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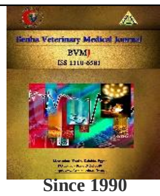

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Original Paper

Synthesis and characterization of nanoemulsion from thymus and cumin oil extracts

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ABSTRACT

Due to the development of several microorganisms that are antibiotic-resistant. Therefore, it became vital to look for alternatives, such as oily plant extracts and nanomaterials made from them that safeguard humans and preserve food against harmful microorganisms. In this study, nanomaterials were synthesized from thyme essential oil, cumin essential oil, and a mixture thereof. The chemical composition of the nanoemulsion was determined by gas mass spectrometry (GC-MS) and the size by zeta potential microscopy and TEM. GC-MS analysis of thyme nanoemulsion showed mainly 7 compounds, of which 1,2,3-propantirylster (32.94%) was the highest. The cumin microemulsion contained mainly 11 compounds, of which oleyl oleate (33.19%) was the highest percentage. The mixture mainly contained 7 compounds of which phenyl palmitolite (31.54%) was the most. Using TEM, the particle size measurements of thyme, cumin, and nano-mixture were 31.70 nm, 31 nm, and 21.9 nm, respectively. There was no aggregation, homogeneous size, and spherical nature. The conductivity, viscosity, polydispersity index (PDI), and zeta potentials were measured to thyme oil nanoemulsion 0.160 ms/cm, 0.8874, 0.260, and -4.70 mV3.99, respectively. The cumin oil nanoemulsion is 0.173 ms/cm, 0.8874, 0.232, and -3.79 mV to 5.79 and mixed oil nano-emulsion, the corresponding values were 0.165 ms/cm, 0.8874, 0.197, and -1.17 mV 10.59from results conclude that we can manufacture nanomaterials from natural materials at very low concentrations, thus reducing their aromatic odor, in addition to the synergistic action between two types of nanomaterials, which may produce new materials with a stronger effect.

1. INTRODUCTION

There are numerous applications for nanobiotechnology in health care, agribusiness, and the ecosystem. Future advances in nanotechnology may enable the treatment of numerous illnesses for which there is currently no remedy. The safety and danger factors associated with the use of nanotechnology in medicinal therapeutics need to be properly assessed (Prasad, 2000).

Nanoemulsion (NEs) typically have widths between 20 and 200 nm and can be translucent or milky white depending on the size of their droplets (Borrin et al., 2016; Acevedo et al., 2017). Compared to traditional emulsions, nanoemulsions are more robust against gravity separation, coalescence, and flocculation they are subject to Ostwald ripening (Chang et al., 2014). Besides, Because of their tiny droplet size and large surface area, they can increase the bioavailability of the bioactive substances (Silva et al., 2011; Chang et al., 2014; Acevedo et al., 2017).

The well-defined nano-formulations with therapeutic significance requires proper NP characterization through determination of particle size and surface charge. The use of dynamic light scattering and zeta potential techniques to determine particle size and surface charge has grown in favor (Bhattacharjee, 2016).

A new age in biological and pharmaceutical uses has been made possible by nanotechnology in terms of better bioavailability, increased therapeutic effectiveness, and increased penetrative power (Ong et al., 2017).

Curcumin, camptothecin, thymol, and eugenol are a few examples of phytomolecules from medicinal and aromatic plants that have distinct bioavailability, effectiveness, and solubility restrictions. It restricts its biological activity and, thus, its use in the field of biomedicine. After these strong phytomolecules were enclosed in the nanocarriers, an increase in biological activity and sustained delivery was seen. Additionally, less hazardous metal nanocarrier production is mediated by MAPs and/or their compounds or oils (Kumari et al., 2019).

The most efficient nanoparticles tested against the tested bacteria were those with a size of 20–25 nm. Additionally, these nanoparticles show no cytotoxicity, pointing to potential applications as antimicrobial additives in the manufacture of both ambulatory and nonambulatory medical equipment (Martinez-Gutierrez et al., 2010)

The antibacterial activity of thyme oil increased with its conversion into a nanoemulsion, and its nano-shape can be employed as a substitute antimicrobial agent in prepared fish or food items. (Ozogul et al., 2020)

The thermodynamic stability of the synthesized formulations was examined, and it was discovered that the thyme oil nanoemulsion with an oil: surfactant ratio of 1:0.5 was the most stable. The size and shape of the droplets were further examined by dynamic light propagation and transmission electron microscopy, who

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measured (52.18 4.53) nm for the thyme oil nanoemulsion (Parisa et al., 2022)

The described nanoemulsion has a polydispersity index value of 0.298 and a mean droplet size of 20.72.6 nm. The EO nanoemulsion's LC50 was 33.65 ppm (Azmy et al., 2021)

After five minutes of sonication, the final droplet diameter was 10.4 ±0.5 nm. The early induction of apoptosis was clearly visible, and the MTT experiment showed that the IC50 value was 1.5 L/mL. Cumin nanoemulsion treatment of tongue cancer cell line resulted in reduced colony formation. Significant antibacterial activity was shown by the nanoemulsion against S. aureus. On treatment with cumin nanoemulsion, a significant cytoplasmic leakage was noticed (Nirmala et al., 2020).

By encapsulating Thymus essential oils (EOs) in various delivery systems (DSs), such as nanoemulsions and biopolymer nanoparticles, it is possible to take advantage of their efficiency as food preservatives. Through different manufacturing processes (high-pressure homogenate emulsification or anti-solvent precipitation), the use of various emulsifiers and stabilizers, and the resultant antioxidant and antibacterial activities of Eos (Jayari et al., 2022)

The presence of monoterpenes, together with a significant amount of phthalides, served as the essential oil's key distinguishing characteristics. The decoction and hydroethanolic extracts also included a total of 7 phenolic compounds, which had intriguing antioxidant capabilities and bacteriostatic action, notably against Gram-positive bacteria. (Spréa et al., 2020)

Plants are the major source of antimicrobials, and they include a range of antimicrobial essential oils. Herbs and spices containing essential oils include sage, rosemary, thyme, basil, oregano, clove, and cardamom. These antimicrobial chemicals are also utilized in combination with edible coatings to prevent the growth of germs on the surface of the food (Mashabela et al., 2022).

Physicians have traditionally relied on antibiotics to treat bacterial illnesses. Antibiotic-resistant bacterial strains, however, pose a danger to the effectiveness of several antibiotic kinds as a result of their misuse. We investigated whether the gram-negative bacterium E. coli might be prevented from growing by 11 regularly used spices.so they decided to try ginger, cinnamon, clove, thyme, oregano, cumin, garlic, black pepper, and rosemary (Gehad and Springe, 2020).

Among other essential oils, the USH method-prepared nanoemulsified Cuminum cyminum L. has strong antioxidant potential and an antibacterial impact. It may be utilized to create innovative natural antioxidants as well as flavouring agents that can be employed in various food item (Nasiris et al., 2020)

The utilization of nanoemulsions has been a key focus in this field since they may be made with readily available food components and technological resources. Oil-in-water nanoemulsions, in particular, are being used as delivery methods for a variety of hydrophobic ingredients in meals, such as nutrients, nutraceuticals, antioxidants, antimicrobials, colors, and tastes. They are composed of tiny oil droplets (200 nm) distributed in water (Das et al., 2020)

The efficacy of nanoemulsions and emulsions of thyme and spearmint essential oils was assessed as an option to chemical control for decreasing *Meloidogyne javanica and Fusarium oxysporum* on coleus plants, *Coleus forskohlii*, in vitro and greenhouse environments. The best outcomes in vitro experiments were obtained with thyme nanoemulsions, with droplet sizes between 25.4 and 32.9 nm, and spearmint nanoemulsions, with droplet sizes between 5.91 and 9.77 nm (Hammad and Hasanin, 2022)

The aim of the study is to find and manufacture natural materials with no side effects that do not affect humans and animals, and at the same time have a high effect on microbes.

2. MATERIAL AND METHODS

2.1 Essential plant oil and extracts

Cumin and thyme oil extracts were provided from the National Research Center (NRC), Giza, Egypt, Tween 80 was provided from Sigma-Aldrich Co. and Double-filtered and deionized water was distilled before use.

2.2 Nanoemulsion preparation, characterization, and cytotoxicity test

The nanoemulsion was prepared in the Nanomaterials Research and Synthesis Unit using oils (20 ml) from cumin or thyme oils, or a combination of both (10 ml from each oil), Tween 80 (30 ml), and distilled deionized water (50 ml). These ingredients were blended for 30 minutes in a homogeneous blender with a 1500-watt, and then distilled water was gradually added to the combined oil phase according to (Rao and McClements, 2011).

2.3 Characterization of oil micro-emulsions:

2.3.1. The prepared oil nano-emulsions were measured using the Zetasizer Malvern Instrument (Corp, Malvern, UK). This instrument measures droplet size, surface charge (zeta potential), size distribution (polydispersity indices PDI), and electrical conductivity.

2.3.2. Transmission Electron microscopy (TEM) monitoring is carried out using [JEM 1400F HRTEM] at ray power of (300) keV to characterize the nanoemulsion and measure electrical conductivity zeta potential (surface charge)

2.3.3. Utilizing GC-MS to identify microemulsion components. The National Institute of Standards and Technology's (NIST's) database, which contains more than 62,000 patterns, was used to interpret the mass spectrum obtained by 1GC/MS-MS. The NIST library's collection of unknown components' spectra. It was determined the components of the test materials' names, molecular weights, and structures (Ezhilan and Neelamegam, 2012).

Cell culture: the Vero Green Monkey cell line was purchased from Mokatam Egypt-based Nawah Scientific Inc. They were cultured in Dulbecco's Modified Eagle Medium DMEM supplemented with 100 mg/mL streptomycin, 100 units/mL penicillin, and 10% heat-inactivated bovine serum at 37 °C in a humidified, 5% (v/v) CO2 air.

Cytotoxicity test: Cell viability was evaluated using the SRB test at various doses (0.01,0.1,1,10,100 ug/ml), according to Allam et al. (2018)

All of the experimental procedures were approved by Animal Ethical committees of Benha University with ethical approval number (BUFVTM34-10-22).

3. RESULTS

3.1. Characterization of micro-emulsions

The nano-emulsion of *Thymus vulgaries* oil, *Cuminum* oil (cumin), and blend oil was measured at 31.70 nm, 31+69 nm, and 21.8 nm, respectively, according to the HRTEM findings (Figure 1; A, B, C). There is no clustering and the nature is spherical with homogeneous dimensions.

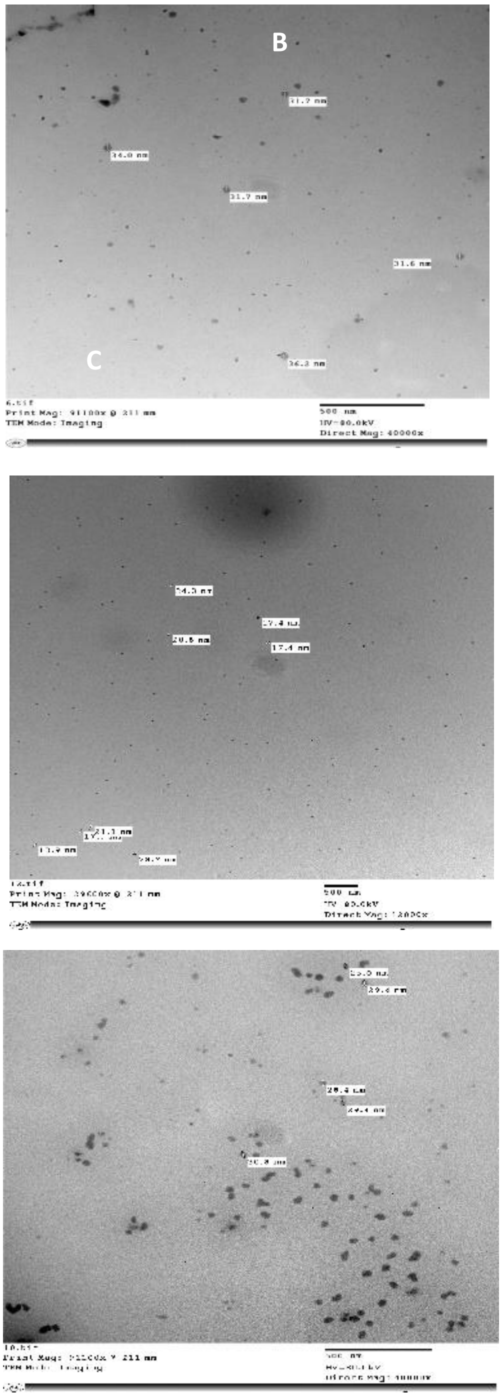

Fig (1). A complete description of each oil: *thyme vulgaris* nanoemulsion (A), cumin oil nanoemulsion (20% oil, in water) (B), and mixed nanoemulsion20% (10% thymus oil + 10 % cumin oil) (oil/water) (C). All have conductivities, viscosities, multiple dispersion indices (PDI), and zeta potentials that were measured to be 0.160 mS/cm, 0.8874, 0.260, and -4.70 mV± 3.99, respectively. Cumin oil nano-emulsion properties, according to 0.173 mV/cm, 0.8874, 0.232, and -3.79 mV ± 5.79. For the oil-mixed nanoemulsion, the corresponding values were 0.165 mV/cm, 0.8874, 0.197, and -1.17 mV± 10.59.

Using the SRB assay ,cell viability was evaluated Following a 72 h incubation period, the vitality percentages of thymus micro-emulsions at various doses (0.01, 0.1, 1, 10, and 100 ug/ml) were 98.34, 98.21, 96.42, 95.80 and 95.80.Additionally, the micro-emulsions of cumin were 98.59 , 98.20 , 96.42 ,95.80 and 90.40 but the micro-emulsions of the blend were 97.3, 98.21, 96.42 ,95.80 and 81.29 meaning that the IC50 was greater than 100 ug/ml. as show in Figure 2.

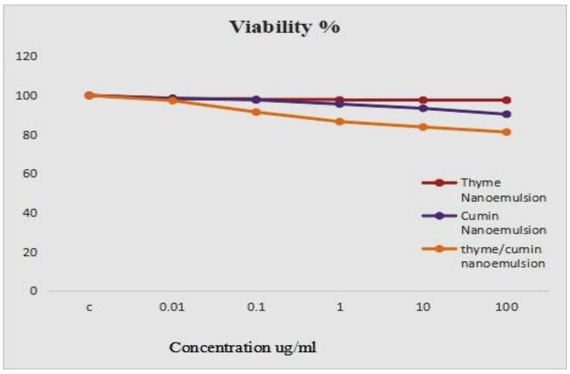

Fig (2). Cell viability percent of cumin nanoemulsion, thymus nanoemulsion, and (thyme + cumin) blend nanoemulsion by SRB-test

3.2. Analysis of Chemical structure of cumin, thyme and blend microemulsion

The cumin microemulsion was analyzed by gas mass spectrometry (GC-MS), and the result was 11 chemical compounds with different ratios and the highest percentage was oleyl oleate (33.19%) as shown in table (1).

Table 1 Analysis of chemical compounds by using GC-MS/MS to cumin nano emulsion

Compound Name	Molecular formula	Area%
Anethole (anise camphor)	aromatic compound. C10H12O	3.48%
Thymol	Phenol Volatile. C10H14O	0.62%
PYRROLIDINE,	C6H14NPS	1.55%
Monoolein	It derives from an oleic acid. C21H40O4	0.69
cis-vaccenic acid	Monocarboxylic acid or derivatives C21H42O2Si	0.25
Behenyl palmitoleate	C38H74O2	0.32
oleyl oleate	C36H68O2	33.19
9-hexadecenoic acid	C34H64O2	30.53
Oleyl palmitoleate	C34H64O2	12.44
Cetyl linoleate	C34H64O2	9.74
1,3-Diolein	C39H72O5	1.08+1.22

The mix oil microemulsion was analyzed by gas mass spectrometry (GC-MS), and the result was 7 chemical compounds with different ratios and the highest percentage was Behenyl palmitoleate (31.54%) as shown in table (2).

Table (2) Analysis of chemical compounds by using GC-MS/MS to mix oil nano emulsion

Compound Name	Molecular formula	Area%
Anethole (anise camphor)	aromatic compound. C10H12O	17.06%
Thymol	Phenol Volatile C10H14O	2.97%
2,5-Dimethoxy-*p*-cymene	C12H18O2	7.32
9-hexadecenoic acid	C34H64O2	19.91
Behenyl palmitoleate	C38H74O2	31.54
1,3-Diolein	C39H72O5	5.7
Oleyl palmitoleate	C34H64O2	11.87

The thyme oil microemulsion was analyzed by gas mass spectrometry (GC-MS), and the result was 7 chemical compounds with different ratios and the highest percentage was 1,2,3 propanetriylester (32.94%) as shown in table (3).

Table (3) Analysis of chemical compounds by using GC-MS/MS in thymus nano emulsion

Compound Name	Molecular formula	Area%
Octadecenoic acid	C21H40O4	1.13
Oleic acid	C21H42O2si	1.30
Ethanaminium	C44H84NO8p	2.95
2-Hydroxy-3-[(9E)-9-OCtadec	C39H72O5	23.36
Glycidyl olcate	C21H38O3	5.63
ENOyloxy	C39H72O5	27.44
1,2,3 propanetriylester	C57H104O6	32.94

4- DISCUSSION

In our research, we were able to successfully synthesize and optimize nanoparticles from thyme and cumin oil and mixture. The best efficiency of nanoparticles can be achieved in large part by controlling the particle size, as the Particle size reduction may improve the bioavailability, solubility, and effectiveness of poorly water- soluble medications. Small particles interact with the cell membrane more effectively than big ones during endocytosis. The TEM picture displayed the droplets of Nanoemulsion which showed spherical shape and size homogeneity and no aggregation these results confirmed by Hassanin et al. (2017) who reported that thyme essential oil nanoemulsion was spherical and ranged in size from 26.6-45.3 nm. Similarly, Sundararajan et al. (2018) found that the essential oil in nanoemulsions had an ordered distribution and a spherical form. Also, Jaiswal et al. (2015) reported that Nanoemulsion usually has particle sizes between 20-200 nm. The size and form of the particles distributed in the continuous phase is the primary distinction between an emulsion and a nanoemulsion.

The zein-instituted nanoparticles synthesized together with *Thymus capitatus (*TC1) and stabilized with maltodextrins were the most stable formulation; they had the following properties: polydispersity index, droplet size, potential, and encapsulation capacity of 0.14, 38.7 mV, 74.7 nm and 99.66%, respectively. Additionally, this formularization improved the antibacterial and antioxidant properties of EO inhibition diameters ranging from 12 to 33 mm vs. a range between 12 to 28 mm for non-encapsulated TC1. The antioxidant activity was improved to 60.69 g/mg vs. 57.67 g/mg for non-encapsulated TC1. This formularization is a viable alternative for the efficient employ of natural antibacterial bioactive compounds against pathogenic and spoilage bacteria (Jayari et al., 2022)

In this research, the LC50 for cumin essential oils was 96.29 ug/mL which come in agreement with Mahran (2022). According to Kamaraj et al. (2011), the methanolic extract of *C. cyminum* seed caused 87.20 1.92% mortality against An. stephensi (LC50 = 500 ppm) and 75.60 2.48% mortality against Culex Quinquefasciatus at a dosage of 500 ppm. This result is different from that of Cumin has also been d (LD50 = 31.8 μg adult1 and LC50 = 3.2 ml L−1, respectively (Benelli et al., 2018).

The chemical compounds found in nanoemulsions in our study according to the mass spectra profile of GC- MS of cumin is coordinated with the results reported by Lopes et al. (2011). The A phenol derived from volatile oils, such as thyme oil. Stabilizers are added to pharmaceutical formulations using it. It was once used as a vermifuge and has been used now for its cleansing, antimicrobial, and antifungal properties. The antibacterial and emulsifying qualities of this ester were assessed in this investigation. The synthesized oleyl oleate's emulsifying ability was inferior to Tween 80 and sodium dodecyl sulphate, and no antibacterial action was found which is consistent with Shinde and Raskar (2019). The antibacterial activity of every synthetic N-(2-oxo-1,2-dihydro-3'H-indol-3-ylidene) pyridine-4-carbohydrazide was tested. The majority of the derivatives effectively combatted Gram-positive and Gram-negative bacteria (Yoon et al., 2018) Antibacterial lipids like fatty acids and monoglycerides, which disrupt bacterial cell membranes and have a variety of direct and indirect inhibitory effects, are antibacterial agents.

5. CONCLUSION

This study emphasized the benefits and difficulties of using nanotechnology in conjunction with medicinal and aromatic plants (MAPs). It would be a useful method for the quick delivery of various phytomolecules as well as the creation of unique nanomaterials, which increases the possibility of researching prospective in MAP molecules.

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