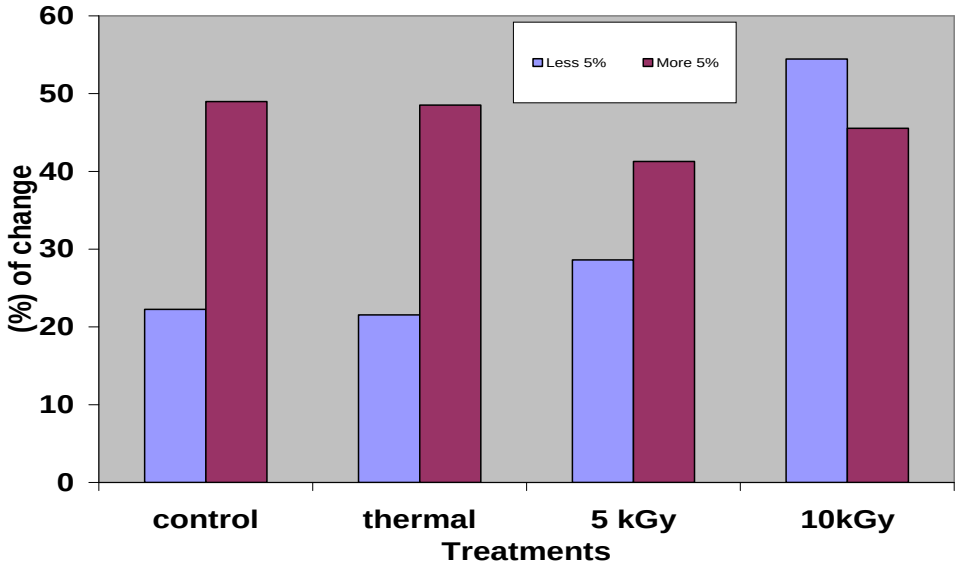
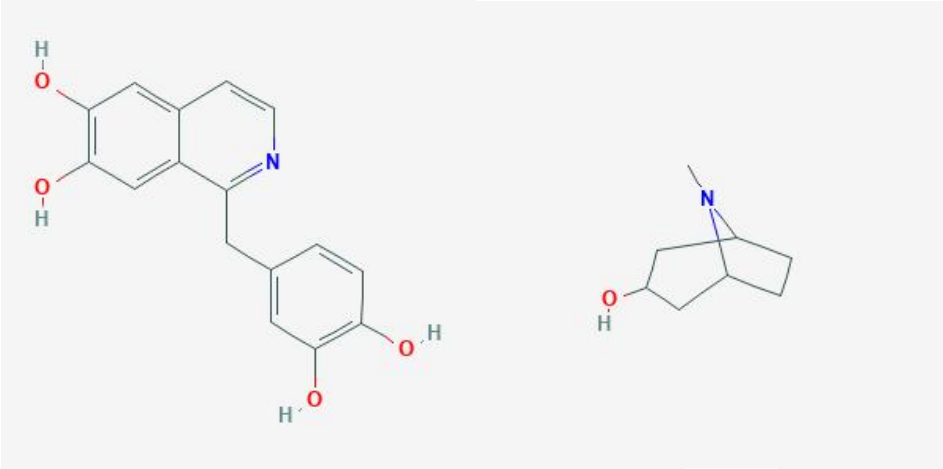
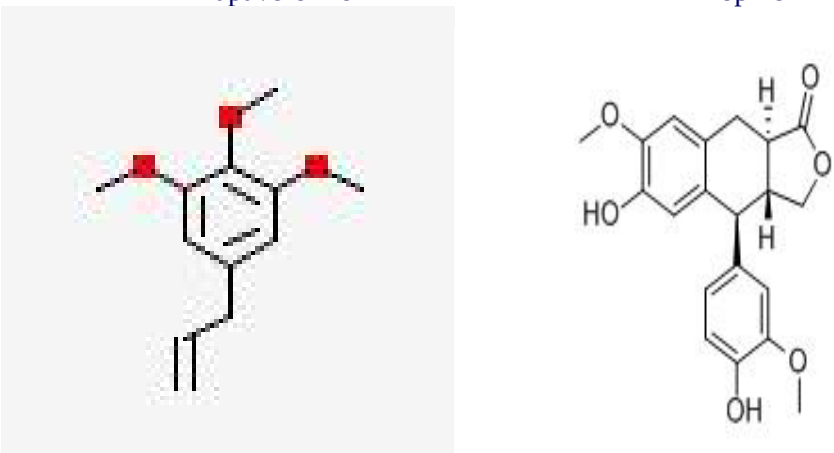


Figure 2. The influence of different methods on numbers depending of concentration of isolated phenols and flavonoids compounds with GC-MS.
Number of compounds with concentration less than 5% and more than 5%.



1-Papaveroline 2-Tropine



3-Elemicine **4**-α-Conidendrin

Figure 3. Some new compounds as resulted in irradiated samples only.

3.4.1. ESR-spectroscopy measurements

The ESR measurements were carried out two weeks after irradiation to avoid any false signal due to grinding according to our preliminary experiment in preparation samples. The ESR spectra of thermal or irradiated treated MLS and non-treated samples are shown in table (6) and figures (4) and (5). The G values were in the range of 2.01027. The ESR signal ascribed to free radical-induced free radicals in cellulose. EN 1787 refers that signals due to the presence of a "cellulose type" radical is attributed to Mn(II) ions under the main paramagnetic feature with a separation of about 6 mT and centered around G = 2.004. It is important to note that the background signal may vary with the type of plants, maturity stage of seeds and the original location sources.

Table (6) Free radical measurements by ESR-parameters of treated Moringa leaves

Treatments	Peak height	ESR -intensity
Thermal -Control. 20 0C -Oven Temp.40.0C -80 ^{0}C	**675** **871** **2117**	**1480.3** **1861.1** **4320.4**
γ- irradiation -5.0 kGy -10.0kGy	**1308** **2047**	**15795.3** **24933.0**

*g-value=2.01152

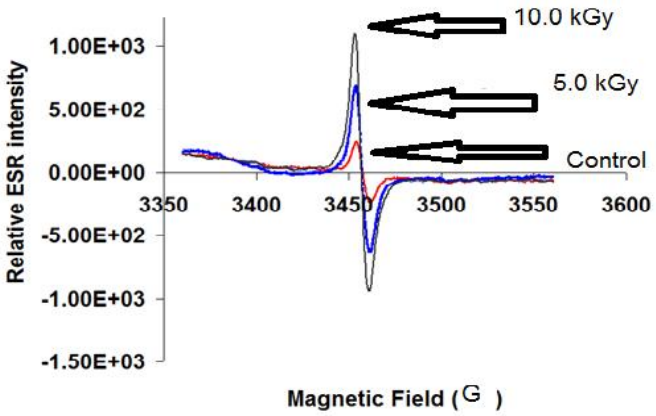


Fig. (4): ESR signals of irradiated MLP at different doses after two weeks of radiation.

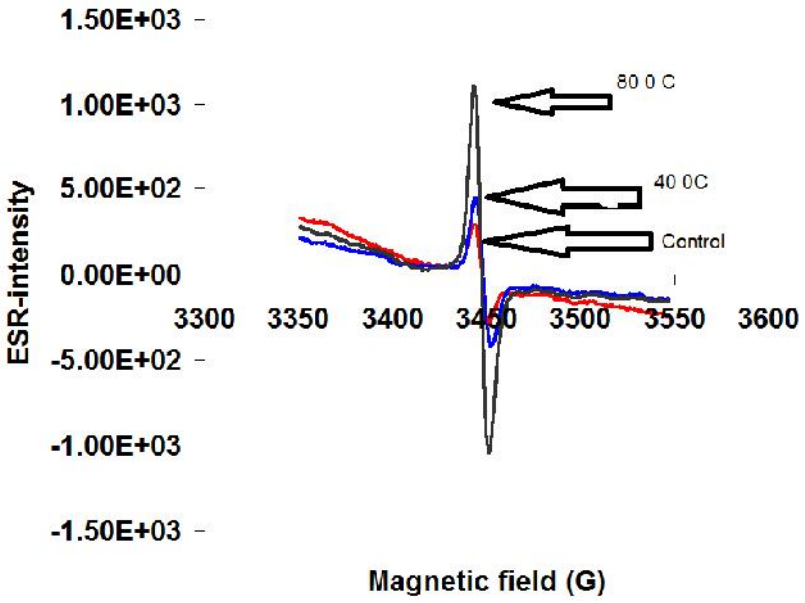


Fig. (5): ESR signals of treated Moringa leaves by thermal treatments at different temperature after two weeks of radiation.

4.0 Discussion

The present study suggests that MLS could be a potential source of phytoantibiotics with strong antioxidant potential. Hence consumption of diet supplemented with MLS could protect against diseases induced by oxidative stress. But, the higher levels of microorganisms on MLS like herbs and spices are heavily contaminated with fine dust or organic fertilizers, consequently became a risk for human health. That can cause disaster especially on the consumption of fast food or cold processed food products as beef Berger. The goal of the present work to get a suitable and efficient alternative method. Our results showed that. γ-irradiation appeared to be the best way for decontamination of spices and herbs as MLS without causing alterations in the condiment quality as reported before (Lee *et al.*, 2005, Kumari, *et al*,2009 and Farag *et al*,2013). Also, results showed that γ-irradiation did not have a negative effect on antioxidant activity, phenolic content and antimicrobial activity of MLS. Some workers investigated changes of MLS quality due to different methods of drying, as reported were more affecting on decreasing, the antibacterial and antioxidant properties depending degree of drying (Hussein *et al*, 2015). A slight increase in DPPH radical scavenging test just was observed after thermal, irradiation treatments. The same observation was observed by Nikousaleh and Prakash (2008) but increased temperature injured the quality especially the antibacterial and antioxidants (Hussein *et al*, 2015).High inhibition was clearly after irradiation MLS at high dose(10.0kGy) and more efficient to inhibit most tested bacteria, fungi than other treatments resulted for inhibition zone by MLS was reported by workers (Caceres *et al*., 1991). The positive response of γ-irradiation as increase the DPPH scavenging, antimicrobial values without negative effect due to considering gamma irradiation as cold treatment .,*i.e* no raising in temperature during irradiation process then no side effect as the high temperature of dry-oven. The explanation of high dose may be due to associated the structural changes of phenolic, flavonoids that induced by irradiation (Khattak *et al*., 2008).In our results, high γ-irradiation dose (10.0kGy)was more effective in inhibition most the microorganisms and increase of DPPH-values. GC-MS analysis proved the presence of 23 phenolics, flavonoids compounds are proved presence in control but raised to 32 by high dose. New phenolic compounds were recorded at intervals retention time with a simple structure of phenolic compounds as arson, benzoic, caffeic acid, Elemicin, Papaveroline, Tropine, and α-Conidendrin. All these compounds have different biological roles as anticancer, antibacterial or antifungal. For instance, Asarone**,** **t**here are two isomers, α (or *Trans*) and β (or *cis*), as a volatile fragrance oil, it is used in killing pests and bacteria *(*Asha and Ganjewala *2009***).** Also, α-Conidendrin considered decomposer of lignin cell bacteria. (Sundman, 1964).

The explanation of that phenomenon may be due to breaking the chemical bonds of complicated phenols, flavonoids or polyphenols; to mono –or di-phenols by irradiation at 10.0 kGy, whereas the increase of hydroxyl radical l radical scavenging activities found by workers (kumara *et al*, 2009 and Dixit *et al*., 2010). These explained the higher efficiency of MLS-products after a higher dose of γ-irradiation (10.0 kGy) on eliminating all microorganisms either spores of fungi or bacteria. These results in paralleled with obtained by workers (Farag *et al*., 2009). Who reported that the same trend was clearly with coriander, cumin, turmeric, and chili. According to EU requirements of herbal medicines must meet several requirements. Besides, some countries must prefer low levels of the total number (Anonymous 2009).

All the obtained results are in harmony, parallel with antibiotic behavior, DPPH –antioxidants capacity determination and GC-MS analysis. Using ESR-proved the presence of signals of free radicals due to cellulose signals, whereas, g value around 2.0 as found by the same author before in irradiated or roasted coffee or irradiated spices (Farag *et al.* (2010).

5.0 Conclusion

It is worth noting that all obtained results of increasing the potentiality of antioxidant, antimicrobial of treated Moringa leave by a high dose of irradiation in palled with DPPH- values and GC-MS analysis. Therefore, increasing the activity of phytoantibiotics of MLS. Could be recommended to protect human health. Its recommended for application using of high irradiated MLS form, in addition to their economic and health-promoting benefits, the application of plant extracts, as natural and safe preservatives, could be highly recommended for the improvement of microbiological and sensory quality of MLS to be more safe and more healthy to keep the immunity system of human to remove the risk bad free radicals.

6.0 References

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