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Effect of mangrove leaves extract (avicennia marina) concentration on the growth of streptococcus mutans and candida albicans

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Abstract

Objective: This study aims to determine the effect of mangrove leaves extract (avicennia marina) concentration on the growth of Streptococcus mutans and Candida albicans which are commonly found in removable denture plates.

Material and Methods: Production of mangrove leaves extract (avicennia marina) is carried out using 96% ethanol solution. Mangrove leaves extract was prepared at concentrations of 2.5%, 5%, 7.5%, and 10% and controlled with denture cleanser materials available in the market. Furthermore, the bacteria activity of streptococcus mutans and candida albicans (ATCC 10231) were tested, then the data were analyzed by one-way ANOVA and post hoc test (Tukey HSD).

Results: Statistical analysis of mangrove leaves extract with 2.5% concentration (6.850 ± 0.127), 5% (7.185 ± 0.007), 7.5% (8.360 ± 0.283), 10% (8.485 ± 0.219), and positive control (9.060 ±0.226) showed significant inhibition zone differences ($p < 0.05$). The extract with concentration of 7.5% had optimal inhibition growth of streptococcus mutans ($p < 0.05$). Mangrove leaves' extract at 10% concentration had not shown inhibitory effects against candida albicans.

Conclusion: Mangrove leaves extract (avicennia marina) is effective for inhibiting the growth of streptococcus mutans, but has not shown inhibitory effects against candida albicans until it reached a concentration level of 10%.

Keywords: Candida albicans, Extract, Mangrove, Streptococcus mutans

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Introduction

Denture stomatitis is often a problem in removable dentures, which is caused by pathogenic microorganisms, particularly streptococcus mutans and candida albicans, that easily attach themselves to removable denture plates, Denture stomatitis consists of mild inflammation and mucosal erythema that occur in removable denture base, and usually also on maxillary, completely removable denture. This condition usually occurs without symptoms, but when symptoms do appear there are many of them, including burning sensation, bleeding of mucous, and dryness in the mouth. The etiology is however considered multifactorial, including trauma, candida albicans, allergies, systemic adverse conditions, surface texture, and permeability of the denture base and the layer of material, which are regarded as some of the main factors causing denture stomatitis. Dentures can generate a number of changes in the ecology of the oral cavity by accumulating microbial plaque on the surface of the denture base.[1]

Another factor that influences biofilm development is the presence of an acquired pellicle (AP) on the denture material surface. The AP is a conditioning film formed immediately after the substratum is exposed to the oral environment by the selective adsorption of peptides and proteins from the saliva. The presence of a saliva pellicle can alter the substratum properties, such as the surface free energy, because the film composition changes the surface reactivity, which provides different receptor sites for the adherence of microorganisms.[2] At the beginning of the formation of salivary pellicle, gram-positive bacteria streptococcus sp. becomes the first bacteria that gets attached to the denture base and forms colonies. One such bacteria is streptococcus mutans.[3] Streptococcus mutans produces extracellular polysaccharide (PSE) that is not owned by other bacteria. The substrate is an access for bacteria and other fungi to adhere to denture base. Bacteria and fungi will proliferate into plaques. These plaques cause denture stomatitis.[4]

Denture stomatitis treatment involves steps such as oral and denture base hygiene, avoiding the use of denture overnight, putting dentures into substances such as chlorhexidine or sodium hypochlorite and antifungal therapy. While asking how to clean dentures better, it must always be based on the data of patient's earlier dental health and devise methods using that data for maintaining the dental hygiene.[5]

Denture cleaning can be done either mechanically or chemically. Mechanical cleaning is done by using toothbrush and ultrasonic. Chemical cleaning can be done by immersing dentures in disinfectant solutions, alkaline peroxide, alkali hypochlorite, chlorhexidine, sodium hypochlorite, enzymes, and herbs.

The place plants are fertile with various biologically active compounds such as alkaloids, flavo-noids, lignins, phenols, sterols, saponins, tannins and terpenes. These metabolites are the major sources of antibacterial, antioxidant and anticancer agents.[6] Plants serve as a reservoir of effective chemotherapeutants and provide valuable sources of natural products in control of several bacterial and fungal diseases.[7,8] Antibiotics are the most important weapons to fight against bacterial infections. Many pathogenic organisms are developing plasmid-mediated resistance to popular drugs. Hence, there is a need for the isolation of novel compounds either from microorganisms or from plants. The potential of mangrove plants as a source of new therapeutic agents is still unexplored. The present study is paying attention to the exploitation of leaves of avicennia marina L for extraction in different organic solvents to screen for antioxidant activity by ABTS method and antibacterial activity by agar well diffusion method.[9] Avicennia marina, avicennia alba, acanthus ilicifolius, ceriops decandra and excoecaria agallocha are found to have better potential given their high antimicrobial properties than others. On the other hand, acanthus ilicifolius and avicennia marina are two mangroves whose leaf extracts were most active in lowest concentrations, indicating the presence of high amount of secondary metabolites and other active components acting synergistically.[10] The ability of mangrove has been proven; therefore, we were interested in determining whether there was an effect of mangrove leaves extract concentration (avicennia marina) to inhibit the growth of streptococcus mutans and candida albicans.

Material and methods

This research was conducted in September–October 2016. Samples of avicennia marina mangrove leaves were taken from Putondo Beach Tourism in the southern coastal village of Cikoang, District Mangarabombang, Takalar, Indonesia. The subject of this study was streptococcus mutans (it was directly brought to Microbiology Laboratory, Pharmacy Faculty of Hasanuddin University) and candida albicans (ATCC 10231), using a denture cleanser (antibacterial and antifungal) on the plate as a positive control. The research was conducted at the Laboratory of Phytochemistry and Microbiology of the Faculty of Pharmacy Hasanuddin University, Makassar Indonesia. This work is an analytical laboratory experimental research. This study used post-test only with control group design.

Mangrove leaves extract (avicennia marina) obtained by maceration of 1 kg mangrove leaves inserted into maceration container, soaked in 2 liters of 96% ethanol, stirred and allowed to stand for 3 × 24 hours and then filtered to produce filtrate, which was collected and evaporated with a rotary evaporator to obtain a thick extract of mangrove leaves. Using Minimal Inhibitory Concentration (MIC), 0.25%, 0.5%, 0.75%, 1%, 2.5% and 5% concentrations of mangrove leaves extract (avicennia marina) were prepared. Determination of MIC in streptococcus mutans was made using SB (sodium broth), the broth was incubated 37°C for 1×24 hours; while for Candida albicans potato dextrose broth (PDB) was used and the broth was incubated at ±28°C for 2×24 hours; observations were made by looking at the turbidity.

To determine the effect of mangrove leaves extract's (at a concentration of 2.5%, 5%, 10%, 15% and a positive control) on streptococcus mutans and candida albicans, the extract was poured into a paper disc and inserted into a petri dish containing Muller Hilton Agar (MHA) and incubated at 37°C for 1 × 24 hours for streptococcus mutans and PDA, and incubated at ± 28°C for 2×24 hours for candida albicans. The observation made was made by looking at the inhibition zone in the area around the paper disc. Analysis of data used one-way ANOVA statistical tests and Tukey's HSD and SPSS 20.0 version (SPSS Inc., Chicago, IL, USA).

Results

MIC Test

The MIC at 5% concentration showed no growth of streptococcus mutans figure 1, but there was growth of candida albicans, which was identified from the level of turbidity figure 2.

Activity Test

One-way ANOVA statistical test of results derived for streptococcus mutans table 1 showed the smallest zone of inhibition at 2.5% concentration (6.850 ± 0.127 mm) and the largest zone of inhibition was found at 10% concentrations (8.485 ± 0.219 mm) and the positive control was 9.060 ± 0.226 mm figure 3. Differences in inhibition zone between concentration and controls were significant ($p = 0.000$; $p < 0.05$).

The results show that there was no inhibition zone of mangrove leaves extract in candida albicans

Table 1 Differences in the inhibition zones of various concentrations of mangrove leaves extract (avicennia marina) to streptococcus mutans

Intervention	Concentration	Inhibition Zone		p-value
		n(%)	Mean ± SD	
Mangrove leaves	2.5%	2 (20%)	6.850 ± 0.127	0.000*
	5%	2 (20%)	7.185 ± 0.007	
	7.5%	2 (20%)	8.360 ± 0.283	
	10%	2 (20%)	8.485 ± 0.219	
Positive control		2 (20%)	9.060 ± 0.226	
Total		10 (100%)	7.988 ± 0.890	

*One-way Anova test; p<0,05: significant

Table 2 Differences in the inhibition zones of various concentrations of mangrove leaves extract (avicennia marina) to candida albicans

Intervention	Concentration	Inhibition Zone		p-value
		n(%)	Mean ± SD	
Mangrove leaves	2.5%	2 (20%)	0.000 ± 0.000	
	5%	2 (20%)	0.000 ± 0.000	
	7.5%	2 (20%)	0.000 ± 0.000	
	10%	2 (20%)	0.000 ± 0.000	
Positive control		2 (20%)	0.000 ± 0.000	
Total		10 (100%)	0.000 ± 0.000	

*One-way ANOVA test; $p < 0.05$: significant

Table 3 Post hoc test of inhibition zones of mangrove leaves extract (avicennia marina) on streptococcus mutans

Intervention (i)	Comparison (j)	Mean Difference (i-j)	*p* Value
Mangrove leaves 2.5%	Mangrove leaves 5%	-0.335	0.509
	Mangrove leaves 7.5%	-1.510	0.003*
	Mangrove leaves 10%	-1.635	0.002*
	Positive Control	-2.210	0.001*
Mangrove leaves 5%	Mangrove leaves 7.5%	-1.175	0.010*
	Mangrove leaves 10%	-1.300	0.006*
	Positive Control	-1.875	0.001*
Mangrove leaves 7.5%	Mangrove leaves 10%	-0.125	0.963
	Positive Control	-0.700	0.079
Mangrove leaves 10%	Positive Control	-0.575	0.149

until 10% concentration, which also occurred in positive control table 2.

Results of post hoc Tukey's HSD for inhibition zone of streptococcus mutans between each concentration and positive control table 3 also show that there is no significant difference between 2.5% and 5% concentrations, between 7.5% and 10%, and also in 7.5% concentration and in the positive control. Therefore, 7.5% is considered as the concentration with optimum inhibition effects.

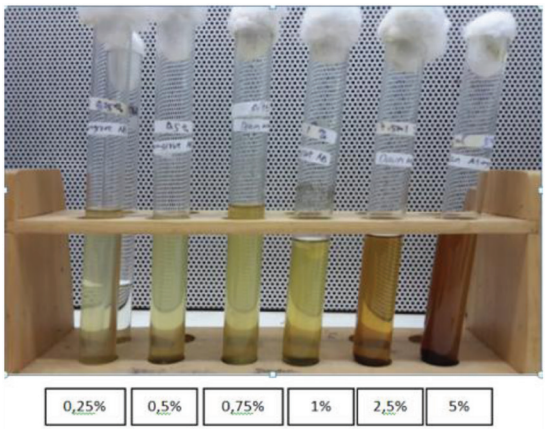

Figure 1 MIC of mangrove leaves extract (avicennia marina) on the growth of streptococcus mutans

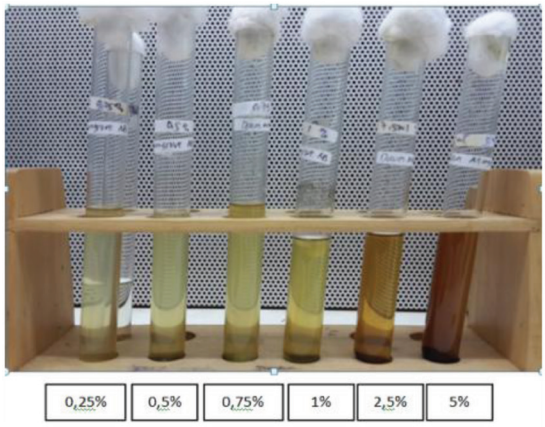

Figure 2 MIC of mangrove leaves extract (avicennia marina) on the growth of candida albicans

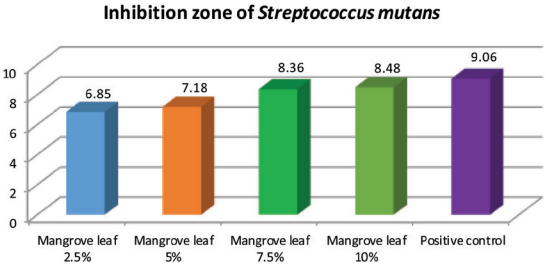

Figure 3 Inhibition zone of mangrove leaves extract (avicennia marina) on streptococcus mutans

Discussion

The analysis of this research showed that the highest inhibitory effectiveness of mangrove leaves extract (avicennia marina) to streptococcus mutans was found at 10% concentration and the lowest was

at 2.5% concentration. The contents of the active compound were alkaloids, flavonoids, terpenoids, steroids, and saponins. These compounds are common ingredients used in modern medicine.[11]

Most often ethanolic extracts are considered as more active than aqueous extracts, probably because biologically active substances are better extracted in this solvent. Ethanol is the most commonly used organic solvent, as the finished products are relatively safer to use. Moreover, nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds and most often obtained through initial ethanol or methanol extraction.[12] The ethanol extract of the plant was found to scavenge superoxide and hydroxyl radicals. The extract was also found to inhibit the generation of nitric oxide radical and lipid peroxides. Recent studies have shown that the plant extract has a remarkable hepato-protective effect. The flavonoids present in the plant were found to have hepato-protective and antioxidant capacities.[13]

Owing to the widespread ability of flavonoids to inhibit spore germination of plant pathogens, they have been proposed against fungal pathogens of humans and were shown to possess capacity to inhibit the growth of the opportunistic pathogens such as candida albicans. Flavonoids have bacteriostatic properties, and at higher concentrations flavonoid were found to be able to kill both gram-negative and gram-positive bacteria.[14]

Alkaloids function as either hydrogen-acceptor or hydrogen-donor in hydrogen bonding critical for the interaction between targets; thus, they potentially affect the curative agents on diseases. Alkaloid has antibacterial ability. The mechanism of alkaloid is that it interferes with peptidogly that enables the constitution of the components of the bacterial cell, so the cell-wall layers are not fully formed and cause the death of these cells. In alkaloids, there is also a group of nitrogen base that reacts with amino acid compounds that make up the cell walls of bacteria and bacterial DNA. This reaction results in changes in the structure and composition of amino acids, which leads to changes in the genetic balance in the DNA chain; thus, the cell is damaged and promotes bacterial cell lysis, causing the death of bacterial cells.[15]

Terpenoids are also referred as terpenes and are the largest group of compounds. Many terpenes have biological activities that can be used for the treatment of human diseases. Terpenoids have a very broad range of biological activities. To review all the biologically active terpenoids would be a difficult task. Owing to space constraints, we will focus on terpenoids with activities against cancer, malaria, inflammation, and a variety of infectious diseases (viral and bacterial). Other chemotherapeutic agents for this diseases have been the subject of many excellent reviews.[16]

Tannin causes denaturation of proteins by forming complexes with proteins through the nonspecific power such as hydrogen bonding and hydrophobic effects such as the formation of covalent bonds, inactivation of microbial adhesion to molecule in attaching to host cells, and stimulation of phagocytic cells that play a role in cellular immune response. Tannins not only heal burns and arrest bleeding but also curtail infection while they continue to heal the wound internally. The ability of tannins to form a protective layer over the exposed tissue keeps the wound from getting further infected. Tannins are also beneficial when applied to the mucosal lining of the mouth.[17]

Saponins are soluble in water and insoluble in ether, and like glycosides on hydrolysis, they can also yield aglycones. Saponins are extremely poisonous, as they cause hemolysis of blood and are known to cause cattle poisoning. Saponins work as an antibacterial agent to destabilize the bacterial cell membranes causing lysis of bacterial cell; therefore, the action mechanism of saponin includes the formation of antibacterial group which disrupts the permeability of bacterial cell membrane, resulting in the damage of cell membranes and causing release of various important components in bacterial cells, that is, proteins, nucleic acids, and nucleotides.[18]

In this study, mangrove leaves extract at 2.5% concentration have already provided significant results; out of the all the concentration levels, it is the 7.5% concentration that yielded optimum inhibition of streptococcus mutans.

Unlike inhibition of candida albicans, the results of this study showed no inhibitory zones of mangrove leaf extracts (avicennia marina) on candida albicans up to 10% concentration; it also occurred in positive controls. The candida albicans biofilm cells that grow on denture acrylic are sensitive to be killed by Hst 5 (the N-terminal 24 amino acid segment of Histatin 3 peptide). Surface-coating acrylic with chlorhexidine or Hst 5 effectively inhibits biofilm growth and has potential therapeutic application.[19] This is due to the highly complex mechanisms of candida albicans infection, including adhesion and invasion. Changes include changes in cell morphology/shape from yeast to filament (hifa), biofilm formation, and immune cell evacuation to the host. The ability of candida albicans to adhere to host cells is an important factor in the early stages of colonization and infection. Phenotypic changes to form filaments allow candida albicans to penetrate the epithelium and play a role in infection and spread to host cells. Candida albicans can also

form biofilms that are believed to be involved in attacks on host cells and associated with antifungal resistance. Therefore, although the basic components of candida albicans cell wall are similar in yeast and filament forms, surface proteom and number of individuals differ. PAMP present in immune cells differs substantially. In addition, conditions in which the cells grow can cause substantial changes in the cell wall, even if cell morphology is unchanged. Due to its highly ordered and responsive nature, the cell wall is a moving target that poses significant challenges to the host's immune system.[20] It is therefore recommended to do further research on mangrove leaf extract (avicenia marina) with concentration levels exceeding 10%.

Conclusion

Mangrove leaves extract (avicennia marina) at 7.5% concentration can yield optimum inhibitory effects on the growth of streptococcus mutans. Mangrove leaves extract (avicennia marina) even at 10% concentration cannot inhibit candida albicans.

Conflict of Interest

The authors report no conflict of interest.

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