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BIOLOGICAL ACTIVITY OF ESSENTIAL OILS AGAINST *STAPHYLOCOCCUS SPP.* ISOLATED FROM HUMAN SEMEN

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ABSTRACT

The antimicrobial and antioxidant activities of 5 commercial plant essential oils (*Citrus paradisi* peel oil, *Citrus reticulata* peel oil, *Juniperus communis* fruit oil, *Eucalyptus globulus* leaf oil, and *Cananga odorata* flower) were determined. Essential oil samples were analysed by GC-FID chromatography (Agilent 6890N). Furthermore, the plant essential oils were tested using antioxidant activity and antimicrobial activity test against 19 species of *Staphylococcus* spp. isolated from human semen and identified with MALDI-TOF MS Biotyper. Antioxidant activities test using DPPH method revealed that *Cannaga odorata* had the higher antioxidant activity and the essential oil showed a positive effect. This activity is probably due the high content of benzyl benzoate and caryophyllene, which are the dominant compounds in this kind of oil. The lowest antioxidant activity was found for *Juniperus communis* essential oil. Antimicrobial activities showed that all tested essential oils inhibited the growth of 19 staphylococci studied. However, *C. paradise, C. reticulata, J. communis* and *C. odorata* had the best antimicrobial effect against *S. hominis, whereas E. globulus* essential oil has the best antimicrobial activity against *S. aureus* and *S. capiti*.

Keywords: antioxidant activity, antimicrobial activity, bacteria, spermatozoa, GC-FID chromatography

INTRODUCTION

Some bacteria are deleterious to the spermatozoa in a concentration-dependent manner. The most extensively studied is the effect of *Escherichia coli* on human spermatozoa survival. This Gram-negative bacterium reduces sperm motility through sperm adhesion and agglutination (**Monga and Roberts, 1994**). *Staphylococcus aureus* (*S. aureus*) is one of the most common pathogens causing both human and animal infections. Transmission of *S. aureus* to humans via contaminated food continues to be a health public concern (**Qiuchun et al., 2019**). *Staphylococcus aureus* (*S. aureus*) is an important zoonotic pathogen, which can infect both humans and animals. It is widely distributed in the nature and is present in air, water and feed; it also exists on the surface of the human body, in the nasal cavity, on animal fur, and in the digestive tract among other sites. *S. aureus* has been responsible for several infectious diseases including tissue and skin infections, pneumonia, sepsis, mastitis, arthritis, and soft tissue infections **(David and Daum, 2010; Tong et al., 2015**). Livestock products can act as a source of *S. aureus* zoonotic infections, and handling or consuming of contaminated food could potentially result in transmission to humans (**Feingold et al., 2012; Papadopoulos et al., 2018**).
Citrus essential oils (O) have been applied in many products, such as foods, beverages, cosmetics and medicines, as flavouring agents as well as for aromatherapy. They are also used for their germicidal, antioxidant and anticarcinogenic properties (**Uysal et al., 2011**). The active constituents of citrus Essential oils (Eos), such as limonene, *α*-pinene, *β*-pinene and *α*-terpinolene exhibit a wide spectrum of antimicrobial activity, as convinced by many studies in other plants (**Cristóbal-Luna, et al., 2018**Citrus fruits are a distinctive berries with the internal parts divided into segments. The number of natural species is unclear, as many of the named species are hybrids. Grapefruit (*Citrus paradisi.* L) belongs to the *Citrus* genus, a taxa of flowering plants in the family *Rutaceae*. The grapefruit is believed to have arisen from the pomelo or shaddock *(Citrus grandis)* or as a hybrid between pomelo and sweet orange. Mandarin fruits (*Citrus reticulata*, family *Rutaceae*) are one of the most abundant edible citruses in the fresh fruit markets. Mandarin peel oil is usually obtained through the cold-pressing process. There are three different types of cold-pressed mandarin oil distinguished by the colour and maturity of the fruits: the so-called “green oil” is obtained from immature fruits and mainly used as fragrance, the “yellow oil” is obtained from mature fruits, being used as flavour and fragrance, while completely matured ones are used to produce the “red oil”, mainly used as flavour (**Reeve and Arthur, 2002**). Main components of mandarin peel oil are limonene (up to 95 wt.%) and other terpenes, like γ-terpinene. Oxygenated components (from 0.2 to 1.5 wt.%) like linalool, decanal and citral have the highest contribution to the aroma fraction (**Lota et al., 2001**). Essential oils are the odorous, volatile products of the secondary metabolism of an aromatic plant, which are often concentrated in a particular organ of the plant such as leaves, stems, bark or fruit and are stored in secretory cells, cavities, canals, epidermic cells or glandulartrichomes (**Gilles et al., 2010**). *Juniperus* (*Cupressaceae*) is a plant widely cultivated in the northern hemisphere. This species grows as trees or shrubs. Traditionally, *Juniperus* has shown several applications, mainly related to its medicinal properties and a highly specific flavour which are associated to its volatile oil components (**Carpenter et al., 2014**). Most of the organs of this plant contain essential oils, but it is mainly extracted from the berries, needles, branches and roots (**Foudil-Cherif and Yassaa, 2012**). The eucalyptus oils can

be found in the leaves of more than 300 species of this genus and less than 20 of these have ever been exploited commercially for the production of essential oils rich in 1,8-cineole by pharmaceutical and cosmetic industries (**Elaissi et al., 2011** Ylang-ylang (*Cananga odorata*) is one of the plants that are exploited at a large scale for its essential oil which is an important raw material for the fragrance industry. The essential oils extracted via steam distillation from the plant have been used mainly in cosmetic industry but also in food industry. Traditionally, *C. odorata* is used to treat malaria, stomach ailments, asthma, gout, and rheumatism. The essential oils or ylang-ylang oil is used in aromatherapy and is believed to be effective in treating depression, high blood pressure, and anxiety (**Tan et al., 2015**).

The aim of this study was to determine the chemical and biological properties of selected essential oils. From a biological point of view, we studied the antimicrobial and antioxidant activity of essential oils against staphylococci from sperm identified with MALDI-TOF MS Biotyper.

MATERIAL AND METHODS

Essential oils

Samples of commercial pure essential oils: *Citrus paradisi* peel oil, *Citrus reticulata* peel oil, *Juniperus communis* fruit oil, *Eucalyptus globulus* leaf oil, and *Cananga odorata* flower oil, obtained via steam distillation were purchased from Aromatika (Russia). The essential oil samples were stored in glass vials with teflon-sealed caps at laboratory temperature in the absence of light.

Gas chromatography

Essential oil samples were analysed by Agilent 6890N (Agilent Technologies, Santa Clara, CA, USA) with FID detector. System control and data analysis were processed using the Agilent ChemStation software Rev. B.04.03-SP1 (Agilent Technologies, Santa Clara, CA, USA). The chromatographic separation was performed in the DB-23 column (0.25 mm i.d., 30 m long, 0.25 µm film thickness) (Agilent Technologies), and 5 µl of the sample was injected. The injector temperature was 250 °C, and the FID temperature is set at 250 °C. The carrier gas (nitrogen) flow was 1.1 ml/min (constant flow) with a split ratio 1:100 and a temperature program from 40 °C to 80 °C at 3 °C/min, from 80 °C to 180 °C at 5 °C/min, and from 180 °C to 220 °C at 8 °C/min and finally held at 220°C for 15 min.

Radical scavenging activity – DPPH method

Radical scavenging activity of essential oils was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to methodic **by Sanchéz-Moreno et al., (1998)** with slight modification. The sample (0.1 ml) was mixed with 3.9 ml of DPPH solution (0.025 g DPPH in 100 ml methanol). Absorbance of the reaction mixture was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. The scavenging activity percentage (AA%) was determined according formula:

$AA\% = [(A_0 - A_{AT})/A_0 \times 100]$

where A_0 is absorbance of control reaction (DPPH radical); A_1 is the absorbance in presence of sample

Antimicrobial activity

Microorganisms

Nineteen different kinds of staphylococci were isolated from human semen. The semen samples were obtained from 30 healthy donors by masturbation into sterile container. Only ejaculates showing normal semen parameters were used. The samples underwent liquefaction at 37 °C for 30 min. Experiments were performed within 1 h from sample collection. The samples were inoculated on Tryptone Soya agar (TSA) and Levine agar (LA). After incubation the bacteria were selected for further confirmation with MALDI-TOF MS Biotyper. Isolated staphylococci were tested for antibiotics resistance against tobramycin 10 msg. Following six human sperm isolates were tested: *Staphylococcus aureus* (4 isolates), *S. capiti* (3 isolates), *S. epidermidis* (4 isolates), *S. haemoliticus* (4 isolates), *S. hominis* (4 isolates). The bacteria species were maintained in Mueller Hinton Agar (Merck, Germany). The mother cultures of each staphylococci were set up 24 h before the assays in order to reach the stationary phase of growth. The tests were assessed by inoculating Petri dishes from the mother cultures with proper sterile media. The main aim was to obtain the microorganism concentration of 10^5 colony forming units $cfu.mL^{-1}$.

Antibiotic susceptibility testing

he antibiotic susceptibility test was performed by using Disc Diffusion Method. Four different forms of sensitivity discs with 10 mcg concentrations were used for studying the *in vitro* sensitivity of isolates: tobramycin.(TOB). These discs were obtained from "Oxoid". The results were interpreted according to **EUCAST (2019).**

Disc diffusion method

We used the agar disc diffusion method for the determination of antimicrobial activities of the essential oil. Briefly, a suspension of the tested microorganism (0.1 mL of 10^5 cells per ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 15 µl of the oil and placed on the inoculated plates. They were inoculated onto the surface of Mueller Hinton Agar (MHA, *Oxoid*, Basingstoke, *United Kingdom).* These plates, after remaining at 4 °C for 2 hours, were incubated anaerobically at 37 °C for 24 h. The diameters of the inhibition zones were measured in millimeters. All the tests were performed in duplicate.

Statistical analyses

All measurements and analyses were carried out in triplicate. Experimental data were evaluated by basic statistical variability indicators using the Microsoft™ Excel® program. Dependency rate between the tested traits was expressed using the linear correlation analysis.

RESULTS AND DISCUSSION

Different kinds of essential oils from various plant material (*Citrus paradisi*, *Citrus reticulata*, *Juniperus communis*, *Eucalyptus globulus*, and *Cananga odorata*) were tested. The most intensive peak in *C. paradisi* essential oil was registered at 15.83 min with area percentage of 87 % (Fig. 1). **Okunowo et al. (2013)** identified 19 compounds in *C. paradise* essential oil and the most abundant compound in sample was D-limonene (75.1 %), following *β*-myrcene (7.3 %), *α*-pinene (2.1 %), caryophyllene (1.9 %), octanal (1.7 %), *β*-phellandrene (1.2 %) and decanal (1.1 %).

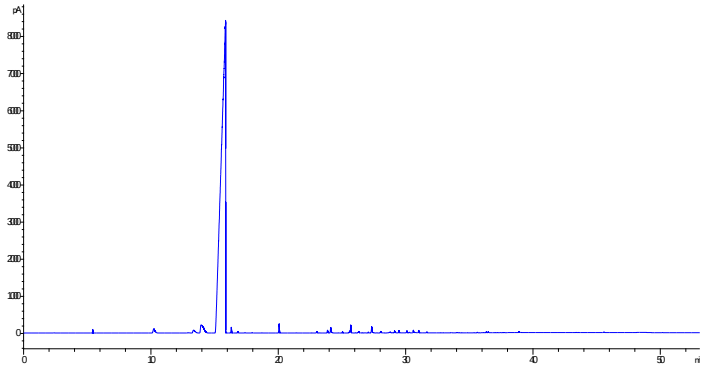

Figure 1 Gas chromatograph trace for *Citrus paradisi* essential oil sample.

The analysis of essential oil from *C. reticulata* showed the dominant peak at 15.89 min. (85 % of area percentage) (Fig. 2). Similar peak was observed at GC of *C. paradisi* essential oil sample (Fig. 1). Limonene (80.3 %), *γ*-terpinene (4.7 %), myrcene (2.1 %), *α*-pinene (1.2 %) and octanal (1.0 %) were identified in *C. reticulata* Blanco (Ponkan) peel oil **(Sawamura et al., 2004).**

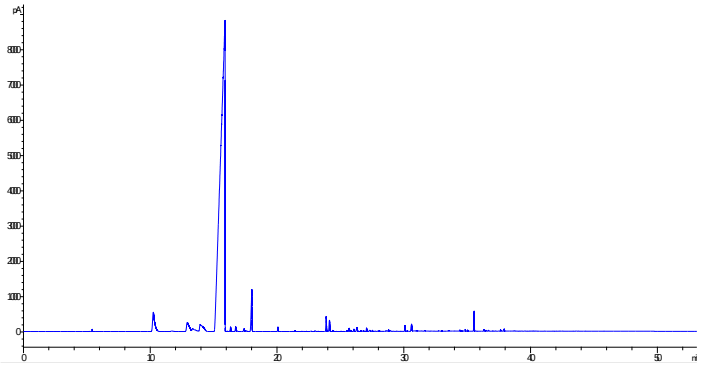

Figure 2 Gas chromatograph trace for *Citrus reticulata* essential oil sample.

The major peaks determined for *J. communis* essential oil sample were identified at 10.75 min (66.6 %), 13.24 min (16.7 %), and 15.3 min (2.9 %) (Fig. 3). **Falasca et al. (2016)** found that the most abundant monoterpene identified in *J. communis* fresh berries was *α*-pinene (13.43-32.34 %), following sesquiterpenes, namely Germacrene D (12.29-20.65%), *β*-Caryophyllene (7.72-11.77%), *γ*-Cadinene (3.86-5.47 %), *α*-humulene (5.21-7.87 %), germacrene B (4.84-9.33 %), and bicyclogermacrene (2.50-4.18 %). **Orav et al. (2010)** found that the

main compounds in essential oil from *J. communis* were α-pinene, germacrene D, (E)-*β*-caryophyllene, and β-myrcene.

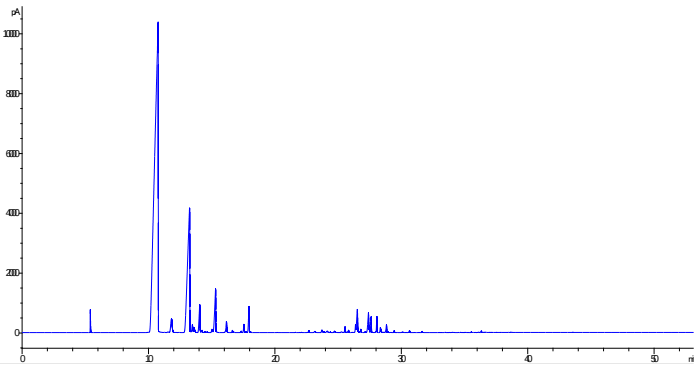

Figure 3 Gas chromatograph trace for *Juniperus communis* essential oil sample.

Essential oil isolated from *E. globulus* leafs showed the dominant peak at 17.95 min (81.7 %) and some minor peaks at 15.5 min (7.3 %) and 18.23 min (6.4 %) (Fig. 4). **Sacchetti et al. (2005)** observed that the major compounds in essential oil from *E. globulus* were 1,8-cineole (52.6 %), *α*-pinene (20.0 %), and *α*-Phellandrene (6.2 %).

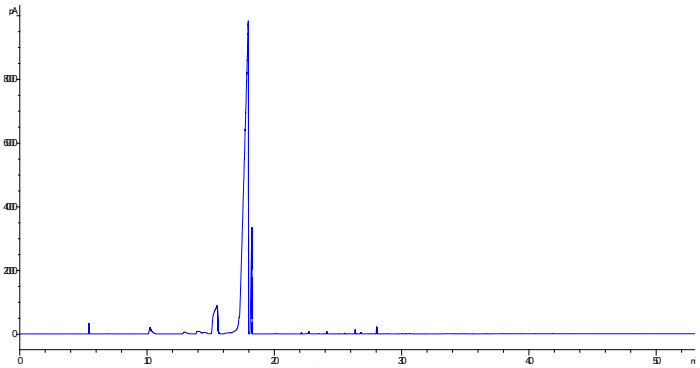

Figure 4 Gas chromatograph trace for *Eucalyptus globulus* essential oil sample.

The last sample of essential oils was isolated from *C. odorata* flower. The gas chromatograph showed the dominant peaks at 24.38 min (22.98 %), 43.92 min (18.28 %), and 30.42 min (7.7 %). Minor peaks were registered at 27.49 min (4.6 %), 29.1 min (4.9 %) and 32.79 min (3.4 %) (Fig. 5). **Sacchetti et al. (2005)** analyzed the chemical composition of essential oil from *C. odorata* and found that the most abundant compounds were benzyl benzoate (33.61 %), linalool (24.5 %), benzyl salicylate (12.89 %), benzyl acetate (9.77 %), and methyl salicylate (2.79 %). The main components of cananga oil were *trans*-carryophyllene (39.03 %), *α*-humulene (11.59 %), *α*- bergamotene (11.29 %) and germacrene (10.94 %) **(Mahfud et al., 2017).**

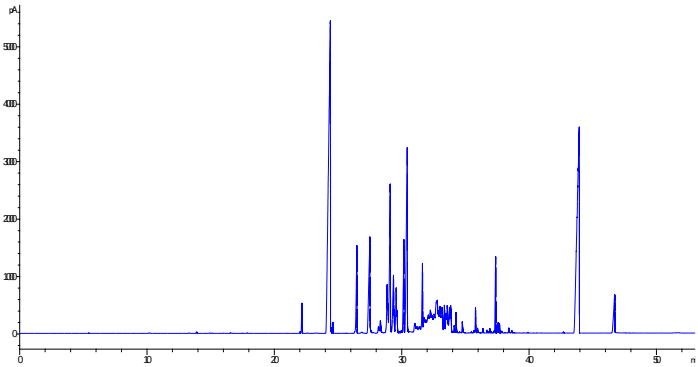

Figure 5 Gas chromatograph trace for *Cananga odorata* essential oil sample.

Misharina and Samusenko (2008) found that essential oils exhibit antioxidant activity that largely depends on their composition in their study on the antioxidant properties of essential oils from lemon, grapefruit, coriander, clove, and their mixtures. Furthermore, **Misharina *et al*. (2011)** found that the antioxidant activity of lemon peel essential oils depends on the system composition and concentrations of essential oils. Individual citral and limonene displayed the lowest antioxidant activity, whereas the activity of their mixture was higher, which explains the synergetic effects in the antioxidant activity of the components. The antioxidant activity of essential oils is revealed in Table 1. In our study the best antioxidant activity varied from 0.997% of *Juniperus communis* to 73.137% of *Cananga odorata*. The essential oil of Citrus paradise showed 58.96% of antioxidant acitivty. *C. grandis* oils and cold-pressed *C. paradisi* oil displayed weak DPPH radicals scavenging capability. DPPH scavenging capacity of cold-pressed *C. paradisi* oil was less than 20% **(Ou et al. 2015).** Distilled *C. paradisi* oil exhibited the potent DPPH scavenging capacity among 4 citrus oils; the EC_{50} value was more than 40 mg/mL. This is consistent with previous studies where 34 kinds of 10 mg/mL citrus oils obtained from Japan and Korea and Italy exhibited weak DPPH radical scavenging effect ranging from 12% to 17.7%. There are studies that reported antioxidant activity of galbuli EO from *J. communis* **(Höferl et al., 2014)** and *J. excelsa* **(Emami et al., 2007).** Our results showed very faint activity of *J. communis*. The DPPH scavenging activity was highest in *E. citriodora* (82.1%), followed by *E. camaldulensis* (81.9%) and *E. microtheca* (81.8%) as compared to positive control BHT **(Ghaffar et al., 2015)**. We found in our study E. *globulus* 31.63 % of antioxidant activity. DPPH assay was used to evaluate the antioxidant property of *C. odorata* extracts to determine the free radical scavenging properties of the extracts. The ethyl acetate extract of the stem bark of *C. odorata* revelled to exhibit the maximum % of DPPH inhibition (79 %) when compared to other investigations **(Kusuma et al., 2014).** Our results showed similar values of the antioxidant activity.

Table 1 Antioxidant activity of essential oils

Essential oil	Antioxidant activity %
Citrus reticulata	**3.40**
Citrus paradisi	**58.96**
Juniperus communis	**1.00**
Eucalyptus globulus	**31.63**
Cananga odorata	**73.14**

Table 2 summarizes the antimicrobial activity of the five EOs studied against 5 isolated *Staphyloccus* species isolated from human semen with MALDI TOF MS Biotyper. Citrus EOs are complex mixtures of different compounds and their antimicrobial activity depends on their chemical composition. Methicillin-resistant *S. aureus* was found to be the most sensitive to the three Eos of *Citrus* sp. because its growth was affected **(Boudries et al., 2017). Martinez *et al.* (2003)** found that mandarin oil (*Citrus reticulata Blanco*) variety Dancy showed an antibacterial activity against *B. subtilis*, *S. aureus* and *L. monocytogenes*. Whereas **Espina *et al*. (2011)** found that the inhibition zones of mandarin EO against *S. aureus*, *E. coli* O157:H7 and *P. aeruginosa* were 18.8, 20.0 (mm) and no inhibition, respectively, which is almost in accordance with our results. In our study the most sensitive *Staphylococcus* was *S. homins* MK-39, resistant to tobramycin against *Citrus reticulata*. Twenty milligram per milliliter cold-pressed *C. paradisi* oil and distilled *C. grandis* oil exhibited very strong inhibitory effects against *S. aureus* (***Ou et al., 2015).*** *The most sensitive* staphylococci *to C. paradisi were S. hominis MK-38 and MK-39 resistant to tobramycin.* ***Zheliazkov et al. (2017)*** *found a strong antimicrobial activity* of *J. communis* EO against *Staphylococcus aureus* subsp. *aureus. The strongest antimicrobial activity of J. communis essential oil was found against S. hominis MK-38.* **Salari et al. (2006)** used *Eucalyptus globulus* leaf extract to evaluate their activity on 56 isolates of *Staphylococcus aureus*. The EOs extracted from all seven *Eucalyptus* spp. showed antibacterial activity against *S. aureus*. The best antimicrobial activity of *E. globulus* in our study were found against *S. aureus* MK-24 and *S. capiti* MK-26 and MK-27. Recent work from Indonesia showed that the stem bark extracts of *C. odorata* exhibited a great antimicrobial property using the agar well disc diffusion assay **(Kusuma et al., 2014).** *C. odorata* showed the best antimicrobial activity against *S. hominis* MK-38 and MK-39.

Table 2 Antimicrobial activity of essential oils in mm

	TOB	*Citrus reticulata*	*Citrus paradisi*	*Juniperus communis*	*Eucalyptus globulus*	*Cananga odorata*
***S. aureus* MK-21**	20	6.33±1.15	4.67±1.15	3.33±1.53	4.67±0.58	11.33±0.58
***S. aureus* MK-22**	15	5.67±0.58	4.33±0.58	3.67±1.53	4.67±1.15	9.67±0.58
***S. aureus* MK-23**	25	4.67±0.58	4.67±0.58	3.33±0.58	5.33±0.58	10.67±0.58
***S. aureus* MK-24**	15	4.33±0.58	4.67±0.58	4.33±0.58	5.67±0.58	11.33±0.58
***S. capiti* MK-25**	30	4.33±0.58	3.67±0.58	3.33±0.58	4.33±0.58	8.33±0.58
***S. capiti* MK-26**	20	4.67±0.58	5.33±1.15	5.67±0.58	5.67±1.15	8.67±1.15
***S. capiti* MK-27**	20	5.67±0.58	4.67±0.58	5.67±1.15	5.67±0.58	9.67±1.53
***S. epidermidis* MK-28**	21	5.33±0.58	2.67±0.58	3.33±1.53	4.33±0.58	9.33±1.15
***S. epidermidis* MK-29**	25	4.67±0.58	2.33±0.58	4.33±0.58	4.67±0.58	9.67±0.58
***S. epidermidis* MK-30**	28	4.67±0.58	3.67±1.53	5.33±0.58	4.67±0.58	8.67±2.08
***S. epidermidis* MK-31**	20	4.67±0.58	2.67±0.58	4.67±0.58	4.67±0.58	7.33±1.15
***S. haemoliticus* MK-32**	20	3.33±0.58	4.67±0.58	3.33±0.58	2.33±0.58	7.67±0.58
***S. haemoliticus* MK-33**	20	3.33±1.53	5.33±0.58	3.67±0.58	2.67±0.58	7.33±0.58
***S. haemoliticus* MK-34**	20	2.67±1.15	4.67±0.58	3.33±0.58	2.67±1.15	7.67±0.58
***S. haemoliticus* MK-35**	25	4.67±1.53	3.67±1.15	3.67±1.15	4.67±1.15	8.67±0.58
***S. hominis* MK-36**	15	7.67±0.58	11.33±1.15	8.33±0.58	4.33±1.15	18.67±1.15
***S. hominis* MK-37**	20	7.33±0.58	11.33±0.58	8.33±0.58	4.67±0.58	18.67±2.08
***S. hominis* MK-38**	20	7.67±0.58	12.67±0.58	9.33±1.15	5.33±0.58	20.33±0.58
***S. hominis* MK-39**	15	8.67±0.58	12.67±2.08	7.67±0.58	4.67±0.58	20.33±0.58

CONCLUSION

In view of their potential as inhibitors of pathogenic microbial growth as well as their antioxidant activity, the *Citrus paradisi* peel oil, *Citrus reticulata* peel oil, *Juniperus communis* fruit oil, *Eucalyptus globulus* leaf oil, and *Cananga odorata* flower essential oils may be recommended for formulation of plant based preservatives for enhancement of shelf life of food items by controlling their losses from bacterial contamination and lipid peroxidation during storage. Additionally, the abundance of *Citrus paradisi* peel and *Citrus reticulata* peel *as* by- products from food industry and *Juniperus communis* fruit, *Eucalyptus globulus* leaf, and *Cananga odorata* flower as renewable raw materials makes the use of essential oils obtained from these plant sources economically adventogenous for practical application, thus they can be considered as potential possible alternatives to synthetic preservatives.

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