Antibacterial and Antioxidant Activities of Some Plant Leaves

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ABSTRACT: Food industries and consumers are concerned about the negative effect of synthetic preservation and there is growing interest in applying natural alternatives additives for their antioxidant and antimicrobial activities. Therefore the objective of the present work was to evaluate total polyphenol, antioxidant activity and antibacterial activity of Moringa (*M. oleifera*), Olive (*Olea europaea*), Rosemary (*R. officinalis*) and Oregano (*O. vulgar*) dried leaves powders and their extracts. The results revealed that the extracted yield of plant leaves was ranged from 19.82% for rosemary to 30.27% for moringa. The result showed that plant leaves extracts have higher phenolic content than plant leaves powder Whereas, total polyphenols content were ranged from 26.41 to 50.13 mg GAE/g extract for oregano and olive, respectively while it was 9.19 and 17.22 mg GAE/g powder for rosemary and olive, respectively. DPPH radical scavenging activity are ranged in ascending order from 18.45 to 31.81 µmol troloxE/g of leaves powder for rosemary and moringa, respectively while from 61.80 to 90.20 µmol troloxE/g of leaves extract for oregano and moringa, respectively. The antibacterial activity against chosen bacterial strains are increased in the following order moringa < olive < rosemary < oregano in the plant powders and its extracts. The diameter of inhibition zone is varies between 4.33 mm for moringa powder to 8.00 mm for oregano powder in case of gram-negative bacteria (*Escherichia coli)* and from 20.66 mm for moringa extract to 29.33 mm for oregano extract in case of gram-positive bacteria (*Staphylococcus aureus).* The results of present work indicated that plant leaves extracts are very effective as antioxidant and antibacterial than plant leaves powders. Also results indicated that there were significant differences between different plant leaves.

Key words: plant leaves, extracts, polyphenols, antioxidant, antibacterial

INTRODUCTION

Lipid oxidation and microbial growth during storage of fatty food can be reduced by applying antioxidant agents to the meat products leading to a retardation of spoilage, extension of the shelf life and maintenance of safety and quality (Krishnan *et al.,* 2014 and Falowo *et al* 2017).Synthetic preservation such as butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA) and propyal gallate (PG) are typically used to protect foods from spoilage, also sodium nitrite and nitrate are part of food preservation system (Nunez De Gonzalez *et al.,*2012). Their use is restricted because of possible carcinogenic effects. Therefore, there has been increasing interest in alternative additives from natural sources (Shan *et al.,* 2009 and Yin *et al.,* 2016). The incorporation of natural antimicrobial inhibits major pathogenic organisms such as *Salmonella, Escherichia coli, Staphylococcus aureus, Listeria monocytogenes* and yeasts and molds (Hasapidou and Savvaidis, 2011). In food antimicrobials are used to preserve the food for long time (control natural spoilage process) and to increase food safety (control growth of pathogenic microorganisms).Meat industry is seeking for natural sources which have consumer acceptance (Karre *et al.,* 2013).

Plants are a rich source of biologically active compounds with potential applications in food industries. The majority of the plant derived compound

including phenols and flavonoids exhibit antioxidant and antimicrobial properties (Rafinska *et al.,* 2019). They are used in the food industry as antioxidant to prevent oxidation process and prolong the shelf life of food (Granato *et al.,* 2017) and (Nikmaram *et al.,* 2018). *Moringa oleifera* leaves have been identified as having great antioxidants and anti-microbial activity (Das *et al.,*2012) due to the presence of ascorbic acid, phenolics , flavonoids and carotenoids (Makkar and Becker,1996; Anwar *et al.,*2007; Moyo *et al.,*2011 and Falowo *et al.,* 2016).

Olive leaves have antioxidant activity (Botsoglou *et al.,* 2014) beside the antimicrobial activity (Markin *et al.,* 2008). Olive leaves possess a strong antibacterial and anti fungal action and these are due to the phenolic compounds (Pereira *et al.,* 2007). The oleuropein and lueotin-7-o-glycoside were the most abundant phenolic compounds present in olive leaves extract (Al-Rimawi *et al.* 2017). Rosemary leaves can be considered as antimicrobial and antioxidant in meat products. Phenolic compounds was found in rosemary as phenolic acid (caffeic acid, ferulic acid, carnosic acid, carnosol, epirosemanol) and volatile compounds as (carvacrol). Flavonoids as (lutiolin) (Velasco and Williams, 2011 and Nieto *et al.,* 2018). Oregano leaves antioxidant activity was observed by Sampaio *et al.* (2012) where the antimicrobial activity was observed by Viuda-Mortas *et al.* (2010). The phenolic compound in Oregano as phenolic acid: caffeic acid, neochlorogenic acid, p coumaric acid, rosmarinic acid, caffeoyl derivatives and volatile compounds as carvacrol, thymol, terpinene,p-cymene was reported by Hac-Szymonczuk *et al.* (2019).Therefore, the aim of this study was to evaluate of moringa, olive, rosemary and oregano leaves powder and its extracts as antibacterial and antioxidant.

MATERIALS AND METHODS

Plant materials

Dried plant leaves of Rosemary (*R. officinalis*) and Oregano (*O. vulgare*) were obtained from supper market at Alexandria city. Fresh Moringa leaves (*M. oleifera*) were obtained after harvest from private farm near Alexandria .Fresh Olive leaves (*Olea europea*) were obtained after pruning from Experimental farm of the City of Scientific Research and Technological Application New Borg El Arab.

Chemicals and reagents

Chemicals and reagents were obtained from El-Gomhouria Company, Alexandria, Egypt and Sigma-Aldrich (Steinheim , Germany).

Bacterial strains

Microorganisms used to determine the antimicrobial activities of plant leaves powders and its extracts were as follows: *Staphylococcus aureus* ATCC 6538P; *Escherichia coli* ATCC 8739; *Pseudomonas aeruginosa* ATCC 9027.All bacterial strains were obtained from the department of microbiology from Ain Shams Culture Collection, MERCN, Cairo. Egypt.

Dried leaves preparation

Air dried leaves (Rosemary and Oregano) were grinded using mixer grinder (Braun Kitchen Machine, Germany) to powder and stored at -18˚C in glass bottle for further use.

Fresh leaves preparation

Fresh leaves of Moringa and Olive were blanched on (90-95 ˚C) (1:4 w/v) in stainless steel cooker for 20 seconds according to Zeitoun *et al.* (2016) before oven drying at 40 ˚C until constant weight ,then grinded to powder and stored at -18˚C in glass bottle until further use.

Preparation of the extract

The extracts were prepared according to Sreelatha and Padma (2009) with some modification. Dried powdered leaves were extracted with cold water 100g/1000ml and stirred for 3h. The extracts were centrifuged at 4000 rpm for 15 min. The supernatant was collected and dried under vacuum oven at 50˚C to dryness .Dried extract were stored in glass bottle at -18˚C until further analysis.

Determination of the total poly phenol

The total phenolic content was determined by the Folin-Ciocalteu method according to El Sohaimy and Masry (2014). 0.4 ml of the powder sample and their extract samples in methanol (1mg/ml) was taken, mixed with 2 ml of Folin-Ciocalteu reagent and 1.6 ml of (7%) sodium carbonate. After shaking it was kept for 90 min in a dark and the absorbance was measured at 750 nm using Shimadzu-UV-160 spectrophotometer. Using gallic acid monohydrates, a standard curve was prepared. The total phenolic content was calculated and expressed as (mg GAE/g of sample).

Free radical scavenging activity

Free radical scavenging activity of the leaves powder and their extracts was determined using the stable DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) radical assay, performed according to Rakesh and Singh (2010).Trolox is used as standard and results are expressed in µM Trolox Equivalents/g. One ml solution of the sample in methanol was added to 0.5 ml of 0.15M DPPH solution in methanol .The contents were mixed vigorously and allowed to stand at 20˚C for 30 min in the dark. The absorbance was read at 517 nm spectrophotometrically.

Antibacterial activity

Bacterial cultural were prepared by transferring one colony into a tube containing 20 ml nutrient broth and grown for 18 h, at 37 ˚C. An agar-well diffusion method was used for determination of antibacterial activities described by Shan *et al*. (2007). All bacterial were suspended in sterile water and diluted to (10^5-10^6) cfu/ml. Wells (8.00 mm in diameter) were cut from the agar with a sterile borer and 65 µl solution of different plant leaves samples were delivered into them . The incubated plates were incubated at 37C˚for 24 h. Antibacterial activity was

evaluated by measuring the diameter of inhibition zone (DIZ) of the tested bacteria.DIZ was expressed in millimeters. All tests were performed in triplicate.

Statistical analysis

All analysis was done in triplicates. Data were analyzed separated by Duncan's multiple range test using Statistical Analysis System 6.21 package (SAS, 1995).

RESULTS AND DISCUSION

Extracted yield

Data of extraction yield are shown in Table (1).Extracted yield of moringa, olive, rosemary and oregano leaves extracted by water were 30.27%, 24.38%, 19.82% and 25.49%, respectively. Moringa leaves were the highest extraction yield (30.27%), while rosemary was the lowest (19.82%). Data of investigated plant leaves were significantly difference. These results are in agreement with the data obtained by Hasaballa *et al.* (2017) who found that moringa leaves gave the highest yield either by water extraction or ethanol (70%) extraction in the range of (30-35%), respectively. Zeitoun *et al.* (2016) reported that the water extraction yield of blanched olive leaves was 23.17%. Awad (2010) reported that the water extracted yield of rosemary was 17.87%. Krishnan *et al.* (2014) reported that the water extraction yield of oregano was 27.20% which close to the present results.

Table (1). Extracted yield (%) of different plant leaves extracted by water based on dry weight

Name of plant	Water extracted yield (%)
Moringa leaves	30.27[a]
Olive leaves	24.38[c]
Rosemary leaves	19.82[d]
Oregano leaves	25.49[b]

Means with different letters within a column are significantly different (P<0.05).

Total polyphenol content

Total phenolic content (mg of GAE/g dw) of different plant leaves powder and its extracts are shown in Table (2 and 3). Data for different plant leaves powder revealed that the highest phenolic content was found in olive leaves powder (17.22 mg GAE/g) followed by moringa leaves powder (11.05 mg GAE/g), oregano leaves powder (9.37 mg GAE/g) and finally rosemary leaves powder (9.19 mg GAE/g). There are significant differences between the results of plant leaves powder for its total phenolic content. On the other hand, data for different plant leaves Extracts showed that the highest phenolic content was found in olive leaves Extracts (50.13 mg GAE/g) followed by rosemary leaves extracts (30.82 mg GAE/g), moringa leaves extracts (27.44 mg GAE/g) and finally oregano leaves extracts (26.41 mg GAE/g). The results of total phenolic content are significantly different between plant leaves extracts.

Table (2). Total phenolic content in different plant leaves powders

Plant leaves powder	Total phenolic (mg GAE/g)of Plant powders
Moringa leaves	11.05[b]
Olive leaves	17.22[a]
Rosemary leaves	9.19[d]
Oregano leaves	9.37[c]

Means with different letters within a column are significantly different (P<0.05).

Table (3). Total phenolic content in different extracts of plant leaves

Plant leaves extracts	Total phenolic (mg GAE/g)of Plants extracts
Moringa leaves	27.44[c]
Olive leaves	50.13[a]
Rosemary leaves	30.82[b]
Oregano leaves	26.41[d]

Means with different letters within a column are significantly different (P<0.05).

Data in Tables (2 and 3) revealed that total phenolic content in different extracts of plant leaves were higher than their plant leaves powder. Significant differences between the results most likely due to genotype and environmental differences, time of talking samples, determination method, diseases, fertility, temperature, location and climate (Shan *et al.,* 2007). These results are in agreement with El Shohimy *et al.* (2015) who reported that phenolic content by water extraction for moringa leaves was 24.67 mg GAE/g, while Hasaballa *et al.* (2017) found that moringa leaves content of total poly phenol was 49.42mg GAE/g extract. Jaywardana *et al.* (2015) found that total phenolic content of moringa was 24mg GAE/g of dry weight. The result of total phenol content of olive leaves was in agreement with a previous study by Botsoglou *et al.* (2014) who reported that total phenolic in olive leaves was 45.20 mg GAE/g and Zeitoun *et al.* (2016) who reported that total phenolic content of olive leaves was 549.30 µg GAE/g extract. This in consistency should attributed to various parameters including the olive leaves cultivars, the season of collection, the leaves age and the extraction solvents.

The result obtained were in agreement with Tawaha *et al.* (2007) who found that the total phenolic compounds of rosemary was 48.90 mg GAE/g and Wojdylo *et al.* (2007) who reported 38 mg GAE/g of dry weight. Krishnan *et al.* (2014) determined the total phenolic content of oregano was 14.09 mg GAE/g of extract which was lower that found in this research work.

Antioxidant activity (DPPH radical scavenging activity) of plant leaves

The antioxidant activity of different plant leaves powders and its extracts are shown in Tables (4 and 5). DPPH radical scavenging activity method ranged from 18.45 to 31.81 µmol trolox E/g of dw of plant leaves powders and from 61.80 to

90.20 µmol troloxE/g of dw of plant leaves extracts. Moringa leaves powders and its extracts gave the highest antioxidant activity, while oregano leaves powders and its extracts gave the lowest antioxidant activity. Olive's activity was the second (24.78 and 84.37 µmol troloxE/g for leaves powder and extract respectively). Rosemary the third gave 18.45 and 74.37 µmol troloxE/g for leaves powder and leaves extract respectively. The results are significantly different.

Table (4). The antioxidant activity of different plant leaves powders

Plant leaves powders	Antioxidant activities µmol trolox/g of dry weight(dw)
Moringa	31.81[a]
Olive	24.78[b]
Rosemary	18.45[c]
Oregano	18.84[c]

Means with different letters within a column are significantly different (P<0.05).

Table (5). The antioxidant activity of different extracts of plant leaves

Plant leaves extracts	Antioxidant activities µmol trolox/g of dry weight(dw)
Moringa	90.20[a]
Olive	84.37[b]
Rosemary	74.37[c]
Oregano	61.80[d]

Means with different letters within a column are significantly different (P<0.05).

The antioxidant activity of plant leaves extracts of moringa ,olive ,rosemary and oregano were higher than plant leaves powders of moringa, olive , rosemary and oregano and they are decreased in the following order moringa > olive > rosemary >oregano for its powder and extract. These variations between them may be due to their different phenolic contents and may be related also to the nature of the phenolics, thus contributing to their electron transfer/hydrogen donating ability (Awad, 2010). Chemical composition of plant extracts mainly depends on the solvent used, uses solvents recommended as safe (GRAS). The obtained extracts should be a source of nutraceuticals for functionalizing foods and may be also used as preservatives or antioxidants which extend the shelf life of food (Rafinska *et al.,* 2019).

These results were in agreement with Hasaballa *et al.* (2017) who found that DPPH were 123.58 and 77.71 µg/ml for water and ethanol extract of moringa, respectively. Similar results were obtained by Sreelatha and Padma (2009) where they found the moringa leaves extracts significantly reduced DPPH radicals. Olive leaves showed results which is closed to the results obtained by Zeitoun *et al.* (2016) (223.81 µg/ml extract) and Talhoui *et al.* (2016) who showing value of 129µg/ml. Botsoglou *et al.* (2014) reported that the antioxidant activity of olive leaves was evaluated by the DPPH assay was 1.45 mmoles TE/g dry leaf. Values

of 1.68 and 1.23 mmoles TE/g dried olive leaf were reported by Mylonaki *et al.* (2008) and Makris *et al.* (2007), respectively. The differences in the radical scavenging activity of extracts may be due to their different phenolic contents in these extracts.

The obtained result of rosemary leaves were in agreement with Awad (2010) who found that antioxidant activity (59.53 µmol TE/g dw). Oregano leaves was the least in antioxidant activity among investigated plant leaves, however it is known for the antioxidant activity which due in part to presence of carvacrol, where higher antioxidant activity was found by Bounatirou *et al .*(2007) and Krishnan *et al.* (2014) who observed 3.30 µmol TE/g.

Antibacterial activity

The antibacterial effects of different plant leaves powders and its extracts on different bacteria strains are shown in Tables (6 and 7) respectively. Microorganisms used were *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Data revealed that there was significant variation in the antibacterial activities (diameter inhibition zones values) between different plant leaves powders and also between its extracts. The inhibition zones varied depending on the bacterial species and the type of the plant leaves. Data also revealed that plant leaves powder showed lower antibacterial activities than its extracts that could be to its low content of bioactive components. The values in two tables are decreased in the following order oregano > rosemary > olive > moringa. The diameter of zone inhibition are ranging between 11.33 to 29.33 mm for different plant leaves extracts and from 4.33 to 15.33 mm for different plant leaves powder. The results showed that all plant leaves powders or its extracts were active against gram-positive and gram-negative bacteria. The results indicated that all the plant leaves examined showed the highest antibacterial activities towards the gram-positive bacteria *Staphylococcus aureus.*

Table (6). Antibacterial activity of different plant leaves powder for chosen bacterial strains

Dried plant leaves	Inhibition zone diameter(mm)		
	Microorganisms		
	Staphylococcus aureus	***Pseudomonas aeruginosa***	***Escherichia coli***
Moringa	9.33^{b}	5.66^{c}	4.33^{b}
Olive	14.00^{a}	7.00^{b}	6.66^{a}
Rosemary	14.66^{a}	7.66^{ab}	7.66^{a}
Oregano	15.33^{a}	8.33^{a}	8.00^{a}

Means with different letters within a column are significantly different (P<0.05).

Table (7). Antibacterial activity of water extracts of different plant leaves for chosen bacterial strains

Dried plant leaves	Inhibition zone diameter(mm)		
	Microorganisms		
	Staphylococcus aureus	***Pseudomonas aeruginosa***	***Escherichia coli***
Moringa	20.66[c]	12.33[b]	11.33[c]
Olive	27.00[b]	16.00[a]	17.00[b]
Rosemary	28.66[a]	17.00[a]	18.66[a]
Oregano	29.33[a]	17.66[a]	19.66[a]

Means with different letters within a column are significantly different (P<0.05).

Gram-positive bacteria are obviously more sensitive to the spice and herb extracts than gram-negative bacteria. The sensitivity difference between the two groups of bacteria was explained because gram-negative bacteria have an outer membrane and a unique periplasmic space not found in gram-positive bacteria Krishnan *et al.* (2014).

The obtained results were in agreement with Hac-Syzmanczuk *et al.* (2019) who demonstrated that antimicrobial activity of oregano water extract towards gram positive bacteria(*Staphylococcus aureus)* were more sensitive than gram-negative. The antibacterial activity of rosemary extract may be due to the synergy between the rosemarinic phenolic acid and the carnosic acid diterpene (Neito *et al.,* 2018).

CONCOLUSION

The results obtained revealed that moringa, olive, rosemary and oregano leaves powder was effective as natural antioxidant and antibacterial. In the same respect the extracts of the investigated leaves showed a higher effect than the powder form. Regarding the difference among all the studies plant leaves it was obvious that moringa had the highest antioxidant effect while olive, rosemary and oregano had the highest antibacterial effect. That will be effective as natural preservatives for food.

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الملخص العربي

النشاط المضاد للبكتريا وللأكسدة لبعض أوراق النباتات

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يهتم قطاع تصنيع الأغذية وكذلك المستهلك بالتأثير السلبى للمواد الحافظة الصناعية حيث يوجد تنامى فى الأهتمام بتطبيق بدائل مضافات طبيعية ذات نشاط مضاد للأكسدة والميكروبات. لذلك كان الهدف من هذا البحث هو تقييم المواد الفينولية الكلية والنشاط المضاد للبكتريا والأكسدة لكلا من الأوراق المجففة ومستخلصات النباتات الآتية: المورينجا والزيتون والروزماري والزعتر. وأوضحت النتائج أن الناتج من إستخلاص أوراق النباتات تراوح بين ١٩,٨٢% للروزماري إلى ٣٠,٢٧% للمورينجا. وأن المحتوى من المواد الفينولية في مستخلص الأوراق كان أعلى منه في مسحوق الأوراق حيث تراوح بين (٢٦.٤١ إلى ٥٠,١٣ مجم مكافئات حمض جاليك/جم مستخلص) للزعتر والزيتون على التوالي بينما تراوح بين (٩,١٩ إلى ١٧,٢٢ مجم مكافئات حمض جاليك/جم مسحوق) للروزماري والزيتون علي التوالي. ولقد تراوحت قيم كاسحات الآصول الحرة(مضادات الأكسدة) تصاعديا من (١٨,٤٥ إلي ٣١,٨١ ميكرو مول مكافئات ترولكس/جم مسحوق) للروزماري والمورينجا على التوالي بينما تراوحت من (٦١,٨٠ إلي ٩٠,٢٠ ميكرومول مكافئات ترولكس/جم مستخلص) للزعتر والمورينجا على التوالي. ولقد تزايد النشاط المضاد للبكتريا ضد السلالات البكتيرية المختارة على هذا النحو من الترتيب: المورينجا ثم الزيتون ثم الروزماري ثم الزعتر سواء لمسحوق الأوراق أو مستخلصاتها. تباين قطر منطقة التثبيط فى حالة البكتريا السالبة لجرام (ايشيرشيا كولاي) بين ٤,٣٣ مم إلى ٨,٠٠ مم لمسحوق أوراق المورينجا والزعتر على التوالي و كذلك في حالة البكتريا الموجبة لجرام(ستافيلوكوكس ايريس) بين ٢٠,٦٦ مم إلى ٢٩,٣٣ مم لمستخلص أوراق المورينجا والزعتر على التوالي. ولقد أشارت نتائج مستخلصات أوراق النباتات المختارة إلى أنها مؤثرة جدا كمضادات للأكسدة وكمضادات بكتيرية أكثر من مسحوق هذة الأوراق وأيضا إلى وجود إختلافات معنوية بين مختلف الأوراق.