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ORIGINAL RESEARCH

Protective effect of *Moringa oleifera* leaf extracts with antioxidant properties in chemotherapy induced oxidative stress

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ABSTRACT
The study investigated antioxidant potency of aqueous and fermented aqueous extracts of dried leaves of *Moringa oleifera* Lam. in different in vitro systems using standard phytochemical methods. Fermented *Moringa oleifera* aqueous extract (FMAE) was prepared using *Lactobacillus plantarum P108* and *Lactobacillus acidophilus P110* co-culture as a starter. The antioxidant activities standard methods were Total phenolics assay, ABTS radical cation scavenging activity, and Superoxide anion radical scavenging activity. The reducing power of both solvent extracts showed strong antioxidant activity in a concentration dependent manner. The FMAE depicted higher percentage inhibition which was comparable with unfermented *Moringa oleifera* aqueous extract (MAE). On the basis of the results obtained, *moringa* leaves are found to be a potential source of natural antioxidants due to their marked antioxidant activity. The results also suggested that FMAE with enhanced antioxidant capacity could provide a functional *moringa* beverage to contribute to the health and nutritional status improvement of consumers.

KEY WORDS: *Moringa oleifera, Lactic acid bacteria, Fermentation, Oxidative stress, Antioxidant*

INTRODUCTION

It has been reported that oxidative injury to living organisms plays an important role in many life style-related diseases such as neurodegenerative, coronary heart, arthritis, emphysema and cancer (Juan and Chou, 2010).

Synthetic antioxidants have been developed and used widely. However, some of them have been found to exert adverse effects and several undesirable disorders have developed due to the side effects of the use of synthetic antioxidants commonly applied in the food and flavoring industries on human health (Williams, Iatropoulos and Whysner, 1999).

Regular consumption of antioxidant-rich-foods may help to reduce the deleterious action of ROS and free radicals, and to balance the oxidative stress related to aging process and serious illnesses. This idea drives a growing trend leading to modify eating habits to improve health and nutritional status of consumer (Frei, 2004).

Moringa oleifera (*M. oleifera*) is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. This rapidly-growing tree (also known as the horseradish tree, drumstick tree, saijhan, sajna or Ben oil tree), was utilized by the ancient Romans, Greeks and Egyptians; it is now extensively cultivated and has

become naturalized in numerous locations in the tropics (Fuglie, 2001).

Data available on phytochemical composition of moringa plants suggest that specific germplasm (mostly Ghanaian or Malaysian cultivars), which have been mainly evaluated for polyphenolic content and innovative applications by food/pharmaceutical industries (Bennett *et al*., 2003). Phenolic compounds prevent or control the formation of free radicals with deleterious health effects and are therefore important to reduce the risk of chronic diseases (Chandrasekara and Shahidi, 2011). They have also been shown to render positive effects on certain types of cancer, including cancer of the stomach, colon, prostate, and breast as well as cardiovascular diseases (CVD), and various inflammatory disorders (Albishi *et al*., 2013).

Biochemical modification by *Lactobacillus* strains contributes directly to many advantageous properties of products (Chandrasekara and Shahidi, 2012). In recent years, fermentation was observed to serve as an efficient approach to enhance the antioxidant properties of legume products (Lee *et al*., 2008).

The aim of this study was to investigate *Moringa oleifera* leaf extracts prepared using different extraction solutions and fermented with *Lactobacillus plantarum* and *Lactobacillus acidophilus* as a starter co-culture in order to improve the content of antioxidant compounds, antioxidant capacity, as well as DNA damage protecting ability by correlation with its enrichment in isoflavone aglycone. Several antioxidant activity methods evaluating different reaction mechanisms were carried out comparing *Moringa oleifera* properties before and after the fermentation process.

RESULTS AND DISCUSSION

Total phenolics assay

As shown, TPC in fermented *Moringa oleifera* leaf aqueous extract (FMAE) and unfermented *Moringa oleifera* leaf aqueous extract (MAE) were 0.0238 and 0.0221 µg/mL respectively. The FMAE had higher TPC than that of MAE.

Several studies have demonstrated that the content of aglycones was increased after microbial fermentation, which may be due to the changes of β-glucosidase activity (Xiao, Rui, *et al*., 2015). (Curiel, 2014) reported that *L. plantarum* was able to efficiently biotransform aryl glycosides to their bioactive aglycones, thus could be used as a functional starter culture to increase the antioxidant activity of plant foods during fermentation. (Chandrasekara and Shahidi, 2012) also demonstrated that phenolic compounds bounded to the insoluble fiber were released during microbial fermentation. (Xiao, Wang, *et al.,* 2015) suggested that higher TPC in fermented soy whey might be due to formation or mobilization of free phenolic and flavonoid molecules during *L. plantarum* fermentation. Therefore, the higher TPC levels found in *Moringa oleifera* fermented with *L. plantarum* and *L. acidophilus* may be beneficial to design new functional foods considering preventive and therapeutic nutritional strategies.

ABTS radical cation scavenging assay

As shown in Fig. 1, FMAE and MAE showed good ABTS radical scavenging activity. At 0.1–1.2 mg/ml, the ABTS radical scavenging activity of MAE ranged from 7 to 70.5 %, while it ranged from 8.4 to 72% for FMAE. FMAE and MAE are free radical inhibitors or scavengers, acting possibly as primary antioxidants. Antioxidant activity of natural antioxidants has been shown to be involved in the termination of free radical reactions (Lee *et al*., 2008).

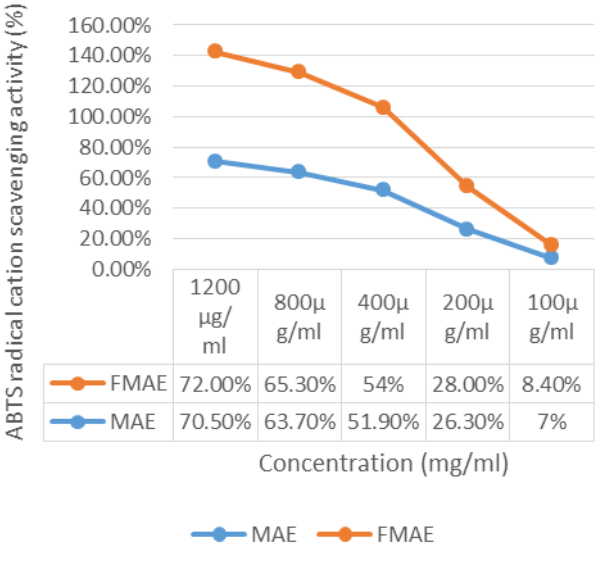

Figure 1: ABTS radical cation scavenging activity

FMAE and MAE might react with free radicals, particularly of the peroxyl radicals, which are the major propagators of the

autoxidation chain of fat, thereby terminating the chain reaction (Shahidi, Janitha and Wanasundara, 1992).

Furthermore, as shown in Fig. 1, microbial fermentation with *L. plantarum* and *L. acidophilus* increased the ABTS radical scavenging activity. For instance, at 0.8 mg/ml, the extract of MAE exerted ABTS radical scavenging activity of 63.7%, whereas a stronger ABTS radical scavenging activity of 65.3% was observed with FMAE. There were more antioxidant components present in FMAE than in MAE.

Kim et al. (2011) reported that increased scavenging on ABTS radical was due to increase in aglycone isoflavone and total phenolic contents. (Marazza, 2012) reported that the isoflavone aglycones formed by fermentation were more active antiradical compounds. Therefore, the greater ABTS radical scavenging activity of FMAE might be due to higher TPC and isoflavone aglycones in the extracts, which are more effective in termination of free radical reactions.

Superoxide anion radical scavenging activity

The superoxide radical scavenging activities of FMAE and MAE are illustrated in Fig. 2.As shown in Fig. 2, FMAE and MAE extracts exhibited moderate superoxide radical scavenging activity in the concentration range of 0.4–2 mg/ml. For FMAE, the higher superoxide radical scavenging activity was 49.8%, while it was 48% for MAE at 2mg/ml.

Superoxide radical scavenging activity was enhanced during fermentation. It has been reported that the antioxidant properties of flavonoids were effective mainly via scavenging of superoxide radicals (Yang *et al.,* 2000).

Superoxide anion radical (O^{2-}) is a precursor to active free radicals that have the potential of reacting with biological macromolecules and there by inducing tissue damage (Pardini, 1995). In the PMS–NADH– NBT system, superoxide anion derived from dissolved oxygen by PMS–NADH coupling reaction reduces NBT. (Verma *et al.,* 2009) showed that (O^{2-}) scavenging activity of polyphenolic fraction of M. oleifera was higher than other fractions. (Obdulio Benavente-García *et al*., 1997) have shown that phenolic compounds, particularly flavonoids and catechins, are important antioxidants and superoxide scavengers. .

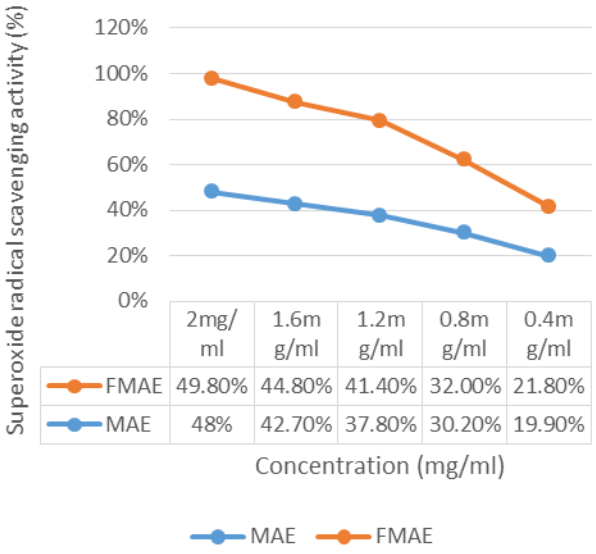

Figure 2: Superoxide radical scavenging activity

CONCLUSION

Biomarkers of oxidative stress reflect environmental pro-oxidant and antioxidant ratio and also serve as a surrogate measure of a disease process. The protective effects of aqueous extract of Moringa leaf may be attributed to the presence of phytoconstituents (polyphenols, tannins, anthocyanin, glycosides, thiocarbamates) that scavenge free radicals, activate the antioxidant enzymes, and inhibit oxidases (Amin and Hamza, 2005; Liu *et al*., 2006).

L. plantarum and *L. acidophilus* provide a potential biotechnological process without causing serious environmental pollution. *L. plantarum* had good growth and could provide probiotic property for the host and had the capacity to improve nutritional and functional properties of the products. Thus, the enhancement of the antioxidant activity of *Moringa oleifera* by fermentation with *L. plantarum* and *L. acidophilus* and the ability to protect DNA oxidation which was demonstrated is of significance. This report evaluates the DNA protective ability of fermented *Moringa oleifera.* Therefore, we propose the strain of *L. plantarum* and *L. acidophilus* as starter co-culture to obtain a functional *Moringa oleifera* beverage rich in phenolics and isoflavone aglycones with enhanced health-promoting properties. Furthermore, use of fermented *Moringa oleifera* as nutraceutical and functional food ingredient or inclusion in therapeutic diet for patients with diseases linked to oxidative stress may be recommended

MATERIALS AND METHODS

Chemicals

Methanol, Folin-Ciocalteau reagent, Potassium persulfate (K2S2O8), Gallic acid (Sigma Chemicals Co, Egypt), while Sodium bicarbonate (Na2CO3), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), Ethanol, β-nicotinamide adenine dinucleotide (reduced form, NADH), Nitroblue tetrazolium (NBT), Phenazin methosulphate (PMS), are obtained from (El Nasr Chemicals Co., Egypt)

Collection of plant sample

Fresh leaves of *Moringa oleifera*, a member of the Moringaceae family was obtained from Moringa Farms, Sadat city, Egypt and authenticated by the Industrial Biotechnology Department, Genetic Engineering & biotechnology Research Institute (GEBRI), Sadat City University.

Extraction procedure

Leaves were thoroughly washed initially with tape water followed by distilled water. Thereafter, leaves were spread in trays lined with tissue papers and allowed to air dry until the weight of the leaves became constant. Dried leaves were crushed in to small particles by using Braun multiquick 5 grinder. Finally Moringa leave particles were packed and stored in a cool dry location for further use.

The different extracts was prepared according to (Nature, 2010) with slight modifications. Leaves were harvested on April 2017. Aqueous extract was prepared so as to simulate conditions of their extraction pertaining to their use for drinking and cooking purposes. The extract was prepared with 50 g powder packed in sterile gauze in distilled water at 70 °C for 30 min. This aqueous extract was concentrated under reduced pressure.

Inoculum preparation

The lactic acid bacteria strains, *L. plantarum* and *L. acidophilus* were isolated and tested for its probiotics properties by (Mahrous et al.,2010) and used for fermentation of Moringa leaves aqueous extract. Before experimental use, cultures were propagated (2%, v/v) twice in MRS medium and incubated at 37 °C for 18 h without agitation in microaerophilic conditions and the activated culture was again inoculated into MRS broth at 37 °C for 16 h..

All solutions were sterilized separately (0.22 mm filtration), and then added to the MRS base. The cells were harvested by centrifugation at 5000 xg at 4 °C for 10 min, and washed twice with sterilized physiological saline and then resuspended in sterilized physiological saline. This cells suspension was ready to serve as inoculum for sample fermentation. The sample was sterilized using laboratory autoclave at 108 °C for 15 min for eliminating the unwanted microorganisms and cooled to room temperature before inoculation.

Total phenolics assay

Total concentration of phenolics in the crude extract was determined by a modification of the method of (Taga, Miller and Pratt, 1984). Dried samples and standards were prepared in 60:40 acidified methanol/water (0.3% HC1). Test solutions (samples or standards) of 100 µL were added to 2.0 mL of 2% Na2CO3. After 2 min, 100 µL of 50% Folin-Ciocalteau reagent were added and allowed to stand at room temperature for 30 min. Absorbance was measured at 750 nm on a Unico 1200, Spectrophotometer, USA. The blank consisted of all reagents and solvents without test compounds or standard. The standard was gallic acid prepared in concentrations of 1.9 µg/mL to 1000 µg/mL. The phenolic concentrations were determined by comparison with the standard calibration curve.

ABTS radical cation scavenging assay

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation (ABTS•+) scavenging activity of the sample was analyzed by using the method previously reported by (Xiao *et al*., 2014) with some modifications. ABTS•+ was generated by the reaction of a 7 mM aqueous solution of ABTS with 2.45 mM aqueous solution of K2S2O8 which was conducted in the dark at room temperature for 16 h before use. The ABTS•+ solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30 °C. About 1 ml of extracts was mixed with 4ml of ABTS•+ ethanolic solution, and the absorbance was recorded at 734 nm after 6 min. Ascorbic acid was used as a positive control. The capability to scavenge the ABTS•+ was calculated using the following equation:

ABTS radical cation scavenging activity (%) = [(Acontrol – Asample) / Acontrol] × 100

Where Acontrol is the absorbance of the blank without extract or Ascorbic acid, and Asample is the absorbance in the presence of the extract or Ascorbic acid.

Superoxide anion radical scavenging activity

The superoxide radical scavenging activity was conducted according to (Li et al., 2014) with some modifications. The superoxide radical was generated in 3 mL of 0.1 M sodium phosphate buffer (pH 7.4) containing 156 μM β-nicotinamide adenine dinucleotide (reduced form, NADH), 52 μM Nitroblue tetrazolium (NBT) and 20 μM Phenazin methosulphate (PMS). After addition of 1.0 mL sample solution (0.4–2 mg/mL), the mixture was incubated at 25 °C for 5 min. The absorbance of the mixture was measured at 560 nm.

The scavenging activity on superoxide radical (%) = (1 − Asample/Ablank) × 100

Deionized water and Ascorbic acid were used as the blank and positive control, respectively.

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