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ESSENTIAL OILS FROM *Melaleuca leucadendra* AND *Pogostemon cablin* Benth AS ACTIVE IN HAND SANITIZER WITH ANTIBACTERIAL AND ANTIVIRAL PROPERTIES

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ABSTRACT

In addition to giving a pleasant scent, essential oils in hand sanitizer formulation could contribute to the increase on its efficacy. Herein, we aimed to evaluate the antioxidant, antibacterial, as well as antiviral activities of eucalyptus oil (EO) and patchouli oil (PO), followed by their application in hand sanitizer formulation. Essential oils were obtained from a simple distillation and followed by fractionation using a rotary evaporator. Antioxidant activities of the essential oils were determined based on a DPPH scavenging assay. The antibacterial activities against *Staphylococcus aureus* as well as *Escherichia coli* were evaluated under the Kirby-Bauer method. The antiviral activities of EO and PO were based on the replication inhibition of Simian retrovirus-2 (SRV-2) in an A549 cells model. Our data suggest that the fractionation increases the purity of EO and PO based on the content of 1,8-cineole (from 54.08% to 85.05%) and patchouli alcohol (from 29.28% to 85.01%), respectively. IC_{50} values of fractionated EO and PO in DPPH scavenging activity were 131.21 and 118.20 ppm, categorized as moderately active antioxidants. The optimum inhibition zones against *S. aureus* along with *E. coli* were achieved by fractionated EO (10.49 and 15.79 mm, respectively). SRV-2 replication could be effectively attenuated even until day-7 post-infection. When applied in hand sanitizer formulation (in a small amount), only antibacterial activity against E. coli was found higher as compared with usual hand sanitizer. Moreover, in hand sanitizer formulation, the efficacy of antiviral activity against SRV-2 was reduced to only last for 5 days post-infection. EO and PO can be used as active ingredients in hand sanitizer formulation owing to their antibacterial, antiviral, and antioxidant activities.

Keywords: *Melaleuca Leucadendra*, *Pogostemon Cablin*, Essential Oils, Antibacterial, Simian Virus

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INTRODUCTION

During the early period of the COVID-19 pandemic, hand hygiene was strongly endorsed to prevent viral fomite-mediated transmission.[1] Effective antivirals against the SARS-CoV-2 are mostly being studied[2], preventive measures and vaccination remain the primary modality in combating the pandemic.[3,4] Consequently, the demand for hand sanitizer has increased with producers innovating their products to add essential oils as the fragrance. The addition of essential oils was stipulated to improve the efficacy of hand sanitizer owing to their antimicrobial activities such as those from *Allium rotundum*[5], *Argania spinosa* (L.)[6], and *Pogostemon paniculatus* (Wild.).[7] Essential oils and plant extracts with complex volatiles composed of various phytochemicals compounds namely sesquiterpenes, monoterpenes, and phenylpropanoids have been used for their taste, aroma, medicinal properties, and as bactericides, antiviral, and preservatives.[8,9] Several studies even have reported the improved efficacy of hand sanitizer, following the addition of essential oils.[10,11] Moreover, a study has proven that essential oil could replace the use of alcohol as a

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bactericidal agent.[12] In Indonesia, and several other countries like Brazil, China, and India, essential oils from *Melaleuca leucadendra* and patchouli (*Pogostemon cablin* Benth.) were major commodities.[13,14] Essential oils from *M. leucadendra* (EO) have been reported to exhibit antiviral, anticancer, antibacterial, antifungal, and antimicrobial effects that contain phenolics, terpenoids, alkaloids, and flavonoids.[13] From the medical perspective, EO has been reported effective as a deodorant, diaphoretic, rubefacient, expectorant, and for the treatment of arthritis, febrifuge, asthma, boils, bronchitis, leprosy, fumigant, diabetes, diarrhea, laryngitis, mastitis, malaria, miasma, pharyngitis, phthisis, malignancies, and many others.[13,15,16] Meanwhile, patchouli oil (PO) has a broad range of composition due to its large number of distinct sesquiterpenes, as opposed to a mixture of various sesqui-, mono-, and di-terpene molecules which is beneficial as anti-influenza, neuroprotective, insecticidal, anti-tumorigenic and anti-inflammatory activities.[17,18] Moreover, the addition of EO or PO in a lotion product does not adversely affect its physical properties, suggesting its applicability in hand sanitizer.[19,20] Thus, researching the effect of the foregoing essential oils as active agents in hand sanitizer formulation in terms of preventing bacterial and viral infections is important.

EXPERIMENTAL

Materials

Dimethyl sulfoxide (DMSO); Mueller-Hinton agar (MHA); 2,5-diphenyl-2H-tetrazolium bromide (MTT); and 2,2-diphenyl-1-picrylhydrazyl (DPPH), and methanol were bought from (Selangor, Malaysia). Pharmaceutical-grade chloramphenicol was purchased from PT. Kimia Farma (Jawa Barat, Indonesia). This study prepared essential oils extracted from *M. leucadendra* and *P. cablin* Benth. leaves (EO and PO, respectively). To compare with commercial therapeutic oil, we employed pharmaceutical grade Alkin® procured from Atsiri Research Center (Universitas Syiah Kuala, Banda Aceh, Indonesia), which was a mixture of high purity EO grade 85%, peppermint oil and a small amount of fractioned PO. Otherwise stated, all materials were analytical grade and used without purification.

Study Design

The objective of this study was to compare essential oils from *M. leucadendra* (EO) and *P. cablin* Benth (PO) as active ingredients in hand sanitizer formulation. Both EO and PO were procured from a community-based home industry in Aceh Province, which later was purified using a rotary evaporator with a fractionation method. Products were compared based on their phytochemical profiles, antioxidant activity (DPPH), and antibacterial activities (*Escherichia coli* and *Staphylococcus aureus*). A sample with the best antibacterial activities was tested for its antiviral activities against the replication of simian type D retrovirus, serotype 2 (SRV-2). Furthermore, studies on the use of Alkin® in hand sanitizer formulation were carried out to observe the effect of essential oil addition on bacterial growth and SRV-2 replication.

Plant Specimen and Essential Oils Extraction

M. leucadendra and *P. cablin* Benth leaves were collected from the surrounding area of Banda Aceh, Aceh Province – Indonesia, which were subsequently cleaned using tap water and air-dried (26±1°C). The distillation was carried out based on the method reported previously (>1 atm; 100ºC).[21] The products from this process were labeled as initial *M. leucadendra* oil (iEO) and initial patchouli oil (iPO). Both products were fractionated using rotary evaporator (evaporation temperature=60—180°C; condensation temperature=2—180°C; rotation speed=100—600 rpm), yielding fractionated *M. leucadendra* oil (fEO) and fractionated patchouli oil (fPO). All products (iEO, iPO, fEO, and fPO) were analyzed for their molecular components using gas chromatography-mass spectrometry (GC-MS) (Shimadzu QP2000A, Kyoto, Japan). *M. leucadendra* and *P. cablin* Benth leaves were collected from a local village in Banda Aceh, Indonesia, the leaves were cleaned with tap water and air-dried (26±1°C). The distillation process was carried out using the steam distillation method.[21] The water was heated to boil and the steam with pressure > 1 atm at 100 ºC to obtain rapid extraction time, which was performed in ARC-PUI Nilam Aceh, Universitas Syiah Kuala using one kettle of stainless-steel boiler with a capacity of 50-150 kg/day. The extracted EOs were placed into molecular distillation and fractioned to yield a high purity grade of EOs. Subsequently, the active component of EOs was analyzed using GC-MS.

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Assay

Antioxidant potentials of the prepared essential oils were assessed in vitro based on a DPPH scavenging assay following the suggestion of previous research.[22,23] Concentrations of essential oils were made at 6.25—100 ppm through a dilution with methanol. Each 5 mL diluted essential oil was poured into a DPPH (0.4 mM; 1 mL) pre-added test tube, and subsequently covered with aluminum foil prior to 30-min incubation at 37°C. The absorbance was measured using a UV-vis spectrophotometer (UVmini-1240, Kyoto, Japan) at 517 nm to measure the % inhibition.

Kirby-Bauer Method

The antibacterial activities were investigated based on the Kirby-Bauer method against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) in the MHA medium. The suspensions of *S. aureus* and *P. acnes* were swabbed according to standard 0.5 McFarland. Next, the medium was divided into 3 parts, where onto each part a 5-mm disc containing 20 µL active component was placed. Chloramphenicol was used as the positive control. The diameter of the inhibition zone yielded in each sample was measured following the 24-hour incubation at 37°C.

Anti-SRV-2 Assay

To determine the maximum concentration for the anti-viral assay, we determined the cytotoxicity of the essential oil against A549 cells. Cytotoxicity was measured based on the MTT assay using A549 cell lines following suggestions from previous literature.[24,25] Firstly, A549 cells were cultured on a T25 bottle with subculture plate micro 96 wells and incubated (24 h; 5% CO^2; 37ºC). Subsequently, the 100 µL extract solutions (five different samples and control) with various concentrations (18.75 to 300 ppm) were respectively added into each well and incubated (48 h; 5% CO^2; 37ºC). After incubation, the microplate wells were added with MTT and incubated for another 4 h using similar conditions. Afterward, the supernatant was removed and added with ethanol (purity 96 %) before being analyzed using an ELISA microplate reader at 595 nm. A549 cells were infected with SRV-2 and cultured in 12 wells (10000 cells/wells), subsequently incubated for 24 h (5% CO_2; 37°C). The sample of 500 µL was then added to each well. The observations of SRV-2 replication were carried out twice on day 5 and -7 based on the viral RNA. Extraction of viral RNA was performed using QI-Aamp Viral RNA Mini Kits (Qiagen, Hilden, Germany). The viral RNA was converted into cDNA using a Sensifast cDNA synthesis kit (Bioline, USA) according to the standard protocols. The RNA was replicated using Reverse Transcription Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR) following the guidelines from the manufacturer. In detail, 1 µL with SRV primary 5737 U1 (5'CCAGATGGCTACCAGAACGAT-3') dan 5943 L (5'-AGGGCTTACCGTGTTGTTG-3') with concentration of 10 pmol was mixed with 10 µL SSofast Evagreen Master Mix (Biorad, USA), 5 µL nuclease-free water dan 2 uL cDNA. The RT-qPCR was carried out in thermocycler IQ5 (Biorad, USA) with a temperature cycle of 95ºC for 3 min (pre-denaturation), 95ºC – 10 s (denaturation), 47ºC – 30 s (annealing), and additional increase in temperature of 72ºC for 30 s. The denaturation step was prolonged for cDNA and repeated for 40 cycles. The detection process was amplified during the annealing process. The RT-qPCR result was indicated as cycle threshold (Ct). The standard SRV-2 virus used was 1-10^6 virus replications. The data was analyzed from standard curves by comparing the Ct value and replication logarithmic of the standard virus.

Hand Sanitizer Preparation

The formulation of hand sanitizer was prepared based on the WHO guidelines.[26] Briefly, the ethanol 96% (833 mL) and essential oil – Alkin® (1 mL) were mixed in a volumetric flask until homogenous. Then, H_2O_2 3% (41.7 mL) and glycerin (14.5 mL) were added and mixed with distilled water until they reached 1000 mL and were homogenous. The preparation was carried out at room temperature (26±1°C). For comparison, we also prepare hand sanitizer without the addition of essential oil.

RESULTS AND DISCUSSION

Molecular Components Based on GC-MS

Essential oil components of EO and PO based on the GC-MS investigation have been presented in Table-1. The fractionation effectively increased the purity of both EO and PO. In the case of EO, the eucalyptol or 1,8-cineole was found in a higher percentage (based on the GC spectrogram peak area) from 54.08% to

85.05% after the fractionation. A previous study obtained the highest yield of 85.32% 1,8-cineole from *Eucalyptus cinerea* during the Spring season through a hydrodistillation.[27] However, a much lower percentage of 1,8-cineole was obtained when a soxhlation was employed as the extraction method.[28] In a review, 1,8-cineole was reported to occupy around 60—85% of its essential oil components throughout all species of Eucalyptus plants.[29] Taken altogether, the fractionation using a rotary evaporator could optimize the purification of EO. Prior to fractionation, EO contained other volatile compounds such as β-myrcene, α-terpinene, and α-terpineol, in each of the compounds has been widely used as a pleasant fragrance with multiple health benefits (*i.e.* antioxidant, antimicrobial, antitumor, and having analgesic effects).[30-32] Following the fractionation (fEO), several changes in the composition occurred; β-myrcene appeared higher, α-terpinene – lower, and α-terpineol – none. Apart from the purification, these changes indicate the possibility of compound transformation during the fractionation step.[33] Similarly, the percentage of PO's main essential oil component, patchouli alcohol, was elevated after the fractionation, from 29.28% to 85.01%. In a previous study using the same fractionation technique, the highest percentage of patchouli alcohol (60.66%) could be obtained by using the temperature range of 125—160°C [34]. More peaks were observed in fPO as compared with iPO indicating the increase of compounds detectable by the GC-MS. Interestingly, a compound that is involved in the biosynthesis of patchouli alcohol, patchoulene, was reduced in fPO, which could be attributed to its second transformation.[35] In addition, α-, β-, and δ-guaienes are other components of fPO with relatively high area percentages. Their antibacterial activities against multiple strains of bacteria have been witnessed in published literature.[36] Another study has even specifically reported the antibacterial activities of patchouli leaves-derived α-guaiene against Gram-positive bacteria, namely *Staphylococcus epidermidis* and *Staphylococcus aureus.* [37] Other compounds such as ledene and cubenol have been found in antibacterial active essential oils of several plants.[6,38]

Table-1: Chemical Components of EO before (iEO) and after the Fractionation (fEO)

#Peak	iEO			fEO		
	Ret. (min.)	Compound	Area (%)	Ret. (min.)	Compound	Area (%)
1	4.28	β-Myrcene	0.26	3.87	Methyl ester	0.05
2	5.59	Eucalyptol (1,8-Cineole)	54.08	7.653	α-Thujene	0.24
3	5.86	α-Terpinene	1.13	7.92	α,-Pinene,(-)-	3.43
4	6.41	3-methyl-6-(1)-Cyclohexene	0.19	8.857	Benzaldehyde	0.12
5	8.52	4-methyl-1-(1-3)-Cyclohexen-1-ol	0.37	9.319	Sabinene	0.19
6	9.34	α-Terpineol	20.24	9.517	1-beta-Pinene	2.83
7	12.95	α-Terpinyl acetate	0.9	9.935	β-Myrcene	2.25
8	13.32	Ylangene	0.14	10.518	Pseudolimonene	0.12
9	13.88	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	0.08	10.62	1-Phellandrene	0.09
10				11.051	α-Terpinene	0.45
11				11.371	β-Cymene	0.6
12				11.79	Eucalyptol (1,8-Cineole)	85.05
13				12.792	ɣ-Terpinene	1.01
14				13.987	α-Terpinolene	0.23
15				18.253	Trans.-Sabinene hydrate	0.27
16				18.91	β-Fenchylalcohol	2.56
17				25.683	dl-Limonene	0.12
18				28.824	trans-Caryophyllene	0.26
19				35.503	(+)-Aromadendrene	0.13

Table-2: Chemical Components of PO before (iPO) and after the Fractionation (fPO)

#Peak	iPO			fPO		
	Ret. (min.)	Compound	Area (%)	Ret. (min.)	Compound	Area (%)
1	12.949	β-Patchoulene	2.91	10.348	β-Patchoulene	0.13
2	12.715	(-)- β-Elemene	1.6	12.092	α -Patchoulene	0.38
3	13.52	Trans (β)-Caryophyllene	4.7	12.17	β-Guaiene	0.18
4	14.124	α-Guaiene	16.21	12.76	β-Copaen-4-α-ol	0.44
5	14.292	Seychellene	6.92	12.879	δ -Guaiene	1.03
6	14.603	α-Patchoulene	7.36	12.97	Patchoulene	0.27

7	14.67	γ-Gurjunene	2.68	13.503	Cedren-13-ol acetate	0.39
8	14.996	α-Guaiene	1.22	14.545	Cyclopentan-3`-spirotricyclo	0.16
9	15.31	β-Selinene	0.75	14.727	Spathulenol DB5-1825	0.53
10	15.62	2-isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydro-naphthalene	4.15	15.422	Cedren-13-ol acetate	0.26
11	15.935	δ -Guaiene	19.8	15.66	α -Guaiene	0.88
12	17.134	Cyclohexanone, 2,3,3-trimethyl-2-(3-methyl-1,3-butadienyl)-,(Z)-	1.16	15.921	Cycloheptan, 4-methylen-1-me	0.31
13	17.797	(-)-Caryophyllene oxide	1.25	16.352	δ-Guaiene	3.71
14	20.406	Patchouli alcohol	29.28	16.601	(-)-Sinularene	0.25
15				16.788	Patchouli alcohol	85.01
16				17.238	Ledene	1.95
17				17.305	Cubenol	2.23
18				17.465	1H-Cycloprop[eazulen-4-ol, decahydro-1	1.13
19				17.58	3-bromo-, (3,β)-Cholest-5-ene	0.55
20				18.079	δ -Guaiene	0.23

Antioxidant Activity

Antioxidant activity is one of the essential biological activities in the development of topical products due to the fact that many skin diseases involve oxidative stress dysregulation.[39,40] Additionally, information pertaining to the antioxidant properties could be useful for the development of nanoparticles through the green synthesis route.[41,42] Scavenging activities of EOs and POs, before and after the fractionation, against free radical DPPH have been presented (Table-3). Both fEO and fPO were proven to have higher antioxidant activities as compared with iEO and iPO, respectively. This further suggests the purification and enrichment of active molecules in the essential oils through fractionation that could have antioxidant properties. When compared with ascorbic acid which acted as the positive control, the IC_{50} values of EOs or POs are indeed significantly lower. Their IC_{50}s fall into a concentration range of 101-250 ppm indicating that the prepared essential oils have moderate levels of antioxidants. In the case of EOs, the IC_{50}s before and after the fractionation were 152.95 and 131.21 ppm, respectively. The increase of the antioxidant activity could be associated with the increase of 1,8-cineole content as the result of the fractionation. Essential oil from *Aframomum danielli* that contained 53.44% 1,8-cineole was reported to yield 45.5 ppm IC_{50} in the DPPH assay.[43] In the foregoing study, the value is the highest among other samples that were majorly constituted by β-pinene.[43] In in-vivo studies, 1,8-cineole even has been proven to reduce oxidative stress by regulating signaling pathways along with radical scavenging [15]. However, a study showed that 1,8-cineole had a prooxidant activity to induce apoptosis in human colon cancer cells via oxidative DNA damage.[16] Hence, it is of important to investigate the genotoxicity of 1,8-cineole before using it as an active ingredient in hand sanitizer.[2] Meanwhile, as for POs, the IC_{50}s were obtained to be 132.36–118.20 ppm which is higher in comparison with that of EOs. A study identified 19 compounds in the PO from the leaves of *Pogostemon paniculatus* (Willd.) Benth. (Lamiaceae) reported that its scavenging activity against free radical DPPH could reach 18.5 ppm.[7] In the aforementioned study, despite patchouli alcohol being dominant (30.65%), several other compounds were observed in high percentages including α-guaiene, β-guaiene, caryophyllene, as well as eicosene with percentages of above 5%.[7] Different values obtained between our present study and the cited study could be attributed to the presence of synergistic interaction or inert compounds.[34,44]

Antibacterial Activities

Antibacterial activities of EOs and POs against Gram-positive *S. aureus* and Gram-negative *E. coli* bacteria have been presented (Table-3). Both fEO and fPO possess higher antibacterial properties than those of iEO and iPO, respectively. Moreover, the inhibition zones of iEO and iPO were more than doubled after the fractionation against *E. coli*. Such improvement could be associated with the dominant volatile compounds of EOs and POs which are 1,8-cineole and patchouli alcohol, respectively. A previous report suggested that PO could act as an antibacterial agent against both *E. coli* and *S. aureus*.[45] In addition, PO was also reported to be active against other pathogens *viz. Bacillus proteus*, *Pseudomonas aeruginosa*, *Helicobacter pylori*,

and *Shigella dysenteriae*.[45-47] As for the case of EO, the pure 1,8-cineole at 50 µL concentration could result in inhibition zones of 14.3 and 12.7 mm against *E. coli* as well as *S. aureus*, respectively.[48] However, the inhibition zone acquired therein was lower as compared with essential oils that contained a predominant 1,8-cineole and small amounts of other compounds (*i.e.* β–pinene, β-myrcene, α-phellandrene, and limonene).[48] In a review, 1,8-cineole was suggested as an important modality to treat the early stage of bacterial infectious diseases.[49] The previous explanation indicates that both EOs and POs could be potentially used as active ingredients to improve the sterilizing ability of hand sanitizer.

Table-3: Antibacterial and Antioxidant Activities of EOs and POs

Sample	Inhibition zone (mm)		DPPH antioxidant activity, IC_{50} (ppm)
	Escherichia coli	*Staphylococcus aureus*	
iEO	7.67	7.6	152.95
fEO	15.79	10.49	131.21
iPO	7.36	6.78	132.36
fPO	16.63	9.32	118.20
Chloramphenicol (1000 ppm)	30.45	25.50	-
DMSO (20%)	6.01	6.01	-
Ascorbic acid	-	-	4.07

Antiviral Activity

For the assessment of antiviral activity, we selected fEO because of its high inhibition zone against both *E. coli* along *S. aureus*. A concentration of 300 ppm for fEO was set as the maximum concentration range because it yielded <50% inhibition of normal A549 cells. The antiviral activity of fEO was assessed based on its ability to inhibit the replication of SRV on days-5 and -7 post-infection, where the results have been presented (Fig.-3). As expected, the highest inhibition of viral replication was reached when the concentration of fEO was 300 ppm (the viral replication was only 2,331). The lowest antiviral activities observed on days-5 and -7 were achieved when the concentration was 75 and 18.75 ppm, respectively. The ratio of fEO and solvent determines the antiviral activity as it affects the availability and diffusion rate of the active ingredient. Previous investigation suggested 1,8-cineol could upregulate interferon regulatory factor 3 (IRF3) which holds a significant role in innate immune response.[50] Not only work in inhibiting viral replication, 1,8-cineol was also found to protect against pneumonia that subsequently occurs during influenza infection.[51] Therefore, fEO has the potential to be used as an active ingredient that can work against bacteria (*E. coli* and *S. aureus*) and viruses (SRV).

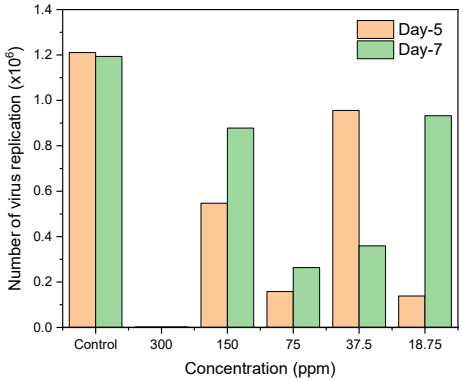

Fig.-3: Antiviral Activities of fEO against SRV-2

EO-Containing Hand Sanitizer

When applied as an active ingredient in a hand sanitizer, the efficacy of the essential oil could be reduced because of a dilution factor by other ingredients. Indeed, usual hand sanitizer uses ethanol as its biocidal ingredient against microbes. However, its efficacy in inhibiting the growth of bacteria following hours post-application may not persist for a long time. Thus, we hypothesized that the addition of EO as an active ingredient to hand sanitizer could enhance its efficacy in preventing microbial growth after its usage. We have performed an investigation of the *M. leucadendra* oil-based hand sanitizer formulation, where we used

commercial *M. leucadendra* oil – Alkin® containing 85% fEO, peppermint oil, and a small amount of fPO. The results of this investigation have been presented in Fig.-4. Based on the antibacterial activities, EO-containing hand sanitizer has a higher efficacy than common hand sanitizer in inhibiting the growth of *E. coli*, but not in the case of *S. aureus*. Antiviral activity was dramatically different as compared with the control, either in hand sanitizer or EO-containing hand sanitizer, when the concentrations were 300 or 75 ppm. Other than the foregoing concentrations, the antiviral activities were found most potent on day 5, but the efficacy was lost after 7 days of exposure.

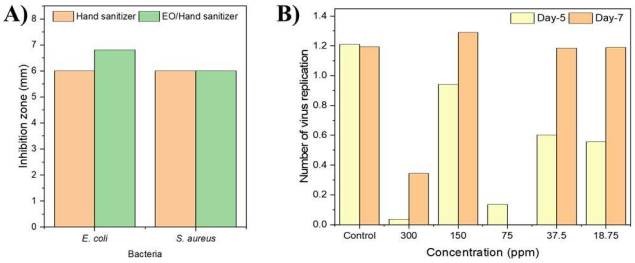

Fig.-4: (A) Antibacterial Activities of Hand Sanitizer and EO-Containing Hand Sanitizer Against *E. coli* and *S. aureus*. (B) Antiviral Activity of EO-Containing Hand Sanitizer Against SRV-2

Several reported studies have suggested that the addition of essential oil could enhance the antimicrobial activities of hand sanitizer. A study using *E. coli* and *S. aureus* models reported that cinnamon oil and lime could improve the efficacy of ethanol-based hand sanitizer.[11] Essential oil from *Piper betle* has also been studied as an active ingredient in hand sanitizer formulation, where the results showed its efficacy in inhibiting *E. coli* and *S. aureus*.[10] Furthermore, a study using clove oil (1.5 and 2.5%), lavender oil (2.5%), and tea tree oil (2.5%) has concluded that the essential oils could replace the ethanol in hand sanitizer formulation with even higher efficacy, even against *Candida albicans*.[12] As a strength of our study, we have studied the antiviral benefit of the essential oils along with the modified hand sanitizer. Nonetheless, several limitations of our study are present, such as we did not determine the bactericidal and virucidal effects of either the essential oils or the hand sanitizer formulation. Regarding the antiviral activity, we did not compare the EO-containing hand sanitizer with usual hand sanitizer. The limitation also includes our inability to investigate the antiviral benefits of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) which have been highlighted in many research due to the ongoing pandemic.[52,53]

CONCLUSION

Essential oils from *M. leucadendra* and *P. cablin* Benth. have moderate antioxidant activities proven by the DPPH scavenging assay. Both of the prepared essential oils could inhibit the growth of *E. coli* (Gram-negative) and *S. aureus* (Gram-positive). The antioxidant and antibacterial benefits of both essential oils were improved as a result of the fractionation process. At low concentrations (<100 ppm), EOs and POs do not affect the viability of A549 cells. Replication of SRV-2 could be inhibited by fEO even until seven days post-infection. The addition of EO into hand sanitizer formulation could enhance its antibacterial activity against *E. coli*, but not *S. aureus*. The antiviral activity was observable when EO was incorporated into the hand sanitizer formulation, especially on day 5 post-infection. Hence, the essential oil could be used as antibacterial and antiviral agents in hand sanitizer. However, due to the different abilities of bacteria and viruses in mitigating toxic agents from the environment, studies using more diverse microorganisms are warranted.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved

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