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Potential of Tulsi Leaves (*Ocimum sanctum L.*) and Charcoal Against *Bacteria Sthaphyloccusepidermidis* and *Pseudomonas aeruginosa*

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Abstract: *Infection is a disease that often occurs in society. Staphylococcus epidermidis and Pseudomonas aeruginosa are pathogenic bacteria exist in the human body. Since ancient times, the use of plants as medicinal materials. The use of natural materials by combining two natural materials has not been developed much. One of the plants that has benefits as traditional medicine is tulsi and charcoal. The research objective was to analyze the antibacterial potential of tulsi leaves, charcoal and their combinations. This research uses Soxhlet and diffusion methods. Tulsi leaves and charcoal either alone or in combination can inhibit the growth of the bacteria Staphylococcus epidermidisand Pseudomonas aeruginosa.*

Keywords: Tulsi leaves (*Ocimum sanctum* L.), *charcoal*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*

1. Introduction

Infection is a disease often occurs in society. Infectious diseases can be caused by microorganisms, including fungi, viruses, bacteria, parasites can be transmitted from human to human or animal to human (Jawetz et al., 2001). Bacteria can cause infections and are well known throughout the body and the most common include *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Streptococcus pyogenes, Staphylococcus epidermidis, Klebsiella pneumonia, Salmonella typhi, Pseudomonas aeruginosa, Neisseria gonorrhoeae, Mycobacterium tuberculosis* and others (Singh et al., 2013).

Pseudomonas aeruginosa is an opportunistic pathogenic bacteria which can cause infection in individuals with decreased immunity (Karsinah, et al. 2005). In baby and frail people *Pseudomonas aeruginosa* may enter the bloodstream and result in fatal sepsis (Jawetz, 2005). *Pseudomonas aeruginosa* is a pathogenic Gram negative

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bacteria that is difficult to treat. Its resistance to treatment leads to treatment failure (Srifeungfung, 2004).

Staphylococcus epidermidis is a normal flora on the skin and will not be pathogenic under normal conditions, if there is a change in conditions, it will become invasive (Jawetz et al, 2001). Since ancient times, the use of plants as medicinal materials, cosmetics has been used. The shift in the pharmaceutical industry in the use of ingredients from synthetic materials to natural ingredients (back to nature) as ingredients for making medicines and cosmetics is increasing.

The use of natural materials by combining two natural ingredients has not been developed much. One of the plants that has benefits as traditional medicine is tulsi (*Ocimum sanctum* L) and charcoal. Tulsi leaves (*Ocimum sanctum* L.) can treat dysentery, skin diseases, as an anti-fungal, antibacterial, and anti-cancer agent (Prakash and Gupta, 2005). Tulsi leaf oil (*Ocimum sanctum* L.) can be used as an antibacterial agent on *Escherichia coli, Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas aeruginosa* (Singh et al, 2005). Tulsi leaf oil (*Ocimumbasilicum*L.) has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, with a minimum bactericidal concentration of 0.5 and 0.25% v / v. From the MBC obtained in this study, it can be said that the essential oil of tulsi leaves has the potential for *E. coli* (Maryati, 2007).

Information on the use of activated charcoal has long been known, but there is not much information on the results of scientific research on bacteria in the human body. This is a challenge for researchers engaged in the health sector to further investigate the efficacy of activated charcoal.

Activated carbon or better known as activated charcoal. Activated carbon is widely used because it has a strong adsorbent ability. The raw materials that are widely used for the manufacture of activated carbon are coconut shell and wood. These materials have better characteristics than non-biomass or fossil materials. This is due, among others, to the ease of processing and quality of results for various purposes of use (Arsad and Hamdi, 2010).

Activated charcoal is widely used to discolor liquids, recover solvents, and remove toxins from water and air. The adsorption properties of activated charcoal have been used for a long time to clean fluids from bacterial endotoxin and exotoxin (Du et al, 1987). According to research (Nolan et al, 1975; Maitra et al, 1981) it has been shown that activated charcoal, when administered in an environment enriched with the endotoxin Escherichia coli, will remove 90–95% of this toxin.

Based on the description above, the researcher aims to analyze the antibacterial potential of basil leaves, charcoal and their combination in the bacteria *Staphylococcus epidermidis* and *Pseudomonas aeruginosa.*

2. Research Method

The research is experimental laboratory. The research was conducted at the Bacteriology laboratory of the Faculty of Health Sciences, Universitas Muhammadiyah Sidoarjo in January-April 2020. The samples used were tulsi leaf extract and charcoal powder obtained from the larangan market in Sidoarjo with concentrations of 0 ppm, 10 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm, 100 ppm. Tulsi leaf extraction was obtained using the Soxhlet method. The test bacteria used were *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* using the diffusion method. At a concentration of 0 ppm (negative control) using sterile distilled water and a concentration of 100 ppm (positive control). Each treatment was repeated 3 times. The working procedure is:

Sample Preparation

Fresh tulsi leaves taken from the stalks and branches then weighed as much as 2000 g, aerated for about 3 days to reduce the moisture content in the leaves. The dry weight is approximately 260 grams, the tulsi leaves are blended into powder and weigh more than 215 grams.

Extraction Process Using Soxhlet Method

30 grams of tulsi leaf powder is taken then put into filter paper that has been shaped into a cylindrical tube and tie it, then assemble the Soxhlet tool. The bag containing the tulsi leaf powder was put into the Soxhlet and 450 mL of n-hexan

organic solvent was added, the Soxhlet equipment was heated with a heating mantle until several cycles occurred. The extraction process is stopped when the n-hexan solvent in the extraction tube is clear.

Making Charcoal Powder

Weighing activated charcoal granules according to the need for one production, which is as much as 200 grams using a digital scale. Smoothing activated charcoal granules using the help of pestle mortar. Grind activated charcoal little by little until smooth. Sieving activated charcoal that has been powdered using a sieve so that the particle size is uniform and facilitates further processing. Storing the activated charcoal powder that has passed the sieve into the storage jar.

Antibacterial Inhibition Zone Test

The antibacterial inhibition test was carried out in vitro with the diffusion method using filter paper or oxoid paper disk. Mueller Hinton Agar (MHA) medium, cooled at 40-45 °C, then poured into a petri dish aseptically in Laminary Air Flow (LAF). After the MHA medium on the petri dish had solidified, bacterial suspension was carried out by spreading it evenly on the surface of the medium using a sterile swab. After the basting, the Blank disc soaked in the respective concentrations of tulsileaf extract, charcoal and its combination was attached to the surface of the MHA medium which had been smeared with bacterial suspension. Then the petri dishes were incubated at 37°C for 1x 24 hours. Bacterial growth and the zone of inhibition that arose around the Blank disc were then measured for diameter using a caliper. Statistics to determine the difference test using the parametric statistical test One Way Anova.

3. Results and Discussion

Table 1. Effect of Basil Leaf extract, Charcoal on Bacteria *Staphylococcus epidermidis*

Treatment	Concentration (ppm)	Mean±SD
	0	0,00±0,00
	10	0,22±0,01
	20	0,30±0,0

Tulsi leaves	40	0,97±0,47
	60	0,99±0,73
	80	1,71±0,02
	100	2,52±0,21
Charcoal	0	0,00±0,00
	10	0,59±0,43
	20	0,93±0,25
	40	1,16±0,06
	60	1,88±0,64
	80	2,06±0,58
	100	1,88±0,64

Table 2. Effect of Tulsi Leaf Extract, Charcoal Against Bacteria *Pseudomonas aeruginosa*

Treatment	Concentration (ppm)	Mean±SD
Tulsi Leaves	0	0,00±0,00
	10	0,14±0,02
	20	0,56±0,07
	40	0,72±0,11
	60	0,84±0,05
	80	1,03±0,20
	100	1,42±0,16
Charcoal	0	0,00±0,00
	10	0,18±0,32
	20	0,45±0,09
	40	0,72±0,16
	60	1,20±0,09
	80	1,40±0,09
	100	2,43±0,32

Table 3. Effect of Combination of Tulsi Leaf Extract and Charcoal on Bacteria *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*

Bacteria	Treatment	Mean±SD
	10:100	0,89±0,80

Staphylococcus epidermidis	20:80	1,35±0,84
	40:60	2,25±0,88
	60:40	2,90±1,35
	80:20	0,62±0,64
	100:10	1,97±065
Pseudomonas aeruginosa	10:100	0,59±0,03
	20:80	0,23±0,03
	40:60	0,33±0,06
	60:40	2,20±0,36
	80:20	1,80±0,30
	100:10	0,43±0,02

Measurement of the zone of inhibition in tulsi leaf extract (*Ocimum sanctum* L.) and charcoal with concentrations of 0 ppm, 10 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm. Combination of tulsi leaf extract (*Ocimum sanctum* L.) with charcoal at concentrations of 10: 100 ppm, 20:80 ppm, 40:60 ppm, 60:40 ppm, 80:20 ppm, 100: 10 ppm against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* bacteria shown in Tables 1 and 2. The results of inhibition zone measurements of tulsi leaf extract (*Ocimumsantum* L.) and charcoal showed varying results in each treatment of *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* bacteria. This is because the levels of the active compound content at each concentration are different. The greater the sample concentration, the greater the amount of active substance contained. So that the higher the concentration, the greater its ability to inhibit bacterial growth (Schlegel, 1994). More or less the content of antimicrobial substances in extracts is influenced by several factors, including the concentration of antimicrobial substances, the number of microorganisms, temperature, species of microorganisms, organic matter, pH (Pelczar and Chan, 1998). Based on research (Tambajong, J., et al. 2017) that the extract of basil leaves (*Ocimum sanctum* L.) can have an effect on the growth of *Staphylococcus epidermidis*bacteria. At a concentration of 0 ppm no inhibition zone was formed, which means that it does not have the ability to inhibit bacteria.

Tulsi leaves (*Ocimum sanctum* L.) have active secondary metabolite compounds that act as antibacterials. According to (Singh et al, 2005) tulsi leaves (*Ocimum sanctum* L.) contain high linolenic acid which functions as antibacterial

activity. Tulsi leaf extract contains secondary metabolites of alkaloids, saponins, steroids, triterpenoids, phenolics, tannins. Flavonoids, resins, fatty acids (Joshi et al, 2009). Flavonoids and phenolics function as antioxidants, essential oils that contain eugenol are useful as antibody activity and damage bacterial cells (Cook and Samman, 1996).

Based on the research results, the inhibition zone formation was greater in Gram positive bacteria *Staphylococcus epidermidis* than Gram negative bacteria *Pseudomonas aeruginosa*. This is due to differences in cell wall structure in determining the bonding and activity of bacterial compounds (Jawetz et al., 2005). The cell wall structure of Gram positive bacteria is simpler than the cell wall structure of Gram negative bacteria. The cell wall of Gram positive bacteria contains a lot of peptidoglycan, lipid content is about 1-4% while the cell wall of Gram negative bacteria contains more lipids around 11-22% with an outer membrane bilayer composed of phospholipids, lipopolysaccharides (Purwoko, 2007) acting as a barrier compound - compounds such as antibiotics (Burt, 2004).

Charcoal is a universal adsorbent that can bind to various molecules. According to research charcoal can be used to remove bacteria and their toxins in vitro and in vivo (Drucker et al, 1977). According to research (Percival and Walker, 1999) activated carbon has the ability to clean *Pseudomonas aeruginosa*and *Eschericia coli* bacteria.

Based on Table 3, the results obtained show that the extract of tulsi leaves (*Ocimumsantum* L.), charcoal and their combination can act as antibacterial agents which can influence the growth of *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* bacteria. Antibacteria are chemical agents capable of inactivating bacteria which inhibit bacterial growth (bacteriostatic) or kill bacteria (bactericide) (Brock, 1994).

The combination of tulsi leaf extract and charcoal has a very strong inhibitory power due to the synergistic ability of tulsi leaf extract and charcoal. The synergistic effect of the combination of active ingredients will produce an active compound that is greater than the effect of a single active compound (Spinella, 2002. The higher the

concentration, the more it will affect the growth of *Staphylococcus epidermidis*and *Pseudomonas aeruginosa* bacteria. This is because the amount of antibacterial contained in each increase in concentration will increase in inhibiting bacterial growth. Based on all table all concentrations were not significantly different. Charcoal is effective in adsorbing microorganisms. The pore size and surface morphology of activated charcoal determine the type of molecule that is absorbed because surface morphology plays an important role in determining the surface availability and the adsorption area. The greater the macroporosity, the higher it will adsorb the bacteria. This is in accordance with research (Ilomuanya et al., 2017). Charcoal derived from agricultural waste can increase macroporosity with the best meso / micro porosity mixture to adsorb *E. coli* bacteria.

4. Conclusion, Implication and Limitation

4.1. Conclusion

Tulsi leaves and charcoal either single or combination can inhibit the growth of *Staphylococcus epidermidis*and *Pseudomonas aeruginosa* bacteria.

4.2. Implication and Limitation

In this research, it is hoped that it can be a source of information and the developments of pharmacognosy about the benefits of tulsi leaves and charcoal, whether used singly or in combination against *S. epidermidis* and *P. aeruginosa.*

The limitation in this research is the use of tulsi leaves (*Ocimum sanctum* L.) and charcoal, bacteria *S. eprdermidis* and *P. aeruginosa*with concentration 0,10,20,40,60,80,100 ppm and 10:100, 20:80, 40:60, 60:40, 80:20, 100:10 ppm.

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