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# Evaluation of Nanotechnology Effects on the Microbiological and Biological Properties of Thyme Essential Oil

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## ABSTRACT

The main objective of the present study was to evaluate the effect of nanotechnology on the properties of thyme essential oil (microbiological and biological properties). This was done by preparing a nanoemulsion of thyme essential oil and comparing its properties with thyme essential oil at normal size. This was done in five stages; first stage is to check the presence of active components of thyme essential oil by GC/Ms. The second stage is preparation nanoemulsion of thyme essential oil using water and non-anionic surfactant (tween 80). Third stage is the characterization of nanoemulsion by TEM and zeta potential. Fourth stage is studying the difference microbiological effect of thyme essential oil and nanoemulsion of thyme essential oil on bacteria (*E. coli* and salmonella), and comparison between them. The final stage is studying the biological effect of thyme essential oil and nanoemulsion of thyme essential oil on breast cancer cell line, and comparison between them. Fractionation and identification of chemical components of thyme oil by GC/MS showed that thyme essential oil contained 14 components and the main component named *Carvacrol* with percentage 43.27%. Characterization of thyme essential oil nanoemulsion confirmed contain it nanoparticles with spherical shape, dispersed in the nanoemulsion, with range of 39.27nm to 86.69nm, and negative charge for zeta potential. microbiological analysis of normal and nano thyme essential oil showed that, normal and nano thyme essential oil have antibacterial effect against *E. coli* and salmonella, and nano thyme essential oil was more effective than normal thyme essential oil as antibacterial. Also, biological studying of normal and nano thyme essential oil confirmed the anticancer effect of them against breast cancer cells line (MCF-7), as well nano thyme essential oil has better effect on breast cancer cells than normal thyme essential oils at all concentrations.

***Keywords:*** nanotechnology, thyme essential oil, nanoemulsion, normal size

## 1. Introduction

Thyme is a fragrant herb that grows throughout the Mediterranean region, which includes Europe, Asia, and North Africa. Taxonomically, thyme is a member of the Dicotyledon class, which is indigenous to the western Mediterranean region, and the family Labiatae (Lamiaceae), specifically the genus Thymus (derived etymologically from the Greek and Latin words "Thymon" and "Thymún"). One of the aromatic therapeutic herbs of the Iberian Peninsula, thyme's essential oil is now one of the most popular in the culinary sector. Thyme's essential oils and chemical constituents are linked to its health benefits, which have been utilized since ancient times. Its essential oils are linked to its economic significance (Nieto, 2020).

The components with the highest economic interest and quality are *Thymol* (68.1%) and *Carvacrol* (10%), which make up the majority of the most active chemicals in the essential oil of these plants. Nevertheless, thyme essential oil (EO) is made up of over 60 components, the majority of which have significant positive benefits, such as antioxidant, antibacterial, anticancer, and antimicrobial qualities (Rota *et al.,* 2008).

Thyme essential oil inhibited the growth of both gram-positive and gram-negative species, including *Salmonella Enteritidis, Yersinia enterocolitica, Salmonella Typhimurium, Escherichia coli*

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*O157:H7, Listeria monocytogenes, Escherichia coli, Shigella flexneri, Staphylococcus aureus*, and *Shigella sonnei*, with inhibition zones ranging from 19.6 to 45.0 mm, (Posgay *et al.,* 2022).

*Thymol* and *Carvacrol* showed promise as chemo preventive or anticancer drugs in a variety of cancer types by exhibiting anticancer effects in cell lines that mimicked human tumors (Nagoor Meeran *et al.,* 2017; Elbe *et al.,* 2020 & Pakdemirli *et al.,* 2020).

*Carvacrol* has been found in various studies to have potent antitumor impacts and to be more cytotoxic than *Thymol*. *Carvacrol* therapy decreased the viability of MDA-MB231 and MCF-7 cell lines in connection to breast cancer (Halat *et al.,* 2022).

The study of molecules at the atomic, molecular, and supramolecular levels (sizes ranging from 1 to 100 nm) in order to identify characteristics that may be used to improve human health is known as nanotechnology. In order to understand biosystems, nanotechnology employs concepts and techniques at the nanoscale. As modern biology and medicine advance, nanotechnology is emerging to produce more materials at the nanoscale that can be applied to biological systems (Roco and Bainbridge, 2013).

Despite its diminutive size, the nanoscale has enormous potential. A nanometer is one billionth of a meter, or $10^{-9}$, of one. A nanometer's size in relation to a meter is equivalent to a marble's size in relation to the earth. A nanometer can also be thought of as the length of time it takes a man to grow a beard before he reaches for the razor (Abiodun‐Solanke *et al.,* 2014).

The Greek word nanos, which means "a dwarf," is the source of the prefix nano. The term "nano" was first used in 1947 during the 14th meeting of the International Union of Pure and Applied Chemistry (IUPAC) to refer to the one-billionth part ($10^{-9}$) of a unit. In various branches of contemporary research, the prefix "nano" has become a common designation in scientific writing to refer to small objects and processes. Nanoscience, nanorobots, nanotechnology, nanoelectronics, nanomagnets, nanoencapsulation, etc. The term "very small" things or processes are all described with the prefix "nano," most frequently at the nanometer scale (Nadeem and Dirk, 2022).

Richard Feynman introduced the world to the concept of nanotechnology in his famous "There is Plenty of Room at the Bottom" speech to the American Physical Society in December 1959. Because of this, he has been given the title "father of modern nanotechnology (Nura *et al.,* 2022).

Two approaches have been established that describe the various possibilities for the synthesis of nanostructures since *Feynman's* discovery of this new field of study that has attracted the attention of many scientists. The two types of these production techniques are top-down and bottom-up, and they vary in terms of cost, speed, and quality (Samer *et al.,* 2020).

The creation of nanoparticles can be accomplished by two methods: bottom-up, which entails assembling individual atoms and molecules into nanostructures, and top-down, which entails shrinking the smallest structures to the nanoscale. Because of the present concerns about climate change, food security, and sustainability, academics are looking at the topic of nanotechnology as a potential new source for significant advancements in the agricultural industry (Bheru, 2020).

Because of their special qualities such as their quantum nature, a surface-to-mass ratio far higher than that of ordinary particles, and their ability to adsorb substances like drugs, proteins, and probes, nanoparticles are used in medical applications. Just as beginning materials can include biological lipids, lactic acid, dextran, chitosan, phospholipids, or chemicals like carbon, silica, metals, and other polymers, so can the composition of nanoparticles (Guo *et al.,* 2013 and Jani *et al.,* 2020).

Given their distinct properties, nanoscale materials have been suggested for a variety of biomedical uses. It is yet unknown, nevertheless, how these nanomaterials behave in tissues and cells and how they biodistribute across living things. Variations in nanoparticle size, shape, and surface chemistry impact fundamental mechanisms of cellular uptake and responses, hence influencing the nanoparticles' suitability for biomedical purposes (White *et al.,* 2022).

Nanotechnology has a lot of potential uses in the field of oncology. Breast cancer diagnosis and treatment is only one of the numerous cancers that are benefiting from this application. The significance of nanotechnology in the prevention of breast and other cancers has been renowned by the National Cancer Institute, which has provided significant funding to advance this area (Haq *et al.,* 2009).

The nanotechnology goal in cancer treatment is the ability to distinguish malignant cells from nonmalignant ones and to selectively eliminate malignant cells (Gmeiner and Ghosh, 2015).

Cell-based assays are further classified according to the functional properties and offer a way to evaluate biological processes, system efficacy, and cellular functions (Garcia *et al.,* 2019).

A nanoemulsion is a dispersion of two or more insoluble fluids with droplet sizes ranging from 50 to 1000 nm, stabilized kinetically. Ultrafine oil-in-water emulsions, or nanoemulsions, with droplet sizes between 50 and 200 nanometers. They are transparent because they do not scatter visible light. These nanoemulsions also maintain their stability over extended periods of time due to the small size of their particles. Food products including flavoring oils, salad dressing beverages, sweeteners, and other processed foods can all be made with nanoemulsions. They successfully protect the qualities from enzymatic reactions and deterioration, (Sharara (2021). Because of their greater surface tension, which increases the ability of essential oils to pass through bacterial cell membranes, nanoemulsions have antibacterial properties (Shetta (2017).

Using plant oils to prepare nanoemulsions is an inexpensive, easily scalable, and environmentally benign process. The immiscible phases of nanoemulsion are oil and water, and the droplets of the mixture range in size from 10 to 100 nm. Nonionic surfactants are used to create nanoemulsions, which are very stable, biocompatible, and safe mixtures of various plant-based oils. When preparing the nanoemulsion, the selected surfactant needs to be able to reduce interfacial tension to a very minimal amount to aid in the dispersion process (Pathania *et al.,* 2018).

The finished product's shelf life is increased by the protection that nanoparticles provide for essential oils against heat and UV deterioration, resulting in increased stability, flavor retention, and functionality. Furthermore, regulated release of essential oils is provided via nanoparticles for extended therapeutic benefits. Essential oil-loaded nanoparticles boost essential oils' ability to diffuse through biological membranes, exhibiting a synergistic antibacterial effect (Nair *et al.,* 2022).

Female newly cancer diagnoses about 23%, in addition 13.7% of cancer-related deaths are caused by breast cancer. The main reason why there is a shortage of chemotherapy medications is because chemotherapeutics accumulate less at tumors compared to other organs, leading to increased toxicities. Numerous approaches have been devised to enhance the care of people with this condition. Natural compound-based nanoemulsions, like the one made from Nigella sativa L. essential oil, may be a good treatment for breast cancer. Through inducing apoptosis in MCF-7 breast cancer cells, this nanoemulsion demonstrates anti-cancer capabilities in vitro. This nanoemulsion may be helpful in encasing active medications to treat breast cancer (Sánchez-López *et al.,* 2019).

## 2. Materials and Methods

### 2.1. Materials

Thyme essential oil obtained from National Research Center (NRC), Giza, Egypt. Tween 80 obtained from Nano Fab Technology comp., Giza, Egypt.

Microbiology strains: *E. coli* obtained from Faculty of Medicine, Mansoura Uni., Egypt, and Salmonella were obtained from Faculty of Agriculture, Mansoura Uni., Egypt.

Growth media: nutrient broth, mueller hinton agar and salmonella shigella (S.S.) agar were obtained from El-Gomhouria comp., Egypt.

Breast cancer cells (MCF-7) which used in in vitro cytotoxicity assay, were obtained from Nawah scientific research center via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. frozen in liquid nitrogen (-180°C).

### 2.2. Methods

#### 2.1.1. Fractionation and identification of chemical components of thyme oil by GC/MS

The HP 6890 Series Gas Chromatograph System with an HP 5973 Mass Selective Detector was the GC/MS system used to analyses the essential oils, at Cairo University Research Park "CURP", Faculty of Agriculture, Cairo Univ., Egypt.

#### 2.1.2. Preparation of thyme essential oil nanoemulsion

The nanoemulsion was formed using the thyme essential oil, surfactant and water. The non-ionic surfactant used is Tween 80.

Nanoemulsion of thyme essential oil were preparing according to Nirmala *et al.* (2020) with a few modifications. The ratio of essential oil to surfactant was 1:3 (V/V), and the three components (thyme essential oil, tween 80 and water) were mixed for three hours at a speed of 700 rpm.

Preparation of thyme oil nanoemulsion was done in two stages. The first step involved creating the emulsions by combining the three essential ingredients (water, surfactant (tween 80) and thyme essential oil) at following percentage (4%, 12% and 84%), in an ultrasonicator bath for 10 min. The second step involved mixing for 3hr at 700 rpm using a magnetic stirrer.

### 2.3. Characterization of thyme essential oil nanoemulsion

#### 2.3.1. Transmission electron microscopy (TEM)

Imaging and determined particles size of thyme essential oil nanoemulsion were performed using transmission electron microscope (TEM) (JEOL JEM-2100), at Electron Microscope Unit. Faculty of Agriculture, Mansoura Uni., Egypt.

the diluted solution was dried at environmental conditions after it had been put onto carbon-coated copper grids. The FEI Tecnai G2-12 - Spirit Biotwin - 120 kV transmission electron microscope was used to obtain the images. At least 10 spots on the TEM grid were investigated. To ensure accurate measurements, we determined the optimal concentration of NPs in a manner analogous to the national institute of standards and technology (NIST) technique. Image analysis was performed using the freely available on the internet Image J software. The sizes of the regions encompassed by the oval selection tool were calculated after it was adjusted to match the scale bar in the TEM images. Using an ideal sphere as a model, we were able to determine the diameter (Souza *et al.*, 2016).

#### 2.3.2. Zeta potential

After a suitable dilution (100-fold) in ultrapure water, the zeta potential for nano essential oils was ascertained by electrophoretic mobility in an electric field (Malvern Zetasizer Nano-ZS90) at 25°C. Viscosity (cP) 0.8872, dispersant RI 1.330, and a potential of ±150 mV were used for the measurements in the electrophoretic cell. Utiliing the Helmholtz Smoluchowski approximation, data analysis were carried out. Three duplicates of each measurement were made according to Dalmolin and Lopez (2018), at Electron Microscope Unit. Faculty of Agriculture, Mansoura Uni., Egypt.

### 2.4. Microbiological effect of normal and nano thyme essential oil

#### 2.4.1. Agar well diffusion method

The antibacterial activity against strains of *E. coli* and Salmonella were determined using the agar well diffusion technique. Different bacterial species were cultured on plates of Mueller Hinton Agar (MHA) and salmonella shigella (S.S.) agar for this experimental purpose. Sterile corkborer was used to make 6 mm- diameter wells in the inoculated agar medium with thyme essential oil (normal and nano). After 24 hours of incubation at 37°C, looking for a zone of inhibition surrounding the wells to see the effect of the oils on the bacteria. Using a scale, the inhibitory zone was measured in millimeters (mm), **(Dulal *et al.*, 2021)**.

#### 2.4.2. Biological effect of normal and nano thyme essential oil on breast cancer cells

The effect of different concentrations of normal and nano thyme essential oil on breast cancer cell (MCF-7) viability were examined by the MTT colorimetric method. Briefly, the cells were put into a 96-well culture plate at a density of 10000 cells/well and incubated with various concentrations of all normal and nano thyme essential oil for 48hr. The cells were treated with normal and nano thyme essential oil at concentrations of (50, 100, 150, 200, and 250 ul/ml). After the culture media was removed, the cells were treated for three hours with MTT solution (5 mg/mL in PBS), and 50 μL of DMSO (Sigma) was used to solubilize the formazan that resulted. The absorbance was measured in a plate reader (BioTeK ELx800) at 540 nm rather than 570 nm, (Kianinodeh *et al.*, 2017).

The term "IC50" refers to the lowest concentration, which inhibited the number of viable cells by 50% comparing to control (Panyajai *et al.*, 2022).

The cytotoxicity assay procedures were established by following standard operating procedure for culturing and cell harvesting. Depending on how dense the cell stock culture used to set up an assay, the IC50 concentration was determined (Hassan and Mujtaba, 2019).

### 2.5. Statistical analysis

According to the procedures outlined by Gomez and Gomez (1984), data were analyzed using ANOVA, Duncan's, and LSD to examine the difference between the means of treatment values.

## 3. Results and Discussion

### 3.1. Fractionation and identification of chemical components of thyme oil by GC/MS

This result indicates to characterize the chemical components of thyme essential oil by use GC/MS analysis. These chemicals, which include fatty acids, esters, alcohols, terpenes, aldehydes, etc., are extensively analyzed by GC-MS.

There may be variations in concentration caused by seasonal fluctuations, adaptive metabolism, plant parts utilized, the distillation process, and other variables (Wang and Yang, 2009).

The result of GC/MS analysis of thyme essential oil showed at Table (1) and Fig. (1), this result indicated that the thyme essential oil which used at current study contained 14 components, the main components of thyme essential oil at this study are *Carvacrol* which represents 47.23 %, and *Cinnamyl carbanilate* 18.52 % of the components, while the rest of other components (12 component) representing 34.25 % of the components of thyme essential oil. The other components included, *4-Isopropylidene- 1-cyclohexene* 12.43 %, *Isoborneol* 5.28 %, *1.6-Octadien-3-ol, 3,7-dimethyl- 4*.44 %, and some other components (9 component) which represent 12.1 % of total components of thyme oil.

Ngongang *et al.* (2022) reported that the GC Mass profiles of essential oil (EOs) for thyme oil was predominently constituted of 21.53% thymol, 17.43% α-Pinene (Dextro) and 15.37 % o-Cymene, this result didn't agree with current result.

These results confirmed by result reported by El Hattabi *et al.* (2016) said that thymol was not detected in the oil of Morocco thyme. Moroccan plants contained mainly 4.6% p-cymene and 78.4% carvacrol in the essential oil.

According to Wesolowska and Jadczak (2019), a number of variables, including the growing environment, stage of growth, and chemotypes, affect the chemical makeup of thyme essential oil. The major components of thyme essential oil are typically claimed to include thymol, linalool, γ-terpinene, p-cymene, carvacrol, and β-caryophyllene.

The primary components of thyme were identified as thymol and carvacrol, according to Halat *et al.* (2022). The primary terpenes detected in thyme, according to GC-MS and HPLC-UV analysis, are 48.19% linalool and 21.3% carvacrol, respectively. Certain components are present in all species of thyme, although in varying concentrations, regardless of the method used to isolate the various essential oil fractions. Factors including as weather, soil, harvest time, and preservation technique contribute to this variety. All above results, confirmed the result of current study. The all above results, confirmed the result of current study.

**Table 1:** Fractionation and identification of chemical components of thyme essential oil by GC/MS.

| Peak | Retention time | Component | Area% |
|---|---|---|---|
| **1** | 9.191 | Delta-3-Carene | 1.15 |
| **2** | 11.036 | 2(10)-Pinene | 2.41 |
| **3** | 11.55 | 3-trsns-(1,1- dimethylethyl)-4-trans-methoxycyclohexanol | 0.89 |
| **4** | 13.223 | Cinnamyl carbanilate | 18.52 |
| **5** | 13.366 | 2-Oxabicyclo [2.2.2] octane, 1,3,3-trimethyl- | 1.11 |
| **6** | 14.501 | 4-Isopropylidene- 1-cyclohexene | 12.43 |
| **7** | 16.248 | 1.6-Octadien-3-ol, 3,7-dimethyl- | 4.44 |
| **8** | 17.729 | 1,7,7-trimethylbicyclo [2.2.1] heptan-2-one | 0.8 |
| **9** | 19.14 | Isoborneol | 5.28 |
| **10** | 19.747 | Benzene, 1-methoxy-4-(1-propenyl)- | 2.49 |
| **11** | 20.422 | .alpha.- Terpineol | 0.61 |
| **12** | 20.785 | Anisole, 2-isopropyl-4-methyl- | 1.26 |
| **13** | 24.053 | Carvacrol | 47.23 |
| **14** | 27.299 | Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethylene-4-vinyl- | 1.39 |

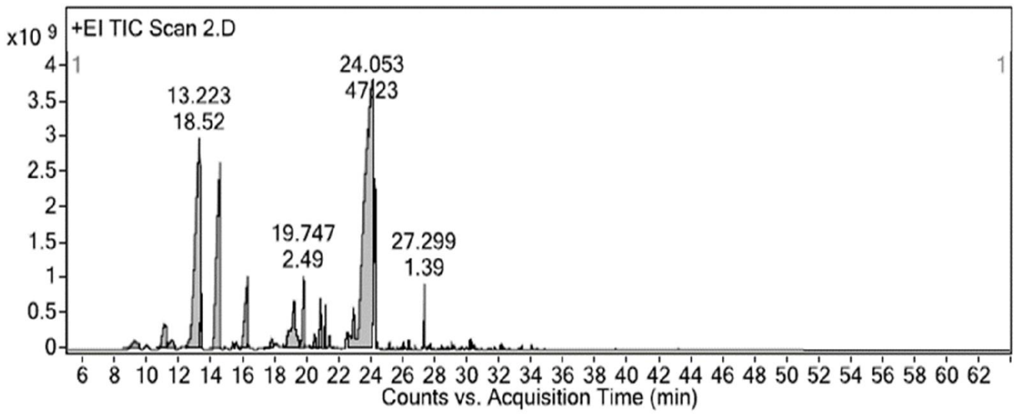


**Fig. 1:** Fractionation and identification of chemical components of thyme essential oil by GC/MS.

**3.2. Characterization of thyme essential oil nanoemulsion**

**3.2.1. Transmission electron microscopy (TEM)**

The original microscope has its roots in transmission electron microscopy (TEM), the first method of using electrons to study samples. The light microscope is unable to match its resolution of around 0.1 nm, which is a thousand times better (Kannan, 2018).

The transmission electron microscopy (TEM) showed that the thyme essential oil nanoemulsion contained particles with spherical shape dispersed in the nanoemulsion. The particles of thyme essential oil nanoemulsion was in the range of 39.27nm to 67.06nm at figure (2), while it in range of 63.40nm to 70.85nm at figure (3), and at figure (4) the particles of thyme essential oil nanoemulsion was in the range of 61.47nm to 86.69nm.

From result in above can conclude that, the thyme essential oil nanoemulsion have particles with spherical shape dispersed in the nanoemulsion, and was in range of 39.27nm to 86.69nm as showed at fig. (2, 3, and 4).

The TEM micrographs used by Hassanin *et al.* (2017) showed that the thyme essential oil nanoemulsion was spherical, rather monodisperse, and measured between 26.6 and 45.3 nm in size.

Consistent with the present finding, Sundararajan *et al.* (2018) observed that the nanoemulsion-loaded essential oils exhibited a spherical form and regular dispersion.

According to El-Sayed and El-Sayed (2020), TEM was used to visualize the prepared nanoemulsion, which contained 10% thyme oil using 10% Tween and 80% water (V/V). The TEM picture showed that the majority of the nanoemulsion droplets were nearly spherical and had roughly homogeneous sizes and shapes. Furthermore, the TEM picture shows that the aqueous phase's thyme essential oil distribution is uniform. Accordingly, the 10% thyme essential oil nanoemulsion displayed nanometer particle size in the range of 20–52 nm, which is an appropriate dispersive capability; this finding supports the findings of the current investigation.

**3.2.2. Zeta potential**

The repulsion forces between the nanoparticles are indicated by the zeta potential values, which are variations in electric charges between the fluid mass in suspension surrounding the nanoparticles and the external ions. Particle flocculation and aggregation might be inhibited by strong repulsive forces, which would stabilize the colloidal system (Guerra-Rosas *et al.*, 2016).

Zeta potential was performed 3 times for the thyme essential oil nanoemulsion at 25°C after dilution (100-fold), which shown in Fig. (5, 6 and 7), where the values of zeta potential was -6.95 mV, -6.88 mV and -6.2 mV.

Particles having a value of ± 30 mV do not aggregate when their zeta potentials are stable in suspension, according to Kumar and Dixit (2017) and Jahan *et al.* (2017).

This result is supported by previous research by Zhang *et al.* (2016) and Zhou *et al.* (2017), they found that non-ionic surfactants, like Tween 80, are expected to have a zeta potential value near to neutral (0 mV).

Jayari *et al.* (2022) reported, the zeta potential values of the delivery system prepared with thymus capitatus essential oil, harvested from Tunisia. Between -11.60 and 38.67 mV, the zeta potential was measured. The z-potential of tween 80-based nanoemulsions was near neutral, which is consistent with the present study's findings and likely caused by the nonionic nature of tween 80 and the surface interaction it has with thymus capitatus essential oil.

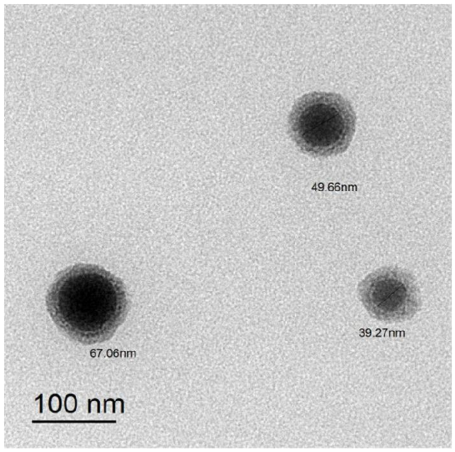


**Fig. 2:** TEM of thyme essential oil

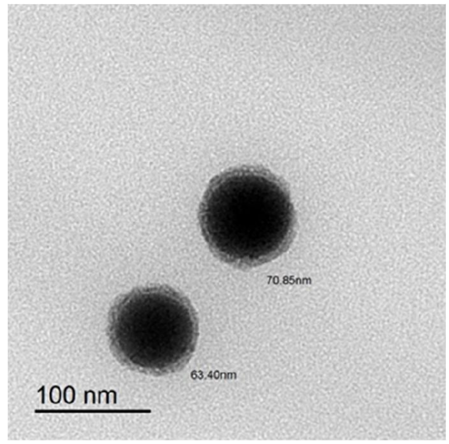


**Fig. 3:** TEM of thyme essential oil

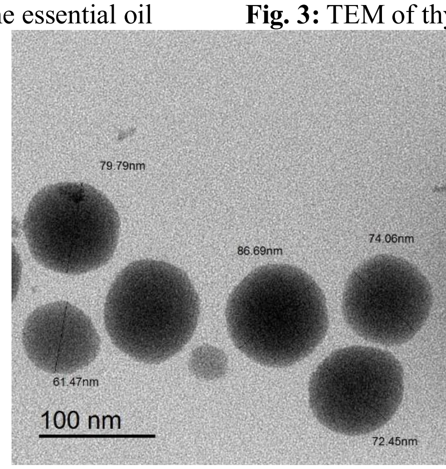


**Fig. 4:** TEM of thyme essential oil

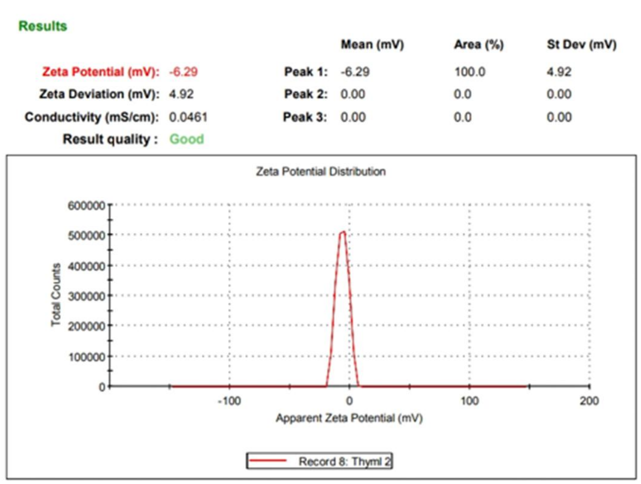


**Fig. 5:** Zeta potential of Thyme oil.

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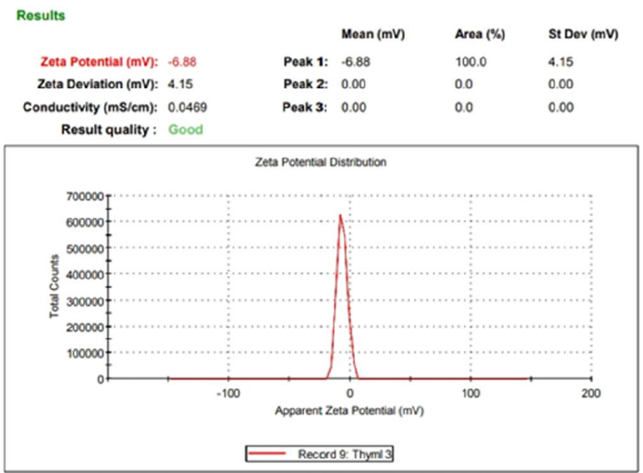


**Fig. 6:** Zeta potential of thyme oil.

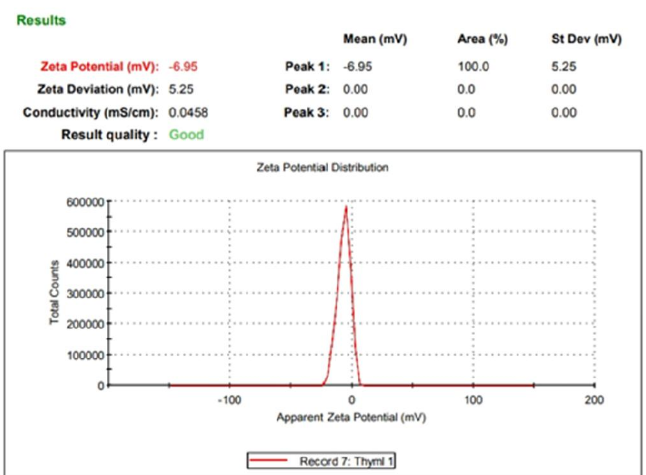


**Fig. 7:** Zeta potential of thyme oil.

### 3.3. Microbiological effect of normal and nano thyme essential oil

The results obtained for the microbiological effect of normal and nano thyme essential oil against bacteria of *E. coli* and salmonella show in Table (2). Normal thyme essential oil inhibited the growth of *E. coli* bacteria with 13mm inhibition zone. While nano thyme essential oil inhibited the growth of E. coli bacteria with 20mm inhibition zone. According to statistical analysis there is significant difference between normal and nano oil.

Also, at Table (2) found effect of Normal and nano thyme essential oil on salmonella, which have inhibition zone estimated as 12mm for normal thyme essential oil, and 15mm for nano thyme essential oil. According to statistical analysis there is nonsignificant difference between normal and nano oil.

Microbiological effect of normal and nano thyme essential oil on *E. coli* and salmonella, due to carvacrol, the main component of thyme essential oil, which has antibacterial effect.

Nano thyme essential oil have a greater effect on *E. coli* and salmonella than normal thyme essential oil, it due to the small size of the nanoparticles, which have a greater effect on bacteria.

Consistent with the present finding, Al-Nabulsi *et al.* (2020) found that thyme essential oil had the most potent inhibitory effect against *E. coli*, with inhibition zones ranging from 24.3 to 50.8 mm at 37 °C.

Olaimat *et al.* (2019) showed, the essential oils of thyme and cinnamon exhibited the highest antimicrobial activity against Salmonella SPP strains with inhibition zones of 22.5–38.5 mm. this result agree with current study.

Also, Ahmadi and Jafarizadeh-Malmiri (2021) found that the presence of two active ingredients in thyme essential oil carvacrol and thymol was linked to the antibacterial activity of the thyme nanoemulsions that were created. These two substances are phenolic compounds that have the ability to change the bacterial strains' cytoplasmic membrane and prevent the creation of proteins and RNA. This outcome is consistent with the study.

In addition, Abdelrasoul *et al.* (2018) reported that a number of research have shown that adding essential oils (EOs) to nanoemulsions improves their antibacterial and physical characteristics when compared to traditional emulsions. The antibacterial activity of the essential oils (EOs) in nanoemulsions was shown to be more effective than that of regular monoterpenes. This is most likely because pure oil, which has a limited water solubility, fails to interface with cell membranes easily, whereas the nanostructures of fat particles are able to bring primary oil to the surface of the membrane. This result confirmed current result.

**Table 2:** Microbiological effect of normal and nano thyme essential oil.

| Bacteria | Normal thyme essential oil | Nano thyme essential oil | LSD at 5% |
|---|---|---|---|
| ***E.coli*** | 13.0 $^{b}$±1.23 | 20.0 $^{a}$±2.02 | 3.77 |
| **Salmonella** | 12.0 $^{a}$±1.66 | 15.0 $^{a}$±1.13 | n.s |

**3.4. Biological effect of normal and nano thyme essential oil on breast cancer cells**

At Table (3) found the result of in vitro test of normal and nano thyme essential oil. this test determined effect of normal and nano essential oil on breast cancer cell line (MCF-7) at 5 concentrations (50,100,150,200 and 250 µl/ml). All 5 concentrations of normal and nano thyme essential oil showed positive effect and decrease the viability of breast cancer cell. That due to the major component of thyme essential oil carvacrol, and anticancer effect of it.

Also, all 5 concentrations of nano thyme essential oil have a greater effect of availability of breast cancer cell than the other 5 concentrations of normal thyme essential oils, that due to the smaller particle size of nano thyme essential oil than the particle at normal thyme essential oil. Which allows it to have a greater ability to destroyed breast cancer cells more than these at normal size.

**Table 3:** Biological effect of normal and nano thyme essential oil on breast cancer cells.

| Concentration µl/ml | Normal thyme essential oil | Nano thyme essential oil | LSD at 5% |
|---|---|---|---|
| **50** | 90.89 $^{a}$±1.578 | 42.16 $^{b}$±3.882 | 7.74 |
| **100** | 83.88 $^{a}$±1.751 | 38.53 $^{b}$±2.153 | 7.43 |
| **150** | 72.20 $^{a}$±3.818 | 36.48 $^{b}$±1.894 | 7.84 |
| **200** | 60.56 $^{a}$±2.481 | 35.38 $^{b}$±3.954 | 5.97 |
| **250** | 54.66 $^{a}$±0.914 | 34.87 $^{b}$±1.053 | 4.79 |

Values of IC50 of normal and nano thyme essential oil were 279.3 µl/ml and 43.2 µl/ml, respectively. that means, the destruction of 50% of cancer cells needs to 43.2 µl/ml of nano thyme essential oil, while, to destruction 50% of breast cancer cell need 279.3 µl/ml of normal thyme essential oil. The need for a small amount of nano thyme essential oil to destroy same count of cells due to, the powerful anticancer effect of nano thyme essential oil.

According to statistical analysis there is significant difference between normal and nano oil, and between all 5 concentrations.

The metabolic activity of cells and, by extension, cell survival, was found to be dose-dependently reduced by thyme essential oil (EOT) in both the MCF-7 and MDA-MB-231 breast cell lines studied by Kubatka *et al.* (2019). This result agree with current result.

According to Sampaio *et al.* (2021), thymol and carvacrol showed antiproliferative and anticancer effects across many signaling pathways. It seems that carvacrol is more effective than thymol in vitro. This result confirmed the above results.

Also, Khan *et al.* (2023) observed that carvacrol has great anti-cancer potential against many different types of carcinomas because it is an active phenolic monoterpenoid. Caspases-3, 6, and 9 were all increased in carvacrol-treated (MCF7) breast cancer cells when compared to control cells that were not treated. This provided a clear illustration of the potential mechanism by which carvacrol-treated cancer cells induce apoptosis. This result confirmed the current study.

Essential oils and their components are often ineffective because of their chemical instability and volatility when exposed to air, light, moisture, and heat (Lammari *et al.,* 2020 and Tiwari *et al.,* 2020). Essential oils that are thermally and/or oxidatively labile can become inefficient or even harmful due to the development of hazardous derivatives when they undergo degradation throughout preparation, transit, storage, and consumption. One new way to get around these problems is by nanoencapsulation. Protecting essential oils and bioactive components from the elements with nanoencapsulation is a novel and exciting way to make them more stable in the long run. In addition to reducing the essential oils' volatility and toxicity, encapsulation increases their bioavailability, bio efficacy, and water solubility through raising the surface-to-volume ratio. This, in turn, permits controlled and site-specific distribution as well as deep tissue penetration. This study confirmed current study.

## 4. Conclusion

From the results obtained, it could be underlining the beneficial effect of convert the thyme essential oil to nanoemulsion. Nanoemulsion of thyme essential oil have antibacterial effect on *E. coli* and salmonella than thyme essential oil at normal size. Also, nanoemulsion of thyme essential oil more effective as anticancer than thyme essential oil at normal size, when it was applied to investigated its effect on breast cancer cell line.

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