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# STUDY OF CHEMICAL STRUCTURE, ANTIMICROBIAL, CYTOTOXIC AND MECHANISM OF ACTION OF *SYZYGIUM AROMATICUM* ESSENTIAL OIL ON FOODBORNE PATHOGENS

***Behrooz Alizadeh Behbahani, Mohammad Noshad, Fereshteh Falah***

## ABSTRACT

In this study, chemical composition (gas chromatography-mass spectroscopy), chemical structure (fourier transform infrared spectroscopy) and antioxidant potential (β-carotene bleaching assay and DPPH/ABTS-radical scavenging activity tests) of *Syzygium aromaticum* essential oil (SAEO) were evaluated. Eugenol (75.11%) was found to be the major compound of SAEO. Eugenol, as the main chemical constituent of SAEO, showed its signature peaks in the wavenumber range of 720 – 1250 $cm^{-1}$, ascribing to the C=C region. The antimicrobial activity of SAEO on *Escherichia coli*, *Staphylococcus aureus*, *Listeria innocua* and *Pseudomonas aeruginosa* were evaluated. The scanning electron microscopy (SEM) was then applied to unravel the antibacterial mechanism of SAEO on *E. coli* as the most resistant strain and *L. innocua* as the most sensitive strain. The MTT assay was also used to investigate the cytotoxicity effect of SAEO on human colonic cancer cell lines (HT29 cell line) and the highest cytotoxic effect was observed at 200 $mg.mL^{-1}$ concentration of SAEO. The SEM micrographs revealed that the SAEO treatment was able to manifestly increase the cell permeabilization and membrane integrity disruption. This means that the entirety of the cell membranes was remarkably affected by the essential oil, which could lead to cytoplasm secretion and subsequent cell death. The data strongly suggest that SAEO had a potential antioxidant, antimicrobial and cytotoxicity activity.

**Keywords:** *Syzygium aromaticum*; Scanning electron microscopy; Cytotoxic effect; Antimicrobial activity; HT29 cell line

## INTRODUCTION

In recent years, foodborne pathogenic and spoilage bacteria have led to the emergence of one of the important food safety challenges, i.e. new foodborne disease outbreaks **(Zhang et al., 2016)**. It is also worthwhile to note that the lipid oxidation reaction could lead to the formation of potentially toxic side-reaction products capable of threating human health **(Zhong et al., 2015)**. In these contexts, chemical synthetic preservatives have been frequently used in the food industry to suppress microbial growth and lipid oxidation reaction; however, their usages have been a controversial topic, owing to their potential to create health problems **(López-Malo et al., 2006; Spickett and Forman, 2015)**. Therefore, there is a necessity to seek new and safe food-grade antioxidant and antimicrobial agents to amend food shelf-lives. Essential oils (EO) derived from aromatic plants have gained a lot of attention in the food industry not only for their natural origin, but also due to their documented benefits in food and human health. These biologically active compounds confer versatile biological characteristics including antimicrobial, insecticidal, antioxidant, analgesic, anti-tumor, anti-inflammatory, and anti-diabetic effects **(Ribeiro-Santos et al., 2017)**. *Syzygium aromaticum* L. (clove) is known as an evergreen tree and its commercial products are cloves, clove oil, and oleoresin. The essential oil of *S. aromaticum* is normally extracted from its stem, unopened buds, and leaves. Clove oil is used in dental formulations, toothpaste, soaps, mouth washes, breath freshner, insect repellent, and cosmetic items owing to different kind of biological properties, such as antibacterial, antifungal, anthelmintic, analgesic, and anti-carcinogenic activities **(Srivastava, Srivastava and Syamsundar, 2005)**. Eugenol is the major chemical compound of *S. aromaticum* essential oil (SAEO) which its antibacterial and antifungal activities have been reported in the literature **(Shokeen et al., 2008; Braga et al., 2007)**. However, as seen in the literature and to the best of authors' knowledge, there is no data available indicating the mechanism of antimicrobial activity of SAEO towards pathogenic and spoilage bacteria. This study was therefore aimed to unravel the antibacterial effect of SAEO through mechanistic approaches to provide more insights into the mode of

antibacterial action. Moreover, the antioxidant activity of the bioactive essential oil was investigated in this study.

## Scientific hypothesis

The essential oil of *Syzygium aromaticum* has outstanding antibacterial effect against food-borne spoilage and pathogenic bacteria, in conjugation with superb radical scavenging activity.

# MATERIAL AND METHODOLOGY

## Materials

*S. aromaticum* were procured from a local market (Ahwaz, Iran). β-carotene, linoleic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt) (ABTS), gallic acid, and quercetin were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Other chemical reagents and materials were of analytical grade and purchased from Sigma-Aldrich Co. (St Louis, MO, USA) or Merck Co. (Germany).

## Essential oil extraction

The plant was firstly verified and then dried at room temperature in a dark place, followed by grinding to obtain powdered forms *via* a laboratory grinder. The powdered plant (20 g) was dispersed in distilled water (750 mL) and the resulting dispersion was then subjected to a hydrodistillation-based Clevenger apparatus for 3.0 h, according to a method introduced by **Behbahani and Shahidi (2019**) with some modification. The extracted essential oil (SAEO) was dried using anhydrous sodium sulfate followed by storing at 4 ºC in glass vials until analyses.

## Essential oil analysis

### *Gas chromatography-mass spectroscopy (GC-MS)*

GC-MS technique was applied to identify the main chemical compounds of SAEO, based on the method of **Behbahani et al., (2017a)**. For this purpose, 0.2 µL of SAEO was injected to the GC column and the separation of chemical compounds of the essential oil was performed at the heating rate of 5 °C.min$^{-1}$ and the ionization energy of 70 eV that was provided by the carrier gas helium with a rate of 1.1 mL.min$^{-1}$. A sequential process consisted of the comparison of normal spectra of chemical compounds with those of alkenes ($C_8$-$C_{28}$), the calculation of Kovats retention index, and the referring of the resulting indices to the natural compounds library was then used to identify the main chemical constituent of SAEO.

### *Fourier transform infrared spectroscopy (FTIR)*

The essential oil was mixed with potassium bromide followed by compressing the mixture to obtain an appropriate pellet. The FTIR spectrum of the essential oil was then recorded in the wavenumber range of 500 – 4000 cm$^{-1}$ with 4 cm$^{-1}$ resolution using a Perkin Elmer model FTIR spectrometer.

### *Total phenol content*

The procedure of **Do et al. (2014)** with minor changes was employed to measure the total phenol content of the SAEO. Briefly, 0.10 mL of the sample was added to 1.0 mL of 10% v/v Folin-Ciocalteu reagent. The resulting solution was vortexed for 5.0 min and it was then charged with 0.30 mL of 10% $Na_2CO_3$ solution. After incubation of the solution at ambient temperature for 2.0 h, its absorbance was read at 760 nm. Gallic acid was used as standard (0 – 0.50 mg.mL$^{-1}$) and the total phenol content of the SAEO was expressed as mg gallic acid equivalent per g dried essential oil.

### *Total flavonoid content*

The total flavonoid content of the sample was determined utilizing the method of **Tohidi, Rahimmalek and Arzani (2017)**, with some modification. The SAEO (0.10 mL; 0.10 mg.mL$^{-1}$) was charged and mixed with 0.30 mL of 5.0% $NaNO_2$ solution. $AlCl_3$ (0.30 mL; 10% w/v) was added and the solution was mixed for 6.0 min, followed by adding 2.0 mL of NaOH solution (1.0 M). The absorbance of the obtained solution was recorded at 510 nm and quercetin was applied as standard (0 – 0.5 mg.mL$^{-1}$). The total flavonoid content was then calculated and recorded as mg quercetin per g dried essential oil.

### *Antioxidant assays*

DPPH-radical scavenging (DPPH-RS) activity: SAEO (100 µL) was charged and mixed with ethanolic solution of DPPH radicals (0.12 mM; 3.90 mL). The resulting solution was kept at ambient temperature in a dark place for 30 min and its absorbance was read at 517 nm. DPPH-RS activity of SAEO was then measured as below **(Behbahani, Noshad, and Falah, 2019)**.

$$\text{DPPH-RS activity (\%)} = [1\text{-A sample/A control}] \times 100$$

Where: A sample and A control are the absorbance of the sample and control (distilled water instead of sample), respectively.

ABTS-radical scavenging (ABTS-RS) activity: The method of **Shan et al. (2005)** was used to evaluate the ABTS-RS activity of the essential oil. ABTS$^{\bullet}$ cations were firstly generated by reacting the same volumes of ABTS (7.0 mM) and potassium persulfate (2.45 mM) solutions following keeping the obtained solution at room temperature, under dark conditions for 16 h. The ABTS$^{\bullet}$ solution was then diluted with methanol to reach 0.70 ±0.20 absorbance at 734 nm. Thereafter, 3.90 mL of the diluted ABTS$^{\bullet}$ solution was mixed with SAEO (0.10 mL) or methanol (0.10 mL; as control sample). The solution was stored for 6.0 min at ambient temperature and the absorbance was measured at 734 nm. The ABTS-RS activity of the essential oil was calculated according to the following equation:

$$\text{ABTS-RS activity (\%)} = [1\text{-As/Ac}] \times 100$$

Where: As indicates the absorbance of the samples and Ac stands for the absorbance of the control.

β-carotene-linoleic acid assay: The inhibitory effect of SAEO against the bleaching of β-carotene-linoleate solution was evaluated *via* a spectrophotometric method based on the below equation **(Dapkevicius et al., 1998)**:

Inhibitory effect (%) = $[(AA_{(120)} - AC_{(120)})/(AC_{(0)} - AC_{(120)})] \times 100$

Where: $AA_{(120)}$ is the absorbance (at 490 nm) of sample after 120 min incubation time, and $AC_{(0)}$ and $AC_{(120)}$ are the absorbance of control sample at time zero and after 120 min incubation time, respectively.

***Antibacterial assays***

The antibacterial effect of the essential oil (SAEO) was evaluated towards *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *Listeria innocua* ATCC 33090, according to the following antimicrobial tests.

Disk diffusion agar (DDA) assay: This antimicrobial test was done based on the method of **Noshad, Hojjati, and Behbahani (2018)** with minor changes. The blank discs were firstly impregnated with 20 µL of SAEO for 15 min at ambient temperature, followed by placing them on the culture media that were previously contaminated with bacterial strains. The media were incubated at 37 °C for 24 h and the inhibition zone areas around discs were determined by a ruler (mm) and expressed as antibacterial activity.

Well diffusion agar (WDA) assay: The WDA test was performed utilizing a procedure introduced by **Behbahani and Fooladi (2018)**. In this antibacterial assay, several holes with 6.0 mm in diameter were firstly created on the surface of Mueller Hinton agar medium (contaminated with bacteria) and then charged with 20 µL of SAEO. The inhibition zones around the holes were measured after incubation of the medium at 37 °C for 24 h, and reported as antibacterial effect.

Minimum inhibitory concentration (MIC) assay: The MIC of SAEO was evaluated using the microdilution assay in 96 well plates, according to the modified method of **Yeganegi et al. (2018)**. Sequential concentrations of SAEO (0.39, 0.781, 1.562, 3.125, 6.25, 12.5, 25, 50, 100, 200 $mg.mL^{-1}$) were prepared in Mueller Hinton broth medium and then sterilized through syringe filters (0.45 µm). In the next step, 200 µL of each concentration was added to the wells containing 20 µL of microbial suspensions with 0.5 McFarland equivalent. The plates were stored at a constant temperature of 37 °C for 24 h and they were incorporated with 20 µL of 5.0% w/v 2,3,5-triphenyltetrazolium chloride solution. The plates were re-incubated and the lowest concentration of SAEO that inhibited the growth of bacterial strains (confirmed by the lack of an amethystine or dark red color in the wells), was considered as the MIC of the essential oil.

Minimum bactericidal concentration (MBC) assay: In this antibacterial test, 100 µL of the solution in microbial growth-free wells (based on the MIC results) was cultured on the surface of Mueller Hinton agar medium, followed by incubation at 37 °C for 24 h. The lowest SAEO concentration that killed the bacterial strains (no visible colony formation) was reported as the MBC of the essential oil **(Alghooneh et al., 2015)**.

***Antibacterial mechanism***

Scanning electron microscopy (SEM) was performed to unravel the mechanism of action of SAEO on cell membranes of the most sensitive and resistant bacteria to the essential oil (i.e., *L. innocua* and *E. coli*, respectively), following the method of **Lv et al. (2011)** and **Moghayedi et al. (2017)**, with minor changes. For this aim, the microorganisms were cultured in a broth medium at their MIC values and incubated at 37 °C under shaking conditions. Next, the microbial suspension was centrifuged for 5.0 min at 5000 g, and the precipitate (contains microorganisms) was washed twice with phosphate buffered solution (0.10 M, pH 7.0). The suspension was filtered using a polycarbonate filter and the filtrate was fixed in a glutaraldehyde solution (2.50% v/v), which was followed by keeping the solution at 4 °C for 2.0 h. The sample was washed several times with double distilled water and it was then dehydrated successively with ethanol solution of various concentrations (30%, 50%, 70%, 80%, 90%, and 100%) for 10 min. In the final step, the samples were dried to completely evaporate the ethanol and coated with gold for SEM analysis. The morphology of *E. coli* and *L. innocua* was checked before and after treatment with SAEO, using a LEO 1450 VP model SEM apparatus.

***Cytotoxicity effect of SAEO***

The cytotoxic effect of SAEO was measured against colon cancer cell line (HT29 cell line) by MTT assay. The cells (Bu Ali Research Institute of Mashhad, Iran) were cultured in DMEM (Dulbecco's Modified Eagle Medium) high glucose medium supplemented with fetal calf serum (10% v/v) and penicillin/streptomycin, and incubated at 37 °C under constant humidity 95% and 5.0% $CO_2$ pressure. HT29 cells were seeded in 96-well flat-bottom plates (approximately 100,000 per well) until 50-60% confluence was achieved. The medium was then replaced with a complete culture medium containing DMEM and fetal bovine serum (200 µL) and various concentrations of SAEO (0.39, 0.781, 1.562, 3.125, 6.25, 12.5, 25, 50, 100, 200 $mg.mL^{-1}$) were added to each well. The blank medium was regarded as control medium. The cell proliferation was quantified by MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay after 24 h incubation time as follows. The MTT solution (30 µL; 5.0 $mg.mL^{-1}$) was added to each well and the plates were incubated for 3.0 h in a $CO_2$-equiped incubator. After removing the medium gently and adding DMSO (200 µL) into the wells, an ELISA/microplate reader at 570 nm reference filter was used to record the absorbance of the mixture. The SAEO concentration ($mg.mL^{-1}$) that was able to inhibit the cell growth by 50%, was calculated and defined as $IC_{50}$. The cell viability curves were plotted with regard to the control cells.

## Statistic analysis

All the experiments were done at three replicates. Data were analyzed through one-was ANOVA by SPSS software at 95% confidence level ($p < 0.05$), and recorded as means ±standard deviation.

## RESULTS AND DISCUSSION

### Chemical composition of SAEO

Six compounds were confirmed in SAEO according to the GC-MS analysis, with eugenol being the main detected constituent among others (75.11%). Caryophyllene (14.05%) was the second bioactive compound identified in SAEO, followed by phenol, 2-methoxy-4-(2-propenyl) (6.09%) and humulene (3.35%). Other minor constituents were delta-cadinene (0.71%) and caryophyllene oxide (0.69%). The results are in congruent with findings of other researchers, who reported that eugenol is the main biologically active compound of the essential oil of *S. aromaticum* **(Cortés-Rojas, de Souza and Oliveira, 2014; Ranasinghe, Jayawardena and Abeywickrama, 2002)**.

### Structural analysis of SAEO

The FTIR spectrum of SAEO obtained *via* the hydro-distillation method is depicted in Figure 1. The region of 3000 – 3 500 $cm^{-1}$ is due to the hydroxyl (OH) groups of phenolic and alcoholic compounds of SAEO. The peaks at around 2847 $cm^{-1}$ and 2937 $cm^{-1}$ are attributed to the frequency asymmetrical patterns of $CH_2$- and $CH_3$- groups of alcoholic compounds in the essential oil **(Mohammed, Abdulkadhim and Noori, 2016)**. Eugenol, as the main chemical constituent of SAEO, showed its signature peaks in the wavenumber range of 720 – 1250 $cm^{-1}$, ascribing to the C=C region. Moreover, the sharp peaks at 1642.22, 1607.06, and 1513.75 $cm^{-1}$ are assigned to the C=C stretching vibration of aromatic moiety of eugenol, in well agreement with the eugenol spectrum reported by **Pramod et al. (2015)**. The peak at 1268 $cm^{-1}$ could be ascribed to the characteristic bands of =C-H in-plane bending absorption of the aromatic rings and $–CH_2$ swing in alkanes; whilst, the peak at 1234 $cm^{-1}$ is likely due to the C-O-C symmetric expansion of aromatic acid esters and C-OH vibrational stretching of phenolic compounds, which the latter peak is often attributed to the absorption of esters and eugenol in essential oils **(Jeyaratnam et al., 2016**. The peaks located at the wavenumbers of 1122 $cm^{-1}$ and 1035 $cm^{-1}$ are probably ascribed to the C-O stretching vibrations and deformation vibration of C-OH groups. In addition, the peaks at 992 $cm^{-1}$ and 743 $cm^{-1}$ could be due to the bending vibration absorption of C-H groups and =CH vibration absorption of benzene rings, respectively. Likewise, the vibration absorption of alkenes could be observed at the wavenumber of 645 $cm^{-1}$ **(Li, Kong and Wu, 2013)**.

### The content of total phenolic and flavonoid compounds

*S. aromaticum* is one of the main vegetal sources of phenolic compounds, such as flavonoids, hydroxyphenyl propens, hydroxycinamic acids, and hydroxybenzoic acids. The total phenolic and flavonoid compounds of the essential oil were found to be 48.14 ±0.12 mg gallic acid.$g^{-1}$ and 23.26 ±0.51 mg quercetin.$g^{-1}$ dry weight of the SAEO. Phenolic acids such as gallic acid and its derivatives (e.g., hydrolysable tannins) are found in high concentration in clove oil. Flavonoid compounds like kaempferol, quercetin, and its glycosylated derivatives are also presented in clove in lower contents **(Cortés-Rojas et al., 2014)**. Total phenolic content of 0.0896 mg gallic acid.$g^{-1}$ dry weight of *S. aromaticum* fruit and 114.41 – 519.33 mg gallic acid.$g^{-1}$ *S. aromaticum* extracts have been reported by **Wojdyło, Oszmiański and Czemerys (2007) and Neaz (2019)**, respectively. These differences could be ascribed to the various extraction techniques and plant parts used for essential oil extraction. Indeed, the oils from the bud, stem, and leaves of clove differ considerably in quality and yield. In addition, the origin, variety, maturity at harvest, and quality of raw materials in conjugation with pre-treatments and extraction modes can influence both yield and composition of the resulting essential oil **(Neaz, 2019)**. Phenolic compounds are strongly contributed to the antioxidant and antimicrobial capacity of bioactive EO.

### Antioxidant activity

DPPH-RS, ABTS-RS, and β–carotene bleaching assays were applied to evaluate the antioxidant activity of SAEO. The relatively stable organic DPPH radical has been extensively used to determine the antioxidative potential of plant extracts and single compounds **(Goupy et al., 2003; Zhang et al., 2019)**.

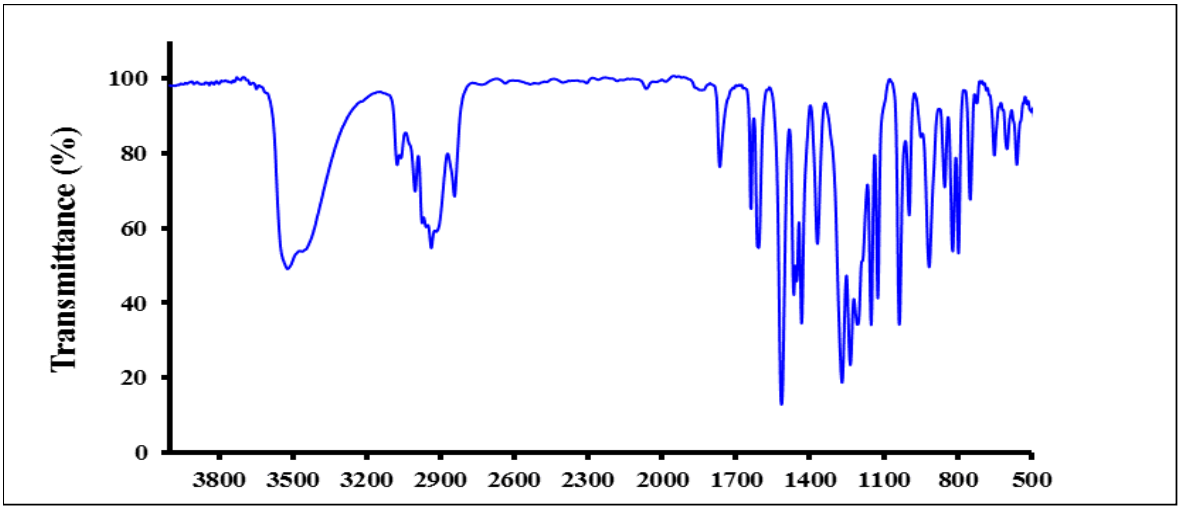


**Figure 1** FTIR spectrum of *S. aromaticum* essential oil.

As can be seen from Table 1, the DPPH-RS activity was increased as the concentration of SAEO increased from 50 to 600 μg.mL$^{-1}$. This means that the SAEO contains bioactive compounds with the ability to neutral DPPH radicals through hydrogen atoms and electron donation **(Wang et al., 2016; Jabbari and Jabbari, 2016)**. The antioxidant capacity measured by ABTS radical test indicated the same trends and relationships as did DPPH-RS assay, and the ABTS-RS activity of the essential oil increased as a function of SAEO concentration. This might be due to the same principle of two DPPH and ABTS assays upon reacting with antioxidant compounds **(Ćavar et al., 2012)**. The SAEO was also able to remarkably suppress the rapid discoloration of β–carotene, and it showed a strong hydroperoxide scavenging power of 66.62%, likely *via* trapping and neutralizing the linoleate free radicals capable of deterioration of β–carotene **(Barros et al., 2007)**. This manifestly high antioxidant activity of SAEO is mainly due to its polyphenol compounds, such as phenolic acids and flavonoids.

## Antibacterial activity

The antibacterial effect of SAEO was evaluated by the qualitative and quantitative assays, such as DDA, WDA, MIC, and MBC. As can be seen from Table 2, SAEO showed strong inhibitory effects against *L. innocua*, *S. aureus*, *P. aeruginosa*, and *E. coli* with the inhibition zone values of 27.10, 29.15, 22.30, and 20.50 mm in DDA test and 32.00, 35.15, 24.40, and 22.65 mm in WDA test, respectively. It is clear that the inhibition zone (i.e. higher antibacterial effect) is higher in WDA assay than that in DDA one, mainly due to the direct contact of SAEO and bacteria in the former; however, in the DDA antimicrobial test, the essential oil should be diffused from the discs into culture medium to exert its inhibitory effect **(Behbahani, et al., 2017b)**. It is also worthwhile to note that the gram positive bacteria (*L. innocua* and *S. aureus*) were inhibited to a more extent by
the essential oil compared to the gram negative bacteria. In addition, the MIC and MBC results revealed that the SAEO was more active against the gram positive bacteria and lower concentration of SAEO was required to inhibit the growth of *L. innocua* and *S. aureus* or kill them. This could be probably ascribed to the presence of a single diffusible mucopeptidic laver in gram positive bacteria that makes them more susceptible to antimicrobial agents; whilst, the complex lipopolysaccharide layer on the outer cell membrane of gram negative bacteria have the potential to remarkably reduce the diffusion rate of lipophilic antibacterial compounds across the cell membrane (**Behbahani and Imani Fooladi, 2018**).

## Antibacterial mechanism

The morphological changes in bacterial cells treated with SAEO were investigated by SEM micrographs. It is clear that the untreated bacteria *E. coli* and *L. innocua* had their typical striated wall structures (Figure 2A, B); however, the morphology of cell membranes of the bacteria underwent severe detrimental changes upon treating with SAEO for 12 h at the MIC values of 1.56 mg.mL$^{-1}$ for *L. innocua* (Figure 2A) and 6.25 mg.mL$^{-1}$ for *E. coli* (Figure 2B). The SEM micrographs revealed that the SAEO treatment was able to manifestly increase the cell permeabilization and membrane integrity disruption. Treated *E. coli* had a malformed and incomplete/sunken shape in concomitant with the lack of cell walls (Figure 2B). This means that the entirety of the cell membranes was remarkably affected by the essential oil, which could lead to cytoplasm secretion and subsequent cell death. Similar findings were reported by **Behbahani, Noshad, and Falah (2019)**, who worked on the antibacterial mechanisms of cumin essential oil against some pathogenic and spoilage bacteria.

## Cytotoxic effect of SAEO

MTT assay is used to evaluate the cytotoxic effects of essential oils owing to its simplicity. Despite the fact that the assay could not always be the best choice, the activation level of cells could be quantified usefully by this method, through an independent mode with bacteria and eukaryotes' proliferation **(Ramak and Talei, 2018)**.

Figure 3 indicates the cytotoxic effects of different concentrations of SAEO on HT29 cell lines after 24 h reaction period. The cytotoxicity effect was dependent on the essential oil concentration; the higher SAEO concentration, the higher was cytotoxicity.

**Table 1** *In vitro* antioxidant activity of *S. aromaticum* essential oil.

| SAEO concentration (μg/mL) | DPPH-RS activity (%) | ABTS-RS activity (%) |
|---|---|---|
| 50 | 29.64 1.10$^{e}$ | 27.18 0.56$^{e}$ |
| 100 | 39.44 0.78$^{d}$ | 38.44 0.50$^{d}$ |
| 200 | 47.62 0.92$^{c}$ | 49.81 0.10$^{c}$ |
| 400 | 57.20 0.95$^{b}$ | 61.14 0.19$^{b}$ |
| 600 | 77.10 1.05$^{a}$ | 79.50 0.17$^{a}$ |

Note: Means with different letters in each column differ significantly ($p$<0.05).

**Table 2** *In vitro* antibacterial activity of *S. aromaticum* essential oil against some pathogenic and spoilage bacteria.

| **Microbial strains** | **Antimicrobial assays** | | | |
|---|---|---|---|---|
| | **Disk diffusion agar (mm)** | **Well diffusion agar (mm)** | **MIC (mg/mL)** | **MBC (mg/mL)** |
| *E. coli* | 20.50 ±0.55 | 22.65 ±0.28 | 6.25 | 50 |
| *P. aeruginosa* | 22.30 ±0.56 | 24.40 ±0.44 | 3.125 | 25 |
| *L. innocua* | 27.10 ±0.40 | 32.00 ±0.23 | 1.56 | 6.25 |
| *S. aureus* | 29.15 ±0.50 | 35.15 ±0.19 | 0.78 | 6.25 |

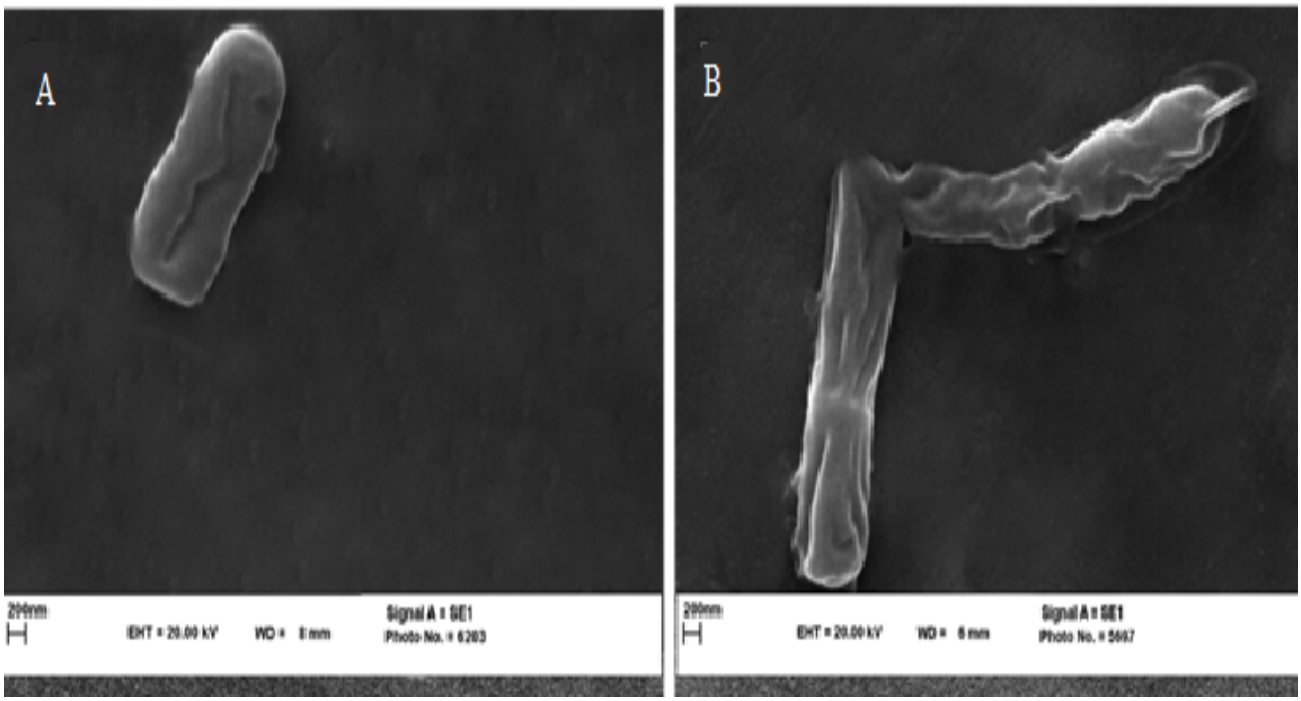


**Figure 2** SEM images of *L. innocua* (A) and *E. coli* (B) cells after treatment with *S. aromaticum* essential oil for 12 h.

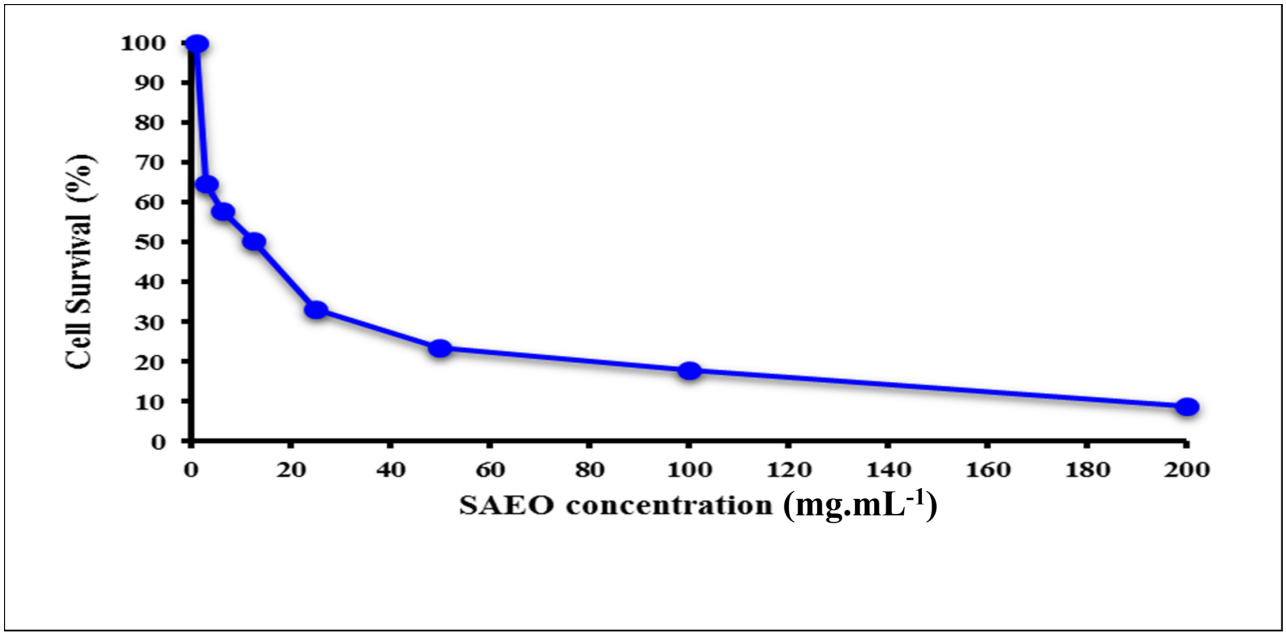


**Figure 3** Cytotoxic effect of various concentrations of *S. aromaticum* essential oil on colon cancer cell line (HT29 cell line) after 24 h reaction period.

It can be seen from Figure 3 that the highest percentage of cell viability was observed at 3.25 mg/mL SAEO concentration, and a relatively low cell survivability was found when essential oil concentration was increased up to 200 mg.mL$^{-1}$. It is noteworthy that the $IC_{50}$ value of the purified active compound was also calculated to check its maximum permissible concentration, and $IC_{50}$ value was observed to be 13.51 mg.mL$^{-1}$. It can be confirmed by the MTT data that low concentrations of SAEO could stimulate cell proliferation substantially ($p$<0.05).

Indeed, essential oils have a lipophilic nature and high affinity for cell membranes. In this way, they could result in manifest changes in the polarization of cancer cell and particularly mitochondrial membranes along with ionic channels and disturbing membrane potential, thereby inhibiting proton pumps and ATP production **(Lesgards et al., 2014; Frolova, et al., 2019)**. It was also reported that essential oils could disrupt the membrane ionic pumps and lead to ion (calcium) and membrane proteins leakage **(Bakkali et al., 2008)**. The cytotoxicity of SAEO and its major components to human skin cell and other herbal extracts through MTT assay have been reported in the literature **(Prashar, Locke, and Evans, 2006; Turan et al., 2018; Ogbole, Segun, and Adeniji, 2017)**.

## CONCLUSION

A sequential procedure consisted of hydrodistillation-based extraction of *S. aromaticum* essential oil followed by GC-MS analysis of the resultant oily solution yielded a bioactive essential oil rich in eugenol. The essential oil of *S. aromaticum* had outstanding antibacterial effect against food-borne spoilage and pathogenic bacteria, in conjugation with superb radical scavenging activity. The antibacterial mechanism of *S. aromaticum* essential oil on the most sensitive (i.e. *L. innocua*) and resistant (i.e. *E. coli*) bacteria was then evaluated by the SEM micrographs, and it was observed that the essential oil caused an increase in cell permeabilization and membrane integrity disruption. In addition, the essential oil showed a dose-dependent cytotoxic effect on the colon cancer cell line (HT29 cell line) and higher essential oil concentration resulted in a higher cytotoxicity effect. *S. aromaticum* essential oil could be therefore used to develop functional food products to possibly suppress radical attacks in human body and treat colonic cancers.

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**Contact address**
*Behrooz Alizadeh Behbahani, Agricultural Sciences and Natural Resources University of Khuzestan, Faculty of Animal Science and Food Technology, Department of Food Science and Technology, Postal Code: 6341773637, Mollasani, Iran, Tel.: +989168729619,
E-mail: B.alizadeh@asnrukh.ac.ir
ORCID: https://orcid.org/0000-0002-1447-5088
Mohammad Noshad, Agricultural Sciences and Natural Resources University of Khuzestan, Faculty of Animal Science and Food Technology, Department of Food Science and Technology, Postal Code: 6341773637, Mollasani, Iran, Tel.: +989173238580,
E-mail:Noshad@asnrukh.ac.ir
ORCID: https://orcid.org/0000-0002-4060-9254
Fereshteh Falah, Ferdowsi University of Mashhad, Faculty of Agriculture, Department of Food Science and Technology, Azadi Street, Postal Code: 9177948974, Mashhad, Iran, Tel: +989174059029,
E-mail: Fereshtefalah11@gmail.com
ORCID: https://orcid.org/0000-0001-8991-8755