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Pomegranate peel nanoemulsion: evaluation of bioactive components and their efficacy to reduce specific pesticide residues

Amr Farouk[1], Ahmed N. Badr[2], Amal S. Hathout[2], Tarek A. Morsy[3], Ahmed .S. M. Fouzy[2], Gomaa N. Abdel-Rahman[2]*

[1] *Flavor and Aromatic Chemistry Department, National Research Centre, Dokki 12622, Cairo, Egypt.*

[2] *Food Toxicology and Contaminants Department, National Research Centre, Dokki 12622, Cairo, Egypt.*

[3] *Dairy Science Department, Faculty of Agriculture, Cairo University, Giza, Egypt.*

Abstract

Pesticides are chemical substances that kill pests and are potentially hazardous to humans. Therefore, this study aimed to focus on preparing pomegranate peel extract and nanoemulsion and its physiochemical characterization, as well as to study their polyphenolic content, antioxidant activity and ability to reduce pesticides. Data showed that twenty-three polyphenolic bioactive components were identified from pomegranate peel nanoemulsion. The characterization of the prepared nanoemulsion was also studied, and results showed that the nanoemulsion particle size was 41.72±12.72 nm, while zeta potential was -27.15±2.76, and the poly-dispersibility index was recorded at 0.21±0.04. The generated nanoemulsion's viscosity was 4.99 mPa•s after 21 days, indicating a greater stability formula. The nanoparticles were found to be spherical, evenly dispersed, discrete, and non-aggregated using a transmission electron microscope. Significantly, the nanoemulsion showed higher content of total phenolic (59.74 mg GAE/g), total flavonoid (27.81 CAE/g), hydrozyable tannin (71.40 mg TAE/g), and total anthocyanin (98.2 mg CGE/g) than those found in pomegranate peel extract. Pomegranate peel nanoemulsion showed significantly higher antioxidant activity than pomegranate peel extract using DPPH• and ABTS assays. The pesticides diazinon, parathion, and chlorpyrifos were reduced by 54.71%, 44.89%, and 29%, respectively, using pomegranate nanoemulsion. The findings of this study might be used to generate innovative methods for reducing pesticide residues using agricultural waste.

Keywords: *Chlorpyrifos; diazinon; parathion; pomegranate peel extract; pomegranate peel nano-emulsion; LC-MS/MS; GC-MS/MS, FTIR, TEM.*

Introduction

The exponential increase in agricultural products and concern for food security is the second priority target of the Sustainable Development Goals [1] and cannot be sustained without the effects of safe chemicals that give nutrients for plant growth [2]. The inescapable socio-technological and agricultural advances brought about by synthetic chemicals in the form of pesticides and fertilizers are not without consequences. These chemically engineered insect repellents, designed to help plants thrive, also harm humans, with over a thousand deaths reported yearly [3]. When used as a pesticide, organophosphates, an ester of phosphoric acid [3], have proved dangerous to agricultural and manufacturing employees and crops. Diazinon was classified as probably carcinogenic to humans (Group 2A), whereas the insecticides parathion was classified as possibly carcinogenic to humans (Group 2B) [4].

Therefore, there is an urgent need for the reduction of pesticide residue. Several strategies are used in

*Corresponding author e-mail: gomaa.nrc@gmail.com.; (Gomaa N. Abdel-Rahman).
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pesticide-residue reduction techniques [5], including physical, chemical, phytochemical, and biological treatments [6]. Modern techniques have implemented the treatment using natural extract [7], fruit peel powders [8], moringa and rice husk [9], and ozonized solutions [10].

Enormous agricultural wastes are generated throughout post-harvest operations and food production, causing considerable health and environmental problems [11]. Scientists focused on exploiting such wastes in many beneficial fields, such as reducing or eliminating pesticide residues and other contaminants from the soil [12]. Pomegranates are an ancient edible fruit grown mainly in the Mediterranean and widely used as fresh fruit, drinks, culinary items (jams and jellies), and dietary supplement [13]. The processing of pomegranate resulted in the development of vast amounts of waste, which, if not handled by the food sector, might pose a threat to the environment. The pomegranate residues, mainly peels (73%) and seeds (27%) are a classic illustration of this phenomenon [14].

Pomegranate peels have been shown to have wound-healing properties [15], immunomodulatory [16], antibacterial [17], anti-atherosclerotic [13], anti-inflammatory [18], and antioxidant effect [19]. Pomegranate has the most significant overall polyphenol content compared to other fruits investigated [20, 21]. As a result of the pomegranate's pharmaceutical and nutraceutical properties, as well as the large annual production of pomegranate as a waste of juice, the peels could have more beneficial applications in food industries than being used as animal feed.

Pomegranate peels are a rich polyphenol component source [22, 23], with a significant function as an antioxidant [24] and a potent ability to interact in chemical reactions and biological functions [25, 26]. Polyphenols in food were found to defend against a wide range of pollutants. In agreement, walnut polyphenol extract was found to protect against malathion and chlorpyrifos-induced toxicity [27]. Nanoemulsions are emulsions made up of nano-sized droplets dispersed in another immiscible liquid that have properties that distinguish them from conventional emulsions and make them suitable for encapsulation, delivery, and formulations of bioactive ingredients in various fields such as drugs, food, and agriculture [28]. Therefore, this study aimed to focus on preparing pomegranate peel extract and nanoemulsion and its physiochemical characterization, as well as to study their polyphenolic content, antioxidant activity and ability to reduce pesticides.

Materials and Methods

1. Chemicals

Ethanol and chloroform were HPLC grade obtained from Merck (Darmstadt, Germany). Chlorpyrifos, diazinon, and parathion were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA 95060, USA).

2. Fruits

Pomegranates (*Punica granatum* L.) (1 kg) were obtained from retailers. The pomegranates were cleaned under impulsive water to remove any adhered pomegranate flesh before sun-drying. A heavy-duty hammer mill (M20 Universal Mill, IKA®-Werke GmbH & Co. KG, Germany) was used to grind the collected materials.

3. Preparation of pomegranate peel extract

Pomegranate peel powder was weighed, transported to conical flasks, combined with ethanol, and incubated in a shaking incubator (MAXQ 481R HP, Thermo Scientific, USA) for the extraction period (24 h/ 120 rpm/20°C). The extract was filtered using Whatman filter paper (No. 4) and evaporated under vacuum (40°C/ 150 Millibar) using a rotary evaporator (Heidolph Instruments GmbH & Co. KG, Germany) [29]. The pomegranate extract was concentrated to 1 mL, where the final product was collected, freeze-dried, and stored in amber vials until further application.

4. Preparation of pomegranate peel nanoemulsion

The nanoemulsion was prepared by combining the concentrated extract with Tween 80 (10%; v/v), then smoothly dropping the product on the walls of the solution, which consisted of maltodextrin (10%) soluble in phosphate buffer solution (PBS; pH 7.2) [30]. During the droplet process, the nanoemulsion was formed using a high-shear unit of Ultra-Turrax (Ika Works T 50 Basic Ultra Turrax, 3150 Commercial Ave Northbrook, IL 60062, USA). Temperature rise was regulated using an ice container surrounding the sample. The Ultra-Turrax was adjusted to 28000 - 30000 rpm at 20°C, followed by ultra-sonicator treatment (13 mm probe diameter/ 20 kHz frequency/ 750 W power output/ 30 min.). The outcome was a stable emulsion containing 20% extract.

5. Identification of phenolic compounds in pomegranate peel nanoemulsion using LC-MS/MS

An LC-MS/MS instrument outfitted with a Triple Quadruple (SCIEX 5500, Framingham, MA 01701, USA) was used to identify phenolic compounds in pomegranate peel nanoemulsion. A C18 column (250 mm i.d.; 4 mm particle size, end-capped) was used. The temperature was maintained at 30°C, and the input flow rate was fixed at 0.4 mL/min. A 40°C was selected as the target temperature for the column oven. The separation and quantification were carried out using solvent A (0.1% formic acid/water) and solvent B (acetonitrile) at a flow rate of 0.4 mL/min and with a split out of 200 mL/min according to the following schedule: 95% A and 5% B for 27 min., 5% B for 28 min., and return to the initial conditions. The injected samples were first passed through a 0.2 mm nylon membrane filter and diluted in 1 mL of 80% ethanol.

6. Determination of stability and physiochemical characterization of the nanoemulsion

6.1. Transmission electron microscope (TEM)

The TEM was used to investigate the morphology of the nanoemulsion. One drop of nanoemulsion was diluted with deionized water, put onto a carbon-coated copper grid, and then stained for one min with a phosphor tungstic acid solution (2%, pH 6.7). The sample was dried at 27°C before viewing the picture using TEM at accelerating voltages of 80 VK [31].

6.2. Dynamic light scattering (DLS)

A DLS with a Zetasizer NanoZS laser diffractometer was used to quantify the particle size distribution of nanoemulsions. Two drops of nanoemulsion were placed in a cuvette before diluting with two mL of water. After that, the samples were sonicated for another five min before the DLS measurement.

6.3. Zeta potential measurements

The zeta potential was measured by phase-analysis light scattering (PALS) with a Zetasizer NanoZS laser diffractometer (Malvern Instruments, UK). Nanoemulsion was diluted with water and sonicated for a total of five min before the zeta potential measurements were performed [32].

7. Determination of the viscosity, pH, titrable acidity, and colour attributes

The viscosity of nanoemulsion was evaluated using a viscometer (Brookfield Engineering Laboratories, Arizona, 85225, USA) equipped with a spindle-type measuring system. The samples were moved to the instrument and equilibrated at 25 °C for 5 min before measurement. Results were expressed as mPa•s and followed along storage after 1, 7, 14, and 21 days at 4 °C [33]. Titrable acidity was calculated as a percentage of citric acid by titrating 10 mL of the nanoemulsion with NaOH (0.1 N) solution to pH 8.1. The pH was measured using a pH meter.

The Hunter LAB (Color quest XE, Hunter Lab, USA) program was used to determine colour. The value of the colour was determined using the symbols L* (which stands for lightness), A* (which represents redness), and B* (which means yellowness). Illuminant D65 was used as the light source, and a 10-degree angle was used for the observation.

8. Determination of total phenolic and flavonoids contents

The total phenolic and total flavonoid contents were carried out according to Dewanto et al. [34]. For total phenolic content, pomegranate peel extract or nanoemulsion (500 µl) was combined with distilled water (500µl) and Folin-Ciocalteu reagent (500µl). The mixture was left for six minutes before adding 1.25 mL of 7% Na_2CO_3. The volume of the solution was increased to 3 mL by adding distilled water. After being left in the dark for thirty min, the absorbance was measured at 650 nm. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/ g. The colourimetric method determined the total flavonoid content for pomegranate peel extract and nanoemulsion. The absorbance was determined at 510 nm wavelength. The findings were expressed as mg quercetin equivalents (QE)/ g.

9. Determination of total anthocyanin content

Total anthocyanin content was determined according to Elfalleh et al. [24]. The results for the total anthocyanin content were determined using a method known as pH differentiation, which was performed with two buffer systems consisting of potassium chloride buffer (pH 1.0, 0.025 M) and sodium acetate buffer (pH 4.5, 0.4 M). Each pomegranate peel extract or nanoemulsion was mixed with 3.6 mL of matching buffers and then measured at 510 and 700 nm compared to a sample of freshwater that served as a blank. The following equation was used to calculate the absorption (A), whereas a molar extinction coefficient of 29600 was used. These data indicated the amount of cyanidin-3-glucoside equivalents contained in mg CGE/g.

$$A = [(A510 - A700)pH\ 1.0 - (A510 - A700)pH\ 4.5]$$

10. Determination of hydrolyzable tannin content

One mL of extract or nanoemulsion (10-fold diluted) and five mL of KIO_3 (2.5%) were vortexed for ten s in a vial. The mixture was measured at 550 nm against the water (blank). The optimal reaction time (to achieve maximum absorbance) was defined as 3 min for the pomegranate peel extract and nanoemulsion and 5 min for the standard solution [24]. The resulting values were in mg of tannic acid equivalent (TAE)/g.

11. Determination of total alkaloid content

The extract or nanoemulsion (1 mL) was first diluted in dimethyl sulfoxide (DMSO), then 1 mL of 2N HCl was added, and finally, the mixture was filtered. The filtrate was put into a separating funnel, and then amounts of bromocresol green (5 mL) and phosphate buffer (5 mL) were added. Chloroform in increments of 1, 2, 3, and 4 mL was added to the mixture and violently stirred. After collecting the chloroform layer in a volumetric flask with a capacity of 10 mL, the volume was finished by adding chloroform. The absorbance of the test solution, the standard solution, and the blank was measured using the UV/Visible spectrophotometer set at 470 nm [35]. The total amount of alkaloid was given as mg of alkaloids equivalent (AE) /g.

12. Determination of antioxidant activity

12.1. Antioxidant activity using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay

An aliquot of 1 ml of extract or nanoemulsion at various concentrations (25, 50, 75, and 100 µg/mL) was introduced to tubes containing 1 mL of 0.078 M DPPH radical in ethanol. The mixture was briskly agitated and allowed to stand for 30 min in the dark at room temperature. The absorbance was determined at 517 nm [36].

12.2. Antioxidant activity using 2, 2′-azino-bis (3-ethyl benzothiazoline6-sulfonate) (ABTS) radical scavenging

The working solution was made by combining the two stock solutions (7 Mm ABTS solution and 4.9 potassium per-sulfate solutions) in equal parts, allowing them to react for 16 h at room temperature in the dark. Before use, the solution was diluted with ethanol to achieve an absorbance of 0.800 to 1.000 nm and then combined with extract or nanoemulsion at various concentrations (25 to 100 µg/mL) or standard solutions. A control solution comprising methanol and ABTS was also created. After 30 min of incubation at 25°C, the absorbance was measured at 734 nm [37].

13. Determinations of Fourier transform infrared spectroscopy (FTIR)

The functional groups in the nanoemulsion were studied using absorption spectroscopy in the infrared region (600-4000 cm^{-1}) at 4 cm^{-1} resolution using a spectrometer to collect the FTIR spectra (Bruker, USA) [23].

14. Evaluation of pesticide reduction by the nanoemulsion

Chlorpyrifos, diazinon and parathion were individually added at concentrations of 100 µg/mL to water containing pomegranate nanoemulsion at a concentration of 100µg/mL. Positive controls were created by using each pesticide only without any pomegranate nanoemulsion. Negative controls were created to keep nanoemulsion distinct and pesticide-free. The tubes were kept in the dark for 2 h at room temperature, the samples were filtered, and the filtrated solutions were GC/MS/MS examined for pesticide residues.

The percentage of reduction was calculated according to the following equation.

$$\boldsymbol{Percentage\ of\ reduction}\ (\%) = \mathbf{1} - \boldsymbol{Treatment/Control\ x}\ \mathbf{100}$$

Gas chromatography analysis

Gas Chromatography system 7890B (Agilent Technology, USA) had a tandem mass spectrometer 7010A Quadrupole. An HP5MS ultra-inert capillary column with dimensions of 30 mm, 0.25 mm, and 0.25 meters was used. The temperature was maintained at 70°C for 1 min, then increased to 150°C at a rate of 50°C/min for 0 min. The temperature was raised to 260°C (6°C/min for 0 min), then increased to 310°C (20°C/min for 1.567 min). As the carrier gas, ultra-high-quality helium (with a purity level of more than 99.999%) was used at a flow rate of 1.654 mL/min. These gases included helium, quench gas, nitrogen, and collision gas. The temperatures of the injector, the transfer line, the ion source, and the quadrupole were 250, 280, 300, and 180°C, respectively.

15. Statistical analysis

All experiments were carried out in triplicate (n= 3) and reported as mean ± SD. The SPSS program version 16 was used and evaluated using the variance

analysis (ANOA one-way) test, whereas P≤0.05 is considered significant.

Results

1. Characterization of polyphenols identified from pomegranate peel nanoemulsion

The LC-MS/MS was used to characterize different polyphenols in pomegranate peel nanoemulsion. Results in Table (1) and Figure (S1) identified 23 polyphenols using LC-MS/MS, including four hydroxybenzoic derivatives, six hydroxycinnamic derivatives, seven ellagitannins, two gallagyl esters, one gallotannin, one dihydro flavonol, and two flavones. These compounds were successfully identified by comparing retention durations and mass spectra with various reference chemicals. Among the hydroxybenzoic acids, four different compounds were detected. The compound that showed an [M-H]- ion at m/z 153 was characteristic of protocatechuic acid. Also, among the hydroxybenzoic acid, vanillic acid 4- hexoside was identified as a [M-H]- ion at m/z 329, whereas this ion had the properties of vanillic acid (Table 1). Amongst the hydroxycinnamic derivatives, the molecule with a [M-H]-ion at m/z 353 led to the identification of chlorogenic acid. Similarly, p-coumaric acid was found by employing the mass number 163, whereas p-coumaric acid-hexoside was found to be another molecule with a [M-H]- ion at m/z 325 (Table 1).

Seven compounds were detected among the ellagitannins in nanoemulsion, including four ellagic acid derivatives with [M-H]- ions at m/z 301, m/z 447, m/z 463, m/z and 625. The derivatives of ellagic acid identified were hexoside, dihexoside, and deoxy-hexoside. Also, among ellagitannins, two punicalagin compounds were detected. Finally, 2 flavones, apigenin-7-O-glucoside with [M-H]- m/z 431 and luteolin-7-O-glucoside with [M-H]- m/z 447, were also detected. The characteristic fragment of caffeic acid derivatives was identified at m/z 137.

TABLE 1. The bioactive components identified in pomegranate peels nanoemulsion

	Chemical Name	R_t	M-H$^-$ (m/z)	Formula
Hydroxybenzoic derivatives	Vanillic acid, 4- hexoside	18.43	329	$C_{14}H_{18}O_9$
	Protocatechuic	29.43	153	$C_7H_6O_4$
	Gallic acid	35.09	169	$C_7H_6O_5$
	Syringic	18.74	197	C_9H10O_5
Hydroxycinnamic derivatives	*p*-Coumaric acid	7.97	163	$C_9H_8O_3$
	p-Coumaric acid hexoside	6.66	325	$C_{15}H_{18}O_8$
	Ferulic acid derivative	11.25	355	$C_{10}H_{10}O_4$
	Caffeic acid derivative	8.94	299	$C_9H_8O_4$
	Chlorogenic acid	9.83	353	$C_{16}H_{18}O_9$
	Rosmarinic acid derivative	13.94	412	$C_{18}H_{16}O_8$
Ellagitannins	Ellagic acid hexoside	6.47	463	$C_{26}H_{26}O_{18}$
	Ellagic acid	33.13	301	$C_{14}H_6O_8$
	Ellagic acid dihexoside	4.54	625	$C_{26}H_{26}O_{18}$
	Pedunculagin I	5.16	783	$C_{34}H_{24}O_{22}$
	Pedunculagin II	12.69	785	$C_{34}H_{26}O_{22}$
	Lagerstannin B	6.47	949	$C_{41}H_{26}O_{27}$
	Ellagic acid deoxyhexoside	39.05	447	$C_{26}H_{26}O_{18}$
Gallagyl esters	Gallagyl-hexoside (Punicalin)	0.58	781	$C_{34}H_{22}O_{22}$
	HHDP-gallagyl-hexoside (Punicalagin)	1.55	1083	$C_{48}H_{28}O_{30}$
Gallotannins	Gallotannin- hexoside	15.97	331	$C_{76}H_{52}O_{46}$
Dihydroflavonol	Dihydrokaempferol-hexoside	41.11	449	$C_{21}H_{22}O_{11}$
Flavones	Luteolin-7-O-glucoside	8.36	447	$C_{21}H_{20}O_{11}$
	Apigenin-7-O-glucoside	8.41	431	$C_{21}H_{20}O_{10}$

2. Characterization of pomegranate peel nanoemulsion

The TEM characterization of the pomegranate peel nanoemulsion revealed their actual size and shape, whereas the droplets in the nanoemulsion appeared black and evenly distributed (Figure 1a). This uniform distribution implied that the nanoemulsions were successfully prepared. The TEM micrographs revealed that the nanoparticles were spherical and had a different average diameter in nm.

The pomegranate peel nanoemulsion was measured to have a mean particle size of 41.72 ± 12.72 nm, demonstrating ultra-fine size as it was less than 100 nm, whereas the mean zeta potential was found to be -27.15±2.76 (Table 2). On the other hand, the mean value of the poly dispersibility index (PDI) was equal to 0.291±0.04 (Table 2). The DLS method examines the size distribution of tiny particles or droplets in suspensions in the 3 nm-5µm range. The particle size distribution for the nanoemulsion was comparable with the PDI values obtained by the dynamic light scattering. The particle sizes determined by the TEM (Figure 1a) were smaller than those specified by the DLS instrument (Figure 1b).

The estimated viscosity values during the storage period (up to 21 days) showed relative stability. The properties of the nanoemulsion reflect the expected changes in its effectiveness and stability. Increasing the viscosity rates to high degrees may explain the occurrence of aggregation of granules and the increase in size with time.

3. Phytochemical screening of the pomegranate peel extract and nanoemulsion

The mean values of total phenolic, total flavonoid, total anthocyanin, hydrolyzable tannin and total alkaloid contents of pomegranate peel extract and nanoemulsion are shown in Figure (2). The pomegranate peels extract and nanoemulsion phenolic content were determined to be 52.91 and 59.74 mg GAE/g, respectively. Total flavonoid concentrations in pomegranate peel extract and nanoemulsion were 27.81 mg CAE/g and 20.04 mg CAE/g, respectively.

The overall anthocyanin concentration of the pomegranate peel nanoemulsion was 89.21.74 mg CGE/g, indicating a greater impact of the nanoemulsion to prevent anthocyanin from degradation. Simultaneously, the anthocyanin concentration of a pomegranate peel extract with no protective shield (no nano-coating) showed a drop in value, recording 51.62.17 mg CGE/g (Figure 2). The pomegranate peel extract contained considerable hydrolyzable tannin content (62.7 mg TAE/g), whereas the pomegranate peel nanoemulsion contained 71.4 mg TAE/g. The total alkaloid content for the pomegranate peel extract was 31.9 mg AE/g, which showed no significant difference from the pomegranate peel nanoemulsion, which recorded 31.7 mg AE/g (Figure 2).

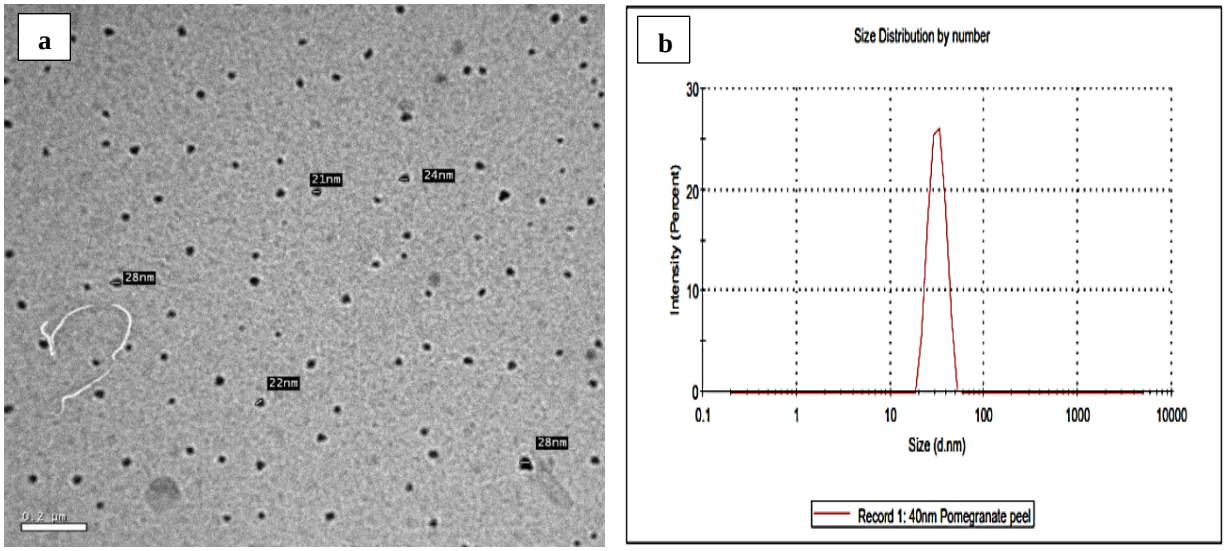

Fig. 1. Characterization of pomegranate peel nanoemulsion using a: the Transmission Electron Microscope; b: particle size distribution.

TABLE 2. Characteristics of pomegranate peel nanoemulsion

Character	value
Physical attribute	
Particle size (nm)	41.72 ± 12.72
Zeta potential (mV)	-27.15 ± 2.76
PDI	0.29 ± 0.04
Viscosity	
1st Day (mPa·s)	4.77±0.125
7th Day (mPa·s)	4.91±0.168
14th Day (mPa·s)	4.89±0.151
21st Day (mPa·s)	4.99±0.174
Color attribute	
L* value	66.81±0.571
a* value	37.58±0.364
b * value	7.41±0.242
Chemical attribute	
pH value	5.12±0.08
Acidity (g citric /L)	1.28±0.11

- Results are expressed in means ± SD (n=3).
- The color attribute of pomegranate peel nanoemulsion was determined, whereas (L*) represents lightness, (a*) represents redness, and (b*) represents yellowness.
- Viscosity determined for emulsion during (21 days) of storage, mPa s: milli-Pascal per seconds

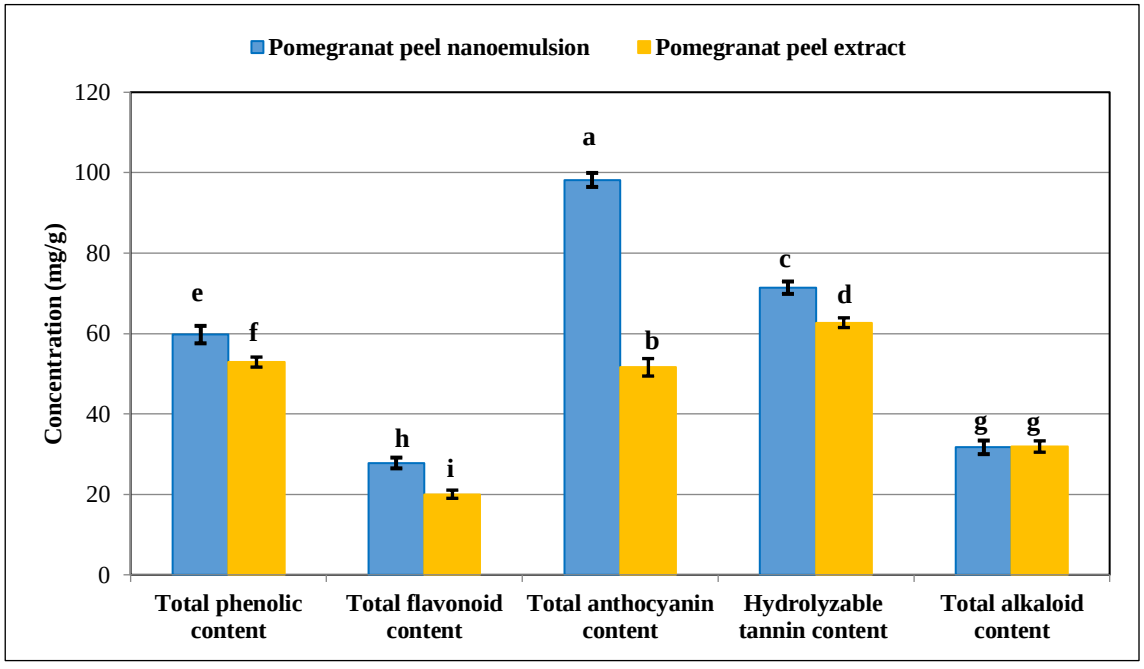

Fig. 2. Phytochemical screening of pomegranate peel extract and nanoemulsion

- For each determined component, the different superscript letter for each column means significant differences P<0.05.

4. Antioxidant activity of the pomegranate peel extract and nanoemulsion

The antioxidant activity of the pomegranate peel extract and nanoemulsion was determined using DPPH (Figure 3) and ABTS (Figure 4) assays. Results revealed that pomegranate peel nanoemulsion recorded significantly higher antioxidant activity than pomegranate peel extract. Data also indicated that the antioxidant activity increased by increasing the concentration of pomegranate peel extract and nanoemulsion.

According to the current study, there is an essential link between antioxidant activity and the quantity of total phenolics and flavonoid contents. Furthermore, the increased total phenolic and flavonoid content in the pomegranate peel nanoemulsion compared to the pomegranate peel extract demonstrated a difference in antioxidant activity. The IC50 values of the antioxidant activity (DPPH, ABTS) were calculated for the pomegranate peel extract and nanoemulsion (Figure 5).

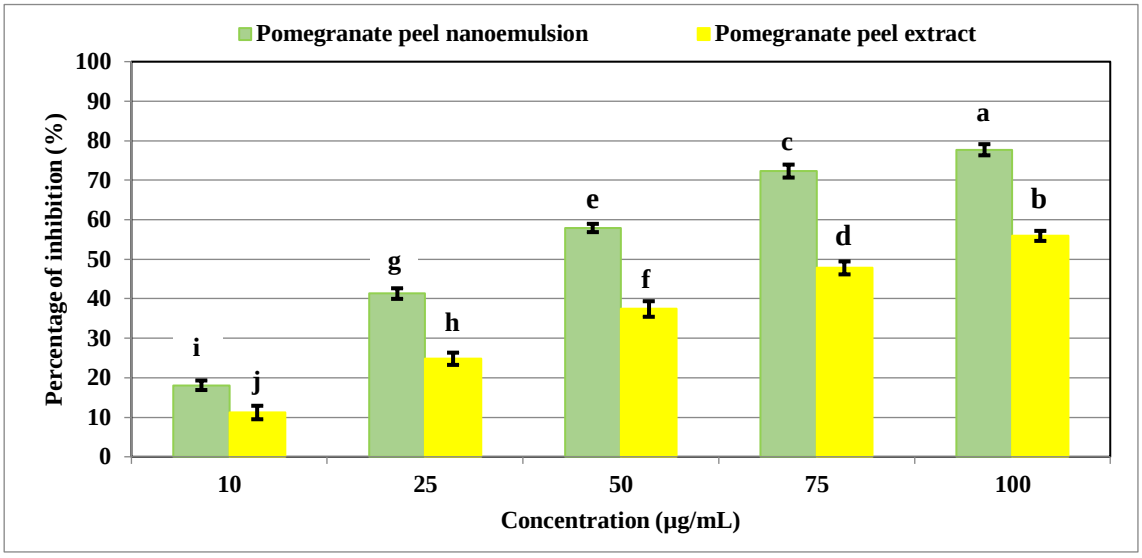

Fig. 3. The antioxidant activity of pomegranate peel extract and nanoemulsion using DPPH assay

- For each applied concentration, the different superscript letter for each column means significant differences P<0.05.

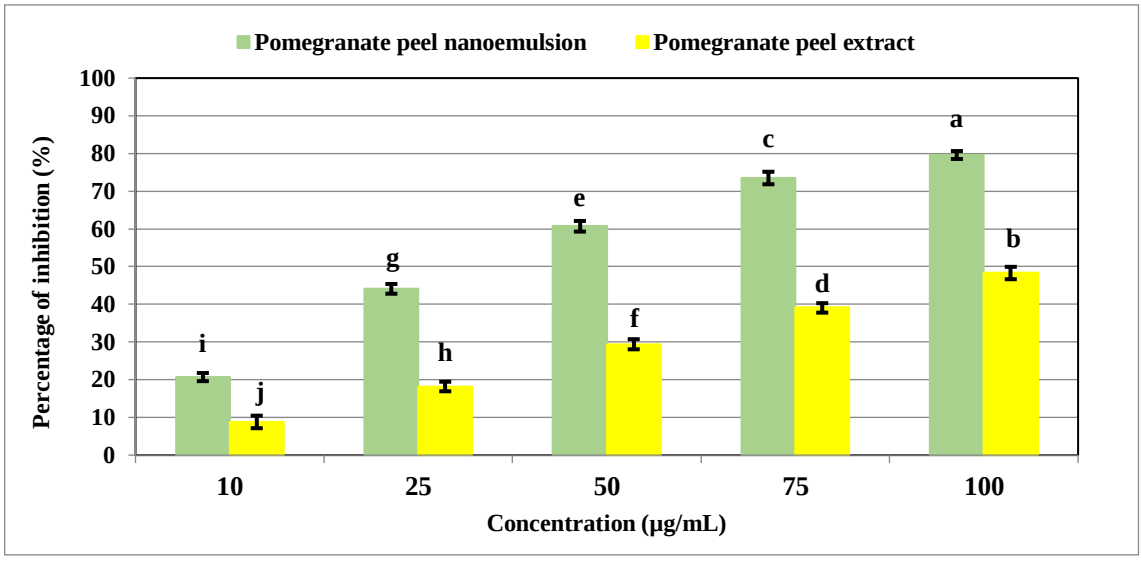

Fig. 4. The antioxidant activity of pomegranate peel extract and nanoemulsion using ABTS assay

- For each applied concentration, the different superscript letter for each column means significant differences P<0.05.

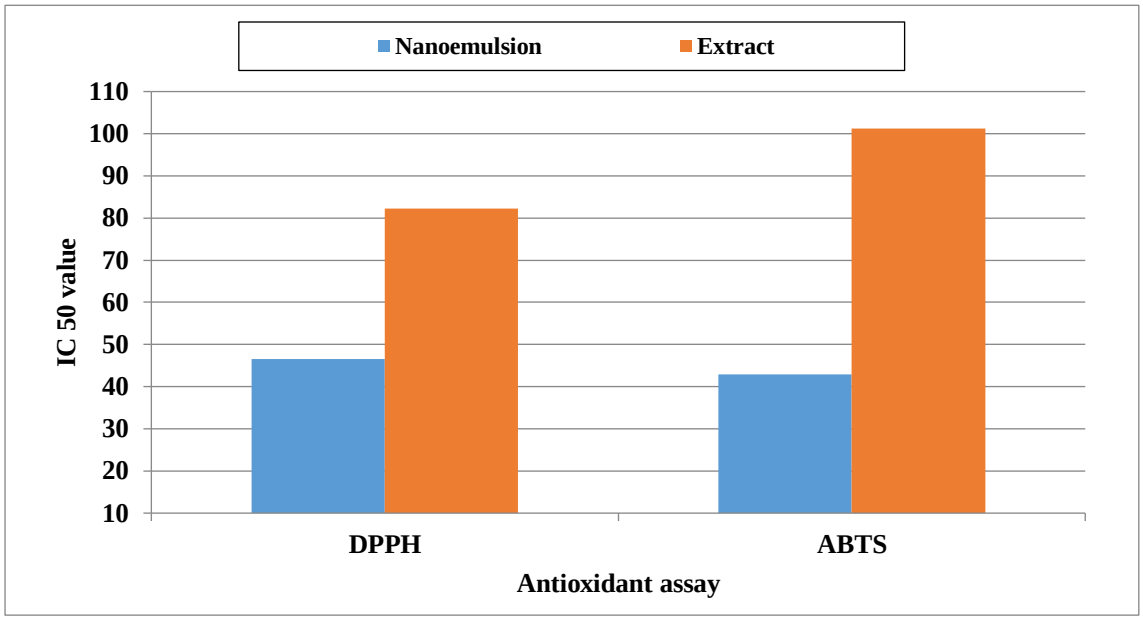

Fig. 5. IC $_{50}$ values calculated for the antioxidant activity of pomegranate peel extract and nanoemulsion of DPPH and ABTS assays

5. The FTIR analysis

The FTIR spectrum of pomegranate peel nanoemulsion is shown in Figure (6). Bands appeared at 3431.71, 2925.48, 1630.52, 1392.35, and 1048.12, cm^{-1} were assigned to O-H stretching (alcohol, intermolecular bonded), O-H stretching (carboxylic acids), C=C stretching (alkene), O-H bending (alcohol), and CO-O-CO stretching (anhydride), respectively.

6. Reduction of pesticides residues by pomegranate peel nanoemulsion

Results in Figure (7) showed the impact of pomegranate peel nanoemulsion on reducing pesticide residues. Results revealed that pomegranate peel nanoemulsion successfully reduced diazinon (54.71%), parathion (44.89%), and chlorpyrifos (29.00%), respectively.

Although pesticide residues are needed to protect plants against pests, their excessive presence poses a significant risk to public health. Natural extracts from agricultural wastes are a substantial source of bioactive components that may be implicated in pesticide residue reduction. Despite this, agricultural waste extracts are gaining popularity among scientists and consumers; however, research lacks pesticide residue-reduction effect, long-term efficiency, and applicability when applied to chemical dangers. Pomegranate peel extract in nanoemulsion form is a realistic and flexible natural solution for pesticide residue reduction, with outstanding efficacy and a broad range of action.

Discussion

Twenty-three polyphenols were identified from pomegranate peel nanoemulsion using LC-MS/MS. However, our results were lower than Ambigaipalan et al. [38], who identified 47 phenolic compounds in the American pomegranate seeds extracts. Among hydroxybenzoic acid, vanillic acid 4- hexoside was detected. Similar observations were reported before by Fischer et al. [39]. Seven compounds were detected among the ellagitannins in nanoemulsion. Similarly, ellagic acid was discovered in pomegranate husks [40] and juices [41]. In agreement, Kazemi et al. [42] detected ellagic acid in pomegranate peel extract. Also, two punicalagin compounds were detected. The pedunculagin was discovered in the Quercus robur leaf and stem bark extracts [43]. The punicalagin concentration was detected in the husk of 16 distinct kinds of pomegranate peel [44]. Punicalagin is the most important phenolic component in pomegranate peels, containing a variety of galloyl and hexahydroxydiphenoyl units esterified with glucose [45]. Similarly, pomegranate juice contained ellagitannins with a gluconic acid core in its centre [39]. Finally, 2 flavones were also detected. These results agree with Man et al. [46], who detected 6 flavones in pomegranate peels.

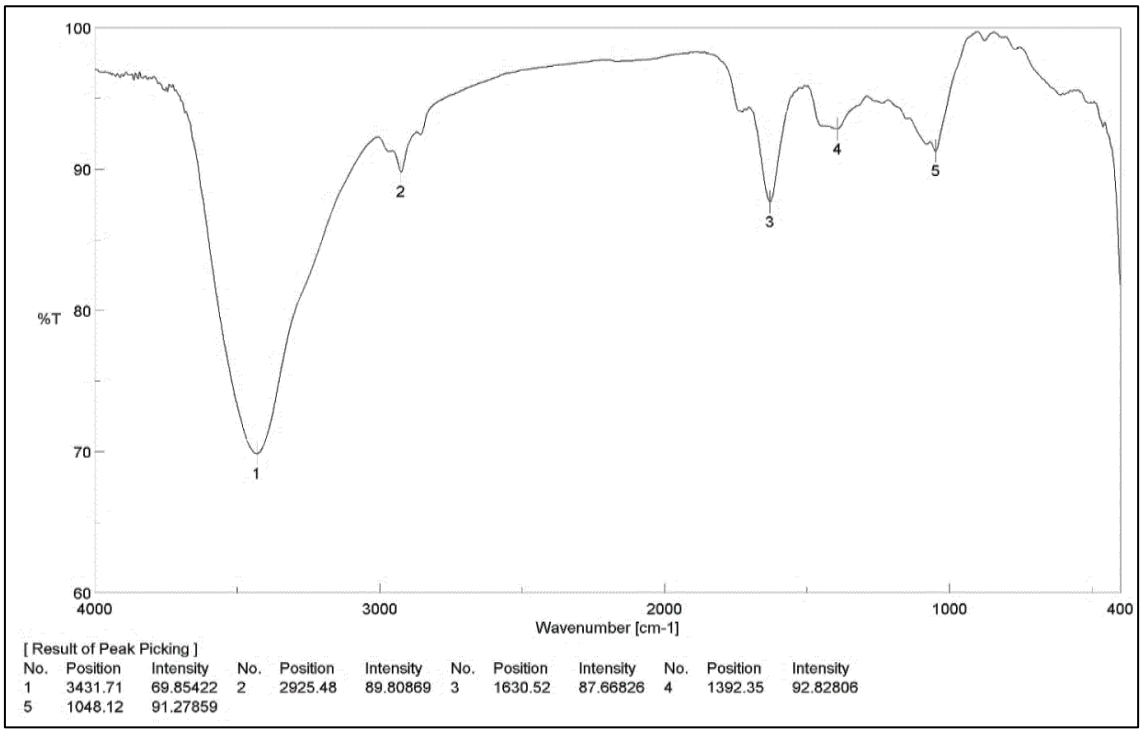

Fig. 6. Fourier transform infrared spectroscopy of pomegranate peel nanoemulsion

1: referred to alcohol, intermolecular bonded groups; 2: referred to a hydroxyl group of compounds such as carboxylic acids; 3: referred to C=C stretching (alkene) groups; 4: referred to alcohols and aldehydes compounds; 5: referred to CO-O-CO stretching (anhydride) groups.

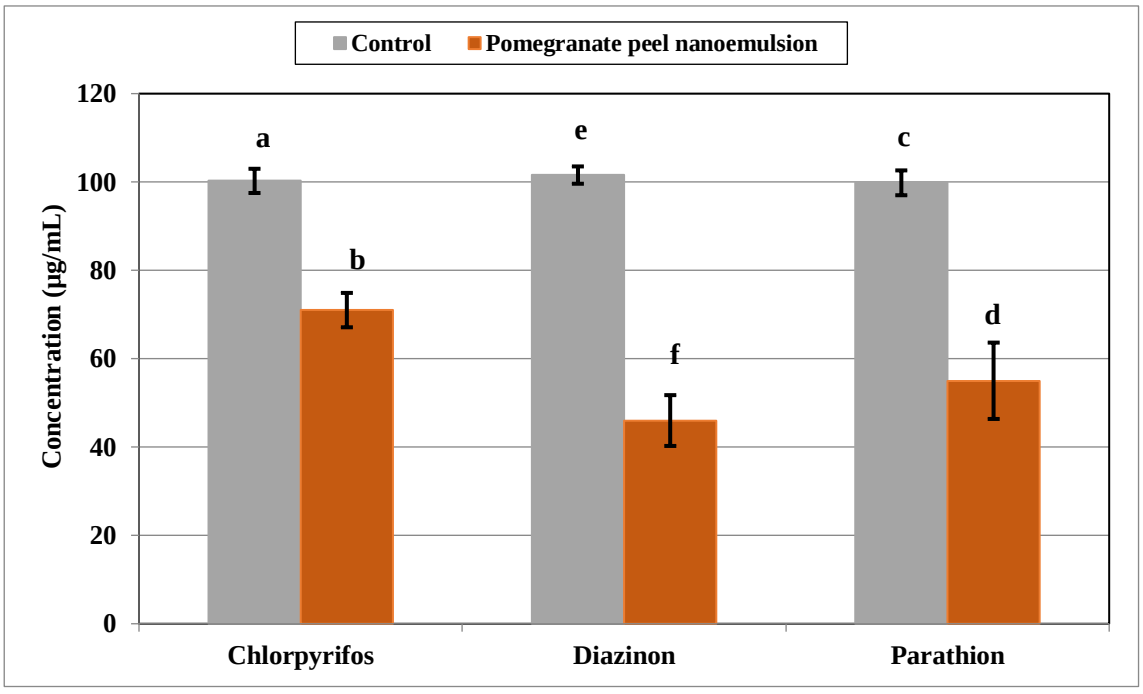

Fig. 7. The impact of pomegranate peel nanoemulsion on the reduction of pesticide residues

- For each pesticide, the different superscript letter for each column means significant differences P<0.05
- Pesticide reduction was determined at a pesticide concentration of (100 µg/mL) in water, whereas the pomegranate peel nanoemulsion was applied at a concentration of (100 µg/mL).
- The ratio between each pesticide concentration and the treatment concentration in water was 1:1.

Materials and biological samples have been imaged using TEM at a resolution of (0.2 nm) [47]. It has also been used to investigate the shape and structure of nanoemulsion [48]. The particle sizes determined by the TEM were smaller than those specified by the DLS instrument. This discrepancy arises because the TEM measurements necessitate air-drying the emulsion. In contrast, Qureshi et al. [49] reported that the size determined by TEM confirmed the size determined by DLS. Generally, these physical characteristics of the formed nanoemulsion could explain its stability and effectiveness in resisting storage changes.

The nanoemulsion's negative zeta potential (surface charge) might be attributed to maltodextrin's adsorbed -OH functional groups on the interface [50]. In agreement, Huang et al. [51] reported that the emulsion prepared had a small particle size, a low polydispersity index, and a high zeta potential. The size distribution profiles of nanoemulsions are noticed as a single and narrow peak in the DLS method because they are monodisperse [52]. The zeta potential of nanoemulsions is used to estimate particle charge or electrical surface charge. When particles with surface charges disperse in the liquid phase, they attract ions with opposing charges, forming a tight attachment known as the stern layer. Nanoemulsions with a high absolute value of zeta potential (negative or positive) are electrically stable, but those with a low zeta potential coagulate [53, 54].

Observing pomegranate peel nanoemulsion viscosity over 21 days revealed higher stability in the formulated nanoemulsion. Similar observations were reported by Silva et al. [55]. Such stability implies that the nanoemulsion has a long shelf life, allowing it to be employed in various applications [56]. The stability of viscosity values determined during the storage time showed low changes. Low viscosity changes are linked to nanoemulsion stability [57]. This might be due to the relation between the aggregation of nanoparticles and the viscosity values of the nanoemulsion [58].

The surfactants utilized, the shape and number density of the droplets, and interactions between the component droplets all influence the rheological characteristics of the nanoemulsions [59]. Significant colour variations were observed based on several parameters for pomegranate peel nanoemulsion (B* 7.41). Similar observations were reported by Moawad et al. [60].

Concerning total phenolic and flavonoid content, our results were considered lower than El-Hamamsy and El-khamissi [61], who showed that ethanolic extract of pomegranate peels contained a significantly higher amount of total phenolics and total flavonoids (161.5 and 70.65 mg GAE/g) respectively. Phenolics are frequently used for their biological effects, particularly their antioxidant properties. On the other hand, flavonoids are antioxidants with biological and chemical actions, the most significant of which are free radical scavenging activities [62]. As a result, pomegranate peels can be considered a key source of antioxidants.

Anthocyanins naturally occur in chemicals that give their colour to fruits, vegetables, and plants. Besides chlorophyll, these are most likely the most significant category of visible plant pigments [63]. Elfalleh et al. [24] reported that pomegranate peels obtained from Tunisia contained anthocyanin at a mean concentration of 73.90 mg/g. The same authors also stated that pomegranate peels contained hydrolyzable tannin content at a mean concentration of 124.08 7mg TAE/g. These results were considered higher than ours.

Furthermore, various characteristics, like harvest ripeness, storage temperature, and relative humidity, influenced anthocyanin content [64]. For hydrozyable tannin content, our results are lower than those obtained by Çam and Hişil [65], who reported that hydrolyzable tannin content in pomegranate peel aqueous extract recorded 82.6 mg TAE/g.

Results revealed that pomegranate peel nanoemulsion recorded significantly higher antioxidant activity. In agreement, Gil et al. [66] indicated that the high antioxidant activity of pomegranate juice could be due to the detection of anthocyanins, ellagic acid derivatives, and hydrolyzable tannins in the pomegranate juices. Meanwhile, Ali et al. [30] stated that pomegranate peel extract's significant antioxidant activity can be due to its high total phenolic and flavonoid content. Similarly, Russo et al. [67] reported a correlation between total phenolic content and antioxidant activity. In agreement, Rashid et al. [68] revealed that secondary metabolites (flavonoids, phenolic compounds, and tannins) detected in pomegranate peel are accountable for their antioxidant activity, due to their ability to scavenge free radicals and chelate transition metals.

The FTIR analysis of pomegranate peel nanoemulsion revealed several functional groups, including alcohol, carboxylic acids, alkene, and anhydride. Similar observations were reported by Hady et al. [69]. Long-chain fatty acids, waxes, carotenoids, and phytosterols are assumed to be present following the methylene ν(C-H) asymmetric stretch between 2800 and 3000 cm^{-1} [70]. The principal sources of carboxylic acid in fruit peels are pectin, cellulose, or lignin [71]. Similarly, the vibration at 1630.52 cm^{-1} implies the presence of phytosterol and fatty acid carbonyls [70]. Most bands

were similar to cellulose, hemicellulose, and lignin [72].

Reduction of pesticide residues could be achieved using several strategies, including physical, chemical, or biological reduction. Physical reduction can be achieved using absorbent materials, including agricultural wastes [73]. Activated carbon effectively removed pesticide residues [74]. Also, microbial reduction of pesticide residues using microorganisms was reported as a successful method [75].

Akhtar et al. [76] reported that the thetriazopho pesticide was effectively removed by rice bran than rice husk. In agreement, Fairooz et al. [77] evaluated the potential of pomegranate peel in removing two pesticides (lambda-cyhalothrin and diazinon) from their aqueous solutions. Recently, Hsu et al. [78] reported that some bioactive components extracted from fruit peels effectively removed pesticide residues such as saponins. Also, using residues and agricultural wastes of the olive-oil production chain effectively reduced pesticide residues [79]. Although pesticide residues are needed to protect plants against pests, their excessive presence poses a significant risk to public health. Natural extracts from agricultural wastes are a substantial source of bioactive components that may be implicated in pesticide residue reduction. Despite this, agricultural waste extracts are gaining popularity among scientists and consumers; however, research lacks pesticide residue-reduction effect, long-term efficiency, and applicability when applied to chemical dangers. Pomegranate peel extract in nanoemulsion form is a realistic and flexible natural solution for pesticide residue reduction, with outstanding efficacy and a broad range of action. On the other hand, phytochemicals, natural compounds found in plants and fruits, could have enhanced reduction of pesticides. According to a previous study, the reduction of pesticides by phytochemicals is influenced by different preconditions [80].

Conclusion

Pomegranate peel nanoemulsion contained different polyphenols and recorded significantly higher total phenolic, flavonoid, anthocyanin, hydrozyable tannin and alkaloid contents. On the other hand, pomegranate peel nanoemulsion recorded significantly higher antioxidant activity than pomegranate peel extract. This could be due to the ability of the nanoemulsion to retain different phytochemicals. The results also indicated that enriching pomegranate peels with phytochemicals might have provided a modern approach to reducing pesticide residues. This study recommends using pomegranate peel nanoemulsion to solve the commonness of pesticide residues in water.

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