British Journal of Bio-Medical Research Vol.03, Issue 05, Pg.1106 - 1120, September - October 2019

Available Online at http://www.bjbmr.org

BRITISH JOURNAL OF BIO-MEDICAL RESEARCH

Cross Ref DOI: https://doi.org/10.24942/bjbmr.2019.577 Volume 03, Issue 05, Sept - October 2019

ISSN:2456-9739

Research Article

Investigating The Anti-Bacterial Effects Of Mentha Longifolia Encapsulated In G2 Dendrimer On Staphylococcus Aureus And Escherichia Coli Bacterium

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ARTICLE INFO

Article History:
Received on 11th Sept 2019
Peer Reviewed on 25th Sept 2019
Revised on 16th October 2019
Published on 29th October 2019

Keywords:
G2 dendrimer, mentha longifolia, Mentha longifolia- G2 dendrimer, Staphylococcus aureus, Escherichia coli, Antimicrobial effect, Nano composition

ABSTRACT

Regarding the recent developments in nanotechnology and tendency to use natural preservatives, the aim of this research was to determine the chemical compositions and antimicrobial effects of mentha longifolia essential oils encapsulated in G2 dendrimer against Staphylococcus aureus and Escherichia coli bacterium. In this research, G2 dendrimer nanoparticles as a new generation of dendrimers was synthesized and conjugated with mentha longifolia essential oils. The essential oils of mentha longifolia were soaked and prepared by Clevenger and after drying encapsulated into G2 dendrimer. Its compounds were determined by GC-Mass spectroscopy, and then the antimicrobial effects of mentha longifolia- G2 dendrimer mixture were studied on a gram-positive bacterium, Staphylococcus aureus and a gram negative bacteria, Escherichia coli. The results obtained from MIC, DLS, and FTIR analysis showed that mentha longifolia- G2 dendrimer nano-composition is a new structural construct that has high usability and has strong antimicrobial effect on Staphylococcus aureus and of Escherichia coli bacterium.

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INTRODUCTION

The harmful effects of chemical and synthetic preservatives have led to extensive research on natural compounds derived from plant and animal sources, in addition to increasing the shelf-life, to protect the harmful effects of chemical preservatives (1). The antimicrobial effects of herbal essential oils on gram-positive and gram-negative bacteria have been confirmed in many studies and in many cases these specifications related to the presence of active monoterpene components (2). The functional mechanism of the essential oils is related to their chemical composition and antimicrobial activity, but in all cases they have no similar mechanism, however, in most cases, the effect of herbal essential oils on the cell wall structure has been confirmed (3). The hydrophobic properties of essential oils cause their permeation into lipid of cell membrane and increases its permeability, which this property produce disturbance in all cell membrane vital activities, ions, vital compounds transpiration, and ultimately causes cell death (4). Toxic effects on the membrane structure and its operation indicate antimicrobial effect of the herbal essential oils and their mononetropenic compounds (5). Mentha longifolia essential oil has an extremely high antimicrobial potential, so it can be used in combination with other preservatives to protect food against microorganisms that cause infection and osmosis (6).

One of the most important ways to produce high-performance antimicrobial drugs against microorganisms is nano-dendrimers (7). Dendrimers are large and uniform molecules that have a regular, highly branched, three-dimensional structure (8). They have a size of about 1 -100nm, which makes them an ideal case for biological studies (9). Due to highly branched structure, dendrimers have large number of functional groups at their external surface (10). This feature enhances the possibility of establishing various connections between the dendrimers and their outer environment, and can be used for purposes such as Adhesives Surface coating or Polymer Cross-link to use (11). Since dendrimers can be used to synthesize by different nuclei and monomers, it is possible to modify and improve the end groups, hence various types of syntheses are possible, and each of which is for specific purposes (12). The trapping nature of a species within the dendrimer may be simple physical trapping, or involves non-linking interactions with specific structures inside the dendrimer (13). It is assumed that the dendrimers have a hollow core of a dense crust, and in fact many of the papers based on drug encapsulation support this hypothesis (14). One of the interesting things that can be done with dendrimers is the physical encapsulation of molecules and atoms inside the dendrimers (15). In the closed space inside the dendrimer, guest molecules can be permanently or temporarily locked (16). Selective release of encapsulated molecules has also been implemented. If end or superficial groups of dendrimer linked to active antimicrobial agents, an antimicrobial susceptibility enhancement is expected from dendrimer superficial groups (17). In most cases, the dendrimers will be able to carry active agents by encapsulating these agents in themselves or by bounding with surface groups (18). For example, most antimicrobial dendrimers with antimicrobial agents attached to their ends are: Ferrocene, Quaternary Ammonium Compounds, Boron compounds, carbohydrates, and lipids (19-23). Staphylococcus aureus is a gram positive and anaerobic bacteria, the most important species in the staphylococcus population. One of the most successful pathogen bacteria. This bacterium produces yellow colonies due to the production of a golden carotenoid pigment called staphyloxanthin (24). This pigment plays a role in pathogenicity as it acts as an antioxidant and protects the bacterium against free oxygen radicals. Staphylococcus aureus, a wide range of infections from simple skin infections (such as acne, boil, carbuncle, Stye and dental abscess) to life-threatening illnesses (such as pneumonia, meningitis, osteomyelitis,

endocarditis, toxic shock syndrome and septicemia) will create (25).

Escherichia coli are gram-negative bacillus from the Enterobacteriaceae family that is commonly found in the warm-blooded Intestine (26). Most of the strains of E. coli are harmless, but some serotypes such as H157O: 7 cause food poisoning and diarrhea. Ecoli on the MacConkey agar generates purple colonies because the bacteria are lactose-positive and ferment sugar and produce acid (27). Acid reduces pH in the MacConkey agar environment and results in purple coloration. The same thing happens in the EMB environment, causing dark purple colonies to form a metallic green color. Therefore, it can be measured in spectrophotometric experiments (28).

Mentha longifolia is a member of the Laminaceae family and is a perennial crop, and wildly grows in the humid areas of the central and southern parts of Europe, southwest Asia, and North Africa. Different parts of this plant are used in the composition of commercial spices as flavors in food. The medicinal properties of mentha longifolia have been proven in eliminating digestive disorders, vomiting, appetite, and liver disorders (29). Nowadays, antimicrobial and antioxidant properties of essential oil and extracts of many plant species such as Piperita, Monta rotundifolia, Montagolum yum and Mentha linguifolia have been proven (30-31). Most research on the antimicrobial effects of essential oils has first been carried out in an in vitro environment and then its functional characteristics have been evaluated in food models (32).

The purpose of this study was to determine the chemical composition and antimicrobial effects of mentha longifolia essential oils encapsulated in G2 dendrimer against Staphylococcus aureus and Escherichia coli bacterium. In order to evaluate the effects of antimicrobial agents, various methods can be used. In all methods, the principles are to measure the effect of certain antibiotic concentrations in inhibiting growth or destroying the tested bacteria. In this research photometric method have been used.

MATERIALS AND METHODS:

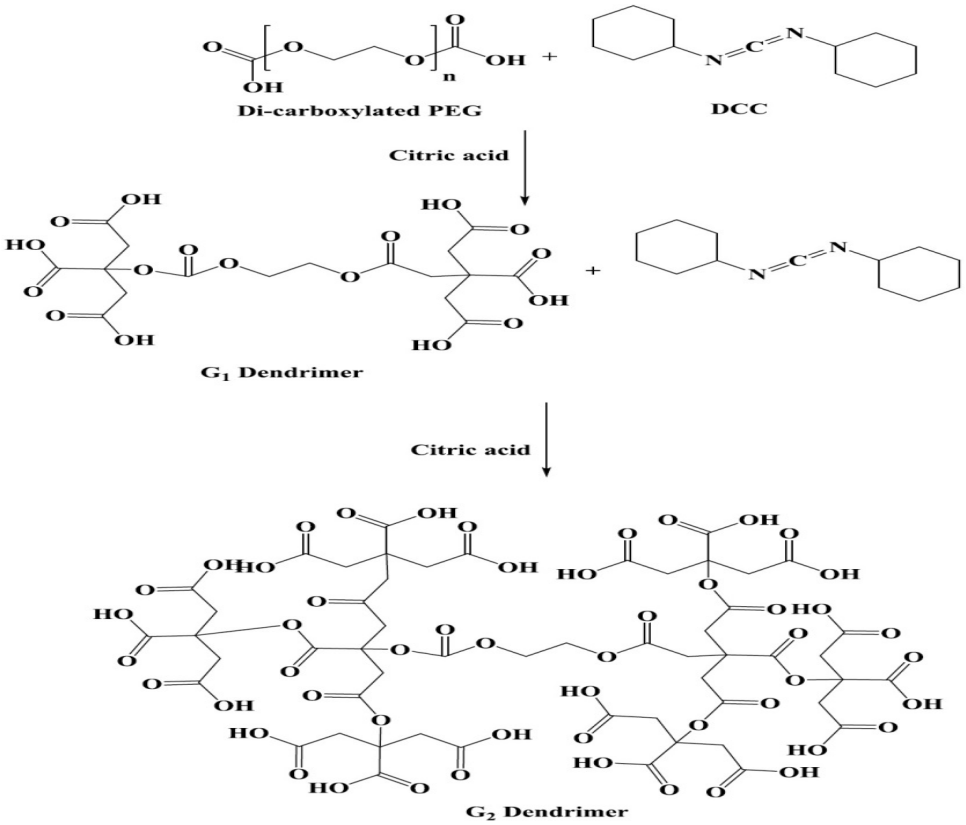
All raw materials were purchased from Sigma Aldrich. Fourier transform infrared (FTIR) spectroscopy (Nicolet 740SX) was used to investigate the chemical structure and functional groups in the structure of dendrimer. Determine the size distribution and zeta potential of particles was investigated using dynamic light scattering (DLS) method and Zetasizer (Malvern Instruments Ltd., Malvern,Worcestershire, UK. The size of dendrimer were acquired by a Zeiss TEM (acceleration voltage = 80 kV, carbon coated copper grid). Essential components were examined using GC Mass (6890N, Agilent).

Essential oils extraction:

In order to extract the essential oils, after drying the plant were powdered and extracted with distilled water by using a Clevenger for 3 hours. Then dried, and stored in dark containers and kept at a refrigerated until its antibacterial properties and the composition of it were determined. The essential oil was injected into the GC-MS and the mass spectrum of the compounds was obtained. Identification of essential oil composition using inhibitory index and studying the mass spectra of each essential oil component and comparing them with reference spectra were done.

Synthesis of G2 dendrimer

G2 dendrimer was synthesized in the following way and was used in this research (Scheme 1).



Scheme 1. Synthesis of dendrimer

1 mL (3.7 mmol) polyethylene glycol (PEG) 600, 10 mL Dimethyl sulfoxide (DMSO) and 0.75 g (2*3.7 mmol) N, N′-Dicyclohexylcarbodiimide (DCC) was added to the reactor and the reaction was continued for 30 mins at room temperature. Then, 0.71 g (2*3.7 mmol) citric acid was added and reaction stirred for 1 h. Afterwards, DCC (2.25 g ,6*3.7 mmol) and DMSO (5 mL) were added and the reaction was continued under the above-mentioned conditions Subsequently, citric acid (2.1 g -6*3.7 mmol) was added and the reaction was continued for 1 week at room temperature. Finally. purification was carried out by using a Sephadex G-50 fine column.

Loading of Essential oil

After purifying the nanoparticle, the powder weighed and 200 mg of the purified product added to 150 mg dendrimer and stirred for 24 h (300 rpm). Finally, resulted product were centrifuged (30 min, 4 °C, 13,000 rpm)

Preparation of Microbial Suspension:

In order to optimize the antibiogram, MIC and propagation of Nano dendrimer- mentha longifolia antibacterial properties, native strains of Staphylococcus aureus (25923) and Escherichia coli were prepared. Some of the colonies of the bacterium were dissolved in sterile physiology serum to reach the opacity of half McFarland. Then, the absorbance of this suspension at 600 nm wavelength is read by the spectrophotometer to have an opacity value of 1.5 × 108cfu / ML.

MIC test (minimum inhibitory concentration): The product is serially prepared in a suitable culture medium, which is usually a Hinton Broth Mueller. Pipes in this series, diluted serially in a culture medium, add 0.1 ml of the desired bacterial suspension (106 × 1 CFU / ml). After 18 to 24 hours of incubation, the tubes containing the microorganism / crop / suspension are tasted to determine minimum product dilution (lowest concentration of the product) that inhibits microorganism growth. This concentration of the product is recorded as the minimum amount of inhibitor concentration. In this study, 2 stock solution were prepared once mentha longifolia in 50 mg of dendrimer and second mentha longifolia loaded in 100 mg of dendrimer nanoparticles

with specific concentrations. Then, with 200 microliter from the stock, solutions with 50, 25, 12.5, 25.6 and 100 ml dilutions were prepared and were poured into the first well 96 wells micro plate, then from each of these wells Serial dilutions were prepared. Finally, to each well was added 100 microliter from the bacteria suspension. After 18 to 24 hours' incubation, it was tasted to determine the highest dilution of the nano dendrimer -mentha longifolia that inhibits the growth of bacteria.

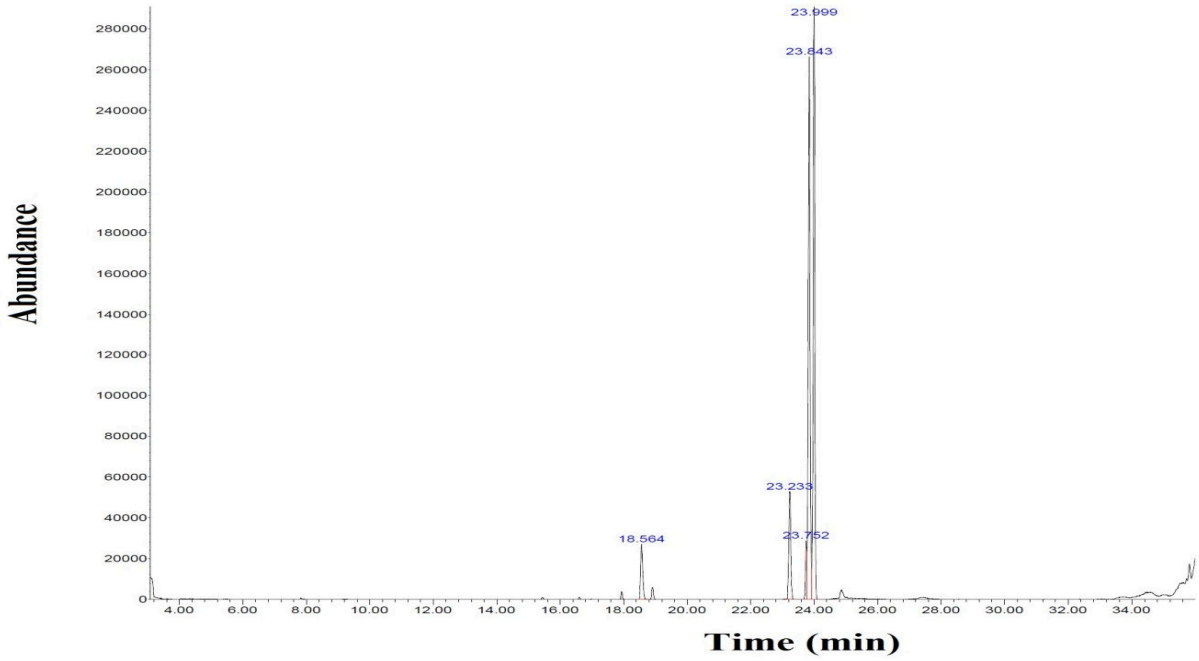
In vivo toxicity of formulations

Three adult Balb/c mice in each group received nanofomulation and after three days, they were sacrificed ethically and Kidney and liver tissues of control and treated Balb/c mice were collected. By preparation of pathological stained lam [H& E staining]. These

Tissues were evaluated for pathological defects.

Results:

Based on the results, 5 compounds of extracted essential oils were identified by water distillation, containing 95% essential oil (Figures 1-6). Figure 1 showed the gas chromatogram of this extract. Among them, 1-benzyl-2-methylbenzene and 4,6-dimethylcyclohex-3-ene-1-carboxylic acid have the highest percentage of components



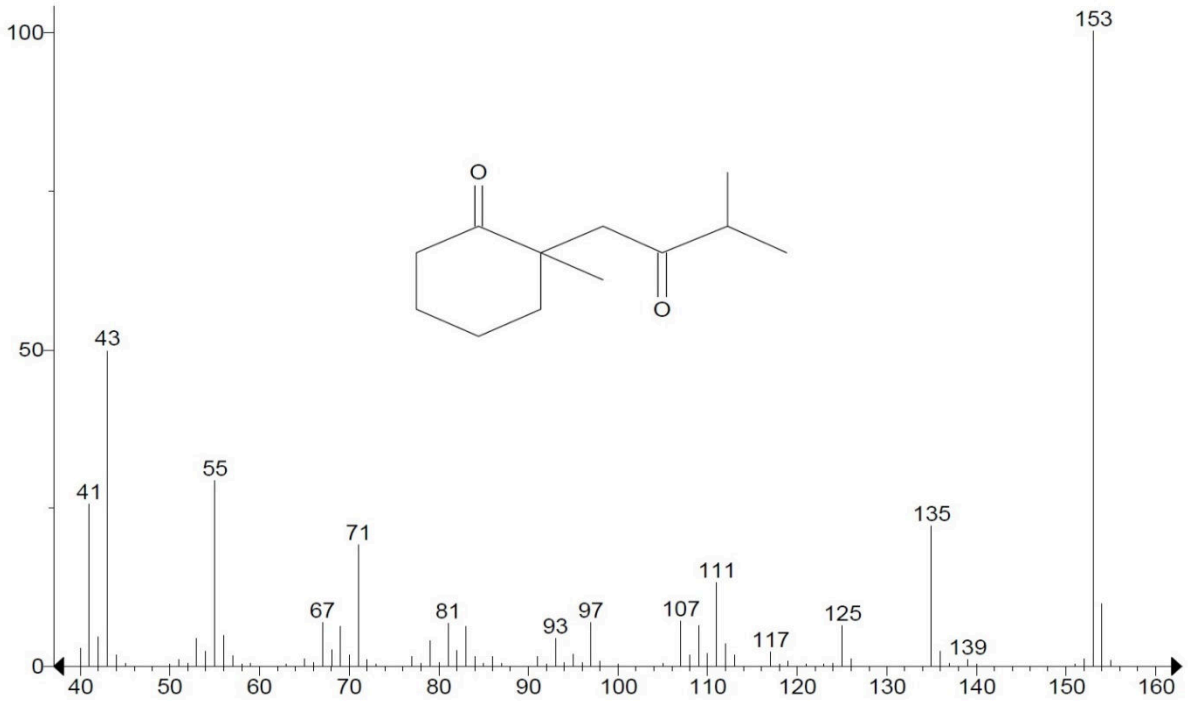


Fig 2. Mass spectrum of 2-methyl-2-(3-methyl-2-oxobutyl)cyclohexan-1-one

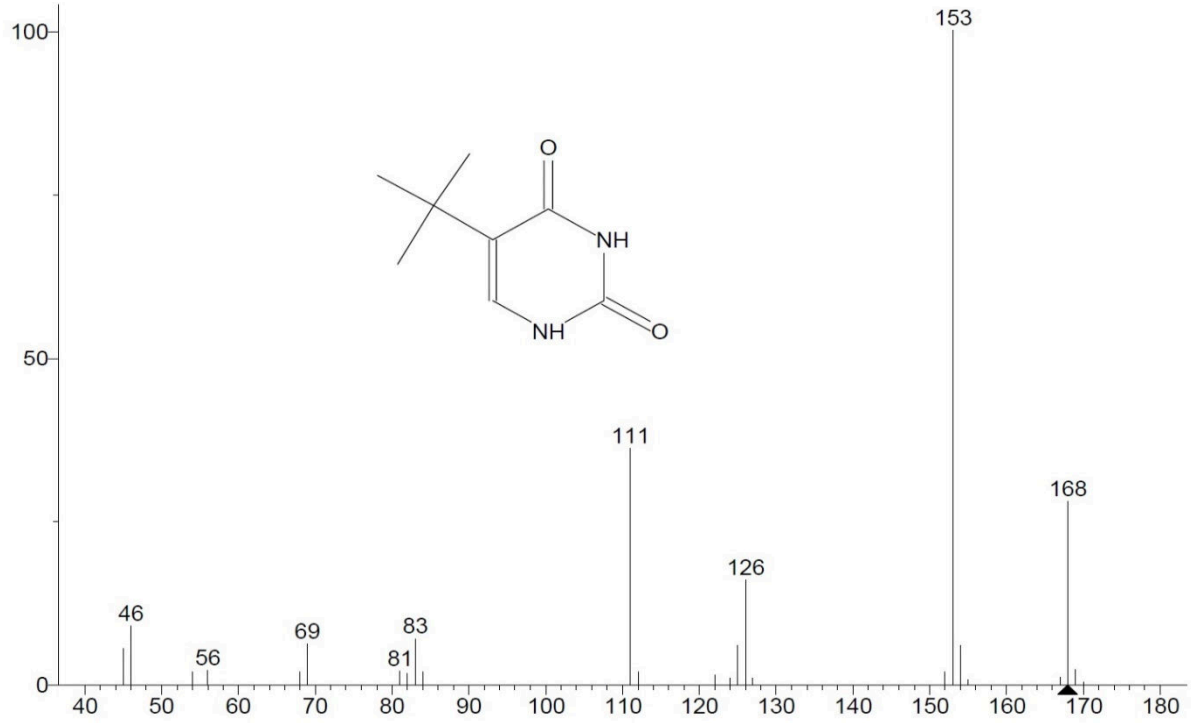


Fig 3. Mass spectrum of 5-(tert-butyl)pyrimidine-2,4(1H,3H)-dione

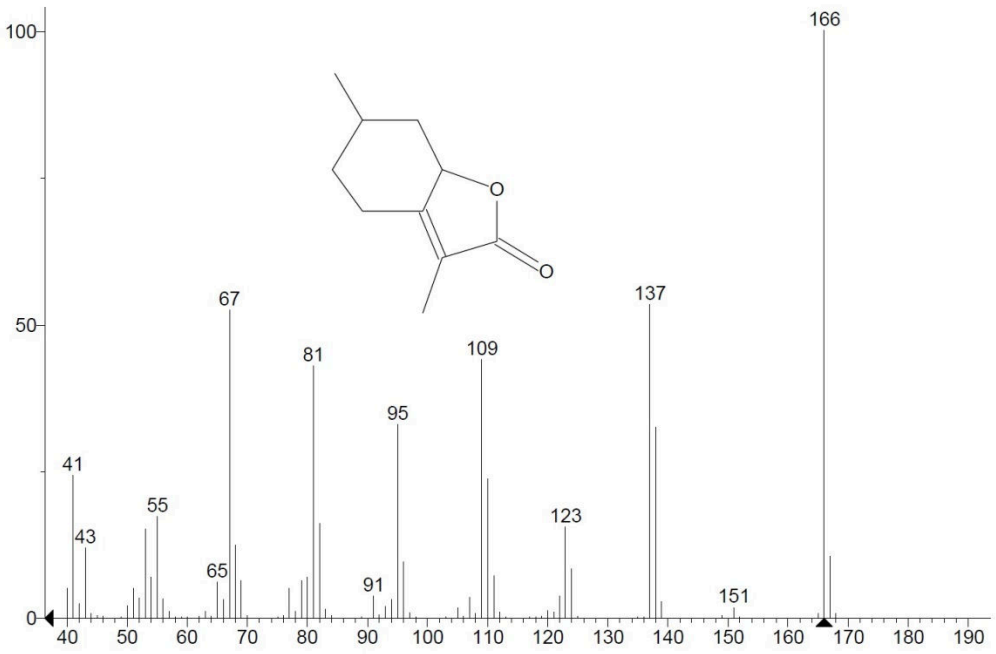


Fig 4. Mass spectrum of 3,6-dimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one

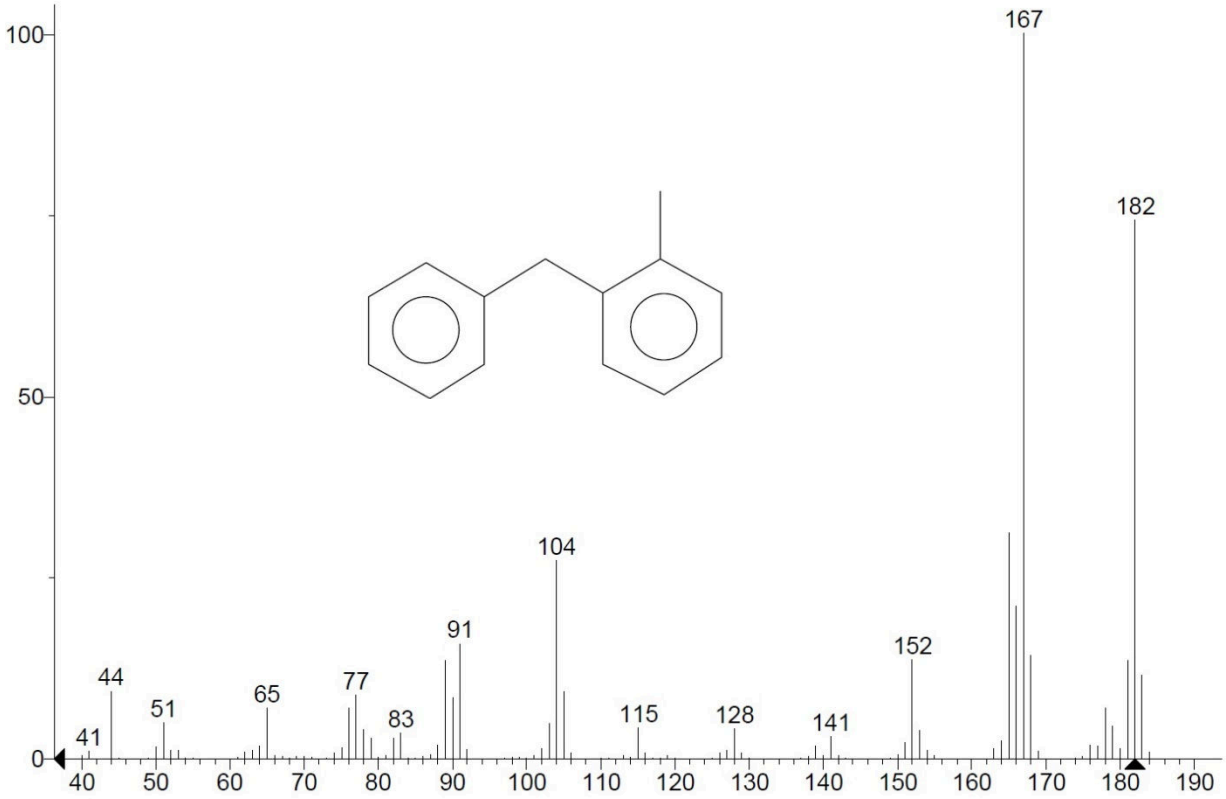


Fig 5. Mass spectrum of 1-benzyl-2-methylbenzene

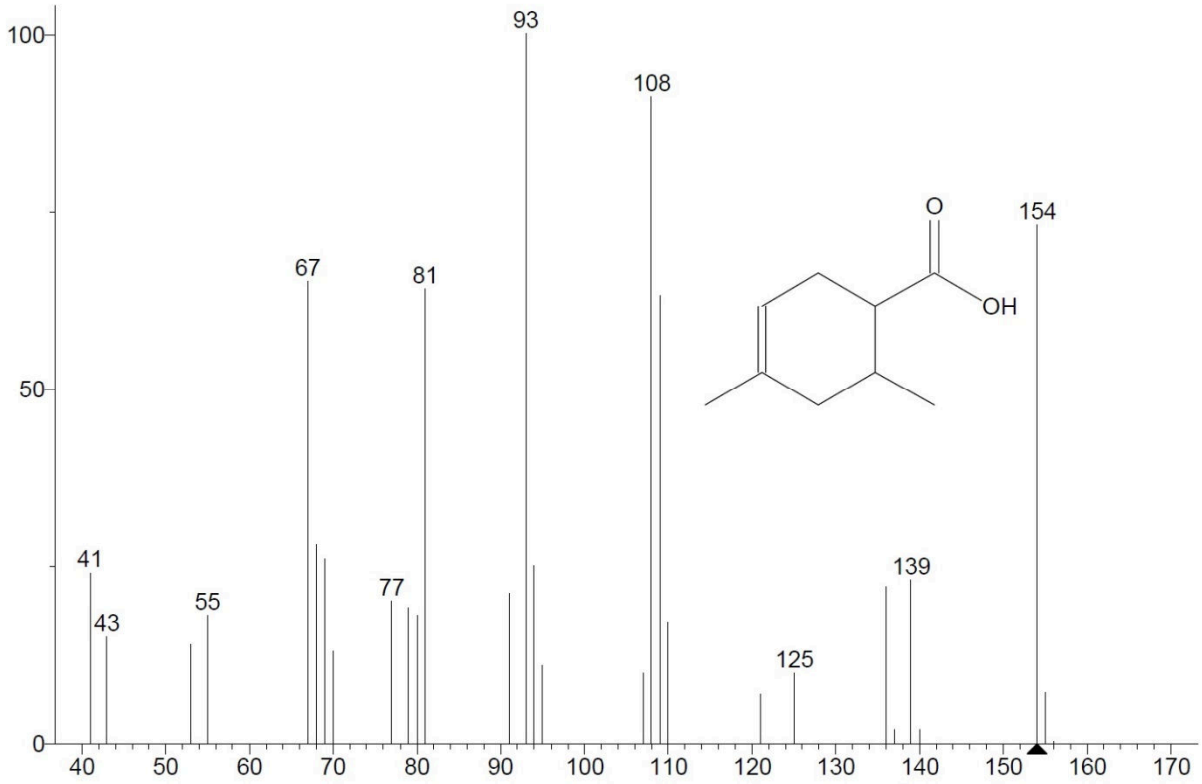


Fig 6. Mass spectrum of 4,6-dimethylcyclohex-3-ene-1-carboxylic acid

Results from FT-IR

The FTIR technique was used to study the functional groups in the dendrimer structure (Figure 7). The peaks observed at 1231.7 cm^{-1}, 1720 cm^{-1} and 3400 cm^{-1} are related to CO, C=O and OH respectively which confirmed the successful synthesis of dendrimer.

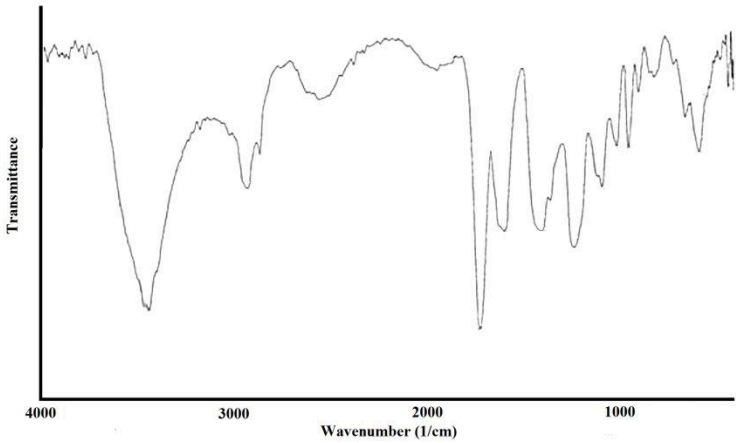


Fig 7. FTIR spectrum of dendrimer

The results of the TEM

The TEM analysis was used to examine the size of synthetic particles (Figure 8). As can be seen, the synthesized particles have a size ranging from 18 to 45 nm.

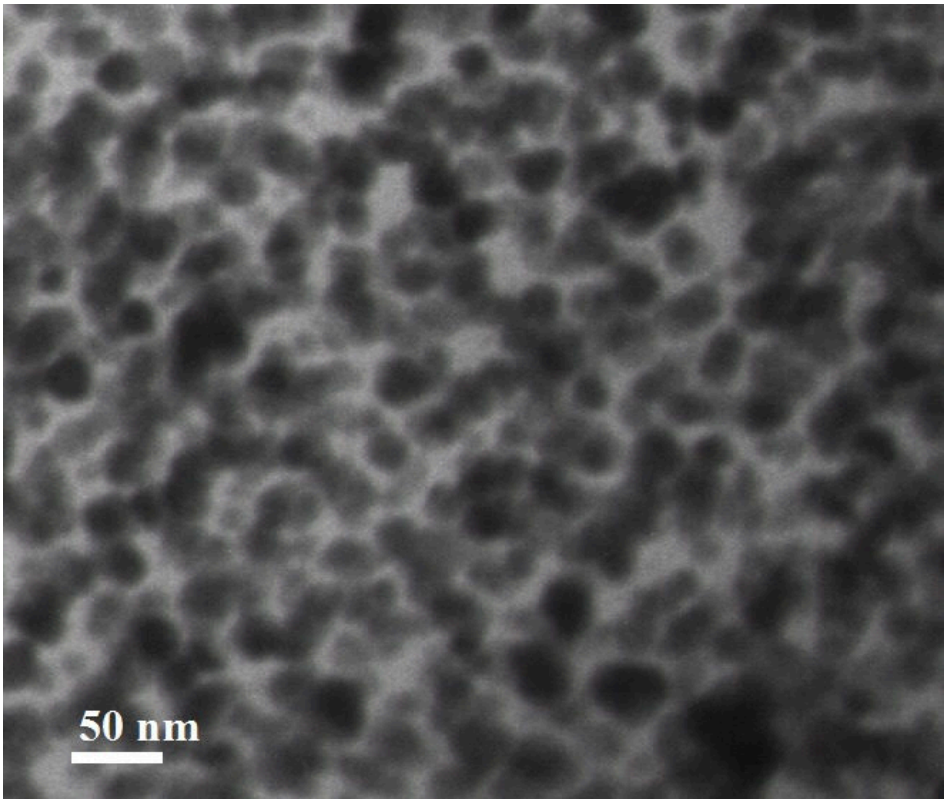


Fig 8. TEM image of dendrimer

The results of Zeta potential test

Identification and determination of load and size of nanoparticles using a Nano ZS zeta sizer, manufactured by Malvern, UK with a measuring range of 0.1 to 10000 nm and a zeta potential measurement range of -200mV to +200 (Figure 9-10). The dendrimer size is about 50 nanometers, while the size of the extract is increased to 70 nm. It was also observed that the load-dendrimer has a negative charge compared with the dendrimer.

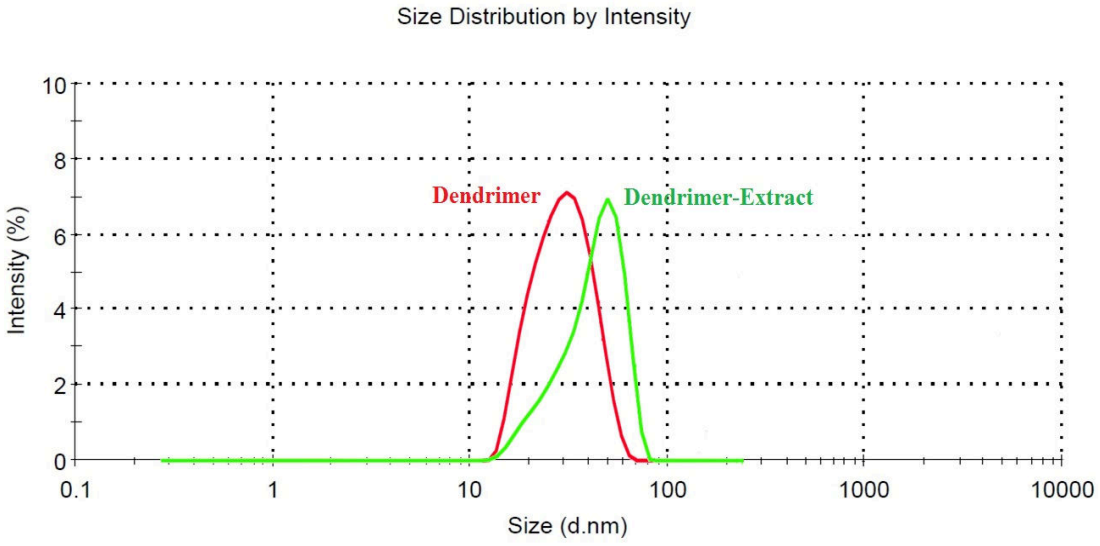


Fig 9. DLS graphs of dendrimer (red) and dendrimer-extract (green)

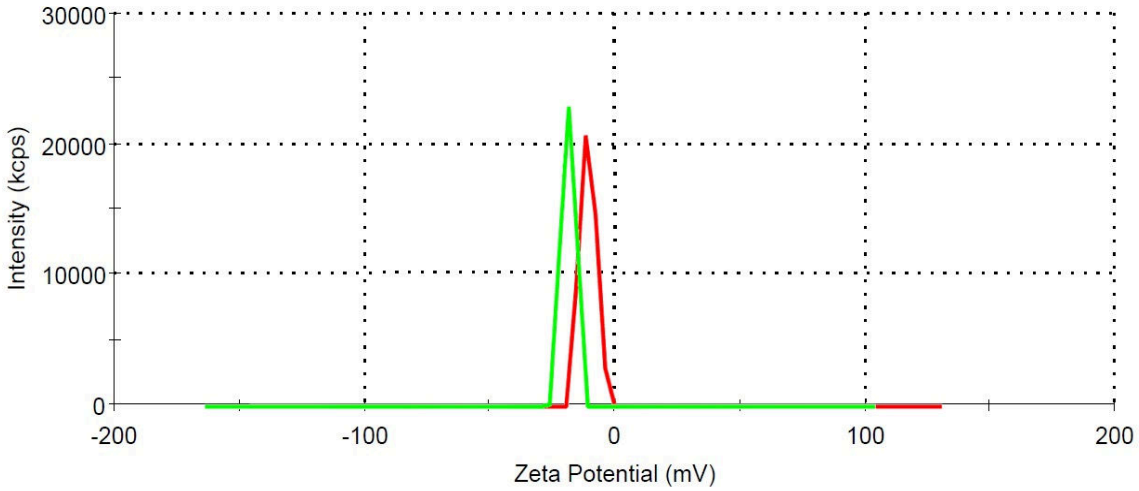


Fig 10. Zeta potential of dendrimer (red) and dendrimer-extract (green)

Results of Antibacterial Properties of Dendrimer- mentha longifolia

The results of the antibacterial test are shown in figures 11-13. The lowest concentration of inhibitors is related to STF and is most closely related to ECOLI. Also, loading of extract in the dedrimer structure caused to increase this concentration.

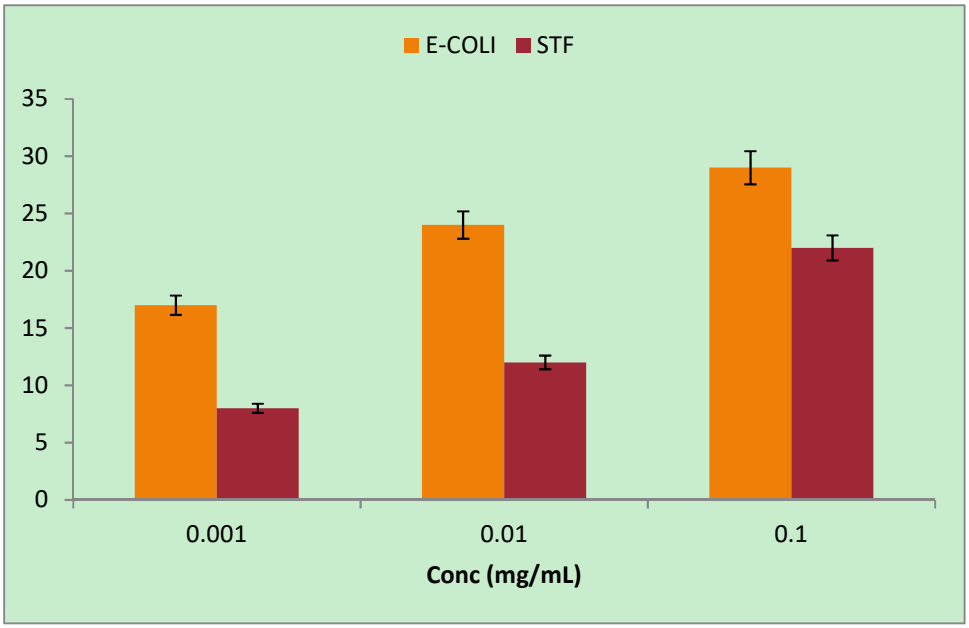


Fig 11. Antibacterial result if extract

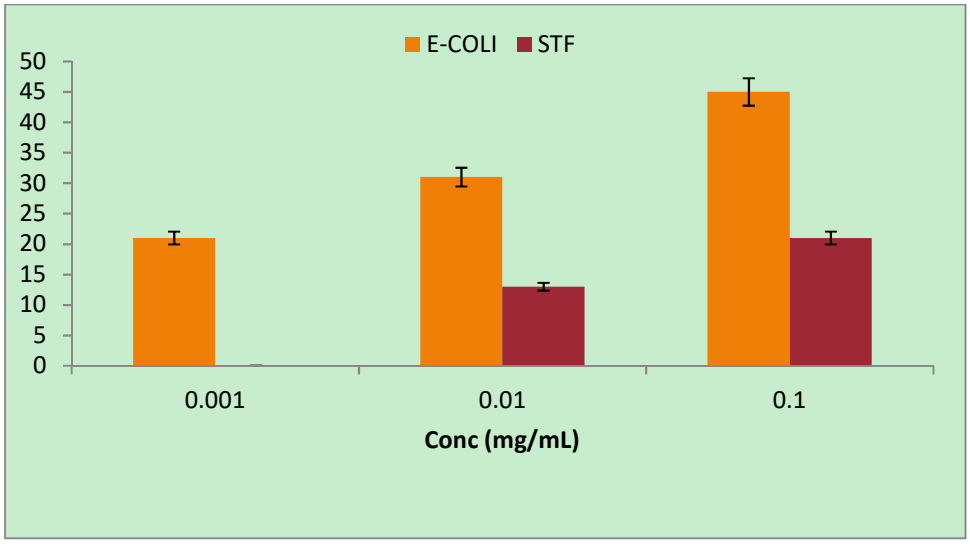


Fig 12. Antibacterial result if extract-dendrimer

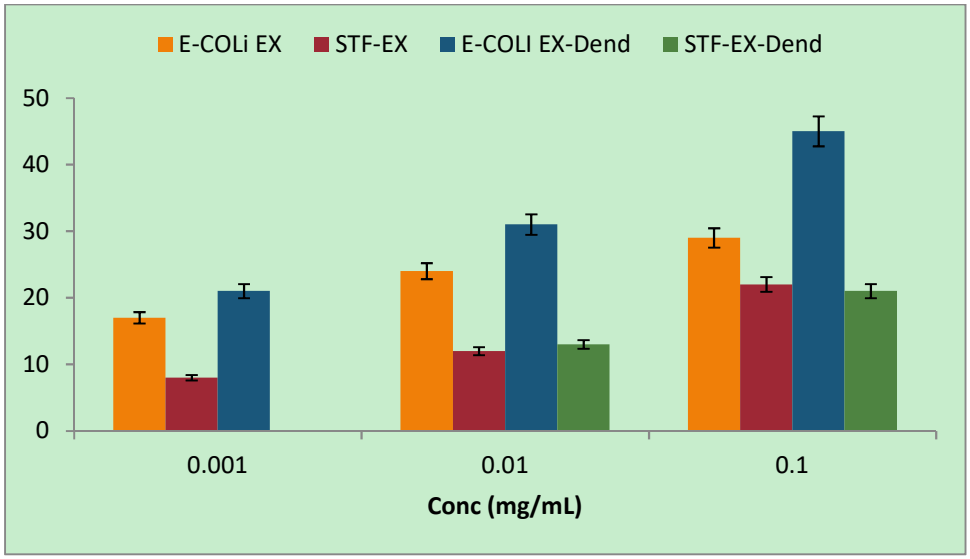


Fig 13. Comparison of antibacterial result

In vivo toxicity evaluation: The results of in-vitro studies showed no toxicity on tissues which confirms the biocompatibility of the structure for the body

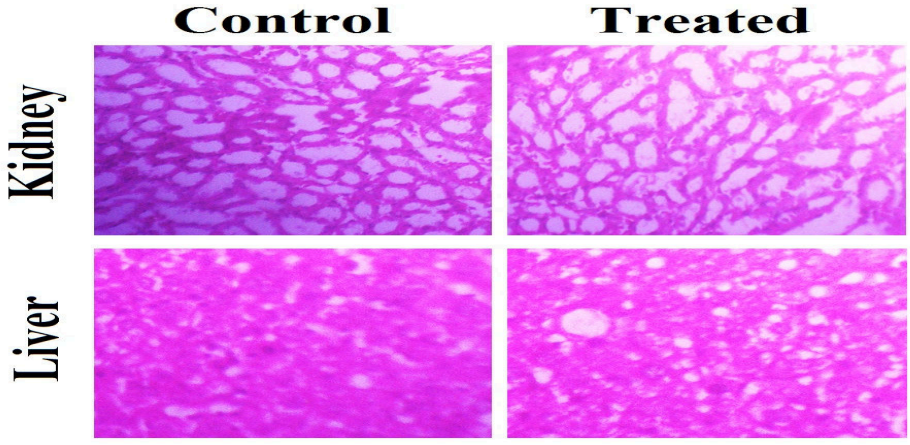


Fig 14. In vivo toxicity evaluation of nano formulation

DISCUSSION

Low levels of infectious doses of many pathogens require extensive research about new combinations with high anti bactericidal potential, which, in order to achieve this goal, use of essential oil and vegetable compositions derived from plants and spices food is very important (33). In this regard, knowing and recognizing the functional mechanisms of these compounds can be very useful in detecting sensitive microorganisms and increasing the efficacy of these compounds in food systems (34). The Most material in the mentha longifolia essential oil used in this study was Pulegone, which its antimicrobial effects have been reported in various studies (35-36). In the study of essential oil of mentha longifolia, the menthol played a very important role in its antimicrobial activity (37). In the present study, amount of this compound was about 0.7 present in essential oil. Investigating the effects of mentha longifolia essential oil on E. coli, and Staphylococcus microorganism's aureus, showed that the role of 1,8 Cineol in this field is very important. In this research work on mentha longifolia loading in dendrimer nanoparticle, two concentrations of 50 and 100 were completely examined. The results of FTIR, TEM, and Zeta Seizer fully confirm the resulting composition. We also examined the amount of anti-bacterial activity (MIC in mg / ml) of mentha longifolia and nano-dendrimer on Staphylococcus aureus and Escherichia coli. The results indicate that the mentha longifolia encapsulated at a concentration of ….mg of dendrimers at different concentrations in two mentioned bacterium had better results. The more important aspect of this study, which is actually a very new topic, is the use of the nanoscale dendrimer-extract form. Dendritic structures and their derivatives due to its unique properties have attracted a great deal of attention in recent studies of drug delivery and antimicrobial properties. Most studies and experiments were on polyamide and amine dendrimers and their derivatives that in many cases toxicity, hemolysis and activation of the complex have been reported. But the linear cyclic anionic dendrimers of citric acid that we used in this experiment have recently been synthesized by Dr Nemazi et al. And are very much considered (38). These biocompatible compounds have a nanoscale dimension up to the second generation and no toxicity and immunological effects have been reported. The use of these dendrimers for drug delivery and antimicrobial purposes has been recently very much considered and studied, but for the first time this dendrimer is used in combination with mentha longifolia and have significant effects in controlling the microbial effects. due to the results of past studies and the current study, it can be predicted that the future of using nano-dendrimers is very promising.

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How to cite this article:
Sahar Javadi, Mehdi Shafiee Ardestani Roya Karimipourkashani. ***Investigating The Anti-Bacterial Effects Of Mentha Longifolia Encapsulated In G2 Dendrimer On Staphylococcus Aureus And Escherichia Coli Bacterium.*** **Br J Bio Med Res , Vol.03, Issue 05, Pg. 1106 - 1120, September - October 2019. ISSN:2456-9739 Cross Ref DOI : https://doi.org/10.24942/bjbmr.2019.577**

Source of Support: Nil **Conflict of Interest:** None declared.