Effectiveness of Oil-based Nanoemulsions with Molecular Docking of its Antimicrobial Potential

Husam Qanash,[a,b] Khulud Alotaibi,[c] Abdu Aldarhami,[d] Abdulrahman S. Bazaid,[a] Magdah Ganash,[e] Nizar H. Saeedi,[f] and Tarek M. Abdel Ghany [g,*]

The biological properties of plant oils are improved by their conversion to nanoemulsions (NEs). This study evaluated the antimicrobial, antioxidant, and anti-hemolytic efficacy of coconut and salad rocket oils and their NEs. The result of the gas chromatography-mass spectroscopy analysis of the oils showed varied constituents such as palmitic acid, trimethylsilyl ester; 2,3-bis(acetyloxy)propyl laurate in salad rocket oil, 2-lauro-1,3-didecoin, n-butyl laurate; laurin, tri-; laurin in coconut oil. NEs diameter of salad rocket and coconut oils was 24.6 and 29.2 nm, respectively. More inhibitory activity of NEs compared with non-NEs form against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Candida albicans*, and *Aspergillus flavus* was detected. Coconut oil and its NEs caused 14.3% (anti-hemolysis 85.7%) and 22% hemolysis (anti-hemolysis 78%), respectively. Salad rocket oil and its NEs caused hemolysis 3.4% and 20.9%, respectively at 1000 µg/mL. Antioxidant activity of salad rocket and coconut oil reflected more IC_{50} (39.3 and 109.4 µg/mL) than its NEs (35.8 and 80.5 µg/mL), respectively. Molecular docking of trimethylsilyl ester and 2-lauro-1,3-didecoin against *S. aureus* (PDB=7BGE) and *C. albicans* protein (PDB=3DRA) revealed optimal binding mode that had the most energy interaction with the binding sites.

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Contact information: a: Department of Medical Laboratory Science, College of Applied Medical Sciences, University of Ha'il, Ha'il 55476, Saudi Arabia; b: Molecular Diagnostics and Personalized Therapeutics Unit, University of Ha'il, Ha'il 55476, Saudi Arabia; c: Biology Department, Faculty of Science, Jazan University, Jazan 45142, Saudi Arabia; d: Department of Medical Microbiology, Qunfudah Faculty of Medicine, Umm Al-Qura University, Al-Qunfudah 21961, Saudi Arabia; e: Biology Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia; f:Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, University of Tabuk, Tabuk, Saudi Arabia; g: Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo 11725, Egypt;
** Corresponding author: tabdelghany.201@azhar.edu.eg (TMA), ahdarhami@uqu.edu.sa(AA)*

INTRODUCTION

Nanoparticles are used in various fields including medicinal, pharmacological, agricultural, chemical, and electrical. Nano-form materials have the advantages of ratio of size to shape, more surface energy, and high surface area (Abdelghany *et al*. 2018; Ganash *et al*. 2018; Al-Rajhi *et al*. 2022a). The properties of nanomaterials (NMs) enable them to penetrate easily inside the materials, which can lead to rapid dissolution and spreading on the materials surface, as well as faster interaction with materials. According to Tadros *et al*. (2004), nano-emulsions (NEs) are kinetically stable structures in which droplets typically have a size range of 50 to 200 nm. Thus, NEs exhibit long-term physical stability without the long-term visible aggregation or coalescence that is characteristic of these

NMs. The long-term stability of NEs may be due to steric stabilization that occurs when applying nonionic polymers or surfactants (Tadros *et al*. 2004). NEs offer additional advantages such as low surfactant content, uniform surface coating, and respectable wettability, diffusion, and penetration capacity compared with microemulsions (Campolo *et al*. 2020; Shehata *et al*. 2022; Al-Rajhi and Abdel Ghany, 2023). NEs enhance the solubility of drugs, regulate drug release, prevent the degradation of drugs, and reduce drug side effects (Elsewedy *et al*. 2021). NEs in medicinal and pharmaceutical applications are utilized in oral, intravenous, and ocular drug administrations (Bernardi *et al*. 2011). In addition, the NEs size of these systems can enhance the effectiveness of functional constituents. However, the construction of stable oil-based NEs may depend on the combination of essential oil/surfactant and/or its ratio thereof and may need great energy inputs like sonication. The process of sonication and large amounts of surfactant usually reduce the size of NEs droplet (Liu *et al*. 2018; Mirgorodskaya *et al*. 2020).

Several oils are applied in pharmaceutical and nutritional fields as natural feed additives. For example, watercress contains great quantities of vitamins like A, B6, and C, riboflavin, and minerals such as iron, calcium, phosphorus, and manganese (Leclercq *et al*. 1998). Recently, Abdul Kareem and Dhaher (2021) mentioned that watercress (*Nasturtium officinale*) oil is a promising natural constitutes with abundant therapeutic and nutritional values. Moreover, the antifungal activity is a safe alternative to synthetic antimicrobials.

Plant essential oils also have been developed as oil-based NEs to fight phytopathogenic bacteria and mycotoxigenic fungi. For example, NEs of ginger oils suppress the growth of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), which is the causative agent of leaf blight in rice (Adamu *et al*. 2021). NEs of thyme and carvacrol oils were applied as antifungal treatments against *Aspergillus fumigatus*, causing alterations in the structure of conidia and fungal hyphae (Hassanien *et al*. 2021). NEs of *Rosmarinus officinalis* oil have been applied (Eid *et al*. 2022) to inhibit *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and methicillin-resistant *Staphylococcus aureus* (MRSA). The antioxidant and antimicrobial activities of *E. sativa* oil and its NEs have been evaluated (Eid *et al*. 2020); the antioxidant and antibacterial activities of NEs are greater than the non-form NEs. *Eruca sativa* (Jarjeer) is an annual herb that belongs to the Brassicaceae family and is used as a food, medical applications such as antibacterial, antioxidant, antidiabetic, antiplatelet, and antihypertensive activities, and to stimulate hair growth (Noor and Iman 2019). Various phytochemicals including carotenoids, sterols, flavonoids, tannins, phenolic acids, terpenes, glycosides, alkaloids saponins, and other secondary metabolites have been detected in the Jarjeer extract (Noor and Iman 2019). The best antimicrobial activity of NEs of *E. sativa* oil has been observed against MRSA, *Staphylococcus aureu*s, *Malassezia furfur*, *Escherichia coli*, without any irritation of the skin when applied (Sanad and Mabrouk 2016). Gulfraz *et al*. (2011) investigated *E. sativa* seeds oil against various species of bacteria. Coconut oil is a natural edible additive composed of important constituents such as vitamin E, lauric acid, caprylic acid, and myristic acid. These constituents exhibit many biological activities such as antitumor, antioxidant, antithrombotic, and hypolipidemic effects (Pengon *et al*. 2018). According to Khor *et al*. (2014), the coconut oil formulation in nanoemulsion form increases its consumption by humans and enhances its pharmaceutical potential. In another study, NEs of coconut oil showed good inhibition against *Staphylococcus aureus* growth; bacterial growth and inflammation were decreased using the prepared coconut oil NEs (Bergsson *et al*. 2001; Hosny *et al*. 2020).

In the current research, oil from *E. sativa* (mill) was used. This plant has different common names: in English it is known as salad rocket, garden, rocket, and arugula; in German it is known as salatrauke; in Spanish it is known as eruca; in French it is known as roquette; in Italian it is known as rucola; and in Arabic it is known as garger. The other oil, *Cocos nucifera*, is known as coconut oil. Regarding the therapeutic and nutrition applications, the current study assessed the antimicrobial, antioxidant, and anti-hemolytic activities of nano-emulsions of two oils including salad rocket and coconut due to the frequent use of these oils as therapeutic and nutritional materials. Docking studies were used to document the antimicrobial activity of the major constituents of the oils.

EXPERIMENTAL

Materials

The used chemicals were in analytical grade form and obtained from Sigma-Aldrich (St. Louis, MO, USA). The chemicals were used to prepare growth media for bacteria and fungi. These included solvents, reagents, polysorbate 80 (Tween 80), and buffers. Rocket salad and coconut oils were purchased from Al- Gomhuria Company (Cairo, Egypt).

Preparation of Oil Nano-emulsion and Characterization by Transmission Electron Microscopy (TEM)

A non-ionic surfactant (Tween 80) was disseminated in distilled water at 2% v/v and then shaken for 10 min *via* magnetic type stirrer to develop a homogeneous solution. Fixed oil (1:100) was added slowly with continuous stirring for 10 min. The developed oil emulsion was sonicated at 20 KHz frequency for 20 min *via* probe ultrasonic homogenizer (Silent Crusher M, Heidolph, Germany) to produce a translucent nano-emulsion (Salvia-Trujillo *et al.* 2013; Moradi and Barati 2019). The formulated oil nano-emulsion was saved in the dark at 4 ºC for further analysis and biological activities throughout 7 days. TEM (JEOL JEM-1200, Tokyo, Japan) was applied to note the size and shape of the prepared nano-emulsion. Each nano-emulsion was stained by phosphotungstic acid, fixed on a copper grid top (400 mesh) enclosed with amorphous carbon film, and observed by TEM.

GC-MS Analysis

The phyto-constituents of oils were discovered *via* gas chromatography attached with a mass spectrometer (GC-MS) (ThermoScientific, Waltham, MA, USA)-MS (ISQ Single Quadrupole Mass Spectrometer)). Helium (high purity, 99.99%) was applied as transferor gas (1 mL/min) at a constant flow level of 1 µL of the oil, which was injected into the GC by split style (split ratio of 1:100) with capillary column TR-5MS of 30 m × 0.32 mm × 0.25 µm. The injector temperature and ion-source temperature were 250 and 280 °C, respectively. The oven was programmed for 2 min at 110 °C, gradually increased for 10 °C/min up to 200 °C/ min, followed by an increase of 5 °C/ min to reach 280 °C/min, and then held at 280 °C for 9 min. At 70 eV, the mass spectra were taken; the time necessary for chromatography was 20 min. The level percentage of each detected constituent was estimated according to the average peak area of each constituent to the total areas. The GC-MS spectra were compared with the database provided from the National Institute of Standard and Technology at https://www.nist.gov/, as previously described (Abdelghany *et al*. 2021).

Agar Well Diffusion

The antimicrobial activity was tested on *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Candida albicans*, and *Aspergillus flavus*. To evaluate the antimicrobial activity of oil and its nano-emulsions (NEs), the agar well diffusion method was applied. Agar wells were punctured with a sterile cork borer (6 mm) and inoculated with the test microorganisms. The tested compound (100 µL) was injected in the well under aseptic condition. Under appropriate conditions (25 °C and 3 days incubation period for fungi; 37 °C and 24 h incubation period for bacteria), the inoculated plates were incubated. The visualized inhibition zones around the loaded wells were measured in millimeters (Abdelghany 2013). Gentamycin and fluconazole were used as positive controls for antibacterial and antifungal, respectively. Dimethyl sulfoxide for oils disbanding was applied as a negative control.

DPPH Radical Scavenging

The antioxidant activity of oil and its NEs was evaluated *via* free radical scavenging activity using 11-diphenyl-2-picryl hydrazyl (DPPH). One mL of 0.1 mM solution of DPPH was added to 3 mL of each concentration (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, 1000 µg/mL) oil, and its NEs were dissolved in ethanol. The reaction mixture was shaken vigorously and left at 25 °C for 30 min. The absorbance of the reaction mixture was read at 517 nm, utilizing a spectrophotometer (UV-VIS). Ascorbic acid was used as a standard compound for antioxidant activity. The log dose inhibition curve was used to calculate the quantity of oil and its NEs required to inhibit 50% (IC_{50} value) of the DPPH free radical (Abdelghany *et al*. 2019) , as follows,

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

where A_c and A_t are the absorbance at using the control reaction and the tested compound, respectively.

Hemolytic Activity

The hemolytic activity of oil and its NEs was evaluated according to Bulmus *et al.* (2003). Five mL of fresh blood sample were collected from a healthy human according to medical ethics guidelines. The sample of blood at 2500 rpm was centrifuged for 10 min. The collected cells after removing the plasma were washed 3 times using 150 mM NaCl, followed by centrifugation as mentioned in the first step. The collected cells after removing NaCl were suspended in phosphate buffer saline (PBS) adjusted at pH 7.4 to obtain final concentration 2% of the collected cells. Oil or its NEs at various quantities (ranged from low dose 50 up to high dose 1000 µg/mL) were added to 2% of cell suspension, which was adjusted to a final volume of 1 mL using PBS. The mixture was kept for 1 h in water bath adjusted at 37 °C, then centrifuged (2500 rpm for 15 min). The wavelength of the collected supernatant was measured at 541 nm. The blank sample contained only PBS; the positive control was deionised water. Hemolysis was calculated by Eq. 2,

$$\text{Hemolysis (\%)} = \frac{(A_{\text{sample}} - A_{\text{blank}})}{A_{\text{postive control}}} \times 100 \quad (2)$$

where *A* stands for absorbance.

Molecular Docking

Molecular docking has become a progressively significant tool for discovery and understanding the mechanisms of drug action. Moreover, the approach of molecular docking can be utilized to predict the preferred interaction and affinity of a ligand in the binding site of a protein. This can make it possible to describe the molecules behavior in the binding site of target proteins and clarify the vital biochemical manners. The structural and chemical characteristics of this specific set of molecules that may have an impact on the apoptotic phenomena were discovered using a computer study. The poses of inhibitors trimethylsilyl ester and 2-lauro-1,3-didecoin within the binding site of *S. aureus* (PDB=7BGE) and *C. albicans* protein (PDB=3DRA) were studied (Pantsar and Poso 2018; Boittier *et al*. 2020) via molecular operating environment (MOE) 2019.0102 program. The receptor structures were identified directly from Protein Data Bank (https://www.rcsb.org/). The downloaded structures were prepared for docking by removing all water molecules and other metal ions or ligands. The primary chain was docked, and the selected chain was then fixed and protonated, utilizing the tools for structure preparation that were already there. In order to build the dummy sites that served as the binding pocket, the MOE site finder generated the active binding sites. The studied compounds were minimized and optimized for the docking process. The dock scoring in MOE software was designed via the London dG scoring function and refined utilizing two different approaches. The greatest five constructions that were existent in the crystal structure and had a lower RMSD value were predicted by the docking process. Using the visualising programme PyMol, the complexes were examined for interactions and their 3D images were captured. Additionally, the RMSD and RMSD-refine fields were utilized to compare the findings of pose-with-pose in the co-crystal ligand location before and next modification, respectively.

Statistical Evaluation

Three replicates of experiment were designed to estimate the standard de*via*tion (SD). IC_{50} depending on GraphPad Prism® software (version 5.0, Boston, USA) of the activity of DPPH radical scavenging was calculated. All investigational outcomes were achieved in triplicate. The standard deviation (SD) and variance were designed via SPSS ver. 22.0 software (version 14, IBM, Armonk, NY, USA).

RESULTS AND DISCUSSION

Phytochemical Constituents

In the current study, salad rocket oil was analyzed *via* GC-MS (Fig. 1) which reflected the presence of 12 compounds based on the molecular formula, mass, area %, and retention time (RT) (Table 1). Based on area percentage, palmitic acid, TMS derivative (44.7%) represent the main compound, while 3,5-di-t-butyl-4-hydroxybenzoic acid, ethyl ester was detected with low area (0.55%) in salad rocket oil. Other detected compounds with different area % were identified (Table 1). Several biological activities were reported by other studies (Parthipan *et al*. 2015) for the detected compounds in the current study. For example, cis-9,cis-12-octadecadienoic acid showed anti-histaminic, anti-inflammatory, anti-arthritic, anti-eczemic, anti-androgenic, anticoronary, anticancer, antihyperchol-esterolemic, besides its applied as hepatoprotective and inhibitor for 5-α reductase. Essential oil from the leaves of the salad rocket contains 67 volatile constituents

(Awadelkareem *et al*. 2022), with the major constituents including 4-methylthio-butylisothiocyanate and 5-methylthiopentanonitrile. One compound containing sulfur, namely 5-hydroxy-3,3,6,6-tetramethyl-4-thiepanone, was detected in salad rocket oil. However, Awadelkareem *et al*. (2022) mentioned that salad rocket oil was characterized by a more content of sulfur containing constituents. In an earlier analysis of salad rocket seed oil, erucic acid (51.2%) was the main detected compound followed by 15.1% oleic acid and 12.5% cis11-eicosenoic acid (Gulfraz *et al*. 2011).

GC-MS analysis revealed 13 compounds in coconut oil (Table 2 and Fig. 2). 2-Lauro-1,3-didecoin was the main compound in coconut oil (53.7%). Other compounds such as n-butyl laurate; laurin, tri- and laurin, 2-mono- were detected in coconut oil but with low and various area %. 2-Lauro-1,3-didecoin exhibited several biological utilizations, for example antioxidant, anti-allergy, antimicrobial, and anti-dandruff, as mentioned previously (Mela *et al.* 2013). Coconut oil in the current decade has become attractive due to its content of monolaurin, which reveals effects against bacteria, fungi, viruses, and protozoa (Pengon *et al*. 2019). Another constituent in coconut oil, namely n-hexadecanoic (area 2.12%) (Table 2), exhibited antioxidant, hypocholesterolemic, and anti-inflammatory property in addition to its inhibition of 5-α reductase (Ponnamma and Manjunath 2012). Oleic acid was a minor constituent of coconut oil (0.59%) (Table 2), which reduces inflammation, tumor necrosis, and interleukins (IL-5, IL-6, and IL-8) (Aira *et al*. 2021).

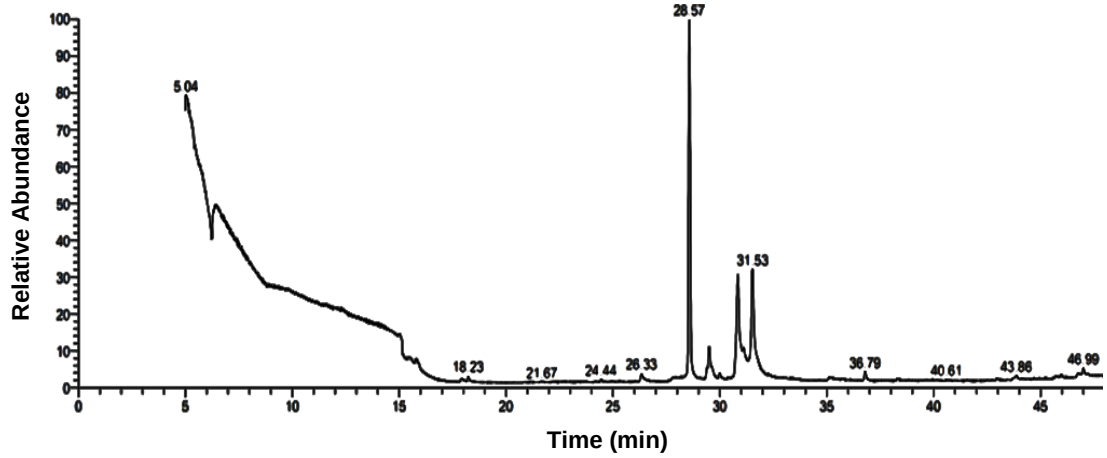


Fig. 1. GC-MS chromatograph of rocket salad oil

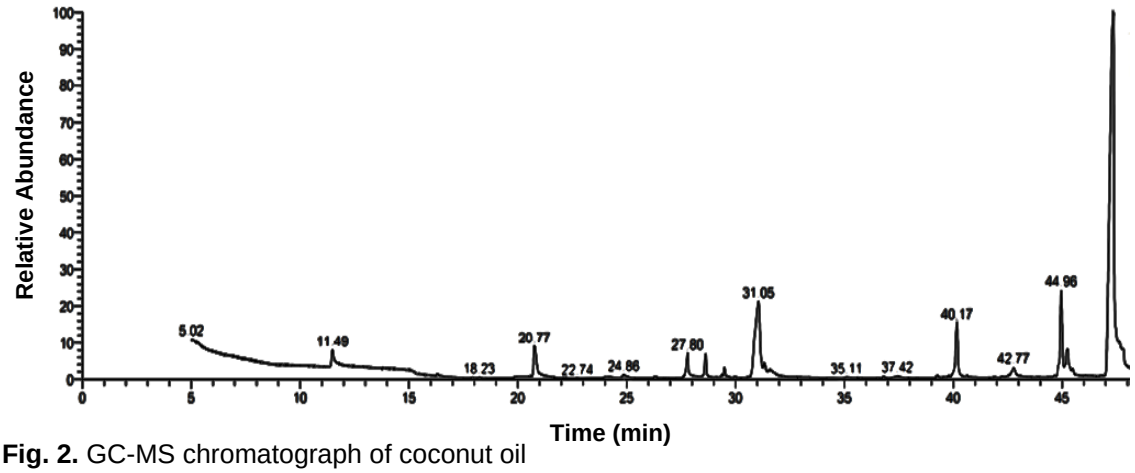


Fig. 2. GC-MS chromatograph of coconut oil

Table 1. Compounds in Rocket Salad Oil Detected by GC-MS

RT	Compound Name	Molecular Formula	Area %	Molecular Weight
6.39	5-Hydroxy-3,3,6,6-tetramethyl-4-thiepanone	$C_{10}H_{18}O_2S$	13.92	202
15.11	1,1,1,2,2-Pentamethyl-2-[(1-pentylnonyl)oxy]disilane	$C_{19}H_{44}OSi_2$	4.39	344
18.23	3,5-Di-t-butyl-4-hydroxybenzoic acid, ethyl ester	$C_{17}H_{26}O_3$	0.55	278
26.33	2,3-BIS(Acetyloxy)propyl laurate	$C_{19}H_{34}O_6$	1.08	358
28.57	Palmitic acid, trimethylsilyl ester	$C_{19}H_{40}O2Si$	44.68	328
29.5	Oleic acid, methyl ester	$C_{19}H_{36}O2$	7.17	296
29.98	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	$C_{19}H_{36}O_3$	0.67	366
30.84	cis-9,cis-12-Octadecadienoic acid	$C_{18}H_{32}O_2$	12.08	280
31.11	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	0.84	322
31.52	9-Octadecenoic acid, (E)-, TMS derivative	$C_{21}H_{42}O_2Si$	12.45	354
36.79	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	$C_{28}H_{25}NO_7$	1.31	487
47.0	Stigmast-5-ene, 3á-(trimethylsiloxy)-, (24S)-	$C_{32}H_{58}OSi$	0.86	486

Table 2. Compounds in Coconut Oil Detected by GC-MS

RT	Compound Name	Molecular Formula	Area %	Molecular Weight
11.49	Octanoic acid, trimethylsilyl ester derivative	$C_{11}H_{24}O_2Si$	1.61	216
20.77	Dodecanoic acid, trimethylsilyl ester derivative	$C_{15}H_{32}O_2Si$	4.18	272
27.8	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	2.12	256
28.62	Palmitic acid, trimethylsilyl ester derivative	$C_{19}H_{40}O_2Si$	2.16	328
29.49	Oleic acid, methyl ester	$C_{19}H_{36}O_2$	0.70	296
31.04	cis-9,cis-12-Octadecadienoic acid	$C_{18}H_{32}O_2$	15.93	280
31.32	Oleic acid	$C_{18}H_{34}O_2$	0.59	282
40.17	Laurin, 2-mono-	$C_{15}H30O_4$	5.45	274
42.77	n-Butyl laurate	$C_{16}H_{32}O_2$	0.88	256
44.96	Laurin, tri-	$C_{39}H_{74}O_6$	8.76	638
45.24	Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{27}H_{52}O_5$	2.87	456
47.35	2-Lauro-1,3-Didecoin	$C_{35}H_{66}O_6$	53.72	582
47.83	4-[7-Acetoxy-2,2-dimethyl-3a-(2-oxo-ethyl)-tetrahydro-[1,3]dioxolo[4,5-c]pyran-6-yl]-3-methyl-but-2-eno	$C_{18}H_{26}O_8$	1.03	370

TEM Characterization of the Prepared NEs

EOs have limited water solubility and great sensitivity to heat, light, and oxygen. Nanotechnology science has helped to solve these problems *via* the conversion to NEs. TEM showed that the mean size of particles of the prepared NEs of salad rocket were 24.6 nm with root-mean-square deviation 10.99, while those of coconut oil were 29.2 nm with root-mean-square deviation 11.52. All droplets of NEs were globular in shape. Through scanned NEs droplets of salad rocket oil, the mean size was 195.3 nm (Eid *et al*. 2020), and the droplet size of coconut oil NEs was between 65 and 195 nm (Hosny *et al*. 2020). As previously noted (Campolo *et al*. 2020), the characterizations of NEs represented by morphological construction and particle size were affected by type and concentration of the used surfactants.

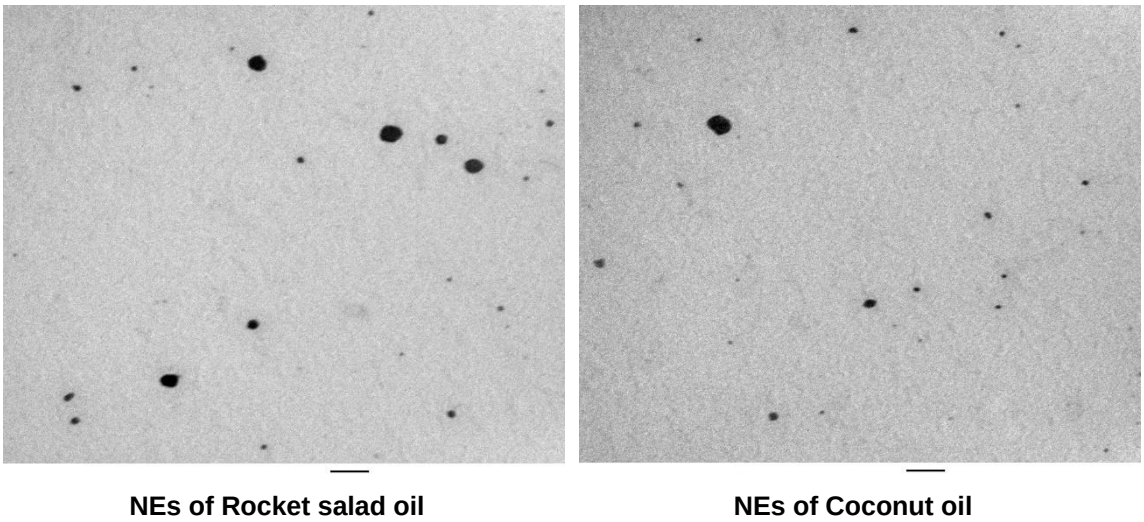


Fig. 3. TEM of NEs of rocket salad and coconut oils. Magnification, 8000 X; Scale bar, 100 nm

Antimicrobial Activity of Rocket Salad Oil, Coconut Oil, and their NEs

The antimicrobial activity of salad rocket and coconut oils and their NEs is reported in Table 3 and Fig. 4. Coconut oil was more effective than salad rocket oil against most tested microorganisms. Both NEs of salad rocket and coconut oils reflected more inhibitory action compared with non-NES against *B. cereus* (25 mm), *S. aureus* (27 mm), *E. coli* (14 mm), *S. typhi* (24 mm), *C .albicans* (22 mm), and *A. flavus* (22 mm). The efficacy of NEs is related to their capability to pass across the wall of cell and membranes, leading to destruction of the cell structures, metabolism disorders, and inhibition of protein synthesis (El-Sayed and El-Sayed 2021). As mentioned in GC-MS analysis, there were several constituents with antimicrobial activities. Numerous investigators have focused on plant oils to discover new compounds required to prevent pathogenic microorganisms, particularly after the outbreak of multidrug resistance microorganisms. The effect of salad rocket oil nanoemulgel on *S. aureus* and MRSA growth was observed, giving a 21 mm and 15 mm inhibition zone (Eid *et al*. 2020). Lately, Abdul Kareem (2021) described the fungistatic potential of watercress oil. Lauric acid and monolaurin in coconut oil have a good antibacterial potential against varied bacterial strains (Bergsson *et al*. 2001). The prepared NEs of coconut oil by Hosny *et al*. (2020) was effective against *S. aureus* due to its rich content of monolaurin. Also, the current outcomes were in agreement with Pengon *et al*. (2019) using coconut oil NEs as an inhibitor of bacterial pathogens.

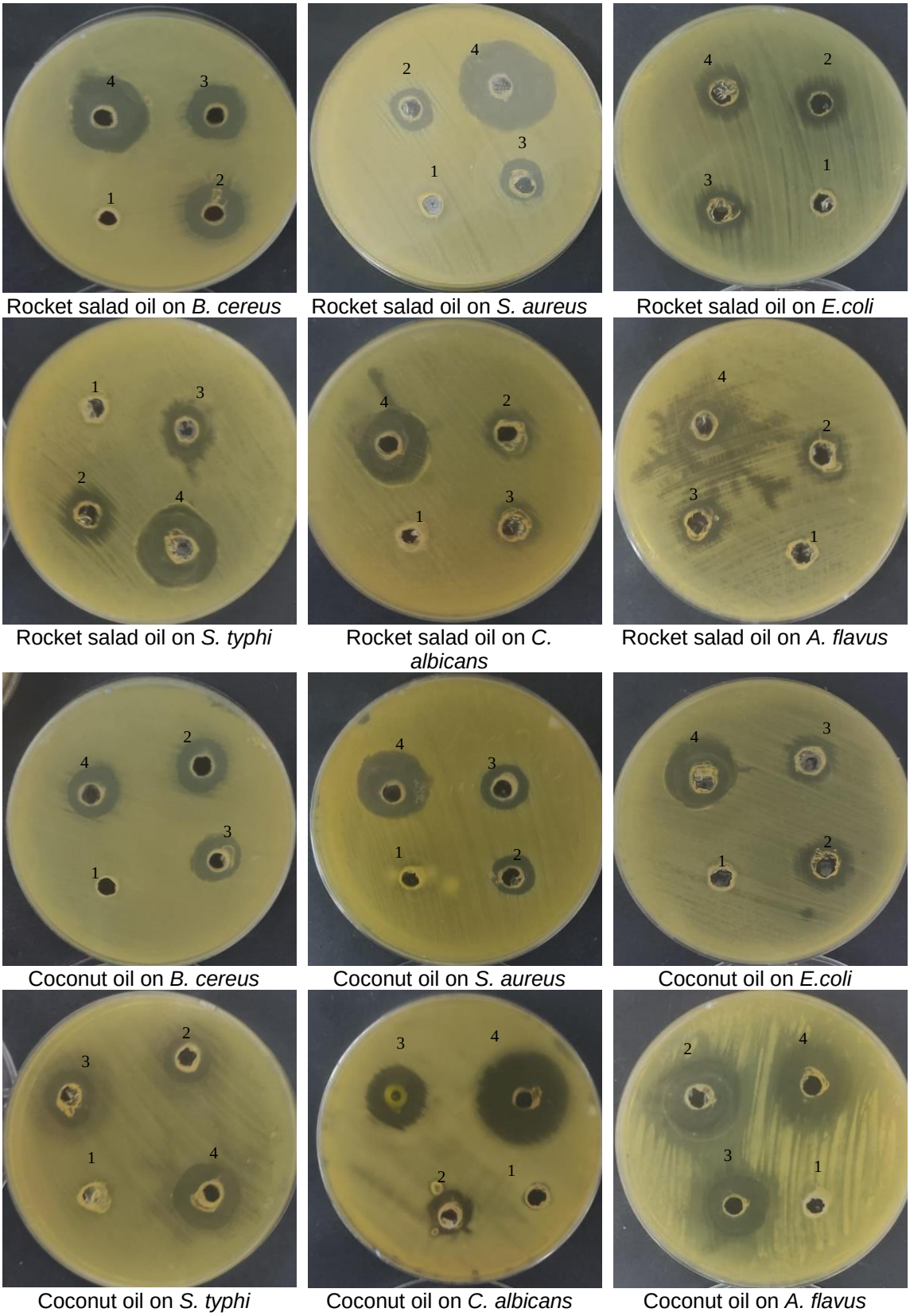


Fig. 4. Antimicrobial activity of rocket salad oil, coconut oil and its NEs. Negative control (1), positive control (2), Oil (3), and NEs of oil (4)

Table 3. Antimicrobial Activity of Rocket Salad Oil, Coconut Oil, and NEs

Test Organisms	Inhibition Zone (mm)				
	Rocket salad oil	NEs Rocket salad oil	Coconut oil	NEs Coconut oil	*Control
B. cereus	16 ±0.40	25 ±0.50	15 ±0.50	18 ±0.33	16 ±0.21
S. aureus	13 ±0.50	27 ±0.33	14 ±0.15	21 ±1.25	13 ±0.25
E.coli	13 ±0.25	14 ±0.15	14 ±0.25	21 ±0.25	16 ±0.26
S. typhi	15 ±0.33	24 ±0.25	15 ±0.33	20 ±0.33	15 ±0.33
C. albicans	13 ±0.33	22 ±0.50	18 ±0.40	27 ±0.5	15 ±0.10
A. flavus	16 ±0.25	22 ±0.33	20 ±0.4	25 ±0.57	14 ±0.33

*****Gentamycin and Fluconazole were utilized as positive controls for antibacterial and antifungal, respectively

Table 4. Anti-hemolytic Activities of Rocket Salad Oil, Coconut Oil, and NEs

Concentration (µg/mL)	Hemolysis (%)			
	Rocket Salad Oil	NEs Rocket Salad Oil	Coconut Oil	NEs Coconut Oil
Control	100±0.005	100±0.005	100±0.005	100±0.005
50	0.2±0.003	1.4±0.004	0.5±0.002	0.8±0.003
100	0.3±0.001	2.0±0.003	1.0±0.002	1.8±0.001
200	0.4±0.002	2.3±0.003	1.6±0.004	3.2±0.007
400	0.4±0.002	2.9±0.004	3.3±0.003	4.7±0.003
600	1.1±0.001	6.4±0.004	3.6±0.006	8.9±0.003
800	1.5±0.003	9.1±0.007	9.0±0.003	10.9±0.004
1000	3.4±0.005	20.9±0.022	14.3±0.011	22.0±0.004

Anti-hemolytic Activities of Rocket Salad Oil, Coconut Oil and its NEs

The anti-hemolytic activities of salad rocket and coconut oil with their NEs are shown in Table 4. Negligible hemolysis was observed at the used concentrations up to 1000 µg/mL of salad rocket oil compared with coconut oil, where the hemolysis was 3.4% and 14.3%, respectively. The NEs of salad rocket oil caused weak hemolysis at low concentrations (50 to 600 µg/mL), but moderate hemolysis (9.1 and 20.9%) was observed at 800 and 1000 µg/mL. The developed NEs of coconut oil revealed that hemolysis % reached 22% at high concentration 1000 µg/mL. The anti-hemolytic activity of the current oils and its NEs may be attributed to its content of flavonoids. Nagamani *et al*. (2016) reported and explained the anti-hemolytic activity of *C. nucifera* where the erythrocytes membrane protection from lysis and damage due to some phenolic molecules having the capability of protection from free radicals generated by H_2O_2.

Antioxidant Activities of Rocket Salad Oil, Coconut Oil and its NEs

Antioxidant activity of rocket salad oil and its NEs are presented in Table 5. Similar values of the antioxidant activity were observed between rocket salad and NEs. At low concentration up to 62.5 µg/mL the antioxidant capability of rocket salad NEs was more than bulk rocket salad oil at the same concentration (62.5 µg/mL) unlike antioxidant activity at high concentrations 125 to 1000 µg/mL. The IC_{50} of jarjeer oil was more (39.3 µg/mL) than the IC_{50} of its NEs (35.8 µg/mL). However, the antioxidant activity of coconut oil and its NEs increment as the concentration increased, but generally at all used concentrations the antioxidant activity of coconut oil NEs was better than coconut oil with IC_{50} 80.5 µg/mL and 109.4 µg/mL, respectively. The medicinal values associated with coconut oil include antioxidant, anti-hypercholesterol, and antimicrobial properties (Ng *et*

al. 2014). Various health benefits have been attributed to *C. nucifera*, such as bactericidal, antihypertensive, antioxidant, antitumor, and diuretic activities (Lima *et al*. 2015).

Table 5. Antioxidant Activity of Rocket Salad Oil, Coconut Oil and its NEs

Concentration (µg/mL)	DPPH Scavenging (%)			
	Rocket Salad Oil	NEs Rocket Salad Oil	Coconut Oil	NEs Coconut Oil
0	0.0±0.006	0.0±0.006	0.0±0.006	0.0±0.006
1.95	19.0±0.005	21.4±0.014	4.1±0.013	8.6±0.003
3.90	25.6±0.004	32.8±0.007	12.3±0.005	14.2±0.008
7.81	32.3±0.004	34.2±0.002	19.1±0.015	21.8±0.005
15.63	39.7±0.002	40.6±0.004	26.5±0.010	29.9±0.006
31.25	45.4±0.002	48.8±0.008	33.8±0.005	41.1±0.007
62.50	52.7±0.003	55.6±0.006	45.7±0.006	48.1±0.004
125	63.7±0.005	61.7±0.004	53.5±0.015	55.7±0.004
250	70.9±0.004	68.1±0.003	59.8±0.005	64.1±0.006
500	77.9±0.003	74.9±0.007	67.2±0.013	70.9±0.003
1000	86.1±0.002	81.8±0.011	74.4±0.009	77.1±0.006
IC_{50}	39.26 µg/mL	35.75 µg/mL	109.35 µg/mL	80.5 µg/mL

Molecular Docking of Trimethylsilyl Ester and 2- Lauro-1,3-Didecoin

Molecular docking is an effective method for determining the type of interaction and binding sites with the interacting molecules. The binding sites and their docking scores of the target compounds were visualised and calculated using the MOE modelling tool. Trimethylsilyl ester and 2-lauro-1,3-didecoin were docked as the main detected components of rocket salad oil and coconut oil, respectively, with the active sites of *S. aureus* (PDB=7BGE) and *C. albicans* protein (PDB=3DRA). More poses might be achieved with improved binding ways and interactions within the receptor pocket. The poses with the greatest acceptable RMSD_refined values, and the ligand's identical binding mechanism were chosen. The results are shown in Tables 6 and 7.

2- Lauro-1,3-didecoin had high binding scores against *S. aureus* and *C. albicans* protein (-7.72557 kcal/mol and -7.44338 kcal/mol respectively), which was better than trimethylsilyl ester (-6.60498 kcal/mol and -6.09047 kcal/mol, respectively). For 2-lauro-1,3-didecoin, one hydrogen bond was observed with *S. aureus* (PDB=7BGE) *via* LYS 74 (3.14Å). Another hydrogen bond was observed with *C. albicans* protein (PDB=3DRA) *via* LYS 266 (3.1 Å). For trimethylsilyl ester, binding interactions with 7BGE recorded one acceptor hydrogen bond *via* ARG 95 (3.06 Å). As well, another acceptor bond of hydrogen with 3DRA *via* GLN 218 (3.23 Å), that is expected to be vital for the activity. The hydrogen bonds between selected compounds and the select proteins are occurred inside Tables 8 and 9. The top fitted poses adopted by the constituents docked are shown in Figs. 5 and 6. The target receptor structure was stabilised by these interactions between the critical amino acid residues of the binding pockets and hydrogen bonds, ions, and hydrophobic bonds. The best docked conformations are all of the docked poses with the lowest binding energy and the highest affinity. The docking tool using MOE was able to replicate experimentally discovered binding modes to determine the specific target-ligand conformation. Investigation of these docked ligands and proteins revealed a very important molecular interaction.

Table 6. Docking Score and Energies of Trimethylsilyl ester and 2- Lauro-1,3-Didecoin with 7BGE Receptors

Molecule	rseq	mseq	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	E_score2
Trimethylsilyl ester	1	1	-6.60498	2.362471	-20.8965	-29.1949	-8.35138	-16.9025	-6.60498
Trimethylsilyl ester	1	1	-6.07463	2.632505	-25.4521	-38.6078	-8.41012	-25.7021	-6.07463
Trimethylsilyl ester	1	1	-5.56526	1.657268	-40.5765	-19.4356	-7.85219	-20.3698	-5.56526
Trimethylsilyl ester	1	1	-5.51936	2.528265	-33.1186	-38.9153	-8.32133	-20.1118	-5.51936
Trimethylsilyl ester	1	1	-5.34679	2.491409	-22.6392	-42.9003	-7.82314	-12.9582	-5.34679
2- Lauro-1,3-Didecoin	1	2	-7.72557	4.436917	29.68322	-20.0131	-6.19171	-32.0396	-7.72557
2- Lauro-1,3-Didecoin	1	2	-7.22997	3.281443	28.2072	-30.8612	-7.32446	-31.6084	-7.22997
2- Lauro-1,3-Didecoin	1	2	-7.21372	3.679614	22.5832	-30.9855	-6.14377	-26.3953	-7.21372
2- Lauro-1,3-Didecoin	1	2	-7.21062	2.453235	45.46341	-14.1364	-7.84649	-31.9192	-7.21062
2- Lauro-1,3-Didecoin	1	2	-6.99802	3.984996	32.61027	-35.3807	-6.63815	-29.7792	-6.99802

Table 7. Docking Score and Energies of Trimethylsilyl Ester and 2- Lauro-1,3-Didecoin with 3DRA Receptors

Molecule	rseq	mseq	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	E_score2
Trimethylsilyl ester	1	1	-6.09047	1.374129	-39.2074	-33.2947	-7.93164	-25.0466	-6.09047
Trimethylsilyl ester	1	1	-5.88313	2.479195	-40.96	-27.8361	-8.58773	-24.6977	-5.88313
Trimethylsilyl ester	1	1	-5.84477	1.456812	-37.0118	-45.0106	-8.0318	-25.2219	-5.84477
Trimethylsilyl ester	1	1	-5.66727	2.781741	-40.1218	-28.8572	-8.76239	-24.2859	-5.66727
Trimethylsilyl ester	1	1	-5.56632	1.489597	-39.4253	-44.236	-7.70293	-24.9744	-5.56632
2- Lauro-1,3-Didecoin	1	2	-7.44338	4.41276	21.23434	0.493912	-6.72056	-40.6945	-7.44338
2- Lauro-1,3-Didecoin	1	2	-7.29684	2.294789	29.44729	-16.9922	-6.53273	-40.8287	-7.29684
2- Lauro-1,3-Didecoin	1	2	-7.19472	3.411809	32.07007	-12.0723	-6.64437	-33.7756	-7.19472
2- Lauro-1,3-Didecoin	1	2	-7.18215	2.170147	22.88751	-9.55386	-7.52212	-39.3124	-7.18215
2- Lauro-1,3-Didecoin	1	2	-7.13228	4.195889	18.78787	-53.5035	-8.20282	-35.9559	-7.13228

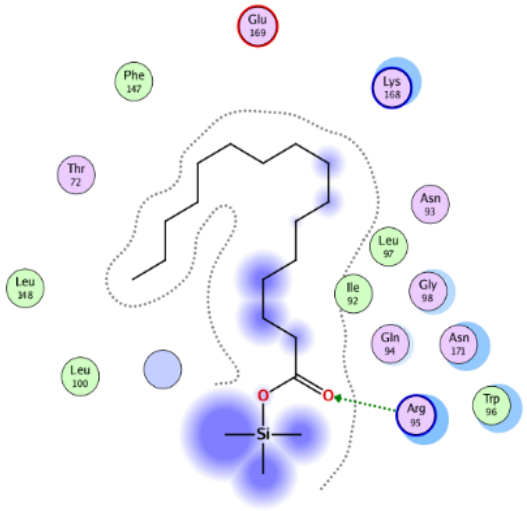
S: Final score, that is the score of the previous stage that was not set to none
Rmsd: root mean square deviation of the pose, in Å, from the original ligand Rmsd_Refine: Rmsd among the pose before refinement and the after refinement
E_Conf: The conformer energy
E_Place: Score from the placement stage
E_Scores 1 and 2: Score respectively from rescoring stages 1 and 2
E_Refine: Score from the refinement stage, evaluated to be the totality of the van der Waals electrostatics and solvation energies under the Born solvation model (GB/VI).

Table 8. Trimethylsilyl Ester and 2- Lauro-1,3-Didecoin Interaction with 7BGE Protein

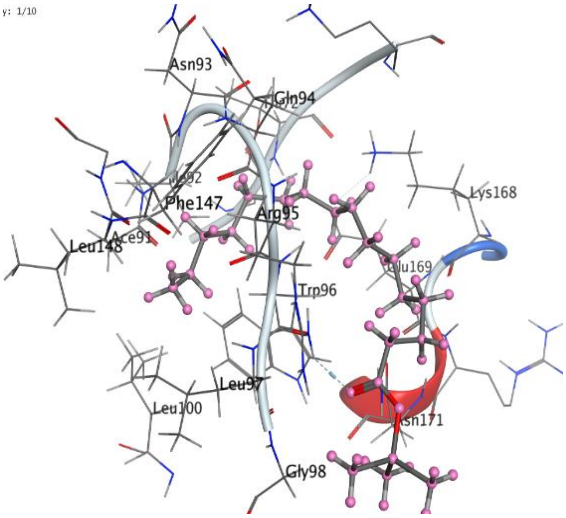
Mol	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
Trimethylsilyl ester	O 3	NH1 ARG 95 (B)	H-acceptor	3.06	-1.9
2- Lauro-1,3-Didecoin	O 74	NZ LYS 74 (B)	H-acceptor	3.14	-4.1

Table 9. Trimethylsilyl Ester and 2- Lauro-1,3-Didecoin Interaction with 3DRA Protein

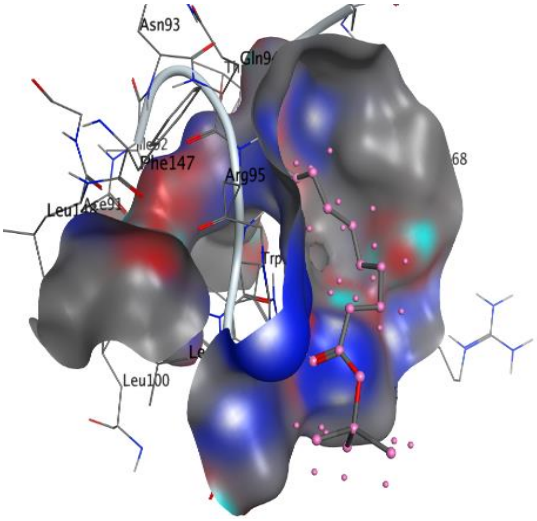
Mol	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
Trimethylsilyl ester	O 3	NE2 GLN 218 (A)	H-acceptor	3.23	-1.7
2- Lauro-1,3-Didecoin	O 69	NZ LYS 266 (A)	H-acceptor	3.10	-5.0



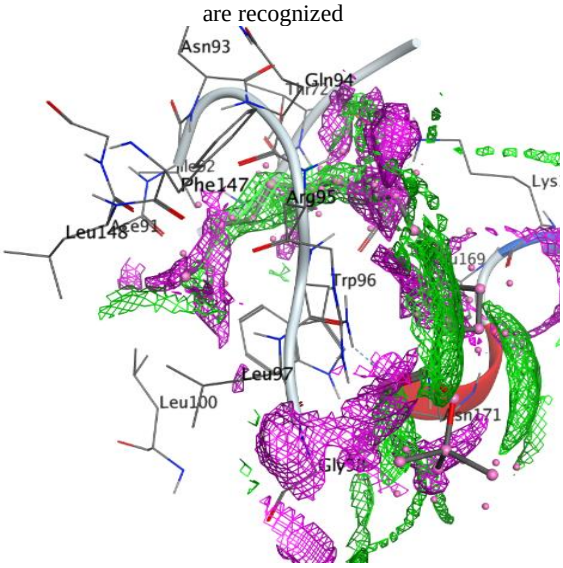
The interaction between trimethylsilyl ester and active sites of 7BGE protein



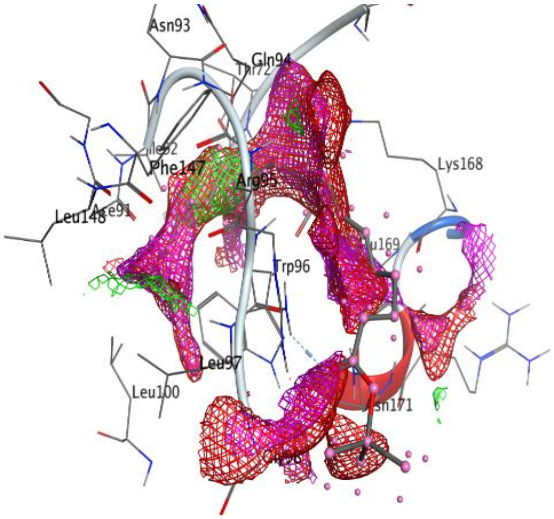
The most likely binding conformation of trimethylsilyl ester and the corresponding intermolecular interactions are recognized



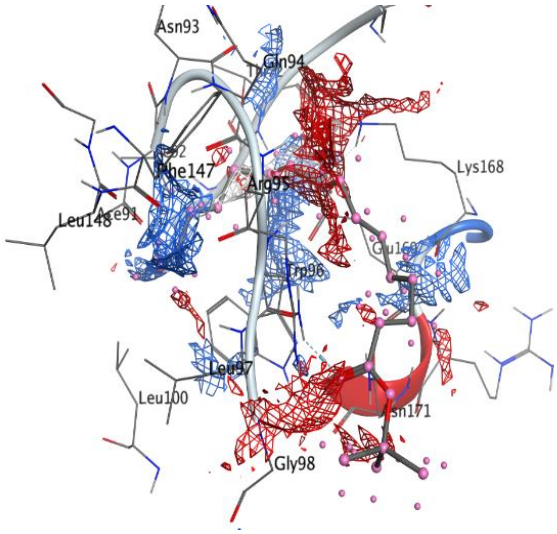
Molecular surface of trimethylsilyl ester with 7BGE



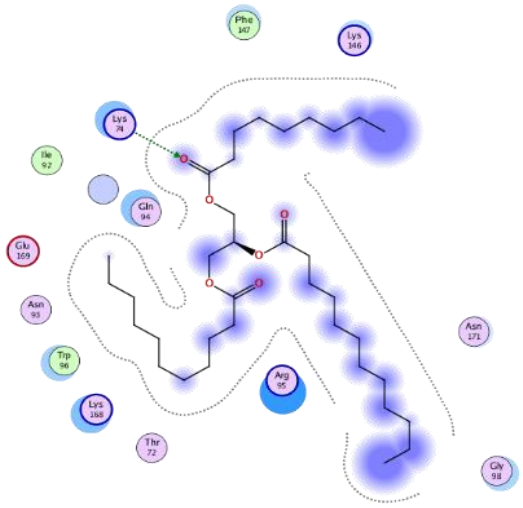
The contact preference of trimethylsilyl ester with 7BGE



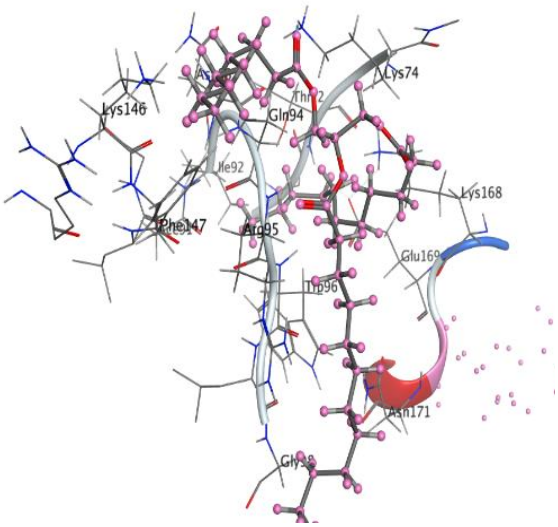
Interaction potential of trimethylsilyl ester with 7BGE



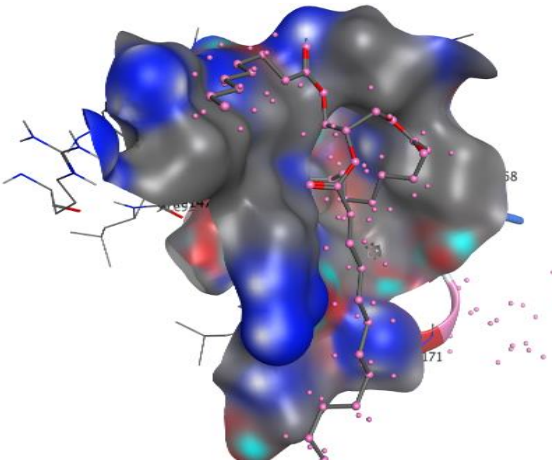
The Electrostatic map of trimethylsilyl ester with 7BGE



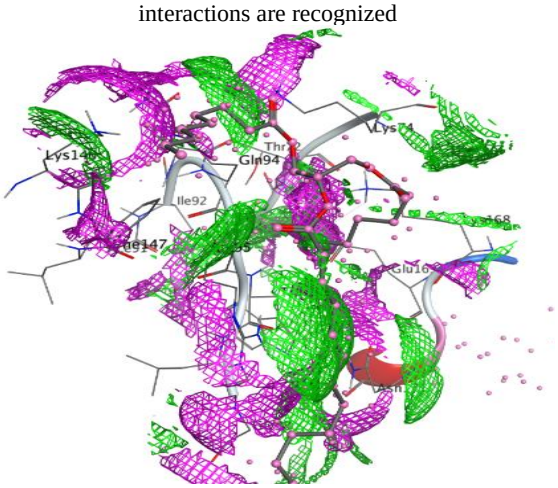
The interaction between 2- Lauro-1,3-Didecoin and active sites of 7BGE protein



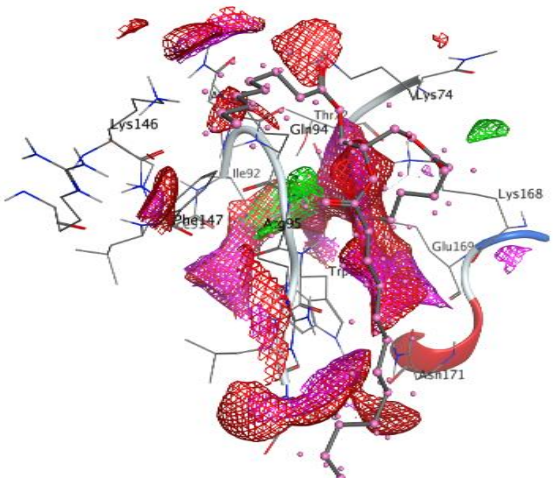
The most likely binding conformation of 2- Lauro-1,3-Didecoin and the corresponding intermolecular interactions are recognized



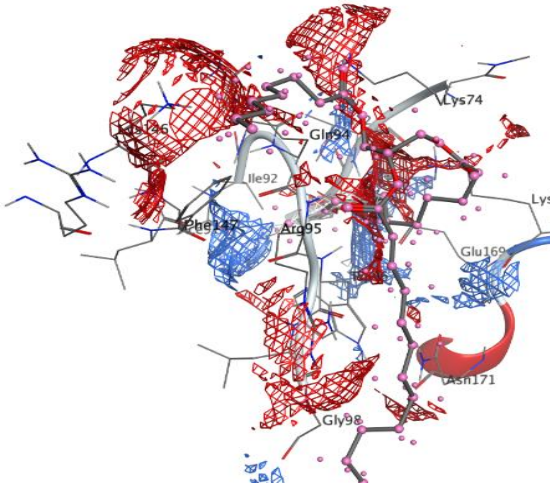
Molecular surface of 2- Lauro-1,3-Didecoin with 7BGE



The contact preference of 2- Lauro-1,3-Didecoin with 7BGE

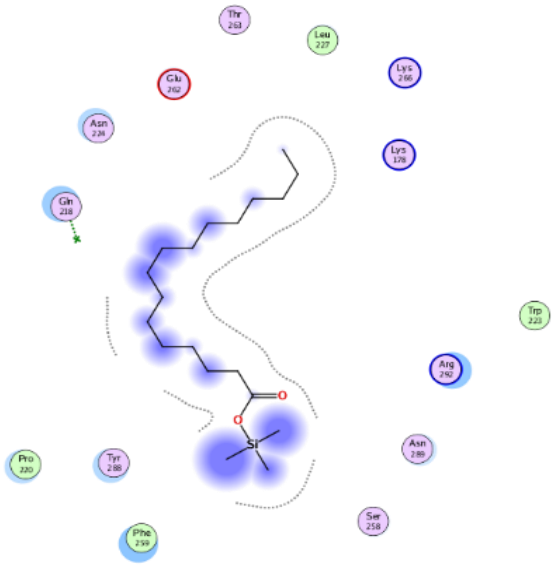


Interaction potential of 2- Lauro-1,3-Didecoin with 7BGE

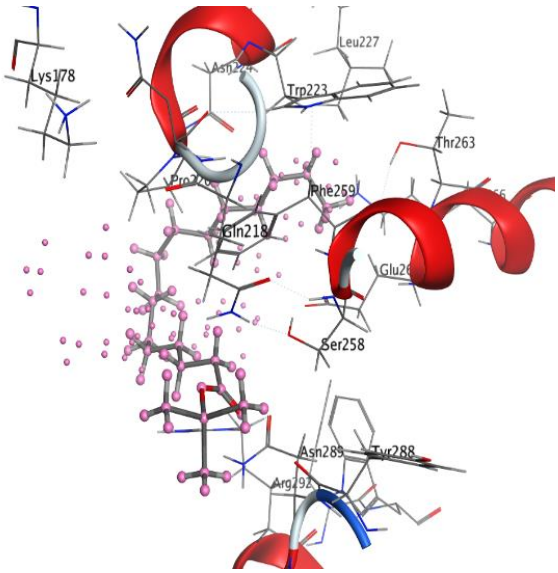


The Electrostatic map of 2- Lauro-1,3-Didecoin with 7BGE

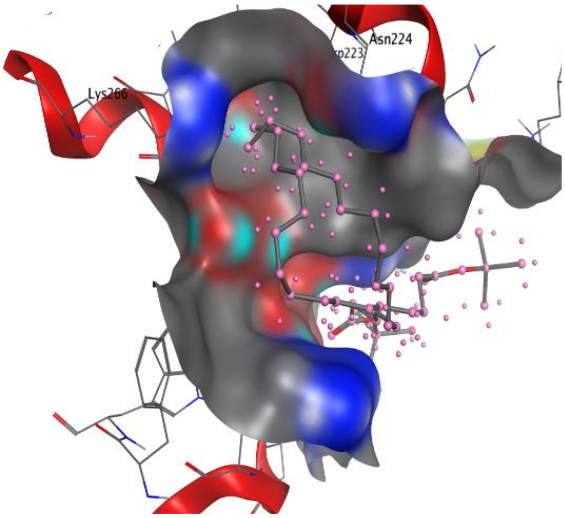
Fig. 5. Molecular docking process of trimethylsilyl ester and 2- Lauro-1,3-Didecoin with 7BGE protein.



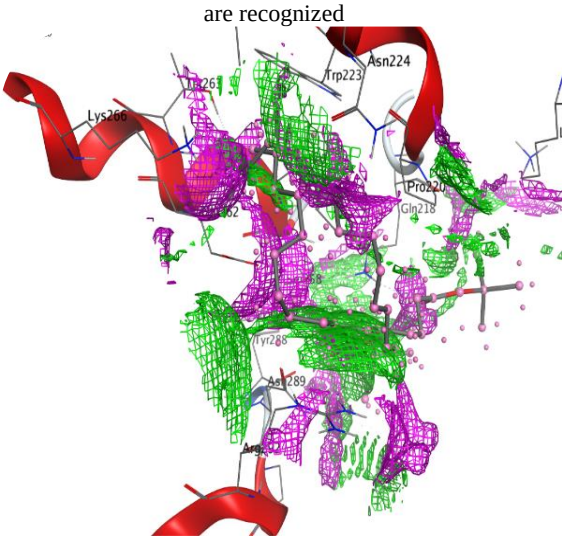
The interaction between trimethylsilyl ester and active sites of 3DRA protein



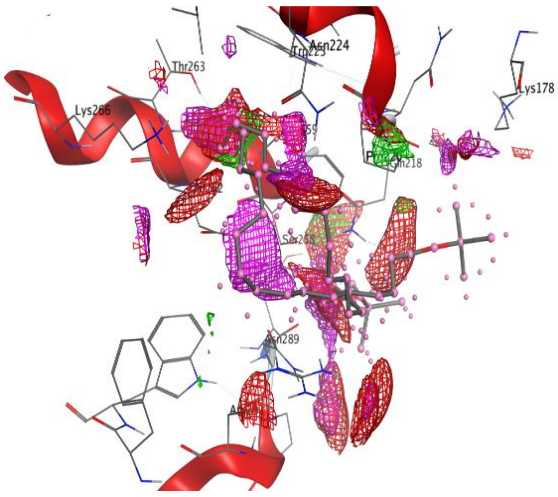
The most likely binding conformation of trimethylsilyl ester and the corresponding intermolecular interactions are recognized



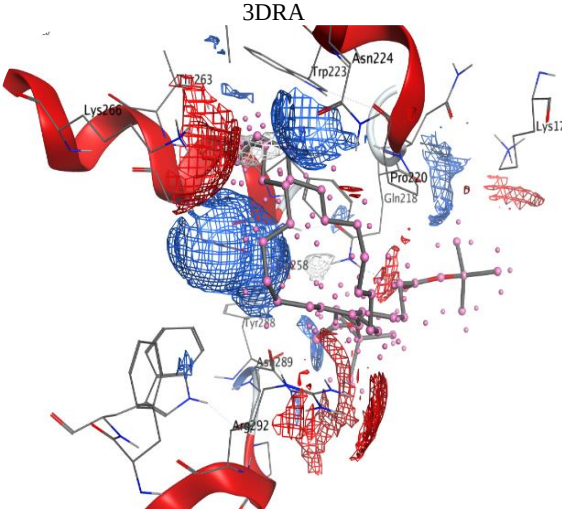
Molecular surface of trimethylsilyl ester with 3DRA



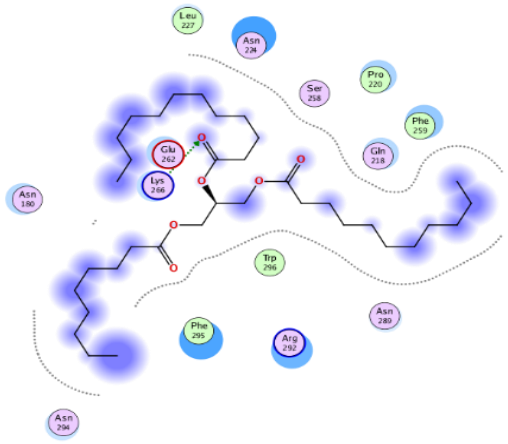
The contact preference of trimethylsilyl ester with 3DRA



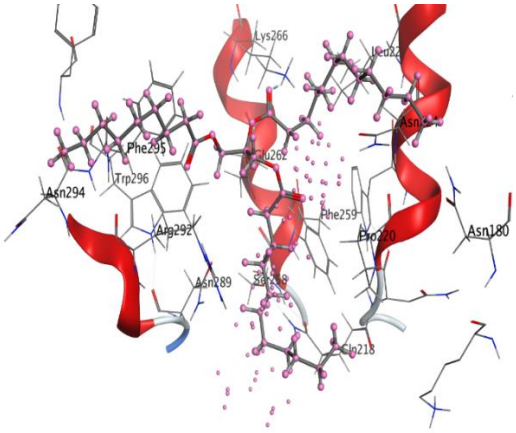
Interaction potential of trimethylsilyl ester with 3DRA



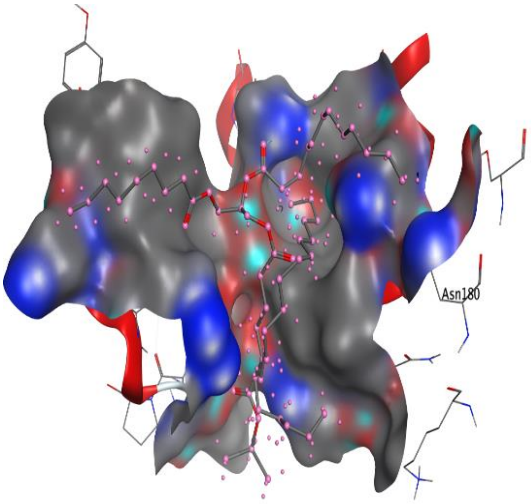
The Electrostatic map of trimethylsilyl ester with 3DRA



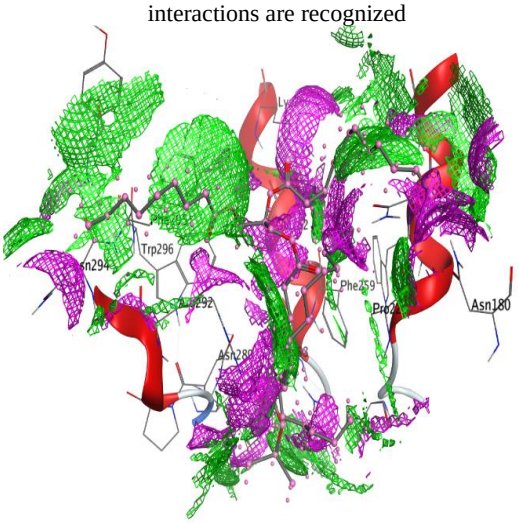
The interaction between 2- Lauro-1,3-Didecoin and active sites of 3DRA protein



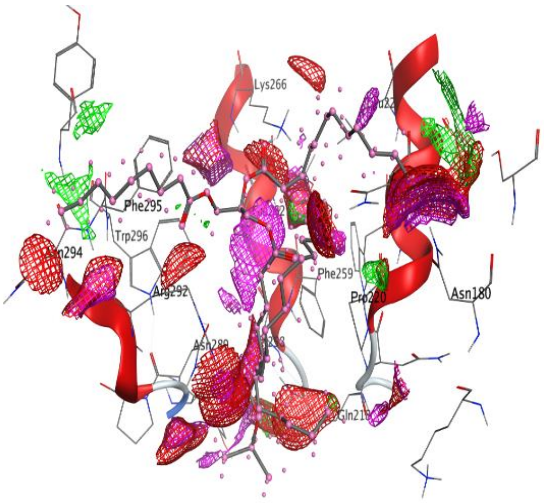
The most likely binding conformation of 2- Lauro-1,3-Didecoin and the corresponding intermolecular interactions are recognized



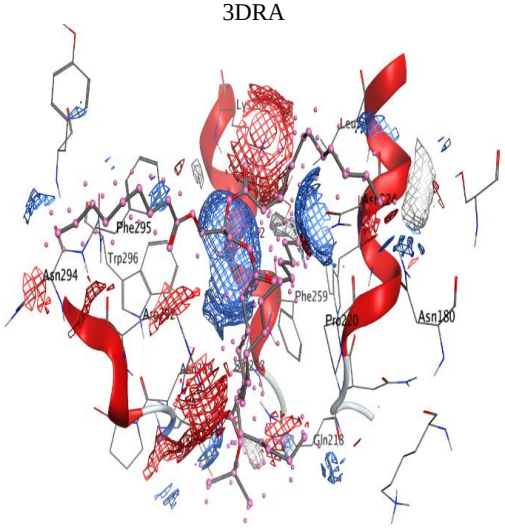
Molecular surface of 2- Lauro-1,3-Didecoin with 3DRA



The contact preference of 2- Lauro-1,3-Didecoin with 3DRA



Interaction potential of 2- Lauro-1,3-Didecoin with 3DRA



The Electrostatic map of 2- Lauro-1,3-Didecoin with 3DRA

Fig. 6. Molecular docking process of trimethylsilyl ester and 2- Lauro-1,3-Didecoin with 3DRA protein

Recently, investigations associated to molecular docking were applied to document the antimicrobial activity of several natural constituents, for instance, chlorogenic acid activity against viruses such as human coronavirus (HCoV 229E) and bacteria such as *Proteus vulgaris* (Qanash *et al*. 2022), neophytadiene against *P. aeruginosa*, luteolin against *E. coli* (Yahya *et al*. 2022), chitosan nanoparticles loaded with *Aloe vera* gel against *Helicobacter pylori* (Al-Rajhi *et al*. 2022b), and interaction of 2-benzenedicarboxylic acid and N-(4,6-dimethyl-2-pyrimidinyl)-4-(4-nitrobenzylidene-amino) benzenesulfonamide with proteins of *C. albicans* and *B. subtilis* (Al-Rajhi *et al*. 2022c).

CONCLUSIONS

1. Gas chromatography-mass spectroscopy analysis reflected the presence of various constituents in salad rocket and coconut oils.
2. Coconut and salad rocket oils as well as their NEs possess considerable antimicrobial and antihemolytic abilities.
3. Antioxidant activity of NEs of salad rocket and coconut oil was more effective with IC_{50} 35.75 µg/mL and 80.5 µg/mL than bulk oils with IC_{50} 39.26 µg/mL and 109.35 µg/mL, respectively.
4. Based on the molecular docking results, trimethylsilyl ester and 2-lauro-1,3-didecoin may be suggested as lead structures for the creation and synthesis of stronger antibacterial medications.

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Conflicts of Interest: The authors declare no conflict of interest

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