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RESEARCH ARTICLE

Hand sanitiser activity test of eucalyptus (*Eucalyptus globulus*) oil extract against *Bacillus subtilis* and *Enterococcus faecalis* bacteria

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Abstract

Background: *Bacillus subtilis* bacteria can cause meningitis, endocarditis, and eye infections, while *Enterococcus* can cause meningitis in neonates and endocarditis in adults. Therefore, it is important to use hand sanitiser to avoid bacterial infections. Eucalyptus is an essential oil-producing plant that can inhibit growth and kill bacteria with its compounds, namely linalool and pinocarveol. **Purpose:** To make a good eucalyptus oil hand sanitiser and test its effectiveness against *Bacillus subtilis* and *Enterococcus faecalis* bacteria. **Method:** Making transparent hand sanitiser with a mixture of eucalyptus oil extract. Physical, chemical and antibacterial tests on *Bacillus subtilis* and *Enterococcus faecalis*. **Results:** The hand sanitiser preparation obtained an acidity of 7, with a specific gravity of 0.8512 g/ml, and a viscosity of 4.14 cP and the cleaning power of *Bacillus subtilis* and *Enterococcus faecalis* was 99.9%. **Conclusion:** Eucalyptus oil extract hand sanitiser is of good quality and effectively kills *Bacillus subtilis* and *Enterococcus faecalis* bacteria.

Introduction

Dirty skin can easily become infected if not cleaned. Hand sanitisers, which are antibacterial preparations, are believed to be able to prevent diseases caused by bacteria, in addition to cleaning the skin (Asri *et al.*, 2016). Many microorganisms, such as bacteria and fungi, are attached to hands every day through physical contact and can cause various diseases. The easiest and most appropriate way to prevent the spread of microorganisms and keep the body from getting sick is to wash your hands with soap and running water. If there is no water, hand sanitiser can also be used (Mahary & AGS, 2021).

Water does not contain antibacterial substances that will infiltrate bacteria found in parts of the human body, such as the skin, so hand washing is not always effective for removing microorganisms (Rini & Nugraheni, 2018). The presence of microorganisms

such as *Escherichia coli* in the environment causes unfavourable consequences to health (Mariana *et al.*, 2017). Most diseases are caused by bacteria (Baumgartner, 2004), and more than 700 species are found in the oral cavity. 90% of those found in root canals are anaerobic bacteria (Ferreira *et al.*, 2002). Many microbes, such as *Enterococcus faecalis* (*E. faecalis*), and others, cause root canal infections (Soraya *et al.*, 2018).

One of the various bacteria that is often found in persistent endodontic infections is *Enterococcus faecalis*. Their ability to form biofilms is a virulence factor that can increase bacterial resistance (Kishen *et al.*, 2006). Bacteria that live as parasites are very harmful, especially those that are pathogenic to humans. Several pathogenic bacteria that are often found in the human body, usually in the digestive tract (intestines) and excretions, are *Bacillus subtilis* (Nopiyanti & Agustriani, 2016).

Eucalyptus essential oil is produced from the distillation process of leaves, flowers, and bark of *Eucalyptus sp* (Astiani *et al.*, 2014). This oil is reported to have antibacterial properties that can inhibit the growth of bacteria. Five main components of Eucalyptus species, including *E. globulus* Labillardiere Indonesia, have been identified through gas chromatography–mass spectrometry (GC-MS) analysis, namely 1,8-cineol (11.49%), terpene acetate (6.84%), pinene (4.48%), and spathulenol (4.15%) (Santoso *et al.*, 2002). Its essential oil can excellently inhibit bacterial growth (Ghalem & Mohamed, 2008).

Alcohol is a synthetic substance that keeps microorganisms from replicating on the surface of the body, by killing or inhibiting the growth of their metabolic action. Hand sanitisers can reduce bacterial exposure to human skin (Kampf & Ostermeyer, 2004). Alcohol is a denatured protein and lipid solvent that can damage cell membranes and confer antimicrobial abilities. The optimal concentration of alcohol is at a concentration of 70-80% (Pratiwi, 2008), but 50-70% is widely used as an antiseptic. Therefore, for convenience when cleaning hands and helping to prevent transmission of diseases caused by bacteria, hand sanitisers are made with the addition of the natural ingredient Eucalyptus oil which has antibacterial activity.

Methods

This experimental research is mostly done through manipulation to find out the effect of the observed object (Sawitri, 2010). Laboratory experiments are studies that test the variance of all or almost all the independent variables that may have an effect, while those that are irrelevant are kept to a minimum. This process manipulates one or more independent variables in any given situation (Kerlinger, 1973).

This research used an experimental method to determine the germicidal power of hand sanitiser preparations with eucalyptus oil extract against *Bacillus subtilis* and *Enterococcus faecalis* using the Association of Official Analytical Chemists (AOAC) method with an exposure time of 60 seconds. It was carried out at the Pharmacy and Food Analysis Laboratory, Health Polytechnic, Health Polytechnic, Ministry of Health, Malang and an accredited test laboratory from March to June 2022. The independent and dependent variables used were the hand sanitiser formula with eucalyptus oil extract and total germicidal power.

Tool

The tools used were: an analytical balance ABS 220-4

(Kern & Sohn GmbH, Balingen, Germany), autoclave ss Xfs-280a (Zhejiang FUXIA Medical Technology Co., Ltd., Zhejiang, China), oven series (BINDER GmbH Tuttlingen, Germany), incubator IP55 (Mettler GmbH, Schwabach, Germany), laminar airflow (Mascotte Iv-s), colony star p/n 8500 (Funke Gerber, Germany), vortex mixer VM-300 (Gemmy Industrial Corporation, Taipei, Taiwan), Erlenmeyer Pyrex 200 ml, Erlenmeyer Pyrex 250 ml, measuring cup Pyrex 100:1 ml (Pyrex Merck KGaA, Darmstadt, Germany), Iwaki beaker glass, 10 ml Iwaki measuring cup, 5 ml Iwaki measuring pipette, 0.5 ml Iwaki measuring pipette (PT. Iwaki Glass Indonesia, Sumedang, Indonesia), cotton (OneMed, Sidoarjo, Indonesia), and aluminium foil (Best fresh).

Ingredients

The materials used were nutrient agar NA (OXOID, UK), NaOH Merck, PO4 buffer Merck, 0.9% NaCl (Merck KGaA, Darmstadt, Germany), aqua dest (Aquistill), alcohol (one med, sidoarjo, Indonesia), eucalyptus oil extract, cultured *Bacillus subtilis*, and *Enterococcus faecalis*.

Formula

The hand sanitiser preparation formula with eucalyptus oil extract

Table I shows the comparison of standard hand sanitiser formulas issued by WHO and modified for a total volume of one litre:

Table I: Comparison of WHO formulation and modified formulation of liquid hand sanitiser preparations with eucalyptus oil extract

Formula (ml)		
Ingredient	X [†]	Y [‡]
Ethanol 96%	833.3	833.3
Hydrogen peroxide 3%	41.7	0
Glycerin 98%	14.5	0
Eucalyptus oil	0	41.7
Aquades added up	1000	1000

[†]Formula X. WHO standard formula (World Health Organisation, 2009).

[‡]Formula Y. Modified hand sanitiser formula

Manufacturing method

Preparation of hand sanitiser with eucalyptus oil extract:

Enter the ethanol into the measuring cup according to the measure in the formula and pour it into the 1000 ml glass beaker. Measure the available eucalyptus oil extract according to the dose in a separate measuring cup. Put the eucalyptus oil extract into the beaker that already contains ethanol, which has been measured

according to the formula. Furthermore, add Aqua Dest distilled water, stir until homogeneous and a liquid hand sanitiser preparation is formed. The mixture is poured into a tight bottle to avoid easy evaporation.

Test

pH measurement

The pH meter or hydrogen potential is a standard for measuring the level of acidity or alkalinity of a hand sanitiser preparation with a scale of 0-14. pH meter measurements using a digital pH meter tool brand Thermo Scientific Eutech model pH 2700.

Specific weight determination

Specific gravity is determined using a clean and dry pycnometer at room temperature, the same as the sample and water comparator. Specific gravity is also used to determine the purity of a substance by calculating its specific gravity and then comparing it with existing theory, if the density is close, it can be said that the substance has high purity.

Viscosity test

Viscosity is a statement of a liquid's resistance to flow. The higher the viscosity, the greater the resistance. This test uses a Brookfield Viscometer (Martin *et al.*, 1993). To measure the viscosity of preparation in a Brookfield viscometer, the material must be stationary in the container while the shaft moves during immersion in the liquid (Nurdianti, 2020). Using a Brookfield Digital Viscometer Model LVDV-E, Middleboro, US. Spindle number 1 with a speed of 60 rpm and at a room temperature of 25°C.

Activity test method with AOAC 960.09

The AOAC method is used to determine the effectiveness of antimicrobials with plate count techniques as well as per cent and log reduction analysis. After preparing the bacterial culture, a sufficient number of test samples for testing activities are placed in the sterile Petri dishes. Several bacterial cultures were tested, inoculated into the previous petri dish, and then immediately stirred. After a predetermined contact time, a small amount of the bacteria and test sample mixture was taken and placed in the dish filled with nutrient agar, incubated, and the log reduction was calculated using the formula (AOAC, 2013).

Results

The results of this research conducted on hand sanitiser showed that eucalyptus oil extract is in liquid form and clear in colour with a distinctive aroma of eucalyptus oil. Furthermore, the hand sanitiser was tested for pH, specific gravity, viscosity, and effectiveness against *Bacillus subtilis* and *Enterococcus faecalis*, and the results are shown in Table II below:

Table II: Test results

Test parameters	Results
pH	7
Specific gravity	0.8512 g/ml
Viscosity	4.14 cP
Germ killing power	99.9%

These results showed that eucalyptus oil extract has a good germicidal effect against *Bacillus subtilis* and *Enterococcus faecalis*. Furthermore, its hand sanitiser has a germicidal effect on the two tested bacteria, with a germ-killing value of 99.9%. It also has a pH value of 7, which was declared neutral. The viscosity of the hand sanitiser with eucalyptus oil extract had a value of 4.14 cP. In examining the specific gravity of the hand sanitiser, the eucalyptus oil extract was found to be 0.8512 g/ml, generally 1 g/ml.

Discussion

Based on the results of this research, the hand sanitiser from the organoleptic side obtained a liquid form with a distinctive aroma of eucalyptus oil and transparent white colour. The pH was determined by measuring the activity of hydrogen ions through or electrometrically using a pH meter. Catalyst ions can affect the stability of the water molecule, which splits into H⁺ and OH⁻ ions which are easier to electrolyse due to a decrease in activation energy. Meanwhile, pH is the degree of acidity used to communicate the level of alkalinity of a solution. The pH scale is not direct or definite, and it is comparable to the standard set controlled by international conventions. The estimation of pH is necessary for a field identified through life or the business of dealing with compounds, such as science, biology, and medicine. It is also used by various fields of science and innovation but less significantly. One of the several ways of measuring pH is the use of a pH meter. The eucalyptus oil extract has a pH value of 7, which was declared neutral.

The specific gravity of a material or substance is the ratio of its weight to water/liquid in volume at the appropriate room temperature. The hand sanitiser test used in this evaluation was weighed at room temperature, and the result was 0.8512 g/ml. This is still said to be in accordance with the density of the liquid, which is 1 g/ml.

The hand sanitiser test was carried out to determine the impact of its microbial killing power on *Bacillus subtilis* and *Enterococcus faecalis*, and the time needed to kill the tested microorganism was 60 seconds. According to the United States Food and Drug Administration (US Food and Drug Administration), hand sanitisers can quickly reduce the number of microorganisms in up to 60 seconds. In addition, the number of microbes on hands changes depending on several factors, particularly the last time they were washed, which will affect the local area of bacteria. The addition of eucalyptus oil extract plays a role in helping the effectiveness of cleaning preparations (Ministry of Health of the Republic of Indonesia, 2017).

The germicidal power test on hand sanitisers showed a germicidal effect on *Bacillus subtilis* and *Enterococcus faecalis*, with a value of 99.9% at 60 seconds of contact time. It is a necessary requirement that good germ-killing power is above 90% at contact time or exposure of 60 seconds.

Hand sanitisers can overcome barriers to hygiene and compliance because they require less time to wash hands effectively, are less damaging to the skin than soap and water, and are more effective at killing many microorganisms. In the formulation for making hand sanitiser, the type of alcohol used was either ethanol, isopropanol or n-propanol, or a combination of both.

Conclusion

In conclusion, the preparation of hand sanitiser with eucalyptus oil extract has good quality in terms of pH, specific gravity, viscosity, and killing effectiveness against *Bacillus subtilis* and *Enterococcus faecalis*. Moreover, it can kill *Bacillus subtilis* and *Enterococcus faecalis* by reducing the number of colonies from the active ingredient. The percentage of germicidal power against the bacteria is 99.9%. Further research on the germ-killing power of hand sanitisers with natural ingredients extracts against different bacteria is recommended to determine their quality and effectiveness.

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Conflict of Interest

The authors declared no conflict of interest.

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