International Journal of Pharmaceutical Science and Research
www.pharmacyjournal.net
ISSN: 2455-4685, Impact Factor: RJIF 5.28
Received: 01-03-2021, Accepted: 26-03-2021, Published: 19-04-2021
Volume 6, Issue 2, 2021, Page No. 01-03

Antibacterial activity of leaf extract of caricature plant (*Graptophyllum pictum* L.) against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Abstract
Caricature plants (*Graptophyllum pictum* L.) contained bioactive compounds that are known to have antibacterial properties, such as alkaloids, flavonoids, saponins and tannins. However the antibacterial activity study of this plant extract is still limited. Current study aims to investigate the effects of ethanol-soluble fraction of leaf extract of the caricature plant on *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains. The ethanol-fractions of 20%, 40 %, 60%, 80%, and 100% (v/v) screened for their antibacterial activity using well-diffusion agar method. Growth inhibition of the extract was compared with distilled water as the negative control and the standard antibiotic, ampicillin, as the positive control. The caricature plant extract significantly inhibits the growth of both tested bacterial strains in a concentration-dependent manner. At the concentration of 100%, the extract caused growth zone of inhibitions of 20.54 mm in *S. aureus* and 19.8 mm in *P. aeruginosa*. The minimal inhibit concentration of the extracts against those bacteria consecutively are 13% and 17%. Sub culturing the bacteria taken from the media with negative growth on the solid NA agar showed that the growth of *S. aureus* still detected, but not for *P aeruginosa*. The ethanol-soluble fractions of the caricature plant leaves extract possess antibacterial activity and depict a bacteriostatic property against *S. aureus* and a bactericidal against *P. aeruginosa*.

Keywords: *Graptophyllum pictum*, caricature plant, daun ungu, antibacterial activity

Introduction

Caricature plant (*Graptophyllum pictum* L.) is an evergreen shrub native to tropical countries such as New Guinea, Philippines and Indonesia. In Indonesia, especially in Java Island, this plant has long been known and used by indigenous people as a traditional medicine to treat hemorrhoids [1]. Pharmacological studies revealed that the plant leaf extract of caricature plant, which in Indonesia is called "daunungu", showed several bioactivities. Methanol-soluble fraction of this plant leaf extract showed an anti-inflammatory property and analgesic activity [2]. A study to test the effect of aqueous extract of the *G. pictum* leaves on the alloxan-induced diabetic Wistar rats showed that this plant is potent to be used as anti-diabetes [3]. Anti-diabetes potential of caricature plant has also shown by the ethyl-acetate extract of this plant leaves [4]. Another study aimed to prove the claim of indigenous peoples in Africa that caricature plants can be used as a delivery aid suggest that leaf extract of this plant possess anti implantation activities [5].

Although there are quite a lot of research on the pharmacological properties of caricature plants, reports on the antibacterial properties of *G. pictum* L. extract are still very limited. One of the *Graptophyllum* species whose leaf extract has been known to have antibacterial properties is *G. granulosum*, instead of *G. pictum*. It is suspected that the bioactive compounds content in *G. granulosum* that have antibacterial properties are flavonoids [6]. The anti-bacterial properties of *G. pictu*m revealed by a study which proved that phytochemicals extracted from this plant could inhibit the plaque growth on acrylic resin complete denture [7].

In an effort to determine the strength of the antibacterial activity of this plant we then tested the ethanol-soluble portion of the caricature plant leaf extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria. This scientific hope is reinforced by the results of phytochemicals screening of *G. pictum* which shows the presence of antibacterial compounds such as: alkaloids, flavonoids, tannins, glycosides, saponins, and steroids [8, 9]. Alkaloids, flavonoids, tannins and saponins are amongst the phytoconstituents that revealed to show antibacterial against *S. aureus* and *P. aeruginosa* [10].

Materials and Methods

Plant sample and extraction

Samples of caricature plants (*G. pictum* L.) collected from Negara Batin Village, East Lampung Regency, the province of Lampung, Indonesia. Taxonomical status of the plant was determined by a botanist at the Botany Laboratory, University of Lampung. The fresh leaves were rinsed with distilled water and cut into small pieces and then sun-dried under black cloth cover. The dried small pieces of leaves (400 g) then macerated using 70% ethanol as the solvent. Macerate were then evaporated using a rotary evaporator at 60^{o}C until a concentrated extract obtained. To make ethanol fraction of the extract, liquid-liquid fractionation were performed using n-hexane and chloroform. The concentrated extracts were diluted with 50 ml of 96% ethanol and then 100 ml of n-hexane were added in a separating funnel. Next, into the ethanol fraction were added 100 ml chloroform. The residual ethanol fraction evaporated at 60^{o}C and dried in an oven at 60^{o}C. The dried leaves extract of caricature plant were then diluted serially with water according to the desired

concentration levels.

Bacterial isolates and media

The bacteria strains of *S. aureus* and *P. aeruginosa* were obtained from Lampung Provincial Health Laboratory, Bandar Lampung, Indonesia. The nutrient agar (NA, Merck) and nutrient broth (NB, Merck) were used for bacterial culturing and assays.

Assay for antibacterial activity

The antibacterial activity of leaves extracts of caricature plant (*G. pictum* L) were compared to standard antibiotic ampicillin as the positive control and distilled water as the negative control. Each ethanol fractions of 20%, 40 %, 60%, 80%, and 100% (V/V) as well as control solution individually loaded on the agar-wells and subjected to antibacterial activity. The zone of growth inhibition surrounding the well after 48 h of incubation at 37^{o}C measured and recorded as the antibacterial activity of the extract.

Determination of MIC and MBC

The minimum inhibitory concentration (MIC) value of the *G. pictum* leaf extracts was determined by the lowest concentration that completely inhibited bacterial growth in antibacterial activity test as mentioned above. The MIC value in question was determined using broth dilution method equipped with positive and negative cultures. The tube containing extracts and the growth medium without inoculums (antibiotic control) and the tube containing the growth medium, physiological saline and the inoculums (organism control). The lowest concentration of the extracts permitting no visible growth (no turbidity) defined as MIC when compared with the control tubes. The lowest concentration that revealed no visible bacterial growth after sub-culturing recorded as the minimum bactericidal concentration (MBC) of the extract.

Statistical analysis

One way ANOVA was applied in the statistical analysis and LSD test was used in post hoc test against the mean values of the data. Statistical significant was set at $p<0.05$.

Results and Discussion

The inhibitory growth zone diameter of the *S. aureus* and *P. aeruginosa* bacteria as the response to the ethanol-soluble fraction of plant leaf extract of the caricature plant, *G. pictum*, are presented in Table 1. In comparison to the negative control, the daun ungu leaf extract showed growth inhibition against the both tested bacteria in a dose-dependent manner. However, the inhibitory effect of caricature plant extracts on bacteria does not, in fact, surpass the effect of ampicillin.

Table 1: Inhibitory growth zone diameter of *S. aureus* and *P. aeruginosa* by extract concentrations of caricature plant (*G. pictum* L)

Treatment	Inhibitory growth zone diameter (mm)	
	S. aureus	***P. aeruginosa***
Distilled water	0^{a}	0 ^{a}
Extract 20%	16.13^{b}	16.16^{b}
Extract 40%	16.87^{c}	17. 03^{c}
Extract 60%	18.05^{d}	18. 05^{c}
Extract 80%	19.28^{e}	19.18^{d}
Extract 100%	20.54^{fg}	19.8^{e}
Ampicillin	21.8^{g}	21.63^{f}

The 20-concentration series of the *G. pictum* leaf extract that used to determine the lowest concentration that could completely inhibited bacterial growth of *S. aureus* and *P. aeruginosa* are presented in Table 2.

Table 2: Results of broth dilution test of the extract of caricature plant (*Graptophyllum pictum* L) of lowest concentration that showed growth inhibition on *S. aureus* and *P. aeruginosa*

Extract dillution series	Visible growth of bacteria	
	S. aureus	***P. aeruginosa***
20%	-	-
19%	-	-
18%	-	-
17%	-	-
16%	-	+
15%	-	+
14%	-	+
13%	-	+
12%	+	+
11%	+	+
10%	+	+
Media control	-	-
Extract control	-	-
Bacteria control	+	+
Growths of bacteria under diluted extract (less than 10%) are not shown here, all of them are still positive.		

The data in Table 2 showed that the MIC value of the *G. pictum* leaf extracts against *S. aureus* and *P. aeruginosa* are 13% and 17% respectively. Sub culturing the bacteria taken from the media with negative growth above on the solid NA agar showed that there were still detected the growth of *S. aureus* but no more growth of *P aeruginosa* detected.

Discussion

The data of current study clearly showed that the ethanol-soluble fraction of *G. pictum* leaf extract Posses strong antibacterial effects against *S. aureus* and *P. aeruginosa.* The antibacterial effect shown by caricature plant extract is in accordance with our initial expectations considering that this plant is rich in bioactive compounds that are antibacterial such as alkaloids, steroids, flavonoids, tannins, coumarins, saponins, anthraquinones, phenolics and volatile oil [11, 12]. The antibacterial activity of the volatile oil extracted from caricature plant leaves were revealed by Jiangseubchatveera *et al*. (2015) in a test against *S. aureus* and *E. coli* [13].

Among bioactive substances obtained from the leaf extract of caricature plant supposed to be anti-bacterial, both on Gram negative as well as Gram positive bacteria, are flavonoids. Several green leafy vegetables known to contain flavonoids are exhibiting antibacterial properties against *S. aureus coli* (Gram +ve) and *P. aeruginosa* (Gram -ve) [14]. Additionally, there are many more examples of plant extracts that have proven to be antibacterial, which is thought to be due to the dominance of flavonoids, for example *Cleome spinosa* Jaqc [15], *Pterospartum tridentatum* and *Mentha pulegium* [16], and *Asparagus suaveolens* aerial parts [17].

There are several mechanisms of phytochemicals presumably cause bacterial growth inhibition, among others: cell division inhibitor, protein and DNA synthesis inhibitor, destruction of bacterial membrane, ATP synthase inhibitor, rigidifing bacterial membrane, damage to the cytoplasmic membrane [18]. Regarding flavonoids, the proposed antibacterial mechanisms are: alteration of the membrane permeability, inhibition of energy metabolism, inhibition of nucleic acid

synthesis, inhibition of cytoplasmic membrane function, inhibition of the attachment and biofilm formation, inhibition of the porin on the cell membrane, and attenuation of the pathogenicity [19].

Conclusion

The ethanol soluble fraction of *G. pictum* leaf extract in this study significantly inhibited bacterial growth in a concentration-dependent manner, ranging from 13% in *S. aureus* and 17% in *P. aeruginosa*. Thus, it can be concluded that the extract of purple leaves has strong antibacterial properties. Furthermore, based on the results of the minimum bactericidal concentration (MBC) test, it can be concluded that the ethanol fraction of *G.pictum* leaf extract is bacteriostatic against *S. aureus* and bactericidal against *P. aeruginosa*.

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