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Studying The Antioxidant And Inhibitory Efficacy For The Flowers Of Roselle Plant And Adding It To Yogurt

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Abstract

This study was conducted with the aim of testing the effectiveness of the Roselle flower plant as an antioxidant and as an anti-growth agent for microorganisms and its use in the manufacture of dairy products, It was bought from the local market and crushed and dried as soon as it reaches the laboratory. The content of total phenolic compounds was determined by using high-performance liquid chromatography (HPLC) device to estimate the concentration of some of the active compounds: Catechine, Gallic acid, Keamferol, Qurcetine and Rutin. A GC.MS gas chromatography-mass spectrometer was used to total diagnose the volatile active compounds. The antioxidant activity was estimated in the alcoholic extract and the aqueous extract of the samples under study. Where it is noticed that the aqueous extract of Roselle flowers has the ability to free radical scavenging and the total antioxidant capacity of the aqueous extract of Roselle flowers reached 29.39%. The aqueous and alcoholic extracts of Roselle flowers showed clear contrast in the inhibition of the pathogenic bacteria under study (S. aureus, E.coil). One of the fermented dairy products, which is yogurt, was chosen to study the effect of adding aqueous extracts to the flowers of the Roselle plant under study. The antioxidant activity of the fermented milk was estimated before and after the addition. A sensory evaluation was performed for yogurt containing 3.0% of the plant extract. The results of the evaluation showed consumer acceptance. No significant differences were showed between the manufactured product and the control treatment in terms of flavor, taste, texture, acidity and external appearance.

Key words :Roselle, HPLC, G-MS, E. coli, S. aureus, Phenols, Flavonoids

Introduction

Food safety, preserving its nutritional value, prolonging the storage life, and reducing spoilage during its handling and storage are among the issues that have aroused the interest of both the producer and the consumer in line with the development and modern technologies in food processing to meet the increasing nutritional needs of human and meet the consumer's desires to move away from what is industrialized for food ingredients and in order to ensure the health and safety of the consumer, the interest in antioxidants has increased in recent times because the process of fat oxidation has negative effects on food quality as it is the main cause of spoilage of fats, oils, and fatty foods, This leads to loss of nutritional value and the appearance of

unwanted flavors (Cook and Samman 1996). The focus has been on the Natural sources found in plants, especially that they are edible and do not have a toxic effect. Phenol compounds are among the main natural antioxidants. Phenols are aromatic compounds that carry one or more groups of the hydroxyl group and are found in almost all parts of the plant such as the leaf, fruits, flowers and have effects. The inhibition against microbiology (Wang et al. 2009; Cai et al. 2004). The results of the studies showed concern about the presence of carcinogenic compounds through the use of synthetic additives in foods (Synthetic preservatives). Thus, the trend was justified towards plants and their extracts and their use in preserving food and preventing the growth of microorganisms in them (Ejechi et al., 1999). Roselle (Hibiscus sabdariffa L), The Roselle plant belongs to the Malvaceae family, the scientific name is Hibiscus sabdariffa L, which is one of the perennials known as Jamaica (in Spanish), red sorrel (in English) or karkadeh (in Arabic), which is native to India and Malaysia but because it can grow in soils with low fertility and low moisture retention (Patel, 2014)and its ability to produce peptides that have activity against pathogenic microorganisms(Algboory & Muhialdin 2021) Roselle has many biological properties, where it acts as an antioxidant, cholesterol, high blood pressure, and as an antimicrobial, anti-inflammatory, diabetes, and anti-cancer because it contains polyphenols and flavonoids (Riaz and Chopra, 2018).Generally Roselle contains flavonols and polyphenols flavanols in simple or polymer form (Da-Costa-Rocha, Bonnlaender, Sievers, Pischel, & Heinrich, 2014). The hibiscus flower is characterized by a red color, which gives the traditional color of drinks. This distinctive color is due to the anthocyanin content, while the acidic taste is due to the content of organic acids such as citric, malic, tartaric, and hibiscus (Martínez et al., 2014). Many studies in various countries of the world have shown the importance of this plant in many industries if the active substances are concentrated in the goblet flowers of the Roselle plant, which are the phenolic compounds, hibicin hydrochloride and glycosides (Resendiz-Lopez, Loara-pina, & Castano-Tostado, 1998). Gujarat contains flavonoids such as quercetin, kaempferol (Sawabe et al., 2005) and myristine, apigenin (Ezugwu, 2002) and phenolic acids such as protocatechuic, o-coumaric, p-coumaric and ferulic, and organic acids such as hydroxyacetic, ascorbic (Ali). Wabel, & Blunden, 2005), Citric, Malick (Wong, Yusof, Ghazali, & Man, 2002), and hibiscus and tartaric acids (Fasoyiro, Ashaye, Adeola, & Samuel, 2005).The researchers confirmed that these two compounds have antimicrobial and antioxidant efficacy, while Al-Abdullah and Waleed (2015) were able to diagnose the two compounds 6-Octadecanoic acid, methyl ester, (z) -, 3-Cyclopentylproionic acid, ethyl ester in the flowers of the Roselle plant. They stated that these two compounds are important to human health, as they are antioxidants that can prevent dangerous diseases such as cancer and others.

Materials and methods:

Collect the sample

Roselle flower plant samples were collected from local markets in Babylon province, Iraq. Then the samples were transferred to the laboratory for the necessary drying using the oven at a temperature of 40 ° C until their weight stabilized, after which the samples were milled with an electric grinder in a fine manner and placed in special numbered and sterilized packages and stored in the refrigerator at a temperature of 5 ° C until use (Sawsan et al. , 2009).Devices and materials used to estimate the concentration of active substances in the flowers of Roselle plant

1-Mass Gas Chromatography-GC

2-High Performance liquid Chromatography

Preparation of plant Extraction

Aqueous extract

Preparation of the aqueous extract, according to the method of Gülçın et al. (2003). 25 g of Roselle flowers were weighed and mixed with 500 ml of boiling distilled water and left for 30 minutes on the magnetic mixture. It was filtered by a Buechner funnel using What man No. 1 filter paper with vacuum, then the filtrate was concentrated in the Rotary Vaccum Evaporator at a temperature of 40 ° C to get rid of the water. After that, the filtrate was left to dry at a laboratory temperature of 25 ° C, then it was placed in opaque bottles and preserved In the refrigerator until use.

3-3-1-2 Alcoholic extract

The extraction was conducted according to the method described by Zhou et al. (2005) by weighing 50 g of herbal powder and adding 250 ml of 80% ethyl alcohol and left for 30 minutes on the magnetic mixture was filtered by a Buechner funnel through filter paper (What man No. 1) with vacuum Then the filtrate was concentrated with the Rotary Vaccum Evaporator at a temperature of 40 ° C to get rid of the solvent. After that, the model was left at room temperature until a dry substance was obtained and it was placed in opaque bottles sealed and kept in the refrigerator until use.

4- Estimation of total phenolic content

3-4-1 Sample Preparation

5 grams of the plant powder prepared in paragraph (3-3) was used and placed in a Soxhlet apparatus and extracted with (300 ml) ethanol at (50- 55 ° C) for 3-4 hours. The extract was filtered through Watman No.1 filter paper, and the extract was concentrated using a rotary evaporator under low pressure at 40 ° C. The extract was weighed after the concentration process (2.6 g) and stored at 4 ° C in a storage flask until the analysis was conducted.

Total phenols were detected according to the method presented by the scientist Zare et al. (2014) using gallic acid and Folin-Ciocalteu reagent, which states that (150 µl) of the alcoholic extract was taken with (500 µl) from Folin's reagent and added It has (1.5 ml) of (20% sodium carbonate) mixed well and the final volume is completed to (10 ml). After two hours of reaction, the absorbance value is recorded at a wavelength of 765 nm. The total phenolate concentration is calculated with respect to the titration curve for calic acid (Fig. 3-2) in units (mg / g dry weight).

3-4-2 Determination of total flavonoid content

The total flavonoid content was determined according to the method presented by the scientist Baba and Malik (2015) in the crude extract that was prepared by (mixing 10 grams of the sample and mixed with 50 ml of 80% ethanol) by the method of measuring aluminum chloride in the presence of the rutin), (50 µl) of the crude extract was mixed with (1 ml) of methanol and (4 ml) of distilled water, then added to it (0.3 ml) of a 20% solution of sodium nitrate) and (0.3 ml) of 20% aluminum chloride solution and the mixture is placed in the incubator for (10 minutes) after which (2 ml) of sodium hydroxide solution (1 mol) is added to the total mixture and the volume is completed to (10 ml) distilled water. Record the absorbance of the sample at the wavelength

of 510 nm. The total flavonoid concentration is calculated with respect to the titration curve for rutin (Fig. 3-3) in units (mg / g dry weight).

3-4-3 Extraction and diagnosis of phenolic compounds and flavonoids by HPLC device

The phenolic compounds and flavonoids were extracted in the laboratories of the Ministry of Science and Technology - Department of Environment and Water according to the method of Mradu et al. (2012). (15 g) is taken from the homogeneous and ground form, and 25 ml of chloroform is added to it to withdraw the chlorophyll, terpenes, and fats present in the plant and leave for a period of (10). Hours) with constant stirring. Then the extract is placed in the sound wave cracker for a period of (20 minutes), after which (25 ml) of Butanol is added to it, then transferred to the separation funnel, then the polar organic layer (butanol) is collected and transferred to the rotary evaporator device to obtain a dry extract (the sample after drying in the device). The process was repeated (3) times to obtain a sufficient amount before the analysis.

4-5-GC-MS analysis

The examination was conducted in the laboratories of the Ministry of Science and Technology - Department of Environment and Water and according to the method provided by Eswaran et al. (2012) where the GC-MS analysis was performed on the automatic sampler of the GC-MS 5977A Series Agilent system and the gas chromatography device connected to the mass spectrometer (GC- MS) using the following conditions: The volume of the injected form is 1 µl prepared by mixing the dry extract with 5 ml of the mixture chloroform methanol in the mixing ratio (1: 1) to be injected into the GC-MS device. Elite-1 capillary fused silica column HP-5MS (30 mm x 0.25 mm) operating in electron effect mode at 70 V; Helium (99.999%) was used as a carrier gas with a constant flow of 1 mL/min and an injection volume of 0,5 µl (split ratio 10: 1) was used, injector temperature 250 ° C; The ion source temperature is 280 ° C. The oven temperature is programmed from 60 ° C (Isothermal for two minutes), with an increase of 10 ° C / min, to 270 ° C, Then 5 ° C / min to 290 ° C, and finished with 9 minutes isothermal at 310 ° C. The mass spectra were taken at 70 eV; 0.5sec scan interval and fragments from 45 to 450 Da. The total running time of the GC is 60 minutes.

3-5 Extraction

The method used by Mashkor et al. (2014) in preparing extracts of plant samples (aqueous and alcoholic) was adopted to estimate the percentage of free radical scavenging and the total antioxidant capacity by taking 100 mg of the plant sample powder, after which 10 ml of methanol was added to it at a concentration of 50% and then placed on a device. Shaker for one hour, after which the samples were placed in a central centrifuge for 10 minutes at a speed of 3000 cycles. 1 minute, then the filtrate was taken and kept in the refrigerator at 4 ° C until use.

1-5-Total Antioxidant Capacity(TAC)

Adopting the method of measuring the green color intensity of the complex compound consisting of Molybdenum phosphate in an acidic medium by taking 0,1 ml of plant extracts (aqueous and alcoholic) prepared by the method (3-5) and placing it in an Eppendorf tube and adding 1 ml of reagent to it) Which was prepared by taking 5.88 ml of sulfuric acid and dissolving it, 1459 mg sodium phosphate and 4.78 mg ammonium molybdate).Then the samples were placed in a water bath at a temperature of 90 ° C for a period of 90 minutes, after which the samples were cooled to room temperature, the intensity of the formed color

was measured at the wavelength of 695 nm, then the readings were calibrated with the standard curve of ascorbic acid (Figure 3-3) Prieto et al., 1999)).

3-5-2 Estimate the percentage of free radical scavenging

The method used is adopted by Mashkor et al. (2014) by following the percentage estimation method for free radical scavenging as follows:

1- Dissolving 40 mg of Diphenyl-1-picrylhydrazyls (DPPH) 22 in 100 ml of methanol with continuous stirring and after complete dissolution gradually add more quantities of methanol with synchronizing the optical absorption of the detector to the wavelength of 516 nm until the optical absorption of the detector becomes 7.0 .

2- Take 100 µl of the extract prepared by the method (3-5) and add 1 ml of DPPH reagent to it and leave it for 24 hours at laboratory temperature

3- The optical absorption reading of samples with wavelength 516 nm is taken and the following equation applied:

$$\textbf{antioxidant activity}(\%) = \frac{Optical\ absorption\ of\ a\ plant\ sample - optical\ absorption\ of\ an\ empty\ sample}{optical\ absorption\ of\ an\ empty\ sample} \times 100$$

3-6 Determination of the inhibitory activity against microorganisms

Detection of the inhibitory activity of plant extracts against E. coli and S.aureus bacteria, and included two phases:

1- Activating pure cultures of microorganisms

The test isolates were activated before the inhibitory activity detection of the plant extract was conducted by transferring the Loop carrier fill to the NB medium from the bacterial culture and then incubating the tubes at 37 ° C for 18 hours (Al-Delaimy & Ali 1970).

2- Disk diffusion method

The method described by Faleiro et al. (1999) was based on spreading the activated test bacteria onto NA medium with a sterile metal diffuser. A hole of 8 mm diameter was made for each dish using a sterile metal drill. Finally, 200 µl of plant extract was added to each pit. The dishes were incubated at 37 ° C for 24 hours, after which the Inhabitation Zone diameter was measured.

3-8 Manufacture of yogurts fortified with medicinal herbal extracts

Yogurt was formulated with Danisco equipped active starter that contains Salivarius Subsp Streptococcus thermophilus and Lactobacillus delbrueckii Subsp bulgaricus. Where a volume of 12% of the skim milk was weighed and dissolved with warm water until complete dissolution, and then the plant extract under study was added by 0.3% from the Roselle plant to the milk prepared in the previous step. The starter rate was 0.2%, then incubated for 45 ° C until inoculation with the initiator at an average of 0.2%, then incubated for 45 ° C until the thrombus occurred and put in the refrigerator at 4 ° C for 24 hours.

3-9 Chemical and rheological examinations of yogurt

3-9-1 Estimation of total acidity

The total acidity of the medicinal herbal yogurt was determined using the method A.O.A.C (2008) by weighing 10 g of the sample in a beaker and adding a few drops of phenolphthalein reagent then crushed with NaoH of 0.1 standards until the pink color appeared. The percentage of total acidity estimated on the basis of lactic acid was calculated according to the following equation:

$$\textbf{Percentage of acidity\%}=\frac{Base\ volume\ consumed\ (ml)\ x\ base\ standard\ x\ lactic\ acid\ equivalent\ weight}{Sample\ weight\ (g)}\times 100$$

Viscosity estimation

The apparent viscosity of the yogurt samples was estimated at 10 ° C using the Brookfield DVII + viscometer produced by (Brookfield Engineering Lab Inc., Stoughton, Mass.) According to the method reported by Donkor et al. (2007).

3-9-3 Texture determination

The texture of the reinforced yogurt samples was estimated using a Brookfield Engineering Lab CT3,4500 texture Analyzer fitted with a 5 kg load cell, according to Joon et al. (2017).

Results and discussion

1.4. Determination of total phenolic and flavonoid content

The results in Table (4-1) showed the total content of phenolic compounds in plants. Where the concentration of Roselle flowers reached 25.44 mg GAE / g. These results are consistent with the results of Sirag et al. (2014). It indicated that the total concentration of phenols in the flowers of Roselle was 07.41 mg GAE / g. Table (4-1), which shows the concentration of flavonoids in the plants under study. The Roselle flowers contained 69.22 mg Rutin / g, and these results agree with Das (2014) that the amount of flavonoids amounted to 27.56 mg Rutin / g.

Table (4-1): Total content of phenolic compounds and flavonoids of Roselle plant

Name	TFC (mg Rutin / gm)	TPC (mg Gallic /gm)
Roselle plant	22,69	44,25

4-2 Diagnosis of the active compounds in the Roselle plant

4-2-1 Diagnosis of Active Compounds by HPLC

Table (4-2) shows the results of analyzing the content of Roselle flowers used in the study from the active compounds using the high-performance liquid chromatography (HPLC) technique for the sample of Roselle flowers and using the standard compounds Catechine, Gallic acid, Keamferol, Qurcetine and Rutin. Where the

presence of Apigenin, Catechine, Gallic acid, Keamferol, Qurcetine, and Rutin was confirmed at concentrations of 11,9, 96,5, 49,7, 68,9, 94,1, 94,8 ppm, respectively. These results are in line with what Zainol (2021) found that herbal tea contains phenolic and flavonoid compounds, including Catechine, Gallic acid, Keamferol, Qurcetine and Rutin at concentrations of 5,94, 1,58, 460, 59, 0, 9.21 ppm, respectively.

Table (4-2): Diagnosis of active compounds in Roselle plant by HPLC

Name	Roselle plant (ppm)
Apigenin	11,9
Catechine	96,5
Gallic acid	49,7
Keamferol	68,9
Qurcetine	94,1
Rutin	94,8

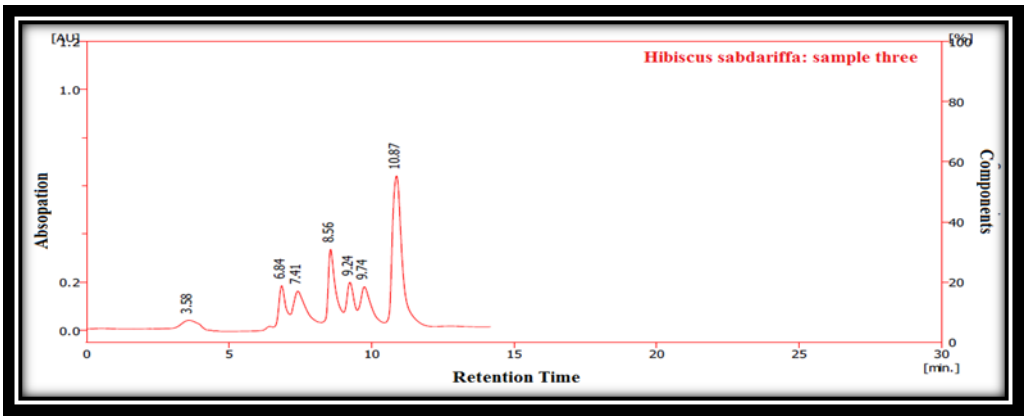

Figure (4-1): Analysis curve by HPLC technique for analyzing active compounds in Roselle plant

4.2.2 Diagnosis of Active compounds with GC-MS

The results in Table (4-3) and Fig. (4-2) showed the most important compounds that were diagnosed in the Roselle flower plant by GC-MS device among the most prominent of these vehicles: Hexadecanoic acid, methyl ester, 6-Octadecanoic acid, methyl ester, (z) -, Hexadecanoic acid, ethyl ester, 3-Cyclopentylproionic acid ethyl ester. These results confirm the findings of Preethi et al. (2010) of diagnosing the two compounds Hexadecanoic acid, methyl ester, Hexadecanoic acid, and ethyl ester in the guava plant using the GC-MS device.

Table (4-3): compounds diagnosed by GC-MS in Roselle plant.

Compound Name	RT	Area %
Hexadecanoic acid, methyl ester	**26,295**	**30,41**
6-Octadecanoic acid ,methyl ester,(z)-	**29,470**	**26,45**
Hexadecanoic acid, ethyl ester	**27,281**	**17,50**

3-Cyclopentylproionic acid , ethyl ester	**26,054**	**7,93**

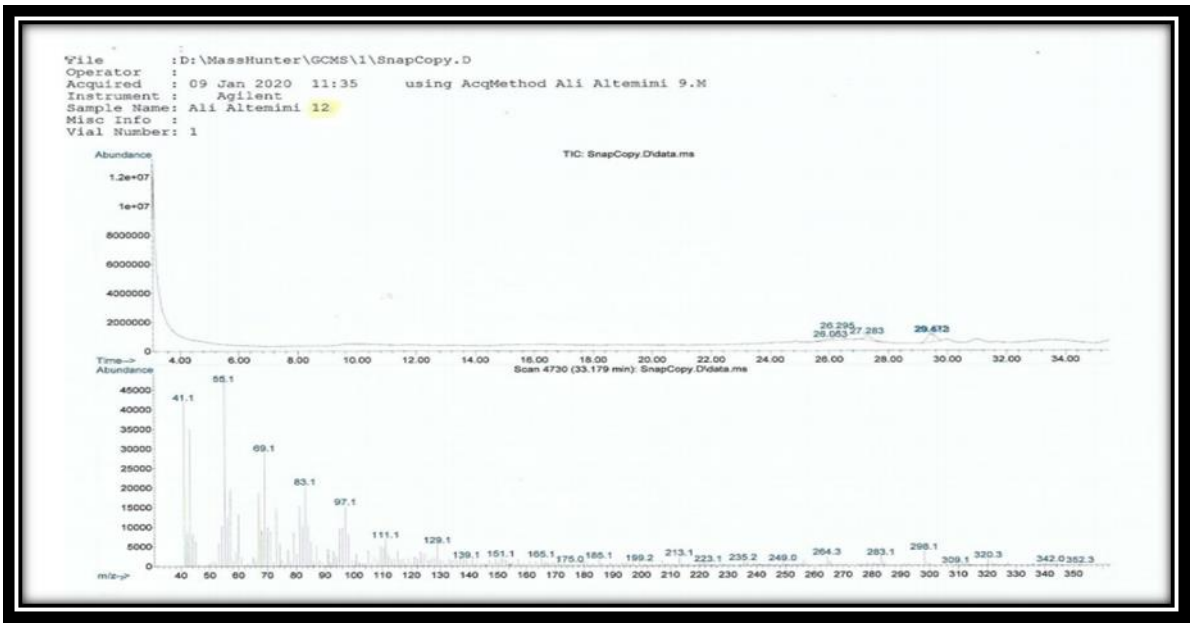

Figure (4-2): Diagnosis of active compounds by GC-MS in Roselle plant

4.3 Antioxidant activity

The antioxidant activity of both the aqueous and alcoholic extract of Roselle flowers was estimated by adopting the method free radicals scavenging and as shown in Table (4-4). The alcoholic extract of Roselle flowers showed antioxidant efficacy, as it gave 6,97%.While the aqueous extracts of the plants under study showed a clear decrease in the antioxidant efficacy, where the percentage of free radical scavenging of the aqueous extract of Roselle flowers reached 25.035%, These results agreed with Mohd-Esa et al. (2010), who found that the effectiveness of suppressing free radicals of Roselle flowers is 91.8%.

4-4 Estimation of Total Antioxidant Capacity (TAC)

Table (4-4) we note the values of the antioxidant capacity of both the aqueous and alcoholic extract of the Roselle flowers, where the antioxidant capacity of the alcoholic extract of the Roselle flowers reached 12.13% and the aqueous extract reached 29.39%.These results were close to what Kırca and Arslan (2008) found in their study of Nigella sativa extracts, as they found that the antioxidant capacity reached 404.29%.

Table (4-4): percentage of free radicals scavenging and total antioxidant capacity in Roselle flowers

Roselle			
Alcoholic extract		**Aqueous extract**	
The total capacity of antioxidants	**free radicals scavenging**	**The total capacity of antioxidants**	**free radicals scavenging**
12.13	**97.6**	**29.39**	**25.0**

4-5 Antimicrobial activity

Table (4-5) and Fig. (4-3a), (4-3b), (4-3c), (4-4d) show the antimicrobial activity of the alcoholic and aqueous extract of Roselle flowers towards the direction of S. aureus, E. coil.

Table (4-5): Diameter of Inhibition zone of the alcoholic and aqueous extract of Roselle against bacteria

concentration			150	200	250	300	350	400
Roselle	alcoholic extract	S.aureus(mm)	31	34	37	39	41	43
		E.coli(mm)	23	24	25	25	27	28
	aqueous extract	S.aureus(mm)	20	22	24	32	38	31
		E.coli(mm)	24	25	28	26	27	27

Where it is noticed that the diameter of Inhibition zone reached 43 mm for the alcoholic extract of Roselle flowers towards S.aureus bacteria, while the diameter of Inhibition zone of the alcoholic extract of Roselle flowers towards E.coil reached 28 mm, and the diameter of Inhibition zone of the aqueous extract of Roselle flowers reached 28 mm Gujarat against S. aureus to 38 mm,

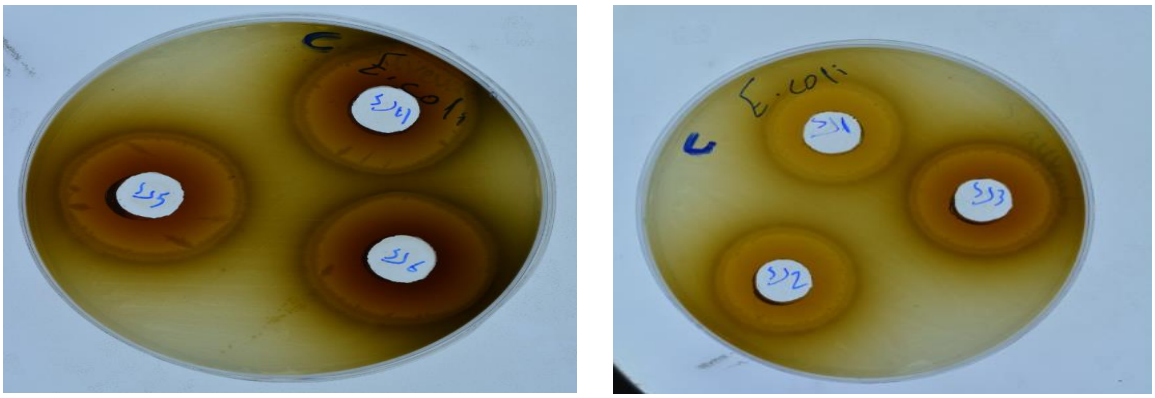

Figure 3-4a: diameter of Inhibition zone of the alcoholic extract of Roselle against E. coli.

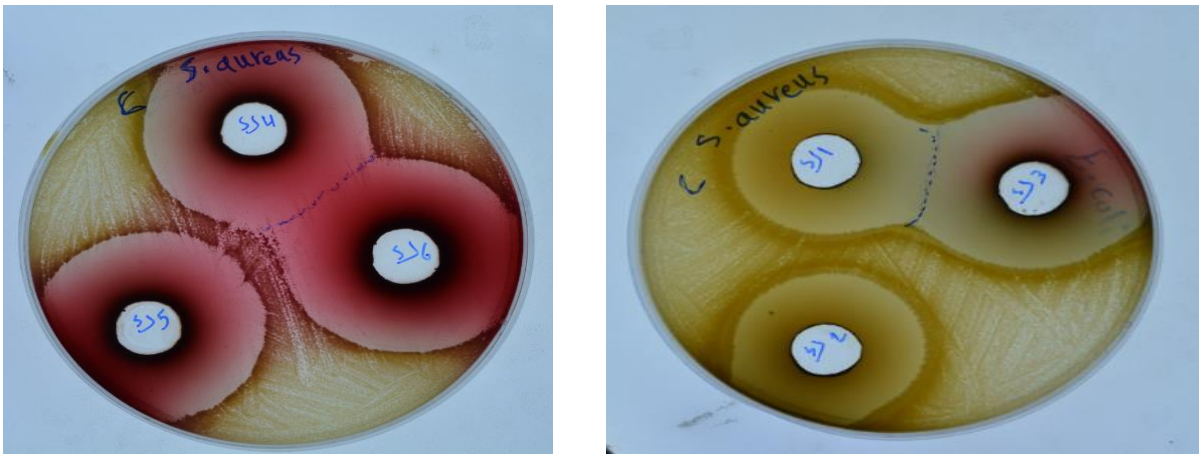

Figure (4-3b): diameter of Inhibition zone of the alcoholic extract of Roselle against S. aureus.

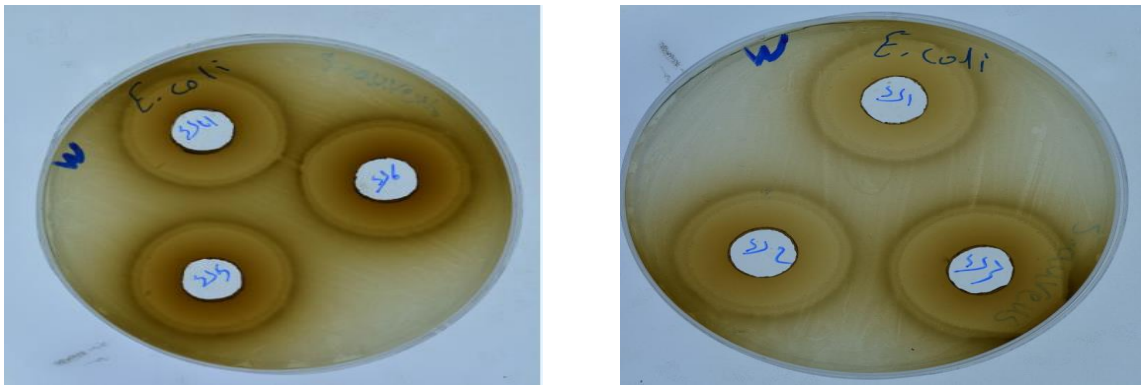

Figure (3-4c): diameter of Inhibition zone of aqueous extract of Roselle against E. coli.

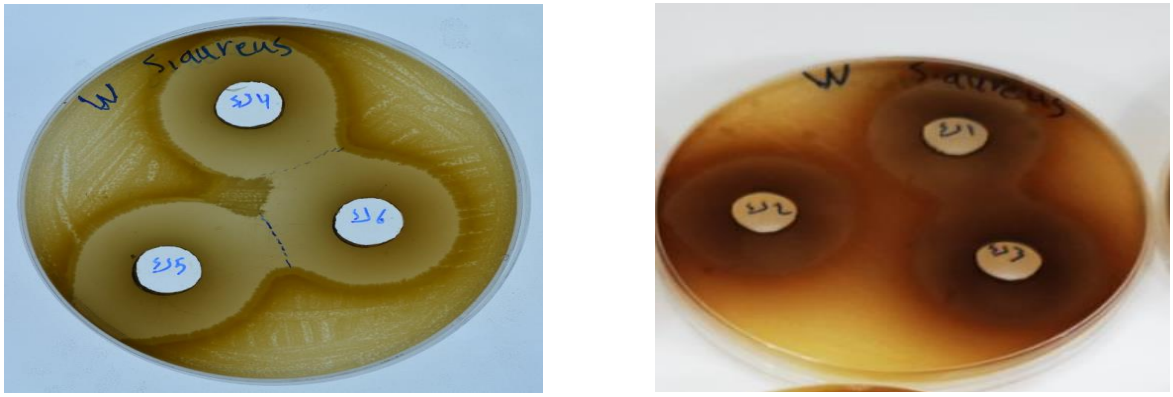

Figure (3-4d): diameter of Inhibition zone aqueous extract of Roselle against S. aureus.

As for the diameter of Inhibition zone , the direction of E.coil bacteria, it reached 28 mm, and these results are close to what Sirag et al. (2013) found in their study on the inhibitory effect of Roselle flowers against S.aureus, as the diameter of Inhibition zone reached 33 mm, and the direction of bacteria. E.coil The diameter of Inhibition zone reached was 23 mm.

4-6 Yogurt fortified with plant extracts

4-6-1 The antioxidant activity of yogurt before and after adding medicinal herbs

Table (4-6) the antioxidant activity values measured by the percentage of free radical scavenging of milk, the control treatment before fermentation, which reached 210.4%, and after fermentation it reached 280%, The reason for this may be clearly because the fermentation of milk leads to some molecular changes in the milk, which leads to the release of various compounds such as peptides, amino acids and fatty acids that have antioxidant capacity. As milk fermentation leads to an increase in antioxidants in dairy products, this effect is achieved with each microbiological culture tested as indicated by Gjorgievski et al. (2014).From Table (4-6) we notice that by adding 0.3% of the extract of Roselle flowers to the milk before fermentation, the percentage of

free radical scavenging reached 308% and is due to the percentage of addition compared to after fermentation, the percentage of free radical scavenging increased to 317%.this was confirmed by Arslaner et al. (2020), where the reason for the high antioxidants was due to the fermentation process and the addition of Roselle flower extract improved the antioxidant activities of the fermented milk samples to varying degrees. There are numerous studies, both in vitro and in vivo, have shown that the Roselle flower extract has a strong antioxidant effect, which explains the highest activities at a concentration of 0.3%. The addition of Roselle flower extract at a concentration of 0.3% significantly improved the antioxidant activity of the fermented milk samples, which is important for human health (Mohd-Esa et al., 2010).

Table (4-6): Determination of the Antioxidant activity (DPPH) of yogurt before and after adding Roselle plant

Treatments	Antioxidant activity before fermentation	The Antioxidant activity after fermentation
Control treatment	**210.4**	**280**
3.0% Roselle concentration	**308**	**371**

4-7 Chemical and rheological properties for yogurt with the adding of Roselle flower extract

4-7-1 The effect of adding Roselle flower extract on the physicochemical and rheological properties of yogurt.

It is noticed from Table (4-7) that the values of viscosity, percentage of acidity and hardness of the control treatment without adding the extract, where the viscosity had reached 1680 centipoise. These are agree with Sadiq (2019) in his study on the effect of fortifying curd with iron coated on its physicochemical, rheological and nutritional properties. As for the percentage of acidity in the control treatment, it clearly increased to 0.9% and came close to what Hussein and Fadel (2017) found in his study of the qualitative and sensory traits of yogurt manufactured by adding some fat substitutes, it is noticed that the hardness was 31.5 g.This is in agreement with what Azari - Anpar et al. (2017) found, in which the hardness of yogurt was 6.76 g. From Table (4-7), the effect of adding Roselle flower extract with a concentration of 0.3% on the rheological traits is noted, where it is noticed that the viscosity reached 1500 centipoise when adding Gujarat flower extract to yogurt. This is confirmed by Arief and Taufik (2016) in their study of the quality and antioxidant activity of Roselle fortified yogurt during cold storage and its effect on viscosity. As for the percentage of acidity, it reached 1.08%, as it is noticed that there is a noticeable effect of the added extract on the percentage of acidity. This is consistent with what Arslaner et al. (2020) found, at 1.08%, while the stiffness ratio increased to 42.1 g. where these results agree with Mudgil et al. (2017) in his study of the effect of adding flax seeds on the rheological traits of yogurt. Where it was observed that flax seed caused an increase in the hardness of the yogurt amounted to 43.05 g due to the presence of the fibers in the flax seed. The fibers improve the growth of the yogurt initiator culture i.e. L. delbrueckii ssp and S.thermophilus. When these bacteria grow well, they can result in desirable texture properties. Hence, the hardness of the milk increases. Besides, the increased hardness may be related to the moisture absorption ability of the flax seed, Where the amount of yogurt hardness was dependent on the combined compound contents, the level of the initiator culture, and the incubation time, the initiator culture level could increase the hardness in the sample yogurt, however the incubation time did not result in a significant change in the hardness.

Table (4-7): Results of the chemical and rheological properties of control yogurt and yogurt with the adding of Roselle flower extract

sample	hardness	viscosity	Acidity
Yogurt product with the adding of 0,3% Roselle extract	**42.1**	**1500**	**1.08**
Yogurt product without adding	**31.5**	**1680**	**0.9**

Conclusions:

From this study, we conclude that the alcoholic Roselle flower extract has antioxidant efficacy, and also the alcoholic Roselle flower extract at a concentration of 3.0% helped increase the free radicals scavenging by 392.8% for yogurt. Also, the alcoholic extract of Roselle flowers showed efficacy against E.coli and S. aureus compared to previous studies. From this study, we recommend that dairy companies and factories use these plants as flavorings and preservatives in the manufacture of yogurt and the possibility of using it in other dairy products.

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