

## ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF GUAVA AND POMEGRANATE LEAVES ETHANOLIC EXTRACTS

Hefnawy, T. H.<sup>1\*</sup> and Gehan A. El-Shourbagy<sup>2</sup>

<sup>1</sup>Biochemistry Department, Faculty of Agriculture, Zagazig University, 44511 Zagazig, Egypt

<sup>2</sup>Food science Department, Faculty of Agriculture, Zagazig University, 44511 Zagazig, Egypt

\*Corresponding author, +201028377005, hefnawytaha2014@gmail.com,



### ABSTRACT

Green leaves are rich in compounds that have a wide range of biological functions, including antioxidant and antimicrobial activities. The first goal was chosen guava leaves and pomegranate leaves were the work of the extract ethanolic 70% and evaluate the antioxidant has been used five tests to assess antioxidant and these tests are (2,2-diphenyl-1-picrylhydrazyl (DPPH), Total phenolic content (TPC), radical scavenging activity, 2,2-azinobis-3ethyl benxothiazoline-6-sulphonic acid (ABTS) cation decolorization activity, and reducing power) were measure and to estimate the antimicrobial was chosen four types of microbes Microorganism They (Salmonella enterico, Staphylococcus aureus, Escherichia coli, and Bacillus subtilis) were evaluated using agar well diffusion and broth-microdilution tests. Among all leaves extracts guava (*Psidium guajava*) and pomegranate (*Punica granatum* L.) exhibited outstanding antioxidant and antimicrobial properties, and the influence of these extracts was investigated at levels of 0.1% and 0.5% (w/w) concentrations on lipid oxidation and microbial criteria in raw beef patties and during storage at 4°C for 12 days compered with butylaed hydroxytoluene (BHT). Color was measured and tested thiobarbituric throughout the storage period of 12 days as Checked microbial measuring and comparing microbial compound that BHT. The results were obtained by a ratio of phenolic compounds in guava and pomegranate extract (22.65 - 22.87 mg / g), respectively, and the proportion of activity antioxidant was (8.80 - 19.08) extract of guava extract pomegranate respectively Overall the extract from pomegranate papers had a clear impact antioxidant and also antimicrobial compered extract of guava leaves

**Keywords :** Guava leaves, pomegranate leaves, ethanolic extract, antimicrobial, preservstive meat, beef.

### INTRODUCTION

Pomegranate (*Punica granatum*) of the oldest known drugs as stated (Thring *et al* 2009) it was used a medicine in Egypt for more than 1550 years BC where he used the crust for the treatment of urinary tract and respiratory system, said this information Ahmed and Ali, 2010 and Deepak *et al.*, 2015), while the (Ahmed and Zaki, 2009 and Hontecillas *et al.*, 2009) pomegranate extract ethanol 70% and has tested it on rats which showed Abstract effect against oxidative stress and that a concentration of (500 mg / kg b.wt.)

Guava (*Psidium guajava* L.) was used as a hypoglycemic agent in folk medicine. The leaves and skin of the fruit have greater effects. (Olajide, *et al.*,1999. It is known that cancers as well as diabetes affects them in a clear and dangerous the increases oxidative stress. Hsieh *et al.*, 2007 and Lutterodt, 1989 studied that guava leaves contain a relevant biological activity of biological functions including antimicrobial materials as well as antioxidant also, Ahmed and Zaki, 2009 studied the evaluation of guava leaf extract antimicrobial as well as the phytochemical where the results showed that the guava leaves contain a number of components of the pilot fixed oil, resins and volatile oil tannin (0.365 - 6 - 3.15 - 8.5%) respectively in addition to fat, cellulose, chlorophyll and mineral salts and a number of other fixed substances (Choudhury *et al.* 2012)

Aima and Danno 2002 and Gutierrez 2008, They studied the biologically active compounds has many biological functions, including antioxidant and antimicrobial activities. Eating fruits (natural antioxidants) help prevent many diseases. (Martha *et al.* 2008 and Deguchi and Miyazaki, 2010), As a result of the foregoing he began thinking in the green leaves of

many plants (because they contain natural anti-oxidant) is likely to be effective antimicrobial and disease

Their bioactive substances and phytonutrients have a wide range of biological functions, including antioxidant and antimicrobial activities (Aima and Danno 2002 and Gutierrez 2008). Some epidemiological evidence has shown that consumption of fruits high levels of natural antioxidants helps to prevent chronic diseases, such as cardiovascular diseases and cancer (Martha *et al.* 2008 and Deguchi and Miyazaki, 2010). Consequently, Leaves green have received substantial attention from researches in recent years as a potential source of natural antimicrobial and antioxidant agents.

Usually it is saved meat products at low temperatures (2-5 °C). (Vayalil 2002) studied that meat products spoil during the cooling is due to two reasons: the first two microbial growth microorganism and the second is oxidative stress, where oxidation of fat found in meat begins by lipid oxidation. (Mancini *et al.*, 2003) that chop meat to get new products from meat where the consumer wants to help to the corruption of those products where the fatty membranes of metal ions displays which helps the occurrence of interaction between prooxidants and unsaturated fatty acid. The second reason is the meat contamination with microbes microorganism, during the slaughtering process or manufacturing process or both as stated (Jakobsen and Bertelsen 2000). It is known that microbes occurring undesirable changes in the meat and spread the bacteria in the meat of lactic acid bacteria (Borch and Arinder, 2002).

The objective of this study was to evaluate the antioxidant and antimicrobial activity of guava and

pomegranate leaves extracts in vitro. Based on these in vitro results, the antimicrobial and antioxidant activities use of these extracts were studied during storage of raw beef patties.

## MATERIALS AND METHODS

### Materials

#### Plant materials

Fresh leaves of guava (*Psidium guaja*), and pomegranate (*Punica granatum*) were purchased from local farms during the harvest season 2014 in Zagazig city Sharkia governorate,– Egypt.

#### Microbial strains

A total of four bacterial strains (*Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus* and *Bacillus subtilis*) obtained from American Type Culture Collection (ATCC) via Cairo Mircen Faculty of Agriculture, Ain-Shams University Egypt.

#### Meat material

Meat sample obtained from a supermarket in Zagazig city, Sharkia governorate, Egypt. The results of the analysis of the sample under study (moisture - protein - fat - salts are 71, 22, 3.9 and 1.1%, respectively.

#### Chemicals and reagents

All chemicals were reagent grade unless specified from Sigma Chemical Co., BDH Chemical Co. and El Gomhoria Co.

### Methods

#### Preparation of guava and pomegranate leaves extracts

Obtained on the leaves of guava and pomegranate of trees, guava and pomegranate from the farm during the 2014 season and moved to the lab where they were securities wash well with distilled water and then placed in a drying oven at 40°C for 48 hours were grinding Securities and save milling output to 0°C. The leaf powder was added to ethanol 70% ,the mixtures were made in sterile 250 mL Erlenmeyer flask wrapped in aluminum foil to avoid evaporation and exposure to light for 3 days at room temperature. The flasks were placed on a platform shaker at 70 rpm. After 3 days of soaking in solvent, were obtained extracted by filtration (Whatman No.2) where it is extracted concentration temperature of 40 °C using rotary evaporator (BÜCH-water bath-B-480, Switerland). Freeze-dryer (Thermo-Electron Corporation-Heto power dry LL300 Freeze Dryer, Czech Republic), using a device that was to get rid of the extra solvent, the residual was weighed, and the extraction yield of each plant material was calculated. The ethanolic extract was then stored at -20°C.

#### Preparation of beef burgers

Beef burgers were prepared according to El-Akary 1986. One kg of meat was mixed with 20g spice mixture, 20 g sodium chloride and 10g, dried onions. Spice mixture consisted of 50% peppers. 30% coriander, 5% cubeb, 5% cloves, 5% cinnamon and 5% red pepper.

Beef burgers were prepared to be of 14 cm diameter, 0.5cm thickness and 30 gm weight. Each

piece was then surrounded with two pieces of butter paper and packed in polyethylene bags. The sealed bags containing beef burger pieces were frozen and stored at -18°C.

### Determination of total phenolic content and antioxidant activities

#### Total phenolic content

It was estimated to total content of phenolic compounds using the Folin-Ciocalteau according to the method of Dewanto, *et al.*, (2002). Where the use of ethanolic extract 70% of the leaves of samples papers where it was taking 100 µl of the sample and placed in the tube test and then added to 2 ml of sodium carbonate solution (2%) and then left for 5 minutes at room temperature 20 °C, then add 100 µl Folin of reagent and mix the ingredients well and then leave for 30 minutes and measured at a wavelength of 750 nm have also been the work of a standard curve using Alglic acid was calculated as the amount of phenols was expressed as mg gallic acid equivalents per g dried leaves

#### DPPH radical scavenging activity

Was estimated free radical scavenging activity by using the method of DPPH where they were to follow the method mentioned using Cheung, *et al.*, (2003). with some modification where they were taking 1 mL of DPPH concentration solution of 0.2 mM was added to 200 µl of processed sample solution then leaves the solution in the dark for 30 minutes on the degree room temperature then measured color output device colorimeter at wavelength 517 nm., follow the same previous steps with the standard solution of ascorbic acid. It is free radical at the expense of on-ascorbic acid equivalents per g dried sample. The DPPH radical scavenging effect was calculated by the following equation:

$$\text{Scavenging effect (\%)} = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100$$

Where  $A_{\text{control}}$  is the absorbance of control without sample,  $A_{\text{sample}}$  is the test sample without DPPH.

#### ABTS cation decolorization assay

An estimate ABTS radical using ABTS capability using scavenge free ABTS radicals, according to the method in Re *et al.*, (1999), where he is prepared solution by taking the size of a 1: 1 from each of the ABTS concentration solution of 7.4 mM and the solution potassium persulphate concentration 2.45 mM and leave the solution for 16 hours in the dark at room temperature and then dilute solution according to density soultions. Then take 1 ml of an aqueous sample solution is added to it 50µl and leave the solution for one hour and then measured color spectrophotometer device at 734nm and is calculated on the basis of the results TE per g dried leaves

The ABTS<sup>+</sup> scavenging effect was calculated by the following equation:

$$\text{Scavenging effect (\%)} = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100$$

Where  $A_{\text{control}}$  is the absorbance of control without sample,  $A_{\text{sample}}$  is the test sample without ABTS<sup>+</sup>.

#### Reducing power

The reductive ability reducing power depends on the reduction of ferric to ferrous by having oxidizer in according to the method of (Kim *et al.*, 2013). in this

way is taken A 250 µl of sample and add to it 250 µl of sodium phosphate buffer concentration (200 mM, pH 6.6) and also added 250 µl of potassium solution concentration (1%) and are a good mixing and then placed in a water bath at 50 °C for 20 min. and then left until it reaches room temperature of 20 °C and added 250 µl of trichloroacetic acid solution 10 % (w/v) and centrifuged at 5000 rpm for 5 min. Take supernatant and saves then takes him to 500 µl and relieves the same size of distilled water and 500 microns Shake well and add 100 µl ferric chloride concentration (0.1%). The resulting color measured on a Spectrophotometer at 700 nm is to zero system by blank (distilled water) and the reducing power activity was expressed as absorbance

#### Antibacterial activity of guava and pomegranate leaves ethanolic extracts

Evaluation the extract ethanolic 70% of the leaves of guava as well as the pomegranate against antimicrobial and then follow the way Abu-Shanab et al., 2004 where he was bacterial strains pure (*S. aureus*, *S. enterica*, *B. subtilis* and *E. coli*) on Mueller Hinton broth (MHB) was the incubated on a rotary shaker at 200 rpm at 37 °C (*S. aureus*, *S. enterica* and *E. coli*) or 28 °C (*B. subtilis*) for 24h and then conducted ease of growth and by taking 1 ml of growth and added to the (MHB) 10 ml and then incubated at 37 °C bacteria (*E.*

*coli*, *S. enterica* and *S. aureus*)) and 28 °C bacteria (*B. subtilis*) until he reached the growth of microbes in the media of the reach a count of  $1.05 \times 10^9$  CFU/ml. After that, several Petri dishes containing (MHB), where microbial placed and contains a different concentration of the extract and to grow for 24 h and the different levels of zones of inhibition were measured using a transparent ruler and the diameter was recorded in mm to conclude the minimum inhibitory concentration (MIC).

#### Application of guava and pomegranate leaves extracts on ground beef patties

##### Beef samples and storage conditions

The mixed beef samples were assigned to one of four different treatments: 1. NC (negative control, meat without additives); 2. PLE (meat with 0.1% and 0.5% (w/w) pomegranate leaves extract) 3. GLE (meat with 0.1% and 0.5% (w/w) guava leaves extract), and 4. PC (positive control, meat with 0.1% and 0.5% (w/w) BHT). Meat mixture was shaped manually using patty maker to obtain round disks 9.5 cm diameter and 0.5 cm thickness. Burgers were packed in polyethylene bags in foam dish. All burger beef treatments were grilled on hot plate with little sunflower oil at 110 °C for 4 min. Then the cooking loss percentage was calculated from following equation according to A.O.A.C. (2000).

##### Fresh burger weight – cooked burger weight

$$\text{Cooking loss (\%)} = \frac{\text{Fresh burger weight} - \text{cooked burger weight}}{\text{Fresh burger weight}} \times 100$$

##### Fresh burger weight

Shrinkage percentage was calculated from this equation according to A.O.A.C. (2000). Shrinkage (%) =  $(a - b) + (c - d) \times 100$

The thickness of uncooked burger (a), the thickness of grilled burger (b), the diameter of uncooked burger (c) and the diameter of grilled burger (d). Sensory evaluation was conducted according to the method described by (Khalid, 2005). Cooked burger samples were served warm to 10 panelists (staff of food science Department, Faculty of Agriculture, Zagazig University, Egypt) without care of age or sex. The panelists were subjected to sensory evaluation using an 8 point hedonic scale for appearance color, juiciness, tenderness flavor and overall acceptability. A numerical basis as assort of evaluation from 1-8 was used where (1=dislike extremely, 2= dislike very much, 3= dislike moderately, 4= dislike slightly, 5= like slightly, 6= like moderately, 7=like very much, 8= like extremely).

#### Color changes in beef patties

Color changes in the patties during storage were monitored with a colorimeter (model DP-390 with chroma meter model CR-300). Color was expressed with  $L^*$  (100 = white, 0 = black),  $a^*$  (positive = redness, negative =5 greenness), and  $b^*$  (positive = yellowness, negative = blueness) values, CIELAB color parameters, of which  $a^*$  (redness) is the most important factor for meat quality and customer acceptance. The colorimetric difference between a sample and a white standard reflectance plate,  $\Delta E^*$ , was calculated using the equation:  $\Delta E^* = [(L^*-L)^2 + (a^*-a)^2 + (b^*-b)^2]^{1/2}$  ( $L^* = 96.37$ ,  $a^* = 0.19$ ,  $b^* = 1.68$ ). Color readings were measured on five randomly chosen spots on the beef

patties and were utilized as an estimate of meat discoloration. (Sayed et al., 2014)

#### Evaluation of lipid oxidation

It was estimated lipid oxidation found in meat samples (beef) and are expressed by measuring as malondialdehyde (MDA) equivalents according to the method (Laguerre et al. 2007), where he was taking 10 g of sample and add 10 ml of distilled water and 5 ml PG-EDTA solution (0.1%) has been good mixing through the use of mixer for 5 minutes and then was added to 75 ml of distilled water and 2.5 ml HCl (4 N) where MDA is separated from the meat protein. The samples were distilled by means of a distillation system, and the first 50 ml of distillate was collected. Next, a 5 ml of the output of distillation and added to 5 ml TBA solution (0.02M) is placed in the tube test and close the well and placed in a water bath to boil for 35 minutes then cooled tubes well and then centrifuged at 5000 rpm for 5 min taken supernatant and measured at 530 nm using a spectrophotometer. The results were expressed as mg MDA per kg meat.

#### Microbial examination

Microbial analysis of minced beef supplemented with GLE and PLE at different concentration compared to a positive control was assessed after different intervals of preservation (0-15 days) at 4°C followed the procedures outlined by Yao and Moellering (1995). The samples (10 g) were transferred aseptically to a stomacher bag containing 90 ml of peptone saline diluent (1.0 g peptone and 8.5 g sodium chloride in 1 liter of distilled water) at room temperature and homogenized for 60 s. A serial 10-fold dilution series

was prepared. Determinations were carried out for different bacterial counts using different specific selective media (Yao and Moellering 1995) as follows total viable count (TVC) was enumerated on Plate Count Agar (PCA, Merck, Darmstadt, Germany) at 25 °C after 72h, psychrotrophs were counted on PCA (Merck, Darmstadt, Germany) at 7 °C after 10 days and coliform bacteria was determined by MacConkey agar (Mast Group, Merseyside, UK) with a double layer of the same medium incubated at 37 °C for 24 h. Microbiological data were transformed into logarithms of the number of colony forming units (CFU/g).

**Statistical Analysis**

All biological trials and measurements were conducted in triplicate and expressed as the mean plus the standard error. ANOVA variance analysis was used for the statistical analysis of data using the general linear models (GLM) procedure of the SAS software

(version 9.1, SAS Institute, Inc., 2003). Least significant differences were used to compare means at  $p < 0.05$ .

**RESULTS AND DISCUSSION**

**Antioxidant activity of guava and pomegranate leaves extracts**

The results of estimating total Abstract 70% ethanol leaves of guava and pomegranate which were estimated in a manner Dewanto, *et al.*, (2002 22.68 - 22.87 mg GAE / g, respectively, as shown in Table 1 it is clear that the proportion of phenolic substances in the extract (guava and pomegranate converging of some). The increase in the proportion of phenolic compounds in the extract have a handle and a high probability that this extract a high antioxidant capability in case of availability of free OH group in the aromatic compounds as a relationship between antioxidant activity as well as antimicrobial

**Table 1. Total phenolic content and antioxidant activity of guava and pomegranate leaves ethanol 70% extracts.**

| Samples | TPC (mg GAE/g sample) | Antioxidant activity                             |  |                                    |
|---------|-----------------------|--|--|------------------------------------|
|         |                       | DPPH anion scavenging activity (mg AAE/g sample) | ABTS cation scavenging activity (mg TE/g sample) | Reducing power (A <sub>700</sub> ) |
| GLE*    | 22.65 ± 0.18          | 8.80 ± 0.01                                      | 60.35 ± 2.70                                     | 0.98 ± 0.02                        |
| PLE*    | 22.75 ± 2.13          | 19.08 ± 1.38                                     | 35.22 ± 0.48                                     | 1.27 ± 0.07                        |

\*GLE: Guava leaves extract, PLE: pomegranate leaves extract.

DPPH radical scavenging activity antioxidant compound or extract in the laboratory in vitro have been made to extract 70% ethanol leaves of guava and pomegranat according to the method of Cheung, *et al.*, (2003). There was significant variation in the radical scavenging activity of the guava and pomegranate leaves ethanolic extracts (8.80 – 19.08 mg AAE/g sample respectively).

ABTS activity was quantified in terms of reduction in ABTS<sup>+</sup> radical cations by antioxidants and expressed as mg TE per g dried leaves green. The ABTS activity of the tested guava and pomegranate varied considerably(60.53 – 35.22 mg TE/g sample).

Antioxidant properties are known to be concomitant with the development of reducing power. Reductones can react directly with peroxides and can also prevent peroxide formation by reacting with certain precursors. The reducing power of pomegranate > guava ( $P \leq 0.05$ ).

Guava has the characteristic aroma related to volatile compounds such as iso-butanol, β-myrcene,

trans-caryo-phyllene, and trans-β-farne-sene, as well as ter-pene, limo-nene, and humulene (Kanjana *et al.*, 2005 and Wua *et al.* 2009). Also, the ethanolic fraction of guava showed the highest polyphenol and flavonoid contents and strong DPPH anion and ABTS cation scavenging activities (Ahn, *et al.*, 2011).

**Antimicrobial activity of guava and pomegranate leaves extracts**

It was measured ethanol extract 70% of guava leaves and pomegranate capacity against antimicrobial activity was firstly measured by an agar which was described in a way (Yao and Moellering 1995) where results show well diffusion method and the results are presented in Table (3). In the agar well diffusion tests, the sensitivity to the extracts was found to differ significantly among the test organisms. No samples showed an antimicrobial effects on *E. coli*, *S. enterica*. Leaf extracts of guava and pomegranate, prevented the growth of *Staphylococcus aureus*. Guava extracts exhibited inhibitory activity against *B. subtilis*

**Table 2 Antimicrobial activity of guava and pomegranate leaves extract ethanolic 70%**

| Samples | E. coil | S. enterica | S. aureus | B. subtilis |
|---------|---------|-------------|-----------|-------------|
| GLE*    | -       | -           | +         | +           |
| PLE*    | -       | -           | ++        | -           |

\*GLE: Guava leaves extract, PLE: pomegranate leaves extract.

For a more accurate determination of antimicrobial activity, a micro-dilution assay was performed. The susceptibility of *S. aureus* and *B. subtilis* against guava leaves extracts was evaluated, and the results are presented as MICs (Table 3). All samples that had MIC values below 10 mg/ml displayed the same MIC values. Only pomegranate leaves extract had a bactericidal effect on *S. aureus*, and its MIC was  $10.0 \pm 0.0$  mg/ml. The main compounds responsible for the antimicrobial activity of pomegranate leaves extracts are

known as phenolics, such as protocatechuic acid, caffeic acid, and *p*-coumaric acid (Rajaa and Ashy (2012) and Suresh et al. (2013). Guava had an inhibitory effect on *B. subtilis*. The MIC values of guava, pomegranate were  $3 \pm 1.4$  mg/ml and  $8.6.0 \pm 0.01$  mg/ml respectively. The ethanol extracts of guava presented broad-range antibacterial ability, suggesting effectiveness against Gram-positive and Gram-negative bacteria (Ahn et al., 2011).

**Table 3. Antimicrobial activity of 70% ethanolic extract of guava and pomegranate leaves. Unit: mg/ml.**

| <b>samples</b> | <b><i>S. aureus</i></b> | <b><i>B. subtilis</i></b> |
|----------------|-------------------------|---------------------------|
| GLE*           | ND                      | $8.6 \pm 0.01$            |
| PLE*           | $10.0 \pm 0$            | $3.0 \pm 1.4$             |

\*GLE: Guava leaves ex-tract, PLE: pomegranate leaves ex-tract.

When considering, the rent test results, we find that the samples that have been effective against antimicrobial they contain TPC at a high rate as the evaluation of antioxidant her tests indicate highly effective antioxidant, suggesting a relationship between the effectiveness of antioxidant and effectiveness against antimicrobial also found that the extract effect ethanol 70% leaves of guava and pomegranate, the microbial inhibitory effects of guava and pomegranate leaves extracts were more effect-tive on Gram-positve than on Gram-negatve bacteria. Similar results have been obtained in other studies ( Malaviya and Mishra 2011).

**Color deterioration during refrigerated storage of beef patties**

The color measurement using a Hunter, according to (Kim et al. 2013). depends on where the measurement of three colors they are yellow, white and red through the shortcuts (L \*, a \*, \* b) and illustrated by appreciation for samples of meat that the color red is the color required in the measurement of samples under study where increasingly accept the consumer on the product the higher the degree of redness of the product were presented results obtained in Figure 1.2 as seen through illustrations color L\*, b\* decreased degree of moral significant ( $P \leq 0.05$ ) were measuring the samples stored on a 4°C for a period (12 days) and that the decline is evident in the color red in the sample Control samples added 70% ethanolic extract of guava leaves and pomegranate as well as the positive control, but note that the red color has fallen in all transactions, but it was more in control

**Lipid stability of beef patties during storage**

Estimated fat oxidation in the samples during storage using the TBARS is described in the (Laguerre et al. 2007), where the production of TBARS through the stages of self-oxidative stress, where consists peroxide compounds, which are oxidized to aldehydes and ketones such as MDA compound where significant difference was found during the storage period (12 days) in the oxidation of fat significantly ( $P \leq 0.05$ )

As expected, it has increased the proportion of fat oxidation in the samples compared to untreated Control samples fortified with 70% ethanol extract of guava leaves and pomegranate, as well as BHT as shown in Figure 3. Among the patties, the samples without antioxidants had the highest TBARS values by the end of storage (day 12) despite a decrease in oxidation rate slope: 0.250),

The results obtained in this study indicate that the addition of extracts rich in phenolic compounds earn products (beef patties) which is augmented by the ability to prevent the oxidation of fat in them. Phenolic Compounds have the ability to stop the formation of free radical by removing some of the cations responsible for the formation process Free radical such as iron as described (McBride et al., 2007). This explains the effectiveness of the ethanol extract 70% of the leaves of guava and pomegranate in reducing the oxidation of fat in the products because they contain phenolic compounds and increase the effectiveness of phenolic compounds whenever fit on a hydroxyl group OH on ring aromatic, where it has the ability to have the hydrogen with the free electrons that prevents formation of free radical and mechanical blocks further degradation to more active oxidizing forms, such as MDA (Biswas et al. 2013 and Ramadan et al. 2015).

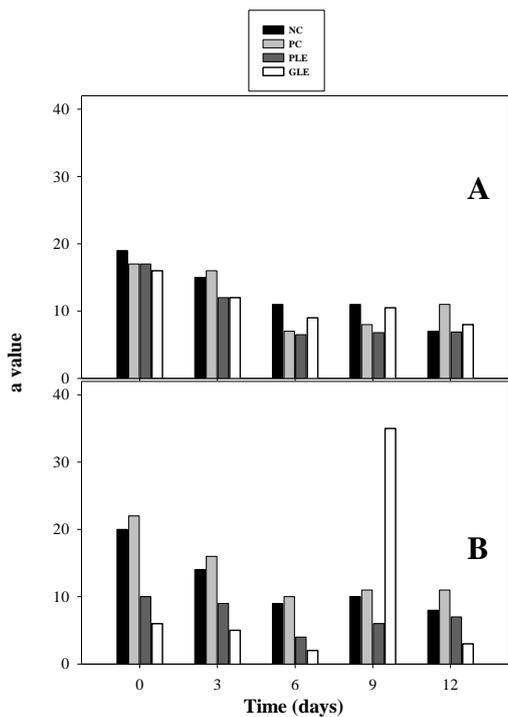


Figure 1 change in the color ( $a^*$  value, redness) of the samples during the storage period (12 days) treated with 0.1% (a) and 0.5% (b) 70% ethanol extract of guava leaves and pomegranate concentration. <sup>1)</sup> NC: *Nega-tive con-trol*. GE: guava extract. PE: pomegranate extract. PC: *posi-tive con-trol* (BHT-).

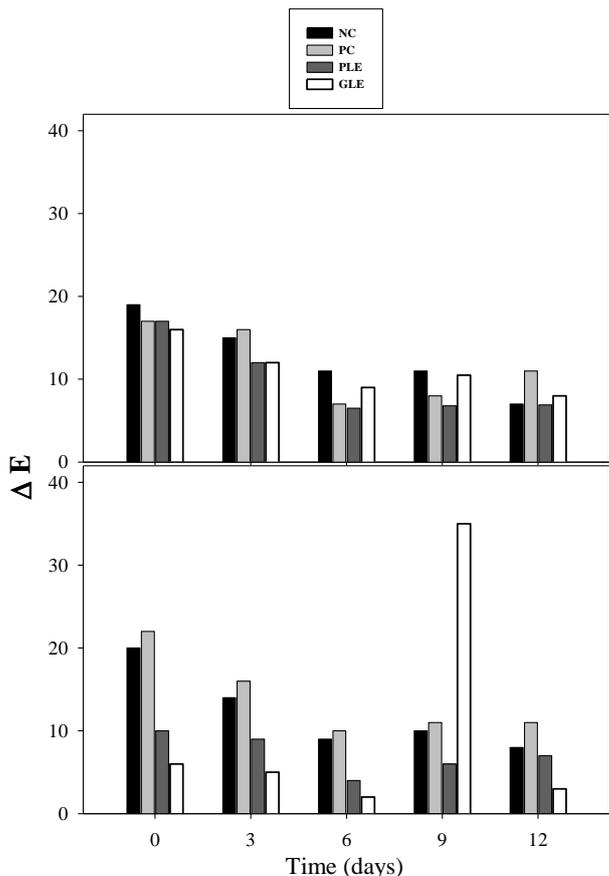


Fig.2. Effect of guava and pomegranate extract on  $\Delta E$  value (color differences) of pattie treated with different concentration of 0.1% (a) and 0.5% (b) extracts. <sup>1)</sup> NC: *Nega-tive con-trol*. GE: guava extract. PE: pomegranate extract. PC: *posi-tive con-trol* (BHT-).

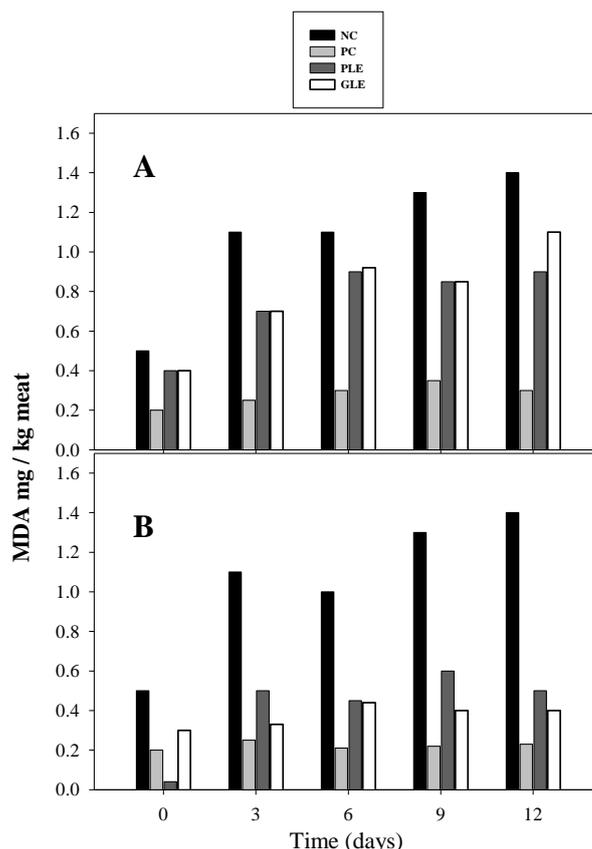


Fig.3. Effect of guava and pomegranate extract on TBARS values (MDA mg/kg meat) of pattie treated with different concentration of 0.1% (a) and 0.5% (b) extracts during refri-gerated storages. <sup>1)</sup> NC: Negative con-trol. GE: guava extract. PE: pomegranate extract. PC: posi-tive con-trol (BHT-).

Table 4. Microbial changes in beef patties samples added with 0.1% of the 70% ethanol extracts of (guava, pomegranate) leaves and stored at 4°C, compared to BHT as a posi-tive control

| microbes              | Treatment <sup>1</sup> | Storage time (day) |            |           |           |            |
|-----------------------|------------------------|--------------------|------------|-----------|-----------|------------|
|                       |                        | 0                  | 3          | 6         | 9         | 12         |
| Total viable counts   | NC                     | 4.56±0.17          | 5.23±0.30  | 6.57±0.53 | 7.45±0.18 | 7.62±0.30  |
|                       | GLE                    | 4.71±0.25          | 5.00 ±0.24 | 6.23±0.16 | 7.00±0.66 | 7.32±0.20  |
|                       | PLE                    | 4.52±0.19          | 5.01±0.16  | 5.73±0.43 | 6.82±0.27 | 7.233±0.17 |
|                       | PC                     | 4.49±0.28          | 4.79±0.40  | 5.23±0.65 | 6.17±0.39 | 6.81±0.46  |
| coliform counts       | NC                     | 2.78±0.19          | 3.52±0.11  | 3.56±0.57 | 3.84±0.45 | 4.43±0.05  |
|                       | GLE                    | 2.66±0.11          | 2.91±0.21  | 3.30±0.13 | 3.34±0.44 | 4.00±0.24  |
|                       | PLE                    | 2.76±0.34          | 3.14±0.23  | 3.00±0.25 | 3.15±0.63 | 3.86±0.15  |
| Lactic acid counts    | PC                     | 2.63±0.07          | 2.92±0.23  | 2.66±0.15 | 2.61±0.16 | 3.45±0.07  |
|                       | NC                     | 4.28±0.22          | 5.36±0.68  | 5.92±0.28 | 6.89±0.11 | 7.18±0.45  |
|                       | GLE                    | 4.35±0.40          | 5.17±0.67  | 5.82±0.57 | 6.33±0.39 | 6.74±0.54  |
| Yeast and mold counts | PLE                    | 4.44±0.28          | 5.23±0.59  | 5.75±0.27 | 6.41±0.40 | 7.12±0.08  |
|                       | PC                     | 4.26±0.32          | 4.93±0.68  | 5.62±0.92 | 6.13±0.20 | 7.22±0.29  |
|                       | NC                     | 4.46±0.19          | 4.98±0.36  | 6.46±0.26 | 7.00±0.34 | 7.24±0.44  |
|                       | GLE                    | 4.18±0.47          | 4.80±0.23  | 6.13±0.18 | 6.42±0.29 | 6.86±0.44  |
|                       | PLE                    | 4.23±0.33          | 4.82±0.18  | 5.60±0.45 | 6.51±0.50 | 6.79±0.25  |
|                       | PC                     | 4.11±0.38          | 4.36±0.64  | 5.00±0.69 | 5.97±0.01 | 6.64±0.04  |

<sup>1</sup>NC: nega-tive con-trol, GLE: guava extract, PLE: pomegranate extract, PC: posi-tive con-trol (BHT).

**Microbiological examination of ground beef patties**

It was conducted examination microbiological through the use of methods (Yao and Moellering 1995), and that the results showed reduction in the number of total viable count (TVC) in the samples treated well compared to the negative control as the results were extracts better than samples positive control or close them significantly ( $P \leq 0.05$ ), as shown in Table (4) and

(5), which shows changes microbiological additives in meat samples compared to the control positive also negative control. The results showed that additions extract led to a reduction in the number of microbes due to the effectiveness of this extract antioxidant as well as antimicrobial and also the greater the concentration of the extract added (0.1 - 0.5%) increased the ability to reduce the number of (TVC) in meat samples compared

to the positive control also negative control. Among the experimental groups, the NC group showed the most rapid increase in the number of (TVC) , followed by samples treated with GLE, PLE, and PC.

The addition of guava and pomegranate leaves extracts resulted in a reduction in growth rate of total viable count (TVC). The TVC of NC was initially approximately 4.56 log CFU/g meat, which increased steadily with storage time and reached close to 7.62 log CFU/g meat at 12 days. By the end of six days, bacterial populations in the samples treated with 0.1% concentration were significantly ( $P \leq 0.05$ ) lower than in the NC by 0.39, 0.81, and 1.43 log CFU/g meat for GLE, PLE, and PC, respectively. In the case of samples

treated with 0.5% concentrations, the difference between the NC and other groups was larger than that of samples treated with 0.1% concentration. The antimicrobial activity of the extracts against microbial growth was the most effective on coliforms. During storage a reduction was seen in not only the rate of increase, but also in the coliform counts in the treated meat samples. The addition of natural extracts did not significantly affect lactic acid bacterial (LAB) counts ( $P > 0.05$ ), and yeast and mold counts showed a similar tendency with TVC. These results fit with the findings of each of the (Ahn *et al.*, 2011).

**Table 5. Microbial changes in beef patties samples added with 0.5% of the 70% ethanol extracts of (guava, pomegranate) leaves and stored at 4°C, compared to BHT as a positive control**

| microbes              | Treatment <sup>1</sup> | Storage time (day) |           |           |           |           |
|-----------------------|------------------------|--------------------|-----------|-----------|-----------|-----------|
|                       | (0.5%)                 | 0                  | 3         | 6         | 9         | 12        |
| Total viable counts   | NC                     | 4.78 ±0.47         | 5.53±0.22 | 6.76±0.28 | 7.36±0.10 | 7.62±0.25 |
|                       | GLE                    | 4.30 ±0.47         | 5.00±0.40 | 5.88±0.14 | 6.52±0.48 | 7.14±0.30 |
|                       | PLE                    | 4.36 ±0.49         | 5.01±0.57 | 5.42±0.47 | 6.27±0.77 | 6.82±0.65 |
|                       | PC                     | 4.42 ±0.24         | 5.12±0.18 | 5.43±0.40 | 6.18±0.88 | 6.71±0.98 |
| coliform counts       | NC                     | 2.51 ±0.26         | 2.66±0.02 | 3.11±0.23 | 3.67±0.09 | 4.52±0.10 |
|                       | GLE                    | 2.32 ±0.25         | 2.19±0.07 | 2.23±0.46 | 2.82±0.38 | 3.57±0.27 |
|                       | PLE                    | 2.42 ±0.30         | 2.36±0.09 | 2.15±0.11 | 2.68±0.42 | 3.50±0.23 |
|                       | PC                     | 2.32 ±0.18         | 1.94±0.17 | 1.92±0.44 | 2.47±0.38 | 3.23±0.40 |
| Lactic acid counts    | NC                     | 4.63 ±0.30         | 5.68±0.23 | 6.26±0.22 | 6.60±0.24 | 6.88±0.57 |
|                       | GLE                    | 4.26 ±0.33         | 5.36±0.34 | 5.86±0.11 | 6.17±0.09 | 6.76±0.39 |
|                       | PLE                    | 4.20 ±0.54         | 5.34±0.24 | 5.72±0.15 | 6.08±0.15 | 6.81±0.67 |
|                       | PC                     | 4.20 ±0.33         | 5.21±0.43 | 5.58±0.30 | 6.32±0.41 | 6.60±0.33 |
| Yeast and mold counts | NC                     | 4.54 ±0.38         | 5.46±0.14 | 6.57±0.23 | 6.80±0.27 | 7.38±0.40 |
|                       | GLE                    | 4.27 ±0.37         | 5.02±0.20 | 5.68±0.13 | 6.32±0.28 | 7.28±0.26 |
|                       | PLE                    | 4.23 ±0.71         | 4.97±0.55 | 5.48±0.68 | 6.15±0.43 | 6.57±0.92 |
|                       | PC                     | 4.45 ±0.24         | 4.69±0.31 | 5.36±0.51 | 6.00±0.45 | 6.61±0.80 |

<sup>1</sup>NC: negative control, GLE: guava leaves extract, PLE: pomegranate leaves extract, PC: positive control (BHT).

## CONCLUSION

Finally, it could be concluded that, the addition of pomegranate leaves extract was as effective against microbial growth and oxidative reactions as synthetic additives. The attic can add ethanol extract 70% of guava leaves and leaves the pomegranate in different ways to meat products as an alternative to antibiotics Industrial oxidative stress antioxidant. You should further experiments in this area to choose the best plant extracts

## REFERENCES

A.O.A.C. (2000). Official Methods of Analysis, 18<sup>th</sup> ed. A.O.A.C. International, MD, USA (pp.1250)  
 Ahmed, M. M. and Ali S. E. (2010). Protective effect of pomegranate peel ethanol extract against ferric nitrilotriacetate induced renal oxidative damage in rats. J. Cell and Mol. Biol. 8(1): 35-43  
 Ahmed, M.M and Zaki, N.I. (2009). Assessment the ameliorative effect of pomegranate and rutin on chlorpyrifos-ethyl-induced oxidative stress in rats. Nature and Science. 7(10): 49-61.

Ahn, S.M., Kim, M.S., Jung, I.C and Sohn, H.Y. (2011). Antibacterial, antioxidative and anti-proliferative activity against human colorectal cell of *pimpinella brachycarpa*. Korean society of food preservation 18(4), 590-596.  
 Aima H, Danno G. (2002). Isolation of antimicrobial compounds from guava (*Psidium guajava L.*) and their structural elucidation. J Biosci, biotechnol and biochem. 66:1727-1730.  
 Biswas, B., Rogers K.; Fredrick M., Daniels, D. and Yadav A. (2013). Antimicrobial Activities of Leaf Extracts of Guava (*Psidium guajava L.*) on Two Gram-Negative and Gram-Positive Bacteria. Inter. J. Microbiology. Volume 2013, Article ID 746165, 7 pages  
 Borch, E. and Arinder, P. (2002). Bacteriological safety issues in red meat and ready-to-eat meat products, as well as control measures. Meat science. 62(3), 381-390.  
 Chen, H. Y. and Yen, G. C. (2007). Antioxidant activity and free radical scavenging capacity of extracts from guava (*Psidium guajava L.*) leaves. Food Chemistry, 101(2), 686-694.

- Cheung, L.M., Cheung, P.C.K., and Ooi, V.E.C. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Food chemistry*, 81(2), 249-255.
- Choudhury, S., Sharan L. and Sinha, M. (2012). Phytochemical and Antimicrobial Screening of *Psidium Guajava* L. Leaf Extracts against Clinically Important Gastrointestinal Pathogens *J. Nat. Prod. Plant Resour.*, 2 (4): 524-529
- Deepak, M.; Bhat Z. and Sunil, K. (2015). Pomegranate (*Punica granatum*) rind extract as a novel preservative in cheese. *Food Bioscience*, 12(1): 47-53.
- Deguchi, Y. and Miyazaki, K. (2010). Anti-hyperglycemic and anti-hyperlipidemic effects of guava leaf extract. *Nutrition and Metabolism* 7: 9-15.
- Dewanto, V., Wu, X., Adom, K.K. and Liu, R.H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agricultural and food chemistry*, 50(1), 3010 – 3014.
- Gutierrez, J., Barry-Ryan, C. and Bourke, P. (2008). The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *International J food microbiology* 124(1), 91-97.
- Hontecillas R., A. Einerhand, M. Diguardo and B. Riera (2009). Activation of PPAR gamma and alpha by punicalagin ameliorates glucose tolerance and suppresses obesity-related inflammation. *J Am Coll Nutr*. 28(2):184-95.
- Hsieh, C.L., Lin, Y.C., Yen, G.C. and Chen, H.Y. (2007). Preventive effects of guava (*Psidium guajava* L.) leaves and its active compounds against a-dicarbonyl compounds-induced blood coagulation. *Food Chemistry* 103 :528–535
- Jaiarj, P., Khoahaswan, P., Wongkrajang, Y., Peungvicha, P., Suriyawong, P. and Sumal, M. L. (1999). Anticough and antimicrobial activities of *Psidium guajava* Linn, leaf extract. *J. Ethnopharmacology*, 67, 203–212.
- Jakobsen, M. and Bertelsen, G. (2000). Color stability and lipid oxidation of fresh beef. Development of a response surface model for predicting the effect of temperature, storage time and modifier atmosphere packaging. *Meat Sci.*, 54: 49-57.
- Kanjana, M.; Kevin, L. G. and Elizabeth, A. B. (2005). Volatile constituents and character impact compounds of selected florids's tropical fruit. *Proc. Fla. State Hort. Soc.* 118:414-418.
- Khalil, M. (2005). Evaluation of antioxidant activity of some plant extracts and their application to ground beef patties. *Food Chemistry*. 69: 135-141.
- Kim, S. J; Cho, A.R. and Han, J. (2013). Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation. *Food Control*, 29: 112-120.
- Kulkarni A.P., Mahal, H.S., Kapoor, S. and Aradhya, S.M. (2007). In vitro studies on the binding, antioxidant and cytotoxic actions of punicalagin. *J Agric Food Chem*. 55(4):1491-500.
- Laguette, M., Lecomte, J. and Villeneuve, P. (2007). Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. *Progress in Lipid Research*. 46( 5): 244–282.
- Lutterodt, G. D. (1989). Inhibition of gastrointestinal release of acetylcholine by quercetin as a possible mode of action of *Psidium guajava* leaf extracts in the treatment of acute diarrhoeal disease. *J. Ethnopharmacology*, 25, 235–247.
- Malaviya, A. and Mishra N. (2011). Antimicrobial activity of tropical fruits. *Biological Forum – An International J*. 3(1): 1-4.
- Mancini, R., Hunt, M. and Kropf, D. (2003). Reflectance at 610 nanometers estimates oxymyoglobin content on the surface of ground beef. *Meat sci.*, 68: 339-344.
- Martha, R., Mitchell, S. and Solis, R. (2008). *Psidium guajava*: A review of its traditional uses, phytochemistry and pharmacology. *J. Ethnopharmacology* 117 : 1–27
- Matsuo, T., Hanamura, N., Shimoi, K., Nakamura, Y. and Tomita, I. (1994). Identification of (+)-gallic acid as a bio-antimutagenic compound in *Psidium guajava* leaves. *Phytochemistry*, 36, 1027–1029.
- McBride, N.T.M., Hogan, S.A. and Kerry, J.P. (2007). Comparative addition of rosemary extract and additives on sensory and antioxidant properties of retail packaged beef. *International J Food Science and Technology*. 42(10)1201-1207.
- Niehus W. and Samuelson B. (1968). Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. *European Journal of Biochemistry*. 6: 126–130
- Olajide, O. A., Awe, S. O. and Makinde, J. M. (1999). Pharmacological studies on the leaf of *Psidium guajava*. *Fitoterapia*, 70, 25–31.
- Rajaa M. and Ashy N. (2012). Inhibitory effect of some plant extracts on clinical isolates of *Staphylococcus aureus*. *Afr. J. Microbiol. Res.* 6(36), 6517-6524.
- Ramadan, H.; Min, B.; Tiwari, A.; Reddy, G.; Adesiyun, A.; Jr, A. H. and Abdela, W. (2015). Antibacterial activity of pomegranate, orange and lemon peel extracts against food-borne pathogens and spoilage bacteria In vitro and on poultry skin. *Inter. J. Poultry Science* 14 (4): 229-239.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*. 26, 1231 – 1237.
- Sayed M. M., Khaled M. Y. and Noha E. M. (2014). The effects of natural antioxidants on colour, lipid stability and sensory evaluation of fresh beef patties stored at 4°C. *J. Agroalimentary Processes and Technologies* 2014, 20(3), 282-292.

- Suresh, M., Babu, K. and Sitaram, B. (2013). In vitro evaluation of antibacterial activity of five indigenous plants extract against five bacterial pathogens of human. *Int J Pharm Pharm Sci*, 5(4), 679-684.
- Thring T. S., P.Hili and D.P. Naughton (2009). Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. *BMC Complementary and Alternative Medicine*. 9: 17-27.
- Vayalil, P.K. (2002). Antioxidant and antimutagenic properties of aqueous extract of date fruit (*Phoenix dactylifera* L. *Arecaceae*) *J Agri. food chem.*, 50(3),610-617.
- Wua, J.W., Hsieh, C.L., Wang, H.Y. and Chen, H.Y. (2009). Inhibitory effects of guava (*Psidium guajava* L.) leaf extracts and its active compounds on the glycation process of protein. *Food Chemistry* 113 : 78–84
- Yao, J. and Moellering, R. (1995). Antibacterial agents. In P.R Murray, E., Baron, E.J, Pfaller, M. A., Tenover, F.C. and Tenover, R.H. (eds.), *Manual of clinical microbiology* (6<sup>th</sup> ed.) (pp. 1281 – 1290) Washington DC: ASM press.
- Abu-Shanab, B, Adwan. G, Abu-Safiya, D, Jarrar. N and Adwan. K (2004). Antibacterial Activities of Some Plant Extracts Utilized in Popular Medicine in Palestine. *Turkish Journal of Biology*. 28, 99-102

### النشاط المضاد للميكروبات والمضاد للأكسدة لمستخلص أوراق الجوافة والرمان الايثانولي حفناوى طه حفناوى<sup>1</sup> و جيهان عبد الله الشوربجي<sup>2</sup>

<sup>1</sup>قسم الكيمياء الحيوية- كلية الزراعة- جامعة الزقازيق-44511 الزقازيق- مصر  
<sup>2</sup>قسم علوم الاغية - كلية الزراعة- جامعة الزقازيق-44511 الزقازيق- مصر

الأوراق الخضراء غنية بالمركبات الفينولية والتي لها العديد من الوظائف البيولوجية، كمضادات الأكسدة والأنشطة المضادة للميكروبات. وكان أول هدف هو تقييم النشاط المضاد للأكسدة والأنشطة المضادة للميكروبات من مستخلص ايثانول 70% لاوراق (الجوافة، والرمان) ولتحديد النشاط المضاد للأكسدة، تم قياس total phenolic content, DPPH radical scavenging activity, ABTS cation decolorization activity and reducing power ولتقييم النشاط المضاد للبكتيريا، تم تقييم التأثيرات المثبطة للمستخلصات ضد بكتيريا (*Escherichia coli*, *Salmonella enterico*, *Staphylococcus aureus*, and *Bacillus subtilis*) باستخدام اختبارات بيئة أجار المرق المغذي. اظهرت مستخلصات أوراق (الجوافة والرمان) قدرتها كمضادات للأكسدة وكذلك ضد نشاط للميكروبات، ولذلك تم دراسة تأثير هذه المستخلصات على أكسدة الدهون والنشاط الميكروبي في منتج البرجر. حيث تم إضافة مستخلصات (اوراق الجوافة واوراق الرمان (BHT) ، الكنترول) بشكل فردي إلى البرجر بتركيزات 0.1% و 0.5% (وزن/وزن) والتخزين على 4°م لمدة 12 يوم. تم قياس اللون وحمض الثيوبوتريك والمواد المتفاعلة (TBARS) القيم بشكل دوري، وتم إجراء التحليل الميكروبي. أدت إضافة المستخلص التالي انخفاض في قيمة TBARS ، و العدد الكلي للبكتيريا في البرجر، وكذلك تحسن لون اللحم والثبات. كان مستخلص اوراق الرمان اكثر فعالية كمضاد للأكسدة وكذلك ضد نشاط الميكروبات من مستخلص اوراق الجوافة.