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RESEARCH ARTICLE

# *In vitro* antioxidant and antimicrobial activity of *Moringa oleifera* leaf as a natural food preservative in chicken burgers

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## ABSTRACT

*Moringa oleifera Lam* (Moringaceae) is a highly valued plant its content phytochemicals, rich in vitamin and minerals and high nutritional value. The objective of this study was to investigate the effect of bioactive compounds in *Moringa oleifera* (MO) leaves and their potential as antimicrobial agents for use as natural food additives to increase the shelf-life of chicken burgers. Seven types of chicken burger were prepared including a control (without antioxidant), with 0.5 % MO polyphenol extract (MOPE) added, 1 % MOPE, 2 % MOPE, 0.5 % whole MO powder (WMOP), 1 % WMOP, and 2 % WMOP. The results showed that chicken burgers treated with MOPE and WMOP at concentrations of 0.5 %, 1 %, and 2 % had significantly ($p < 0.05$) lower total plate counts (TPCs) at the end of the storage period (6 days) compared to the control. Burgers with added MOPE and WMOP had significantly ($p < 0.05$) higher total phenolic content, flavonoids content, and antioxidant activity compared to the control. Acceptability of chicken burger was not affected by the addition of MOPE or WMOP. Our findings suggest that adding MOPE and WMOP at concentrations of 1 % and 2 % could be an effective natural food preservative in chicken burgers.

**Keywords:** Antioxidant activity; Chicken burgers; Food preservation; Microbial analysis; Moringa oleifera

## INTRODUCTION

*Moringa oleifera* Lam (Moringaceae) is a highly valued plant its content phytochemicals, rich in vitamin and minerals and high nutritional value. The tree species of the Hindustan center of crap origin, and the tree is one of the 14 species of the Moringaceae family called *Maringa oleifera* (MO) (Melo et a., 2013; Moyo et al., 2011). MO is widely distributed in the Al-Ahsa oasis in the east of Saudi Arabi (Basuny, 2016; Madane et al., 2019). The MO tree is considered a "miracle tree" or "wonder tree" of significant socioeconomic importance due to its nutritional, pharmacological, and industrial applications (Vergara-Jimenez et al., 2017). All parts of the MO tree, including the leaves, seeds, bark, roots, sap, and flowers, are used as medicinal and food products (Leone et al., 2016). The MO tree is known to be drought resistant and has valuable nutrient and medicinal properties (Moyo et al., 2011), and it could become an increasingly important crop in arid and semiarid region (Mangale et al., 2012). MO was reported with high numbers of bioactive compounds such as terpenoids, flavonoids, glucoseinolates, alkaloids, glycosides and carotenoids which play an important role for prevents several chronic diseases (Chhikara et al., 2020). Moreover, a previous study by Melo, et al (2013) shown that MO leaves are a good source of protein which an essential nutrient for human health.

In the food industry, an increasing number of consumers prefer natural products as healthy choices and for the health benefits their ingredients confer. Plant-based polyphenols may be suitable for applications as a natural preservative to increase the shelf-life of meat (Papuc et al., 2017). It has been reported that antioxidant-rich ingredients can reduce the extent of microbial spoilage during the storage of meat and meat products (Andrés et al., 2013; Aziz and Karboune, 2018; Tayengwa et al., 2020). Chicken burgers are among the most popular processed meat product worldwide (Pereira et al., 2017). This product is highly accepted and consumed by large portions of the population, mainly due to its convenience and low price (Pereira et al., 2017). In Saudi Arabia, the number of local burger restaurants has

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increased rapidly over the last five years; however, chicken and other meats have limited stability, possibly due to the effect of peroxidation of lipid content and microbial activity, which can have both safety and health-related repercussions in humans. A previous study reported that viable psychrotrophic and/or mesophilic microorganisms have been found during meat processing (Han et al., 2009).

The bioactive compounds and functional properties of MO leave grown in Al-Ahsa (Saudi Arabia) have not been well studied. Thus, the objective of this study was to determine the effects of MO leaves and their bioactive compounds, antioxidant activity and antimicrobial agents on chicken burgers use as natural food additives to increase the shelf-life.

## MATERIALS AND METHODS

### Sample preparation and chemicals

Fresh MO leaves (500 g) were harvested and collected manually from fully ripe trees randomly selected during the year 2019 from the local Al-Ahasa farm in Saudi Arabia (Fig. 1). The MO leaves were cleaned thoroughly to remove extraneous dirt and washed under running tap water then rinsed with distilled water to dry in air dryer at room temperature (25°C) for 24 hours before being ground into a powder (the whole MO powder (WMOP) were added to chicken burger). Aliquots (100 g) of powder was mixed in 80 % (v/v) methanol and the mixture was shaken (100 rpm) using a shaker for 10 min and finally centrifuges at 3000x rpm for 10 min, this step was done three times and the supernatants were collected. The combined of were concentrated by in a rotary evaporator (IKA RV 10 Control, Werke GmbH and Co. KG, Germany) at 40 °C with a vacuum pump and the extracts were freeze-dried (used it as MO polyphenol extract (MOPE) in chicken burger). The dried extracts were dissolved in 80 % (v/v) methanol for analysis to identify and quantify phytochemical compounds by gas chromatography–mass spectrometry (GC–MS). All chemicals used in this study such as methanol, Folin–Ciocalteu reagent, 2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid and catechin were obtained from Sigma-Aldrich (Saudi Arabia).

Fig 1. Moringa oleifera tree growth in Al-Ahsa oasis in Saudi Arabia.

### Identification and quantification of phytochemical compounds in MO leaves by Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS was performed with an Agilent GC/MS 7000D GC/MS triple quadrupole 5977B series. Samples were separated on a 30 m × 0.2 mm ID × 0.25 μm DF BR-5MS capillary column (5 % diphenyl 95 % dimethyl polysiloxane). The injector transfer line and ion source temperature were set at 290 °C. The ionizing energy was 70 eV. Electron multiplier voltage was obtained from autotune. The oven temperature was initially held at 60 °C (hold for 1.5 min) and then the temperature was raised to 280 °C at a rate of 3 °C/min. The total run time was 20 min, and the injection volume was 1 μL. The interface temperature was held at 290 °C and mass spectra were acquired from m/z 30 to 600 at a rate of 3 s. The identification and qualification of phenolic compounds in MO extract was achieved by comparing the gas chromatographic retention times. The mass spectra were matched with those standards available in mass spectrum libraries. (Adams et al., 2010). The number of compounds in the MO extract was expressed as a percentage of the peak area relative to the total peak area.

### Preparation of chicken burger

Minced breast chicken was provided by a local poultry market (Al-Ahasa, Saudi Arabia) and was used to make chicken burgers. The ingredients used to make the chicken burger was shown in Table 1 which including minced breast chicken (88 %), whole egg (5 %), salt (1 %), black pepper (1 %) and breadcrumbs (5 %). The chicken burger was treated with MOPE and WMOP using different concentrations (0.5 %, 1 %, and 2 %). The control without antioxidants were used. Each sample was prepared separately in a bowl chopper to make chicken burgers and were aerobically packed in bags and stored at 4 °C. Raw chicken burger samples were used for analysis total phenolics, flavonoid contents, antioxidant activity, pH, and microbial count at 0, 2, 4, and 6 days of storage under refrigerated conditions (4 °C). The sensory attributes of cooked chicken burger samples (control, MOPE and WMOP) were also determined. All experiments were carried out in triplicate.

### Determination of total phenolic compounds in chicken burger

The total phenolic compound content was assessed using the Folin–Ciocalteu method (Sun et al., 2002). Briefly, 10 g of chicken burger samples (MOPE and WMOP at

**Table 1: Ingredients of chicken burgers with control and treatments**

| Ingredients(%) | Control | 0.5% MOPE | 1% MOPE | 2% MOPE | 0.5% WMOP | 1% WMOP | 2% WMOP |
|---|---|---|---|---|---|---|---|
| Brest Chicken | 88 | 88 | 88 | 88 | 88 | 88 | 88 |
| Breadcrumbs | 5.0 | 4.5 | 4.0 | 3.0 | 4.5 | 4.0 | 3.0 |
| Whole egg | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Salt | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Black pepper | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| MO polyphenols extract | 0.0 | 0.5 | 1.0 | 2.0 | 0.0 | 0.0 | 0.0 |
| Whole MO powder | 0.0 | 0.0 | 0.0 | 0.0 | 0.5 | 1.0 | 2.0 |

Treatments: control= chicken burgers (without antioxidant); 0.5%MOPE =chicken burgers with 0.5% MO polyphenol extract; 1% MOPE=chicken burgers with 1% MO polyphenol extract; 2% MOPE =chicken burgers with 2% MO polyphenol extract; 0.5% WMOP= chicken burgers with 0.5% whole MO powder; 1% WMOP =chicken burgers with 1% whole MO powder; 2% WMOP = chicken burgers with 2% whole MO powder

concentrations 0.5 %, 1 %, and 2 % and the control (without antioxidants)) were homogenized with 50 mL of 80 % methanol (v/v) and kept for 5 minutes then mixed again before centrifuges at 3000x rpm for 10 minutes. A 1.5 ml of the supernatants were collected and added into tubes mixed with 0.5 mL of Folin–Ciocalteu reagent. After incubation for 3 min at room temperature, 4 mL of sodium carbonate was added, and the tubes were placed on a shaker for 25 min at room temperature. The absorbance was read at 650 nm using a UV-1800 spectrophotometer (Shimadzu, China). The total phenolic content was calculated using the calibration curve constructed with gallic acid as a standard, and the results are expressed as gallic acid equivalents (GAE) mg/g dried MO leaves (mean ± SE; n = 3, triplicate analysis).

### Determination of the total flavonoid content in chicken burger

The total flavonoid content was measured using the aluminum chloride assay (Ostertag et al., 2010). Briefly, 10 g of chicken burger samples (MOPE and WMOP at concentrations 0.5 %, 1 %, and 2 % and the control (without antioxidants)) were homogenized with 50 ml of 80 % methanol (v/v) and kept for 5 minutes then mixed again before centrifuges at 3000x rpm for 10 minutes Then, the supernatants were mixed with 0.3 mL of sodium nitrite (5 %). After incubation for 5 min at room temperature, 0.3 mL of 10 % $AlCl_3$ was added. After an additional 6 min, 2 mL of 1 M sodium hydroxide was added, and the total volume was brought to 10 mL with distilled water. The absorbance of the reaction mixture was measured at 512 nm using a UV-1800 spectrophotometer (Shimadzu, China). The total flavonoid content is expressed as catechin equivalents (mg CHE)/g (mean ± SE; n = 3, triplicate analysis).

### Determination of the DPPH radical scavenging activity of chicken burgers

The antioxidant activity chicken burgers determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Amarowicz et al., 2004), which is based on the DPPH free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. For this assay, 1 mL chicken burger samples (MOPE and WMOP at concentrations 0.5 %, 1 %, and 2 % and the control (without antioxidants)) were mixed with 1 mL of 0.1 mM DPPH in methanol, and the absorbance was measured after 30 min of incubation at room temperature in a dark room at 515 nm using a UV-1800 spectrophotometer (Shimadzu, China). The result was calculated as a percentage using the following equation:

$$\text{DPPH Scavenged } (\%) = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{\text{Absorbance of Control}} \times 100$$

### Antimicrobial analysis

The chicken burger samples were divided into 10 g portions Each treatment (MOPE and WMOP) was administered at different concentrations (0.5 %, 1 %, and 2 %) and compared with the chicken burger control (without antioxidants). Each sample was homogenized with 90 mL of sterile peptone water (0.1 %) and diluted to different concentrations. A 1 mL dilution was inoculated onto Petrifilm (3M, Saudi Arabia) to obtain the total plate count (TPC) and incubated at 37 °C for 0, 2, 4, and 6 days of storage under refrigerated conditions at 4 °C (Lorenzo et al., 2015). In addition, 1 mL dilutions were inoculated in selected agar to determine the chicken burger samples effects on *Escherichia coli* and *Staphylococcus*. The selected agar was Macconkey agar and Baired Parker medium (Sigma, Saudi Arabia), and plates were incubated at 37 °C for 24 h. Microbial analysis was performed after 0, 2, 4, and 6 days of storage under refrigerated conditions (4 °C). Microbial colonies from the plates were counted and are expressed as (log cfu/g).

### Determination chicken burger pH

pH levels were determined according to AOAC (1995). Briefly, one gram of chicken burger samples (MOPE and WMOP) at different concentrations (0.5 %, 1 %, and 2 %) and the control (without antioxidants) were homogenized in 10 mL of distilled water and mixed it. Samples were filtered by No 2-filter paper before the pH measurement. The pH was measured by a pH meter (Benchtop pH Meters Hanna Instruments, Italy). The pH was calibrated using buffers of pH 4.0, pH 7.0, and pH 10.0 prior to analysis.

### Sensory evaluation of chicken burgers

A panel of twenty judges experiences in the characteristics of chicken products was performed the sensory evaluation at King Faisal University. Different sensory attributes, including texture, flavor, taste, color, and overall acceptability, were determined using an 8-point descriptive scale (Keeton, 1983), where 8= Like extremely and 1= dislike extremely. Chicken burgers were cooked for 5 min on a grill until the center of the chicken reached 80 °C and the chicken burger were warmed in a microwave oven for 50 second immediately before served to the panelists to evaluate. Water was given to cleansing mouth between samples. The evaluation was carried out on MOPE and WMOP samples and the control (chicken burgers without antioxidant).

### Statistical analysis

The results of all experiments are expressed as the average of triplicate experiments with standard error (SE). Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) software, version 26 (IBM SPSS statistics, United States). Data were analyzed using ANOVA with all samples, including the control and MO treatment samples (MOPE and WMOP) at different concentrations (0.5 %, 1 %, and 2 %) which were compared using Tukey's test at a 5% significance level ($p < 0.05$) for the effects of total phenolic compound content, total flavonoid content, antioxidant activity, pH effect, microbial activity of chicken burgers, and sensory evaluation of cooked chicken burgers.

## RESULTS AND DISCUSSION

### Identification and qualification of polyphenols in MO extract

The composition of the methanolic (80 %) extract of MO leaves was identified and qualified by GC–MS analysis. The GC–MS chromatograms showed 19 peaks, which are presented in Table 2 and Fig. 2. The mean compounds have identity in MO extract are 1-(+) ascorbic acid 2,6-dihexadecanoate (2.16 %), 1,3-benzenedicarboxylic acid (16.21 %), dimethyl phosphite (0.25 %), 1-methylpentyl hydroperoxide (22 %), furohydroxamic acid (1.41 %), succinic acid (8.70 %), 3-hydroxybenzaldehyde (9.30 %), trans-(2 chlorovinyl) dimethylethoxysilane (8.12 %), 2'-hydroxypropiophenone (0.80 %), 2-phenylbenzo[b]thiophene (13.65 %), glutaric acid, (0.56 %), 4-hydroxy-3-methoxybenzyl (3.44 %), lupeol (0.56 %), benzoic acid (0.23 %), valeric acid (1.21 %), hexacosane (0.16 %), dimethyl(4-(2-phenylprop-2-yl)silane (0.12 %), acetic acid, (0.55), and dihydro-actiridioide (3.22 %). These compounds constituted 98.39 % of the entire MO chromatogram. The four major compounds present in the methanolic (80 %) extract of MO leaves were 1-methylpentyl hydroperoxide, 2-phenylbenzo[b]thiophene, succinic acid, and 3-hydroxybenzaldehyde. These compounds are known

**Table 2: Compounds identified by GC–MS in methanolic (80%) extracts of MO. Values are means of triplicate experiments (n = 3)**

| Peak | Compound | RT [a] | Concentration (%) |
|---|---|---|---|
| 1 | 1-(+) Ascorbic acid 2,6-dihexadecanoate | 1.07 | 2.16 |
| 2 | 1,3-Benzenedicarboxylic acid | 1.61 | 16.21 |
| 3 | Dimethyl phosphite | 2.05 | 0.25 |
| 4 | 1-Methylpentyl hydroperoxide | 2.33 | 22.0 |
| 5 | Furohydroxamic acid | 2.74 | 1.41 |
| 6 | Succinic acid | 3.63 | 8.70 |
| 7 | 3-Hydroxybenzaldehyde | 4.17 | 9.30 |
| 8 | Trans-(2-Chlorovinyl) dimethylethoxysilane | 5.13 | 8.12 |
| 9 | 2'-Hydroxypropiophenone | 5.60 | 0.80 |
| 10 | 2-Phenylbenzo[b]thiophene | 5.91 | 13.65 |
| 11 | Glutaric acid | 6.50 | 6.30 |
| 12 | 4-Hydroxy-3-methoxybenzyl | 7.12 | 3.44 |
| 13 | Lupeol | 7.79 | 0.56 |
| 14 | Benzoic acid | 8.32 | 0.23 |
| 15 | Valeric acid | 8.87 | 1.21 |
| 16 | Hexacosane | 9.24 | 0.16 |
| 17 | Dimethyl(4-(2-phenylprop-2-yl) silane | 9.94 | 0.12 |
| 18 | Acetic acid | 10.39 | 0.55 |
| 19 | Dihydro-actiridioide | 11.34 | 3.22 |
| | Total compounds | - | 98.39 |

[a]RT: Retention Time (min).

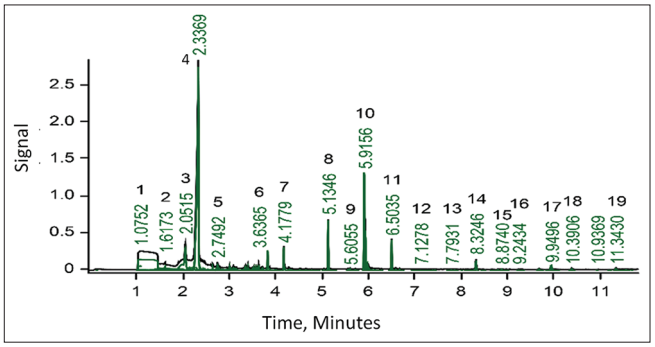


**Fig 2.** Typical GC-MS chromatogram of phenolic compounds of MO methanolic extract; phenolic compounds are: (1) 1-(+) Ascorbic acid 2,6-dihexadecanoate, (2) 1,3-Benzenedicarboxylic acid, (3) Dimethyl phosphite, (4) 1-Methylpentyl hydroperoxide, (5) Furohydroxamic acid, (6) Succinic acid, (7) 3-Hydroxybenzaldehyde, (8) Trans-(2-Chlorovinyl) dimethylethoxysilane, (9) 2'-Hydroxypropiophenone, (10) 2-Phenylbenzo[b]thiophene, (11) Glutaric acid, (12) 4-Hydroxy-3-methoxybenzyl, (13) Lupeol, (14) Benzoic acid, (15) Valeric acid, (16) Hexacosane, (17) Dimethyl(4-(2-phenylprop-2-yl)silane, (18) Acetic acid, (19) Dihydro-actiridioide.

to be biologically active and provide many health benefits to humans (Shahidi and Ambigaipalan, 2015). Some of the phytochemical compounds present in many plants have previously been reported to have antimicrobial and antifungal activities, as well as substantial natural antioxidant effects (Chibane et al., 2019; Lee and Lee, 2010). In addition, some of the bioactive compounds, including phenolic acids and flavonoids, have been confirmed to be anti-inflammatory and have anti-cancer effects because of their antioxidant

activities that reduce and neutralize free radicals (Babbar et al., 2011; Hua Zhang and Tsao, 2016). Here, methanolic (80 %) extracts of MO leaves were reported to contain high levels of phenolic compounds, similar to previous findings (Okumu et al., 2016).

### Total phenolic content, flavonoid content and antioxidant activity of MO leaves

The total phenolic content, flavonoid content, and antioxidant activity of control chicken burgers and MOPE- and WMOP-treated burgers at different concentrations (0.5 %, 1 %, and 2 %) are presented in Table 3. Chicken burgers prepared with 1 % and 2 % MOPE or WMOP showed significantly higher ($p< 0.05$) total phenolic compound content (1 % MOPE 45.2 mg/g; 2 % MOPE 73.4 mg/g; 1 % WMOP 34.4 mg/g; 2 % WMOP 62.3 mg/g) and total flavonoid content (1 % MOPE 12.5 mg/g; 2 % MOPE 18.2 mg/g; 1 % WMOP 15.1 mg/g; 2 % WMOP 31.1 mg/g) than those of the control or 0.5 % MOPE- and WMOP-treated samples.

The aqueous chicken burger with MOPE presented a significantly higher total phenolic content and total flavonoid content than chicken burgers with WMOP ($p< 0.05$). The high content of phenolic compounds in the MOPE was due to the polarity of the solvent used for extraction of the polyphenols in MO leaves, introducing them into the chicken burgers and subsequently increasing the of antioxidant activity and antimicrobial activity (Hadadi et al., 2020; Naz et al., 2017).

DPPH is a free radical that can be used to determine the free radical scavenging ability of plant extracts (Ilaiyaraja et al., 2015) and many fruit and vegetables such as olive oil (Giuffer et al., 2018), bergamot fruit (Giuffrè, 2019) and tea (He et al., 2020). In this study, we analyzed the antioxidant activity of chicken burgers supplemented with either MOPE or WMOP, and these treated samples showed higher antioxidant activity than the control (without additives). Additionally, a significant difference ($p < 0.05$) in antioxidant activity was observed between the 1 % and 2 % MOPE and WMOP samples compared to the control (without additives) (Table 3). According to the antioxidant activity results, MOPE had a high radical scavenging activity in chicken burgers, which increased according to the concentration added; 0.5 % MOPE addition (45 % ± 2.05), 1 % MOPE addition (56 % ± 1.33) and 2 % MOPE addition (85 % ± 1.67) (Table 3). This may be due to the high levels of total phenolic compounds and flavonoids in MOPE, which are responsible for increased antioxidant activity. Previous studies have reported that antioxidants can be used as natural additives in foods such as meats and meat products to increase the shelf life of products, and improve meat quality and safety (Velasco and Williams 2011; Lorenzo et al., 2018). In addition, foods containing these natural bioactive compounds have human health benefits and are considered natural functional foods, leading them to receive increasing levels of interest from the food industry (Vieira da Silva et al., 2016; Martirosyan and Miller, 2018).

**Table 3: Total phenolic compound content, total flavonoid content, and antioxidant activity of MO leaves in chicken burgers (mean ± SE)**

| Samples | Total Phenolic Compound Content (GAE mg/g) | Total Flavonoid Content (CHE mg/g) | Antioxidant Activity (DPPH) (%) |
|---|---|---|---|
| Control | 0.01 ± 0.02$^{a}$ | 0.03 ± 1.55 $^{a}$ | 0.01 ± 0.03$^{a}$ |
| 0.5% MOPE | 24.2 ± 1.60$^{a}$ | 10.2 ± 1.52$^{a}$ | 46 ± 2.05$^{b}$ |
| 1% MOPE | 45.2 ± 1.69$^{b}$ | 12.5 ± 1.50 $^{b}$ | 56 ± 1.33$^{c}$ |
| 2% MOPE | 73.4 ± 1.4$^{c}$ | 18.2 ± 2.54 $^{c}$ | 85 ± 1.67$^{d}$ |
| 0.5% WMOP | 22.2 ± 1.43$^{a}$ | 11.2 ± 1.26$^{a}$ | 42 ± 1.52$^{e}$ |
| 1% WMOP | 34.4 ± 1.91$^{d}$ | 15.1 ± 1.72$^{d}$ | 51 ± 2.15$^{f}$ |
| 2% WMOP | 62.3 ± 1.88$^{e}$ | 31.1 ± 1.83$^{e}$ | 74 ± 2.01$^{g}$ |

Treatments: control= chicken burgers (without antioxidant); 0.5%MOPE =chicken burgers with 0.5% MO polyphenol extract; 1% MOPE=chicken burgers with 1% MO polyphenol extract; 2% MOPE =chicken burgers with 2% MO polyphenol extract; 0.5% WMOP= chicken burgers with 0.5% whole MO powder; 1% WMOP =chicken burgers with 1% whole MO powder; 2% WMOP = chicken burgers with 2% whole MO powder. Mean values with different letters within the same column among treatments are statistically different ($p< 0.05$).

### Changes in chicken burger pH during different storage times and treatments

The pH value of the control and treated chicken burgers with MO leaves stored under refrigerated conditions at 4 °C

**Table 4: Change of pH of chicken burgers with different levels of MO leaves and control**

| Treatment | Storage Time (Days) | | | | Sig |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | |
| Control | 6.22 ± 0.03$^{a}$ | 6.33 ± 0.15$^{a}$ | 7.21 ± 0.20$^{a}$ | 7.35 ± 0.06$^{a}$ | *** |
| 0.5% MOPE | 6.28 ± 0.03 | 6.43 ± 0.01$^{ab}$ | 6.51 ± 0.12$^{b}$ | 6.72 ± 0.08$^{b}$ | *** |
| 1% MOPE | 6.19 ± 0.07$^{b}$ | 6.53 ± 0.08$^{ab}$ | 6.73 ± 0.10$^{bc}$ | 6.80 ± 0.09$^{b}$ | *** |
| 2% MOPE | 6.30 ± 0.02$^{ba}$ | 6.45 ± 0.60$^{b}$ | 6.51 ± 0.02$^{ab}$ | 6.91 ± 0.01$^{ab}$ | *** |
| 0.5% WMOP | 5.98 ± 0.04 | 6.13 ± 0.01$^{ab}$ | 6.42 ± 0.05$^{bc}$ | 6.50 ± 0.20$^{bc}$ | *** |
| 1% WMOP | 5.92 ± 0.03$^{ab}$ | 6.22 ± 0.06$^{ac}$ | 6.39 ± 0.04$^{b}$ | 6.94 ± 0.04$^{ab}$ | ** |
| 2% WMOP | 5.72 ± 0.04$^{ab}$ | 6.22 ± 0.25$^{ab}$ | 6.30 ± 0.02$^{ab}$ | 6.60 ± 0.05$^{bc}$ | *** |
| Sig | ** | ns | ** | ** | |

Treatments: control= chicken burgers (without antioxidant); 0.5%MOPE =chicken burgers with 0.5% MO polyphenol extract; 1% MOPE=chicken burgers with 1% MO polyphenol extract; 2% MOPE =chicken burgers with 2% MO polyphenol extract; 0.5% WMOP= chicken burgers with 0.5% whole MO powder; 1% WMOP =chicken burgers with 1% whole MO powder; 2% WMOP = chicken burgers with 2% whole MO powder. Mean values with different letters within the same column among treatments are statistically different, ***$p < 0.001$, **$p < 0.05$. ns = not significant; Sig = significance.

were evaluated after 0, 2, 4, and 6 days of storage, as shown in Table 4. The results show that the pH increased significantly ($p< 0.001$) in both the control and treated chicken burgers with increased storage duration (0, 2, 4, and 6 days). The pH values increased from 6.22±0.03 to 7.35±0.06 (control), from 6.28±0.03 to 6.72±0.08 (0.5 % MOPE) 6.19±0.07 to 6.80±0.09 (1% MOPE), from 6.30±0.02 to 6.91±0.01 (2% MOPE), from 5.98±0.04 to 6.50±0.20 (0.5% WMOP), from 5.92±0.03 to 6.94±0.04 (1% WMOP), and from 5.72±0.04 to 6.60±0.05 (2 % WMOP). The increase in pH of the samples may be due to the metabolism of microbes and bacterial growth in the samples over time. The increase in pH of the samples may be due to the metabolism of microbes and bacterial growth in the samples over time (Zhang et al., 2016a).

Our results also showed a statistically significant reduction in pH (MOPE and WMOP) treated in chicken burgers compared to the control samples for all storage durations (0, 2, 4, and 6 days) ($p< 0.05$), which may be linked to the high levels of antioxidant and bioactive compounds in MO leaves that were added to the chicken burgers. Similar observations were reported in previous studies which showed an increase in the pH of chicken meat nuggets and chicken sausages after treatment with MO flowers and leaves for different storage times (Madane et al., 2019; Jayawardana et al., 2015).

### Antimicrobial activity of MO leaves

Table 5 presents the mean and SE values of the TPC of the control and MO-treated (MOPE and WMOP) chicken burgers at different concentrations (0.5 %, 1 %, and 2 %) after 0, 2, 4, and 6 days of storage under refrigerated conditions (4 °C). After being stored for 0, 2, 4, and 6 days, a significant increase ($p< 0.05$) in microbial activity count was seen in the control, MOPE, and WMOP groups with increasing storage period. The TPC in the control chicken burger (without antioxidants) increased with storage time from 4.63 ± 0.01 $\log^{10}$ cfu/g, (0 day) to 134.36 ± 0.07 $\log^{10}$ cfu/g (6 days). A significant reduction in TPC was observed ($p< 0.001$) in MOPE and WMOP samples at concentrations of 1 % and 2 % after 2, 4, and 6 days of storage compared to the control and 0.5 % concentration samples (MOPE and WMOP). This may be because increased levels of bioactive compounds and antioxidant capacity have been shown in this study, which help to reduce the level of microbial activity in meat and meat products, increasing the shelf-life of products (Banerjee et al., 2012; Papuc et al., 2017; Zhang et al., 2016b). Our results also show that chicken burgers treated with MO (MOPE and WMOP at concentrations of 0.5 %, 1 %, and 2 %) had significantly lower TPCs ($p< 0.05$) than the control at the end of the storage period (6 days). It has been reported that bioactive compounds and polyphenols in MO leaves have potential antimicrobial activities against pathogenic bacteria (Jayawardana et al., 2015). It is well understood that some bioactive compounds, known as pterygospermin, contained in MO leaves have antimicrobial properties that can reduce the level of pathogenic bacteria in meat and meat products, and can play an important role in increasing the shelf-life of meat products which can be used as natural food preservative (Bukar et al., 2010). Previous study reported that, the high phenolic compounds from different plant sources strongly reduce the level of microorganism poultry and meat product (Ghomari et al., 2019). One of the possible mechanisms that phenolic compounds can break down the cell wall, influence the synthesis of DNA and RNA and destroy protein translocation (Shan et al., 2007). In addition, our study showed that no *E. coli* or *Staphylococcus* were detected in any of the samples (data not shown), which may be because a high level of hygiene and food safety is required and applied to poultry and meat products in Saudi Arabia.

**Table 5: Total plate count (TPC; log 10 cfu/g) of chicken burgers with different concentrations of MO and control over time**

| Treatment | Storage Time (days) | | | | Sig |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | |
| Control | 4.63 ± 0.01$^{a}$ | 37.03 ± 0.11$^{a}$ | 94.50 ± 0.10$^{a}$ | 134.36 ± 0.07$^{a}$ | *** |
| 0.5% MOPE | 5.32 ± 0.05$^{b}$ | 11.93 ± 0.09$^{b}$ | 32.36 ± 0.10$^{b}$ | 126.73 ± 0.05$^{b}$ | *** |
| 1% MOPE | 3.13 ± 0.15$^{ab}$ | 20.03 ± 0.12$^{ab}$ | 25.50 ± 0.09$^{ab}$ | 90.40 ± 0.06$^{ab}$ | *** |
| 2% MOPE | 2.11 ± 0.03$^{a}$ | 12.45 ± 0.09$^{b}$ | 60.63 ± 0.02$^{c}$ | 84.66 ± 0.02$^{ab}$ | *** |
| 0.5% WMOP | 4.12 ± 0.05$^{b}$ | 11.13 ± 0.05$^{ab}$ | 40.55 ± 0.03$^{b}$ | 103.36 ± 0.01$^{c}$ | *** |
| 1% WMOP | 3.23 ± 0.09$^{bc}$ | 10.22 ± 0.08$^{bc}$ | 45.73 ± 0.03$^{bc}$ | 90.43 ± 0.03$^{ab}$ | ** |
| 2% WMOP | 4.86 ± 0.08$^{b}$ | 7.20 ± 0.25$^{b}$ | 18.02 ± 0.07$^{b}$ | 36.16 ± 0.05$^{b}$ | *** |
| Sig | ns | ** | ** | ** | |

Treatments: control= chicken burgers (without antioxidant); 0.5%MOPE =chicken burgers with 0.5% MO polyphenol extract; 1% MOPE=chicken burgers with 1% MO polyphenol extract; 2% MOPE =chicken burgers with 2% MO polyphenol extract; 0.5% WMOP= chicken burgers with 0.5% whole MO powder; 1% WMOP =chicken burgers with 1% whole MO powder; 2% WMOP = chicken burgers with 2% whole MO powder. Mean values with different letters within the same column among treatments are statistically different. ns = not significant; Sig = significance; ***$p < 0.001$, **$p < 0.05$.

### Sensory analysis

A sensory evaluation was carried out to determine the optimal level of MO leaf (MOPE and WMOP) addition to chicken burgers using different concentrations (0.5 %, 1 %, and 2 %) and the control (without antioxidants). There were no significant differences in the sensory characteristics of all chicken burgers (Table 6). Control, MOPE, and WMOP chicken burgers at all concentrations exhibited not significantly ($p> 0.05$) marked changes in texture, flavor, taste, color, or general acceptability.

**Table 6: Effect of MO leaves and control on the sensory attributes of chicken burgers (mean± SE)**

| Samples | Overall acceptance | Texture | Flavor | Taste | Color |
|---|---|---|---|---|---|
| Control | 8.16±1.33 | 7.91±1.88 | 8.33±1.55 | 7.5±1.73 | 8.33±1.37 |
| 0.5% MOPE | 7.83±1.97 | 7.75±1.60 | 8.16±1.52 | 7.75±2.05 | 8.25±1.48 |
| 1% MOPE | 7.75±1.60 | 7.16±1.69 | 7.91±1.50 | 7.82±1.33 | 8±1.34 |
| 2% MOPE | 6.66±1.87 | 6.91±2.4 | 7.5±2.54 | 6.41±1.67 | 7.41±2.10 |
| 0.5% WMOP | 7.1±0.83 | 8.33±1.43 | 8.16±1.26 | 7.83±1.52 | 8.08±1.44 |
| 1% WMOP | 7.58±1.83 | 8.25±1.91 | 7.33±1.72 | 7.58±2.15 | 7.91±1.08 |
| 2% WMOP | 6.33±1.49 | 7.41±1.88 | 6.41±1.83 | 7.33±2.01 | 7.6±1.27 |

Treatments: control= chicken burgers (without antioxidant); 0.5%MOPE =chicken burgers with 0.5% MO polyphenol extract; 1% MOPE=chicken burgers with 1% MO polyphenol extract; 2% MOPE =chicken burgers with 2% MO polyphenol extract; 0.5% WMOP= chicken burgers with 0.5% whole MO powder; 1% WMOP =chicken burgers with 1% whole MO powder; 2% WMOP = chicken burgers with 2% whole MO powder. *n*=20. None of the mean values within the parameter were significantly different.

## CONCLUSION

To our knowledge, this is the first study to examine the antioxidant activities and antimicrobial properties of MO leaves grown in Al-Ahsa, Saudi Arabia and added to chicken burgers. Our results showed that MO leaves are a significant source of polyphenols, which exhibit high levels of antioxidant activity. In addition, the results indicated that MOPE and WMOP, at concentrations of 1% and 2%, have strong antimicrobial activity in chicken burgers that increases the shelf-life of chicken burgers up to 6 days of refrigeration storage at 4 °C. MOPE and WMOP can be used as a natural food preservative in chicken burgers and may play an important role in extending the shelf-life of other meat products, providing natural antioxidant activities that are valuable to the food industry and offer potential health benefits to consumers.

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## CONFLICTS OF INTEREST

The authors had no conflict of interest to declare.

### Author contributions

Conceptualization R.A., and H.A.; investigation and analysis of the samples R.A., and H.A.; writing -original draft preparation, R.A., and H.A.; writing- review and editing R.A. All authors have read and agree to the published version of the manuscript.

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