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Antimicrobial and Antioxidant Activity of Some Plant Extracts against Different Food Spoilage and Pathogenic Microbes

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ASS, AAZ and MAMZ designed the study and wrote the protocol. Author ASS performed the statistical analysis and wrote the first draft of the manuscript. Authors ASS and GMH managed the analyses of the study. Authors ASS and SA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Guava (*Psidium guajava*), pomegranate (*Punica granatum*), olive (*Olea europaea L.*), and moringa (*Moringa oleifera*) extracts which are assumed to contain active components and which are renewable sources in fighting infections of microbes. This study aimed to investigate its antioxidant and antimicrobial activity.
Methodology: The agar well diffusion technique, minimum inhibitory concentration (MIC), total phenolic content (TPC), total flavonoid content (TFC), and the free radical scavenging activity of the plant extracts were applied.
Results: All extracts exhibited different results against the microorganism used in the research. The minimum inhibitory concentration (MIC) values for bacteria and fungi ranged from 25 to 300

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mg/mL. The antioxidant activity was evaluated by using DPPH radical scavenging assay. In addition, the amount of total phenolic content (TPC) of the extracts ranged from 48.08 to 324.08 mg/g, while total flavonoid content (TFC) ranged from11.53 to 65.85 mg/g.
Conclusion: It could be noticed that the guava and pomegranate extracts had strong antioxidant and antimicrobial effects, while olive extract had a moderate effect, but moringa showed a very weak effect against tested microbes. Therefore, the herbal extracts of guava and pomegranate could be used as novel, safe, and effective food preservatives instead of chemical ones.

Keywords: Antimicrobial activity; antioxidant activity; guava extract; pomegranate extract; olive extract; Moringa extract.

1. INTRODUCTION

Food, the main source of energy for humans, could be easily contaminated by many microbes, which play an essential role in food spoilage and human foodborne illness [1,2]. Food poisoning is one of the major reasons for disease and death in developing countries [3-6]. The majority of food poisoning cases are related to microbial contamination, particularly with Gram-negative bacteria such as *Salmonella typhi*, *Escherichia coli*, and *Pseudomonas aeruginosa* [7-9]. Additionally, some Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus cereus* have also been known as the common factors causing foodborne illnesses and food spoilage [10].

Food spoilage could be traditionally avoided by using different chemical preservatives [11-13]. Although the high efficiency of chemical preservatives in the prevention and control of foodborne illnesses and food spoilage, they have some disadvantages such as the presence of chemical residues in the treated foodstuffs, the emergence of resistant microbial strains, the negative impacts of these chemicals on the human health [14]. Therefore, to avoid such disadvantages, various studies have focused on developing effective and safe food preservatives. Nowadays, the utilization of plant extracts as natural antimicrobial agents for food preservation became a promising trend in the food industry [15]. Such plant extracts are natural ingredients, therefore, it is considered as safe and degradable preservatives when applied in food products [15-18]. The antimicrobial effect of plant extracts has been investigated by several researchers [19-22]. The antibacterial activity of guava, ginger, and garlic extracts was evaluated against some pathogenic microbes [5]. It was observed that ginger extract was effective against *S. aureus* while guava and garlic extracts were effective against all tested microorganisms. The Pomegranate extract showed high antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhi,* and *E. coli*; therefore, it can be applied for the prevention of foodborne diseases or as a preservative in the food industry [23,24]. However, to the best of our knowledge, few studies investigated the antimicrobial effects of plant extracts on Gram-positive, Gram-negative, yeast, and mold strains.

Therefore, the present work aims to investigate the antimicrobial effects of four plant extracts, namely: Guava (*Psidium guajava*), Pomegranate (*Punica granatum*), Olive (*Olea europaea L.*), and Moringa (*Moringa oleifera*) against various microbial strains (Gram-positive, Gram-negative and fungi strains) which are incriminated in food spoilage and foodborne illness.

2. MATERIALS AND METHODS

2.1 Preparation of Plants Extracts

Guava leaves, Pomegranate peels, Olive leaves, and Moringa leaves were collected from a private farm, Beheira, Egypt. The plant was identified by the Department of Botany, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria, Egypt. The selected plant parts were washed with distilled water and dried at 40°C/ 24 hr [25]. Then the dried samples were ground, passed through sieve no. 20, and finally, stored in light-dark bottles at 4°C for further use. Water, ethanol, and methanol extractions were carried out according to Fernandes et al. [26]. The extracts were lyophilized by a vacuum freeze dryer (model FDF 0350, Korea) and kept in light-dark bottles at 4°C for further analysis.

2.2 Microorganisms and Culture Conditions

Bacterial strains used were; three Gram-positive strains; *Staphylococcus aureus* EMCC 1351,

Streptococcus pyogenes EMCC 1772, and *Bacillus subtilis* EMCC 1009, and five Gram-negative strains; *Salmonella enterica* EMCC 1350, *Escherichia coli* BA 12296, *Klebsiella pneumonia* EMCC 1637, *Pseudomonas aeruginosa* EMCC 1256, and *Proteus mirabilis* EMCC 1312. Tested yeast strains were; *Pichia memberanifaciens* EMCC 90, *Rhodotorula glutinis* EMCC 175, *Schizosaccharomyces octosporus* EMCC 93, *Aureobasidium pullulans* ATCC 42023, and Hansenula anomala CBS 5759. Tested mold strains were *Aspergillus niger* EMCC 102, *Aspergillus flavus* EMCC 101, *Aspergillus parasiticus* EMCC 886, and *Aspergillus ochraceus* EMCC 515. All strains were obtained from Microbiological Resources Center (MERCIN), Ain Shams University, Egypt. The strains were maintained in 60% glycerol/ LB culture at -80°C.

2.3 Antimicrobial Activity

The antimicrobial activity was performed by agar well diffusion assay for all sample extracts [27]. Wells were made using a 0.55 cm cork-borer. The used concentration was 100 µl/well. All the bacterial strains were incubated at 37°C for 24 hr while the fungal strains were incubated at 28°C for 72 hr. The inhibition zone was determined by calculating the diameter around the well, including the well diameter. For each microbial strain, also controls were maintained as pure solvents (Sterile distilled water) instead of the extract. The readings were taken in three different fixed directions, and the average value was calculated.

2.3.1 Determination of minimum inhibitory concentration (MIC)

The MIC was performed by agar well diffusion assay for all sample extracts [27]. The bacterial strains were grown in nutrient broth at 37°C, whereas the fungal strains were grown in Sabouraud dextrose at 28°C for 24h. Five concentrations of reconstituted plant water extracts (300, 200, 100, 50, and 25 mg/ mL) were studied to evaluate the MIC of each against a specific pathogenic strain [28,29]. The inhibition zone diameter (IZD) was calculated by calculating the diameter around the well, including the well diameter. The measurements were taken in three different fixed directions and the average value was calculated.

2.4 Determination of Bioactive Compounds

2.4.1 Determination of total phenolic contents (TPC)

The TPC of the extracts were estimated by using Folin-Ciocalteau reagent [30,31]. The absorbance at 650 nm was recorded versus the prepared blank using a spectrophotometer (Labo America, USA). A standard curve was prepared using different concentrations of Gallic acid monohydrate in the range of (5-1000 µg/ml). TPC was expressed as mg GAE/g extract and calculated using the following linear equation based on the calibration curve: y = 0.0025x - 0.0732

2.4.2 Determination of total flavonoid contents (TFC)

The TFC of the plant extracts were determined by a modified colorimetric method described by Sakanaka et al. [32], using catechol as a standard at concentrations of (20–200 µg/ ml). The mixture was mixed well and absorption was measured at 510 nm using a spectrophotometer (Labo America, USA). TFC was expressed as mg CE/g extract and calculated using the following linear equation based on the calibration curve: y = 0.0088 x - 0.0515

2.5 Antioxidant Activity

The free radical scavenging activity of plant extracts was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH [33]. The absorbance was read at 517nm using a spectrophotometer (Labo America, USA). Ascorbic acid solutions as standards in the concentration range of (5-500 µg/ml) were used to establish a standard curve. DPPH radical scavenging activity was expressed as mg AAE/g extract. The percentage of DPPH radical-scavenging activity was calculated using the following equation:

$$\text{DPPH } \% = \frac{\left(Abs_{\ control} - Abs_{\ sample}\right)}{Abs_{\ control}} \times 100$$

For control, all reagents were added except for the plant extract.

2.6 Statistical Analysis

The data were reported as means ± standard deviation (n=3). Values were statistically investigated using a one-way analysis of

variance (ANOVA test) by SPSS 25 software package for windows. Differences between groups were considered significant at a statistical probability (*P* values) less than 0.05 using the Duncan Multiple Range test [34].

3. RESULTS AND DISCUSSION

3.1 Antimicrobial Activity

Antimicrobial activities expressed as inhibition zone diameters, of GE, PE, ME, and OE against tested bacterial strains and yeast and mold strains are displayed in Tables 1 and 2, respectively. According to preliminary experiments, it was found that the antimicrobial effects of aqueous extracts were better than those obtained by ethanolic and methanolic extracts (data are not shown). The IZD values of selected aqueous extracts against bacterial strains ranged from 12 to 35 mm. The GE showed the highest IZD for *E. coli* and the lowest for *Salmonella spp.* Likewise, PE presented the highest IZD against *E. coli* and the lowest against *Salmonella spp*. On the contrary, ME had no antibacterial activity against tested strains. OE had the highest IZD against *Proteus* and the lowest against *Streptococcus pyogenes*. Regarding antifungal activity of selected extracts applied against some yeast and mold strains, GE showed the highest IZD against *A. niger* and the lowest against *R. glutinis*. PE presented the highest IZD against *A. niger* and the lowest against *S. octosporus*. In the contrast, results showed that ME had no antifungal activity. The OE had the highest IZD against *A. niger* and the lowest against *A. ochraceus*.

Table 1. Inhibition zone diameter of the plant aqueous extracts against some bacterial strains

Microbial strains		Inhibition zone diameter (mm)			
		GE	PE	ME	OE
G+	*Bacillus subtilis*	24±1.22	22±1.47	NA	15±1.86
	Staphylococcus aureus	23±1.83	23±2.29	NA	13±1.74
	Streptococcus pyogenes	20±2.28	22±2.48	NA	12±1.95
G-	*Salmonella spp.*	19±1.64	21±1.48	NA	NA
	Escherichia coli	35±2.36	29±1.36	NA	18±1.19
	Klebseilla pneumonia	20±1.73	25±1.97	NA	14±1.86
	Pseudomonas spp.	23±1.19	23±2.11	NA	14±1.74
	Proteus spp.	20±2.25	23±1.97	NA	19±1.53

**All results are expressed as the means ± standard deviation; n=3; Concentrations of the extract are in mg/mL; Diameter included 5 mm well diameter; GE, guava extract; PE, pomegranate extract; OE, olive extract; ME, moringa extract; NA: Not active; G+, gram-positive bacteria; G-, gram-negative bacteria.*

Table 2. Inhibition zone diameter of the plant aqueous extracts against some yeast and mold strains

Microbial strains		Inhibition zone diameter (mm)			
		GE	PE	ME	OE
Yeasts	*Aureobasidium pullulans*	16±1.93	23±2.11	NA	NA
	Schizosaccharomycesoctosporus	18±1.72	22±1.99	NA	NA
	Hansenulaanomala	NA	NA	NA	NA
	Pichia memberanifaciens	21±1.27	23±1.19	NA	NA
	Rhodotorulaglutinis	14±1.90	23±1.99	NA	NA
Molds	*Aspergillus niger*	42±1.29	32±1.99	NA	26±1.54
	Aspergillus parasiticus	30±1.76	NA	NA	NA
	Aspergillus ochraceus	NA	28±2.01	NA	25±1.88

**All results are expressed as the means ± standard deviation; n=3; Concentrations of the extract are in mg/mL; Diameter included 5 mm well diameter; GE, guava extract; PE, pomegranate extract; OE, olive extract; ME, moringa extract; NA: Not active*

Table 3. Minimum inhibition concentration (MIC) of the plant aqueous extracts against some bacterial strains

Microbial strains		MIC diameter (mm)									
		GE 300	GE 200	GE 100	GE 50	GE 25	PE300	PE 200	PE 100	PE 50	PE 25
G+	*Bacillus subtilis*	24±1.39	20±1.28	18±1.53	15±1.77	13±1.69	22±1.95	20±1.58	18±1.62	16±1.84	14±1.68
	Staphylococcus aureus	23±1.73	20±1.99	17±1.81	15±1.48	13±1.28	23±1.72	20±1.41	18±1.78	16±1.56	13±1.89
	Streptococcus pyogenes	20±1.83	19±1.51	17±1.28	15±1.43	12±1.22	22±1.61	19±1.78	15±1.84	13±1.91	10±1.58
G-	*Salmonella spp.*	19±1.19	18±1.68	15±1.72	12±1.65	9±1.87	21±1.91	20±1.82	18±1.98	15±1.46	12±1.54
	Escherichia coli	35±1.25	32±1.19	29±1.95	22±1.84	17±1.61	29±1.48	23±1.26	20±1.62	15±1.19	12±1.86
	Klebseilla pneumonia	20±1.38	19±1.23	16±1.68	14±1.73	12±1.57	25±1.61	23±1.59	20±1.76	16±1.85	11±1.76
	Pseudomonas spp.	23±1.70	21±1.66	19±1.95	17±1.91	14±1.30	23±1.55	21±1.46	18±1.61	15±1.92	13±1.88
	Proteus spp.	20±1.58	17±1.46	15±1.81	13±1.57	10±1.62	23±1.79	18±1.50	15±1.67	13±1.18	11±2.19

**All results are expressed as the means ± standard deviation; n=3; Concentrations of the extract are in mg/mL; Diameter included 5 mm well diameter; GE, guava extract; PE, pomegranate extract; NA: Not active; G+, gram-positive bacteria; G-, gram-negative bacteria.*

Table 4. Minimum inhibition concentration (MIC) of the plant aqueous extracts against some yeast and mold strains

Microbial strains		MIC diameter (mm)									
		GE 300	GE 200	GE 100	GE 50	GE 25	PE 300	PE 200	PE 100	PE 50	PE 25
Yeasts	*A. pullulans*	16±1.30	14±1.19	10±1.66	NA	NA	23±1.83	20±1.51	18±1.77	15±1.92	11±1.39
	S. octosporus	18±1.94	13±1.91	NA	NA	NA	22±.78	20±1.88	17±1.21	14±1.36	12±1.86
	P. memberanifaciens	21±1.82	16±1.68	NA	NA	NA	23±1.97	20±1.71	17±1.45	14±1.59	11±1.63
	R. glutinis	14±1.38	11±1.82	NA	NA	NA	23±1.02	16±1.94	14±2.06	12±1.98	10±1.09
Molds	*A. niger*	42±1.92	35±1.77	28±1.93	20±1.89	14±1.29	32±1.95	25±1.91	18±1.68	13±1.26	11±1.29
	A. parasiticus	30±1.29	22±1.56	20±1.89	15±1.92	11±1.22	NA	NA	NA	NA	NA
	A. ochraceus	NA	NA	NA	NA	NA	28±1.89	24±1.99	21±1.25	18±1.34	14±2.03

**All results are expressed as the means ± standard deviation; n=3; Concentrations of the extract are in mg/mL; Diameter included 5 mm well diameter; GE, guava extract; PE, pomegranate extract; NA: Not active.*

Antibacterial activity values in the current study were in agreement with those reported by Al-Zoreky and Agourram et al. [23, 35] who found a higher antibacterial activity of PE against *S. aureus, L. monocytogenes, Yersinia enterocolitica, E. coli, B. cereus, S. xylosus, Salmonella, P. aeruginosa,* and *P. fluorescens.* Moreover, Nair et al. [36] found that PE could be used as a natural alternative for synthetic fungicides. Eltayeb and Abdel-Rahim [37] reported that PE was efficient against many types of yeasts such as *C. valida*. Additionally, Fernandes et al. [38] observed that GE has antimicrobial activity against tested strains like *S. aureus, E. coli, P. aeruginosa,* and *C. glabrata.* Besides, Moringa leaf acetone extract showed a weak antibacterial efficacy against *E. cloacae, E. coli, S. aureus, P. vulgaris,* and *M. kristinae* at high concentrations [39]. Moreover, aqueous and acetone extracts of ME didn't offer any antifungal activity against tested strains as *P. notatum, C. albicans, A. niger,* and *A. flavus* [39]. Furthermore, Aliabadi et al. [40] showed good antibacterial activity of aqueous OE at a concentration of 0.6% (w/v) against *P. aeruginosa, E. coli, K. pneumonia,* and *S. aureus* and oppositely disagreed with Markin et al. [41] who found that *B. subtilis* was inhibited only at a concentration of 20% (w/v) using OE. The presence of bioactive compounds such as flavonoids, phenolics, and tannins in the plant extracts may inhibit the microbial growth of several bacterial strains such as *E. coli* [42].

Moreover, the differences in the effectiveness of tested plant extracts as antimicrobial agents might be due to the variations in the contents of their bioactive compounds such as polyphenolics [43]. These results encourage the use of aqueous PE and GE extracts in food preservation as they showed the best antimicrobial activity and the best economical usage as the cost of water used in the extraction is lower than the cost of ethanol or methanol.

3.2 Minimum Inhibitory Concentration (MIC)

The MIC is an important parameter that measures the resistance and sensitivity of microbes against specific compounds [44]. MIC is defined as the minimum concentration of compound applied to inhibit the growth of a specific microbial population under standardized conditions [45,46]. The MIC of GE and PE ranged from 25 to 300 mg/ml against bacterial strains, yeast, and mold strains as shown in Tables 3 and 4, respectively. The MIC of selected extracts versus bacterial strains showed efficacy with clear zone diameter ranged from 9 to 35 mm. GE showed the highest MIC against *E. coli* and the lowest for *Salmonella spp*. Whereas PE presented the highest MIC against *E. coli* and the lowest for *S. pyogenes.*

Regarding yeast and mold strains, GE showed the highest MIC against *A. niger* and the lowest against *A. pullulans*. PE presented the highest MIC for *A. niger* and the lowest for *R. glutinis*. The obtained results were in agreement with those reported by Fernandes et al. [38] who found that MIC of GE was 25 mg/ml against *E. coli,* and *S. aureus*. A similar trend was observed by Sanches et al. [47] who found that the MIC of GE against several microbes such as *S. aureus* was 25 mg/ml. Moreover, the MIC of PE was consistent with the findings observed by Al-Zoreky [23] who found that the MIC was 25 mg/ml. However, previously published studies showed some variations in the MIC values of plant extracts which ranged from 0.00062 to ≥0.250 g/ml [48,49].

Thaipong et al. and Zahidah et al. [50,51] proposed that the strong antimicrobial activity of GE could be due to the high content of phenolic compounds such as guavin B, quercetin, ferulic acid, caffeic acid, gallic acid, and ascorbic acid. Likewise, the antimicrobial activity of PE may occur due to the high content of ellagitannins (Punicalaginanomers a and b, pedunculagin, punicalin), phenolics (ellagic acid, proanthocyanidins, gallotannins, punicalagin, anthocyanins, hydroxybenzoic acids, hydrolyzable tannins, hydroxycinnamic acids, gallagic acid), and flavonoids [52,53].

3.3 Total Phenolic Content (TPC)

Phenolic compounds are important as antioxidant and antimicrobial agents that have many benefits for preventing many diseases and promoting human health [54]. The TPCs of GE, PE, OE, and ME in mg GAE/g extract are displayed in Table 5. The TPC values ranged from 303.68 to 324.08 mg GAE/g extract for GE, 256.48 to 271.28 mg GAE/g extract for PE, 90.48 to 120.48 mg GAE/g extract for OE, and 48.08 to 73.68 mg GAE/g extract for ME. ME aqueous extract showed the lowest TPC value, whereas GE methanolic extract showed the highest. Used solvent revealed a significant effect (p<0.05) on the TPC of the extracts. It could be noticed that GE had the highest TPC among all tested

extracts followed by PE and OE, whereas ME had the lowest value. The TPC values of GE were similar to those obtained by Zahidah et al. [50] with a value of 368.61 mg GAE/g extract. Likewise, these results were in agreement with the values reported by Agourram et al. [35] who found that the TPC of PE was 212.3 mg GAE/g for aqueous extract. Wissam et al. [55] reported that TPC of OE ranged from 26.8 to 102.1 mg GAE/g and these differences in the values could be due to the temperature, time, and solvents used. Also, Vongsak et al. [56] found that the TPC of ME was 47.6 and 73.6 mg of GAE/g for aqueous and methanolic extracts, respectively. Zahidah et al. [50] reported that the high TPC may be the main factor responsible for the strong effectiveness as antioxidant and antimicrobial. Also, polyphenols have many vital effects like preventing cardiovascular diseases, anti-aging, anti-diabetic, and anticancer [57]. Tachakittirungrod et al. [58] explained that the values of the phenolic compounds differ according to the extracts' properties as well as the polarity of the solvents during the extraction process.

3.4 Total Flavonoid Content

Flavonoids are natural compounds that have a polyphenolic structure; thus, they have antimicrobial and antioxidant activity and help prevent many diseases like Alzheimer's, cancer, atherosclerosis, etc. [59-61].

The TFC of various plant extracts are shown in Table 6. The TFC values of all investigated extracts ranged from 11.53 to 65.83 mg CE/g extract. The highest TFC value of GE was achieved using methanolic extract and the lowest value obtained using ethanolic one. On the contrary, the highest TFC value of PE was recorded with ethanolic extract, and the lowest value was recorded with the methanolic one. Also, the highest TFC value of OE was obtained using the ethanolic extract while the lowest value was obtained using the aqueous one. Additionally, the highest TFC value of ME was recorded using the methanolic extract while the lowest value was obtained using the aqueous one. The solvents used in the extraction process revealed a significant effect (p<0.05) on the TFC of the extracts.

Our findings were consistent with the findings of Fernandes et al. [38] who found that the TFC of GE was 22.58 mg CE/g. Also, Derakhshan et al. [62] showed that the TFC of PE ranged from 1.8 to 54 mg CE/g. Likewise, Şahin et al. [63] found that the TFC of OE was in the range of 32.34-36.34 mg CE/g according to the thermal treatment. Moreover, Vongsak et al. [56] reported that the TFC of ME was in the range of 9.1 - 67.1 mg CE/g with different solvents. Marinova et al. [64] stated that the TFC in vegetables and fruits were in the range of 15.0 – 190.0 mg CE/g.

The importance of TFC is mainly because of redox properties, that may have been responsible for the antimicrobial and antioxidant activity against several types of microbes and free radicals [65]. Moreover, the activity of TFC as an antioxidant agent has a close relationship with the hydroxyl groups in its structures, which responsible for scavenging the lipid peroxy-radicals, singlet oxygen, superoxide anion, and stabilization the free radicals [50]. Díaz-de-Cerio et al. [66] explained that the content of bioactive compounds can vary depending on the used solvent as they found that using a mixture of solvents resulted in a higher content of bioactive compounds compared to using an individual solvent probably due to the high solubility of compounds in the mixture solvents.

3.5 DPPH Radical Scavenging Activity

The DPPH is an important parameter to evaluate the antioxidant activity of an extract [67]. The IC_{50} represents the concentration of plant extract needed to scavenge 50% of the DPPH radicals. Table 7 shows the IC_{50} values of different examined plant extracts. The lower the IC_{50} value, the higher the antioxidant activity. From the displayed results, all plant extracts (obtained by different solvents) showed higher antioxidant activity. The IC_{50} of L-ascorbic acid, as the positive control, was 4.28 µg/ml. The highest IC_{50} value of GE was obtained using ethanolic extract and the lowest value obtained using the methanolic one. In contrast, the highest IC_{50} value of PE was recorded using methanolic extract, and the lowest value recorded using ethanolic one. Also, the highest IC_{50} values of OE and ME were achieved using aqueous extract, and the lowest values obtained using ethanolic and methanolic extracts, respectively. Additionally, the highest IC_{50} value of ME was recorded using aqueous extract, and the lowest value was obtained using methanolic one. The solvents used in the extraction process revealed a significant effect (p<0.05) on IC_{50}. The antioxidant activity findings were consistent with the TPC and TFC results (Tables 5 and 6), where a positive correlation between total phenolic content and antioxidant activity was

Table 5. Total phenolic content of the plant extracts using different solvents (mg GAE/g extract)

Solvents	GE	PE	OE	ME
water	318.48±8.08^{a}	266.08±4.99^{a,b}	90.48±9.12^{b}	48.08±1.02^{c}
ethanol 70 %	303.68±6.02^{b}	271.28±7^{a}	120.48±5.01^{a}	62.08±4.05^{b}
methanol 70 %	324.08±4.03^{a}	256.48±6.04^{b}	106.88±11.02^{a,b}	73.68±9.03^{a}

**All results are expressed as the means ± standard deviation; n=3. Means followed by different superscript letters within each column differ significantly (P < 0.05) according to Duncan's multiple range test (p < 0.05); GE, guava extract; PE, pomegranate extract; OE, olive extract; ME, moringa extract.*

Table 6. Total flavonoid contents of the plant extracts using different solvents (mg catechol/g extract)

Solvents	GE	PE	OE	ME
water	64.38±0.12^{b}	25.28±0.05^{b}	46.76±0.16^{c}	11.53±0.13^{c}
ethanol 70 %	59.15±0.83^{c}	26.88±0.27^{a}	65.28±0.18^{a}	19.38±0.18^{b}
methanol 70 %	65.85±0.15^{a}	25.06±0.1^{b}	58.81±0.11^{b}	21.76±0.16^{a}

**All results are expressed as the means ± standard deviation; n=3. Means followed by different superscript letters within each column differ significantly (P < 0.05) according to Duncan's multiple range test (p < 0.05); GE, guava extract; PE, pomegranate extract; OE, olive extract; ME, moringa extract.*

Table 7. The inhibition concentration values (IC_{50}) value with the plant extracts by different solvents

Solvents	GE	PE	OE	ME
water	22.81±0.1^{a}	22.62±0.11^{b}	71.4±0.05^{a}	92.04±0.04^{a}
ethanol 70 %	22.96±0.06^{a}	22.52±0.11^{b}	29.73±0.13^{c}	48.97±0.12^{b}
methanol 70 %	22.79±0.13^{a}	23.67±0.12^{a}	33.36±0.16^{b}	38.4±0.1^{c}

**All results are expressed as the means ± standard deviation; n=3; Concentrations of the extract are in µg/mL; Means followed by different superscript letters within each column differ significantly (P < 0.05) according to Duncan's multiple range test (p < 0.05); GE, guava extract; PE, pomegranate extract; OE, olive extract; ME, moringa extract*

observed. Generally, PE and GE showed higher antioxidant activity as compared to OE and ME. The antioxidant activity of the plant extracts in the current study was higher than those reported by Ayoola et al. [68] and Singh et al. [69] for GE and PE, respectively, but lower than those observed by Wissam et al. [55] and Vongsak et al. [56] for OE and ME, respectively. Dehkharghanian et al. [70] showed that the differences in solvents' polarity might affect the composition of the extracts and, therefore, their antioxidant activity. Moreover, the amount of plant secondary metabolites depends on several factors (such as genes, environment, storage, and handling), which may cause variations in the phenolic contents of different herbal sources [71]. Generally, it could be concluded that PE and GE could be applied in food processing and preservation as natural, safe, and effective preservatives.

4. CONCLUSION

The antimicrobial and antioxidant activity of guava, moringa, olive, and pomegranate herbal extracts against several strains of food spoilage and pathogenic micro-organisms (including Gram-positive, Gram-negative, yeasts, and molds) were investigated. Among all examined herbal extracts, it could be noticed that the guava and pomegranate extracts have the best antioxidant and antimicrobial effects, while olive extract showed a moderate effect, but Moringa exhibited a very weak effect against tested microbial strains. It could be suggested that the herbal extracts of guava and pomegranate could be used as novel, safe, and effective food preservatives instead of the chemical ones. Further studies on the application of these extracts in food preservation are required.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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