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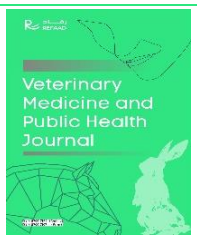

A Study of the Antioxidizing and Inhibiting Effects of the Alcoholic Extract of Sage *Salvia Officinalis* on Microorganisms

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Abstract:

Objectives: A study of the antioxidizing and inhibiting effects of the sage *salvia officinalis* on microorganisms.

Methods: Chemical constituents of sage leaves the percentage of the 98% ethanol extract of sage was 5.8% prepared in concentrations of 50, 100, 150 and 200 mg/ml, Antioxidizing and inhibiting effects on microorganisms, and sensory characteristics of the sage added biscuits.

Results: Scanning electron microscope images, the structure of powder sage leaves which appear as long tubes and round crystals, Hight percent concentration of minerals carbon and oxygen while low concentrations of (Zn, Al, Na, Mg, Si, K, Co, Ca, and Ce) were also found. the percentage of the moisture, protein, fat and ash (11.09, 6.89, 13.14, 9.64) % respectively, the inhibition effect of sage leaves ethanol extract prepared in concentrations of 50, 100, 150 and 200 mg/ml on three bacteria and fungi species of disc method. maximum effect on all three bacteria species *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas* sp. was found with the concentration 50 and 100 mg/ml. compared with 150 and 200 mg/ml. It has been found that the 150 mg/ml concentration has the most inhibition effect on the three species of fungi studied *Aspergillus niger*, *A. carbonarius*, *A. flavus*. All extract concentration inhibited *A. flavus*, it was that the antioxidant activity, iron ion bonding, reducing power and the ability capture the hydrogen peroxide of sage ethanol extract increases with high concentrations. Peroxide values decreased with increased sage added biscuits percentage (0, 0.5, 0,75 and 1) w/w% in stored for periods. However, the sensory characteristics of the sage added biscuits samples were good and close to the standard biscuit control.

Conclusions: Finally, we conclude that the sage plant has medicinal and nutritional benefits due to its antioxidant and antimicrobial activity.

Keywords: *Sage plant; Antioxidant; Microbial inhibitor.*

1 Introduction

Salvia officinalis (Family Labiatae) which is locally known as Maramiya (sage) is a herb with height ranging from 40-80 cm and had grayish leaves and blue flowers. It is a perennial plant but flowers appear in spring and early summer (Fleming, 2000). This herb is traditionally used as a tea or as a flavor to other herbal or black teas. sage has also been used as medicinal herb to treat wounds and internal sores. In Britain, the herb is prescribed to enhance perception and memory improvement. It is effective to restore health in patients suffering from paralysis and trembling of organs, it also helps soothing joint pain and treating bloody diarrhea and convulsions Hamidpour *et al*., (2014). sage tea has been known to have a remarkable antioxidant effect and the herb was recommended to be used as a natural antioxidant agent in the manufacture of medicines as it is safe and has minor side effects (Committee on herbal Medicinal Products (HMPC) 2009). Several researches have revealed that aromatic oils and organic extracts of many herbs possess strong antioxidant and antibacterial effect. The powerful antioxidant effect of sage extract is referred to its poly phenols compounds and volatile oils (Abdel Kader *et al*., 2014). In the Iraqi society, the use of herbal and alternative medicine is increasing and people tend to avoid chemical treatment. Therefore, the present study aims at investigating the antioxidant and microbial inhibiting effect of the extract of dry sage leaves.

2 Materials and Methods

2.1 Materials

Plant materials: the dry sage plant was purchased from the local market of Basra Governorate and grinded and stored in plastic containers away from light in a dry place at room temperature until use.

Culture media: Nutrient broth medium to activation of bacterial isolates prior to implantation and Nutrient agar medium for development of bacteria and PDA medium to activate and develop molds.

Microbiology used in the study: The bacteria *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas*sp., *Aspergillus niger*, *A. flavus* and *A. carbonarius* from the Faculty of Science / Life Sciences at the University of Basra.

Materials Preparing the biscuits: all-purpose flour, baking powder, NaCl salt, sugar (sucrose), butter, distilled water and the ground sage plant.

2.1 Methods

Estimation of the chemical structure of the sage plant: The chemical estimates were made at the rate of three replicates and were calculated on the basis of dry weight, which included the determination of the moisture, ash and fat according to (AOAC. 1984) and protein estimation by (Pearson, 1971). Analysis of the electron microscopy Scanning (SEM) The mineral elements were estimated and a number of images were taken using the nanotechnology technology of the sage plant. The Field Emission Scanning Electron Microscope (FESEM) (SupraTM55VP) model from the German company Carl Zeiss using the electronic detector SE2, with a voltage of 1000 volts with an amplitude of 5800 x and 3.2 mm According to (European Medicines Agency, 2010).

Preparation of plant extract ALimpic *et al* (2015): Take 5 g of crushed dry matter and add 50 ml of ethanol at 98% concentration at room temperature for 24 hours. after that, filter with What man No.1 and concentrate on rotary vacuum evaporator at 40 ° C. In a glass dish and dry in oven at 40 ° C then skimmed and put in sealed dark sealed containers and kept in cooling 4+° C until use.

Concentration Preparation and Calculation of Produced Concentrations: Concentrations of the ethanolic extract of the sage (5, 10, 15, 20) mg / ml for iron ion bonding and hydrogen peroxide uptake and (50, 100, 150, 200) mg / ml for antioxidant efficacy and reducing power melted in ethyl alcohol 98% The percentage of the product was calculated according to the following law:

Percentage of yield = (dry ethanol content of the extract / sample weight) × 100

Estimation of antimicrobial efficacy: Using the anti-bacterial agar disc diffusion method (Firuzi *et al*., 2013) using an appropriate temperature and time period for microorganisms studied, using paper tablets with diameters 6 mm and diameter of the inhibition zone in mm.

Antioxidant Property Evaluation: Antioxidant efficacy of the ethanolic extract of the sage was estimated ferric thiocyanate method by the suggested (Osawa and Namiki ,1981) and was calculated according to equation:

[% Antioxidant efficacy= 100 - Absorption reading of the model \ Absorption reading of control sample x 100].

Chelating of ferrous ion was followed by Su *et al*., (Su *et al*., 2008) in (Rohman *et al*., 2010) the susceptibility of the extract was calculated by attaching the iron ion to the following equation:

Link ability% = -1 [Absorption reading of the model / absorption reading of the control sample] × 100

Hydrogen peroxide scavenging: measured by Turkoglu *et al*., (2010) the following equation was applied to calculate the effect of ethanolic extract of the sage in the capture of the peroxide root:

% Effectiveness of peroxide capture = [Absorption reading of the control sample - Absorption reading of the model / absorption reading of the control sample x 100]

Measuring the power of reduction: It was estimated according method to the Wu *et al*., (2003) and the absorbance reading was adopted to measure the reduced capacitance of the ethanolic extract of the sage.

Preparing the biscuits: Prepare standard biscuits in ratios (w/w) using 60.5% all-purpose flour, 1.5% baking powder, 1% NaCl salt, 1% sugar (sucrose), 7.5% butter, 28.5% distilled water, and the ground sage plant is added to it. The mixture was replaced with a ratio of (0, 0.5, 0.75, 1) % (w/w). The biscuits were shaped and baked in a convection oven at 180°C for 10 minutes and left to cool. Then they were stored in plastic containers at laboratory temperature for (0, 4, 9) days (Batista *et al*.,2019).

Sensory evaluation of laboratory biscuits: Sensory evaluation of the biscuit was done after adding the sage leaves cut into small pieces to flour and by (0,0.5, 0.75, 1) w / w% by nine arbitrators from the Department of Food Science according to the form of sensory evaluation (Table 1) mentioned in (AACC: American Association of Cereal Chemists, 1983).

Peroxide value: The peroxide value was calculated in the laboratory biscuits stored (0, 4, 9) day according to the method (AOAC. 1984).

Statistical analysis: Full randomized design (C.R.D.) was used for two-factor global experiments (3 × 4 × 9) to study nine sensory characteristics of the laboratory biscuits by four samples and three replicates. The statistical data were statistically analyzed using SPSS (2009) and tested using the least significant difference L.S.D. at a significant level of 0.05.

Table 1: Formal assessment form for laboratory biscuits (American Association of Cereal Chemists. 1983)

QUALITY ELEMENTS	Class limits	A	B	C	D
Color	1-10				
Appearance	1-10				
Taste	1-10				
Arrhythmia of pulp tissue	1-10				
Figure	1-10				
External crust thickness	1-10				
Smell	1-10				
Chewing	1-10				
The papyrus	1-20				
Total	**100**				

3 Results

Chemical constituents of sage leaves are shown in Table 2. The results showed that the percentage of protein, moisture, fat and ash were as follows (9.64, 13.14, 6.89, 11.09%).

Table 2: Chemical composition of dry sage leaves

Plant Type	% Fat	% Protein	% Moisture	% Ash
Sage plant	13.14	11.09	6.89	9.64

Scanning electron microscope Figures (1,2 and 3) illustrate the structure of ground sage leaves which appear as long tubes and round crystals. Percent concentration of minerals in sage powder are shown in a stylist 1 and 2. Carbon and oxygen constituted 75.71%, 79.63% and 19.90%, 23.92 % in the tubes and crystals respectively. Low concentrations of Zn, Al, Na, Mg, Si, K, Co, Ca, and

Ce were also found. In their study on the minerals of sage and Ginger in Nigeria, Lamari *et al*. (2011) found low concentration of Co, Cr, Sc, Sb and Rb which constituted 0.845, 1.67, 0.520, 0.107 and 0.430 respectively. The European Committee of Herbal Medicinal Products EMA (2010) found that extract of sage leaves is rich in iron and magnesium, which reached 885 ppm and 4.1 kg/g respectively, together with Na, K, Ca, Mn, Zn and Cu.

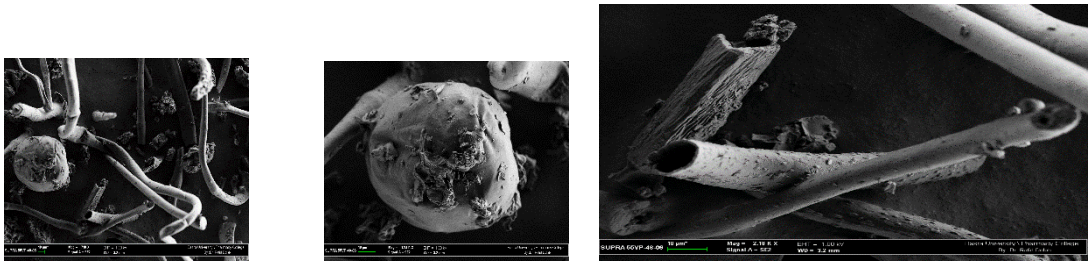
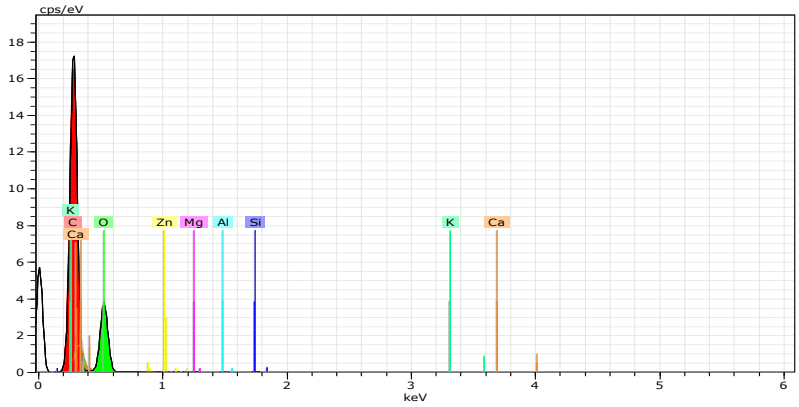
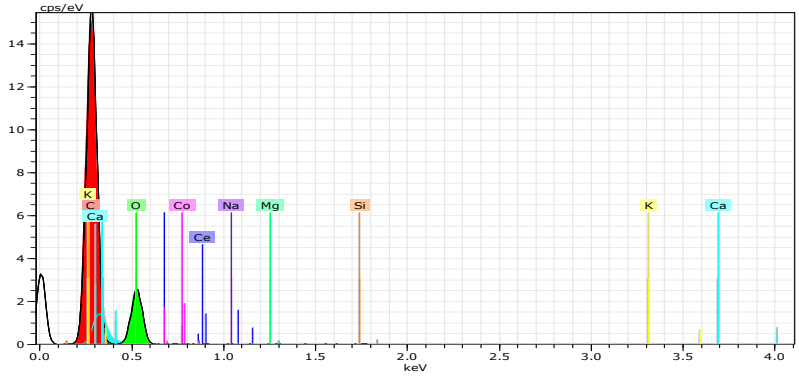


Figure (1, 2 and 3): shows the morphology of the sage leaves under the scanning microscope.



A stylist 1: illustrates the mineral elements present in the crystalline forms of the sage leaves.



A stylist 2: illustrates the mineral elements present in the tubular shapes of the sage leaves.

The results exhibited that the percentage of the 98% ethanol extract of sage was 5.8% which was found to be effective in extracting all reactive constituents of the leaves. (ALimpic *et al*., 2015) found that the 96% ethanol extract of sage leaves was rich with phenolic compounds which reached 144.6 mg/ml while the whole plant contained 127.71 mg/ml and the stems 119.02 mg/ml, followed by 50%, 30% and 10% concentration. These authors also found that the phenolic compounds concentration was higher in the methanol extract compared to ethanol extracts (35.82-144.60) mg/ml.

Table 3 shows the inhibition effect of sage leaves ethanol extract prepared in concentrations of 50, 100, 150 and 200 mg/ml on bacterial growth. Maximum effect on all three bacteria species was found with the concentration 50 and 100 mg/ml. Diameter of inhibition field on *E. coli* reached 20 mm with 50 mg/ml concentration and 20 mm on *Pseudomonas* sp. with 200 mg/ml concentration.

Table 4 exhibits results of the effect of sage extract on fungi. It has been found that the 150 mg/ml concentration has the most inhibition effect on the three species of fungi studied. Maximum inhibition diameter was 26 mm on the fungus *A. flavus*, followed by the 200 mg/ml concentration

with an inhibition diameter of 21 mm on the same species. The least inhibition effect was that of the 100 mg/ml concentration on *A. niger* and *A. carbonarius*. All extract concentration inhibited *A. flavus*.

The use of antioxidant activity of sage ethanol extract at 50, 100, 150 and 200 mg/ml concentrations as well as the artificial antioxidant BHT at 0.05% concentration has shown that the ethanol 200 mg/ml yielded maximum effect with 79% activity while the BHT 0.05% gave 92.62 activity (Figure 5). Both 100 and 150 mg/ml concentrations exhibited similar antioxidant effect with 61% and 67% respectively while the 50 mg/ml concentration showed 52% activity.

Table 3: Diameters of the inhibition zones (mm) for the growth of the bacteria with four concentrations of the ethanol extract of the sage.

diameter Growth inhibition zones (mm)			Name of bacteria
Pseudomonas	*Staph. aureus*	*E.coli*	concentration Extract mg / ml
9	13	20	50
8	9	8	100
10	8	—	150
20	10	—	200
—	—	—	98% ethanol (control)

Table 4: Diameters of the inhibition zones (mm) for the growth of fungus with four concentrations of the ethanol extract of the sage.

diameter Growth inhibition zones (mm)			Name of fungi
A. carbonarius	*A. flavus*	*A. niger*	concentration Extract mg / ml
—	9	9	50
—	14	—	100
13	26	7	150
12	21	—	200
—	—	—	98% ethanol (control)

The results presented in Figure 4 indicate the ability of sage alcoholic extract to bind ferrous ion compared to the compound EDTA-2Na and for the concentrations 5, 10, 15 and 20 mg/ml. It has been observed that the ability of iron binding increased with increasing concentration and reached its highest value of 95.71% at 20 mg/ml compared to EDTA which gave a binding ability of 90.95 for the same concentration.

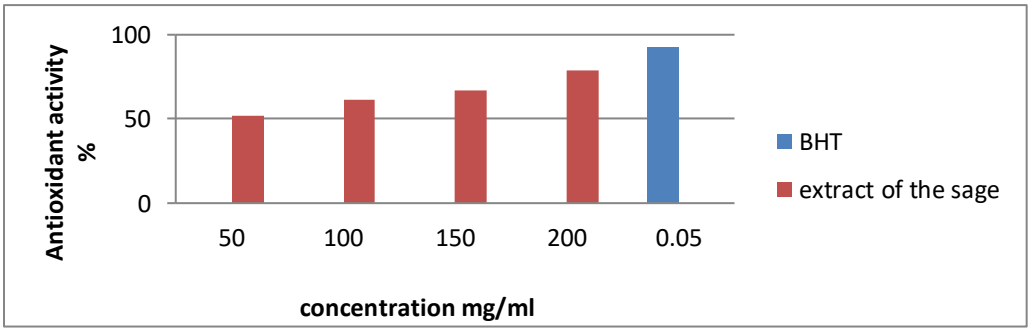


Figure 4: shows the antioxidant efficacy (%) of the ethanol sage extract compared to the industrial antioxidant BHT.

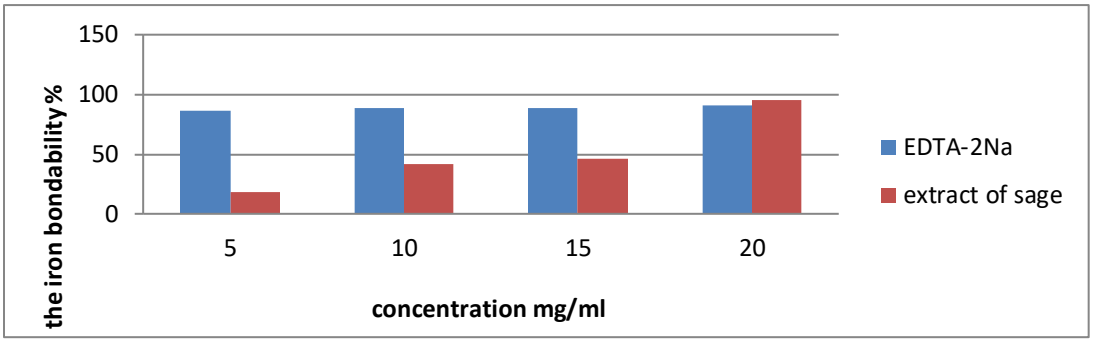


Figure 5: Iron ion bonding (%) for the ethanol sage extract compared to the EDTA-2Na compound.

Figure 6 illustrates the reduction power of alcohol extracts of sage at concentrations 50,100,150 and 200 mg/ml measured at 700 nm wavelength and compared to ascorbic acid solution prepared at same concentrations. It was observed that reduction power increased with concentration and reached 2.238, 2.749, 2.874 and 2.832 nm for all concentrations respectively while the standard ascorbic acid 2.013, 2.263, 2.694 and 2.879 nm respectively. Maximum value was obtained for 200 mg/ml concentration and was nearly similar to that of standard ascorbic acid.

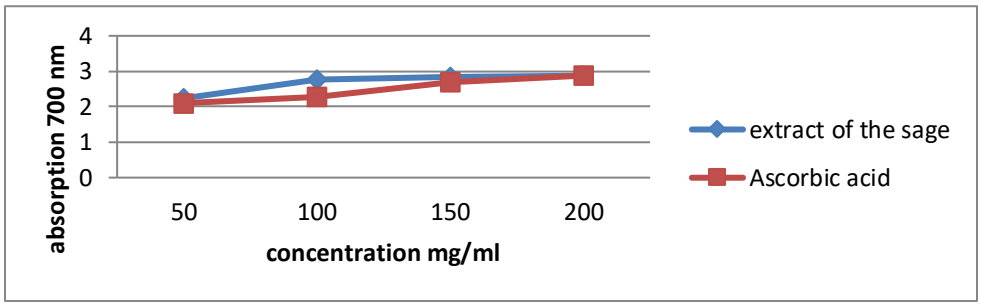


Figure 6: The reduced power of the ethanol extract of the sage plant compared with the standard ascorbic acid.

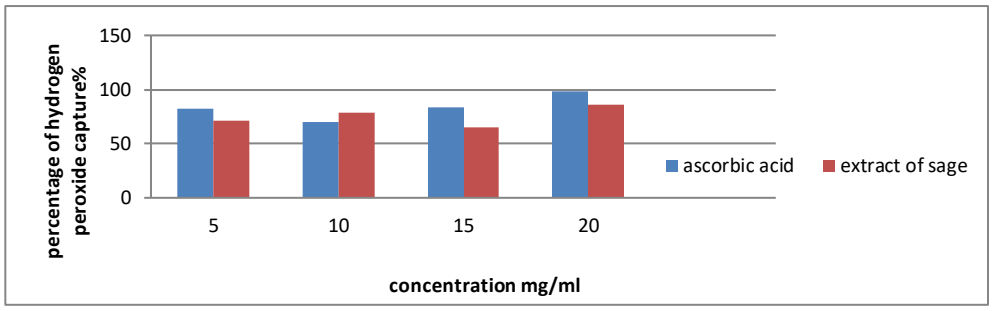


Figure 7: The ability of the ethanol extract to capture hydrogen peroxide (%) compared to ascorbic acid.

The results in Figure 7 show the ability of sage alcohol extract prepared with concentrations 5, 10, 15 and 20 mg/l for susceptibility of hydrogen peroxide (H_2O_2) in comparison to standard ascorbic acid. This extract is susceptible to peroxide radical at all concentrations but appeared higher at 20 mg/ml and reached 86.17% while it was 71.34% for the 5 mg/ml concentration. These values were lower than that of standard ascorbic acid, which reached 97.97% and 82.41% for 20 and 5 mg/ml concentration respectively.

Statistical analysis of sensing evaluation of sage added biscuits (Figure 9) did not show significant differences ($P \leq 0.05$) for all sensing features studied except for the taste of standard biscuits which showed significant variation between biscuits types (A,B,C,D) which reached 7.2, 7, 8 and 6.3 respectively.

Table 5: Results of the sensory

QUALITY ELEMENTS	Class limits	A	B	C	D	L.S.D.
Color	1-10	7.6	8.11	8.4	7.8	0.32
Appearance	1-10	7.8	7.4	8.2	7.8	0.60
Taste	1-10	7.2	7	8	6.3	0.20
Arrhythmia of pulp tissue	1-10	7.8	8.2	8.4	8.11	0.33
Figure	1-10	8.4	8.3	8.5	8.6	0.40
External crust thickness	1-10	8	8.6	8.2	8.2	0.60
Smell	1-10	7.7	7.8	7.7	8	0.40
Chewing	1-10	7.7	8.3	8	8.4	0.50
The papyrus	1-20	10.7	12	12.11	11.22	0.60
Total	**100**	**72.9**	**75.71**	**77.51**	**74.4**	

evaluation of the laboratory biscuits factory. A: - 0.5% Sage B: - 0.75% Sage
C: - 0% Sage (control) D: - 1% Sage , L.S.D. :-Least Significant Difference Value.

The values in Figure 8 refer to the effect of adding sage leaves percentage (0, 0.5, 0,75 and 1) w/w% on peroxide values of experimental biscuits 0, 4 and 9 days at room temperature. Peroxide values decreased with increased added sage concentration in contrast to control samples.

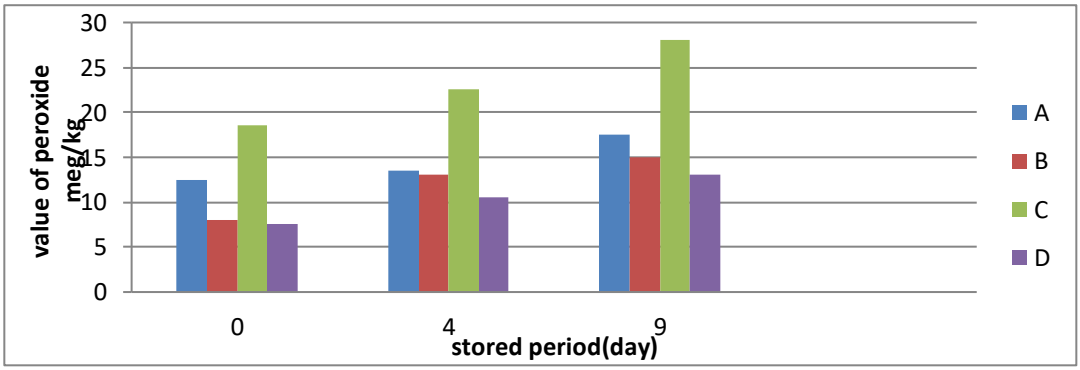


Figure 8: Effect of sage addition at different rates on the peroxide values in laboratory biscuits.
A: - 0.5% Sage B: - 0.75% Sage C: - 0% Sage (control) D: - 1% Sage

4 Discussion

Chemical constituents of sage leaves It is noticed that concentration of fats is rather high, particularly volatile oils which are considered as reactive compounds. Protein reached 11.09%, which is moderate concentration, followed by ash that contains high parentage of essential minerals.

The inhibition effect on bacterial growth in contrast, no inhibition effect was found on *E. coli* with 150 and 200 mg/ml concentrations. Similar outcome was observed by (Abdel Kader *et al*., 2014) who found that a lower concentration of 75 mg/ml of sage oil gave inhibition field diameter of 18 mm on *E. coli*. They concluded that phenolic compounds present in sage oil cause such effect but these compounds did not affect other bacteria like *Pseudomonas* and *Staphylococcus*. Several studies, however, have shown that aromatic oils and organic solvents extracts of herbs and spices have

Because only dry leaves of sage were analyzed, water content was low and reached 6.89%. Present results of analysis revealed high nutritional value of sage, a reason it was prescribed as tea to increase minerals in the body and also to treat joint pain and infections.

remarkable inhibition effect on some microorganisms (Albayrak *et al*., 2010; Firuzi *et al*., 2013). The inhibition activity of sage extracts on fungi is attributed to the flavonoid compounds such as Apigenin which high effect on both bacteria and fungi (Then *et al*., 2004). Other inhibiting compounds include Carvacol, Thymol, Limonene, *a* and β -Thugone and Menthol which exert considerable antifungal and antibacterial effect on G- and G+ (Akloul *et al*., 2014). Similarly, (Ismail, 2010) demonstrated the inhibition effect of aqueous extract of sage on the fungus *Fusarium* particularly

with high extract concentrations and found an inhibition percentage of 17.2, 26.5, 40.8 and 49.1% for 125, 250, 500 and 1000 ppm concentrations respectively.

It was observed that the antioxidant activity of sage ethanol extract increases with high concentrations, a finding coincided with the results of (Al-Hilfi, 2009) who attributed such relationship to the high concentration of antioxidant compounds in alcoholic and aqueous extracts for all studied plants. The present findings also appear similar to those of (Abdel Kader *et al*., 2014) who found that the DPPH antioxidant activity of the methanol extract of sage at IC_{50} scale was 27.53 which is nearly similar to that of BHA which was 14.48, while that of vitamin C was 2.70 which is rather high compared to that of volatile oil (62.65). It has been concluded that the strong antioxidant effect of sage extract is due to the presence of poly phenolic compounds and volatile oils where poly phenols concentration reached 31.25 mg/g and the total flavonoids reached 18.46 mg/g. . The low value of IC_{50} is an indicator of strong antioxidant activity. The strong antioxidant activity of alcoholic extract of sage is mainly because of the presence of the poly phenols, flavonoid glycosides, derivatives of rosmarinic acid , super onion oxide (SOD) , xanthenes enzyme oxidize, caffeic acid and β-catechol (Lu and Foo, 2001; Then *et al*., 2004; Ismail, 2010). In their work on the common medicinal herb *Salvia tomentosa* (sage) in Turkey (Dincer *et al*., 2013) found that its antioxidant activity ranged between 1.77 and 2.29 mg/g dry weight and its content of phenoleas and falvonoids ranged between 49.27 - 66.15 and 36.27 – 40.83 mg GAE g ^ (-1) (dry weight).

The ability of sage alcoholic extract to bind ferrous ions is attributed to its content of phenolic acids such as chlorogenic acid, caffeic acid, ferulic acid and rosmarinic acid, which are biologically active compounds that are responsible for the antioxidant effect of sage. Other compounds include poly phenols, flavonoids, flavon glycosides, xanthenes oxidize, β-catechol, SOD enzyme super oxide anion radicals (Lu and Foo, 2001a ; Lu and Foo, 2001b ; Then *et al*., 2004). The present results are in accordance with those of (Yusof *et al*., 2013) who observed increasing of iron binding activity with increasing concentration of methane extract of Pandan plant. In their study on iron binding activity of three species of Turkish sage, (Erdogan –orhan *et al*., 2010) found significant effect ranged between (2.5 - 12.04) % for the concentrations 0.5 and 0.1 mg/ml in comparison to BHT which showed 21.71 and 26.94 for the same concentrations.

Maximum value of the reduction power was obtained for 200 mg/ml concentration and was nearly similar to that of standard ascorbic acid, this is attributed to several phenolic and flavonoids compounds in sage where total phenols reached 31.25 mg/g and flavonoids reached 18.46 mg/g (Abdel Kader *et al*.,2014). Many compounds with antioxidant activity have the ability to reduce ferric ions $Fe^{+3}$to Fe^{+2} and therefore increasing its reduction power (Moein *et al*., 2008). The present results were in agreement with those of (El-shennaway *et al*., 2017) who estimated the reduction power of three aromatic oils of sage; lemongrass, sage and thyme at concentrations 5% and 10% comparison to ascorbic acid and found it reached (32.28 , 63.71) (23.14 , 49.23) and (18.14 , 34.71) m mol ascorbic acid respectively.

This sage ethanol extract is susceptible to peroxide radical at all concentrations in comparison to standard ascorbic acid. The high peroxide susceptibility of is attributed to its antioxidant compound *a* and β-thujone in its volatile oil form which is capable of grabbing free radicals. Other reactive compounds include apigenin (poly flavonoids), rosmarinic acid (strong antioxidant) and methyl carnosate carnosic acid (Überegger *et al*.,2002). The present results coincide with the findings of (Al-Hilfi, 2009) who found that all alcoholic and aqueous extracts of herbs and spices (turmeric, black pepper and chamomile) have high hydrogen peroxide susceptibility, which is higher in methanol extracts than aqueous extracts. The results also agreed with the findings of (Al-Athary, 2016) who observed high peroxide susceptibility of all aqueous and alcoholic extracts of ginger , black pepper , cinnamon , pink and turmeric which was lower than that of ascorbic and alcohol extracts (ginger, black pepper, cinnamon, cloves and turmeric) were lower than the sample of ascorbic acid 98% compared to study reached at 97.97% for concentration (20 mg / ml). The high efficiency of the sage extract is due to its containment of 33.77 mg / g poly phenol compounds and 18.14 mg / ml flavonates supported by volatile oils and therefore has a rapid anti-oxidant effect (Abdel Kader *et al*., 2014).

The taste of standard biscuits which showed significant variation between biscuits types (A, B, C, D) which reached 7.2, 7, 8 and 6.3 respectively. This variation is due to the amount of sage added to different types of biscuits where the control sample gave highest values of most features

including appearance, taste, texture and fluffiness, which reached 8.2, 8, 8.4, and 12.11 respectively. It was also noticed that there were no significant differences between biscuit samples for appearance and smell where sample D (1% sage) had highest value of appearance, smell and chewing which reached 8.6, 8 and 8.4 respectively in comparison to sample C where it reached 8.5, 7.7 and 8 respectively and this can be referred to the strong odor of sage aromatic oils. Sample B (0.75% sage) had highest value of color and outer texture which reached 8.11 and 8.6 respectively in comparison to the control sample with values of 8.4 and 8.2 respectively. These results are in agreement with those of (Dave *et al*., 2014) who observed that the sensation features of standard biscuits were significantly higher than those with honey added to with different percentages. The high sensation values of all studied characters of sage added biscuits (75.71, 74.4, 72.9) with addition percentage 0.75, 0.5 and 1% in comparison to control (77.51) would indicate that sage added biscuits can be made with high sensation quality.

Peroxide values decreased with increased added sage concentration in contrast to control samples. This effect is due to increment of antioxidant phenolic compounds of sage, which impede fat oxidation in biscuits. The effect of 1% concentration was higher than that of 0.5 and 0.75 where peroxide values reached 5.7, 8, 5.12 and 5.18 meq/kg at the start of storage, but these values increased with longer storage periods. The values were lower in the samples with sage added in contrast to control sample which has undergone rapid increase of peroxide values at the end of storage period, peroxide value was 18.5 meq/kg at the beginning and increased to 22.5 meq / kg after four days and then to 28 meq/kg after 9 days at room temperature. This is mainly due to rise of room temperature in summer which was 40 °C. Also, the butter used in making biscuits has been stored in polyethylene containers at room temperature which led to oxidizing rotting in addition to other factors such as humidity, minerals and microbial contamination (Dave *et al*., 2014). Our results indicate that sage leaves and its alcoholic extract can act as a natural source of antioxidants and microbial inhibitors which would prolong storage period of experimental biscuits.

5 Conclusions

We conclude that the sage plant has medicinal and nutritional benefits due to its antioxidant and antimicrobial activity, it was that the antioxidant activity of sage ethanol extract increases with high concentrations, also recorded inhibitory efficacy for the studied Bacteria, yeasts and fungi with varying inhibition rates.

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References:

AACC: American Association of Cereal Chemists. (1983). *Approved Methods of the AACC, 8th ed.*, st. paul, MN, USA.

Abdel Kader, M., Ahcen, B., Rachid, D. & Hakim, H. (2014). Phyto chemical study and Biological Activity of sage (Salvia officinalis L.). International *Journal of Biological, Agriculture and food Engineering*, 8(11).

Akloul, R., Benkaci_Ali, F., Zerrouki, M. & Eppe, G. (2014). Composition and biological activities of the essential oil of Nigella sativa seeds isolated by accelerated microwave steam distillation with cryogenic grinding. *American Journal of Essential oil and natural products*, 1(3), 23-33. https://doi.org/10.1080/0972060x.2013.813275

Al-Athary, R.A.A. (2016). *Effect of some plant extracts on quality characteristics of beef patties during refrigerated and frozen storage*. A thesis Master, College of Agriculture, food science and biotechnology, university of Basrah, (Arabic) pp118.

Albayrak, S., Aksoy, A., sagdic, O. & Hamzaoglu, E. (2010). Compositions, Antioxdant and Antimicrobial Activity of Helichrysum (Astera ceae) species Collected from turkey, *Food chem*., 119, 114-122. https://doi.org/10.1016/j.foodchem.2009.06.003

Albayrak, S., Aksoy, A., Albayrak, S. & sagdic, O. (2013). In vitro Antioxidant and Antimicrobial Activity of some Lamiaceae species. *Iranian Journal science and Technology*. A1, 1-9.

Al-Hilfi, S. A. H. (2009). *Extraction, Separation and Identification of phenolic compounds and its derivatives from plant sources and use it as antioxidant and antimicrobial in food system*. A thesis doctorate, College of Agriculture, food science and biotechnology, university of Basrah, (Arabic) pp187.

ALimpic, A.Z., Duletic _ LAusevuc, S.N., Matevski, V.S. & Marin, P.D. (2015). Antioxidant Activity of *Salvia jurisicii* kosanin ethanol extract. *Botanica Serbica*, 39(1) :53-58.

AOAC. (1984). *(Association of Official Analytical Chemists). Official methods of analysis*. 14th ed., Washington, Dc, USA.

Batista, A. P, Niccolai, A., Bursic, I., Sousa, I., Raymundo, A., Rodolfi, L., Biondi, N. & Tredici, M. R. (2019). Microalgae as functional ingredients in savory food products: Application to wheat crackers. *Foods*, 8 (12), 611. https://doi.org/10.3390/foods8120611

Committee on herbal Medicinal Products (HMPC) (2009). "Assessment Report on *Salvia officinalis* L., Folium and *Salvia officinalis* L., *Aethero Leum*" European, Medicines Agency, Retrieved 6-6-2016 Edited.

Dave, N., Patel, I. & Patel, V. (2014). Antioxidant Activity, Textural Properties and Storage Stability of Honey Fortified Biscuits. *Advances in Bioresearch*, 5 (1), 93-101.

Dincer, C., Tontul, I., Burakcam, I., Özdemir, K.S., Topuz, A., Nadeem, H.S., Tugrul Ar,S. & Göktürk, S.R. (2013). Phenolic Composition and antioxidant a ctivity of salvia tomentosa Miller: effects of cultivation harvesting year, and storage. *Academic Journals*, published by scientific and Technological, Research Council of Turkey, journals tubitake. gov. tr.

El-shennaway, M. & Abozid, M. M. (2017). Chemical Composition, Antioxidant and Antifungal Activities of Three Essential oils against Fungal pathogens causing Damping off and Root-rot diseases in pea. *Der pharma chemica*, 9(8), 85-93.

European Medicines Agency (EMA). (2010). Committee herbal medicinal products (HMPC),Assessment report on *salvia officinalis*., Folium and *salvia officinalis*. Aetheroleum. http://www.emea. europa.eu.(1-39)pp.

Ensikat, H.J., Ditsche- Kuru, P. & Barthlott, W. (2010). Scanning electron microscopy of plant surfaces: simple but sophisticated method for preparation and examination. Microscopy: Science, Technology, Applications and Education, 248-255.

Erdogan–orhan, I., baki, E., Senoi, S. & Yilmaz,g. (2010). *Sage –called plant species sold in turkey and their antioxidant activities*.

Firuzi,O., Miri, R., Asadollahi, M., Eslami , S. & Jassbi, A. (2013). Cytotoxin, Antioxidant and Antimicrobial Activity and Phenolic contents of Eleven Salvia species from Iran. *Iranian Journal of pharmaceutical Research*, 12(4), 801-810.

Fleming, T. (2000). *PDR for Herbal Medicines, Montrale: Medical economics company*, p 655_657.

Hamidpour, M., Hamidpour, R. & Hamidpour, S. (2014). chemistry, Pharmacology and Medicinal Property of sage (salvia) to prevent and Cure Illness such as obesity, Diabetes, Depression, Dementia, Lupus, Autism, Heart Disease, and Cancer. *Journal of Traditional and complementary Medicine*, 4 (2), 82-88 edited. https://doi.org/10.4103/2225-4110.130373

Ismail, F. K. (2010). Evaluation of inhibitory activity of extracts of some plants in the growth of fusarium. *Journal of Agricultural Sciences of Iraq*, 41 (2): 165-172.

Lamari, Z., Larbi, R. & Yagoubi, B. (2011). Trace element content of ginger and sage medicinal plants from Algeria. *Sci. Res.*, 3(9), 542-544.

Lu, Y. & Foo, L. (2001a). Antioxidant Activity of poly phenols from sage (*Salvia officinalis*). *Food chemistry*, 75(2), 197- 202. https://doi.org/10.1016/s0308-8146(01)00198-4

Lu, Y. & Foo, L. (2001b). Salvianolic acid L, apotent Phenolic Antoxidant from *Salvia officinalis*. *Tetrahedron Letters*, 8223-8225. https://doi.org/10.1016/s0040-4039(01)01738-5

Moein, M. R., Moein, S. & Ahmad-izadeh, S. (2008). Radical Scavenging and Reducing power of *Salvia mirzayanii* sub fractions. *Molecules*, 13, 2804-2813.

Osawa, T. & Namiki, M. (1981). Anovel Type of antioxidant isolated from leaf wax of Eucalyptys leaves. *Agric Biol. Chem*, 45, 735-739.

Pearson, D. (1971). *The chemical analysis of food. 6th ed*, Chemical Pubishing Company,INC. New York. 604.

Rohman, A., Riyanto, S., Yuniurti, N., Saputra, W.R., Utami, R. & Mulatsih, W. (2010). Antioxidant activity, total phenolic, and total flavanoid of extracts and fraction of red fruit (Pandanus conoideus Lam). *International Food Research Journal*, 17, (97-106).

SPSS (2009). *Statistical Package for the Social Sciences Window Ver*. 17.O. Chicago, SPSS, Inc.

Su, M. S., Shyu, Y. T. & Chien, P. J. (2008). Antioxidant activities of citrus herbal product extracts. *Food Chemistry*, 111, 892-896. https://doi.org/10.1016/j.foodchem.2008.05.002

Then, M., Szöllosy, R., văsăr helyi_ perédi, K. & Szentmihălyi, K. (2004). Poly Phenol_, Mineral Element content and total Antioxidant power of sage (*Salvia officinalis* L.) Extracts. Future for Medicinal and Aromatic plants. *Acta Hort, publication* supported by can. In. Dev. Agency (CIDA), 123-129. https://doi.org/10.17660/actahortic.2004.629.16

Turkoglu, I., Turkoglu, S., Celik, S. & Kahyaoglu, M. (2010). Antioxidant and antimicrobial activities of Turkish endemic Achillea species. *African Journal of Microbiology Research*, 4(19), 2034-2042.

Überegger, E., Vichi, S., Bortolomeazzi, R. & Pozzale, L. (2002). Antioxidant Activity of sage (*Salvia officinalis*) and (*S. fruticosa*) and Oregano (*Origanum onites* and *O. Indercedens*) extracts related to their Phenolic compound content. *Journal of the science of food and Agriculture*, 82 (14), 1645-1651. https://doi.org/10.1002/jsfa.1240

WU, H.C., Shiau, C.Y., Chen, H.M. & Chiou, T.K. (2003). Antioxidant activities of carnosine anserine, some free amino acids and their combination. *J. Food and Drug Anal.*,11(2), 148-153. https://doi.org/10.38212/2224-6614.2720

Yusof, N.B., Hasan, M.H., Armayni, U.A., Bin Ahmad, M.S., Mohsin, H.F. & Wahab, I.A. (2013). The ferrous Ion chelating Assay of Pandanus Extracts. *The open Conference Proceedings Journal*, (4), pp1. https://doi.org/10.2174/22102892013040100155