Nanoemulsions of Some Edible Oils and their Antimicrobial, Antioxidant, and Anti-hemolytic Activities

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Plant oils have been applied for numerous purposes. Developing the composition of oils through nanotechnology has become a requirement, whether from a medical or industrial point of view. In this study, nanoemulsions (NEs) of olive and peanut oils were evaluated. GC-MS was used to determine the saturated and unsaturated fatty acids contents in both oils. Based on the area %, cis-8,11,14-eicosatrienoic acid (54.0%), myristic acid (30.7%), and arachidonic acid (23.1%) were the greatest constituents in peanut oil**,** while arachidonic acid (23.2%), cis-11,14,17-eicosatrienoic acid (22.7%), and cis-11-eicosenoic acid (11.4%) were the greatest constituents in olive oil. TEM examination indicated that the diameter of peanut oil NEs (14.6 nm) was less than that of olive oil NEs (24.5 nm). Olive oil and its NEs exhibited more antioxidant activity than peanut oil and its NEs had IC_{50} values of 158.6, 102.5, 435.1, and 291.5 µg/mL, respectively. Negligible hemolysis was observed using olive oil, unlike peanut oil, while hemolysis based olive oil NEs was increased compared with hemolysis based peanut oil NEs, particularly at high concentrations of 600 to 1000 µg/mL. Molecular docking investigation offered the structure–activity correlation and binding modes of cis-8,11,14-eicosatrienoic acid with *Salmonella typhi* (5ZXM) enzymes.

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INTRODUCTION

The development of efficient natural additives that may be used in place of synthetic alternatives has received much attention because there is growing interest from consumers as well as food and pharmaceutical industrial companies in the exploration of extremely safe natural ingredients (Al-Rajhi *et al*. 2022a). Although essential oils (EOs) have many applications in the fields of food, medicine, and cosmetics, there is still a problem of instability that prevents these uses. Scientists have been made aware of employing nanotechnology to solve these difficulties as a result of the growth of nanoscience (Abdelghany *et al*. 2018; Ganash *et al*. 2018; Al-Rajhi *et al*. 2022b; Yahya *et al*. 2022). Not all oils were covered in the publications that discussed the evolution of emulsions into nano-emulsions (NEs). The characteristics of NEs, which range in size from 20 to 100 nm, include long-term physical stability, high bioavailability, a high surface-to-volume ratio, and simple digestion. Due to this, two EOs – peanut and olive oils – were used to prepare NEs for the current investigation. NEs have attracted a growing interest in numerous fields, including materials sciences, chemistry, and in medical and

pharmaceutical sciences (Atanase 2022).

Tropical and sub-tropical nations are well suited for the production of peanuts (*Arachis hypogaea* L.) (Bhatti *et al*. 2020). India is the world's largest important yielder of peanuts, followed by China, West Africa, and the USA.

According to Rodrigues *et al*. (2011), peanut oil is used in the production of margarines, surfactants, medicines, and cosmetics. From peanut oil's biological activities, including its ability to repress the bacteria growth such as *Staphylococcus aureus*, *Listeria ivanovii, L. innocua*, *Bacillus cereus*, *Enterococcus hirae*, and *Pseudomonas aeruginosa* (Sebei *et al*. 2013), antioxidant potential resulting from its great content of total phenolic compounds (Matthäus and Özcan 2015; Zio *et al*. 2021), and anti-aging due to γ-tocopherol, vitamin E, α-tocopherol and phytosterols (Matthäus *et al*. 2015).

According to Lin *et al*. (2017), olive oil has appealing biological properties that include improving ROS removal, lowering the risk of cardiovascular disease, and improving memory and cognitive performance in the aged. Olive oil's antibacterial potential against different species including *B. cereus*, *B. subtilis*, *E. coli*, and *S. aureus* was recently reported by Wang *et al*. (2021). Olive oil had the highest concentrations of squalene and *β*-sitosterol, which were major contributors to its antibacterial effects (Wang *et al*. 2021).

For confirmation of the efficacy of the any NEs preparation, its activities were compared with bulk oil. Moghimi *et al*. (2016) demonstrated that the NEs of sage (*Salvia officinalis*) reflected higher antibacterial activity than bulk oil. Another study was performed on the antioxidant and antibacterial activity of olive oil NEs, indicating stronger activity than bulk oil (Lu *et al*. 2020). Also, the influence of a number NEs of oils on the microbial contamination of muscle foods was reviewed recently by Aziz *et al*. (2022). It was shown that olive oil NEs reduced the proliferation of mesophilic and psychrophilic bacteria, besides lactic acid bacteria under storage conditions. At the same time, no changes were observed on muscle foods as a result of NEs treatment. Nano-emulsions (NEs) of olive oils planned for the intravenous drug delivery was established (Karami *et al*. 2019) and NEs displayed low hemolysis (4.6%); therefore, it was considered safe for intravenous administration. Recently, a successful trial for oral *S. aureus*, *S. epidermidis*, *Chromobacterium voilaceum* treatment was documented using oils NEs (Ullah *et al*. 2022). Also, NEs were prepared and experimented against phytopathogenic bacteria by means of *in vitro* and *in vivo* investigations. Abdelrasoul *et al*. (2018) converted monoterpenes to NEs to enhance their antibacterial potential for suppression of *Pectobacterium carotovorum* and *Ralstonia solanacearum.* Compared with the bulk peanut oil, the proliferation of A549 lung cancer cells was more influenced by its NEs (Parastoo *et al.* 2021). In olive oil, there are only a few kinds of fatty acids, but the quantities of each strongly effect the nutritive and characteristics of the oil such as oil stability (Ghanbari *et al.* 2012). Also, polyunsaturated fatty acids have been found to play an essential role in the rancidification of numerous oils. Olive oil nutritional value, as well as its health utilities are attributed to the presence of a great quantity of oleic acid as a monounsaturated fatty acid and valuable minor components (Al-Bachir and Sahloul 2017). From the earlier literature, peanut oil commonly consists of triglycerides, including eight fatty acids. Oleic acid and linoleic represent nearly 80% of these fatty acids. Moreover, the content of phospholipids in the crude peanut oil can represent from 0.6 to 2%, depending on the peanuts maturity (Akhtar *et al*. 2014; Yang *et al*. 2022).

Based on the development of nanotechnology and vital role of EOs, the current investigation was designed to formulate NEs from two edible oils, namely olive and peanut oils, with determination of its antioxidant, antimicrobial, and hemolytic potentialities.

EXPERIMENTAL

Materials

All chemicals were analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA), except microbial growth media and oils (Al- Gomhuria Company, Cairo, Egypt) and dimethyl sulfoxide (DMSO) (Riedel-de-haen, Seelze, Germany).

Fatty Acid Analysis of Olive and Peanut Oils *via* GC-MS

The extraction solvent was composed of chloroform and methanol (2/1, v/v). One mL of oil was extracted by 20 mL of the solvent, followed by the addition of salt solution to separate the lower and higher phases. The lower phase was separated with a separation funnel, followed by concentration of the fatty acids. Fatty acid methylation was performed by adding H_2SO_4-methanol 2% (v/v) to the vial containing fatty acid extract. The vial was heated at 80 °C under slow shaking. Then, 0.25 mL of 1 M NaOH was added to neutralize the solution under slow shaking. The sample was subjected to fatty acid analysis (Liu *et al*. 2018).

Nano-emulsion Preparation and Visualization by Transmission Electron Microscopy (TEM)

Polysorbate 80 (Tween 80) surfactant was dispersed into a homogeneous suspension at 2% v/v in distilled water to prepare 100 mL. Olive or peanut oil (1:100) was added slowly to the suspension with constant stirring for 10 min. The EMs were prepared according to the method described previously (Salvia-Trujillo *et al*. 2013; Moradi and Barati 2019), with some modifications. The prepared EMs were sonicated by probe ultrasonic homogenizer (Silent Crusher M, Heidolph, Germany) for 20 min to obtain translucent NEs. Phosphotungstic acid was mixed with one drop of oil emulsion, which was then fixed on a copper grid. The shape and size of the prepared NEs of the olive and peanut oils in the dispersion NEs system (*via* Brownian diffusion) were examined via transmission electron microscopy (JEOL JEM-1200, Tokyo, Japan) at 200 kV with a tungsten source.

Microbial Inactivation Assay of Bulk Oils and its NEs

The well diffusion method was used to evaluate the antimicrobial activity of olive, peanut oils, and other specimens. Agar plates were inoculated with the test bacteria (*Salmonella typhi*, *Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus*) and fungi (*Candida albicans* and *Aspergillus flavus*). Agar plugs were removed *via* sterilized cork borer (6 mm), and 100 µL of the tested compounds were added into the well. Under appropriate temperature (37 °C for bacteria and 30 °C for fungi), the agar plates were incubated for 24 h and 3 days for bacteria and fungi, respectively. The visualized clear zone diameters around each well were measured. The activity of tested compounds was compared with a positive control represented with standard antibiotic (Gentamycin) and antifungal (Fluconazole). Another control (as negative control) was represented by DMSO without oils. The experiments were repeated three times (Al-Rajhi *et al*. 2022c).

DPPH Scavenging for Determine the Antioxidant Activity

The reaction mixture composed of 2 mL of 1,1-diphenyl-2-picryl hydrazyl (DPPH) dissolved in DMSO with separate doses of tested compound ranged from 3.9 up to 1,000 μg/mL. Using a vortex, the tubes containing the reaction mixture were shaken vigorously, then kept for 24 h without light. Then, the absorbance (517 nm) of the reaction mixture was measured by spectrophotometer (UV-VIS Milton Roy). DPPH solution free from the tested compound was applied as a control, while DMSO was utilized as a blank. A comparison with a standard antioxidant was conducted, and thus, ascorbic acid was utilized with the same procedures required to determine the antioxidant of tested compound. The inhibition of 50% (IC_{50} value) (by undetermined quantity of the tested compound) of the DPPH free radical was determined through log dose inhibition curve (Qanash *et al*. 2022). The DPPH scavenging activity (%) was estimated *via* calculation of the following expressions

$$\text{DPPH scavenging activity } \% = \frac{A_c - A_t}{A_0} \times 100 \quad (1)$$

where A_c is the absorbance of the control and A_t is the absorbance of the tested compound.

Assay of Hemolytic Activity of Bulk Oils and its NEs

There were a few modifications of the protocol of Bulmus *et al*. (2003) to determine the hemolytic activity of olive and peanut bulk oils and its NEs. Briefly, in a sterile tube, a blood sample (five mL) was taken from healthy humans (non-suffering from any disease) and then centrifuged at 2,500 rpm for 10 min in order to separate the plasma from cells. Then, the cells were collected and washed three times using 150 mM of NaCl, followed by centrifugation to remove NaCl and collect the cells. Cells suspension was prepared as 2% in phosphate buffer saline (PBS) with pH 7.4. Different doses of tested samples were added to the cells suspension then completed by the PBS to 1 mL, followed by incubation at 37 °C for 1 h in water bath. Finally, at the same prior condition, the cells suspension was centrifuged, and the absorbance of the collected supernatant was recorded at 541 nm. The positive control and blank sample were represented by deionized water and PBS, respectively. The hemolysis % was estimated *via* the next expression:

$$\text{Hemolysis } \% = \frac{\text{Absorbance of supernatant} - \text{Absorbance of PBS}}{\text{Absorbance of deionised water}} \times 100 \quad (2)$$

Experimental: Molecular Docking

In the current research, molecular docking was carried out to explore the probable molecular mechanisms underlying the antibacterial activity of cis-8,11,14-eicosatrienoic acid. The molecular interactions were studied for binding affinities of selected compounds of oil with bacterial receptors that play key roles in cell growth and DNA duplication. The crystal structures of the proteins identified for the *Salmonella typhi* (5ZXM) were supplied from the bank of protein data (http://www.rcsb.org/pdb, accessed on 20 June 2021). The protein's surrounding water molecules were eliminated, and hydrogen atoms were then added. The MMFF94x force field was used to assign the parameters and charges. Cis-8,11,14-eicosatrienoic acid (the main detected fatty acid in peanut oil) was docked in the active site using the DOCK module of MOE after alpha-site spheres were created using the site finder module of MOE. The London dG scoring function, placement: triangle matcher, retain: 10, and refinement: forcefield were used to determine the dock scoring in the MOE programme. RMSD values, binding energies and binding modes with the selected residues

were considered to identify the leading conformations of the docked ligands (Gurung *et al*. 2021; Qanash *et al*. 2022).

Statistical Analysis

All experiments were achieved in triplicate, and the findings are described as the mean to calculate standard deviation of the obtained results.

RESULTS AND DISCUSSION

Fatty Acids Analysis of Oils

GC-MS analysis of the olive and peanut oils indicated its richness in fatty acids (Tables 1 and 2). GC-MS examination of peanut oil reflected the occurrence of 16 and 19 saturated and unsaturated fatty acids, respectively (Table 1).

Table 1. Detected Peanut Oil Fatty Acids by GC/MS

Retention Time	Compound Name	Type	Area	Area (%)
2.959	Enanthic acid	Saturated	10.31	1.31
3.283	Butyric acid	Saturated	3.625	0.46
4.807	Caproic acid	Saturated	3.229	0.41
4.992	Caprylic acid	Saturated	1.122	0.14
5.705	Capric acid	Saturated	0.524	0.07
6.731	Undecanoic acid	Saturated	0.763	0.10
7.103	Lauric acid	Saturated	1.172	0.15
8.214	Tridecanoic acid	Saturated	0.886	0.11
9.753	Myristic acid	Saturated	108.03	30.70
9.827	Myristoleic acid	Unsaturated	0.619	0.08
9.983	Pentadecanoic acid	Saturated	0.404	0.05
11.372	cis-10-pentadecanoic acid	Unsaturated	0.572	0.07
11.553	Palmitic acid	Saturated	1.582	0.20
11.661	Palmitoleic acid	Unsaturated	0.470	0.06
12.125	Heptadecanoic acid	Saturated	2.013	0.26
13.13	cis-10-heptadecanoicacid	Unsaturated	0.464	0.06
13.532	Stearic acid	Saturated	1.458	0.18
14.258	Olieca acid	Unsaturated	3.682	0.47
14.466	Eliadic acid	Unsaturated	0.582	0.07
14.878	Linoleic acid	Unsaturated	0.608	0.08
15.406	Linoleliadic acid	Unsaturated	0.458	0.06
15.678	Linolenic acid	Unsaturated	6.246	0.79
15.94	gamma-Linolenic acid	Unsaturated	0.408	0.05
16.083	Arachidic acid	Saturated	0.736	0.09
16.15	cis-11-Eicosenoic acid	Unsaturated	0.536	0.07
6.248	cis-11,14-Eicosadienoic acid	Unsaturated	3.086	0.39
16.485	cis-11,14,17-Eicosatrienoic acid	Unsaturated	15.40	1.95
16.574	cis-8,11,14-Eicosatrienoic acid	Unsaturated	425.56	53.98
16.866	Arachidonic acid	Unsaturated	182.04	23.09
17.039	cis-5,8,11,14,17-Eicosapentaenoic acid	Unsaturated	6.216	0.79
17.311	Heneicosanoic acid	Saturated	0.566	0.07
19.062	Behenic acid	Saturated	0.910	0.12
19.353	Erucic acid	Unsaturated	1.063	0.13
19.465	cis-13,16-Docosadienoic acid	Unsaturated	2.559	0.32
19.749	cis-4,7,10,13,16,19-Hexaenoic acid	Unsaturated	0.463	0.06

Cis-8,11,14-eicosatrienoic acid, myristic acid, and arachidonic acid represented 54.0%, 30.7%, and 23.1%, respectively. Other important fatty acids included lauric acid, erucic acid, and caprylic acid. Lauric acid and caprylic acid have antibacterial and antiviral activity (Fischer *et al*. 2012; Matsue *et al*. 2019). They have low inhibitory potential toward commensal lactic acid bacteria, while high inhibitory potential toward *Clostridium* and *Bacteroides* (Matsue *et al*. 2019). The mechanism of antimicrobial activity was previously reported (Bergsson *et al*. 1998): the cell wall or membrane of bacterial pathogens are disrupted by these acids. Seven saturated and 10 unsaturated fatty acids were recognized in olive oil (Table 2). Most of the unsaturated fatty acids were detected with high area%, such as arachidonic acid (23.2 %), cis-11,14,17-eicosatrienoic acid (22.7 %), and cis-11-eicosenoic acid (11.4 %). All fatty acids detected in olive oil were detected also in peanut oil. Kanlayavattanakul and Lourith (2011) reported that the natural sources containing linoleic acid exhibited anti-inflammatory activity and were used in skin ulcer treatment. According to Kapseu (2009), the stability of peanut oil is due to the composition of fatty acids including more than 47% of monounsaturated fatty acids.

Table 2. Detected Olive Oil Fatty Acids by GC/MS

Retention Time	Compound Name	Fatty acid type	Area	Area%
3.306	Butyric acid	Saturated	241.3	11.9
4.746	Caproic acid	Saturated	21.45	1.1
5.026	Caprylic acid	Saturated	40.15	2.0
5.862	Capric acid	Saturated	12.52	0.6
6.525	Undecanoic acid	Saturated	13.81	0.7
13.312	cis-10-heptadecanoicacid	Unsaturated	145.91	7.2
13.906	Stearic acid	Saturated	10.78	0.5
14.057	Oleic acid	Unsaturated	21.28	1.1
14.088	Elaidic acid	Unsaturated	46.87	2.3
14.082	Linoleic acid	Unsaturated	24.58	1.2
17.114	cis-11-Eicosenoic acid	Unsaturated	230.12	11.4
16.409	cis-11,14-Eicosadienoic acid	Unsaturated	21.43	1.1
16.485	cis-11,14,17-Eicosatrienoic acid	Unsaturated	458.3	22.7
16.555	cis-8,11,14-Eicosatrienoic acid	Unsaturated	11.58	0.6
16.981	Arachidonic acid	Unsaturated	468.63	23.2
17.25	cis-5,8,11,14,17-Eicosapentaenoic acid	Unsaturated	147.64	7.3
17.29	Heneicosanoic acid	Saturated	104.35	5.2

TEM Characterization of the Prepared NEs

Despite the many applications of the essential oils, there are some problems that impede the performance of these applications, such as limited water solubility of oils and excessive sensitivity of oils to storage or industrial conditions associated to oxygen, heat, and light. Conversion of oils to NEs may represent a great solution to overcome these problems. The diameter of the NEs was 14.6 nm for peanut and 24.5 nm for olive (Fig. 1). The NEs of olive oils that were created in another study (Karami *et al*. 2019) had a spherical shape and diameter of 40 nm. The size of NEs may be influenced by quantities and type of the applied surfactants (Campolo *et al*. 2020).

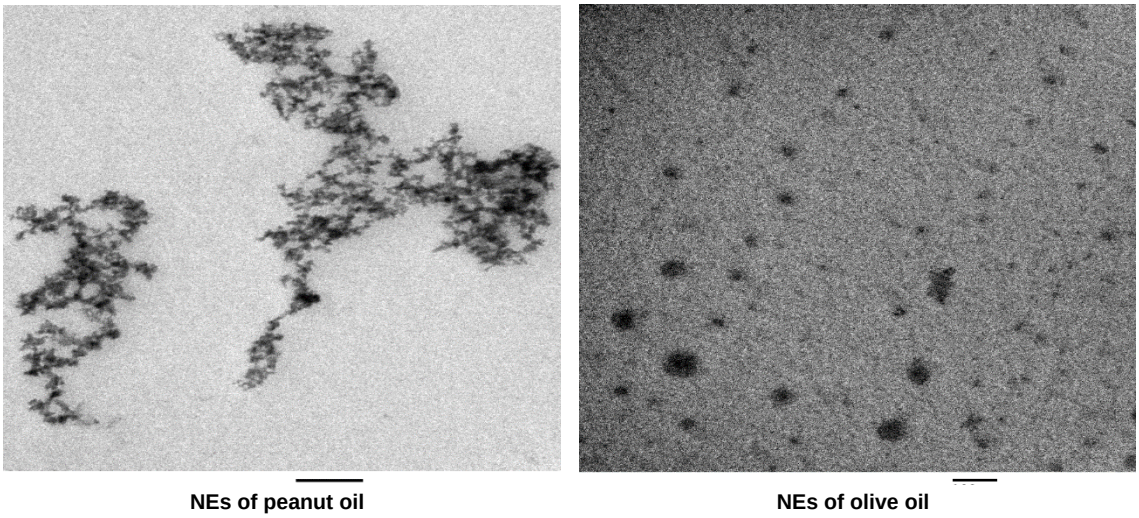


Fig. 1. TEM of NEs of olive and peanut oils. Magnification, 10000 X; Scale bar, 100 nm

Biological Activity

The tested microorganisms varied in their susceptibility towards the same oil and its NEs (Table 3 and Fig. 2). There was a slight change in the inhibition zone of *B. cereus*, and *A. flavus* treated with peanut oil compared with its NEs. The sensitivity of other tested organisms particularly *S. typhi* was higher (inhibition zone was 20.17 mm) when treated with NEs than when treated with peanut bulk oil (Inhibition zone was 13.17 mm). These outcomes are similar to those of Moghimi *et al*. (2016), who reported that bulk oil of sage (*Salvia officinalis*) had less bacteriostatic potential than NEs using *E. coli* as a test bacterium.

The conversion of olive oil to NEs enhanced its antimicrobial activity against all tested organisms; the inhibition zones of *B. cereus*, *S. aureus*, *E.coli, S. typhi*, *C. albicans*, and *A. flavus* were 25.8, 20.2, 20.3, 19.3, 20.8, and 18.2 mm using NEs compared to bulk oil with 21.3, 13.2, 13.3, 14.8, 16.8, and 15.5 mm, respectively (Table 3). In previous reports, NEs of some oils were tested against food-borne and human pathogens compared to bulk oils. For example, NEs of sage oil showed better bactericidal potential against *Shigella dysentery*, *Salmonella typhi*, and *Escherichia coli* (Moghimi *et al*. 2016), with MIC being four-times higher for the bulk oil than for the NEs.

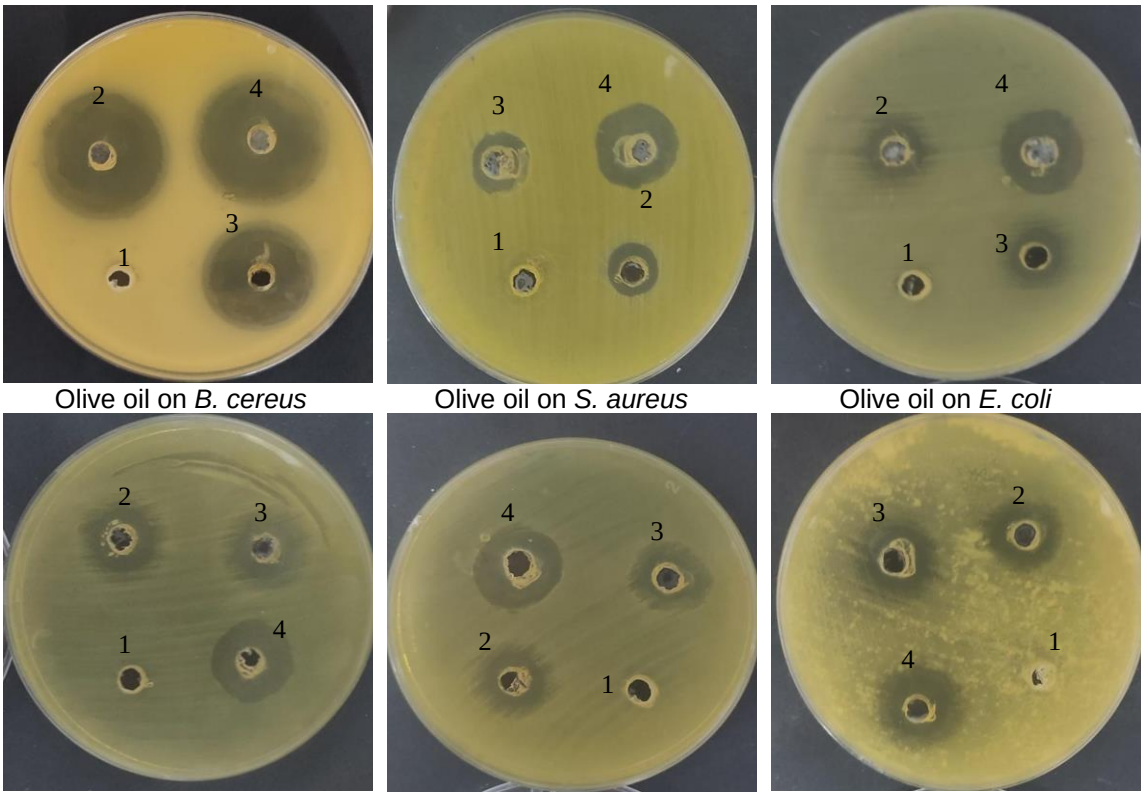
While many other studies demonstrated that the antibacterial activity of EOs was enhanced when it was converted into NEs (Salvia-Trujillo *et al*. 2015; Zahi *et al*. 2015; Maté *et al*. 2016; Lu *et al*. 2018), in some reports there was no alteration in the antimicrobial activity when the essential oils were converted into NEs (Chang *et al*. 2012; Xue *et al*. 2015). The mechanism of the bactericidal potential may be due to the electrostatic interaction among positively charged NEs and the negatively charge of the walls (Majeed *et al*. 2016).

Table 3. Antimicrobial Activity of Peanut Oil, Olive Oil, and NEs

Test Organisms	Inhibition Zone (mm)				
	Peanut Oil	NEs Peanut Oil	Olive Oil	NEs Olive Oil	Control
B. cereus	15.17±0.29	15.83±0.58	21.33±0.58	25.83±0.29	24.83±0.29
S. aureus	12.33±0.58	14.50±0.87	13.17±0.33	20.17±0.29	13.0±0.50
E. coli	15.67±0.58	17.17±1.76	13.33±0.29	20.33±0.29	13.83±0.58
S. typhi	13.17±0.29	20.17±0.29	14.83±1.26	19.33±0.58	14.83±0.29
C. albicans	15.33±0.29	20.67±0.58	16.83±0.76	20.83±0.29	15.33±0.58
A. flavus	15.67±0.58	15.83±0.76	15.50±0.50	18.17±0.29	14.17±0.76

The antioxidant activity of olive and peanut oils and their NEs are shown in Table 4. The increased concentration of olive and peanut and their NEs was accompanied by increased antioxidant activity. The achieved results indicated that NEs of the two oils, olive and peanut, exhibited more antioxidant activity with IC_{50} 102.5 µg/mL and 291.5 µg/mL than bulk oils with IC_{50} 158.6 µg/mL and 435.08 µg/mL, respectively. Olive oil and its NEs reflected the highest antioxidant activity compared to the antioxidant activity to oil and its NEs.

According to previous literature, the richen lipids with unsaturated fatty acids exhibited good antioxidant activity compared with the less saturated fatty acids containing lipids (Banskota *et al*. 2019). These results were unlike those in the literature, probably due to the presence of certain fatty acids.



Olive oil on *B. cereus* | Olive oil on *S. aureus* | Olive oil on *E. coli*

Olive oil on *S. typhi* | Olive oil on *C. albicans* | Olive oil on *A. flavus*

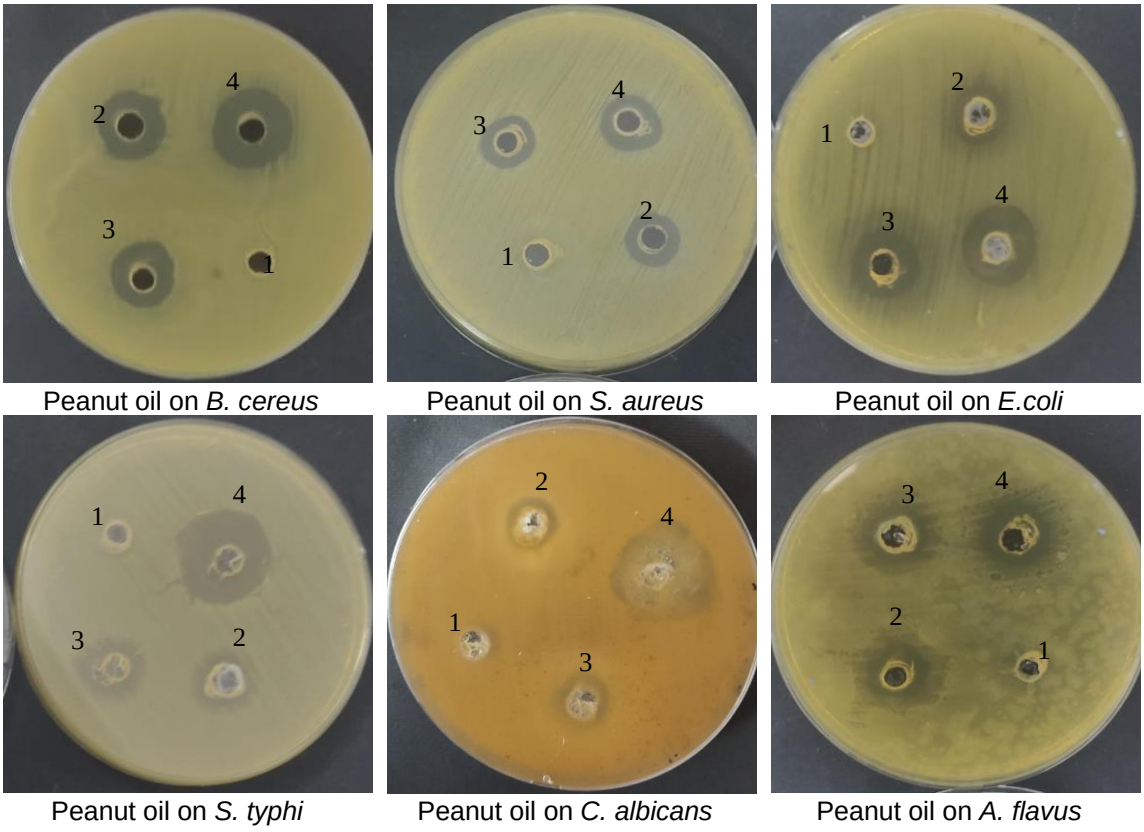


Fig. 2. Antimicrobial activity of olive oil, peanut oil and its NEs. Negative control (1), positive control (2), Oil (3), and NEs of oil (4). Positive control represented with standard antibiotic (Gentamycin) and antifungal (Fluconazole). The negative control was represented by DMSO without oils.

Table 4. Antioxidant Activity of Olive Oil, Peanut Oil, and NEs

Concentration (µg/mL)	DPPH Scavenging (%)			
	Olive Oil	NEs olive oil	Peanut oil	NEs Peanut oil
0	0.0±0.006	0.0±0.006	0.0±0.006	0.0±0.006
1.95	6.8±0.020	9.0±0.004	1.0±0.004	2.5±0.004
3.90	13.4±0.007	15.9±0.005	8.2±0.003	9.4±0.001
7.81	22.1±0.003	23.8±0.007	14.8±0.003	16.6±0.010
15.63	31.2±0.003	33.1±0.004	19.3±0.002	23.9±0.002
31.25	38.2±0.004	40.5±0.007	24.7±0.008	30.9±0.009
62.50	41.7±0.004	46.4±0.005	31.9±0.007	38.0±0.003
125	48.8±0.002	52.4±0.005	37.9±0.013	41.4±0.006
250	52.8±0.003	58.6±0.001	43.9±0.002	48.2±0.003
500	58.8±0.008	64.7±0.003	52.4±0.005	54.2±0.005
1000	66.9±0.004	71.5±0.003	58.7±0.011	60.8±0.004
IC_{50} µg/mL	158.6	102.5	435.08	291.5

A gradual rise in hemolysis% was recorded using olive oil at all used concentrations up to 1000 µg/mL (Table 5). Olive oil caused 2.7% hemolysis, while its NEs caused 19.8% hemolysis and NEs of peanut oil caused less 9.5% hemolysis than peanut oil (26.8% hemolysis) at 1000 µg/mL. Up to 200 µg/mL of the two oils and its NEs, the hemolysis % did not exceed 2%, so the inhibition of RBC lysis was near 98% with using NEs of olive oil (0.2 % hemolysis) followed by olive oil (0.9 % hemolysis), followed by NEs peanut oil (1.1 % hemolysis), and followed by NEs peanut oil (2.0% hemolysis). Some fatty acids of the oils prevent the membrane damage of RBC resulting from oxidative stress through scavenging of the generated hydrogen peroxide and peroxide radicals. The developed NEs of olive oils by Karami *et al*. (2019) exhibited only 4.6% of hemolysis with safe application when used for intravenous delivery.

Table 5. Hemolytic Activity of Olive Oil, Peanut Oil, and NEs

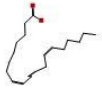
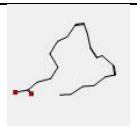
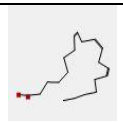
Concentration (µg/mL)	Hemolysis (%)			
	Olive Oil	NEs olive oil	Peanut oil	NEs Peanut oil
Control	100±0.005	100±0.005	100±0.005	100±0.005
50	0.3±0.002	0.4±0.003	0.8±0.006	0.8±0.006
100	0.5±0.002	0.5±0.009	2.0±0.007	2.0±0.007
200	0.9±0.001	0.2±0.009	2.0±0.004	1.1±0.009
400	1.2±0.002	3.1±0.007	10.9±0.004	1.4±0.002
600	1.9±0.002	10.9±0.003	23.2±0.004	3.4±0.002
800	2.3±0.002	12.2±0.004	25.4±0.007	6.4±0.004
1000	2.7±0.001	19.8±0.011	26.8±0.006	9.5±0.007

Molecular Modeling Study

Every synthetic effort aims to identify candidates having the best biological performance. However, the implementation of an *in silico* approach can eliminate the time-consuming and expensive process of synthesizing a large number of organic compounds and their biological screening. Molecular docking is a versatile *in silico* technique that helps to target the biologically effective templates among the libraries of compounds.

The docking process was carried out by simulating the interaction of cis-8,11,14-eicosatrienoic acid with *Salmonella typhi* (5ZXM), which were chosen from the literature in order to examine the binding mechanism and conformation structure that affect how protein interact with investigated compound.

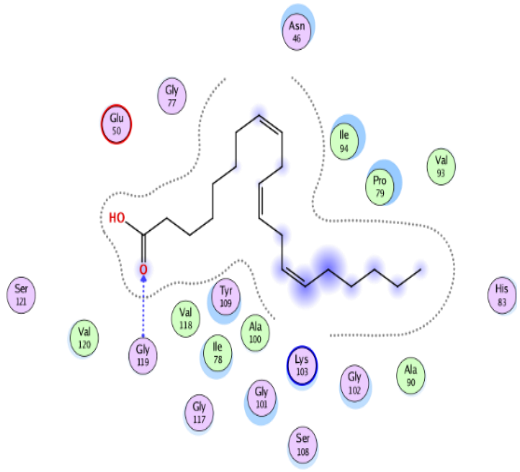
Table 6. Docking Scores and Energies of Cis- 8,11,14-Eicosatrienoic acid with 5ZXM Receptors

mol	rseq	mseq	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	E_score2
	1	1	-7.81966	3.557131	-3.72375	-60.8839	-10.1151	-41.2794	-7.81966
	1	1	-7.65453	2.406948	-2.36783	-64.2811	-9.60792	-38.959	-7.65453
	1	1	-7.61212	1.158137	-8.98535	-63.1923	-9.35917	-34.147	-7.61212
	1	1	-7.39527	2.069693	-5.77203	-56.2697	-9.71895	-32.5104	-7.39527
	1	1	-7.38209	1.510009	-1.08094	-39.4237	-9.56942	-36.6065	-7.38209

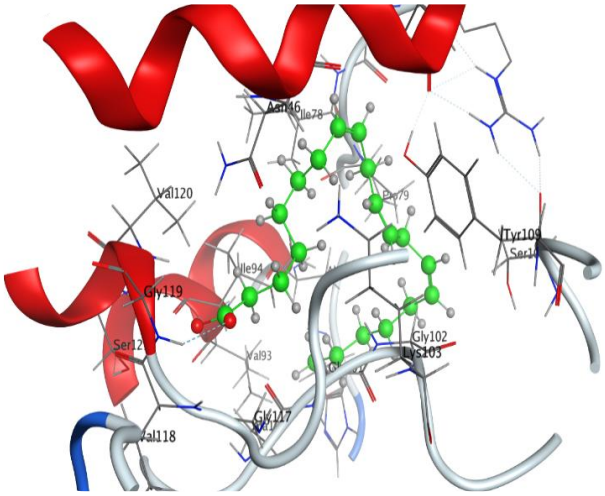
Notes: rmsd means the root mean square deviation of the pose, in Å, from the original ligand. This field was present if the site definition was identical to the definition of ligand. S means the final score, which is the score of the last stage that was not set to none. rmsd_refine means the root mean square deviation between the pose before refinement and the pose after refinement. E_place means the score from the placement stage. E_conf means the energy of the conformer. If there was a refinement stage, this is the energy calculated at the end of the refinement. Note that for force field refinement, by default, the energy was calculated with the solvation option set to Born. E_score 1, E_score means the scores from rescoring stages 1 and 2. E_refine is the score from the refinement stage, calculated to be the sum of the van der Waals electrostatic and solvation energies, under the generalized Born solvation model (GB/VI).

Table 7. Interaction of Cis- 8,11,14-Eicosatrienoic acid with 5ZXM Protein

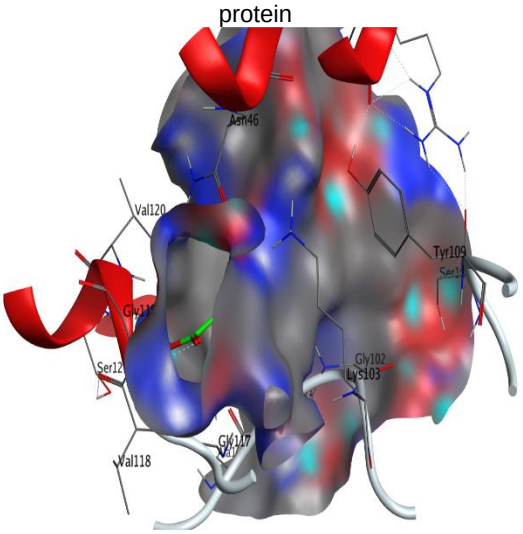
Ligand	Receptor	Interaction	Distance	E (kcal/mol)
O 24	N GLY 119 (A)	H-acceptor	3.03	-0.5



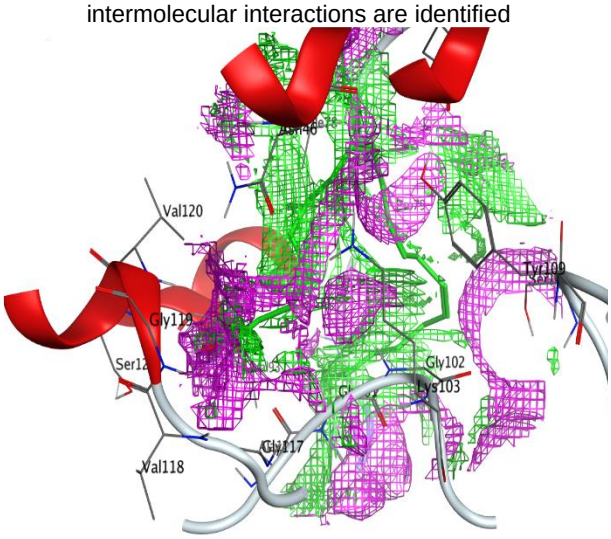
The interaction between Cis- 8,11,14-Eicosatrienoic acid and active sites of 5ZXM protein



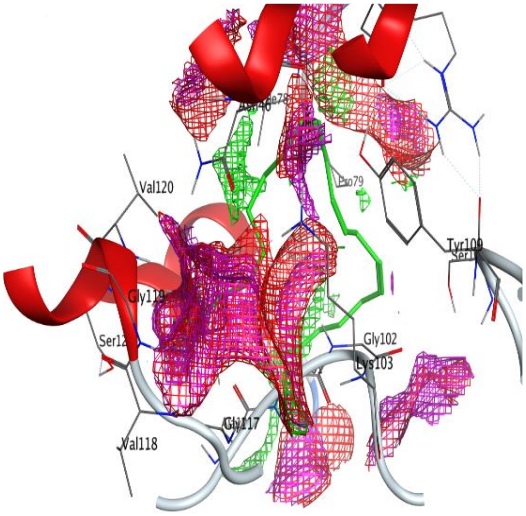
The most likely binding conformation of Cis- 8,11,14-Eicosatrienoic acid and the corresponding intermolecular interactions are identified



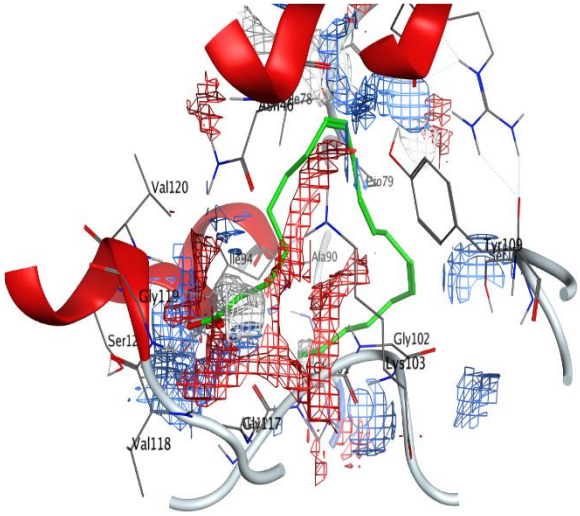
Molecular surface of Cis- 8,11,14-Eicosatrienoic acid with 5ZXM



The contact preference of Cis- 8,11,14-Eicosatrienoic acid with 5ZXM



Interaction potential of Cis- 8,11,14-Eicosatrienoic acid with 5ZXM



The Electrostatic map of Cis- 8,11,14-Eicosatrienoic acid with 5ZXM

Fig. 3. Molecular docking process of Cis- 8,11,14-Eicosatrienoic acid with 5ZXM protein

Docking of the ligand with (5ZXM) active sites indicated that there was H-acceptor interaction between the O 24 atom in the ligand with GLY 119 amino acids residue, with a distance of 3.03 Å. The free binding energy (S, kcal/mol) of the hydrogen bonds that were created between the receptors and the subject substance were utilised to rank the binding affinity. Figure (3) shows the 2D and 3D-docking modes of cis-8,11,14-eicosatrienoic acid with 5ZXM protein. The results that were collected are shown in Table 6, illustrating the ranking poses originated by the scoring functions. Hydrogen bond between cis-8,11,14-eicosatrienoic acid with coenzymes of the selected protein was offered in Table 7. Several investigations documented the biological efficacy of plant and microbial natural molecules *via* molecular docking approaches as reported on the chlorogenic acid against human coronavirus (HCoV 229E) and *Proteus vulgaris* (Qanash *et al*. 2022), bacteriostatic activity against *Helicobacter pylori* of chitosan nanoparticles loaded by extract of *Aloe vera* gel (Yahya *et al*. 2022), anticancer potential of kaempferol and chrysoeriol against breast and human prostate-cancer-associated proteins, respectively, besides activity of luteolin and neophytadiene against *E. coli* and *P. aeruginosa*, respectively (Al-Rajhi *et al*. 2022b), N-(4,6-dimethyl-2-pyrimidinyl)-4-(4-nitrobenzylideneamino) benzenesulfon-amide and benzene di-carboxylic acid against *C. albicans* and *Bacillus subtilis* (Al-Rajhi *et al*. 2022c), Molecular docking among chlorogenic acid and *E. coli* DNA (7C7N); rutin and human prostate-specific antigen (3QUM) and breast cancer-associated protein (1JNX) were also investigated (Al-Rajhi *et al*. 2022d). In sum, peanut and olive oil-based NEs can offer therapeutic and nutritional value for topical applications. In the future, there is the probability of their utilizations as carriers of low water-soluble drugs in NEs.

CONCLUSIONS

1. Peanut and olive oil-based nanoemulsions (NEs) were created using ultrasonication.
2. Various fatty acids with different levels were recognized *via* gas chromatography/mass spectrometry (GC/MS) in peanut and olive oil.
3. Compared with that of bulk oils, oil NEs of the two oils gave good antimicrobial and antioxidant activities**.**
4. NEs of olive oil caused more hemolysis than bulk oil, unlike peanut oil.
5. Molecular docking reports provided the structure–activity relationship and binding modes of cis-8,11,14-eicosatrienoic acid with *S. typhi* (5ZXM) enzymes. Compounds having little binding energies, minor RMSD values, and significant bindings with the selected residues were of interest.

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