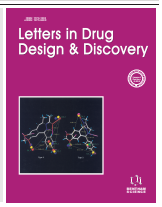
Letters in Drug Design & Discovery, 2019, *16*, 408-416

RESEARCH ARTICLE



Anti-Microbial and Free Radical Scavenging Activities of *Nigella sativa* Colloidal-Emulgel

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Abstract: ***Background*:** *Nigella sativa* L. (*N. sativa*) has been reported to have biological activities such as anti-bacterial, anti-inflammatory, anti-oxidant and anti-fungal activities.

***Objective*:** This study aims to develop *N. Sativa* colloidal-emulgel with the evaluation of its anti-bacterial, anti-oxidant and *in-vivo* irritation and sensation testing.

***Method*:** Colloidal-emulgel formulations were prepared for *N. sativa* using different surfactants (Sodium Lauryl Sulphate (S.L.S) and sucrose ester). *N. sativa* emulsion formulations were prepared using heat inversion technique. After that, the optimum formulation was mixed with Carbopol to produce the colloidal-emulgel. The droplet size, size distribution, and rheological behavior were measured for emulgel formulations. Anti-bacterial and anti-oxidant activities were also reported in the *in vivo* studies for sensitivity, irritancy and spreadability.

***Results*:** It was found that the sucrose ester was able to produce the optimum emulsion formulation with droplets size of less than 1 µm. In the anti-bacterial test for *Staphylococcus aureus*, it was found that emulgel has an inhibition zone of 2.5 cm in diameter, but the oil alone being 1.3 cm. According to MRSA, the inhibition zone for emulgel was 1.1 cm, but for oil, it was 0.5 cm in diameter. Emulgel does not show any irritation or sensitivity. Also it has a homogeneous appearance with a smooth texture. In addition, it shows fair mechanical properties, and easy spreadability with acceptable bio-adhesion.

***Conclusion*:** It is concluded that *N. sativa* emulgel has been prepared with dermatological and cosmeceutical benefits.

ARTICLE HISTORY

Received: February 23, 2018
Revised: May 26, 2018
Accepted: June 11, 2018

DOI:
10.2174/1570180815666180620150922

Keywords: *Nigella sativa* L., emulgel, heat inversion technique, sucrose ester, anti-bacterial, anti-oxidant.

1. INTRODUCTION

The significance in joining two immiscible liquids into one phase is to have a good drug carrier especially for the delivery of lipophilic drug was the main aim behind discovering emulsion [1], but emulsion formulation has many disadvantages such as low viscosity and bad spreadability, therefore, researchers have tried to solve these problems by mixing emulsions with hydrogel (Emulgel), which obtained good results and improved the viscosity and compliance. Emulgels are emulsions which might be of either type of oil in water or water in oil, gelled after mixing it with a gelling agent. Emulgel is a topical drug delivery system which should be paid extra attention in pharmaceutical knowledge.

The importance of incorporating emulsion with the gel is to produce better stability product and make a dual control release system. Because of lack of insoluble excipients and excessive oily bases, the studies showed superiority of emulgel over other drug delivery systems in producing better drug release dosage form. Because it has gel phase, it produces topical delivery system which is not greasy and has better patients preference [2]. Hydrophobic moiety has difficulties to be delivered through the skin; therefore, it is very important to find a pharmaceutical solution for this problem. Emulgels solve this problem by providing better loading capacity, controlled and dual release of drug with short half-life and higher stability [3]. Many drugs of anti-viral, antimicrobial as well as non-steroidal anti-inflammatory drugs are

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1875-628X/19 $58.00+.00

studied for their topical delivery by using emulgel formulation and some of them are available in the market [4].

Nowadays, pharmaceutical industries intensely focus their attention on the usage of natural bioactive materials as medicinal agents. Therefore, *N. sativa* oil was used in the formulations of oral and cosmeceutical products. *N. sativa* also called black-caraway or "Kalonji", is a well-known seed all over the world [5, 6]. It contains many useful chemical constituents, which can be found in its fixed oil, such as thymoquinone, thymohydroquinone, dithymoquinone, thymol, nigellicine, carvacrol, nigel-limine, nigellicine, nigellidine and alphahederin [7-9]. Due to these numerous important ingredients, it was found that it affects different areas of our body and has many pharmacological effects as antibacterial, antiviral, anti-inflammatory, wound healing effect, also for acne vulgaris, skin cancer, pigmentation, and many cosmeceutical applications [10-12].

Many studies have discussed the anti-bacterial efficacy of black seeds, examples of these included: Thymoquinone, which is a part of black seeds oil found to have a bactericidal activity against most bacteria included in the study (A range from 8 to 32 µg/ml for MICs values) mainly Gram-positive cocci types such as (*Staphylococcus epidermidis* CIP 106510 and *Staphylococcus aureus* ATCC 25923). For *Staphylococcus aureus*, at a concentration of 300 mg/ml, a clear inhibition in the growth was observed. According to the approach that they used for testing, a modified paper disc diffusion method was used for that. According to *E.* Coli and Enterobacter, there was no effect on these types of bacteria [13]. For the effect of Black seeds on *H-pylori*, it was found that they possess a good activity compared with triple therapy [14]. Also, it was found that the black seed extract has several multidrug-resistant clinical bacterial effects [15].

The aim is to improve the efficacy, safety, and ease of administration of a therapeutic drug which directly moves along with enhancing patient compliance, for present research study on *N. sativa* emulgel.

2. MATERIALS AND METHODS

2.1. Materials

*S*ucrose ester (DK ESTER F-160) was kindly gifted by SISTERNA company-Japan. Glycerol and Carboxyvinyl polymer (Carbopol 940) were purchased from, CBC Co., Ltd., Japan. Sodium Lauryl Sulphate was obtained from AL-Shamas company, Palestine. *N. sativa* seeds from Mahmas Alqouds, Palestine. (DPPH) 2, 2-Diphenyl-1-picrylhydrazyl, Folin-Ciocalteu's reagent and Gallic acid and Trolox ((S)-(-)-6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma Aldrich, Denmark.

2.2. *N. sativa* Oil Extraction

A total of 100 g of plant seeds powder was weighed and then 400 ml (50% ethanol in triple distilled water) and 200 ml (*n*-hexane) were used for exhaustively extracting the powdered seeds. The mixture was placed in a shaker for 72 hours, which was set at 200 rpm at room temperature. Afterward, suction flask and Buchner funnel were used for the filtration of the mixture. The obtained filtrate representing the organic phase and aqueous phase was placed in a separatory funnel for obtaining two separated layers. The upper one represented organic phase containing the plant oil and the lower layer which represented the aqueous phase.

The organic phase was placed in the rotary evaporator for 1 hour at 40ºC to evaporate organic solvents and completely dried seeds oil was obtained and then stored at 25ºC till use. The remaining solid material was re-extracted again with 50 ml hexane and 125 ml of 50% ethanol in triple distilled-water and the re-extraction and same steps were carried out as mentioned above.

The % yield was calculated using the following equation:

$$\%\ \text{yield} = \frac{M}{Bm} \times 100$$

Where M is the mass of the extracted oil (g) and Bm is the initial plant biomass (g).

2.3. Antioxidant Test for *N. sativa* oil

Free radical scavenging assay was used for the antioxidant activity:

Free radical scavenging activity of *N. sativa* oil extract was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH) according to the following procedure:

2.3.1. Trolox Standard and Plant Working Solutions

1 mg/ml in methanol stock solution was prepared for plant extract and Trolox (standard reference compound). From this Stock solution, serial dilutions were prepared with the following concentrations (1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80 µg/ml).

2.3.2. Spectrophotometric Analysis

A freshly prepared solution of DPPH (free radical compound) at a concentration of 0.002% (w/v) was mixed with methanol along with each of the working concentrations in a ratio of 1:1:1. Methanol was used as blank for the UV/Visible Spectrophotometer (Jenway, Germany). DPPH with methanol was the first solution of the series concentrations. They were incubated in a dark place at room temperature for about 30 minutes. Then, the spectrophotometer at a wavelength of 517 nm was used to determine their optical densities.

2.3.3. Percentage of Inhibition of DPPH by Plant Different Extracts and Trolox

The following formula was used to calculate the percentage of antioxidant activity of the Trolox standard and the plant's extract:

DPPH inhibition activity (%) = (B-S)/B ×100%

Where B is the optical density of the blank and S is the optical density of the sample.

The antioxidant IC_{50} (Half-maximal inhibitory concentration) was calculated for both the standard deviation as well as the plant extract using BioDataFit edition 1.02 (data fit for biologist) [16].

2.4. Preparation of Colloidal-emulgel

Emulgel formulations were prepared by incorporating emulsion formulation with hydrogel.

2.4.1. Preparation of Colloidal-emulsion

N. sativa oil mini-emulsions were papered by using two different compositions. The first composition was composed of oil, glycerol and sucrose ester as non-ionic surfactant while the second composition was composed of oil, glycerol and sodium lauryl sulfate (SLS). The emulsions were prepared by using different surfactant concentrations, as shown in Table **1**. Our aim was to compare between both groups on the basis of droplet size in order to achieve the optimum emulsion formulation producing the smallest droplet size.

Emulsions were prepared using heat inversion technique, in which, surfactant, glycerol and *N. sativa* oil were weighed accordingly. The glycerol was heated at about 75 ± 5 ^{o}C. Then the surfactant was added to the hot glycerol and gently mixed until it was completely dissolved and produced a homogeneous mixture. *N. sativa* oil was heated up to the same temperature as the surfactant/oil mixture before it was gradually poured into the mixture with continuous stirring. The preparations were mixed for about 10 minutes until it cooled out to the room temperature. Finally, the droplets size was analyzed for these formulations in order to select the optimum formulation.

2.4.2. Droplet Size and Size Distribution Analysis of Emulsion

A laser diffraction particle and droplet size analyzer (SALD-2300, SALD-MS23, Shimadzu Corp., Japan) were used to determine the emulsions' droplets size and size distribution. The mean and standard deviations were taken in triplicate.

2.4.3. Selection of Emulsion Formulation

The selection of emulsion formulation was done based on the droplets size. The formulation that aquires the smallest droplet size with the lowest size distribution was selected.

2.4.4. Hydrogel Formulation

Hydrogel was prepared through the addition of Carbopol 940 in water and constantly stirred by using homogenizer to produce a uniform dispersion. The hydrogel pH was adjusted to pH 6 using 2M NaOH which was added under constant stirring. The mixture underwent continuous stirring and left overnight for 24 hours for complete gelation.

2.4.5. Emulgel Formulation

The colloidal-emulgel formulations were prepared by incorporation of the hydrogel matrix at different concentrations (0.7, 0.8 and 1%) at 100 rpm with the optimum colloidal-emulsion for 10 minutes until emulgel is formed [17]. Droplets size, size distribution and zeta potential were measured as mentioned earlier.

2.5. Physical Characterization of Colloidal-emulgel

The homogeneity, spreadability, consistency as well as the visual appearance and phase separation Colloidal-emulgel formulations were inspected. In addition, pH meter (CG 820, Schott Gerate GmbH, Hofheim, Germany) was used to measure the pH values of the prepared colloidal-emulgels.

2.6. Rheological Measurement for Colloidal-emulgel

The rheological behavior of emulgel formulations was prepared with different concentration of Carbopol 940 (0.7, 0.8 and 1% Carbopol) as a thickening agent, which was evaluated at ambient temperature using a rotational viscometer (Brookfield DVI, USA). All measurements were made in triplicate. The viscosity was determined within the shear rate range (0–100 rpm).

2.7. *In Vivo* Study

2.7.1. Study Design

An observational crossover study was conducted on a total of 20 healthy volunteers aged more than 20 years with

Table 1. The composition of emulsion formulations.

Formulation	*N. sativa* Oil (%)	Sucrose Ester (%)	SLS (%)	Water (%)
1	20	1	-	79
2	20	2	-	78
3	20	3	-	77
4	20	4	-	76
5	20	5	-	75
6	20	-	1	79
7	20	-	2	78
8	20	-	3	77
9	20	-	4	76
10	20	-	5	75

normal or dry skin for two weeks. The study was approved by the Institutional Review Board (IRB) at An-Najah National University, Palestine. The primary objective was to assess the organoleptic properties of the emulgel such as odor, feel, spreadability, easy to pick up from container, smoothness, and emolliency. A blank emulgel formulation was used as a control, which does not contain *N. sativa* oil.

Exclusion criteria:

- Volunteers with oily or sensitive skin.
- Volunteers using other moisturizing creams or other cosmetics.

2.7.2. Application and Assessment

Participants were divided into two groups. In the first week, one group applied *N. sativa* colloidal emulgel formulation and the other group applied the blank emulgel formulation on their hands. Each group rated the emulgel from 1-5 (1: the least, 5: the best) by filling a specific questionnaire. In the second week after three days washout period, the two groups were switched to complete the assessment of the emulgel formulations. The results were recorded and analyzed by paired sample T-test.

2.7.3. Tests of Irritancy and Sensitivity

This study was carried out based on volunteer basis. Health human volunteers were selected to check the safety for topical use. The safety of the formulation was determined using patch test. The patch test was carried as follows:

The patch test was performed as described by More *et al*., 2013 with some modification. 10 healthy volunteers between 20 and 60 years of age were selected for testing skin irritation. The dorsal skin was cleaned with 70% alcohol before application of the formulation. One gram of the formulation containing 10% oil was applied to 4 X 5 cm marked regions on the forearm. The regions were covered with the surgical dressing after application and kept in contact with the skin for 48 hours (Single patch test). The surgical dressing was removed after 48 hours and the forearm was washed with physiological saline. Then the signs of erythema and edema were evaluated based on Table **2**. Emulgel without *N. sativa* oil was used as negative control [18].

Table 2. Classification system for skin reaction.

Reaction	Score
Erythema	-
No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness)	4
Edema	-
No edema	0
Very slight edema	1
Well defined edema (edges of the area well defined by define rising)	2
Moderate edema(raising approximately 1mm)	3
Severe edema(raised more than 1mm and extended beyond the area of exposure)	4
Total possible score of primary irritation	8

2.8. Anti-bacterial Test

2.8.1. Microorganisms

The organisms used for the bacterial test were *Escherichia coli*, *staphylococcus aureus*, MRSA, Shigella, and Candida.

2.8.2. Culture Media

The culture media used were Muller Hinton agar and Bifco LB Broth, Lennox both were produced from Becton, Dickinson, and company Sparks, France.

The Bifco LB Broth was used to inoculate the bacterial cultures, which were then incubated for 24 hours with shaking at 37 ^{o}C. The Mc Farland turbidity standards were used to standardize the inoculum. The Mc Farland 0.5 standard was used for the turbidity comparison, which offers turbidity similar to a bacterial suspension containing $1.5X10^{8}$ CFU\ml.

Agar well diffusion test: A plate containing Muller Hinton agar was used for the inoculation of a standard inoculum of bacterial culture. 6 mm diameter wells were punched inthe agar. Four holes were made. A was filled with *N. sativa* emulgel, B was filled with *N. sativa* oil pure extract, C was filled with emulgel without *N. sativa* oil, and finally, D was filled with Fusidic acid cream as reference. The plates were incubated at 37 ^{o}C for 24 hrs. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition [19].

3. RESULTS

3.1. The Yield of *N. sativa* Seed Extraction

100 g of *N. sativa* seeds were prepared for extraction. From these 100 g of seeds, 26.34 g *N. sativa* Oil was obtained. So the yield was equal to 26.34%.

3.2. Anti-oxidant Results of *N. sativa* Oil

Based on the clinical studies, the anti-oxidant activity of natural compound is an indication of the reduction in the incidence of causing chronic diseases such as cancer and heart disease. The results are shown in Table **3**, and the table readings are explained in Fig. (**1**).

3.3. Droplet Size, Distribution Size Analysis of Emulsion and Selection of Formulation

The effects of various surfactants were studied for their potential to produce an emulsion with small droplet size and

Table 3. %age Inhibition activity for Trolox standard and *Nigella* seeds oil.

Concentrations (µg/ml)	% Inhibition by Trolox	% Inhibition by *Nigella seeds oil*
1	32.90 ±2.22	18.25 ±2.34
2	58.12 ±3.31	22.35 ±3.20
3	69.85 ±2.33	32.90 ±2.36
5	75.80 ±2.20	33.40 ±2.22
7	80.12 ±2.17	34.0 ±2.38
10	83.22 ±3.25	41.25 ±2.16
20	87.25 ±2.07	45.20 ±2.33
30	88.50 ±3.31	55.12 ±2.41
40	88.65 ±2.47	59.10 ±3.38
50	92.30 ±3.23	60.43 ±3.61
80	96.31 ±3.31	67.40 ±2.43
100	97.12 ±2.62	73.50 ±3.39
IC_{50}	2.29 ±0.22	19.95 ±1.12

narrow size distribution. Two different surfactants (sucrose ester and sodium lauryl sulfate) were investigated to optimize the optimum emulsion formulation.

Both surfactants showed different behavior in producing an emulsion. As a comparison between them, sucrose ester was able to produce an emulsion with droplets size below 1 µm, while, sodium lauryl sulfate produced an emulsion with droplets size above 1 µm as shown in Table **4**.

3.4. Colloidal-emulgel Formulations

Colloidal-emulgels containing *N. sativa* oil were prepared by using different concentrations of Carbopol 940 (0.7, 0.8 and 1%). Carbopol provides ling and swelling properties to the formulation, due to its contribution as a thickening agent [20]. The self-emulsification technique was used to emulsify the optimum colloidal-emulsion formulation which contained sucrose laureate in distilled water and then under continuous stirring, Carbopol hydrogel was added in order to form the colloidal-emulgel. Colloidal-emulgel formulations were subjected for the measurement of their droplets size, size distribution and viscosity properties.

Table 4. Mean droplet size and size distribution results of emulsion formulations.

Formulation	Mean Droplet Size (µm) ±SD	Size Distribution ±SD
1	0.921 ±0.07	0.158 ±0.010
2	1.104 ±0.09	0.168 ±0.007
3	1.430 ±0.14	0.175 ±0.009
4	1.488 ±0.16	0.170 ±0.014
5	1.485 ±0.12	0.165 ±0.008
6	2.099 ±0.17	0.368 ±0.023
7	2.853 ±0.20	0.397 ±0.016
8	3.536 ±0.19	0.461 ±0.008
9	2.941 ±0.15	0.367 ±0.012
10	2.274 ±0.12	0.326 ±0.019

3.5. Influence of Various Carbopol Concentrations on Droplets Size and Size Distribution

The mean droplets size results showed that the droplets produced were of submicron size and low polydispersity with narrow size distribution. The results of the optimum colloidal-emulsion formulation were used to be compared with the results obtained for the mean droplet size and size distribution of colloidal-emulgel formulations. Fig. (**2**) shows the comparison between the mean droplets size of colloidal-emulgel formulations containing different concentrations of Carbopol 940 with the colloidal-emulsion (initial). Fig. (**3**)

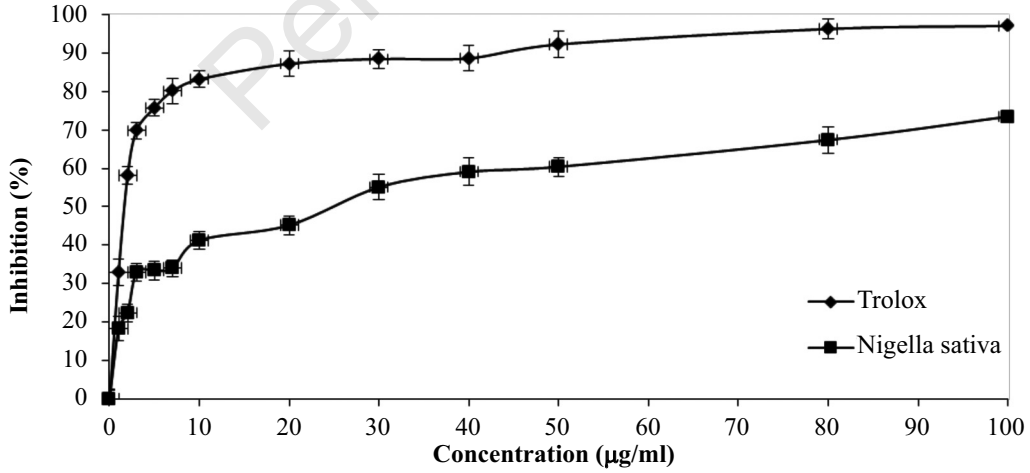

Fig. (1). Inhibition activity of Trolox standard and *Nigella* seeds oil.

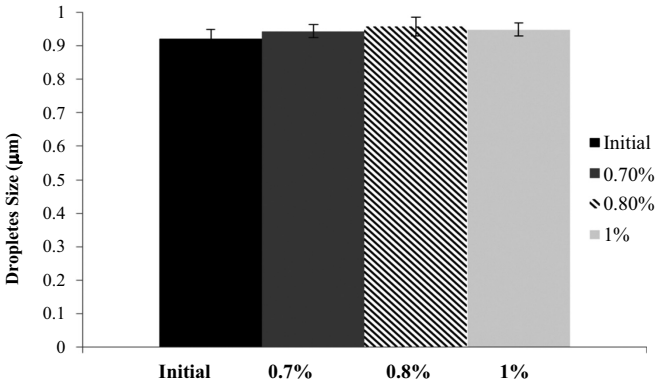

Fig. (2). Droplets size measurement of initial emulsion and emulgel formulations containing different concentrations Carbopol (0.7, 0.8, 1%).

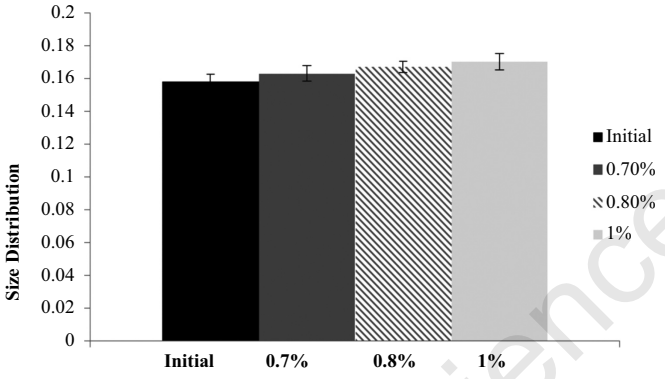

Fig. (3). Size distribution measurement of initial emulsion and emulgel formulations containing different concentrations of Carbopol (0.7, 0.8, 1%).

shows the size distribution for colloidal-emulgel formulations containing *N. sativa* oil. The result for *N. sativa* oil ranged from 0.163 to 0.170.

3.6. The Rheological Behavior of Colloidal-emulgel Formulations

Usually, the flow properties of semisolid pharmaceutical products are evaluated and controlled by the rheological characterization, which is important to ensure effectiveness and quality of the formulation. Rheological analysis of colloidal-emulgel formulations is shown in Fig. (**4**). The rheological behavior of the colloidal-emulgel is pseudo-plastic, *i.e*., the viscosity decreases with an increase in the shear rate.

3.7. General Organoleptic and Sensorial Properties Analysis

Based on the odor, smoothness and emolliency, there is a significant difference between *N. sativa* colloidal emulgel and the blank emulgel, where the *p*-value is less than 0.05. *N. sativa* colloidal emulgel showed a good emolliency and smoothness to the skin. With regard to spreadability and easy pick up from the container, three different *N. sativa* colloidal emulgels were evaluated, which contained different Carbopol concentrations (0.7, 0.8 and 1%). It was found that emulgel containing 0.7% Carbopol was easily taken from the jar. While emulgels with 0.8 and 1 % were difficult to adhere to the fingers and picked up from the jar. The spreadability was good for all of the formulations with no significant difference between them, but as emulgel, which contains 0.7% Carbopol was chosen as it has the lowest amount of Carbopol.

3.8. Test of Irritability and Sensitivity

The result of patch test showed no sign of irritation and sensitization for *N. sativa* emulgel (Table **5**). The edema and erythema parameters were found in average between 0-1.

Table 5. Cutaneous reaction after the application of *N. sativa* emulgel in patch test on health volunteers.

Parameters	Single Batch Test
Erythema	Nil
Edema	Nil
Irritation	Nil
Skin allergy	Nil

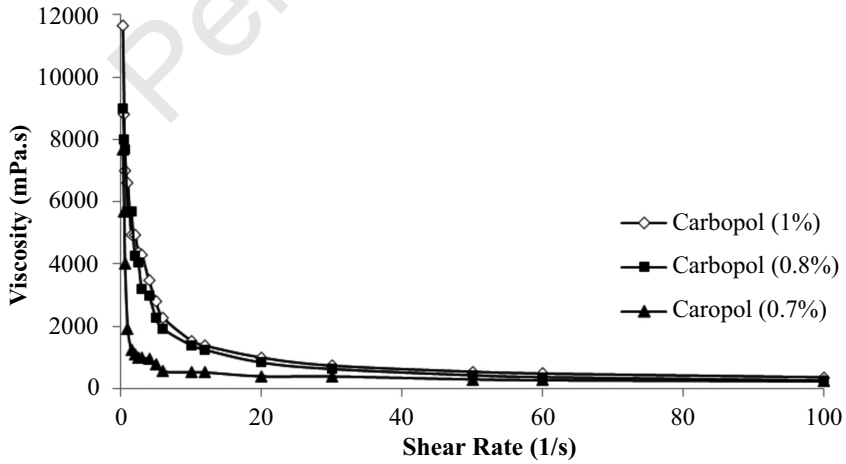

Fig. (4). Rheological behavior of different emulgel formulations.

Table 6. Activity of different samples against *Staphylococcus aureus* and MRSA.

Sample Type	*Staphylococcus aureus* Inhibition Zone (cm)	MRSA Inhibition Zone (cm)
Fusidic acid	3.5	3.4
N. sativa emulgel	2.5	1.1
N. sativa oil	1.3	0.5
Emulgel without *N. sativa*	0	0

3.9. Anti-bacterial Test

The antimicrobial results obtained from this study showed that *N. sativa* oil has potential bioactivity against the growth of microbes (*Staphylococcus aureus* and MRSA) as shown also by the positive control (Fusidic acid). While both *N. sativa* oil and Fusidic acid showed no activity against Shigella, *E.coli* and Candida growth. The *in vitro* antimicrobial activities were calculated in terms of the zone of inhibition diameter (cm) and the results are recorded in Table **6**. In addition.

4. DISCUSSION

Different combinations of surfactant, glycerol, and oil were screened to produce colloidal-emulgel with small droplet size below 1 μm. On the basis of surfactant concentration and results of droplet size and size distribution of the obtained emulsion, the optimum emulsion formulation was selected. Colloidal-emulgel was achieved by various concentrations of Carbopol 940. Then it was subjected to evaluate their droplet size, size distribution and rheological properties to select the optimum Carbopol concentration. The prepared colloidal-emulgels were white in color with homogeneous appearance and smooth viscous creamy. They showed an easy spreadability with suitable bio-adhesion at fair mechanical properties. The pH value of formulations ranged from 5.5 to 6.6. This pH value is considered as acceptable for application to the skin in which it avoids the risk of skin irritation [21, 22].

The results showed high anti-oxidant activity of the oil when compared with Trolox as standard, which is more than half of the anti-oxidant activity of Trolox. The high level of the antioxidant activity in *N. sativa* oil was represented by low IC_{50} (19.95 ±1.31) when compared with Trolox IC_{50}.

Sucrose ester showed high emulsification properties due to its good miscibility properties. Same findings were stated by Szuts and his research team [23], mentioning that sucrose laurate was good in preparing solid dispersion due to its good miscibility properties.

The heat treatment used during the production of colloidal-emulsion leads to a change in the molecular characteristics of the surfactant, which helps in dissolving sucrose ester in the glycerol. Therefore, sucrose ester becomes progressively dehydrated during heating because it is non-ionic surfactant with a hydrophilic head group. Based on this reason, during heating, the surfactant molecules have changes in their packing, interfacial tension, and oil/water solubility. Same results were stated in previous studies which used a higher temperature to facilitate the production of micro-emulsion and nanoemulsion formulations when the same changes occurred to the non-ionic surfactants [24-27]. In addition, a shift in the production from an emulsion to colloidal-emulsion at ambient temperature was prevented by the kinetic energy barrier in the oil-glycerol-surfactant system. But the kinetic energy barrier was reduced as the temperature was raised, which helped in changing the emulsion from one state to another. Same results were mentioned by Rao and McClement, 2011 who specified this change during the production of oil in water micro-emulsion and nanoemulsion [26].

Oil in water dispersion required HLB value between 9 to 18, so the selection of the optimum HLB value of emulsifying agent depends on its hydrophilicity [28]. Sucrose ester as a non-ionic surfactant with 16 HLB value was chosen because of its capability in producing colloidal-emulsion formulation with small droplets size, narrow size distribution. Usually as the degree of sucrose esterification increased and/or the fatty acid chain length increased, the sucrose ester HLB value reduced. Their ability in producing colloidal-emulsion was due to its good droplets entrapment and stabilization efficacies, which are explained by the low amounts of di-, tri-, and polylaurates and higher amount of monolaurate as well as the lauric acid short chain length [28]. Sodium laurel sulfate (HLB 40) showed emulsion with bigger droplets size compared to sucrose ester, which, is due to its HLB value which is not in the range of producing O/W emulsion. In addition, the non-ionic surfactant is mild on the skin and does not cause skin irritation, while sodium laurel sulfate may cause skin irritation and it is an ionic surfactant. Therefore, it could be concluded that sucrose ester as a non-ionic surfactant was able to produce a colloidal emulsion with small droplets size and narrow size distribution.

The best-selected formulation was formulation 1 as it produces the smallest droplets size 0.921±0.07 μm with the lowest size distribution 0.158 ±0.010. In addition, formulation 1 is composed of sucrose ester as nonionic surfactant showed no irritation effect when compared to sodium lauryl sulfate surfactant as SLS was demonstrated to be dermal irritant [29]. Therefore, sucrose ester was the chosen surfactant to prepare this emulgel.

The mean droplets size of *N. sativa* colloidal-emulgel formulations ranged from 0.943 to 0.957 μm. It was noticed that the addition of different Carbopol concentrations showed no significant change in the mean droplets size. Same results were reported by Yilmaz & Bolchert. They found that the mean droplets size of colloidal-emulgel

prepared by the addition of Carbopol 940 as thickener agent to nanoemulsion formation was not significantly changed as compared to nanoemulsion formulation [30]. But there was only a slight increment in the mean droplets size with the increment of Carbopol concentration, which was due to the increment in the viscosity by increasing Carbopol concentration thus resulted in the enlargement of the droplet size. The same findings were stated by different authors, who stated that higher concentration of the polymer resulted in the increase of the viscosity of the medium that resulted from a high degree of cross-linking. Hence giving larger size of droplets [31, 32].

There were no significant changes in the size distribution. *N. sativa* colloidal-emulgel formulations showed narrow size distribution, which indicates that colloidal-emulgel formulations have good stability. This has been approved by Jeong and colleagues, who stated that emulsions with narrow size distribution have better stability when compared to broad size distribution [33].

Based on a preliminary screening, we found that emulgel formulations exhibit concentration-dependent behavior: The higher the concentration of Carbopol, the more viscous the emulgel. At low shear rate, there was a drastic increase in the viscosity from 1.6 to 6.6 Pa·s as the concentration increased from 0.7 to 1%. Those results are presented online with Eid *et al*., (2014), who reported that nanoemulgel viscosity increases with the increase in Carbopol concentration [34].

N. sativa colloidal-emulgel showed higher zone of inhibition when compared with *N. sativa* oil. This is due to the small droplet size of oil in emulgel which enhances penetration and microbial inhibition. This finding was in line with Marslin *et al*., (2015), who studied the effect of *Withania somnifera* cream incorporated with silver nanoparticles on the microbial growth. They found an increase in the penetration of *Withania somnifera* cream silver nanoparticles, which led to an increase in the inhibition of the bacterial growth [35]. Lkhagvajav *et al*., (2011) reported that the antimicrobial activity of colloidal silver nanoparticles was improved by the method of preparation and the particle size [36]. Moreover, Mokarizadeh *et al*., (2017) stated that nanostructured lipid carriers were used to improve the antimicrobial activity of a natural preservative [37]. In addition, the improvement of the activity was due to well packing of bacteria by the colloidal oil droplets, which increased the exposure of the bacteria to *N. sativa* oil and, consequently, increased the concentration of *N. sativa* oil entering into bacteria. A similar finding was found by Assali *et al*., (2017), who mentioned that the antimicrobial activity of ciprofloxacin single-walled carbon nanotubes was improved due to the aggregation of the drug around the bacteria which increases the drug residence time, leading to more penetration through the bacteria [38].

CONCLUSION

The study demonstrated the good antimicrobial activities, antioxidant activity, no skin irritation and the desired physical properties of the colloidal-emulgel formulations containing the *N. sativa* oil. *N. sativa* oil colloidal-emulgel showed an enhancement in the antimicrobial activity when compared to the crude *N. sativa* oil, which is due to better penetration of colloidal-emulgel that was prepared in small droplets size less than 1 μm and narrow size distribution using sucrose ester as surfactant. Also 0.7% Carbopol 940 showed superiority in hydrogel rheological charactersitics, spreadability on the skin and pick up from the jar when compared to 0.8 and 1% Carbopol.

AVAILABILITY OF DATA AND MATERIALS

All data in this study is included in the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Institutional Review Board (IRB) at An-Najah National University, Palestine.

HUMAN AND ANIMAL RIGHTS

No animals were used in this study. The reported experiments were performed in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013 (http://ethics.iit.edu/ecodes/node/3931).

CONSENT FOR PUBLICATION

A written informed consent was obtained from all the volunteers that are the basis of this research.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

This study was not funded by any institution. Sincere thanks are extended to all the participants in the study.

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