The Effects of Main Formulation and Process Parameters on Characteristics of Frankincense Essential Oil Microemulsions

Molaie, Soheila; Memar Maher, Behnaz*[+]

Department of Chemical Engineering, Ahar Branch, Islamic Azad University, Ahar, I.R. IRAN

Anarjan, Navideh

Faculty of Engineering, Tabriz Branch, Islamic Azad University, Tabriz, I.R. IRAN

Hamishehkar, Hamed

Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz z, I.R. IRAN

ABSTRACT: *The frankincense essential oil was successfully incorporated into nano-sized microemulsion systems through low energy self-emulsification technique. The effects of main formulation parameters, namely, surfactant, co-surfactant, essential oil, and water concentrations, as well as the mixing rate and temperature on mean particle size, polydispersity (PDI), turbidity and antioxidant activities of colloidal frankincense essential oil nanoparticles were investigated. The results show that all studied independent parameters affect the most characteristics of frankincense essential oil microemulsions, significantly. The antibacterial activities of essential oils were also considerably increased as incorporated into nano-sized microemulsions. It resulted that the most desired frankincense essential oil microemulsions, with desired characteristics (less particle size, size distribution, turbidity, and greater antioxidant activity) could be obtained using high concentrations of surfactant (0.7 g), medium concentrations of co-surfactant, essential oil and water (0.2 g, 0.1 g, and 9.2 mL, respectively), and medium levels of mixing rate and temperature (500 rpm and 40 °C). Thus, by tuning the formulation or process parameters the most desired nano-sized essential oils can be prepared as natural preservers or health-promoting agents for various food and beverage applications.*

KEYWORDS: *Frankincense essential oil; Microemulsion; Formulation parameters; Process parameters; Antioxidant activities.*

INTRODUCTION

Essential oils are a mixture of various non-volatile and volatile compounds originating from different parts of plants. Many of the compounds in essential oils have been shown to possess bioactive properties, e.g., antimicrobial, antiviral, antifungal, antioxidant, and antiseptic that can be used in food applications,

*** To whom correspondence should be addressed.*

+ E-mail: b_maher@iau-ahar.ac.ir

1021-9986/2022/11/3733-3743 11/$/6.01

pharmaceutical and medical products. The fact that essential oils do not contain artificial ingredients has led to particular interest within the food industry since many consumers are interested in purchasing foods that contain natural components [1-4].

The frankincense (*boswellia serrate)* essential oil has attracted considerable attention lately due to its effectiveness in treatments of fevers (antipyretic), cardiovascular diseases, skin and blood diseases, bronchitis, mouth sores, asthma, cough, jaundice, hair-loss and etc. The antioxidant, anti-cancer, anti-inflammatory, Anti Diabetic, anti-arthritic, anti-atherosclerotic (anti-coronary plaque), anti-hyper-lipidemic (controls blood lipids), analgesic (pain-reliever) and hepato-protective (protects the liver) were confirmed by Modern medicine [5].

The frankincense contains, monoterpenes (α-thujene); diterpenes (macrocyclic diterpenoids such as incensole, incensole oxide, iso-incensole oxide, diterpene alcohol [serratol]); triterpenes (such as α- and β-amyrins); pentacyclic triterpenic acids (boswellic acids); tetracyclic triterpenic acids (tirucall-8,24-dien-21-oic acids) [5].

Regardless of essential oils prevailing use as a natural antioxidant and antimicrobial agent, the widespread use of essential oils in foods is currently limited due to their low water solubility, the tendency to interact with other food matrices' constituents, and strong aroma and taste. Incorporation of essential oils into proper delivery systems can overcome these problems. Various well-designed nano-sized colloidal delivery systems such as nanodispersions, nanoemulsions, and microemulsions are offered by nanotechnology in order to effectively deliver these functional lipid compounds. It should be noted that the appearance of the food system should be kept changeless after the addition of essential oil, which can also be achieved by proper nano-sized colloidal delivery systems [3].

Microemulsions are efficient nano-sized delivery systems for essential oils. Unlike nanoemulsion systems, the microemulsions are thermodynamically stable at wide ranges of compositional and environmental conditions. This system can be simply formed by mixing water, oil and surfactants in a little applied external energy, like stirring or heating. Consequently, the nano-metric diameter size, self-stability, less fabrication cost, less energy input, transparency water-dispersibility of microemulsions and presence of both lipophilic and hydrophilic domains, make the microemulsions appropriate systems to incorporate a wide range of lipophilic water-insoluble nutraceutical and pharmaceutical bioactive compounds and improve their solubility, bioavailability and stabilities [6, 7].

In most previous research, frankincense was formulated together with myrrh oil in order to increase their bioactivities. For instance, the solid lipid nanoparticles loaded with frankincense in combination with myrrh oil have been prepared in order to increase their water solubility and decrease their chemical instability [8]. However, their nano-based delivery system was usually based on high-energy techniques and needed various compounds in order to be stabilized. Moreover, the frankincense essential oil has not been incorporated into nano-based delivery systems individually, and their single characteristics have not been evaluated yet.

Thus, the aim of the present study was the preparation of frankincense essential oil microemulsions through low energy techniques and also the evaluation of various formulation parameters, namely, surfactant, co-surfactant, essential oil, and water concentrations, or formulation parameters, specifically, mixing speed and temperature on their mean particle size, size distribution (PDI), turbidity and antioxidant activity.

EXPERIMENTAL SECTION

Materials

Frankincense essential oil (*Boswellia serrata* essential oil, 100 %) was provided by Najian Co. (NG, Tabriz, Iran). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Non-ionic surfactants Tween 80 (HLB= 15), and glycerol (HLB 4.5) were purchased from Merck (Darmstadt, Germany). Double distilled water was provided by Dr. Mojalali Co. (Tehran, Iran). *Escherichia coli* (*E. coli*, PTCC 1276) and *Staphylococcus aureus* (*S. aureus*, PTCC 1431) were obtained by microbial Persian Type Culture Collection (PTCC, Tehran, Iran). Mueller Hinton Broth (MHB) medium was purchased from Biolife (Biolife Co., Milan, Italy).

Methods

Formulation of microemulsions

In order to prepare the frankincense essential oil microemulsions, Tween 80 was first mixed with glycerol and stirred magnetically (IKA Plate, RCT digital, and Deutschland, Germany) for 5 min, at various rotating speeds. The essential oil was then added to the Tween

Table 1: The selected levels of formulation and process variables in preparation of Frankincense essential oil microemulsions.

Sample name	Surfactant (g)	co- surfactant (g)	Frankincense (g)	Water (mL)	stirring speed (rpm)	Temperature (°C)
1	0.7	0.2	0.1	9.2	500	40
2	0.5	0.2	0.1	9.2	500	40
3	0.3	0.2	0.1	9.2	500	40
4	0.5	0.3	0.1	9.2	500	40
5	0.5	0.2	0.1	9.2	500	40
6	0.5	0.1	0.1	9.2	500	40
7	0.5	0.2	0.15	9.2	500	40
8	0.5	0.2	0.1	9.2	500	40
9	0.5	0.2	0.05	9.2	500	40
10	0.5	0.2	0.1	11.2	500	40
11	0.5	0.2	0.1	9.2	500	40
12	0.5	0.2	0.1	7.2	500	40
13	0.5	0.2	0.1	9.2	700	40
14	0.5	0.2	0.1	9.2	500	40
15	0.5	0.2	0.1	9.2	300	40
16	0.5	0.2	0.1	9.2	500	60
17	0.5	0.2	0.1	9.2	500	40
18	0.5	0.2	0.1	9.2	500	20

80/glycerol mixture and was stirred for extra 15 minutes. The mixture was titrated drop-wise to a certain volume of distilled water, which was placed into a water bath, under a magnetic stirrer till a homogeneous translucent appearance occurred. The mixture was turned from translucent into completely transparent systems after 1 day of equilibration at room temperature [9].

The concentrations of frankincense essential oil, surfactant (Tween 80), co-surfactant (glycerol), distilled water as well as the magnetic stirrer speed and water-bath temperature were shown in Table 1. The homogenization speed was set constant all through the fabrication steps.

Analysis

Mean particle size and size distribution

The average particle size and size distribution (polydispersity, PDI) of frankincense essential oil microemulsions were measured based on dynamic light scattering (DLS) technique, using zetasizer (Nano-ZS, Malvern Instruments, Malvern, UK), one day after sample preparation. Measurements were done at $25°C$. Mean particle diameter was reported as z-diameter. The PDI ranged from 0 to 1, in which the smaller values of PDI shows the colloidal system with most homogenous particles from size view point. All measurements were performed in triplicate [10].

Turbidity

The turbidity of frankincense essential oil microemulsions was measured using an UV-Visible spectrophotometer (PG Instruments Ltd, T70+UVNIS, UK) according to [11]. The absorbance of samples at 600 nm was reported as their turbidities. The temperature was set at 25 °C and the deionized water was used as a blank solution.

In-Vitro antioxidative activity (DPPH assay)

Most of the essential oils have antioxidant effects in various food formulations. Thus, the in-vitro anti oxidative activity of nanoemulsions can be correlated to the loaded

essential oil in formulated microemulsions. Due to the highest antioxidant activities come from more encapsulation efficiencies of samples. 1 mL of sample was added to 3.9 mL of fresh methanolic solution of DPPH. The absorbance of these solutions and methanolic solution of DPPH without any microemulsions, at λ=517 nm, were measured using a UV–Visible spectrophotometer (PG Instruments Ltd, T70+UVNIS, UK) and coded as A1 and A0, respectively. The radical scavenging activity of samples was calculated according to the following equation:

$$\text{Scavenging activity (\%)} = (1 - A1/A0) \times 100\%,$$

The methanol was used as a blank in these measurements. All assays were performed in triplicate [12].

Antibacterial analysis

The Minimum Inhibitory Concentration (MIC) of essential oils either in pure form or in microemulsions (the best sample, sample no. 1), as well as glycerol and Tween 80 were investigated using the agar dilution method. Aliquots of samples were sequentially diluted in a 96-well plate containing MHB medium to produce various concentrations for either pure or emulsified essential oils. The final concentration of each strain of bacteria was adjusted to 5×10^6 CFU/mL based on 0.5 McFarland standard. The plates were incubated for 24 h at 37 ℃ and the minimum concentration of samples giving no microbial growth in the plate was recorded as their MIC values [13].

Morphology

The morphology and microstructure of the microemulsion (sample 1) was also determined by transmission electron microscopy (TEM, Hitachi H7500, Japan). One drop of diluted microemulsion (1:10) was placed on the film grid, stained by a 1% aqueous solution of phosphotungstic acid, and was observed after drying.

Chemical analysis

The chemical composition of frankincense essential oil was analyzed using Agilent 7890B/5977A GC–MS system (Waltham, Massachusetts, US). The sample (1.0 μL) was injected with a PAL RSI 120 at 250 °C with a split ratio 5:1. Volatiles were separated on a TG-5MS fused silica capillary column (30 m, 0.251 mm, and 0.1 mm film thickness). The initial temperature of the oven was set at 40 °C and was held for 1 min, and was increased to 250 °C at the rate of 4°C/min, and was again held for 10 min. The carrier gas was helium at the flow rate of 1 mL/min. The used MS detector was Electron Impact (EI) ionization at 70 eV with the ion source temperature of 230 °C and scan time segments from 40 to 350 am.

The compound's retention times were compared to the database of the stored known compound spectrums in the GC-MS NIST 08 library. All identified components were quantified using their percent relative peak area.

Statistical analysis

All experiments were carried out at least in triplicate using freshly prepared samples, and the results are reported as the mean ± Standard Deviation (SD). One-way analysis of variance (ANOVA) was carried out by Minitab 17 with the level of significance at p-value< 0.05.

RESULTS AND DISCUSSION

General results

The frankincense essential oil microemulsions were successfully synthesized in the ranges from 12 to 633 nm. The characteristics of microemulsions are summarized in Table 2. As can be seen in Table 2, most of the studied independent parameters had significant effects on the characteristics of prepared microemulsions (p-value<0.05).

Effects of surfactant concentration

Previous research concluded that the surfactant concentration has the most significant effects on the characteristics of colloidal organic nanoparticles as compared to other formulation parameters [14]. As can be shown in Table 2, decreasing the surfactant concentration led to an increase in the mean particle size of gained microemulsions. Increasing the mean particle size of organic nanoparticles with stabilizing surfactant concentration has also been reported in various studies [15]. However, some researchers have resulted in a different trend for changing the mean particle size of nanoparticles by increasing the surfactant concentration. For instance, the increase of surfactant raised the particle size of astaxanthin nanodispersions at high organic phase concentrations [14].

The size distribution or PDI values of frankincense essential oil microemulsions were also affected by

Table 2: The characteristics of frankincense essential oil prepared at different formulation and process conditions.

Sample name	z_ average (nm)	PDI	Turbidity	Antioxidant activity (%)
1	12.63±2.403 [a]	0.226±0.0113 [a]	0.006±0.0050[a]	64.5±0.74 [a]
2	98.1±5.312 [b]	0.230±0.0115 [a]	0.014±0.0086 [b]	63.8±0.331 [a]
3	171.15 ±9.504 [c]	0.528±0.0264 [b]	0.022±0.0096 [b]	61.8±1.27 [b]
4	48.33±4.292 [a]	0.175±0.00875[a]	0.011±0.0040 [a]	68.7±0.141 [a]
5	98.1 ±5.312 [b]	0.230±0.0115 [b]	0.014±0.003 [a]	63.8±0.331 [b]
6	182.05 ±9.175 [c]	0.244[b]±0.0122[b]	0.027±0.0106 [b]	61.9±0.850 [c]
7	131.74±8.292 [a]	0.215±0.01075[a]	0.018±0.009 [a]	65.5±0.0.92 [a]
8	98.1 ±5.312 [b]	0.230±0.0115 [a]	0.014±0.009 [a]	63.8±0.331 [b]
9	12.78 ±2.432 [c]	0.375±0.01875[b]	0.011±0.022 [a]	55.9±1.37 [c]
10	633.5 ±17.171 [a]	0.907±0.04535[c]	0.004±0.0029 [a]	62.7±0.78[a]
11	98.1±5.312 [b]	0.230±0.0115 [a]	0.014±0.0009 [b]	63.8±0.331[a]
12	12.47 ±2.536 [c]	0.329±0.01645[b]	0.017±0.0050 [c]	64.7±0.72[a]
13	73.74 ±4.160 [a]	0.237±0.01185[a]	0.012±0.0075 [a]	65.5±0.48 [a]
14	98.1±5.312 [b]	0.230±0.0115 [a]	0.0140.0070[a]	63.8±0.331 [b]
15	106.8 ±6.185 [c]	0.265±0.01325[b]	0.018±0.0078 [a]	59.8±0.83 [c]
16	43.81 ±3.609 [a]	0.224 ±0.0112 [a]	0.011±0.0082[a]	57.1± 1.650[a]
17	98.1 ±5.312 [b]	0.230 ±0.0115 [a]	0.014±0.0005 [b]	63.8 ±0.331[b]
18	251.9 ±12.337[c]	0.308±0.0154 [b]	0.029±0.0045 [c]	65.6±0.387 [c]

surfactant concentration, in which decreasing the surfactant content increased the PDI or size distribution of microemulsions and decreased the homogeneity of system. At less surfactant concentrations, the coverage of newly produced nanoparticles by surfactant molecules would be incomplete, and, consequently, their stabilization would be incomplete. Thus, the imperfect stabilized nanoparticles tend to aggregate and produce nanoparticles in larger and varied sizes. Increasing the PDI by decreasing surfactant concentration has also been reported in most previous researches [14, 16]. However, they also observed a polymodal size distribution for astaxanthin nanoemulsions by increasing the surfactant concentration due to the production of free micelles [17]. The surfactant concentration not only has an important effect on the size of nanoparticles, it can control their shape and morphology. It was reported that the obtained nanoparticles at higher surfactant concentrations are usually in spherical shapes, however, at less surfactant concentrations, the nanoparticles are in agglomerated or rode-like appearance [17]. Normally, the colloidal systems with less PDI values are more physically stable due to their less ripening process. Besides, uniform-sized colloidal systems are more desired due to their homogenous dissolution rate and better intestinal absorption and bioavailabilities [18]. Contrary to present results, increasing the PDI by surfactant concentrations was observed in some previous studies [19]. They reported bimodal size distributions for their prepared colloidal vitamin E systems by increasing the surfactant-to-oil ratio.

Decreasing the transparency or increasing the turbidity of frankincense essential oil microemulsions by raising their surfactant content can also be explained by decreasing their mean particle sizes, in which the colloidal systems with smaller particle sizes show higher transparencies. The smaller particles have weak light scattering and then make the system more optically transparent. These results are consistent with what has been found previously by *Jaberi et al.* [16]. Thus, it can be concluded that higher surfactant content leads to the

production of smaller essential oil nanoparticles and, consequently, more optically clear dispersion systems. Thus, it would be possible to include a greater amount of a lipophilic ingredient into clear beverages devoid of any adverse effect on their appearance [19]. After formation of nanoparticles, their size growth may be due to Ostwald ripening or coalescence processes, depending on surfactant, co-surfactant and oil phase concentrations.

The in-vitro antioxidant activity of gained frankincense essential oil microemulsions was also decreased by decreasing the surfactant concentration of the system. Prior researches confirmed the high impact of antioxidant compounds nano-sized delivery systems' particle sizes on their antioxidant activities. It was found that the smaller the particles, the better protection and superior antioxidant activity. The radical scavenging activity of frankincense essential oil nanoparticles was increased with surfactant content. These results are consistent with previous observations in which higher surfactant concentration could assist the dissolution of frankincense essential oil in the aqueous phase, resulting in higher antioxidant activity [20, 21].

Effects of co-surfactant concentration

Co-surfactants are mostly surfactant molecules with smaller hydrophobic or hydrophilic groups. Thus, despite their surface activity, they cannot form micelles themselves. However, they are miscible in surfactants and can strengthen the emulsification activity of surfactant and form stable micelles, because mixing co-surfactant and surfactant decreases the mean head group area between them at the micelle surface and surge their packing parameter and thus providing less interfacial tension and more surface activity and enhanced emulsifying and stabilizing ability [22]. Thus, the effects of co-surfactant concentrations on studied characteristics of frankincense essential oil microemulsions are similar to the surfactant concentration, in which increasing the co-surfactant content decreased the mean particle size, PDI, turbidity but increased their antioxidant activities. The similar observations were also reported by [23] in the synthesis of vitamin-E enriched nanoemulsions produced through spontaneous emulsification. Glycerol could dehydrate the hydrophilic head of surfactant molecules and provide the most favorable curvature and decrease their cloud point. Furthermore, co-surfactants can amend the rheological characteristics, density, viscosity, interfacial tension, and refractive index [23, 24].

Effects of essential oil (frankincense) concentration

Since in the formation of organic nanoparticles through spontaneous emulsification, the mixture of oil-surfactant-water in an oil droplet formation region is more imperative than the properties of pure oil, and the properties of the oil phase can be changed over time due to the structural organization of oil-surfactant-water mixture, it seems that a good correlation between the characteristics of nanoparticles and oil phase concentration may not be observed.

In the present study, as can be seen in Table 2, the frankincense concentration also affected the mean particle size, homogeneity, and antioxidant activity of gained frankincense essential oil microemulsions. However, in the studied frankincense concentration range (0.5-0.15%), the turbidity of the system remained constant. The equal turbidity of the samples prepared at different essential oil concentrations was unexpected despite significant changes in their particle sizes. It seems that the determining parameter in the turbidity of these samples was a factor other than their particle sizes, maybe the intrinsic color of frankincense essential oil. The mean particle size of the produced colloidal system was increased by raising the essential oil concentration. As the essential oil increases (at constant emulsifier content), the available surfactants decrease, and thus imperfect stabilization occurs. Thus, the coagulation, coalescence, and Ostwald ripening of newly produced nanoparticles were increased leading to extensive increases in their mean sizes [14]. A decrease in the essential oil content of colloidal systems may also reduce the interfacial tension and thus form finer droplets [19]. A considerable increase in particle size of various lipophilic active compounds nanoparticles was also seen in most previous research by increasing the initial load of active compounds [14, 25].

While the mean size of microemulsions was decreased by decreasing the essential oil content of the system, the heterogeneity of the system was increased. The oil phase may affect the size distribution of gained nanoparticles through various mechanisms. For instance, the viscosity of the oil phase controls the movement rate of surfactant from the organic toward aqueous phases. Thus, the viscosity of the system increases by raising the oil content of the system, leading to slower movement of surfactant, and

thus larger molecules in varied sizes would be created. Besides, at less essential oil concentrations, the probability of free micelles (without inner oil phase) by surfactant and/or co-surfactant molecules will be increased, and thus the varied sized particles would be created [14, 23].

Since the antioxidant activity of the system is mostly related to the bioactive ingredients of frankincense essential oil, the DPPH scavenging activity of samples was increased by increasing the concentrations of active compounds. It was observed that the mean particle size of the nanoparticles did not affect their antioxidant activities in various essential oil concentrations (other formulation and process parameters were kept constant). In some cases, a considerable decrease was shown in antioxidant activity of smaller nanoparticles, due to their more exposure surface area to oxygen and free radicals and thus higher proneness to degradation [10, 26].

Effects of aqueous phase volume (water concentration)

While the mean particle size of frankincense essential oil nanoparticles was decreased by reducing the aqueous phase volume, their turbidities and antioxidant activities were increased. No certain pattern was also seen in the PDI of the system by decreasing their water contents. While in most previous researches, a decrease in mean particle size was reported for organic colloidal systems by increasing the aqueous phase ratio of system [14, 16], decreasing the mean particle size of colloidal systems by reduction of their water content have also resulted in some previous researches [27]. Increasing water content eliminates rigid film, leading to coalescence of droplets and increased particle size.

Unexpectedly, the turbidity of the system was increased by decreasing the water-content despite of gaining smaller nanoparticles. This observation can be related to more dissolved co-surfactant and surfactant molecules as compared to suspended ones, at higher water contents. Accordingly, the intensity of scattered light by suspended molecules decreases, leading to more clear appearance of the system [14, 25, 28].

Effects of mixing speed of the phases

Previous researches resulted that the characteristics of functional lipid nanoparticles prepared through the self-emulsification process are mostly influenced by the formulation parameters rather than process ones [10, 29]. However, in the present research, the effects of two main process parameters, namely, mixing speed and temperature were also evaluated on mean particle size, PDI, turbidity, and antioxidant activity of frankincense essential oil nanoparticles. It was known that the stirring guarantees homogenous distribution of the oil phase and surfactant/co-surfactant in the aqueous phase that assists the spontaneous creation of smaller particles. Generally, in the studied mixing speed range, increasing the mixing rate improved the characteristics of nanoparticles. For instance, more homogenous smaller particles with greater antioxidant activities were obtained at a higher mixing rate of phases. However, the turbidity of the system was not affected by the mixing rate. Previous studies also concluded that the mean droplet size of nanoemulsions produced by a low-energy technique decreases by increasing stirring speed [17, 19, 26, 29]. Increasing the mixing rate provides more mechanical energy for the system in order to break up and even distribute and maintain the concentration gradients at interfaces [23]. The greater antioxidant activity of the nanoparticles synthesized at higher mixing rates can be related to the smaller sizes of these particles as compared to others.

Effects of mixing temperature

The mixing temperature also affected the mean particle size, PDI, turbidity, and antioxidant activity of frankincense essential oil nanoparticles, in which as the mixing temperature rises (from 20 to 60 °C), the mean particle sizes, PDI, turbidity, and the antioxidant activity of the system decrease. These results are in good agreement with previous research in which they observed that the formation of nanoparticles through low-energy techniques was facilitated by increasing the mixing temperature [23]. It seems that despite of the role of energy input in high-energy preparation techniques, which is mainly breaking up the particles, in low-energy techniques at low temperatures the kinetic energy barrier in the oil-surfactant-water system, prohibited it from changing from opaquecro-sized dispersion to clear an nano-sized one. Thus, raising the temperature reduces this energy barrier assists these transition states, and leads to continuous size reduction of the particles, resulting in the creation of smaller particles in similar sizes [28]. Moreover, decreasing the viscosity and surface energy of the system by increasing the temperature also expedites

Table 3: The MIC of surfactant (Tween 80) and co-surfactant (glycerol), macro-sized frankincense essential oil and nano-sized frankincense essential oil (frankincense essential oil microemulsions prepared at surfactant, co-surfactant, frankincense essential oil and water concentrations of 0.7 g, 0.2 g, 0.1 g and 9.2 mL, respectively, and mixing rate of 500 rpm and temperature of 40 °C).

	MIC (mg/mL)			
	frankincense essential oil microemulsions	macro-sized frankincense	Tween 80	Glycerol
E. coli	0.4	4	>400	>400
S. aureus	0.4	4	>400	>400

the disintegration of the oil–water interface and construction of smaller particles. On the other hand, by increasing the temperature, the solubility characteristics and molecular geometry of non-ionic surfactants can be changed due to dehydration of their hydrophilic head-groups. Increasing the hydrophobicity of non-ionic surfactants together with the decreasing of the oil–water interfacial tension promotions the formation of smaller particles at higher temperatures, as well [19]. The decreasing of the turbidity by rising the temperature can be explained by decreasing their particle sizes.

Due to volatility of frankincense essential oil as an active compound of the system, increasing the mixing temperature causes a considerable decrease in the active compound composition of nanoparticles and thus decreasing their antioxidant activities [10, 14, 26].

The decreasing of turbidity by increasing mixing temperature can be related to the gain of smaller-sized frankincense essential oil nanoparticles at higher process temperatures.

Antibacterial activity of frankincense essential oil

Sample no 1, prepared using surfactant, co-surfactant, frankincense essential oil and water at concentrations of 0.7 g, 0.2 g, 0.1 g and 9.2 mL, respectively, and mixing rate of 500 rpm and temperature of 40 °C was selected as the desired nano-sized frankincense essential oil and its antibacterial activity was evaluated against *E. coli* and *S. aureus*. The pure frankincense essential oil, surfactant and co-surfactant were also used at similar concentrations. The data can be seen in Table 3. According to Table 3, the obtained MIC of frankincense essential oil microemulsion (0.4 mg/mL for both *E. coli* and *S. aureus*) was about 10 times greater than pure essential oil. The Tween 80 and glycerol did not show any inhibition activity against both *E. coli* and *S. aureus*. Thus, using frankincense essential oil in nano-sizes or incorporating them into nano-sized delivery systems can enhance their antibacterial activities considerably. The previous research also observed enhanced antibacterial activities for their nano-sized products such as D-limonene and oregano oil nanoparticles than their original pure macro-sized ones in similar concentrations [30- 32].

It was proved that the nano-size antibacterial compounds could upsurge the passive cellular absorption mechanisms and reduce mass transfer resistances, due to their subcellular sizes.

Morphology of frankincense essential oil microemulsions

The TEM image of frankincense essential oil microemulsions was shown in Fig. 1 in order to visualize their morphology. The tested sample was sample no. 1, which was prepared at surfactant, co-surfactant, frankincense essential oil, and water at concentrations of 0.7 g, 0.2 g, 0.1 g, and 9.2 mL, respectively, and mixing rate of 500 rpm and temperature of 40°C. The droplets were spherical in morphology and were in the range of less than 50 nm. However, some larger particles were also found in the tested sample. The TEM image confirms the mean particle size and PDI obtained by DLS analysis. Due to the presence of larger nanoparticles, some physical instabilities or size growth can occur in gained microemulsions due to the ripening process.

Chemical analysis

The main chemical components of frankincense essential oil were obtained using GC and the main detected components were shown in Table 4. As can be seen in Table 4, α-Thujene, α-Pinene, β-Pinene, Limonene, and Estragole are the main components of frankincense essential oil, with a total peak area of 87%.

The most identified main compounds for frankincense essential oil in this study were also previously reported *by Mikhaeil et al.* for frankincense

Table 4: The main chemical compounds of frankincense essential oil.

No	Retention time (min)	Percentage (%)	Compounds
1	26.320	29.3	α-Thujene
2	23.934	26.5	α-Pinene
3	26.120	13.2	β-Pinene
4	23.047	9.5	Limonene
5	16.256	8.5	Estragole
Total			87%

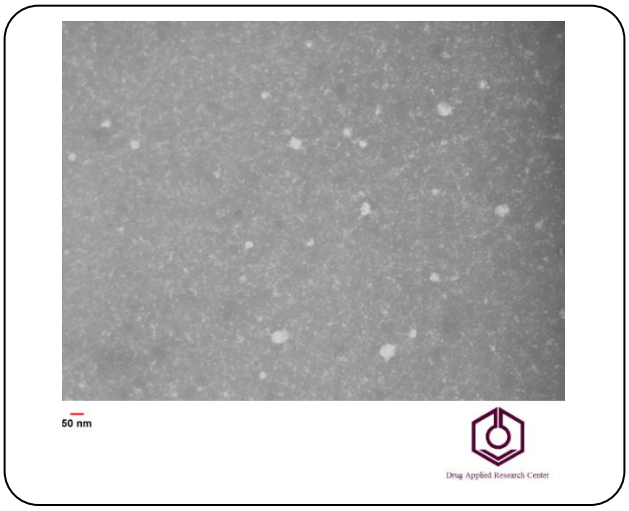

Fig. 1: TEM image of frankincense essential oil microemulsions.

essential oil obtained from steam distillation of oleogum resin of Boswellia carterii Birdwood [33]. Moreover, all detected components in Table 4, were in the list of observed volatile compounds for twenty commercial frankincense essential that were studied by *Vanvuuren et al.* [34].

CONCLUSIONS

The frankincense essential oil microemulsions were successfully prepared through a low energy self-emulsification process, using Tween 80 as surfactant and glycerol as co-surfactant in varied size ranges (approximately from 12 to 633 nm), at various formulation and process parameters. All selected independent parameters affected the most characteristics of frankincense essential oil microemulsions, significantly. According to the obtained results, the most desired frankincense essential oil nanoparticles obtained using high concentrations of surfactant, and medium concentrations of co-surfactant, oil phase, and water, as well as medium levels of mixing speed and temperature. All prepared frankincense essential oil nanoparticles show in-vitro DPPH radical scavenging activities. The antibacterial activities of essential oils were also enhanced through their size reduction into nano-range particles.

Received: Aug. 19, 2021 ; Accepted: Dec. 13, 2021

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