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Research Article

Wild Tembelek plant (*Lantana camara*) as a potential bioactive natural product againts *Streptococcus pyogenes* in Indonesia

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Abstract

Infectious diseases are common problems in most countries. Streptococcus pyogenes is the infectious agent that causes diseases such as pharyngitis, impetigo, toxic shock syndrome, and necrotizing fasciitis. Tembelek (Lantana camara) is a wild plant that can easily be found in every ecosystem in Indonesia whether in nature or settlements and known as a plant that has an antibacterial effect but the knowledge about its potential against Streptococcus pyogenes in this past five years remain scant. The aim of this study was to determine the potentiality of Lantana camara leaves and flowers extract against Streptococcus pyogenes. In this experimental study, in vitro using Post-test Only Control Group Designed, has been done and confirmed by the Indonesian Institute of Sciences. Lantana camara leaves and flowers extracts were obtained by maceration using ethanol. The extracts were diluted into eight concentrations and their antibacterial activity against Streptococcus pyogenes was tested using the Kirby-Bauer disc then proceeded Minimum Inhibitory Concentration (MIC) test and phytochemical assay. The data processed using SPSS software version 22. The results showed that flowers extract had the most significant inhibition zone (11.85 ± 0.119 mm) compared with the leaves extract (9.54 ± 0.07 mm) at the highest tested concentration was 640 mg/ml. The MIC of both extracts was 250 mg/ml. Flavonoids, phenolic, steroids, and saponins were found in both extracts whereas, alkaloid was found only in flowers extract. In conclusion, the Tembelek plant has an antibacterial effect against Streptococcus pyogenes. Future study is needed related to its mechanism of antibacterial effect.

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INTRODUCTION

Infectious diseases are common problems in most countries. According to 2016 data from Global Burden of Disease, they caused more than 10 million deaths in the world (Hay 2016); Basic Health Research data from 2018 showed that infections were still one of illness with high morbidity and mortality rate in Indonesia (Kemenkes 2018). The aforementioned data shows that infection is still a prevalent national pandemic and needs to be managed both in preventive and curative aspects.

Various types of microbes, including normal flora, either pathogens or opportunistic pathogens, cause infections. Streptococcus pyogenes, also known as Group A Streptococcus (GAS), is part of skin microbiota that responsible for lots of illnesses found daily in the clinical world, such as pharyngitis and impetigo. It can also cause fatal illnesses such as Toxic Shock Syndrome, which has a mortality rate of 12,1% (Strom, 2017). The drug of choice (DOC) to treat GAS infections is penicillin group (Peters, 2017). The problem is, the resistance rate towards this bacteria is increasing caused by its own beta-lactamase synthesis defense system, which disables penicillin-type medications such as amoxicillin (Katzung, 2012). Resistance to macrolide (Erythromycin), Clindamycin, and Lincosamide have also been reported (Berwal, 2019; Kumar, 2017). There needs to be an alternative medication to eradicate Streptococcus pyogenes. Herbal medicine, which has bioactive products and antimicrobial effects, is a potential alternative medication that can also reduce antibiotic resistance. It is also thought that herbal medicine has a cheaper price and fewer side effects than synthetic drugs used in allopathic medicine (Hay 2016).

Tembelek (Lantana camara) is a plant that, for generations, has been used by Indonesian ancestors to treat many diseases such as itchiness, lepra, hypertension, measles, ulcer, asthma, tetanus, and rheumatic. This plant has also been known to have antibacterial potency, especially its flower and leaf (Kumar, 2012). For example, ethanol extract of its leaf can effectively halt the growth of some pathogenic bacteria, such as Staphylococcus aureus, Escherichia coli, Salmonella typhi, and Pseudomonas aeruginosa (Agrawal, 2012; Obinna, 2013); it is also proven that it has a bactericidal effect to three strains of Mycobacterium tuberculosis (Kirimuhuzya, 2009). Overall, the extracts of Lantana camara exerts a broader inhibitory activity on Gram-positive bacteria than Gram-negative bacteria (Shakya, 2016). Unfortunately, until now, there is no international publication of its bioactive product's and antibacterial qualities' effects on Streptococcus pyogenes in Indonesia. Considering the prevalence of some diseases caused by Streptococcus pyogenes infection remain high in Indonesia, there needs to be research to analyse the effects of this plant's bioactive products as an antimicrobial agent against Streptococcus pyogenes.

METHODS

This is experimental in vitro study using a Post-test Only Control Group Design. The study was conducted in two places, mainly laboratory of Madang Campus Medical College Sriwijaya University and Microbiology Unit of Palembang Health Laboratory Center. The study was conducted from September 2015 until October 2015. This study was approved by Mohamad Hoesin General Hospital ethical committee, Palembang and Medical College Sriwijaya University ethical committee, Palembang.

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Plant Extract Preparation

The test sample was wild Lantana camara leaf extract, which was determined in the Biological Botany Research Centre of Indonesian Institute of Sciences in Cibinong, Bogor. It was extracted using the maceration method. Maceration is an extractive technique that is conducted at room temperature. It consists of immersing a plant in a liquid (water, oil, alcohol, etc.) inside an airtight container, for a variable time based on the plant material and liquid used. There were 32 grams of dry leaf powder and 51 grams of flower powder which was macerated using ethanol solution with a ratio of 1:1 for 3x24 hours in the macerator; it was then filtered to let only the liquids out. The macerate was then left to develop deposits, which was then separated carefully. The deposit was then macerated once again by means of the second filtration and then separated just like before. The resulting filtrate was then evaporated using rotavapor until the remaining solvent disappeared, and a thick extract was obtained. This method was chosen because it requires only simple equipment, which made it easily replicable and applicable in a majority of Indonesian laboratories. After the ethanol extract was acquired, it was preserved in a beaker. The extract was then dried using a hairdryer to obtain the dry extract, which is going to be used in tests against Streptococcus pyogenes.

Bacterial Specimen Preparation

The standard strain of bacteria which is still sensitive to standard therapies: Streptococcus pyogenes NCIMB 13285, which were obtained from the Palembang's Centre for Health Laboratory (CHL). The bacteria were incubated in a nutrient medium, which was placed inside a 37o C incubator for 24 hours.

Antibacterial Test Preparation

This test was done using disk diffusion by the Kirby-Bauer method. Flower and leaf extract were diluted serially: 640 mg/ml, 320 mg/ml, 160 mg/ml, 80 mg/ml, 40 mg/ml, 20 mg/ml, 10 mg/ml, dan 5 mg/ml. Then, a petri dish filled with blood agar, bacteria, and the extract was incubated in a 37o C incubator for 24 hours. This test was repeated five times.

Minimum Inhibitory Concentration (MIC) test was done using the broth dilution method. MIC test demonstrates the lowest level of antimicrobial agent that greatly inhibits growth. The bacteria were prepared by making a suspension with a turbidity of 0,5 Mc Farland. Flower and leaf extract were diluted serially: 1000 mg/ml, 500 mg/ml, 250 mg/ml, 125 mg/ml, dan 62,5 mg/ml. The positive control was 20 µg/ml amoxicillin; the negative control was Dimethyl sulfoxide (DMSO). Then, both the extract and the control were given another bacterial suspension and incubated in a 37o C incubator for 24 hours. The lowest, clearest concentration was the MIC. The experiment on each tested specimen was repeated five times, according to Federer formula.

Phytochemical Test Preparation

Phenol

In the phytochemical screening, the filtrate was tapped to a G60 F254 silica gel plate and was rubbed with ethyl acetate: formic acid: toluene: water = 6:1,5:3:0,5. Then, it was dried and examined under visible light, UV 366 nm. It was then sprayed using FeCl3, dried, and examined under visible light, UV 366 nm. Phenolic (+) will show as blackish dark green.

Alkaloid

The sample was given 2 N sulfuric acid drops and tested using Wagner reactors. Any changes were examined after 30 minutes. Test results were deemed positive if it showed as yellowish, and there was a brown deposit post-Wagner reactor.

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Flavonoid

In the phytochemical screening, the filtrate was tapped to a G60 F254 silica gel plate and was rubbed with hexane: ethyl acetate: formic acid = 6:4:0,2. Then, it was dried and examined under visible light, UV 366 nm. It was then sprayed using sitroborat, dried, and examined under visible light, UV 366 nm. Flavonoid (+) will show as yellow.

Saponin

A small sample was separated into a test tube and was given hot water. Changes in how bubbles formed were examined; the reaction was deemed positive if the bubbles were stable for 10 minutes and didn't go away after given a drop of HCl 2 N.

Steroid and Triterpene

The results were deemed positive if there is a green ring on the steroid and purple ring on the triterpene using the Liebermann-Bucchard test.

Data Analysis

The statistical data were analyzed using SPSS Software 22nd version. Normally distributed data were analyzed using t-test study to compare the mean inhibitory zone diameters of leaf extract with flower extract. Furthermore, one-way ANOVA study was conducted to observe whether there is a significant difference between mean inhibitory zone diameters of leaf and flower extract with a significance threshold of 0.05.

RESULTS

We performed in vitro culture of a standard strain of Streptococcus pyogenes to examine the effect of Lantana camara extracts with the results as following:

Table 1. Inhibitory zone diameters of the Tembelek leaf (*Lantana camara*) extract against *Streptococcus pyogenes*

Concentration (mg/ml)	Inhibitory zone diameters (mm)					Mean ± SEM
	I	II	III	IV	V	
5	6	6	6	6	6	6
10	6,1	6,3	6,52	6,4	6,2	6,3 ± 0,0736
20	6,8	7	7,27	7,21	7,1	7,076 ± 0,083
40	7,4	7,6	7,63	7,74	7,5	7,574 ± 0,058
80	7,9	8	7,95	8,05	8,01	7,98 ± 0,026
160	8,3	8,2	8,22	8,32	8,2	8,25 ± 0,026
320	9,3	8,8	9	8,92	9,1	9,024 ± 0,085
640	9,8	9,5	9,44	9,4	9,55	9,54 ± 0,07
Amoxicillin	40	41,1	39,4	32,5	36,46	37,89 ± 1,55
DMSO	6	6	6	6	6	6

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Table 2. Inhibitory zone diameters of the Tembelek flower (*Lantana camara*) extract against *Streptococcus pyogenes*

Concentration (mg/ml)	Inhibitory zone diameters (mm)					Mean ± SEM
	I	II	III	IV	V	
5	6	6	6	6	6	6
10	6	6	6	6	6	6
20	6	6	6	6	6	6
40	6	6	6	6	6	6
80	6	6	6	6	6	6
160	6	6	6	6	6	6
320	6,6	7,92	7,8	7,55	7,7	7,514±0,236
640	12,2	11,18	11,5	12	11,74	11,85±0,119
Amoxicillin	34	35,1	35	33,01	35,5	34,52±0,45
DMSO	6	6	6	6	6	6

Table 3. Minimum Inhibitory Concentration (MIC) of Tembelek leaf extracts (Lantana camara)

Concentration (mg/ml)	Turbidity level
1000	-
500	+
250	+
125	++
62,5	++
Amoxicillin	-
DMSO	++

Two hundred and fifty mg/ml was the lowest concentration which inhibited bacterial growth; this concentration also had a small growth in suspension. The 125 mg/ml concentration still showed a lot of bacterial growth in suspension, which was shown on the turbidity of the tube. DMSO, which was used as a negative control, shows a lot of bacterial growth; amoxicillin had no bacterial growth in suspension.

A similar result happened to the flower extract: 250 mg/ml was the lowest concentration which inhibited bacterial growth; this concentration also had a small growth in suspension. The 125 mg/ml concentration still showed a lot of bacterial growth in suspension, which was shown on the turbidity of the tube. DMSO, which was used as a negative control, shows a lot of bacterial growth; amoxicillin, as the positive control, had no bacterial growth in suspension.

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Table 4. Minimum Inhibitory Concentration (MIC) of Tembelek flower extracts (Lantana camara)

Concentration (mg/ml)	Turbidity level
1000	-
500	-
250	+
125	++
62,5	++
Amoxicillin	-
DMSO	++

Table 5. Phytochemical Screening of Tembelek Leaf Extract (Lantana Camara)

Alk	Flv	Fn	Trp	St	Sp
-	+++	+++	-	+++	+

Table 6. Phytochemical Screening of Tembelek Flower Extract (Lantana Camara)

Alk	Flv	Fn	Trp	St	Sp
+++	++	++	-	++	+

Alk = Alkaloid, Flv = Flavonoid, Fn = Fenol, Trp = Triterpen, St = Steroid, Sp= Saponin; + weak, ++ medium, +++ strong

Phytochemical Screening

The biochemical contents of the flower and leaf extract of wild *Lantana camara* is shown in Table 5 and Table 6. In the extracts, secondary metabolites such as flavonoid, phenol, steroid, and saponin, was found. Alkaloid was found in the flower extract.

DISCUSSION

Antibacterial Activities on the Extracts

This research proved that the extracts showed antibacterial activities against Streptococcus pyogenes. This is in line with a research which found that The Lantana camara showed good activity against dermatophyte bacterial strains Streptococcus pyogenes and Staphylococcus aureus (Agrawal, 2016). The flower extract, in particular, had a more potent inhibitory effect. Even though there still less publication about its antimicrobial effect against Streptococcus pyogenes, but it is proofed that every part of the plant has different capabilities in inhibiting the bacteria's growth as shown in a study done by Mahdi P.B. et al.: the leaf extract was proven to have more potent antibacterial effect than other parts of the plant towards Salmonella typhi (Badakhshan 2009). The extract also showed its antibacterial effects towards E.coli, P.aurigenosa, and B.subtilis; it is, however, ineffective against S.aureus (Ganiewala, 2009).

In this study, the inhibitory zone diameters of both extracts are significantly different from those of amoxicillin as a positive control group. This result may be affected by the extraction technique used in this study and thus implying a limitation of this study. The maceration technique used in this research is the simplest technique and, thus, was used by the researchers. The limitation of the maceration technique used is a low absorption capability of secondary plant metabolites, resulting in a low quantity of metabolite obtained.

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The leaf extract's antibacterial activity was passively caused by the presence of secondary metabolites in the plant (Agrawal, 2012). A phytochemical test was needed to know what kinds of secondary metabolite compounds were present in the plant. The test results, using Wagner method, showed that the contents were flavonoid, phenol, steroid, and saponin; other tests, using Mayer method, found that there was alkaloid in the leaf extract (Naz, 2013): this means that, in the flower extract, there were alkaloid, phenol, flavonoid, steroid, and saponin.

Alkaloid is a kind of organic compound which inspired the development of antibiotics such as the quinolone-type medications. Researches to develop new antibiotics were mostly centered on it, too (Cushine, 2014; Pervaiz, 2016). Alkaloids such as Berberine and Harmane are able to insert themselves to the DNA, which aids in inhibiting bacterial growth and disturb peptidoglycan components build in the bacterial membrane (Cushine, 2014). Phenol is a secondary metabolite from the plant, which has lots of benefits for humans. Phenol's ability to eradicate bacteria cannot be understated—bacteria are even unable to build resistance towards phenol (Keman, 2018). It has an antibacterial effect because phenol is able to cause protein coagulation, which in result, causes instability in the bacteria's membrane, causing lysis (Rempe, 2017). The flavonoid, in some plant types, is known to have antibacterial effects by inhibiting nucleic acids synthesis, which disrupts cytoplasm membrane's function, inhibits energy metabolism, prevents sticking, and biofilm creation, inhibits porin on the cell membrane, and disrupts the changing of membrane permeability (Xie, 2015). Saponin's mechanism as an antibacterial agent is by reducing surface tension, making the cell membrane more fragile. Saponin also creates a complex compound with the cell membrane by means of a hydrogen bond to disrupt the permeability of the cell wall, causing its death (Muharrami, 2019).

CONCLUSION

Wild Tembelek (Lantana camara) has a potential antibacterial effect against Streptococcus pyogenes. The flower extract is more potent in inhibiting the bacteria's growth compared to the leaf extract. In both extracts, bioactive natural products as secondary metabolites of plants such as flavonoid, phenol, steroid, and saponin can be found; alkaloid is only found in the flower extract. Considering its potential antibacterial effect and bioactive natural products, this plant, especially the flower parts, could be used to synthesize a novel alternative treatment against Streptococcus pyogenes infection in Indonesia. Future study, especially in vivo study, is needed to further examine the mechanism of the antibacterial effect and the toxicity of Wild Tembelek plant.

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