International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Antibacterial Activity Test of Basil Seed Extract (Ocimum sanctum L.) Against Growth Staphylococcus epidermidis

Marselina Chindi Claudia Diana Su'u[1], Kartini Lidia[2], Su Djie To Rante[3], Desi Indriarini[4]

[1]Medical Education Study Program, Faculty of Medicine and Veterinary Medicine, Universitas Nusa Cendana
[2]Department of Biomedicine, Pharmacology and Therapeutics, Faculty of Medicine and Veterinary Medicine, Universitas Nusa Cendana
[3]Department of Surgery, Faculty of Medicine and Veterinary Medicine, Universitas Nusa Cendana
[4]Department of Biomedicine, Microbiology, Faculty of Medicine and Veterinary Medicine, Universitas Nusa Cendana

ABSTRACT

Background: *Staphylococcus epidermidis* is a normal flora found in almost all parts of the human body that under certain circumstances can cause infection. It is the most common major nosocomial pathogen associated with medical devices and is currently one of the leading causes of increased morbidity and mortality in hospitals. *Staphylococcus epidermidis* infections are usually treated with antibiotics, which are generally resistant, so alternative natural ingredients are needed to inhibit their growth. One alternative that can be used is basil seeds (*Ocimum sanctum L.*).

Objective: To determine the antibacterial activity of basil seed extract (*Ocimum sanctum L.*) against the growth of *Staphylococcus epidermidis*.

Methods: This type of research uses a true experimental design with a post-test only control group design. In this study there were treatment groups given basil seed extract with concentrations of 20%, 40%, 60%, 80%, and 100%, positive control clindamycin and negative control sterile distilled water with *Staphylococcus epidermidis* as the test bacteria. The antibacterial testing method used disc diffusion. The results were analyzed using the Kruskal-Wallis statistical test.

Results: The results of the antibacterial potential test of basil seed extract against the growth of *Staphylococcus epidermidis* showed that basil seed extract with concentrations of 20%, 40%, 60%, 80% and 100% did not form an inhibition zone around the disc paper.

Conclusion: Basil seed extract (*Ocimum sanctum L.*) has no antibacterial activity against the growth of *Staphylococcus epidermidis*.

Keywords: *Staphylococcus epidermidis*, basil seeds, antibacterial

INTRODUCTION

Staphylococcus epidermidis is a normal flora present in almost all parts of the human body that under

certain circumstances can cause infection. This bacterium is the most common major nosocomial pathogen in medical device-related infections and is currently one of the causes of increased morbidity and mortality in hospitals. The impact of nosocomial infections is also felt by medical and non-medical personnel, namely increased workload, feeling threatened in carrying out work and potential malpractice claims.[1,2,3]

Staphylococcus epidermidis infections are usually treated with antibiotics, which are generally resistant to several types of antibiotics. In addition, antibiotic-type drugs are relatively expensive so natural ingredients are needed that can be used as an alternative to kill the growth of *Staphylococcus epidermidis*. One alternative natural ingredient that has been proven to inhibit the growth of *Staphylococcus epidermidis* is basil (*Ocimum sanctum L.*).[4,5]

Research conducted by Nur'Aini and Francisca in 2020 found that basil leaf extract with a concentration variation of 20%, 40%, 60%, 80% and 100% has antibacterial activity against *Staphylococcus epidermidis* ATCC 12228 with inhibition zones formed respectively 10.23 mm, 10.32 mm, 10.58 mm, 13.37 mm, and 15.83 mm.5 Research in 2021 by Ika Kusuma and Citra Ninggrum, basil leaf extract of Ocimum x africanum Lour species has antibacterial activity against *Staphylococcus epidermidis* with an average inhibition area diamater value at concentrations of 3%, 5%, 7% which are 10.88 mm, 14.81 mm, and 16.83 mm respectively.6 Another study conducted by Sesilia et al in 2022 proved that basil leaf extract (*Ocimum sanctum L.*) contains alkaloid, tannin, flavanoid and poifenol compounds and basil flower extract (*Ocimum sanctum L.*) contains alkaloid, saponin, tannin, flavanoid, and polyphenol compounds.[7]

In addition to basil leaves, part of the basil plant that can be utilized is basil seeds. Basil seeds are produced from basil flowers, where basil flowers are often just waste from the plant. Basil seeds are proven to contain secondary metabolites such as alkaloids, flavonoids, tannins, polyphenols, and essential oils that have antibacterial potential.[7]

Several studies have tested *Staphylococcus epidermidis* using basil leaves, until now testing of *Staphylococcus epidermidis* using basil seeds has not been found so that researchers are interested in examining the antibacterial activity test of basil seed extract (*Ocimum sanctum L.*) against the growth of *Staphylococcus epidermidis*.

METHODS

Basil seed extract preparation

Basil seeds obtained from Osiloa Village, Tarus, Kupang Regency, East Nusa Tenggara that have been mashed and filtered are macerated with 96% ethanol solvent with a ratio of basil seed powder to solvent 1: 10 for 3 days while stirring for 15 minutes every day. After that, the pulp and filtrate were suctioned by filtering to obtain the basil seed extract. The results of maceration were evaporated with a rotary evaporator to obtain a thick extract of basil seeds.

Ethanol-free and phytochemical tests

The ethanol-free test of basil seed extract is carried out by reacting potassium dichromate ($K_2Cr_2O_7$) with ethanol in an acidic atmosphere. If the solution is ethanol-free or does not contain ethanol, a mixed color will form from the extract solution and potassium dichromate solution added to sulfuric acid, but if the solution contains ethanol, a blue color will form. After that, the thick basil seed extract was subjected to phytochemical tests to determine the content of antibacterial secondary metabolites contained in the extract. Phytochemical screening of extracts includes tannins, saponins, alkaloids, flavanoids and polyp-

henols.

Test bacteria preparation

Staphylococcus epidermidis was tested for bacterial confirmation using gram staining, catalase test and mannitol fermentation test. Making nutrient agar media by cooking and then sterilizing it and pouring it into a petri dish and allowing it to solidify. Then, making bacterial suspensions using 0.9% NaCl and 1-2 ose of bacteria until it reaches the 0.5 Mc Farland standard.

Antibacterial activity test

Test the antibacterial activity of basil seed extract using disc diffusion method. Antibacterial activity of basil seed extract is indicated by the presence of an inhibition zone or clear zone around the disc paper. The clear zone is measured with a caliper to determine the antibacterial activity of the basil seed extract. The antibacterial test treatment stage uses a sterile cotton stick that has been dipped in a suspension of *Staphylococcus epidermidis* bacteria, then flattened onto nutrient agar media. Then, into the Petri dish, 1 paper disc with a diameter of 6 mm was placed using sterile tweezers. The disc paper had previously been dipped in each concentration of basil seed extract for 30 minutes. Next, all media were incubated in an incubator at 37°C for 24 hours. The same process was carried out for the negative control which was sterile distilled water and the positive control which was clindamycin. The final stage was the calculation of the diameter of the inhibition zone using a caliper. Inhibition zone measurement data were then recorded as the results of the study.

Data analysis

Analysis of univariate data using shapiro wilk to determine whether the data is normally distributed or not. Homogeneity test using levene statistic to determine whether the data is homogeneous or not. Then proceed with non-parametric kruskal wallis test and mann-whitney post hoc test.

RESULTS

Extraction result of basil seeds

The results of the extraction of 1.2 kg of basil seeds that have been dried and cleaned from the flowers and macerated with 96% ethanol solvent for 3 days obtained a thick extract of basil seeds as much as 35 grams or 35 ml. Calculation of the yield of basil seed extract was carried out to determine the amount of yield of the extract. The results of the calculation of the yield of basil seed extract can be seen in table 1.

Table 1: Yield of Basil Seed Extract

Powder weight	Extract weight	Yield	Interpretation
1 Kg	35 grams	3.5%	Not optimal because the yield value < 10%

Ethanol free and phytochemical test results

The results of the ethanol-free test showed that basil seed extract did not contain ethanol (orange reaction or mixed color of basil seed extract, $K_2Cr_2O_7$, and H_2SO_4). Phytochemical screening of basil seed extract was carried out qualitatively on tannin, saponin, flavanoid, polyphenol and alkaloid compounds. The results of phytochemical screening can be seen in table 2.

Table 2: Phytochemical Screening Results of Basil Seed Extracts

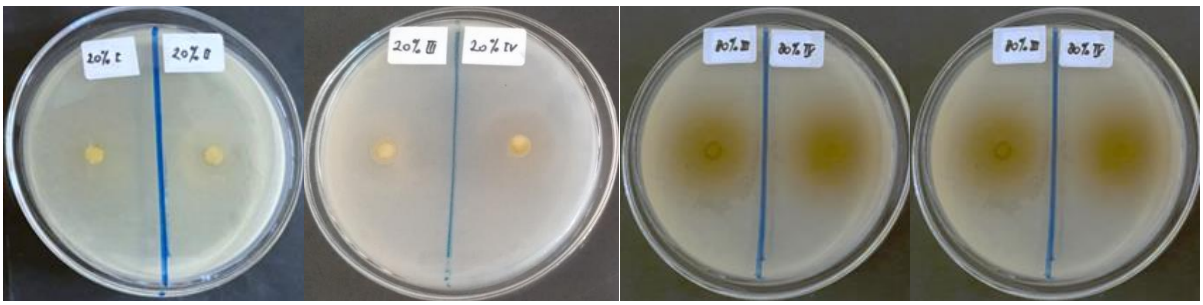
Compound group	Reagent	Test result
Tannins	$FeCl_3$	(+) Blue-black
Saponins	Aquades	(+) Forms foam/froth
Flavanoids	Metal Mg, HCl	(+) Light orange
Polyphenols	Aquades and $FeCl_3$	(+) Blue-black
Alkaloids	Mayer	(-) No white precipitate formed

Antibacterial activity test results

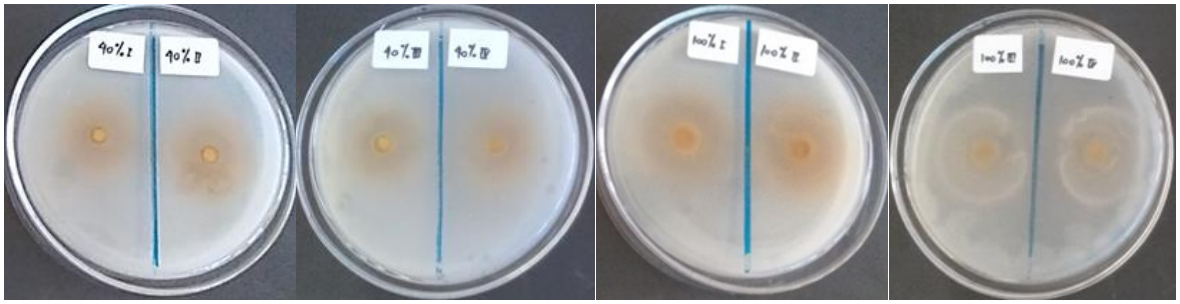
The results of the antibacterial activity test of basil seed extract (*Ocimum sanctum L.*) against the growth of *Staphylococcus epidermidis* ATCC 14990 with basil seed extract concentrations of 20%, 40%, 60%, 80%, 100%, the positive control used is clindamycin antibiotic and the negative control used is sterile aquades obtained in basil seed extract concentrations of 20%, 40%, 60%, 80%, 100%, and negative control there is no clear zone formed around the disc paper, while the positive control shows a clear zone formed around the disc paper with an average inhibition zone value of 38.5 mm (table 3).

Table 3: The diameter of the inhibition zone of basil seed extract against the growth of Staphylococcus epidermidis

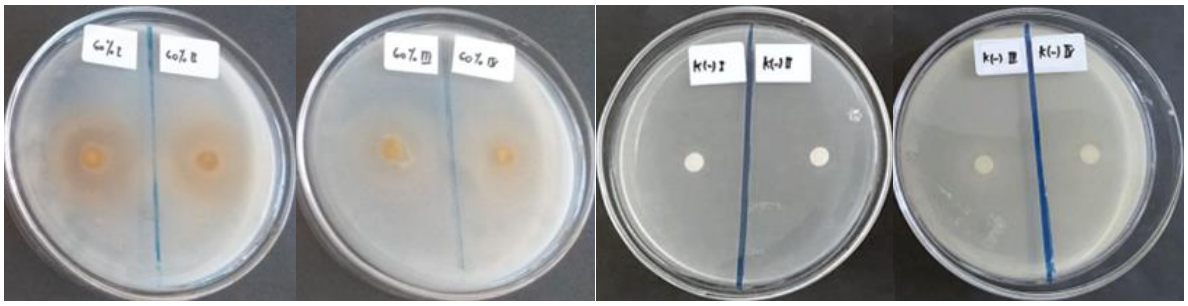
Consentrations	Diamater of inhibition zone (mm)					Potency
	R1	R2	R3	R4	Mean	
20%	0	0	0	0	0	-
40%	0	0	0	0	0	-
60%	0	0	0	0	0	-
80%	0	0	0	0	0	-
100%	0	0	0	0	0	-
control (+)	39,32	39,3	37,7	37,7	38,5	Very strong
control (-)	0	0	0	0	0	-



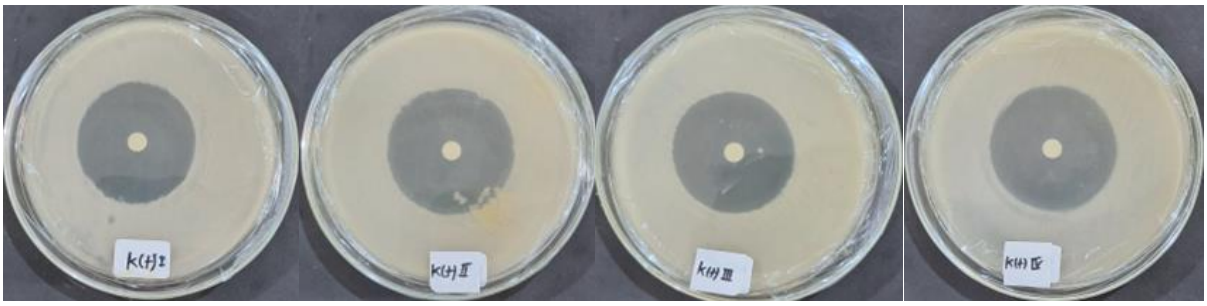
(a) (d)



(a) (e)



(b) (f)



(g)

DISCUSSION

Based on the research that has been done, it is found that there is no antibacterial activity of basil seed extract (*Ocimum sanctum L.*) against the growth of *Staphylococcus epidermidis*. This can be seen from the absence of an inhibition zone or the absence of a clear area that does not grow bacteria around the disc paper at concentrations of 20%, 40%, 60%, 80% and 100% basil seed extract. The negative control also did not form an inhibition zone, while the positive control using clindamycin antibiotic obtained an average inhibition zone of 38.5 mm with very strong inhibition potential according to Davis and Stout criteria.[8]

The results of this study are different from research conducted by Rina and Awalludin (2017) which states that the combination of basil seed extract and basil leaves using the dilution method can inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* at concentrations of 20% and 40% characterized by the absence of bacterial colonies on Muller Hilton Agar (MHA) media.[9] Another study conducted by Permadi and Susanti (2021) methanol extracts of basil leaves and basil seeds which are products of basil flowers can inhibit the growth of *Bacillus sublitis*.[10]

The presence or absence of antibacterial activity is influenced by the extraction processes itself such as the type of solvent used, the duration of maceration time, and maceration temperature. The type of

solvent is an important factor in the extraction process. In principle, a material will dissolve easily in a solvent with the same polarity. Polar compounds will dissolve in polar solvents and non-polar compounds will dissolve in non-polar solvents. The type of solvent has a big effect on yield, total phenolics, and total carotenoids. Where the greater the yield produced by a material, a higher bioactive content contained in a material. Polar solvents have the ability to dissolve phenolic compounds better so that the phenolic content in the extract is higher.[11] In this study, the secondary metabolite compounds drawn such as tannins, saponins, alkaloids, flavanoids and polyphenols are dominantly polar.

Besides the type of solvent, the maceration time is an important factor that affects the extraction. The longer the maceration time given, the longer contact between the solvent and the material, so that it will increase the number of cells in the material that break and release solutes into the solvent which results in more active ingredients dissolved. This condition will last until a balance condition occurs between the concentration of compounds in the material and the concentration of compounds in the solvent. The increase in maceration time will increase the yield value and total phenol produced by a material. However, maceration time that exceeds the optimal duration will damage the solute in the material and can increase the loss of compounds in the solution due to evaporation.[12,13]

Besides the type of solvent and maceration time, maceration temperature is also influential in inhibiting bacterial growth. Extraction by maceration method is generally done at room temperature. However, the use of room temperature has the disadvantage that the extraction process is far from perfect, so that the compound becomes less soluble.[14]

In a study conducted by Arfira Khofifa, et al. which compared the types of solvents n-hexane, methanol, and ethyl acetate and maceration times of 24 hours, 36 hours, and 48 hours showed the maceration method with methanol solvent and maceration time of 48 hours produced the highest extract yield and had the greatest inhibition of *Staphylococcus aureus* growth with an inhibition zone diameter of 19.70 mm which was categorized as very strong.[11] Research conducted by Ulfa Kinasih comparing 70% and 96% ethanol extracts of basil leaves (*Ocimum sanctum L.*) against the growth of *Escherichia coli* ESBL obtained 70% ethanol solvent of basil leaves with a concentration of 500,000 ppm has the highest inhibition power in inhibiting *Escherichia coli* ESBL with an average diameter of 7.375 mm and in 96% ethanol solvent of basil leaves with a concentration of 500,000 ppm has the highest inhibition power in inhibiting *Escherichia coli* ESBL with an average diameter of 7.25 mm.15 Based on these two studies, it is known that methanol and 70% ethanol solvents have a higher level of polarity when compared to 96% ethanol, ethyl acetate, and n-hexane which are non-polar. Another study conducted by Setyo Widodo et al, which compared maceration times of 12 hours, 24 hours, 36 hours, 48 hours and 72 hours to the antioxidant activity of mundu leaf extract, found that the best maceration time was 24 hours with a yield of 24.67% and total phenol 143.82 mg.GAE/g. The lowest yield was obtained from the treatment with maceration time for 72 hours at 16.76%.[13] The limitation in this study is that this study did not conduct quantitative tests of the content of chemical compounds in basil seed extract.

CONCLUSSION

Basil seed extract (*Ocimum sanctum L.*) obtained through the extraction process as much as 35 grams from 1 Kg of dry simplisia powder. The active substances contained in basil seed extract (*Ocimum sanctum L.*) are tannins, saponins, flavanoids and polyphenols. At all concentrations of basil seed extract (*Ocimum sanctum L.*), namely concentrations of 20%, 40%, 60%, 80% and 100%, it does not form a clear zone, which means that there is no inhibition zone against the growth of *Staphylococcus*

epidermidis, so there is no antibacterial activity of basil seed extract (*Ocimum sanctum L.*) against the growth of *Staphylococcus epidermidis*. Suggestions for further research include the need to quantitatively analyze phytochemical compounds to determine the active ingredient compounds contained in basil seed extract, further research using more polar solvents (methanol, 70% ethanol, water) and further research with maceration times of more or less than 72 hours so that it is more effective in binding secondary metabolite compounds.

REFERENCES

1. Foster TJ. Surface Proteins of Staphylococcus epidermidis. Front Microbiol. 2020;11(July):1–10.
2. Le KY, Villaruz AE, Zheng Y, He L, Fisher EL, Nguyen TH, et al. Role of Phenol-Soluble Modulins in Staphylococcus epidermidis Biofilm Formation and Infection of Indwelling Medical Devices.JMolBiol[Internet].2019;431(16):3015–27.Availablefrom: https://doi.org/10.1016/j.jmb.2019.03.030
3. Chairani, Riza S, Putra Y. Hubungan Pengetahuan dan Sikap Pencegahan Infeksi Nosokomial dengan Kepatuhan Perawat dalam Mencuci Tangan di Ruang Rawat Inap Terpadu Rumah Sakit Umum Daerah Aceh Besar Tahun 2022. J Healthc Technol Med [Internet]. 2022;8(2):1293–302. Available from: https://jurnal.uui.ac.id/index.php/JHTM/article/download/2397/1239
4. Chabi R, Momtaz H. Virulence factors and antibiotic resistance properties of the Staphylococcus epidermidis strains isolated from hospital infections in Ahvaz, Iran. Trop Med Health. 2019;47(1):1–9.
5. Purnamaningsih A, Romana Sri Supadmi F. Antibacterial Activity Test of Kemangi Leaf Extract (Ocimum sanctum L.) on Bacteria Staphylococcus epidermidis ATCC 12228. Media Ilmu Kesehat. 2020;9(3):225–30.
6. Kusuma IM, Ningrum CW. Potensi Antibakteri Ekstrak Etanol Daun Kemangi (Ocimum x africanum Lour.) terhadap Staphylococcus epidermidis. Sainstech Farma. 2021;14(2):87–90.
7. Pakadang SR, Waris MAA, Sari KA, Karim D. Perbandingan Karakteristik Potensi Antibakteri Ekstrak Daun Dan Bunga Kemangi (Ocimum sanctum L) Terhadap Propionibacterium acnes. Media Farm. 2022;18(1):60.
8. Davis WW, Stout TR. Disc Plate Method of Microbiological Antibiotic Assay. Appl Microbiol. 1971;22(4):659–65.
9. Septia R, Susanto A. Efektifitas Kombinasi Ekstrak Biji dan Daun Selasih (Ocimum basilicum L.) Sebagai Antiseptik Alami Terhadap Bakteri Pada Tangan. J Insa Cendekia. 2017;5(1):8–16.
10. Permadi, Susanti LA. Aktivitas Antibakteri Ekstrak Metanol Tanaman Kemangi (Ocimum sanctum) Terhadap Pertumbuhan Bakteri Bacillus subtillis. Akademi Farmasi Surabaya; 2021.
11. Khofifah A, Antara NS, Wartini NM. Pengaruh Jenis Pelarut Dan Waktu Maserasi Terhadap Ekstrak Daun Jarak Pagar (Jatropha cur-cas Linn) Dalam Menghambat Escherichia coli dan Staphylococcus aureus. J Rekayasa Dan Manaj Agroindustri. 2022;10(2):144.
12. Angga Prasetya IWG, Ganda Putra GP, Wrasiati LP. Pengaruh Jenis Pelarut dan Waktu Maserasi terhadap Ekstrak Kulit Biji Kakao (Theobroma cacao L.) sebagai Sumber Antioksidan. J Rekayasa Dan Manaj Agroindustri. 2020;8(1):150.
13. Widodo S, Yusa NM, Timur Ina P. Pengaruh Waktu Maserasi Terhadap Aktivitas Antioksidan Ekstrak Daun Mundu (Garcinia dulcis (Roxb.) Kurz). J Ilmu dan Teknol Pangan. 2021;10(1):14.

14. Ananta DA, Ganda Putra GP, Arnata IW. Pengaruh Suhu dan Waktu Maserasi terhadap Aktivitas Antioksidan Ekstrak Kulit Buah Kakao (Theobroma cacao L.). J Rekayasa Dan Manaj Agroindustri. 2021;9(2):186–97.
15. Ulfa K. Perbandingan Daya Hambat Ekstrak Etanol 70% Dan 96% Daun Kemangi (Ocimum sanctum L) Terhadap Pertumbuhan Escherichia coli ESBL [Internet]. Sekolah Tinggi Ilmu Kesehatan Nasional Surakarta; 2021. Available from: http://librepo.stikesnas.ac.id/id/eprint/769