

Phytochemical and comparative study of anti microbial activity of *Lepisanthes amoena* leaves extract

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Abstract

The genus *Lepisanthes* belongs to one of the groups of plant family Sapindaceae. A number of species from this genus are widely used in traditional and folk medicine systems in different parts of the world. *Lepisanthes amoena* extract was obtained from the leaves by extracting from methanol and 95% ethanol solvent. The highest yield of *Lepisanthes amoena* extract was found in methanol extract (8.75%). This study was designed to test the antimicrobial potential of *Lepisanthes amoena* (Sapindaceae) leaves against selected oral and skin pathogen, such as *Streptococcus mutans*, *Candida albicans* and *Propionium acnes*. A qualitative phytochemical analysis was performed for the detection of alkaloids, terpenoids, steroids, flavonoids, saponin and carbohydrate. The antimicrobial activity were tested by agar well diffusion method. Almost all extracts exhibited good inhibitory effect against tested pathogens. The expression level of inhibitory effect on the growth of tested organism was reduced by increasing the concentration of extract. The ethanol extract of *Lepisanthes amoena* leaves exerted greater antimicrobial activity than corresponding methanol extract at the same concentrations The zones of inhibition produced by ethanol extracts against test organisms were in the order: *Propionium acnes* > *Streptococcus mutans* > *Candida albicans*, while methanol extracts were *Streptococcus mutans* > *Candida albicans* > *Propionium acnes*. *Lepisanthes amoena* have been found to be effective against pathogenic microorganisms involved in oral and skin infections. The result of the study justified the use of the plant in the treatment of diseases of microbial origin in herbal medicine.

Keywords: *Lepisanthes amoena*, phytochemical, antimicrobial

1. Introduction

There are many plants that have been reported to possess antibacterial activity towards. Among them is come from Sapindaceae family. Sapindaceae are one of the most important forest species to be conserved and valued in Indonesia due to their medicinal value and multiple uses. They exist as trees and shrubs, and tendril-bearing vines with about 140 to 150 genera and 1400 to 2000 species worldwide (Adeyemi *et al.*, 2011). They are geographically distributed in the temperate and tropical regions of the world. Some plants from this family, such as *Sapindus mukorossi*, *Dodonaea viscosa*, *Allophylus africanus*, *Sapindus trifoliatus* and *Paullinia pinnata* have been used traditionally as oral and skin health care. (Dhar *et al.*, 1989; Chabra *et al.*, 1991; Pirzada *et. al.*, 2010; Parganiha *et al.*, 2011; Rao *et al.*, 2012). One of species in this family that found in East Kalimantan (Indonesia) is *Lepisanthes amoena*. This plant distributes in Indonesia (Java, Sumatra, Kalimantan, Lesser Sunda Islands), Malaysia (Peninsular, Sabah, Sarawak), Brunei and sometimes cultivated as an ornament. The fruits are edible but astringent and the wood is very hard. (Verheij *et al.* , 1992). The plant has a variety of traditional uses, such as utilized in folklores medicine in East Kalimantan by Dayak's tribe for the treatment of facial skin. They scrub the young leaves until the foam appear and paste to their facial skin.

In this regard, the present work focused on providing scientific information on the phytochemical composition and antimicrobial activity of methanol and ethanol extracts of *Lepisanthes amoena* leaves on oral and skin pathogens

such as *Candida albicans*, *Streptococcus mutans*, and *Propionium acnes*.

2 Material and Method

1.1 Plant material

The study was carried out on the leaves of *Lepisanthes amoena*. The sample was obtained from East Kalimantan and was identified by a botanist from Forestry Faculty of Mulawarman University, Indonesia. The old leaves were picked from stem and air dried over a period of two weeks. 100 g each of the dried leaves were used for the extraction.

1.2 Preparation of Leaves Extracts

The dried leaves were crushed to coarsely powder. One hundred gram of leaves powdered was macerate with methanol and 95% ethanol, respectively. The extract solution were filtered with filter paper and evaporated using rotary evaporator to yield solid extract. Each of the plant extracts was dissolved in acetone to obtain the stock solution of sample extract at concentration 10 mg/ml (w/v). The sample extracts were reconstituted in acetone for the bioassay analysis.

1.3 Preliminary phytochemical screening

The chemical tests were performed for testing different chemical groups present in extracts. For the presence of alkaloids, the extracts were tested with the alkaloidal reagents (Dragendorff's). Frothing test for saponins and NaOH and conc. HCl tests for flavonoids were carried out. Lieberman-Burchard's test was applied for detecting steroidal/triterpenoidal nucleus, while the presence of carbohydrate was tested with Molisch reagent solution. (Dey and Harborne, 1987; Trease and Evans, 1989).

1.4 Microbial samples

The bacterial and fungal cultures were obtained from the microbial culture collection obtained from the laboratory stock of the Forest Product Chemistry, Forestry Faculty of Mulawarman University, East Kalimantan, Indonesia. The following microorganisms were used for the study : *Streptococcus mutans*, *Candida albicans* and *Propionium acnes*.

1.5 Culture media and antibiotic

Nutrient Agar media was used for the growth of bacterial and for fungi. Chloramphenicol was used as the standard antibiotic.

1.6 Antimicrobial activity

Microorganisms were inoculated into Nutrient broth in test tubes and grown to stationary culture for 24 h at 37°C. Antimicrobial activity of various extracts was determined by the agar well diffusion method. In this method, isolate of each microbe was sub cultured on the media for each microorganism at 37°C for 24h. A plate of each microorganism was taken with a sterile loop and transferred into normal saline (0.9%) under aseptic conditions. Density of each microbial suspension was adjusted equal to that of 10⁶ cells/ml (standardized by 0.5 McFarland standard) and used as the inoculum for performing agar well diffusion assay. The culture plates were prepared by pouring 20 ml of Nutrient agar medium into sterile petri plates. The 100µl of inoculum test microorganism were then swabbed over the agar media using sterile cotton swabs to get uniform distribution of the bacterial cultures. The agar plates were allowed to dry and wells of 8 mm were made with a sterile borer in the inoculated agar plates. The wells were filled with the sample extracts.

A series volume of each extract was propelled directly into the wells of the inoculated specific media agar plates for

each test organism, to get 100 µg, 200 µg and 300 µg concentrates per well. The two control wells received 10 µl acetone (negative control) and chloramphenicol (0.5 mg/ml) dissolved in acetone (positive control). The plates were allowed to stand for 10 minutes for diffusion of the extract to take place and incubated at 37°C for 24h. Sterile acetone served as the negative control and chloramphenicol served as the positive control.

The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone of inhibition was greater than 8 mm. The experiments were performed in triplicates and the mean values of the diameter of inhibition zones were calculated.

3 Result

Percentage yield of the powdered *Lepisanthes amoena* leaves crude extracts obtained using different solvents is shown in Table 1.

The percentage yield of methanol soluble extract and ethanol soluble extract of *L. amoena* leaves was 8.75% and 5.83% respectively. The highest yield was obtained in methanol extracts. The phytochemical screening of the *Lepisanthes amoena* leaves revealed the secondary metabolites which are of medicinal interest as presented in Table 2. The preliminary phytochemical studies revealed the presence of alkaloid, flavonoid, carbohydrate, steroid, triterpenoid and saponins.

From the table 2, it shows that methanol and ethanol (95%) extracted some of more polar compounds. This can be seen by the presence of flavonoids and saponin. Triterpenoid are absent in both extract. The results of the antibacterial activity of the methanol and ethanol extracts of the leaves of *Lepisanthes amoena* against *Streptococcus mutans*, *Candida albicans* and *Propionium acnes* are shown in Table 3. The negative control (acetone) did not produce any inhibitory activity against the test organisms.

The zones of inhibition produced by methanol extracts against test organisms were in the order: *Propionium acnes* > *Streptococcus mutans* > *Candida albicans*. The zones of inhibition produced by ethanol extracts against test organisms were in the order: *Streptococcus mutans* > *Candida albicans* > *Propionium acnes*. The ethanol extract was more active against all the microorganisms tested except against than the methanol extract. The zones of inhibition produced by 300µg/well of ethanol extracts against the *Candida albicans* and *Streptococcus mutans* were much higher (16.1 mm and 14.8 mm, respectively). The activity of both extracts against the bacterial strains used were, however, lower than that of chloramphenicol as control positive.

4 Discussion

Percentage yield of the powdered *Lepisanthes amoena* leaves crude extracts are varied depending on the solvent used. The percentage yield of methanol soluble extract and ethanol soluble extract of *Lepisanthes amoena* leaves was 8.75% and 5.83% respectively. Differences in the yield of extracts from *Lepisanthes amoena* leaves might be ascribed to the differing polarities of the solvents used.

Polarity of the solvent used for extraction may influence the availability of extractable components of varying nature defined by their chemical composition from the plant material. The presence of phytochemical in the leaves of *Lepisanthes amoena* are summarized in Table 2. From the table 2, it shows that the leaves contain alkaloids, saponins, flavanoids and steroids. Phytochemical screening showed that methanol and ethanol (95%) extracted some of the more polar compounds. This can be seen by the presence of saponins and flavonoids in the leaves extract.

The presence of carbohydrates in extracts seems to indicate that the leaves could be exploited as source for edible food or raw materials for industries that utilize carbohydrates to produce food or drugs (Nnamdi *et. al.*, 2010).

There are various reports on phytochemical present in the genus *Lepisanthes* in the literature (Saburi *et. al.*, 1999; Din *et. al.*, 2002). But this was the first report on the qualitative phytochemical analysis present in *Lepisanthes amoena* and its comparative antimicroorganism

The antimicroorganism assays in this study were performed by the agar well-difussion methods. The *in vitro* antimicroorganism activities of the crude plant extracts of are shown in Table 2. The results of the antimicrobial activity presented in Table 3 shows that all extracts exhibited appreciable antimicroorganism properties inhibiting the

growth of all pathogen. The susceptibility of the microorganism to the crude extracts on the basis of zones of growth inhibition varied according to extracting solvent. This has been reported by some researcher that extraction with different solvents affect antimicrobial activity (Kuspradini, 2010, Tatiya, 2011, Bakht, 2012). In this investigation most of the sample in this study showed inhibition of the growth test microorganism. The variation in the effectiveness of the extract against different microorganisms might be depends upon the chemical composition of the extracts and membrane permeability of the microbes for the chemicals and their metabolism. The antimicrobial activities of these crude extracts were due to the presence secondary metabolites or compounds like saponins, flavonoids, steroids, alkaloids this has already been confirmed by many researchers. It has been documented that flavonoid, saponins and alkaloids are plants metabolites well known for antimicrobial activity (Tschesche, 1971; El-Mahmood, 2009; Kuspradini, 2009)

The anti microorganism evaluation of the leaves of *Lepisanthes amoena* showed that the ethanol extract was more active than the methanol extract against all the microorganisms tested, except *Propionium acnes* at concentration 30mg/ml. In our previous study, the ethanolic extracts from Indonesian plants also could inhibit the *Streptococcus sobrinus* bacteria and its correlated enzyme (Kuspradini, 2007; Kuspradini, 2009). The inhibition zones of methanol extract and ethanol extracts were ranging from 8 – 12.4 mm and 10.3 – 13.1 mm, respectively. This finding implies that the active compound have polar characteristics. Chloramfenicol which served as positive control produce zone inhibition measuring range at 19.6 mm – 25.3 mm. Highest activity was demonstrated by the control standard antibiotic (chloramfenicol), while the negative control (acetone) produced no observable zone. The high activity of chloramfenicol is because the antibiotic is in pure state and has refined processes that have established it as a standard antibiotic (Abubakar, 2009). Their effects on the growth of all tested microorganism were most likely due to the release of chemicals from the crude extracts into the medium. The different reactions of tested microorganism to the different extracts indicated that each solvent extracted different chemical components of plants.

The highest zone of inhibitions (16mm) noted in ethanol extract against *Candida albicans* in 100µg concentrates. The ethanol extract of *Lepisanthes amoena* leaves exerted greater antibacterial activity than corresponding methanol extract (Tables 3) at the same concentrations. These observations may be attributed to the nature of biological active components (alkaloids, flavonoids, and saponins) which could be enhanced in the presence of ethanol.

Taking into consideration with the presence of various phytochemicals in the leaves of *Lepisanthes amoena*, antimicroorganisms activity in the plant extracts can be attributed to at least one of the compounds. The extracts of the plants proved active against *Streptococcus mutans*, *Candida albicans* and *Propionium acnes*. Thus, this plant can be used in the treatment of the ailments involved in oral and skin infections. It may, therefore, be concluded from the above investigation that the crude extracts obtained from the leaves of *Lepisanthes amoena* may be used to treat the bacterial oral infections caused by *Streptococcus mutans* and the bacterial skin infection caused by *Propionium acnes* which has shown comparable inhibition zone with the standard antibiotic drugs used to treat skin and oral infections, and also the fungal oral and skin pathogens especially *Candida albicans* which has shown greater inhibition zones.

These findings confirmed that the leaves may have potential use in pharmaceutical, cosmetic, and food products. *Lepisanthes amoena* are potential medicinal plants from the phytochemical screening and antimicrobial activities. Toxicity investigation of these plants with isolation, identification, characterization and elucidation of bioactive compounds are ongoing and will be communicated later. Therefore, the findings are of the great impact in going further in research relevance.

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Tables

Table 1. Extraction yields of methanolic and ethanolic extracts of *L. amoena* leaves

No.	Solvent	Yield (%)
1.	Methanol	8.75
2.	95% Ethanol	5.83

Table 2. Preliminary phytochemical constituents of the *Lepisanthes amoena* leaves

No	Constituents tested for	Observation	
		methanol	ethanol
1.	Alkaloid	(+)	(+)
2.	Flavonoid	(+)	(+)
3.	Carbohydrat	(+)	(+)
4.	Steroid	(+)	(+)
5.	Triterpenoid	(-)	(-)
6.	Saponin	(+)	(+)

Table 3. Antibacterial activity of *Lepisanthes amoena* leaves using well diffusion method

No.	Test microorganism	Concentrate/well (μg)	Activity (μl)**	
			methanol	ethanol
1.	<i>S. mutans</i>	100	8.8	11.8
		200	9.4	13
		300	11.3	13.1
		Positive control*		19.6
2.	<i>Candida albicans</i>	100	7.4	10.8
		200	8	11
		300	9.3	12.7
		Positive control*		22.3
3.	<i>Propionium acnes</i>	100	10.2	10.3
		200	10.8	11.5
		300	12.4	11.8
		Positive control*		25.3

* Chloramphenicol (5 μg)

**Diameter in mm along with well diameter

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