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The Effect of Basil (*Ocimum X Africanum L.)* Extract on The Growth of Microbes in the Hand

Siti Aminah[a], Sri Wantini[b], [a,b]Department of Health Analyst, Poltekkes Kemenkes Tanjung Karang, Sumatera, Indonesia, Email: [a]aminahkurun.ak@gmail.com, [b]sriwantini2101@gmail.com

Basil leaves (*Ocimum x africanum L.*) contain essential oils that contain antibacterial properties (Sharma, 2003, in Parag et al., 2010). In addition to essential oil, basil leaves also contain antibacterial flavonoids. Flavonoids may hinder nucleic acid synthesis, hinder the purpose of the cytoplasmic membrane, and hinder cellular energy metabolism. Many hand sanitiser products to clean hands, to avoid the entry of germs into the body, are made from chemicals, but still very few hand sanitiser products use natural ingredients. The goal of this study is to rate the ability of basil extract (*Ocimum x africanum.L*) to kill microbes on the hands. The research was established in the laboratory of the Microbiology Department of Health Analysis from April to October 2017. The quantitative research type, experimental study designs, inhibition test method, Kirby Bauer disk diffusion method, extracts of basil (Ocimum x africanum.L) concentration of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% to 100% were used with 3 repetitions. Data was analysed by Anova (analysis of variance) at a level of 95%. Results showed the mean inhibition zone formed at concentrations of 10 to 100% from 6.33 to 14.44 mm, the effective concentration of 50% obtained in killing microbes on the hands.

Key words: *Extracts Of Basil (Ocimum x Africanum .L), Microbes On The Hands.*

Introduction

Basil (*Ocimum x africanum*) is the largest species of basil throughout the world, whether in the form of fresh or for the production of essential oils. Among the genus *Ocimum L.*, basil is one of the species of interest because of the aroma and taste. Asians use this herb as medicine and food

ingredients from generation to generation. These plant oils are also used widely in the pharmaceutical industry and the perfume industry (Kicel, 2005).

Basil leaves (*Ocimum x africanum L.*) contain antibacterial essential oils (Sharma, 2003, in Parag et al., 2010). In addition to essential oil, basil leaves also contain antibacterial flavonoids. Flavonoids may hinder nucleic acid synthesis, hinder the function of the cytoplasmic membrane, and hinder cell energy metabolism (Sumarni, Sabang, and Ratman, 2018).

Benefits of the basil plant, among others, are: quickly eliminating odours on the palm and fingers of the hand when compared with a handwashing soap, as a skin fungus cure (Surahmaida & Umarudin, 2019). Basil plant parts are leaves, flowers, stems, and roots. Seeds are known to have therapeutic potential and have been utilized as: expectorant, anti-diabetic, analgesic, anti-cancer, anti-fertility, anti-asthmatic, and anti-stress. Triphala along with juice of basil leaves is used in eye drops and is suggested for glaucoma, chronic conjunctivitis, eye diseases, and cataracts. The juice of fresh leaves is also given to patients to treat dysentery, chronic fever, dyspepsia, and bleeding. Basil leaves can also reduce vomiting as a prophylactic against malaria (Dadang and Prijono, 2008).

To have a high health status requires the achievement of certain health level marked by the inhabitants who live in a healthy environment, practising clean and healthy behaviours. Clean and healthy living behaviours include always washing hands before and after doing certain activities, to avoid the germs that enter through the hands. Washing hands with soap is one of the actions of sanitation; hands and fingers cleaned with soap and water breaks the chain of germs. Washing hands with soap is one of the most effective ways to preclude diarrhoeal diseases and respiratory infections, which are both a major cause of death of children. Every year, 3.5 million children worldwide die before reaching the age of five due to diarrhoeal disease and acute respiratory infection. Washing hands with soap can also preclude worms that live in the gut, skin and eye infections, SARS, and avian influenza (Ministry of Health RI, 2014).

The types of bacteria found in the hands are *Bacillus cereus, Serratia liquefacients, Serratia marcescens, Staphylococcus saprophyticus, Staphylococcus aureus, Staphylococcus epidermidis, Enterobacter aerogenes, Citrobacter freundii, Salmonella sp, Pseudomonas aeruginosa, Neisserriamucosa* (Pratami, 2013). Today many soap products and kinds of hand cleaners (hand sanitisers) are offered to wash hands to avoid the entry of germs into the human body. Research on the hand cleaning products has been widely carried out including research results Fitr L. (2010), that an antiseptic soap rod with a concentration of 50% can hinder the growth of Staphylococcus aureus and Escherichia coli, which is equivalent to 2% phenol ability; another study by Aminah

(2015) showed concentration 80% unlisted antiseptic liquid soap BPOM has effectively killed *E. coli* in the palm.

Another study by Aminah (2016) states 10% concentration of antiseptic hand sanitiser liquid in soap form is not capable of killing microbes on the hands, and three times did not effectively clean bacteria on the hands when compared to using antiseptic hand sanitiser gel.

Testing of basil leaf extract through research has been carried out among others by Rahmawati (2010), finding a basil leaf extract at a concentration of 80% has the highest inhibitory effects on *Staphylococcus aureus*. At a concentration of 100%, experience found inhibition zones are almost indistinguishable at concentrations of 20% to 100%. Another study by Novitasari (2014), stated the essential oil of basil leaves has an detrrent effect on the growth of *Staphylococcus aureus.* Research by Muthmainnah, et al (2014), about the basil oil as an anti-bacterial and testing of the *Staphylococcus aureus* producing the highest activity was shown by basil oil 0.75 mL in 10 mL liquid soap, which with an average diameter of 9.8 mm inhibition.

Another research by Susanto, et al (2012) on the effects of basil essential oil as an agent for inhibiting formation of *Streptococcus mutans* biofilm shows the results of the concentration of essential oil of basil 0.0625%, 0.125%, 0.25%, 0.5%, 1% can be inhibiting *S. mutans* biofilm of 4.451%, 40.121%, 80.416%, 88.586%, and 94.812%.

Based on this, the basil plant is beneficial and faster, among other things, in eliminating odours on the palm and fingers. *O. × africanum Lour*. is also known as lemon basil (English), basil (Indonesia), Camangi (Makassar), serawung (Sunda), lufe-lufe (Ternate), and kelampes (Central Java) (Mead 2014).

Species *O. × africanum Lour*. is the result of natural hybridisation between *O. basilicum L.* and *O. americanum L.* originating from Thailand; species *O. × africanum Lour.* is more similar to *O. americanum L.* (Paton and Putievsky 2014). The characteristic species of *O. × africanum Lour*. which is a typical aromatic herb with a strong lemon scent, and some hybrids smell of anise (Paton and Putievsky, 2014). The annual, sometimes woody herb, 20-70 cm tall, square stems and many branches, single leaf sitting opposite alternate, petiole 3-25 mm long, leaf shape from rounded-elongated, size of 2.5-5 cm x 1-2.5 cm, pinnate leaf bone, pointed leaf tip, leaf edge sawing; inflorescence carriage with a length up to 15 cm, elongated protective leaf with a length of 2-3 mm, green-purple petals 4-5 mm long with a crown of white-pink coloured 4-7 mm long, the male genitals and female genitalia are in one flower, pollen stem length of 1-3 mm. Fruit-shaped box, each fruit contains 4 seeds, bean-shaped ellipsoid colored dark brown to black, measuring 1.9 mm × 1.0 mm (Figure 1) (Paton et al. 1999; Simon et al., 1999; Patel et al. 2015).

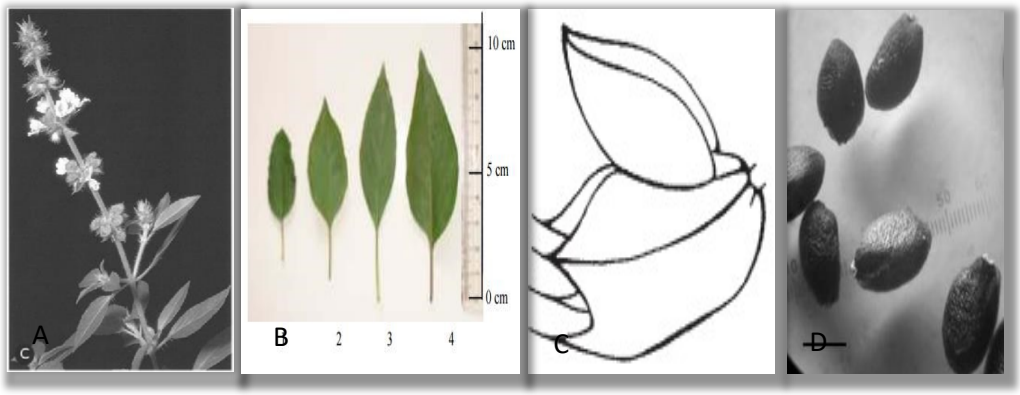

Figure 1. Morphology of *Ocimum x africanum L.*

Ocimum × *africanum Lour* is propagated by seeds obtained from ripe fruit. Seed traits that can be used for the propagation of plants are: black, and dry (Patel, et al., 2015). In normal circumstances, these plants germinate 1-2 weeks and grow until reaching a height of 1 m. This species is easily cultivated, tolerant of hot and cold weather, and can be grown in the highlands at an altitude of 500-2000 m above sea level. It grows in habitats with nutrient-poor soil and likes damp (Paton et al. 1999).

Some studies reveal that *O.* × *africanum Lour*. contains bioactive compounds such as eucalyptol, linalool, camphor, estragol, eugenol, methyl (E)-Cinnamate, caryophyllene, α-bergamotene, β-bisabolene, αfarnesene, sphatulenol (Vieira and Simon 2006), navadensin, salvigenin, cirsimaritin (Vieira et al. 2003), estragol, geranial and neral (Stanko et al. 2011; Santos et al. 2015).

Microscopic *Ocimum x africanum*

1. Leaf size (cm) 0.5 to 3.5 x 0.5 to 2.0
2. Lemon aroma
3. Long petals (mm) 4 to 5.5
4. Pale purple colour, white crown
5. Crown length (mm) 4-6
6. Inflorescence type of carriage (Verticillaster)
7. Long grain size (mm) 1.9

Chemical Ingredients

Anthocyanins – derived from the word *anthos* (flowers) and *Kyanos* (dark blue) – are types of secondary metabolites that belong to the flavonoid compounds that are produced in plants. Flavonoid effect nerve cells through selective actions at different points by giving a signal to the cell that acts to protect the brain from damage (Denny and Buttriss, 2005).

Anthocyanins in plants can be identified by the appearance of pigments of red, purple, blue, and orange-reddish in leaf cells. Colour appearance caused by the effect of anthocyanins depends on the chemical nature and concentration, pH vacuoles, and interaction with other pigments. Colouration by anthocyanin can be found in vegetables, fruits, flowers, and cereal grains (Konczak and Zhang 2004).

Anthocyanin contained in the plant has long been utilized as traditional medicine (Horbowicz et al., 2008) because it can serve as an antibiotic, antifungal, hepatoprotective, antimicrobial and anti-inflammatory (Nyarko et al. 2002; Bassole et al., 2005; Euloge et al. 2012; Kumari and Jain 2012). In the countries of North America, Europe, and China, plants containing anthocyanins have also been used as a remedy for various diseases, especially heart disease, some types of cancer, fever, liver disorders, diarrhoea, kidney stones, diabetes, urinary tract infections, to increase visual acuity, and improve blood circulation (Konczak and Zhang, 2004). Also, plants having anthocyanin can also be used as natural dyes in the food industry (Konczak and Zhang, 2004).

The species of *Ocimum sp*. is a plant in which is scattered a wide range of bioactive compounds, one of which is the compound anthocyanin. Anthocyanins can only be found in the leaves of purple Ocimum. *Ocimum anthocyanin* in the leaves, which are located on the leaf epidermis, serves to absorb some of the light energy when the leaf exhibits photosynthetic active radiation. *Ocimum* utilises its leaves as a defence when light intensity is above the optimal range. The content of anthocyanin in the leaves is not affected by the amount of nitrogen fertiliser when growing and at harvesting time (Politycka and Golcz, 2004).

Tannins are phenolic derived flavonoid compounds called empire and gallotannin acid: they have binding function, precipitate, shrinking protein, used as pathogen defence and determine the nutritional value of a plant. Tannin is widespread in plants: it can be found in the young tissue, leaves, fruits, stems, and roots. Plants that contain tannins will taste astringent when consumed and cause a dry feeling in the mouth. Tannins can be seen on decaying vegetation and soil surface that has a pool of water – the effects of tannins on water show as yellow, blackish green, to black (Ashok and Upadhyaya, 2012).

Essential oils of basil leaf are effective antibacterially against *Staphylococcus aureus* with a minimum deterrent concentration (MIC) of 0.5% v. The use of essential oils is thought to be less acceptable, so it needs to be formulated in a gel form. This research aimed to decide the effect of essential oils of basil leaves on the physical properties of gel preparation and its activity against S. aureus. Basil essential oil acquired by steam distillation method and water. Gel made in four formulas with carbopol base. Control formula without the addition of essential oils, the formula 1-3 with a concentration of essential oils 2g/ 102g, 4g / 104g, and 6g / 106g. Evaluation gel formulation includes pH test, organoleptic test, homogeneity, test the effect of storage on evaporation, dispersive test power, viscosity test, antibacterial activity test, and hedonic test. The higher the concentration of essential oils in the gel viscosity and pH decreases, the power spread, the antibacterial activity is increasing. At a concentration of essential oils 2g / 102g obtained radical zone of 9.21 ± 2.09 mm and at a concentration of 6g / 106g got a radical zone of 10.51 ± 0.89 mm (Novitasari, et al 2014).

Basil (*Ocimum x africanum L.)* contains saponins, flavonoids, polyphenols and tannins that reportedly could inhibit bacterial growth so that basil leaf extract is indicated to have antimicrobial power. This research goals to decide whether or not the hindrance of extracts of basil (*Ocimum x africanum L.*) on the growth of *Staphylococcus aureus* and *Escherichia coli* are research laboratory experimental research with the research subject is an extract of basil (*Ocimum x africanum L.*) Bacteria used *Staphylococcus aureus* ATCC 6538 and Escherichia coli ATCC 11229. The method is using the Kirby Bauer disk Oxoid. *Staphylococcus aureus* and *Escherichia coli*, respectively BAP medium (Blood To Plate) and Mc. Conkey 24-hour-old standardised 0.5 standard Mac. Farland, then applied using a sterile cotton stick on Muller Hinton media. Oxoid blank disc is used as a negative control, amoxicillin antibiotic discs on *Staphylococcus aureus*, and *Escherichia coli* chloramphenicol at as a positive control. Oxoid disk that contains extracts of basil (*Ocimum x africanum L.*) With a concentration of 20%, 40%, 60%, 80%, 100% placed on it. Incubation at a temperature of 370 C for 1x24 hours later measured inhibition zone is formed. Data were analyzed with a non-parametric test of Kruskal-Wallis followed by a Mann-Whitney test. The results of the analysis obtained basil leaf extract at a concentration of 80% have the highest inhibitory effects on Staphylococcus aureus. At 100% inhibitory concentration decreased. Whereas in the growth of *Escherichia coli* only very few inhibitory zones are found, which can hardly be distinguished at a concentration of 20% to 100% (Rahmawati, 2010).

Skin disease is still one of the problems for the people of Indonesia. One of the distressing skin diseases is ulcers, and the disease is caused Because of Gram-positive bacterial infections *Staphylococcus aureus*. Staphylococcus aureus bacterium is a pathogenic bacterium, the most common human being who lives as a saprophyte in the human skin membrane and digestion. One of the anti-bacterial which is currently being studied is, which has been studied extensively,

namely antibacterial properties of essential oils of basil leaves. Essential oils of basil leaves have an antibacterial activity of the power quite high. This study shows that the oil content of basil in a liquid bath soap can inhibit bacterial growth *Staphylococcus aureus*. The addition of basil oil added 0.25 ml shower gel 10 ml, show good activity that is with an average diameter of 7.8 mm inhibition. The highest activity was indicated by basil oil 0.75 ml in 10 ml liquid soap, which with an average diameter of 9.8 mm inhibition. However, liquid soap formulations are most preferred by respondents is basil oil soap 5%, which is 0.5 ml added to 10 ml of liquid soap with a diameter of 8.3 mm resistor (Muthmainnah, et al., 2014).

Basil (*Ocimum x africanum.L*) is a plant that is often utilized for traditional medicine. The content of eugenol in the essential oil of basil leaves has an antibacterial effect against gram-positive bacteria. Streptococcus mutans is a Gram-positive facultative anaerobic bacteria in the oral cavity, which are found in plaque biofilms. This research goal is to determine the effects of essential oils basil (*Ocimum x africanum L*) as an inhibiting agent *Streptococcus mutans* biofilm formation. This research was started from procuring basil leaves and then carried out steam distillation to obtain essential oils. The concentration of essential oils used in this study were 1%, 0.5%, 0.25%, 0.125%, 0.0625% using PEG 400 solvent 2.5%.

The biofilm-forming inhibitor test was performed on microplate round bottom 96 wells with 0.5% violet crystal staining. The reading of absorbance values using a microplate reader with a wavelength of 595 nm. The data were processed using the formula of the hindrance of the formation of biofilms. IC50 values express power inhibition of biofilm formation were analyzed using probit methods. The outcomes indicated that the concentration of essential oil of basil 0.0625%, 0.1255, 0.25%, 0.5% and 1% can inhibit *Streptococcus mutans* biofilm formation amounted to 4.451%, 40.121%, 80.416%, 88.586%, and 94.812%. The conclusion of this study is the essential oil of basil (*Ocimum basilicum L*) affects an agent for inhibiting biofilm formation by *Streptococcus mutans* with IC50 at a concentration of 0.168% (Susanto, et al., 2012).

Natural microbes in hand, normal flora in hand

The skin is a barrier / first protection for the body, protects the body from microorganisms not enter into the body. Hands certainly much contact with microorganisms in the environment. Microorganisms that naturally exist in the human hand or the normal flora in the hands categorised into two types of microorganisms resident (stationary) and transient (temporary):

1. Microorganisms Resident: composed of microorganisms that are under the surface of the stratum corneum cells and are also found in the skin surface. The dominant species are as *Staphylococcus epidermisdis*, and oxacillin-resistant bacteria are unusually high. Another

resident bacteria, including *S. hominis* and coagulase-negative staphylococci bacteria followed by *corynoform (Propionibacteria, Corynebacteria, dermobacteria, and micrococci*). Fungi are common as resident skin flora is *Pityrosporum sp (Malassezia)*. Resident flora has two main protective functions: Antagonistic Microorganisms and Competition nutrients in the ecosystem. In general, resident flora to be associated with little chance of infection, but may cause infections in sterile body cavities, eyes, or non-intact skin.

2. Microorganisms transient (transient flora): colonise the surface layers of the skin and are easily removed with routine hand hygiene. Transient microorganisms can not multiply in the skin, but they live and breed sporadically surface of the skin. The health workforce usually obtains transient microorganisms for direct contact with the patient or the patient's environment that borders. Microorganisms may form the persistent colonies as S. aureus, Gram-negative bacillus, or yeast (yeast). Normal human skin is an aerobic bacterial colonies numbering ranges from 1 x 10 6 colony forming units (CFU) / cm2 on the scalp, 5 x 10 5 CFU / cm2 in the abdomen, 1x10 4 CFU / cm2 on the forearm. (World Health Organisation, 2006).

Methods

Quantitative research design experimental studies, the independent variable is the extract of Basil (Ocimum x africanum L) concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% and the dependent variable is the growth of microbes on the hands. Test examination method the inhibition by Kirby Bauer diffusion method. Each concentration was repeated 3 times. The study design was Completely Randomised Design is the design made on the number of experiments that not too much with the homogeneous experimental unit and external factors that influence can be controlled. Data analysis using ANOVA (analysis of variance).

Place of research conducted in the laboratory of Microbiology Department of Health Analyst Poltekkes TJK in April- November 2017. Subjects were extracts of Basil (*Ocimum x africanum.L*) with a concentration of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%.

Criteria basil leaves (*Ocimumx africanum.L*) used has characteristics as follows: green to brownish-green color, the aromatic smell is typical with a mildly spicy flavor. Leaf-blade shape elongated oval, round or circular egg eggs lengthwise, bones pinnate leaves, shallow jagged edges or uneven and bumpy, meat thin leaves, surface smooth-haired, leaf length of 2.5 cm to 7.5 cm, a width of 2.5 cm. Probandus selected are students after conducting cleaning activities their hand. Propandus palms Samples were taken in the form of outward appearance on the palm with repetition three times.

Data obtained by collecting the leaves of basil (*Ocimum x africanum L*) by the criteria of the sample, then determined after it is dried to form a powder, then extracted and created a concentration of 10%, 20%, 30%, 40%, 50%, 60 %, 70%, 80%, 90%, 100%. Testing the microbial sensitivity test Diffusion Kirby Bauer method, see whether there is a zone of inhibition around the discs from each concentration tested. Then the results were analyzed by analysis of data using ANOVA (analysis of variance).

Tools and Materials

Autoclave, incubator, hot plate, oven, test tubes, tube rack, volume pipette, measuring pipette, flask, flask, funnel glass, glass beaker, light spirits, a loopful, copy paper, aluminum foil, cloth, and paper labels.

Extract Basil (*Ocimum x africanum L*), Blood agar plate, Mannitol salt agar, DNAsre order, Plasma citrate rabbit, Mac concey agar, Endo Agar, eosin methylene blue agar, TSIA, SIM so, Simmon citrate agar, Urea agar, MrVp agar, glucose, lactose, maltose, mannitol, sucrose agar, Mueller Hinton Broth (MHB), Mueller Hinton Agar, Phenol 2% Disk blank sterile, Nutrient order Slant (NAS), sulfuric acid (H2SO4), Barium Chloride Dyhidrat (BaCl2. 2H2O) 1%, 0.85% NaCl, NB (Nutrient Broth), alcohol, phenol 2%, sterile distilled water.

Research Procedure

1. Basil Plant Determination

The determination was carried out by bringing basil plant biology laboratory MIPA UNILA, the result of the decision indicates horticultural basil that will be tested from varieties Ocimum x africanum.

2. Sterilisation Equipment

Glass tools are used all wrapped in aluminum foil, then sterilised in the oven with a temperature of 160 for 1 hour (Soemarno, 2000).

Preparation of Test Solution

a. Making Test Solution

1) Simplisia
Ocimum x africanum L basil leaf samples in fresh conditions. The leaves are green to brownish-green color, and the aromatic smell is typical with a mildly spicy flavor. Subsequently chopped and dried with aerated under indirect sunlight. The dried simplisia marked with if broken already fragile and pollinated and stored in a dry place.

2) Making the Basil Leaf Extract
   a) Simplisia inserted into a measuring cup 1000 ml to 10 parts, and poured as much as 75 parts of ethanol, then put in a glass beaker, and a closed aluminum foil, were left for 5 days in a place protected from light while stirring repeatedly, then filtered (maserat 1).
   b) Simplisia dregs soaked with ethanol as much as 25 parts measuring cup, left for 2 days at room temperature, then filtered again (maserat 2).
   c) Maserat Maserat 1 and 2 are mixed and then conducted to separate the solvent evaporation by phytochemicals from basil. The extract does examination viscosity level was evaporated over the water bath until thick with a temperature of 40 ° C, then the weighing is done to achieve a constant weight to ensure that the extract has not contained solvents longer considered to be a concentration of 100%.
   d) Then the solution is diluted to a concentration 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% using sterile distilled water.
   e) Making the Mac Farland 0.5 standard; Solution of sulfuric acid (H2SO4) 1% as much as 99.5 ml mixed with barium chloride dihydrate (BaCl2.2H2O) 1% 0.5 ml. Reagents were mixed shaken until homogeneous. Each will be used should be shaken prior (Soemarno, 2000).
   f) Preparation of 0.85% NaCl; weighed as much as 0.85% grams of crystalline NaCl then put in a measuring flask added by using 100 ml of distilled water and stirred until homogeneous. NaCl solution is poured into the flask and sterilised by autoclave at a temperature of 121 1 atm pressure for 15 minutes (Soemarno, 2000).
   g) Mueller Hinton order; For Mueller Hinton weighed 17 grams, then put in Erlenmeyer plus 500 ml of distilled water, covered with cotton which is wrapped in aluminum foil, heated on a hot plate until dissolved, then sterilised using an autoclave temperature of 121 for 15 minutes a pressure of 1 atm, removed and allowed for a few minutes until the temperature of the media 50 and poured into a petri dish disk thickness 4mm (Sumarno, 2000).
   h) Nutrient Broth; Weighed as much as 0.8 grams of Nutrient Broth media and then put into the flask, add 100 ml of distilled water, covered with cotton wrapped with paper coffee,

heated on a hot plate until dissolved, then inserted into the tube 5 ml of each tube closed with cotton and sterilised by autoclave temperature of 121 for 15 minutes at a pressure of 1 atm.

i) Nutrient order Slant (NAS); For Nutrient media is weighed 2.8 grams and then put in a 100 ml Erlenmeyer added distilled water, covered with cotton, heated on a hot plate until dissolved then poured into long tubes of 10 ml, 5 ml. Tube covered with cotton and sterilised by autoclave temperature of 121 for 15 minutes at a pressure of 1 atm, lifted, and then the tube is tilted.

j) Microbial identification on the palms; Identification of microbes on the palms is done by performing probandus smear on your palms, then proceeds outward grown on Blood agar plate media, Nutrient agar, Mac concey agar, eosin methylene blue agar, Endo agar. They are then incubated at 370C for 24 hours, the atmosphere aerobe. Studying the growth of bacterial colonies growing on the seed media. From separate colonies do Gram, then recorded the results. From the bacterial colonies do TSIA, SIM, Simmon citrate, urea, glucose, lactose, maltose, mannitol, sucrose, then incubated temperature of 370C for 24 hours, the atmosphere aerobe. Reading culture results, then determining the bacteria present on the palm.

k) Suspension Preparation of *E. coli*; take two ose colony eyes from *E.coli* culture from Nutrient media that slant and put into tubes containing Nutrient Broth, then shaken until homogeneous, incubation at 37for 4 hours If more turbid then the suspension is added with 0.85% NaCl until turbidity similar to Mac Farland 0.5 standard = M.farland. / 108CFU / ml CFU = Colony Forming Unit.

l) Power Test Inhibitory; Sterile swab is inserted into the tube containing the bacteria suspense that has been equated with standard MacFarland turbidity, left for a while so that the liquid can seep into the lift and pressed cotton in the tube wall while playing. The swab was rubbed on the media Mueller Hinton Agar (MHA) until the entire surface sealed with suspense staining bacteria. Swab on the commute from the outward appearance 1 and 2, the plate is rotated 45 degrees. Left for 15 minutes so that the suspension of bacteria seep into the media. One plate is used for three concentration/treatment, put one plate each time the repetition media Mueller Hinton Agar (MHA), and painted until the entire surface sealed with suspense staining bacteria. Swab on the commute from the outward appearance 1 and 2, the plate is rotated 45 derajat. Left for 15 minutes in order suspense bacteria seep into the media. Blank disk that has been soaked with distilled water sterile as a negative control, and soaked into a solution of phenol 2% as a positive control disk that has been soaked with the test solution dilution of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% is taken with sterile tweezers and placed on media Mueller Hinton Agar (MHA)s. As a negative control disk soaked in sterile distilled water and placed on the MHA and the positive control disk soaked in phenol 2%. MHA media was incubated

at 37 and immersed into a solution of phenol 2% as the positive control disc blank that has been soaked with the test solution dilution of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% taken with sterile tweezers and placed on media Mueller Hinton Agar (MHA). As a negative control disk soaked in sterile distilled water and placed on the MHA and the positive control disk soaked in phenol 2%. MHA media was incubated at 37 and immersed into a solution of phenol 2% as the positive control disc blank that has been soaked with the test solution dilution of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% taken with sterile tweezers and placed on media Mueller Hinton Agar (MHA). As a negative control disk soaked in sterile distilled water and placed on the MHA and the positive control disk soaked in phenol 2%. MHA media was incubated at 37for 24 hours. Measured by the total inhibitory zone formed around the disk and recorded. The inhibitory zone formed against the positive control disk is measured. Readings formed inhibition zone marked by the absence of bacterial growth around the disk were measured with calipers in mm.

Data analysis

The data obtained are presented in tabular form, and then the data were analyzed using Anova (analysis of variance). If Fhitung> Ftabel, then proceed Least Significant Difference test at 95% confidence level.

Results and Discussion

Univariate Analysis

The results of the effectiveness test the effectiveness of extracts of basil (*Ocimum x africanum L*) with concentration 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% were able to inhibit the growth of *E. coli* on palms characterised by forming a growth inhibition zone around the disc inhibition zone formed by each concentration tested with the results in table 1:

Table 1. The diameter of the inhibition zone basil leaf extract (Ocimum x africanum.L) against the growth microbes on the hands.

Concentration (%)	Repetition	Repetition	Repetition	Amount (mm)	The mean (mm)
	I	II	III		
10	6:20	6:30	6:50	19:00	6:33
20	6:40	6:30	6.60	19:30	6:43
30	6.90	7:40	7:00	21:30	7:10
40	7.70	7:40	7.80	22.90	7.63
50	7.90	8:40	9:40	25.70	8:56
60	9.60	9:50	10:10	29.20	9.73
70	10.80	11:40	11:20	33.40	11:13
80	11.80	12:30	12:10	36.20	12:06
90	12:20	13:20	13:30	38.70	12.90
100	14:40	14.60	15:00	44.00	14.66
control +	8:20	9:00	8.70	25.90	8.63
control -	0	0	0	0	0

Control (+): Phenol 2%; Control (-): sterile Aquadest

At a concentration of 10% average inhibition zone of 6.33 mm, and the concentration of 100% of the average zone of inhibition of 14.66 mm.

Bivariate analysis

The bivariate analysis was used in this study is a statistical test ANOVA (analysis of variance), and if there is a significant difference, then proceed with the least significant difference test or Post Hoc LSD (Least significance different) at 5% level.

a. One-way ANOVA test

Requirements to Test One-way Anova is normal data distribution and variance of the same data. Results of tests of normality in this study are significant value for each concentration was obtained $p > 0.05$, meaning 10 concentration distribution of the data is normal, then significance tests homogeneity of variances showed $p > 0.05$ significant variance same data. Then proceed to the analysis of data on one-way ANOVA analysis.

Measurement of the average diameter zone of inhibition of microbial growth on the hands (*E.coli*) contained in Table 2 and then carried One-way ANOVA test at level α = 0.05., With the result p = 0.000 (p <0.05) significantly contained mean difference in the diameter of the inhibitory zone extracts of basil (*Ocimum x africanum.L*) followed by a test of Least Significant Difference (LSD) or Post hoc LSD (Least Significance Different) (p <0.05) to determine the real difference between the concentration diameter.

Table 2. Results of one-way ANOVA analysis of inhibition zone diameter basil leaf extract (Ocimum x africanum.L) against the growth of microbes on the hands (E.coli)

Concentration	N	Mean ± sb	*P-value*
10%	3	6.33 ± 0.15	0,000
20%	3	6.43 ± 0.15	
30%	3	7.10 ± 0.26	
40%	3	7.63 ± 0.20	
50%	3	8.57 ± 0.76	
60%	3	9.73 ± 0.32	
70%	3	11.13 ± 0.30	
80%	3	12.07 ± 0.25	
90%	3	12.90 ± 0.61	
100%	3	14.67 ± 0.31	
K (+)	3	9.56 ± 2.72	

b. Least Significant Difference Test (LSD)

Least Significant Difference test results presented in Table 3. Least Significant Difference test results prove that basil leaf extract concentration (*Ocimum x africanum.L*) 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80 %, 90%, and 100% gives a real difference, meaning that at each concentration tested, give a different effect on the average diameter zone of inhibition of microbial growth on the hands (*E.coli*). The effective concentration of the extracts obtained basil *Ocimum x africanum* in killing microbes on the palms is at a concentration of 50%.

Table 3. Least Significant Difference test extracts of basil (Ocimum x africanum.L) against the growth of microbes on the hands (E.coli)

	mean difference	CI 95%		*p-value*
		Minimum	Maximum	
100% vs. 90%	1.77 *	1.11	2.42	0,000
100% vs. 80%	2.60 *	1.94	3.25	0,000
100% vs. 70%	3.53 *	2.88	4.19	0,000
100% vs. 60%	4.93 *	4.28	5.59	0,000
100% vs. 50%	6.10 *	5.44	6.75	0,000
100% vs. 40%	7.03 *	6,38	7.69	0,000
100% vs. 30%	7.57 *	6.91	8.22	0,000
100% vs. 20%	8.23 *	7.58	8.89	0,000
100% vs. 10%	8.33 *	7.68	8.99	0,000
100% vs. K (+)	6.03 *	5.38	6.69	0,000
90% vs. 80%	0.83 *	0.18	1.49	0,015
90% vs. 70%	1.77 *	1.11	2.42	0,000
90% vs. 60%	3.17 *	2.51	3.82	0,000
90% vs. 50%	4.33 *	3.68	4.99	0,000
90% vs. 40%	5.27 *	4.61	5.92	0,000
90% vs. 30%	5.80 *	5.14	6.45	0,000
90% vs. 20%	6.47 *	5.81	7,12	0,000
90% vs. 10%	6.57 *	5.91	7.22	0,000
90% vs. K (+)	4.27 *	3.61	4.92	0,000
80% vs. 70%	0.93 *	0.28	1.59	0.007
80% vs. 60%	2.33 *	1.68	2.99	0,000
80% vs. 50%	3.50 *	2,84	4.15	0,000
80% vs. 40%	4.43 *	3.78	5.09	0,000
80% vs. 30%	4.97 *	4.31	5.62	0,000
80% vs. 20%	5.63 *	4.98	6.29	0,000
80% vs. 10%	5.73 *	5.08	6.39	0,000
80% vs. K (+)	3.43 *	2,78	4.09	0,000
70% vs. 60%	1.40 *	0.74	2.05	0,000
70% vs. 50%	2.57 *	1.91	3.22	0,000
70% vs. 40%	3.50 *	2,84	4.15	0,000
70% vs. 30%	4.03 *	3.38	4.69	0,000
70% vs. 20%	4.70 *	4.04	5.35	0,000
70% vs. 10%	4.80 *	4.14	5.45	0,000

70% vs. K (+)	2.50 *	1.85	3.15	0,000
60% vs. 50%	1.17 *	0.51	1.82	0,001
60% vs. 40%	2.10 *	1.45	2.75	0,000
60% vs. 30%	2,63 *	1.98	3.29	0,000
60% vs. 20%	3.30 *	2.65	3.95	0,000
60% vs. 10%	3.40 *	2.75	4,05	0,000
60% vs. K (+)	1.10 *	0.45	1.75	0,002
50% vs. 40%	0.93 *	0.28	1.59	0.007
50% vs. 30%	1.47 *	0.81	2.12	0,000
50% vs. 20%	2.13 *	1.48	2,79	0,000
50% vs. 10%	2.23 *	1:58	2.89	0,000
50% vs. K (+)	-0.67 *	-0.72	0.59	.834
40% vs. 30%	0.53	-0.12	1.19	.104
40% vs. 20%	1.20 *	0.55	1.85	0,001
40% vs. 10%	1.30 *	0.65	1.95	0,000
40% vs. K (+)	-1.10 *	-0.16	-0.35	0,004
30% vs. 20%	0.67 *	0.01	1.32	0.045
30% vs. 10%	0.77 *	0.11	1.42	0,023
30% vs. K (+)	-1.53 *	-2,18	-0,88	0,000
20% vs. 10%	0.10	-0.55	0.75	0,753
20% vs. K (+)	-2.20 *	-2.85	-1.5	0,000
10% vs. K (+)	-2.30 *	-2.95	-2.95	0,000

Description: * real different

Leaf extracts of basil (*Ocimum x africanum.L*) have content of flavonoids, saponins, and tannins (Batari, 2007) is antibacterial so that it can inhibit microbial growth on the palms. The ability of the extracts of basil that has been tested on concentration 10-100%, showing different capacities to inhibit microbial growth on the palms at each concentration tested with a mean inhibition zone of 6.33 up to 14.66 mm. Effective concentrations ranging from the concentration of 60% to 100% with a mean inhibition zone formed 9,73mm up to 14,66mm compared with controls (+) phenol 2% inhibition zone formed 8,63mm. This is due to the higher concentration of basil leaf extract tested, the greater the ability to inhibit the growth of microbes on the hands, for each concentration gives a different effect on the mean diameter of microbial growth inhibition zone on the hands (*E.coli*). In addition to flavonoids, saponins, and tannins contained in extracts of basil leaves are also chemical constituents eugenol, β-caryophyllene, β-bisabolenes (Kicel, 2005) so the ability to kill microbes on hands quite high as Antibacterial agents when compared to controls (+) phenol 2%. Mechanisms owned flavonoids as anti-bacterial substances such as by inhibiting the synthesis

of nucleic acids, inhibits the function of the cytoplasmic membrane, and inhibits the metabolism of energy (Cushine, et al., 2005). Saponins can increase permeability bacterial cell membranes so that it can change the structure and membrane function and result in denaturation of membrane proteins resulting in cell and lysis damage.

Limitations of this study was just testing out the extracts from the leaves of basil Ocimum x africanum against the growth of microbes on the hands, not yet got to the stage interesting active substances contained in the extracts are flavonoids and tannins, that can be a key element in the process of making the product of hand sanitiser collaborate with majors pharmacy.

Similar research has been conducted by Rahmawati (2010) on the activation test antibacterial activity of the extracts from basil (*Ocimum sanctum.L*) against the bacteria *E. coli* atcc 11229 and ATCC 6538 *Staphylococcus aureus* in vitro shows the results of the concentration of 80% has the highest inhibitory effects on *Staphylococcus aureus* and can not be distinguished ability inhibition at concentrations of 20 to 100% in *E.coli*.

Research by Novitasarin (2014) in antiseptic hand gel formulation of essential oils basil (*Ocimum Basilicum L*) with a base carbopol and evaluation of antibacterial activity against *Staphylococcus aureus* basil essential oil states have an inhibitory effect on the growth of *Staphylococcus aureus*.

Another study by Susanto, et al (2012) on the effects of essential oils basil (*Ocimum basilicum L*) as an agent for inhibiting biofilm formation by *Streptococcus mutans*, shows the concentration of essential oil of basil 0.0625%, 0.125%, 0.25%, 0.5 %, and 1% can inhibit *Streptococcus mutans* biofilm of 4.451%, 40.121%, 80.416%, 88.586%, and 94.812%.

Leaf extracts of basil (*Ocimum x africanum.L*) can kill microbes in the palm of the hand with the highest concentration of 100% inhibition zone formed by 14.66 mm, because of the content of flavonoids, saponins, and tannins.

Conclusion

Extracts of basil (*Ocimum x africanum.L*) able to kill the growth of microbes on the hands, at a concentration of 10-100% with a mean diameter of inhibition zone is formed of 6.33 mm - 14.66 mm. And the effective concentration of the extract of leaves of basil (*Ocimum x africanum.L*) are capable of killing microbes in the palm is 50%

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