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COMPARATIVE ANTIBACTERIAL ACTIVITY OF NUTMEG SEED AND *CINNAMOMUM BURMANII* STEM BARK EXTRACT AGAINST *STAPHYLOCOCCUS EPIDERMIDIS*

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ABSTRACT

Objective: This study was aimed to compare the antibacterial effect of nutmeg seed and *Cinnamomi burmanii* stem bark extract against *Staphylococcus epidermidis* ATCC 13228. **Methods:** Nutmeg seed and *C. burmanii* stem bark were macerated in the 70% ethanol as the solvent. Then both extract's secondary metabolites were analyzed using a standard method. The antibacterial activity of both samples extract was evaluated on the growth of *S. epidermidis* in vitro using the agar diffusion method. Then, minimum inhibitory concentration (MIC) values of both extracts were determined using the broth dilution. **Results:** *C. burmanii* stem bark extract demonstrated higher antibacterial potential than the nutmeg seed extract against *S. epidermidis* in the MIC/MBC values of range 0.15625 to 0.3125 %w/v for *C. burmanii* and 0.3125 to 0.625%w/v for nutmeg seed. **Conclusion:** *C. burmanii* stem bark extract was recommended as antibacterial agent for *S. epidemidis* infection.

KEYWORDS: *Cinnamomum Burmanii*, nutmeg, extract, antibacterial, *Staphylococcus epidermidis.*

INTRODUCTION

Staphylococcus epidermidis is a common Gram-positive, coagulase-negative bacteria found normally in all individual skin.[1] It is a bacterium that may colonize many parts of the human body, including the skin, nose, head, and mucous membrane. Under some situations, *S. epidermidis* acts as an opportunistic pathogen, worsening conditions in immunosuppressed patients such as those with implanted medical devices, chronically hospitalized, drug users, and those with acquired immunodeficiency syndrome.[2] This bacterium expresses polysaccharide intracellular adhesin (PIA), it is identified as a bacterial biofilm-forming organism. As a result, one of its virulence factors is PIA production, which is associated with opportunistic infections.[3] *S. epidermidis* is thought to cause 35% to 60% of infections in synthetic urinary sphincters and penile prosthesis.[4]

The rise of drug-resistant strains during the last few decades has provided a problem for the treatment and management of *S. epidermidis*. Three lineages of *S. epidermidis* of the multi-drug-resistant (MDR) clonal complex 2 have been found globally and adapted to hospitals. These lineages have a broad spectrum of antibiotic resistance, including β-lactams, rifampicin, and vancomycin.[5] Most clinical isolates are resistant to β-lactam antibiotics, and some even to glycopeptide antibiotics.[6,7] *S. epidermidis* produces penicillinases, enzymes that break the penicillin β-lactam ring, leading to antibiotic resistance.[8] As a result, there is an urgent need to discover novel and efficient treatments against resistant S. epidermidis strains. Recently, the global issue of bacterial resistance to synthetic antibiotics has prompted scientists to examine the use of antibiotic compounds found in nature, such as medicinal plants.[9]

Herbal medicine is currently used in the global health system and is becoming increasingly popular in industrialized countries.[10] Plant-derived compounds have been used as medicines for ages. These advantages are attributed to their high concentration of bioactive chemicals. Several medicinal plants have been reported to have therapeutic properties such as antiinfective, antioxidant, and anti-tumor activity. These therapeutic benefits are attributed to their bioactive component content.[11,12] Traditionally, herbal medicinal plants have been used as a treatment. Among these, herbs and spices are often regarded as safe therapeutic drugs that have been shown to be useful against specific illnesses.[13] Spices and herbs are some of the most often utilized antimicrobial ingredients in foods; they not only provide taste and pungent stimuli but also create antibacterial properties. Nutmeg seed (*Myristica fragrans*) is a

spice herb that has the potential to be employed as a natural antimicrobial agent in oral care products.[14] The ethanolic extract of nutmeg seeds showed antibacterial efficacy against enterohemorrhagic *Escherichia coli*, which is very susceptible to β-pinene.[15] Aside from nutmeg, *Cinnamomii burmanii* is also utilized in traditional medicine and the taste business.[3,4] Trans-cinnamaldehyde is one of the discovered bioactive compounds in *C. burmannii*.[8] Another research found that *C. burmannii* bark extracts had significant quantities of phenolics and shown strong antibacterial activity, with inhibitory effects greater against Gram positive bacteria than Gram negative bacteria. The researchers assumed that the antibacterial activity of *C. burmannii* bark extract was strongly connected to their phenolic component.[9] The goal of this study was to explore the antibacterial spectrum of nutmeg seed and *C. burmanii* ethanolic extract against *S. epidermidis*.

MATERIALS AND METHODS

Materials

The growth mediums utilized in this study were Mueller-Hinton Agar (MHA-Oxoid) and Mueller-Hinton Broth (MHB-Oxoid). The chemicals used are normal saline solution, barium chloride solution (Merck), sulfuric acid solution (Merck), Neomycin Sulfate as the standard, Neomycin Sulfate, Dragendorf reagents, Mayer reagent, Lieberman - Burchard reagent, vanillin (Merck), sulfuric acid solution (Merck), barium chloride solution (Merck), n-butanol, technical toluene (Brataco), ferric chloride reagent (Merck), and distilled water. *Staphylococcus epidermidis* ATCC 13228 was used as tested bacterium, obtained from Laboratory of Microbiology, Faculty of Pharmacy, Padjadjaran University. C. *burmannii* barks and nutmeg seed were collected from herbal center in Bandung, West Java, Indonesia. Both samples have been identified in the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjadjaran, Bandung Sumedang Km 21 Jatinangor Sumedang, West Java, Indonesia.

Extraction and Phytochemical Screening Analysis

I Kg g of each C. *burmannii* barks and nutmeg seeds were washed using clean water and dried. The dried samples were powdered and weighed then macerated with ethanol 70% as the solvent and the macerates were collected every 24 h for 3 d. The collected maserates were evaporated by a rotary evaporator in 40°C untill the thick extract achieved in a constantly weigh. The extracts were screened using Fansworth method to detect the content of

secondary metabolites such as alkaloids, polyphenols, flavonoids, tannins, quinone, triterpenoid, monoterpenoid, sesquiterpenoid, steroid, and saponins.[10]

Preparation of Bacterial Suspension

Preparation of *S. epidermidis* suspension was conducted by taking one Ose of *S. epidermidis* colony from slant agar, then suspended into sterile physiological saline. Bacterial turbidity was measured using a spectrophotometer and compared with a standard 0.5 Mc Farland.

Antibacterial Activity

The antibacterial activity of *C. burmannii* barks, nutmeg seed, and tetracycline HCl were tested utilizing the agar diffusion method using a perforation method. The ethanolic extract and Neomycin Sulfate were diluted to obtain evaluated test concentrations of 20-80 %b/v. A sterile petri plate was filled with 20µL of bacterial suspension, followed by 20 mL of MHA solution. The medium was homogenized, solidified, and then aseptically drilled using a perforator. Each hole is then filled with the extract and tetracycline at varying concentrations in 50 uL. All media tests were incubated at 37 °C for 18-24 h. The diameter of inhibition value was measured using a calliper.

Minimal Inhibitory Concentration (MIC) Determination

The minimal inhibitory concentration of *C. burmannii* bark ethanol extract was determined using microdilution. The extract was solubilized in dimethyl sulfoxide (DMSO) before being serially two-fold diluted in MHB medium to achieve concentrations ranging from 10 to 0.3125% w/v. The bacterial strains were suspended in sterile normal saline (0.9%) under aseptic conditions, homogenized, and adjusted to an optical density of 0.05 at 530 NM (corresponding to 1 X 10^6 CFU/mL). Each concentration was evaluated with 10 µL of standardized bacterial cell cultures. The tested medium was then incubated for 20 h at 37^0 C. The MIC was calculated using the lowest concentration of extract that produced no turbidity in the medium.

RESULTS AND DISCUSSION

Phytochemical Analysis Result

The phytochemical examination of *C. burmanii* stem bark and nutmeg seed extract revealed varying phytochemical concentrations, as shown in Table 1. The existence of numerous secondary metabolites, which may be responsible for their therapeutic properties, such as antibacterial activity.[11] The presence of such metabolites has been shown to have

antibacterial properties.[12] Several investigations have found that alkaloids, flavonoids, polyphenolics, tannins, saponins, quinones, and steroids in their extract are the metabolites responsible for antibacterial mechanism, mainly for anti-staphylococcal activity.[13-15]

Table 1: Phytochemical screening.

Detected Compounds	Results		
	Nutmeg seed		*C. burmanii*
Alkaloids	-		+
Flavonoids	+		+
Tannins	+		+
Polyphenolics	-		+
Monoterpenoids and Sesquiterpenoids	-		-
Steroids	+		+
Triterpenoids	+		-
Quinones	+		-
Saponins	-		+

Notes: (+) = presence; (-) = absence

Antibacterial Activity Result

C. burmanii extract has a higher antibacterial activity against *S. epidermidis* than nutmeg seed extract or neomycin sulphate. However, in comparison with neomycin, as seen in Table 2. There was a strong correlation between extract concentrations and inhibitory diameters. The ability of *C. burmannii* barks to limit *S. epidermidis* growth showed antibacterial activity, which might be used to treat Staphylococcal infections, mainly *S. epidermidis*.

Table 2: Antibacterial Activity.

Extract concentration (%w/v)	Inhibitory diameter of *S. epidermidis* ATCC 13228 (mm)		
	C. burmanii	*Nutmeg seed*	Neomycin Sulfate
80	19,50±0.0100	14,20±0.0001	19,50±0.0001
60	18,55±0.0025	13,00±0.0000	16,55±0.0001
40	17,95±0.0025	12,70±0.0001	14,70±0.0001
20	16,30±0.0025	12,20±0.0000	13,20±0.0001

Note: Perforator diameter = 9 mm

MIC Result

It was established what the minimum inhibitory concentration (MIC) of the examined extracts needed to inhibit the growth of *S. epidermidis*. The MIC value, which was determined by broth dilution procedures, was displayed in Table 3 and demonstrated the significant antibacterial activity of the ethanol extracts against the microorganisms under

study. The nutmeg seed extract did not exhibit the same antibacterial activity against *S. epidermidis* as the stem bark extract of *C. burmanii* (MIC/MBC values: 0.3125 to 0.625%w/v for nutmeg seed, 0.156250 to 0.3125%w/v for *C. burmanii*). The antibacterial qualities of this extract can add to our understanding of herbs that can be used to treat *S. epidermidis*.

Table 3: MIC Result.

Extract Concentration (%w/v)	Turbidity	
	Nutmeg	*C. burmanii*
10	+	-
5	+	-
2.5	+	-
1.25	-	-
0.625	-	-
0.3125	-	-
0.15625	-	+

Notes: (+) = Turbid; (–) = Clear

CONCLUSION

It was shown that the ethanolic extract of *C. burmanii* stem bark had significant antibacterial activity against *S. epidermidis*.

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