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Antimicrobial and toxicity tests of flavonoid total *Dendrophthoe pentandra* (L) miq from false ashoke tree (*Polyalthia longifolia*)

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Abstract:

Research on antimicrobial and toxicity tests of flavonoid total *Dendrophthoe pentandra* (L) Miq from false ashoke tree (*Polyalthia longifolia*) have been done. This study aims to determine the activity of toxicity of total flavonoid of *Dendrophthoe pentandra* (L) Miq as mistletoe of false ashoke tree (*P. longifolia*) based on Brine Shrimp Lethality Test (BSLT) method using *Artemia salina* Leach shrimp larvae obtained Lethal Concentration (LC_{50}) of 30.06 mg / L which means strong toxic activity. Antimicrobial test of flavonoid total has used the diffusion method in order to obtain the inhibit zone diameter on *Streptococcus mutans* at the total flavonoids concentration of 3%, 6% and 9% were 6; 9 and 17.25 mm, in *Escherichia coli* is 3.55; 4.25 and 9.15 mm and at *Candida albicans* is 8.30; 4 and 5.30 mm where *S. mutans* and *E. coli* are greater but *C. albican*s less affect but still have good inhibitory activity so effective to be developed as antimicrobial agents.

Keywords:

false ashoke leaves, antimicrobial, toxicity, diffusion and BSLT

Introduction

As a biodiversity mega country, Indonesia has a wealth of biodiversity. A number of studies were conducted to examine the potential of plants in Indonesia as raw materials of the drug. There are approximately 7000 species of plants including medicinal plants of ± 28.000 species of plants that can be found in Indonesia. Medicinal plants are groups of plants that can be used as medicine or raw materials of medicine. Utilization of medicinal plants is usually in the form of simplicia from plant parts such as roots, stems, leaves, and fruit or seeds (Fatmawati, 2008).

Parasitic plants are also known as medicinal plants called with mistletoe or in Indonesia called Benalu. The mistletoe from coffee tree is usually used to treat measles. The mistletoe from lime tree is used as a medicinal herb for tonsil diseases, while mistletoe of tea and mango reported can be used as a cure for cancer (Purnomo, 2000). Clinical effects on mistletoes are thought to be due to the presence of bioactive compounds contained inside of amino acids, carbohydrates, flavonoids, alkaloids, and saponins that can neutralize the effects of toxic substances thereby reducing cell damage (Pitojo, 1996).

The trees are plenty to see as plants for noise pollution dampers and have benefits as medicinal plants for skin diseases, fever, hypertension and helmenthiasis (Rastogi et al., 1995). Several recent studies have shown that *P. longifolia* trees function as antidiabetic drugs (Lakshmi et al., 2011), anti-ulcer (Malairajan et al, 2008) and effective for Hela and MCF-7 cells (Manjula et al., 2010). Mistletoe on *Polyalthia longifolia* trees can be meet on their branchs. For the mistletoe of that trees, no one has tested it's activity as medicinal plants. Therefore, the authors wish to examine the toxicity and antimicrobial activity of the total flavonoids of mistletoe leaves of *P. longifolia* trees and their phytochemical screening.

As we know from the family, P. Longifolia is one family of Annonaceae which is family of soursop tree and nona tree which have been tested have antimicrobial activity and high toxicity because it has content of active compounds which is as phytopharmaca drug.

Materials and Methods

Materials

Mistletoe Leaves of *P. longifolia* tree, Methanol pa Merck, Brataco Technical Methanol, Technical N Hexana , Technical Ethyl Acetate, Mayer Reagent, Dragondorf Reagent, Wagner/ Bouchardat Reagent, Aquadest, DPPH p.a Sigma Aldrich, $FeCl_3$ p.a Merck, DMSO p.a Merck, *Artemia salina* Cyst , Aquadest, Sea Salt Packaging, Mueller Hinton Agar, Nutrient Agar, Nutrient Broth, *E. coli* culture, *S. mutans* culture and *Candida albicans* culture.

Sample Preparation

Mistletoe leaves of *P. longifolia* that grow around the USU Library were collected purposively (not

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comparing with other regions) as samples. Mistletoe leaves cleaned and weighed further in the dry-aired. After drying, the sample was smoothed using a blender. Then as much as 200 g of sample was added to Erlenmeyer glass added with 1 L of methanol. Maserated for 24 hours at room temperature. The maserate was then added with methanol back to the sample of mistletoe leaves to the clear-colored solvent then collected the filtered maserate, evaporated with a Rotary Evaporator under vacuum until a viscous extract was obtained. The viscous extract was evaporated until the solvent evaporates completely and obtained a concentrated extract of sample. (RI, 2000). Furthermore, the concentrated extract of mistletoe leaves was dissolved with ethyl acetate filtered then the filtrate was evaporated until the solvent completely evaporated so as to obtain the ethyl acetate extract of mistletoe leaves which was then reconstituted with methanol until completely dissolved and in partition with n-hexane formed two layers, the bottom layer was taken and evaporated until the total flavonoid is obtained.

Phytochemical Test

Alkaloid Test

A total of ± 5 drops of filtrate of methanolic mistletoe leaves extract were each dropped on four test tubes. The first reaction tube was dropped by 2 drops of Mayer reagent (positive for forming a white or turbid precipitate), the second reaction tube was dropped 2 drops of Dragendorf reagent (positive for orange precipitate formation), the third reaction tube drops 2 drops of Wagner reagent (positive if it formed brown red precipitate) and the fourth test tube drops 2 drops of Bouchardat reagent (positive if formed a red precipitate). Observed changes that occur (Harborne, 1987).

Phenolic Test

A total of ± 5 drops of filtrate of methanolic mistletoe leaves extract was dropped on a test tube then added each with 3 drops of 1% FeCl3 solution (positive if blackish brown). Observed changes that occur (Harborne, 1987).

Flavonoid Test

A total of ± 5 drops of filtrate of methanolic mistletoe leaves extract was dropped onto a test tube with ± 3 drops of ethyl acetate plus 2 drops of FeCl3 1% to form a green to brownish color (positive to green to brownish). Observed the color changes that occur (Harborne, 1987).

Terpenoid and Steroid Test

Liebermann Bouchard Test

A total of ± 20 mL of partial methanolic mistletoe leaves extract was fed into the beaker glass then evaporated until the solvent was exhausted then cooled and then dropped ± 5 drops of anhydrous acetate and ± 5 drops of concentrated sulfuric acid (positive terpenoid if brownish to violet and positive steroid form if color was formed green to blue). Observed changes that occur (Harborne, 1987).

Thin Platform Test

As much as ± 3 mL of methanolic mistletoe leaves extract spilled on a thin plate heated on a hotplate in ± 2 drops of concentrated sulfuric acid (positive terpenoid if reddish) (Harborne, 1987).

Saponin Test

A total of ± 5 drops of methanolic mistletoe leaves extract was inserted in a test tube then with 20 mL of aquadest. The cooled filtrate was then shaken strongly for 10's and stays for 10 min (positive if foam was formed). Observed changes that occur (Harborne, 1987).

Toxicity Test by Brine Shrimp Lethality Test Method (BSLT)

Preparation of Shrimp Larvae

The mother liquor of the container was insulated in two parts, filled with 38 g of sea salt dissolved in 1 L aquadest obtained by artificial seawater. A total of 20 mg of Artemia salina eggs were inserted in a closed partition and one partition was left open, then lighted over an open section to draw *Artemia salina* shrimp to the light-affected part so that it was separated from its shell. The eggs of Artemia salina would hatch into larvae within 24-48 hours of hours and were used toxicity test from the total flavonoid of mistletoe leaves extract. Made standart solution with 100 mg of flavonoid total of mistletoe leaves extract plus 3 drops DMSO, dissolved to 10 mL with artificial sea water obtained concentration of standart solution was 10.000 mg / L then diluted to 3 concentration that is 10,100 and 1000 ppm. Concentration of 0 ppm as negative and positive control with the addition of DMSO (McLaughlin et al., 1998).

Toxicity Test

A total of 10 larvae were inserted into each test tube that had filled 5 mL of concentrated extract solution of mistletoe leaves extract with concentration of 1000, 100 and 10 ppm respectively. Three repetitions made. After 24 hours, observed the number of dead larvae for each concentration.

Antimicrobial Test

Testing Antimicrobial Activity with Agar Diffusion

Prepared 10 mL of Mc solution. Farland (10^8 CFU / mL) was then taken *Streptococcus mutans* with sterile ose needle then put into 10 mL of aquadest in the test tube then in suspension, mixed with vortex until homogeneous and closed tube with cotton and seal wrap. *Streptococcus mutans* on a petri dish containing sterile MHA then perforated MHA on

petri that has been applied with a uniform hole using Cop Borer then put 50 µL of test sample with concentration 3%, 6%, 9% and DMSO as blank then sealed with seal wrap and incubated at temperature 35 ± 2^0 C for 24 hours. Further, measured the diameter of the drag area around the hole by using the sliding term. Do the same thing on *Escherichia coli* and *Candida albicans* (Ditjen POM, 1995).

Results

Sample Preparation

Fresh mistletoe leaves as sample were cleaned and then dried were weighed as the initial weight of 1000 g. Then the sample was allowed to dry until the leaves could be kneaded in the room (± 1 week) and weighed again as the final weight of 230 g or by 23%. 200 g of leaf parasite powder was macerated with methanol solvent for 1 night and then filtrate and macerated until the filtrate of the sample was colorless or clear then the filtrate was evaporated with a rotary evaporator at 60° C and concentrated with a water bath obtained by methanol condensed extract of 24, 5 g or 12.25% and then dissolved with ethyl acetate and evaporated. Ethyl acetate extract was dissolved with methanol and fractionated with n-hexane and the bottom layer was evaporated until dried as total flavonoid then weighed 4.98 g or by 2.49%.

Tests of Phytochemical

Table 1
Phytochemical screening of metanolic leaf extract

Groups	Reagents	Results
Alkalod	Meyer	-
	Buchardat	-
	Dragendorf	-
	Wagner	-
Fenolik	Flavonoid (Ekstrak Et.asetat $FeCl_3$ 1%)	++++
	Fenolik (Ekstrak Metanol $FeCl_3$ 1%)	++++
Saponin	Aquadest	-
Terpenoid/ Steroida	Lieberman Bouchard	+++
	CeSO4 1% dalam H2SO4 dengan Plat TLC	+++

Test toxicity activity with brine shrimph lethality test (BSLT)

Table 2
Observations with BSLT

Concentration (ppm)	Lived Larvae Number	Died Larvae Number
		24 hours
1000	10	10
	10	10
	10	10
100	10	2
	10	1
	10	1
10	10	0
	10	1
	10	1
Blank +	10	0
	10	0
Blank -	10	0
	10	0

From Table 2 Observation with Brine Shrimph Lethality Test at concentration 1000 ppm be seen total shrimp larvae mortality of total flavonoid from mistletoe leaves extract to the three test tubes containing shrimp larvae suffered total mortality while for the concentration of 100 and 10 ppm be seen mortality rate of low shrimp larvae.

Table 3
Observation of toxicity activity test with BSLT during 24 hours

Concentration (ppm)	Lived Larvae Number	Died Larvae Number	A	B	C	B+C=D	B/D	E
1000	10	10						
	10	10	10	10	0	10	1	100
	10	10						
100	10	2						
	10	1	1,3	11,3	8,7	20	0,57	57
	10	1						
10	10	0						
	10	1	0,6	12	18,1	30,1	0.40	40
	10	1						

Note:
A: Average of Dead Larvae, B: Accumulation of Dead Larvae, C: Life Lifecycle Accumulation, D: Total Mortality and E: Percent of Mortality

From Table 3 Observation of Toxicity Activity Test with Brine Shrimph Lethality Test for 24 hours% mortality obtained at total flavonoid concentration 10 ppm is 40%, 100 ppm was 57% and 1000 ppm was 100%. If Percent Mortality was Y axis and antilog as X axis then obtained graph as follows.

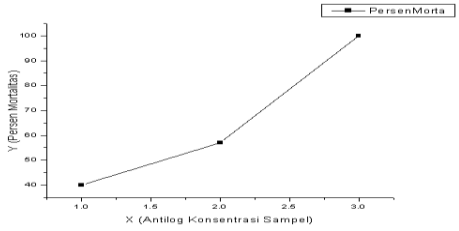

Fig 1. Graph of toxicity activity of BSLT where X as antilog of total flavonoid of mistletoe leaves and Y as % mortality.

Test of antimicrobial activities of flavonoid total from mistletoe leaves

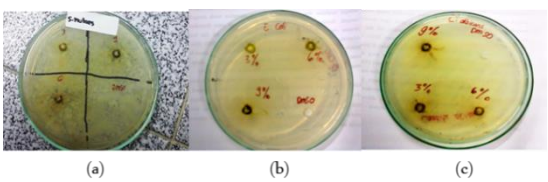

Fig 2. Test of Antimicrobial Activity (a*) S. mutans* (b) *E. coli* and (c) *Candida albicans.*

From Fig 2 be seen inhibition zone of Negative Bacteria of *S.mutans, E. coli and C. albicans* to concentration of 3%, 6% and 9% of flavonoid total from mistletoe leaves extract and DMSO as blank.

Table 4.
Results of measurement of inhibitant zone flow test of antimicrobial activity flavonoids total from mistletoe leaves extract

Konsentrasi (%)	Diameter Zona Hambat (mm)		
	S. mutans	*E. coli*	*C. Albicans*
3	6	3.55	8.30
6	9	4.25	4
9	17.25	9.15	5.30
Blanko	0	0	0

From the table above we saw the graph below:

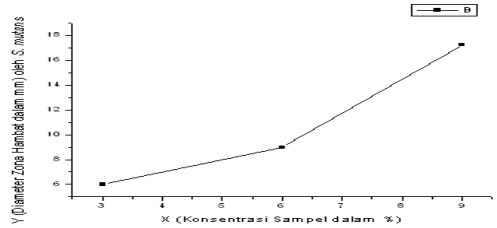

Fig 3. Graph X as concentratio flavonoid total versus Y as inhibitory activity by *S. Mutans*

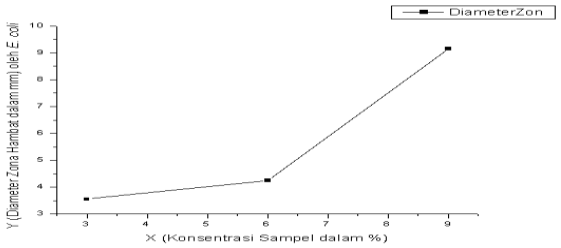

Fig 4. Graph X as concentration flavonoid total versus Y as inhibitory activity by *E. coli*

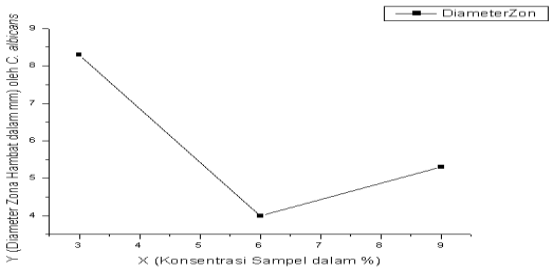

Fig 5. Graph X as concentration flavonoid total versus Y as inhibition activity by *Candida albicans*

Discussion

The phytochemical test of fresh mistletoe leaves, Alkaloid test was performed, among others, methanol extract of mistletoe leaves with addition of Meyer, Dragondorf, Wagner and Bouchardat reagents did not give any color change (negative alkaloids) nor did saponin produce constant foam (negative saponin). For Phenolic test of methanol extract with 1% $FeCl_3$ addition of discoloration of blackish brown (positive phenolic) and with ethyl acetate extract plus $FeCl_3$ 1% giving a blackish brown color (positive flavonoids) as well as terpenooid test with CeSO4 giving red color on TLC plate and with Lieberman Bouchad reactor giving a change in the blackish red color.It could be seen from Table 1 that the Positive Sign ++++ shows strong and contrasting color changes indicating that mistletoe leaves contain secondary metabolite compounds namely Phenolic, Flavonoid and Terpenoid.

Table 2 Observations with BSLT at 1000 ppm concentrations showed total mortality of shrimp larvae after 24 hours. The reaction tube containing the shrimp larvae with the concentration of 100 ppm extract experienced an average mortality of 1.3 on 10 shrimp larvae and at 10 ppm experienced a mean mortality of 0.6 scale of 10 shrimp larvae in each test tube. From Table 3 observation test, the activity of toxicity with BSLT for 24 hours obtained % mortality at total flavonoid concentration of mistletoe 10 ppm was 40%, 100 ppm was 57% and 1000 ppm was 100%.

If LC_{50} (Lethal Concentration 50) is the antilog of death of shrimp larvae in 50% then obtained LC_{50} of 30,06 mg / L. According to Meyer et al. (1982), an extract was considered toxic if it has a value of LC50 <1000 ppm whereas for pure compounds said toxic if LC50 <200 ppm. LC50 was the concentration of a chemical compound in air or in water that can cause 50% of death in a population of test animals or certain living things. While the LD_{50} (Lethal Dossage 50) is a dose of a chemical compound which can cause 50% of the death of the test animal administered to any prescribed individual or, more precisely, a statistically obtained single dose of a substance that can cause 50% of animal deaths. As sample which was analogyzed of shrimp larvae (*Artemia salina*) has the same cell division with cancer cell division, after shrimp cysts hatched at 24-48 hours, shrimp larvae grew rapidly so that it was assumed as abnormal cell growth.

Table 4, be seen that the inhibition zone of samples with *E. coli* as gram negartif bacteria, *S. mutans* as gram positive bacteria and *Candida albican*s as fungi. According to Davis et al. (1971), the provision of antibacterial activity strength of an extract was based on an inhibit zone where, if <5 mm, the inhibitory power was weak, if 5-10 mm inhibitory power was moderate, 10-20 mm inhibitiveness was strong and> 20 mm its inhibitory power was very strong. Table 5 explained that inhibitory power of *S. mutans, E. coli* and *Candida albicans* increased with concentration of total flavonoid of mistletoe leaves, it proved that the antimicrobial was effective to be developed as an antibacterial agent. Flavonoids played a role in inhibition of DNA-RNA synthesis by intercalation or hydrogen bonding with the accumulation of nucleic acid bases, and the role of inhibiting energy metabolism. These compounds disrupt energy metabolism by inhibiting the respiratory system

because it required enough energy for the active absorption of various metabolites and for macromolecular biosynthesis (Nuria et al., 2009).

Conclusion

The results of research conducted on mistletoe leaves of *Polyalthia longifolia* tree could be concluded as followed:

To test the toxicity activity of mistletoe leaves from *Polyalthia longifolia* trees using Brine Shrimp Lethality Test (BSLT) obtained LC_{50} of 30.06 mg / L which means toxicity activity against shrimp larvae (*Artemia salina* Leach) was quite toxic. To test the antimicrobial activity of mistletoe leaves from *Polyalthia longifolia* trees using diffusion method, in order to obtain inhibit zone on *S. mutans* the concentration of extract are 3%, 6% and 9% respectively are 6; 9 and 17.25 mm, on *E. coli* is 3.55; 4.25 and 9.15 mm and at *C. albicans* is 8.30; 4 and 5.30 mm where *E.coli* and *S. mutans* inhibitory power were the greater than *C. albicans*, but once the three microbes were concluded effective to be developed as antimicrobial agents.

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