World Journal of Dairy & Food Sciences 12 (2): 102-107, 2017
ISSN 1817-308X

DOI: 10.5829/idosi.wjdfs.2017.102.107

Determination of Polyphenolic Compounds and Antioxidant Activity of Olive Leave, Moringa Leave and Marigold Petals Extracts

[1]Dalia A. Kotb, [2]M.R. Shahein, [1]M.A. Abd El Whab and [2]M.M.K. Metwally

[1]Desert Research Center, Minister of Agriculture and LandReclaimization, Egypt
[2]Faculty of Environmental Agriculture Sciences, Arish University, Egypt

Abstract: Using of natural substances which are found in medicinal plants and dietary plants as antioxidants have attracted a great deal of attention in recent years. So that this study was designed to evaluate the antioxidative activities of Olive leave, Moringa leave and Marigold petals by assessed phenolic compounds and their antioxidant activity. The phenolic compounds identified by qualitative and quantitative HPLC-DAD chromatographic analysis, peaks were monitored at three signal 280 nm, 320 nm and 360 nm. Total phenolic, total flavonoid contents and DPPH scavenging radical activity (%) were estimated. The main phenolic compound detected were Chyrsin, Caffeic acid, Oleuropein, Protochatchuic acid and Qurecetin. Total phenolic contents were 189.5 mg/g, 66.5 mg/g and 27 mg/g as gallic acid equivalent for Olive leave, Moringa leave and Marigold petals, respectively. Also, total flavonoid content was 239.43 mg/100g, 34.7 mg/100g and 7.4 mg/100g as catachin equivalent and antioxidant activity was 338.85%, 11.15% and 8.43% for Olive leave, Moringa leave and Marigold petals extracts, respectively. These results suggested that of Olive leaves extracts had extremely rich antioxidant and Moringa leave extract consider a good source for antioxidant activity, while Marigold petals had low antioxidant activity compared to Olive or Moringa leaves .

Key words: Polyphenolic compounds · HPLC · Antioxidant activity · DPPH · Olive Leaves · Moringa leaves · Marigold Petals

INTRODUCTION

Many herbal medicines and foodstuff believe to have preventive effects on chronic diseases due to their radical scavenging or antioxidant properties [1]. Currently available synthetic antioxidant show low solubility and moderate antioxidant activity comparing with dietary herbs and their extracts. Although, medicinal plants are widely considered to be lower risk comparing with synthetic drugs, they are not completely free from the possibility of toxicity or other side effects. However, there is a considerable interest in identifying natural antioxidants as an alternative to synthetic medicines isolated from plants that protect them against free radical damage. Antioxidant nutritional agents have consequently attracted major attention and rightfully deserve to study carefully for possible beneficial roles [2-4].

Subsequently, it is strong recommendation of the three medicinal plants which serve as a safe and cheap source of natural antioxidant. They are also easily cultivated and grown in various regions of the world in particular, growing in Mediterranean region [1, 5-7]. These plants named Olive (*Olea europaea*), Moringa (*Moringa oleifera*) and Marigold (*Calendula officinalis*).

Olive leave could be an interesting source of natural antioxidants. Their extraction could represent an interesting way to increase in value of this by-product. Their extraction could represent an interesting way to increase in value of this by-product. Notably, Ancient Egyptians used Olive leaves for mummification and as a remedy against various diseases. Olive leave extract has been shown to have a variety of biological activities including; antioxidative, antimicrobial, antiviral and anti-inflammatory agents, lipid stabilizers and blood pressure regulators in animals. The main phenolic compounds in olive leaves are the glycosylated forms oleuropein. Many of these properties have been described as result from the anti-oxidant character of oleuropein. [5, 8- 10].

Corresponding Author: Dalia A. Kotb, Desert Research Center, Minister of Agriculture and Land Racemization, Egypt.
E-mail: d.kotb@yahoo.com.

Moring multipurpose tree, is called a miracle tree, utilization of this plant can help poor countries to fight against poverty, hunger, malnutrition and diseases. Leave of Moringa species contain high levels of nutrients and antioxidants. A rapidly growing numbers of published studies have shown that aqueous, hydroalcohol, or alcohol extracts of *M. oleifera* leave possess a wide range of additional biological activities including antioxidant, tissue protective liver, kidneys, heart, testes and lungs, analgesic, antiulcer, antihypertensive, radioprotective and immunomodulatory actions [1, 7, 11, 12].

Calendula usually known as "marigold", Since *C. officinalis* has grown in North Africa; it is also named as "African marigold" is a reputed medicinal plant with ornamental properties. The yellow or orange-colored flowers are used as natural food dye, spice and tea as well as tincture, ointment or cosmetic cream. Recently, Marigold has become quite important in phytotherapy due to its healing effects against dermatological diseases. Marigold flower extract has several ingredients with reported antioxidant with profound antioxidant potential and having the ability to trigger cellular antioxidants, can be exploited for its use against a number of disorders including cardiovascular diseases, inflammation and cancer. Along with this it has been reported to possess several pharmacological, antiviral, antibacterial, cytotoxic, antitumor activity, antigenotoxic effect and also possessed wound-healing activity [13, 14].

The present study was undertaken to compare the potential of Olive leave, Moringa leave and Marigold petals as a source of antioxidants agents which were considered as route to prevent oxidative stress in the body in case of it used as a drug in fortified food of dairy industry.

MATERIALS AND METHODS

Materials: Olive leave were obtained from Faculty of Environmental Agricultural Sciences, Arish Univ., North-Sinai Egypt. Moringa leave obtained from National Research Center, Giza, Egypt and Marigold petals were obtained from Desert Research Center's Farm, El Fayom Governorate, Egypt.

Chemicals: Folin-Ciocalteu reagent, Gallic acid and potassium persulfate were bought from Fisher Chemic Ltd Chennal -India. 2, 2'-Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS), 2, 2-Diphenyl-1-pycrylhydrazyl (DPPH) purchased from Sigma- Aldrich, potassium persulfate from Fischer chemic Ltd chennal, India. All other chemicals from El-Nasr Co, Cairo, Egypt.

Methods

Extraction of Bioactive Compounds: The leave dried at 40°C ± 0.5 for 24h and milled into powder, 80% ethanol was added to olive leaves powder (10:1 v/w). The mixture was left to stand under agitation for 48 h and then was filtered Whatman No.2 filter paper The extract was concentrated by evaporation at 280 rpm /45°C in rotary evaporator (BÜCHI -Germany) to dryness at 45 °C and the residue obtained was stored in glass vials, at – 20 °C until to analysis [15].

The *Moringa oleifera* L. 80% ethanol was added to Moringa powder (10:1 v/w). The mixture was left to stand under agitation for 48 h and then was filtered through Whatman No. 1 filter paper. The extract was concentrated by evaporation at 280 rpm /45°C in rotary evaporator (BÜCHI -Germany) to dryness at 45 °C and the residue obtained was stored in glass vials at – 20 °C until to analysis [16, 17].

Fresh marigold flower were used for extraction of the active components. The petals were dried at 30 °C for 48h. About 450 ml of ethanol 70% was added to 700g Calendula petals for 48 h.; the extracts were filtered using filtered through Whatman No. 1 filter paper and concentrated by evaporation at 280 rpm /40°C in rotary evaporator (BÜCHI -Germany) to dryness at 40 °C and the residue obtained was stored in glass vials at – 20 °C until to analysis [18, 19].

Determination of Total Phenolic Compounds (TPC): TPC was calculated in the methanolic extracts of Olive leave, Moringa leave and Marigold petals according to the Folin–Ciocalteu method [20]. All values were expressed as mean (mg of Gallic Acid Equivalents (GAE)/g of dry weight).

Determination of the Total Flavonoid Compounds (TFC): The total flavonoid content was measured with aluminum chloride colorimetric assay [21]. The total flavonoid contents of the dry herbs were pressed as milligrams of catachin equivalents (CE) per 100 g dry weight (mg CE/100 g DW).

Detemination of Antioxidant Activity by DPPH Radical Scavenging: The 2, 2-Diphenyl-1-pycrylhydrazyl (DPPH) assay was used to determine the antioxidant activity of

plant extracts which contain natural compounds. The free radical scavenging activity of extracts was measured using the method described by Dehshahri *et al.* [22]. The extracted diluted in 1 ml ethanol 90% with 500 µg from both Moringa, Marigold and 20 µg of Olive extracts. Then, 4ml methanolic DPPH solution (60 mol/L). Blank was prepared with ethanol only and added to DPPH. Absorbance was measured at 515 nm.

The radical scavenging activity of DPPH was calculated using the following equation:

DPPH scavenging activity (%) = $[(A_0 – A_1)/ A_0]$ X100

where:
A_0 = the absorbance of the blank reaction
A_1 = the absorbance in the presence of methanolic extract

Fractionation Polyphenolic Compounds by HPLC: A reversed-phase high-performance liquid chromatographic (HPLC) technique was developed to identify and quantify the major phenolic compounds contained in the extracts [28]. HPLC analysis was carried out using Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector. The analytical column was an Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 ml/min for a total run time of 60 min and the gradient program was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The injection volume was 50 µl and peaks were monitored simultaneously at 280 nm, 320 nm and 360 nm for the benzoic acid and cinnamic acid derivatives, respectively. All samples were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI) before injection. By Central Lab.of Food Industry and Nutrition Division - National Research Centre, Giza, Egypt.

RESULTS AND DISCUSSION

Bioactive Compounds in Studied Plant Extracts

Total Phenolic Compounds: *In vitro*, antioxidant activity of the extracts showed that Olive leaves extract was too rich with polyphenols in terms of TPC the values were 189.50, 66.5 and 27mg/g as gallic acid for Olive leave followed by Moringa leave extract then Marigold, respectively.

Total Flavonoid Compounds: The values of TFC were 239.4, 34.7 and 7.4 mg/100g as catachin for Olive leave followed by Moringa leave extract then Marigold, respectively.

DPPH Scavenging Radical Activity: Data presented in table 1 showed that scavenging activity (DPPH) was 338.85, 11.15 and 8.43% for Olive, Moringa and Marigold, respectively.

It was concluded that the Olive leave extract had the highest antioxidant activity, this may be due to their highest phenols and flavonoids values, which agree with Moyo *et al.* [23] whom reported that flavonoids possess strong antioxidant and have a free radicals scavenging activity. Moreover, the synergistic effect of phenolic compounds may contribute significantly to the ability of the extracts to adsorb and neutralize free radicals or decompose peroxides and disturb the autoxidation reaction. The ability of free radical scavengers as antioxidant could be due to their redox properties, presence of conjugated ring structures and carboxylic group which had been reported to inhibit lipid peroxidation. Previous studies had found that polyphenols act as a synergistic behavior in their radical scavenging capacity when mixed together, this similar to that occurs in olive leaves extract, with a high content of oleuropein and other active polyphenols compared to the individual phenolics alone [10, 24].

Fractionation of Phenolic Compounds of Studied Plants by HPLC: Detection of some polyphenolic compounds of Olive leave, Moringa leave and Marigold petals extracts by using HPLC-DAD analysis was carried out at different signals (280, 320 and 360 nm) for qualitative and quantitative analysis.

Tables 2, 3 and 4 represent the qualitative analysis of 18 phenolic compounds in Olive, Moringa and Marigold.

- At 280 nm eight compounds were found (gallic acid, protochatchuic acid, catachine, syrngic acid, vanillic acid, cinnamic acid, chyrsin and oleuropein).
- At 320 nm four compounds were found (caffeic acid, ferulic acid, sinapic acid, rosmarinic acid) from six compounds of standards.
- At 360 nm tow compounds were appeared (rutin and quercetin) from three of standards [7, 22 , 25-27].

Table 1: Total phenolic compounds and Antioxidant activity of Olive, Moringa and Marigold extracts

Parameter	Olive	Moringa	Marigold
TPH	189.50	66.50	27.00
TFC	239.429	34.714	7.422
DPPH	338.85	11.15	8.43

TPH: Total phenolic content concentration (mg/g) as gallic acid equivalent

TFC: Total Flavonoids content concentration (mg/100g) as catachin equivalent

DPPH: Inhibition % / 0.1 mg extract

Table 2: Retention time (min) using HPLC of Olive, Moringa, Marigold and nine standards of phenolic compounds, using HPLC-DAD at 280 nm

	Compound	Olive	Moringa	Marigold	Standards
1	Gallic acid	5.37	5.3	ND	5.8
2	Protochatchuic acid	10.0	10.0	ND	10.09
3	Catachin	ND	17.7	ND	18.6
4	Syrngic acid	ND	23.0	23.3	22.9
5	Vanillic acid	ND	24.3	ND	24.9
6	Coumarin	ND	ND	ND	37.2
7	Oleuropein	38.7	39.6	ND	39.5
8	Cinnamic acid	42.7	42.9	42.45	42.8
9	Chyrsin	51.9	51.9	51.9	51.6

Table 3 Retention time (min) of Olive, Moringa and Marigoldand six standards of phenolic compounds, using HPLC-DAD at 320 nm

	Compound	Olive	Moringa	Marigold	Standards
10	Gentisic acid	ND	ND	ND	17.6
11	Chlorgenic acid	ND	ND	ND	20.6
12	Caffeic acid	21.13	21.15	21.9	21.6
13	Ferulic acid	32.13	ND	ND	32.3
14	Sinapic acid	33.54	33.44	ND	33.5
15	Rosmarinic acid	40.06	41.76	ND	40.04

Table 4: Retention time (min) of Olive, Moringa, Marigold and three standards of phenolic compounds, using HPLC-DAD at 360 nm

	Compound	Olive	Moringa	Marigold	Standards
16	Rutin	ND	35.8	34.8	35.4
17	Quercetin	43.15	43.14	ND	43.2
18	Kaempferol	ND	ND	ND	46.2

Table 5: Quantity determination of the phenolic compound (µg/g extract) of Olive, Moringa and Marigold by using HPLC- DAD at 280nm, 320 nm and 360 nm

	Compound	Sig	Olive	Moringa	Marigold
1	Gallic acid	280 nm	699.4535	21.35657	ND
2	Protochatchuic acid		8448.625	877.8573	ND
3	Catachine		ND	2144.311	ND
4	Syrngic acid		ND	27.45396	126.1332
5	Vanillic acid		ND	119.3396	ND
6	Coumarin		ND	ND	ND
7	Cinnamic acid		70.66248	56.98245	21.2324
8	Chyrsin		2794.973	393.9059	624.0929
9	Oleuropein		1147.503	662.1564	ND
10	Gentisic acid	360 nm	ND	ND	ND
11	Chlorgenic acid		ND	ND	ND
`12	Caffeic acid		1344.333	3199.989	139.8908
13	Ferulic acid		1894.619	ND	ND
14	Sinapic acid		588.2554	504.3867	ND
15	Rosmarinic acid		606.9275	313.7431	ND
16	Rutin	320 nm	ND	106.4706	67.6693
17	Qurecetin		218.3412	1241.935	ND
18	Kaempferol		ND	ND	ND

Cinnamic, acid chyrsin and caffeic acid were found in three extract but gallic acid protochatchuic acid, oleuropein, sinapic acid, rosmarinic acid and quercetin found in olive and moringa. While, rutin and syrngic acid were found in Moringa and Marigold extracts. Moreover, catachin and vanillic acid found in Moringa extract only. With various amounts as mentioned in Table 5.

Noticeable from above mentioned results Moringa recorded the highest account number of polyphenols, but Olive gained the highest amount of polyphenols. Meanwhile, Marigold possessed the lowest amount and number of polyphenols.

CONCLUSION

This study carried out to evaluate antioxidant in Olive leave, Moringa leave and Marigold petals to use them as source of antioxidant as a drug or in dairy industries. Olive extract had rich of phenolic compounds despite most phenolic compounds identified in Moringa extract by HPLC but Olive extract had highest antioxidant activity. Meanwhile, Marigold had low phenolic compounds and antioxidant activity. These extracts with 0.1, 0.2, 0.3 g/L had been used in the manufacture of bio-yoghurt in a separate paper.

ACKNOWLEDGMENT

I would like to express my sincere gratitude to Prof. Dr. M. M. Osman professor of Dairy Microbiology, Dairy Science Dept., Faculty of Agriculture, Suez Canal Univ., for his help and advice throughout the study.

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