Egypt. J. Chem. **Vol. 67**, No. 9. pp. 297 - 308 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Bioactivity of Ethanolic Leaf Extract for Pomegranate, Guava and Green Garlic as Antioxidant and Antimicrobial Agents

Noha G. Sulieman[1], Mohamed, F. Khallaf [2], Manar T. Ibrahim [2], Amir A. Ibrahim [1], Gamal F. Mohamed[1] and Mohamed T. Fouad [3]

1 Food Technology Department, National Research Centre (NRC), 12622 Dokki, Egypt

2Food Science Department, Faculty of Agricultural, Ain Shams University, Cairo, Egypt

3 Dairy Department, National Research Centre (NRC), 12622 Dokki, Egypt

Abstract

The aim of study was to investigate the antioxidant and antibacterial activities of pomegranate leaves (PLE), guava leaves (GLE) and green garlic leave extracts (GGLE). All plants were extracted with 80% ethanol and used to determine total phenolic and flavonoid contents. The antioxidant capacity was determined by DPPH and ABTS radical scavenging assay. Polyphenol profile of investigated extracts was determined by HPLC assay. The antimicrobial activity was analyzed using the well diffusion method, where antimicrobial activities were determined by inhibition zone method. Results showed that the highest phenolic content (27.84GAE/g) in PLE, GLE (17.43 GAE/g) then GGLE (10.45 GAE/g), while total flavonoids were 70.65 mg CE/g, 59.76 mg CE/g and 5.56 mg CE/g for samples, respectively. The highest DPPH (92.00%) was recorded for PLE, GLE and GGLE, respectively. The ABTS was highest in PLE (94.54%), GLE then GGLE. All the plant extracts were effectively inhibited the growth of pathogenic strains used in the study. HPLC analyses revealed that, the most abundant phenolic component is gallic, cateachin and ferulic for PLE, GLE and GGLE, respectively. The results indicated that the tested Egyptian local plants may be potential sources of natural antioxidant and antimicrobial activities in food industries to replace synthetic ones.

***Keywords*:** Antibacterial activity; antioxidant property; bioactive compound; DPPH; green garlic leaves; pomegranate leaves; guava leaves.

1. Introduction

Novel strategies for intervention have been investigated as a means of fulfilling the growing customer demand for goods that are safe, convenient, and healthful with little processing, as well as a distribution system centered around products with sufficient shelf life, antimicrobial activity, and antioxidant activity [1].

Phytochemicals are often referred to non-nutritive compounds thought to be produced by plants as protection against such dangers like harmful ultraviolet radiation, pathogens and herbivorous predators. The consumption of a plant-based or phytochemical-rich diet has been associated with a reduced risk of chronic human illnesses such as certain types of cancers, inflammation, cardiovascular and neurodegenerative diseases [2-3]. Thus, when assessing phytochemicals' potential health benefits for humans, their chemistry and biology are crucial. Flavonoids, anthocyanins, and tannins are examples of phenolic compounds, which constitute the primary class of antioxidant phytochemicals with intriguing characteristics and significant biological and free radical scavenging potential [4]. Pomegranates are considered by practitioners of traditional medicine to possess inherent antiviral, antifungal, and antibacterial properties.

The main causes of food quality decline are lipid oxidation and microbial growth. Natural antibacterial and antioxidant chemicals are crucial not only for food preservation, but also for human consumption. Recently, research into natural plant products for the identification of active chemicals has emerged in order to locate naturally occurring antioxidant and antibacterial agents for use in food to replace synthetic additions that are carcinogenic [5].

In recent years, consumers prefer food of easy preparation, good quality, safe, natural and low

*Corresponding author e-mail: nohasameh259@gmail.com (Noha gouda)
Received date 25 December 2023; revised date 20 February 2024; accepted date 25 February 2024
DOI: 10.21608/EJCHEM.2024.258117.9069

processed but with longer shelf-life. With food preservation technologies, more long-lasting products are obtained, maintaining their initial nutritional and sensory characteristics [6-10].

Synthetic preservatives have been employed in food production for many years, with antimicrobial preservatives being the most commonly utilized, however studies now show that ingestion of chemical additives can lead to allergies, intoxications, cancer, and other degenerative disorders [8,11]. As a result, consumers depreciate them, motivating the need to seek out new options [12-13].

Food manufacturing generates a huge amount of waste in the form of skins, seeds, and leaves, the disposal that could be hazardous to the environment and expensive for the companies concerned. Many fruit residue are rich in phenolic compounds, and can be extracted and used as antioxidant and antimicrobial preservatives in the food industry. Pomegranate (*Punica granatum*) is a member of the *Punicaceae* family and is also known as "ponus" and "granatus" from the Latin terms. Pomegranate is an Iranian native that is currently grown in various Chinese areas. This section of pomegranate has a high concentration of polyphenols, including flavonoids (catechin and anthocyanins), hydrolysable tannins (ellagic acid, punicalagin, gallic acid, gallic penicillin, and pedunculagin), and overall antioxidant capacity. The extract of pomegranate has a high concentration of phenolic components (ellagitannins, flavonoids, punicalagin, ellagic acid, vitamin C, and minerals). Pomegranate is a nutrient-dense fruit that is high in beneficial compounds that are employed in medicine [14-15]. Consuming the phenolic phytochemical, that likewise functions as an antibacterial, is thought to have several favorable effects on the skin, eyes, and immune system. It is considered to be the most antioxidant substance after gallic acid [16].

The *Myrtaceae* family includes guava (Psidium guajava L.), which is a fruit that is widely consumed in tropical regions such as South America, Bangladesh, Pakistan, India, and Indonesia. Numerous phytochemicals, including quercetin, avicularin, apigenin, guaijaverin, kaempferol, hyperin, myricetin, gallic acid, epicatechin, catechin, chlorogenic acid, epigallocatechin gallate, and caffeic acid, have been linked to the health benefits of guava plant leaves. Studies have been conducted on the biological effects of guava leaf extracts, which include lipid-lowering, hepatoprotective, anticancer, antidiabetic, antioxidant, and antidiarrheal properties [17].

Plant byproducts, such as fruit or vegetable pomace, seeds, husk, bran, coat, skin, and leaves, are a valuable source of bioactive chemicals and may be used as functional food components, according to recent research [18]. Because GL extract contains a wide range of compounds, including flavonoids like quercetin and guaijaverin, which are well-known for their antimicrobial, antioxidant, and anti-inflammatory properties, as well as vitamins, rutin, naringenin, gallic acid, catechin, epicatechin, kaempferol, and isoflavonoids, many reports point to the benefits of using GL extract as a functional food ingredient [19]. Without affecting the rheological or sensory qualities of the meal, GL is a great source of active chemicals for functional ingredient adds. Owing to that GL is high in proteins, minerals, and vitamins, it is easier to use them as a direct source of nutrition. Many physiological and metabolic processes in the human body have been shown to be improved and stabilized by the presence of various bioactive chemical substances in GLs. Garlic (Allium sativum) is a vegetable belongs to the Allium, a class of bulb-shaped plants belongs to family *Liliaceae* [20]. It is a significant spice crop in the nation and has therapeutic qualities in addition to being a herb used for food and spice. Its origins are on the Mediterranean coast and western Asia. Natural antioxidants found in garlic have the ability to lower lipid peroxides, reactive oxygen species, and low-density lipoprotein [21]. Due to the presence of diallyl sulfides, other sulfur compounds, and allicin, garlic exhibits a wide range of physiological effects and activities in different metabolic pathways [22]. Phytochemicals that are present in garlic include tannins, alkaloids, flavonoids, and phenolic compounds [23]. Garlic extract prolongation results in the formation of antioxidant phytochemicals, such as distinct lipid-soluble and water-soluble organosulfur components and flavonoids, which fend off oxidative damage and are crucial in the prevention of aging and various diseases, such as cancer, neurodegenerative, and inflammatory conditions [24].

Garlic is valued for its bactericidal, antiparasitic, antiviral and antifungal properties. Compounds present in garlic have a beneficial effect on the human organism, mainly by the reduction in oxidative stress, which strengthens the immune system response to various diseases. Due to its composition, especially with the content of allicin and other sulphur compounds, it can be referred as a natural antibiotic [25-26].

This study was established to independently assess and evaluate the three aforementioned extracts; i.e. pomegranate (PLE), guava leaves (GLE), and green garlic leaf extract (GGLE) due to the growing interest in the usage of natural antioxidants and antimicrobials. There haven't been any reports up to this point that focus on the chemical structure and its biological activity. The goal of the current study was to assess the antibacterial, antioxidant, and phenolic compound properties of PLE, GLE, and GGLE. These extracts were selected due to their noteworthy antioxidant qualities and positive impacts on human health.

2. Materials and methods

2.1. Chemicals and reagents

Standard of phenols, butylated hydroxyl toluene (BHT), DPPH (2, 2-Diphenyl-1-picrylhydrazyl), ABTS (2, 22 -azino-bis (3ethylbenzothiazoline-6-sulphonic acid), potassium persulphate acid, Folin-Ciocalteu reagent and aluminium chloride and were obtained from Sigma Chemical Co., Germany. Methanol, ethanol, DMSO, ampicillin were purchased from El-Nasr Co., Cairo, Egypt. All used solvents and chemicals were of analytical grade.

2.2. Bacterial strains and culture preparation

Seven strains of foodborne bacteria were obtained from microbiology lab, National Research Centre, Dokki, Egypt including: four strains of Gram-negative bacteria (E. coli 0157: H7 ATCC 6933, Y. enterocolitica ATCC 9610, Salmonella typhimurium (ATCC® 14028™) and P. aeruginosa ATCC 9027) and three strains of Gram-positive bacteria (B. cereus ATCC 33018, S. aureus ATCC 20231 and L. monocytogenes ATCC 7944). Cultures were maintained in their appropriate agar slants at 4 °C and used as stock cultures. Mueller–Hinton broth (MHB) and Mueller–Hinton agar (MHA) were used in the present study.

2.3. Plants source

Fine-quality fresh green pomegranate leaves (*Punica granatum* L.), guava leaves (*Psidium guajava* L.) and fresh green garlic leaves (*Allium sativum*) were collected during March 2022 from the west farm of Faculty of Agriculture, Cairo University, Giza, Egypt. All plant samples (Fig. 1) were kept in polyethylene bags at 4±1°C until extraction.

2.4. Extraction treatment

Pomegranate leaves (PL); guava leaves (GL) and fresh green garlic leaves (GGL) were cleaned from extraneous matter and properly washed then dried in hot air-oven for 24 h at 40°C. The dried leaves were ground with grinder into a powdery form and separately kept in a closed dark glass bottle and stored at 4°C until further analysis.

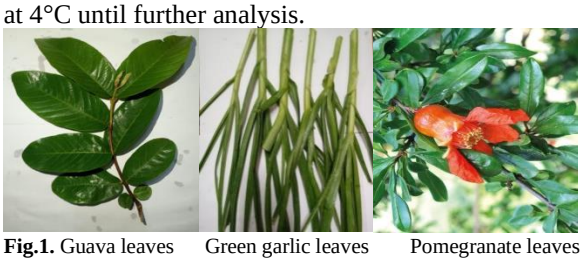

Fig.1. Guava leaves Green garlic leaves Pomegranate leaves

According to the extraction method of [27], hundred gram of PL, GL and GGL powder were extracted overnight with 1000 ml of 80 % methanol solution in a shaking incubator (100 rpm) at room temperature. Then the extracts were centrifuged at 3500 rpm for 15 min. The supernatants were filtered through a Whatman No.1 filter paper, then extracted solution were concentrated to dryness in a rotary evaporator (Eyela, Rikakikai, Tokyo, Japan) at 40°C and dried in oven overnight at 40°C to form powder, which was separately stored at -20˚C until analysis. The extraction yield of each sample was calculated.

2.5. Proximate composition

Chemical composition such as carbohydrate content, moisture, ash, crude lipid, and total nitrogen (as assessed by the Micro-Kjeldahl method) were determined according to the methods described in the AOAC [28].

2.6. Determination of total phenolic and flavonoid contents

The total phenolic and flavonoid contents of the PLE, GLE and GGLE were spectrophotometrically quantified by (Thermo Fisher Scientific, Genesys, Madison, USA) measurement of the absorbance according to the Folin Ciocalteu and using aluminum chloride methods, respectively as reported by [29].

2.7. Antioxidant activity (DPPH and ABTS) free radical assays

The antioxidant activity of PLE, GLE and GGLE tested using DPPH free radical scavenging was evaluated [30]. Total antioxidant activity was measured in vitro with ABTS assay [31]. Each was replicated three times.

2.8. HPLC analysis of phenolic compounds

The high performance liquid chromatography (HPLC) analysis was carried out according to [32]. The separation and determination were performed on Agilent 1260 series -C18 column (4.6 mm × 250 mm i.d., 5 μm). The column was eluted by water (solvent A) and 0.02% tri-floro-acetic acid in acetonitrile (solvent B) at a flow rate of 1 ml/min. The obtained peaks were simultaneously monitored at 280 nm.

2.9. Antibacterial assay

The antibacterial spectrum activity was performed by the agar-well diffusion method as described by [33]. Briefly, 1 mL culture of the activated indicator strain (105 cells /mL) was inoculated into 20 mL of Mueller-Hinton agar (Becton Dickinson, USA) and poured in Petri dishes. After solidification of the agar, wells of 5 mm in diameter were cut from the agar with a sterile borer and 50μL (0.02mg/ml) of ethanolic extract of PL, GL and GGL were delivered in each well. After holding the plates at room temperature for 2 h to allow diffusion of the extract into the agar, the plates were incubated at 37 °C for 24 h. After that, inhibition of the bacterial lawn and the diameters of the inhibition zones were measured. A microbial

susceptibility control test was performed with discs having Penicillin G (10 units) as positive control and methanol as negative control. The tests were performed in technical triplicate for each bacterial strain.

2.10. Statistical analysis

Results were expressed as means and standard deviation from triplicate determinations. Analysis of variance (ANOVA) was performed for comparing. Significant differences were defined as $P<0.05$; according to [34].

3. Results and discussion

3.1. Proximate composition

The proximate composition of fresh PL, GL and GGL was given in Table (1). GGL provided the highest total protein content (14.17%), followed by PL (6.50%) and GL (4.36%). The moisture content of GL, GGL and PL was found to be 50.47, 18.64 and 13.12%, respectively. GL had remarkably higher fat (5.84%), compared to GGL (2.74%) and PL (1.14%). Ash content was 7.09, 5.27 and 3.35 for GGL, GL and PL, respectively. The carbohydrate content was the highest in PL (64.67%), moderate for GGL (55.18%) and GL had the lowest one (22.72%). Fiber content was 11.34, 11.22 and 2.18 in GL, PL and GGL, respectively. The proximate compositions are within the normal limits and agree with those found [35].

Pomegranate leaves composition varies in relation to the type of cultivation, growth region, climate, maturity and cultural practices [36], observing that the organoleptic characteristics and the benefits offered by its consumption are due to the presence of polyphenolic compounds such as anthocyanins responsible for the reddish colour, as well as tannins which give astringent taste. Organic acids, citric and malic acids responsible for the acidified taste, while ellagitannins and a lesser extent anthocyanins confer properties as antioxidants [37].

As in pomegranate peel, tannins are also found in leaves, as well as some glycosides such as apigenin which is a flavone uses as anxiolytic [38] and naringin which has been studied for its effect as an anticancer. Other present polyphenols are glycosylated flavones (luteolines), punicalinas, punicalaginas, corilagina and punicafolina [39]. The proximate composition of Guava leaves was in line with [40-41, 17]. The proximate results are also close to those reported for green garlic leaves by [42-45]. However, slight differences in proximate composition may be due to the differences in the season, geographical location, species and variety.

Table (1): Proximate composition and extract yield of PL, GL and GGL on dry wt. basis

Item (%)	PL	GL	GGL
Moisture	13.12^{b} ±0.16	50.47^{b} ±0.27	18.64^{b} ±0.14
Protein	6.50^{a} ±0.15	4.36^{b} ±0.14	14.17^{a}± 0.41
Fat	1.14^{a} ±0.12	5.84^{b} ±0.16	2.74^{c} ± 0.36
Ash	3.35^{b} ±0.23	5.27^{a} ±0.18	7.09^{a} ± 0.19
Fiber	11.22^{a}±16	11.34^{a} ±0.12	2.18^{c}±0.15
Total carbohydrates	64.67^{a} ±0.17	22.72^{b} ±0.12	55.18^{a}±0.17
Extract yield (gm/100gm)	26.00^{b} ±0.13	20.43^{a} ±0.17	14.22^{b}±0.21

There is no significant difference ($P>0.05$) between the row having the same superscripts in the same.
* Calculated by difference.

3.2. Extraction yield

Ethanolic extraction yield was given in Table (1). Pomegranate leaves provided higher yield (26.00%) than guava leaves (20.43%) and Green Garlic leaves (14.22%). The extraction yield depends on the chemical nature of the sample. The variation in the yields might be ascribed to the different availability of extractable component, resulting from the different chemical composition of plants. Similar results were achieved for pomegranate leaves [46-47]; guava leaves [48-49] and GGL [44].

3.3. Total phenolic content (TPC)

Phenolic compounds are widely distributed in plants and have gained much attention, due to their antioxidant activities and free radical scavenging capacities, which potentially have beneficial implications for health [50]. Total phenolic of the extracts (Table 2) showed PLE very rich with polyphenols; i.e. 27.84, 17.43 and 10.45 (mg gallic/g dw) for PLE followed by GLE then GGLE, respectively which was in agree with [57-59].

Table (2): Total phenolics and total flavonoids content of PLE, GLE and GGLE

Item	Total phenolic (mg GAE/g)	Total flavoniods (mg CE/g)
PLE	27.84	70.65
GLE	17.43	59.76
GGLE	10.45	5.56

It was also found that, pomegranate is a drought-tolerant and salt-tolerant crop. The PL contain high levels of phytochemicals have many health benefits [51]. The amounts of phenols determined in PLE in the present study are in good agreement with [15]; [52-53] as described in the HPLC assay (Table 3). Pomegranate leaves contains many bioactive compounds such as alkaloids, ellagic acid, punicalagin

among other ellagitannins, anthocyanins, flavonoids, tannins and other phytochemicals that may play an essential role in human health and the prevention and treatment of many illnesses. Different varieties normally have different amount and types of bioactive compounds [54-55]. Thus, the bioactive profile is influenced by the cultivar, growing region, climate, maturity, cultivation practice, and storage conditions [56].

3.4. Total flavonoids content (TFC)

The selection of extraction conditions is very important [62]. All amounts were reported in Table (2) and values showed a great variation in various studied plants. The pomegranate leaves extract exhibited the highest level of TFC (70.65mg CE/g) compared to GLE (59.76 mg CE/g) and GGLE (5.56 mg CE/g). These results are in agreement with the previous ones [15, 52-53]. The results strongly show that total flavonoids content from GLE are similar to those reported by [57-59]. The amount of flavonoids determined in GGLE was agreement with [60-61].

3.5. Antioxidant activity

3.5.1. DPPH assay

Scavenging activity was measured by the decrease in absorbance as the DPPH radical received an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule [63]. The scavenging ability of the PLE, GLE and GGLE samples on DPPH free radical was shown in Fig. (2). The results showed a dose dependent scavenging power. Especially, the scavenging ability of PLE increased from 56.61 to 92.00 %, at 200 µg/mL indicating that it has generally better scavenging ability, even than BHT (88.45%). DPPH of GLE increased from 43.00 to 79.71%, while the value increased from 4.61 to 14.12 % in GGLE, at 400µg/mL.

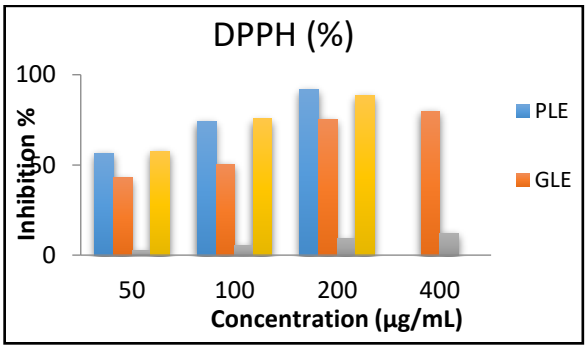

Fig.2. Effect of PLE, GLE, GGLE and BHT on DPPH inhibition

These results were found [64-66]. Results are in contrast with the study of [57-59] for DPPH of GLE. The green garlic leaves showed a higher content of total polyphenols and a higher antioxidant activity than cloves [45]. Usually, higher total phenol and flavonoids contents lead to better DPPH scavenging activity [62]. As known, polyphenols have a metal chelating potential and their redox properties can be justified by their chemical structure [67]. For this reason, the high polyphenolic content in PLE, GLE and GGLE may explain the high antioxidant activity.

3.5.2. ABTS - radical scavenging assay

The principal objective of this test is to measure the capacity of different substances to scavenge the ABTS + radical cation. The results showed a dose dependent scavenging ABTS + radical cation (Fig. 3). Scavenging ability of PLE was 94.54 %, which was more than BHT (91.00%) at 200 µg/mL indicating it has generally better scavenging ability. ABTS of GLE was 82.10%, while in GGLE was 14.12 % at 400µg/mL. It is obvious that tested samples are effective to provide their capacity to scavenge the ABTS+ radical cation. The results PLE are within the previous studies [15, 64-66] who reported that pomegranate leave extract is a rich source of natural antioxidants.

Results exhibited that GLE had higher scavenging activity, while GGLE was the weakest as same as shown in the DPPH-radical scavenging assay (Fig. 3). This is confirms that GLE was the best extract after PLE to scavenge free radicals among the tested extracts. Results are in contrast with [57-59]. The obtained results regarding the ABTS inhibition of GGLE are similar to that reported [60-61].

The positive and significant correlation between TPC and ABTS antioxidant activity strengthens the results observed in the DPPH scavenging method used in this study. This investigation confirms the hypothesis that an increase in total phenolic compounds will increase the antioxidant activity of extracts.

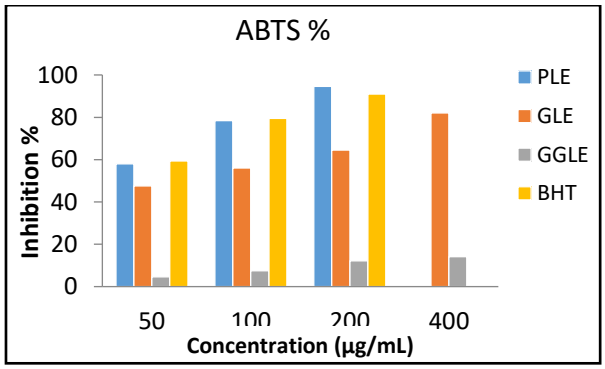

Fig.3. Effect of PLE, GLE, GGLE and BHT on ABTS inhibition

3.5.3. Identification of phenolic compounds

High performance liquid chromatography (HPLC) analysis was used to identify 13 phenolic compounds

in PLE, 16 phenolic compounds in GLE and 14 phenolic compounds in GGLE (Table 3 and Figs. 4-6).

Results illustrated in Fig. (4). identified the components of PLE (µg/g) were gallic(3743.09), protocatechuic (31.99), p-hydroxybenzoic (81.82), caffeic (479.65), vanillic (209.91), ferulic (66.89), sinapic (5.93), p-coumaric (8.80), apigenin-7-glucoside (23.70), rosmarinic (2173.95), cinnamic (31.80), qurecetin (10.58) and apigenin (14.48).

The HPLC chromatogram (Figure 4) also reveals the predominant phenolic compound was gallic acid (3743.09µg/g), while sinapic (5.93µg/g) was the lowest. Such results are in close agreement with those reported by [15, 64-66].

The assayed components of GLE (µg/g) were gallic acid (2576.49), protocatechuic (83.32), p-hydroxybenzoic (39.12), cateachin (889.72), caffeic (35.26), vanillic (0.91), ferulic (82.80), sinapic (28.43), rutin (28.63) p-coumaric (77.65), apigenin-7-glucoside (77.78), rosmarinic (8.74), cinnamic (8.74), qurecetin (297.29), kaempferol (12.94) and chrysin (0.41%).

The HPLC chromatogram as shown in Fig. (5). also reveals the dominant phenolic compound in GLE as gallic acid (2576.49), while the peak chrysin acid was very low (0.41%) [60-61].

The components assayed for GGLE (µg/g), were as follows: gallic acid (37.78), protocatechuic (12.02), p-hydroxybenzoic (4.59), caffeic (1.06), syringic (0.84), vanillic (6.84), ferulic (90.39), sinapic (12.69), apigenin-7-glucoside (50.85), rosmarinic (20.16), cinnamic (4.24), qurecetin (1.36), apigenin (3.18) and kaempferol (4.39).

Fig. (6). showed that main phenolic compound in GGLE was ferulic acid (90.39), while syringic was very low (0.84) [44-45].

Table (3): Phenolic compounds of PL, GL and GGL extracts obtained by HPLC (µg/g)

Compound	PLE	GLE	GGLE
Gallic	3743.09	2576.49	37.78
Protocatechuic	31.99	83.32	12.02
p-hydroxybenzoic	81.82	39.12	4.59
Gentisic	ND	ND	ND
Cateachin	ND	889.72	ND
Chlorogenic	ND	ND	ND
Caffeic	479.65	35.26	1.06
Syringic	ND	ND	0.84
Vanillic	209.91	0.91	6.84
Ferulic	66.89	82.80	90.39
Sinapic	5.93	28.43	12.69
Rutin	ND	28.63	ND
p-coumaric	8.80	77.65	ND
Apigenin-7-glucoside	23.70	77.78	50.85
Rosmarinic	2173.95	23.38	20.16
Cinnamic	31.80	8.74	4.24
Qurecetin	10.58	297.29	1.36
Apigenin	14.48	ND	3.18
Kaempferol	ND	12.94	4.39
Chrysin	ND	0.41	ND

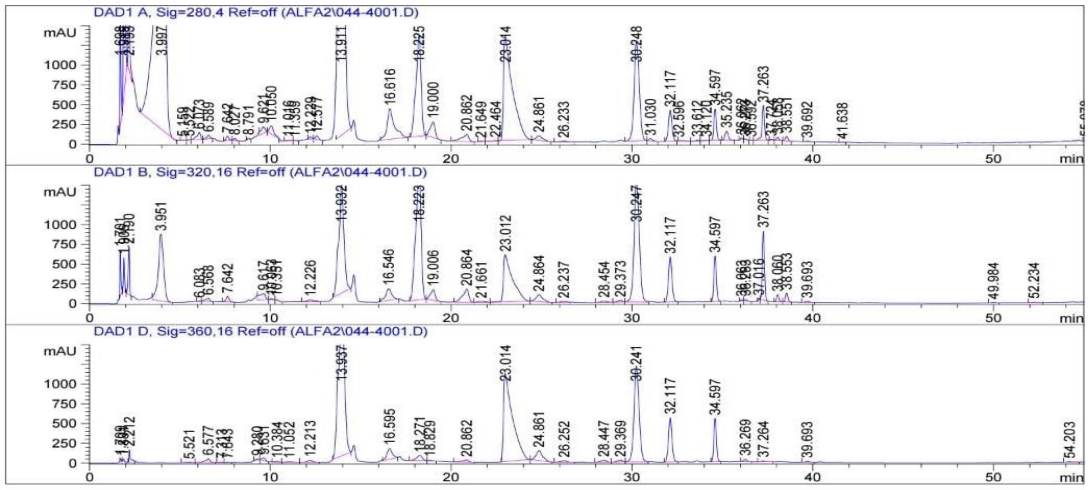

Fig. (4): HPLC analysis of PLE.

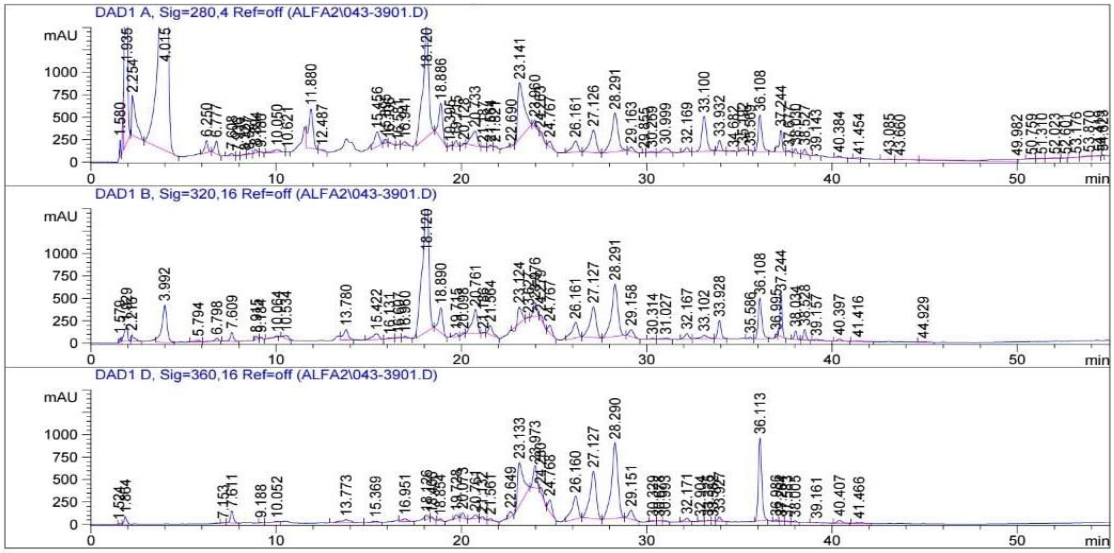

Fig. (5): HPLC analysis of GLE.

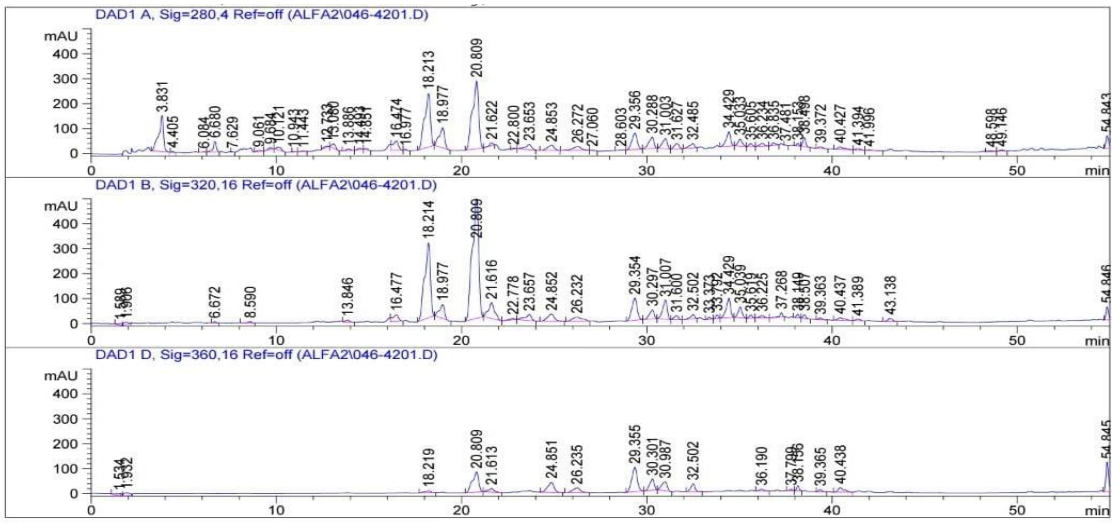

Fig. (6): HPLC analysis of GGLE

3.6. Antibacterial activity

Antibacterial activity of ethanolic extract of PL, GL and GGL was tested by agar diffusion method against seven pathogenic bacteria. All the plant extracts were inhibited the growth of such pathogenic strains (Table 3).

The obtained results revealed that PLE recorded a large inhibition zones against tested bacteria ranged from 17 - 20 mm [68] studied pomegeranate leaf extracts had the capacity to inhibit bacterial growth of the clinical strains screened (0.03125 to 10 mg/mL). The MIC values obtained for both Gram-positive and Gram-negative bacteria were similar. *Klebsiella pneumoniae* presented the lowest MIC values (0.6 mg/mL), and as such, revealed the highest susceptibility to the three tested pomegranate leaves' extracts. While, GLE indicated inhibition zones against tested bacteria ranged between 13-18 mm [69], but GGLE showed inhibition zones within 11-17 mm. The antibacterial activity of garlic extract were against some gram positive (*S. aureus, S. epidermidis, and Strep. pyogenes*) and gram negative (*Ps. aeruginosa*) bacteria, correlating with the report of [70] that garlic has antibacterial activity against a wide range of bacteria. PLE showed higher inhibition zone on all pathogenic bacteria compared to GLE, GGLE and penicillin.

Table (4): Antibacterial activity (mm) of PL, GL and GGL extract of the inhibition zones

Microorganism	Gram reaction	Inhibition zone diameter (mm)			
		Penicillin	PLE	GLE	GGLE
Staphylococcus aureus	(+)	15	20	18	12
Bacillus cereus	(+)	17	18	15	14
Listeria monocytogenes	(+)	16	17	13	12
Escherichia coli	(-)	18	19	15	11
Salmonella Typhimurium	(-)	17	19	16	13
Pseudomonas aeruginosa	(-)	16	17	14	13
Yersinia enterocolitica	(-)	15	19	15	17

The difference of antimicrobial effects of investigated plant parts may be due to the phytochemical properties and various contents. [71] reported that phenolic acids such as protocatechuic, vanillic, ferulic and caffeic acids could be used as antimicrobial agents because of presence of carboxylic, hydroxyl groups in para and ortho position of benzene ring and also to a methoxyl (OCH3) group in the meta position.

4. Conclusion

This work emphasizes the significant difference in chemical composition between PLE, GLE and GGLE and their significant influence on biological activities. The concentrations of phenolic and flavonoids were determined to be higher in PLE than GGLE; all extracts are endowed with potent antioxidant and antimicrobial activities, especially PLE. Antioxidant activity (DPPH and ABTS assays) was the highest in PLE, followed by GLE then GGLE. HPLC analysis indicated 13 phenolic compounds in higher concentrations in the chromatographic profile being gallic acid in a highest level. GLE had also chromatographic profile gallic acid as dominant one. Regarding to GGLE, the dominant phenolic compound was ferulic. All extracts inhibited the growth of pathogenic bacteria under investigation. Such findings assured that Egyptian plants (PLE, GLE and GGLE) are rich sources of bioactive compounds and can be successfully used in food industry. For their contents of natural polyphenolic compounds that possess antioxidant and antimicrobial properties.

5. Conflicts of interest

"There are no conflicts to declare".

6. Acknowledgments

The authors gratefully thank the staff from the Department of Food Science, Faculty of Agriculture, Ain Shams University and the National Research Centre (NRC), especially the Department of Food Technology, Department of Food Industry and Nutrition Research.

7. References

[1] Elhadef, K.; Smaoui, S.; Hlima, H.B.; Ennouri, K.; Fourati, M.; Mtibaa, A.C.; Ennouri, M. and Mellouli, L. (2020). Effects of ephedra alata extract on the quality of minced beef meat during refrigerated storage: A chemometric approach. Meat Science, 170:1-18. https://doi.org/10.1016/j.meatsci.2020.108246.

[2] Kong, J.M.; Chia, L.S.; Goh, N.K.; Chia, T.F. and Brouillard, R. (2003). Analysis and biological activities of anthocyanins. Phytochemistry, 64(5): 923-933. https://doi.org/10.1016/S0031-9422(03)00438-2.

[3] Beretta, G.; Rossoni, G.; Alfredo, S.N. and Maffei, F.R. (2009). Anti-ischemic activity and endothelium-dependent vasorelaxant effect of hydrolysable tannins from the leaves of Rhus coriaria (Sumac) in isolated rabbit heart and thoracic aorta. Planta Med., 75(14):1482-1488. https://www.researchgate.net/publication/26312678.

[4] Elfalleh, W.; Tlili, N.; Nasri, N.; Yahia, Y.; Hannachi, H.; Chaira, N.; Ying, M. and Ferchichi, A. (2011). Antioxidant capacities of phenolic compounds and tocopherols from tunisian pomegranate (*Punica granatum*) fruits. J. Food Sci., 76(5):C707-C713. https://doi.org/10.1111/j.1750-3841.2011.02179.x.

[5] Odeja, O.; Ogwuche, C.E.; Elemike, E.E. and Obi, G. (2016). Phytochemical screening, antioxidant and antimicrobial activities of Acalypha ciliata plant. Clinical Phytoscience, 2(1):1- 12. DOI: 10.1186/s40816-016-0027-2.

[6] Zhou, G.H.; Xu, X.L. and Liu, Y. (2010). Preservation technologies for fresh meat - a review. Meat Sci., 86(1): 119-28. https://doi.org/10.1016/j.meatsci.2010.04.033.

[7] Dave, D. and Ghaly, A.E. (2011). Meat spoilage mechanisms and preservation techniques: A Critical Review, Am. J. Agri. & Biol. Sci., 6(4):486-510.

[8] Sauceda, E.N.R. (2011). Uso de agentes antimicrobianos naturales en la conservación de frutas y hortalizas. Ra Ximhai., 7(1):153- 70. https://WWW.redalyc.org/articulo.oa?id=46116742014.

[9] Sivakumar, D. and Bautista-Baños, S. (2014). A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. Crop Protection, 64: 27-37. https://doi.org/10.1016/j.cropro.2014.05.012.

[10] Hussein, H.A.; Fouad, M.T.; El-Razik, K.A.A.; El-Maaty, A.M.A.; Ambrosio, C.D; Scaloni, A. and Gomaa, A.M. (2020). Study on prevalence and bacterial etiology of mastitis, and effects of subclinical mastitis and stage of lactation on SCC in dairy goats in Egypt. Tropical Animal Health and Production,52:3091–3097. https://doi.org/10.1007/s11250-020-02331-5.

[11] El-Shenawy, M.A.; Zaghloul, R.A.; Abbass, I.H.; Esmail, A.I. and Fouad, M.T. (2016). Incidence of some epidemiologically relevant food-borne pathogens in street-vended sandwiches. Journal of Pharmaceutical, Biological and Chemical Sciences, 7 (2):468-474. https://www.researchgate.net/publication/301554689.

[12] Hayek, S.A.; Gyawali, R. and Ibrahim, S.A. (2013). Antimicrobial natural products. Microbial pathogens and strategies for combating them: science, technology and education (A. Méndez-Vilas, Ed.), 910-921.

[13] Gyawali, R. and Ibrahim, S.A. (2014). Natural products as antimicrobial agents. Food Control, 46: 412-429. https://doi.org/10.1016/j.foodcont.2014.05.047.

[14] Miguel, M.G.; Neves, M.A. and Antunes, M.D. (2010). Pomegranate (*Punica granatum* L.): a medicinal plant with myriad biological properties - A short review. Journal of Medicinal Plants Research, 4(25):2836–2847. https://www.researchgate.net/publication/228966344.

[15] Elfalleh, W.; Hannachi1, H.; Tlili, N.; Yahia, Y.; Nasri, N. and Ferchichi, A. (2012). Total phenolic contents and antioxidant activities of pomegranate peel, seed, leaf and flower. Journal of Medicinal Plants Research, 6: 4724-4730. http://www.academicjournals.org/JMPR.

[16] Martínez, L.; Ros, G. and Nieto, G. (2018). Hydroxytyrosol: Health benefits and use as functional ingredient in meat. Medicines, 5(1): 1-13. https://doi.org/10.3390/medicines5010013.

[17] Kumar, M.; Tomar, M.; Amarowicz, R.; Saurabh, V.; Nair, M.S.; Maheshwari, C.; Sasi, M.; Prajapati, U.; Hasan, M.; Singh, S.; Changan, S.; Prajapat, R. K.; Berwal, M. K. and Satankar, V. (2021). Guava (*Psidium guajava* L.) leaves: Nutritional composition, phytochemical profile, and health-promoting bioactivities. Foods, 10(752): 1-20. https://doi.org/10.3390/foods10040752.

[18] Punia, S.; Sandhu, K.S.; Grasso, S.; Purewal, S.S.; Kaur, M.; Siroha, A.K.; Kumar, K.; Kumar, V. and Kumar, M. (2020). Aspergillus oryzae fermented rice bran: A byproduct with enhanced bioactive compounds and antioxidant potential. Foods, 10(1):70. https://doi.org/10.3390/foods10010070.

[19] Shaheena, S.; Chintagunta, A.D.; Dirisala, V.R. and Kumar, N.S.S. (2019). Extraction of bioactive compounds from Psidium guajava and their application in dentistry. AMB Expr., 9:208. https://doi.org/10.1186/s13568-019-0935-x.

[20] Block, E. (1985). The chemistry of garlic and onions, Sci Am., 252(3):114–119. https://www.researchgate.net/publication/19185535.

[21] Saravanan, G. and Prakash, J. (2004). Effect of garlic (*Allium sativum*) on lipid peroxidation in

experimental myocardial infarction in rats. J. Ethnopharmacol. 94(1):155–158. https://doi.org/10.1016/j.jep.2004.04.029.

[22] Amagase, H.; Petesch, B.L.; Matsuura, H.; Kasuga, S. and Itakura, Y. (2018). Recent advances on the nutritional effects associated with the use of garlic as a supplement: intake of garlic and its bioactive components. J. Nutr., 131(3):955S–962S. https://academic.oup.com/jn/article-abstract/131/3/955S/4687054.

[23] Olusanmi, A.T. and Amadi, J.E. (2010). Studies on the anti-microbial properties and phytochemical screening of garlic (*Allium sativum*) extracts. Ethnobot Leaflets, 14: 537-545.

[24] Chanda, S.; Kushwaha, S. and Tiwari Raj, K. (2011). Garlic as food, spice and medicine: A perspective. Journal of Pharmacy Research, 4(6):1857-1860. https://www.researchgate.net/publication/281521870.

[25] Ruhee, R.T.; Roberts, L.A.; Ma, S.; Suzuki, K. (2020). Organosulfur compounds: A review of their Anti-inflammatory effects in human health. Front. Nutr., 7: 64.
https://doi.org/10.3389/fnut.2020.00064.

[26] Bastaki, S.M.A.; Ojha, S.; Kalasz, H. and Adeghate, E. (2021). Chemical constituents and medicinal properties of Allium species. Mol. Cell Biochem., 476: 4301–4321.
https://doi.org/10.1007/s11010-021-04213-2.

[27] El Anany, A. M. (2015). Nutritional composition, anti-nutritional factors, bioactive compounds and antioxidant activity of guava seeds (*Psidium*, *Mytaceae*) as affected by roasting processes. J. Food Sci. Technol., 52(4):2175-2183.
DOI: 10.1007/s13197-013-1242-1.

[28] AOAC. (1995). Official Methods of Analysis. 16th Edn., Association of Official Analytical Chemists Inc., Arlington, Virginia, USA.

[29] Bakari, S.; Hajlaoul, H.H.; Daoud, A.; Mighri, H.; Ross-garcia, J.M.; Gharsallah, N. and Kadri, A. (2017). Phytochemicals, antioxidant and antimicrobial potentials and LC-MS analysis of hydroalcoholic extracts of leaves and flowers of Erodium glaucophyllum collected from Tunisian Sahara. Food Science and Technology, 38(2):310-317.
DOI: https://doi.org/10.1590/fst.04517.

[30] Cheurfa, M. and Allem, R. (2016). Evaluation of antioxidant activity of different extracts of Aloysia triphylla leaves (L'Herit.) from Algeria in vitro. Phytothérapie,14(3):181-197. http://dx.doi.org/10.1007/s10298-015-0969-4.

[31] Ben-Nejma, A.; Znati, M.; Nguir, A.; Daich, A.; Othman, M.; Lawson, A.M. and Ben Jannet, H. (2017). Phytochemical and biological studies of Tripplex inflata f. Muell.: Isolation of secondary bioactive metabolites. The Journal of Pharmacy and Pharmacology,69(8):1064-1074. http://dx.doi.org/10.1111/jphp.12735.PMid:28464303

[32] Kim, K.H.; Tsao, R.; Yang, R. and Cui, S.W. (2006). Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. Food Chem., 95: 466–473.
https://doi.org/10.1016/j.foodchem.2005.01.032.

[33] Ghazya, O.A., Fouad, M.T.; Saleha, H.H.; Kholif, A.E. and Morsy, T.A. (2021). Ultrasound-assisted preparation of anise extract nanoemulsion and its. Food Chemistry, 341:1-7.
https://doi.org/10.1016/j.foodchem.2020.128259.

[34] Rao, V. N. M. and Blane, K. (1985). Statistical Programs (PC-STAT) for Microcomputers. Version IA, Department of Food Science and Technology, The University of Georgia, Athens, GA., USA.

[35] Sreedevi, P.; Vijayalakshmi, K. and Venkateswari, R. (2017). Phytochemical evaluation of punica granatum l. leaf extract. Int. J. Curr. Pharm. Res., 9(4):14-18.
http://creativecommons.org/licenses/by/4.0/.

[36] Heshi, A.; Garande, V.; Wagh, A. and Katore, H. (2001). Effect of preharvest sprays of chemicals on the quality of pomegranate fruit (*Punica granatum* L.). Indian Journals, 21(1): 25-27.

[37] García-Viguera, C., & Pérez, A. (2004). La granada. Alimento rico en polifenoles antioxidantes y bajo en calorías. Alimentación. Nutrición y Salud, 11(4): 113-120.
http://hdl.handle.net/10261/17946.

[38] Zand, R.; Jenkins, D. and Diamandis, E. (2000). Steroid hormone activity of flavonoids and related compounds. Breast Cancer Research and Treatment, 62(1):35-49. http://dx.doi.org/10.1023/A:1006422302173.

[39] Nawwar, M., Hussein, S., and Merfort, I. (1994). Leaf phenolics of Punica granatum L. Phytochemistry, 37(4): 1175-1177.
https://doi.org/10.1016/S0031-9422(00)89552-7.

[40] Chiari-Andréo, B.G. ; Trovatti, E.; Marto, J. ; Almeida-Cincotto, M.G.J ; Melero, A. ; Corrêa, M.A.; Chiavacci, L.A. ; Ribeiro, H.; Garrigues, T. and Isaac, V.L.B. (2017). Guava: phytochemical composition of a potential source of antioxidants for cosmetic and/or dermatological applications. Braz. J. Pharm. Sci., 53(2):e16141 http://dx.doi.org/10.1590/s2175-97902017000216141.

[41] Morsy, W.A.; Younan, G.E. and El-Gabry, H.E. (2019). Effect of dietary guava (*psidium guajava* l) leaf extract supplementation on productive performance, blood parameters and carcass traits of growing rabbits .Egyptian J. Nutrition and Feeds, 22(2): 183-192.
DOI: 10.21608/EJNF.2019.103478.

[42] Yusuf, A.; Fagbuaro, S.S. and Fajemilehin, S.O.K. (2018). Chemical composition, phytochemical and mineral profile of garlic (*Allium sativum*). Journal of Bioscience and Biotechnology Discovery, 3(5):105-109. https://doi.org/10.31248/JBBD2018.073.

[43] Kope´c, A.; Skoczylas, J.; Jedrszczyk, E.; Francik, R.; Bystrowska, B. and Zawistowski, J. (2020). Chemical Composition and Concentration of Bioactive Compounds in Garlic Cultivated from Air Bulbils. Agriculture, 10(40): 1-15. www.mdpi.com/journal/agriculture.

[44] Shekhar, S.; Prakash, P.; Singha, P.; Prasad, K.; Singh, S.K. (2023). Modeling and Optimization of Ultrasound-Assisted Extraction of Bioactive Compounds from Allium sativum Leaves Using Response Surface Methodology and Artificial Neural Network Coupled with Genetic Algorithm. Foods, 12(9),1925:1-21. https://doi.org/10.3390/foods12091925.

[45] Skoczylas, J.; Jedrszczyk, E.; Dziadek, K.; Dacewicz, E.; Kope´c, A. (2023). Basic Chemical Composition, Antioxidant Activity and Selected Polyphenolic Compounds Profile in Garlic Leaves and Bulbs Collected at Various Stages of Development. Molecules, 28(18), 6653:1-19.
https://doi.org/10.3390/molecules28186653.

[46] Kaneria, M.J.; Bapodara, M.B. and Chanda, S.V. (2012). Effect of Extraction Techniques and Solvents on Antioxidant Activity of Pomegranate (*Punica granatum* L.) Leaf and Stem. Food Anal. Methods, 5:396–404.
DOI:10.1007/s12161-011-9257-6.

[47] Swilam, N. and Nematallah, K.A. (2020). Polyphenols profle of pomegranate leaves and their role in green synthesis of silver nanoparticles. Scientific Reports, 10:14851. https://doi.org/10.1038/s41598-020-71847-5.

[48] Seo, J.; Lee, S.; Elam, M.L.; Johnson, S. A. ; Kang, J. and Arjmandi, B H. (2014). Study to find the best extraction solvent for use with guava leaves (*Psidium guajava* L.) for high antioxidant efficacy. Food Science and Nutrition, 2(2): 174–180. Doi: 10.1002/fsn3.91.

[49] Ruksiriwanich, W.; Khantham, C.; Muangsanguan, A.; Phimolsiripol, Y.; Barba, F.J.; Sringarm, K.; Rachtanapun, P.; Jantanasakulwong, K.; Jantrawut, P.; Chittasupho, C.; Chutoprapat, R.; Boonpisuttinant, K. and Sommano, S.R. (2022). Guava (*Psidium guajava* L.) Leaf Extract as Bioactive Substances for Anti-Androgen and Antioxidant Activities. Plants, 11(24): 3514.
https://doi.org/10.3390/plants11243514.

[50] Petti, S. and Scully, C. (2009). Polyphenols, oral health and disease: A review. Journal of Dentistry, 37: 413-423.
DOI: 10.1016/j.jdent.2009.02.003.

[51] Hooks,T. and Niu, G. (2021). Performance and Phytochemical Content of 22 Pomegranate (Punica granatum) Varieties. Hortscience, 56(2):217–225.
https://doi.org/10.21273/HORTSCI15551-20.

[52] Setiadhi, R. and Sufiawati, I. (2017). Fractionation of Red Pomegranate (*Punica granatum* L.) Seed Ethanolic Extracts for Identifying Active Compounds. TIMNAS7-JSMID4 - The 7th International Meeting and The 4th Joint Scientific Meeting in Dentistry. Shangri, 277–280.
https://www.researchgate.net/publication/329493232.

[53] Fellah, B.; Bannour, M.; Rocchetti, G.; Lucini, L. and Ferchichi, A. (2018). Phenolic profiling and antioxidant capacity in flowers, leaves and peels of Tunisian cultivars of *Punica granatum* L. J. Food. Sci. Technol., 55(9): 3606–3615. https://doi.org/10.1007/s13197-018-3286-8.

[54] Li, X.; Wasila, H. and Liu, L. (2015). Physicochemical characteristics, polyphenol compositions and antioxidant potential of pomegranate juices from 10 Chinese cultivars and the environmental factors analysis. Food Chem., 175:575–584.
https://doi.org/10.1016/J.FOODCHEM.2014.12.003.

[55] Hmid, I.; Elothmani, D. and Hanine, H. (2017). Comparative study of phenolic compounds and their antioxidant attributes of eighteen pomegranate (Punica granatum L.) cultivars grown in Morocco. Arab J. Chem., 10(2):S2675–S2684.
https://doi.org/10.1016/j.arabjc.2013.10.011.

[56] Fernandes, L.; Pereira, J.A. and Lopéz-Cortés, I. (2017). Physicochemical composition and antioxidant activity of several pomegranate (Punica granatum L.) cultivars grown in Spain. Eur. Food Res. Technol., 243(10):1799–1814.
https://doi.org/10.1007/s00217-017-2884-4.

[57] Nantitanon, W.; Yotsawimonwat, S. and Okonogi, S. (2010). Factors influencing antioxidant activities and total phenolic content of guava leaf extract. Food Science and Technology, 43(7): 1095-1103.
https://doi.org/10.1016/j.lwt.2010.02.015.

[58] Birdi, T.; Daswani, P.; Brijesh, S. and Antia, N. (2010). Newer insights into the mechanism of

action of *Psidium guajava* L. leaves in infectious diarrhoea. BMC Complement. Altern. Med., 28:10-33. https://www.researchgate.net/publication/44805959.

[59] Irondi, A.E.; Agboola, S.O.; Oboh, G.; Boligon, A.A.; Athayde, M.L. and Shode, F.O. (2016). Guava leaves polyphenolics-rich extract inhibits vital enzymes implicated in gout and hypertension in vitro. J. Intercult Ethnopharmacol, 5(2):122-130.

www.jicep.com

[60] Abdel-Gawad, M.; Abdel-Aziz, M.; El-Sayed, M.; El-Wakil, E. and Abdel-Lateef, E. (2014). In vitro antioxidant, total phenolic and flavonoid contents of six allium species growing in egypt. J. microbiol biotech food sci., 3(4): 343-346.

[61] Snoussi , P.; Trabelsi, N.; Dehmeni, A.; Benzekri, R.; Bouslama, L.; Hajlaoui, B.; Al-sieni, A. and Papetti, A. (2016). Phytochemical analysis, antimicrobial and antioxidant activities of Allium roseum var. odoratissimum (Desf.) Coss extracts. Industrial Crops and Products. 89: 533-542.

https://doi.org/10.1016/j.indcrop.2016.05.048.

[62] Felhi, S.; Saoudi, M.; Daoud, A.; Hajlaoui, H.; Ncir, M.; Chaabane,R.; El Feki, A.; Gharsallah, N. and Kadri, A. (2017). Investigation of phytochemical contents, in vitro antioxidant and antibacterial behavior and in vivo anti-inflammatory potential of Ecballium elaterium methanol fruits extract. Food Science and Technology, 37(4): 558-563. http://dx.doi.org/10.1590/1678-457x.26316.

[63] Cheurfa, M. and Allem, R. (2016). Evaluation of antioxidant activity of different extracts of Aloysia triphylla leaves (L'Herit.) from Algeria in vitro. Phytothérapie,14(3):181-197. http://dx.doi.org/10.1007/s10298-015-0969-4.

[64] Farag, R.S.; Abdel-Latif, M.S.; Sekina, S.; Emam, S.S. and Tawfeek, L.S. (2014). Phytochemical screening and polyphenol constituents of pomegranate peels and leave juices. Agriculture and Soil Sciences (LRJASS) 1(6): 086-093. http://www.landmarkresearchjournals.org/lrjass/index.php.

[65] Balamurugan, C.; Karuppasamy, R.; Sivaraj, C.; Saraswathi, K. and Arumugam, P. (2020). *Punica granatum* L. (Pomegranate) leaves extract: The study of antioxidant and antibacterial activity. Journal of Pharmacognosy and Phytochemistry, 9(6):397-402. https://doi.org/10.22271/phyto.2020.v9.i6f.12915.

[66] Benchagra, L.; Berrougui, H.; Islam, M.O.; Ramchoun, M.; Boulbaroud, S.; Hajjaji, A.; Fulop, T.; Ferretti, G. and Khalil, A. (2021). Antioxidant effect of Moroccan Pomegranate (*Punica granatum* L. Sefri Variety) extracts rich in punicalagin against the oxidative stress process. Foods, 10(9): 2219. https://doi.org/10.3390/foods10092219.

[67] Schvab, M.C.; Ferreyra, M.M.; Davies, C.V.; Stefani, A.; Cayetano, M.C.; Gerard, L.M. and Gonzalez, R.F. (2015). Effects of orange winemaking variables on antioxidant activity and bioactive compounds. Food Science and Technology, 35(3):407-413. https://doi.org/10.1590/1678-457X.6571.

[68] Marcelino, S.; Mandim, F.; Taofiq, O.; Pires, T.C.S.P.; Finimundy, T.C.; Prieto, M.A. and Barros, L. (2023).Valorization of Punica granatum L. Leaves Extracts as a Source of Bioactive Molecules. Pharmaceuticals, 16(342):1-18. https://doi.org/10.3390/ph16030342.

[69] Lahlou, Y.; El Amraoui, B.; El-Wahidi, M. and Bamhaoud, T. (2022). Chemical composition, antioxidants and antimicrobial activities of moroccan species of psidium guajava extracts. Rocz Panstw Zakl Hig; 73(1):65-77. https://doi.org/10.32394/rpzh.2022.0199.

[70] Cutler, R.R and Wilson, P. (2004). Antibacterial activity of new stable aqueous garlic extract of allicin against methicillin resistance Staphylococcus aureus. British Journal of Biomedical Sciences. 61(2): 68-78. DOI: 10.1080/09674845.2004.11732646.

[71] Heleno, S. A.; Martins, A.; Queiroz, M.J.R.P. and Ferreira, I.C.F.R. (2015). Bioactivity of phenolic acids: metabolites versus parent compounds: a review. Food Chemistry, 173:501-513. http://dx.doi.org/10.1016/j.foodchem.2014.10.057.