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Nanoemulsification of Thyme Essence by a Mixture of Emulsifiers and Evaluation of the Antibacterial Activity Against *E. coli* DH5α

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Introduction

Essence or essential oils are important secondary metabolites in plants that exhibit strong antibacterial, antiviral, and antifungal effects (Ferreira et al., 2010, Lingan, 2018). Thyme is a plant with the scientific name *Thymus vulgaris* of the mint family, which is seen as a bush and its branched stems are covered with white hairs. The aerial part of thyme plant contains essential oil, saponin compounds, tannins and phenols. Thymol and carvacrol as the main components and monoterpene hydrocarbons such as para-cymene, alcohols such as linalool and alpha-terpinene are the main components of thyme essential oil (Antih et al. 2021). Thyme essence is used in traditional and modern medicine to treat many diseases (Javed et al., 2013). The present study was conducted with the aim of nanoemulsifying *Thymus vulgaris* essential oil to increase its stability and investigate its antibacterial effects on E. coli DH5α bacteria. The physical and chemical properties of the prepared nanoemulsion were evaluated. Antibacterial effect of nanoemulsion on *E. coli* were evaluated. The results showed that the physical and chemical properties of nanoemulsions prepared in 10 and 30 % of essence were suitable and the antibacterial effects of these nanoemulsions were observed at low concentrations. According to the stability of the produced thyme essential oil nanoemulsions, it can be concluded that the antibacterial effects of this compound have increased compared to the original essence.

Material and Methods

Preparation of essential oil of thyme plant and GC Mass analysis: The essential oil of the thyme plant after drying at 30 °C was extracted by distillation with water and using a celevenger device. In order to investigate the composition profile of thyme essential oil was used the Agilent 7890 A GC-Mass device, coupled with an Agilent MSD-5975C mass spectrometer and a flame ionization detector (FID) along with an Agilent MSD-5977B quadrupole mass selective detector and a column HP-5MS.

Preparation of nano emulsion of thyme essential oil and determination of its physicochemical properties: Nanoemulsion was prepared in the form of oil in water (O/W), and for this purpose, an O/W microemulsion was first prepared. To prepare the oily phase, 30 % (v/v) pure essential oil was added dropwise to 20 % (v/v) Tween 80 under uniform stirring by a magnetic stirrer. In order to determine the physical and chemical properties of nanoemulsions, the surface charge of the samples was determined by a Zeta potential analyzer and their morphology was determined by an electron microscope TEM.

Investigating the antibacterial effect of thyme essential oil on DH5α E. coli bacteria: In order to compare the antibacterial effects of essential oil and nanoemulsions produced from thyme plant, volumes of 0, 5, 10, 20, 30, 40, 50, 60 and 100 µl of thyme essential oil for nanoemulsion containing 10 % diluted in DMSO and for 30 % essential oil volumes 5, 10, 30, 50, 70 and 100 µl were dissolved in colony count culture medium and then *Escherichia coli* bacteria with a dilution of 10-6 cultured on media. Also, the stability of nanoemulsions after 30 days and its effect on the growth of bacterial colonies were investigated.

Result and Discussion

The results of GC Mes showed that the main components of essential oil included p-Cymene (23.3%), Thymol (22.93%), γ-Terpinene (10.61%) and Carvacrol (5.31%). Thyme contains 0.8 to 2.6 percent of essential oil, most of which is phenolic compounds such as thymol and carvacrol, monoterpene hydrocarbons such as paracymene and gamma terpinene, and alcohols such as linalool and alpha-terpinene (Singletary, 2016). In this study, ratios of 1 and 2 % Tween 80 were used for 10 % and 30 % nanoemulsion, respectively. The amount of SDS used as a charged emulsifier was 0.5 % and 1 % for nanoemulsion 10 % and 30 %, respectively. Surfactants and emulsifiers increase the amount of essential oil emulsification, which has a direct effect on the particle size and its stability (Zhang et al, 2014). The ratio of oil to surfactant, oil to water is a fundamental criterion for the formation and stability of emulsion droplets (Das et al., 2020). Oils and lipophilic components can be encapsulated in nano-droplets, to protect sensitive and degradable bioactive molecules against environmental conditions. In addition, nanoemulsion can preserve the physicochemical properties of essential oils, and enable the transformation of solutions into fine powders (Saberi et al., 2013). The results showed that the zeta potential became more negative after the passage of time and remained in the same acceptable range (-100 to +100), which indicates the preservation of the stability of nanoemulsions. Also, the result of DLS analysis of nanoemulsion containing 30 % freshly prepared essential oil showed that the prepared suspension had a uniform distribution. TEM images related to the prepared nanoemulsion particles showed that the particles were uniformly spherical in shape and the average particle size for the nanoemulsion containing 10 and 30 % essential oil was 43 and 30 nm, respectively. The amount of zeta potential is related to the surface charge density of nanoparticles. The main reason for the stability of colloids is the surface charge on the particles. Therefore, the higher the surface charge of the colloid particles, the more stable the colloid is, and its particles condense or precipitate more slowly (Costa et al., 2009; Pangi and Beletsi, 2003). The size of the nanoparticles formed in both concentrations of essence 10 and 30 % was below 100 nm, which has well maintained the size at the nano level. The results of the antibacterial effect of nanoemulsion containing 10 % freshly prepared essential oil on *E. coli* DH5α showed that in volumes of 0, 5, 10, 30, 50, 70 and 100 microliters of essential oil, the growth rate after counting the number of colonies Bacteria were 100, 93, 74, 61, 31, 21, 8 and 0 %, respectively. The results of the antibacterial effect of nanoemulsion containing 30 % of freshly prepared essential oil on DH5α *E. coli* bacteria showed that in volumes of 0, 5, 10, 20, 30, 40, 50, 60 and 100 microliters of essential oil, the growth rate after counting the number of bacterial colonies were 100, 86, 71, 55, 27, 12, 0, 0 and 0 %, respectively. Nanoemulsions of 10 and 30 % in the volume of 25 and 20 µl, respectively showed almost 50 % inhibition (IC_{50}).

The results of the effect of thyme essential oil on the growth rate of *E. coli* DH5α showed that in volumes of 10, 20, 30 and 40 microliters of pure essential oil, the number of bacterial colonies grown were 56, 48, 40 and 28%, respectively and in a volume of 20 µl, it showed almost 50 % inhibition (IC50).

The nanoemulsions prepared in this study have kept their antibacterial properties compared to essential oil and this result showed that their nanoemulsification did not prevent the antibacterial properties of essential oil. Even after 30 days, nanoemulsions maintained antibacterial properties. The cell wall of Gram-negative bacteria consists of a thin peptidoglycan layer and an outer membrane consisting of a phospholipid bilayer, which is almost impermeable and limits access to the cell membrane. Small droplets of nanoemulsions are able to bring essential oils to the surface of the cell membrane, increase access to the microbial cells and make disruption of the cell membrane possible (Donsi, 2018). The integration of emulsifier droplets with two phospholipid layers of the cell membrane causes the targeted release of the essential oil in the desired locations (Salvia-Trujillo et al., 2014).

Conclusions

According to the results of this study, in higher concentrations of essential oil, nanoemulsion showed better physicochemical and antibacterial properties compared to lower concentrations of nanoemulsion essential oil. Also, in a higher percentage of nanoemulsion essential oil in higher treatment concentrations, antibacterial properties were better than pure essential oil. Considering the growing popularity of using herbal essential oils in order to reduce secondary effects, reduce costs and their wide role, the use of nanoemulsions of essential oils can be a big step forward in various industries, including pharmaceuticals, nutrition, perfumes and fragrances.

Keywords: Antibacterial, E. coli DH5α, Nanoemulsion, Thymus vulgaris.

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Declaration of conflict of interest

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Statement on ethics

The authors guarantee that they followed the scientific ethics of the article.