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Evaluation of antibacterial action of saponins from golden hahnii roots on *Escherichia coli* and *Staphylococcus aureus*

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Abstract

***Sansevieria-trifasciata*-is commonly known as both in and out door house plant. It is also used intraditional medicine for treatment of influenza, cough and respiratory inflammation. The roots and leaves have secondary metabolites such as saponins that exbihit remedy for cough, snake bite, sprain, bruise, boil, abscess, respiratory inflammation and hair tonic. The plant sample was collected and dried. The preliminary test for saponnins was carried out and subsequently saponnin was extracted. A compound was isolated using thin-layer chromatography (TLC) and the antibacterial activity was tested. The *S. trifasciata*-root extracts and the isolated compound demonstrated potent activity against *Escherichia coli* and *Staphylococcus aureus.* The zone of inhibition of growth produced rangesbetween 18.67 and 24 mm at 200 ppm concentration level respectively. It is the best eluent that separated the triterpenoid-saponins was chloroform:methanol:water (20:60:4) with 3 spots having R_f of 0.125, 0.75 and 0.812.**

Keywords: *Sansevieria trifasciata,*roots extract, saponins, antibacterial,*Escherichia coli*,*Staphylococcusaureus*.

INTRODUCTION

Sansevieria-trifasciata is not just known as both out andin door plant but has medicinal properties. It is used in traditional medicine for influenza, cough and respiratory inflammation. Its roots and leaves have secondary metabolites such as saponins that exbihit remedy for cough, snake bite, sprain, bruise, boil, abscess, respiratory inflammation and hair tonic (Stover, 1983).

Commercially, saponins have been extracted from plants and are used for tumor cell growth inhibitor and lowering blood cholesterol level. It is also used as an antitussive and an expectorant in traditional medicine. Pharmacologically, saponins can be used as spermicide (contraception), anti-inflammation, cytotoxic and antimicrobial agents (Afolayan, 2013; Odeh and Amom 2014; Dey, 2014).

Antibacterial compound is used for controlling harmful

bacteria growth. Microorganism growth control is required so as to prevent disease and infections from spreading. It prevents materials from putrefaction and destruction by microorganisms, and to eradicate infected host micro-organisms. Antimicrobial activities include antibacterial, antimicotic and antiviral effects. An antibacterial is a chemical agent which is able to inactivate bacteria. Bacterial inactivation can be as bacteria growth inhibition *(bacteriostatic)* or bactericide.

Antibacterial test can be done to observe bacterial activity against antibacterial (Komolafe, 2014; Sheela, 2012)

Saponins coarse extract from *Averhoabilimbi* stem has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Faradisa, 2008). *E. coli* is found inhuman and animal respiratory tracts, and becomes opportunist pathogen out of intestine, such as in urinary tracts. It causes wound infection, cholecytitis, appendicitis, peritonitis, sinusitis, meningitis, endocarditis and diarrhea. Microbes used to observe saponins antibacterial capacity of *S. trifasciata* are *E. coli* and *S. aureus. E. coli* may cause diarrhea, whilst *S. aureus* causes cough on human.

METHODS

Research was conducted in the Laboratory of Agricultural Product Technology, Polytechnique Lampung (Polinela Rajabasa, Bandar Lampung) for six months (March-August, 2015). Data collection includes sample preparation, preliminary test, saponins extraction, antibacterial activity test, and compound separation by thin layer chromatography (TLC).

Sample preparation

A total of 500 g of *S. trifasciata* roots were cleansed, then dried by oven at 60°C until a constant weight was obtained. Then ground and polished to a soft powder.

Preliminary test

Foam test

0.5 mg of sample is introduced into a tube containing distilled water then shaken vigorously for 5 min and observed the foam arising until stable and high measured foam (foam height of 1 to 3 cm). stable foam after drops of HCL 1 N then show positive reaction (Faradisa, 2008).

Liebermann- Burchard (LB) test

0.5 mg of sample was weighed and put into test tube I,plus 5 ml of CHCl 3 then heated 5 min above the water bath while being shaken and then cooled. Taken 1 ml mixture of the first test tube and put into a test tube II. Into the test tube II was tested (LB) (1 ml of anhydrous Acetic Acid and 1 drop of Concentrated Sulfuric Acid). Then observed changes that occur until approximately 30 min (Indriani, 2006).

Saponin extraction

Saponin extraction was performed using a maceration method with a 70% ethanol solvent of 300 mL, into an erlenmeyer fed 25 g of sample and shaked every 2 h for 24 h, then filtered to produce filtrate and residue. The residue was macerated again with 300 mL of ethanol solvent and this immersion treatment was repeated 3 times. The obtained filtrate was concentrated with the vacuum rotary evaporator. The concentrated extract was placed in a 250 mL separated funnel and suspended with 35 mL aquades, washed with 1: 1 diethyl ether, shaken and allowed to form two layers. The water layer was extracted and extracted with n-butanol 1: 1. Butanol extracted and concentrated by rotary vacuum evaporator. Furthermore, the extract tested the antibacterial effectiveness and isolated by TLC (Kristianingsih, 2005).

Antibacterial activity test

Medium preparation

The preparation of the medium was carried out by 2 g of nutrient to be dissolved in 100 mL of distilled water in a beaker glass, then heated to boiling and put into 10 test tubes (10 mL each for 8 test tubes and 5 mL for 2 test tubes) and covered with cotton. Then sterilized in autoclave temperature 121°C for 15 min. Then the tube containing 5 mL of nutrient solution to be placed in posisimiring and silenced for 24 h at room temperature (Faradisa, 2008).

Pure cultures of S. aureus and E. coli

Pure cultures of *S. aureus* and *E. coli* stroked aseptic loopful on solid media for oblique and media tubes covered with cotton. Furthermore, cultures of *S. aureus* and *E. coli* were incubated for 48 h at 37°C (Faradisa, 2008).

Preparation of bacteria solution S. aureus and E. coli

Taken 1 oz pure cultures of *S. aureus* and *E. coli* to dissolve in 10 mL of sterile distilled water (Faradisa, 2008*).*

Antibacterial activity test

The nutrient solution was put into a Petri dish and each was mixed with 0.1 mL of *S. aureus* and *E. coli* bacteria, then homogenized. Paper discs soaked in the extract for 15 min with various concentrations of 100, 200, 300, 400 500, 600, 700 and 800 (mg / L). Positive control for *S. aureus* bacteria using penicillin 25 mg / mL, whereas the positive control for the *E. coli* bacteria using streptomicyn 6.25 mg/mL and the solvent distilled water (negative control). After the paper disc soaked in the extract for 15 min placed on the surface of the media using sterile forceps and pressed a little. Furthermore, it is incubated at 37ºC until the barrier area appears for 24 h. The measurement of the obstacle zone is done by measuring the diameter of the clear area using the sliding term.

Isolate compound separation by TLC

Analytical thin layer chromatography (TLC)

Concentrated extract spotted at a distance of 1 cm from the bottom edge of the TLC plate using a capillary tube. Then dried in air and eluted to a distance of 8 cm in a glass vessel with a diameter of 6

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cm. Eluent used was a mixed solution of chloroform: methanol: distilled water with a concentration variation (13:4:1, 65;50:10, 20:60:4, 20:60:10). The chromatograms observed with UV light at λ256 nm and λ 366 nm. The color was observed and calculated Rf with the size. Eluen which gives the best results used for preparative TLC separation (Kristianingsih, 2005).

Preparative TLC

30 mg of concentrated extract is dissolved in methanol and then bottled at a distance of 1 cm from the bottom edge of the TLC plate using a capillary tube. Eluent used is the best eluent on analytical TLC. After the scrubbing process was finished the spots were scraped, then added 3 mL of n-butanol and centrifuged for 15 min. After the precipitated silica centrifuged and the supernatant was separated. The supernatant was evaporated the solvent with a stream of nitrogen gas to form solids solids are taken and used in the testing of antibacterial activity against *E. coli*and *S. aureus*. (Kristianingsih, 2005),

Antibacterial test of saponin compound result of TLC isolation

then homogenized. Paper discs soaked in isolates for 15 min with the concentration of the test results crude extract best effectiveness. After that it was placed on the surface of the media using sterile tweezers and pressed slightly. Furthermore incubated at 37ºC until a bottleneck area for 24 h. Measurement of inhibition zone is done by measuring the diameter of the clear area using a caliper.

RESULTS AND DISCUSSION

Antibacterial activity test was done to observe if saponins extract of *S. trifasciata* roots could inhibit positive and negative Gram bacteria, such as *S. aureus* and *E. coli*, as saponins are known to be able to inhibit bacterial cell wall synthesis and destroy plasma membrane of positive and negative Gram germ cell. Therefore, tannins assay towards positive Gram bacteria (*S. aureus*) and negative Gram bacteria (*E. coli*) needs to be conducted to rule out tannins as the causative agents of the observed activity.

Antibacterial effectiveness test of coarse extract of *S.trifasciata* roots was done on *S. aureus* (gram positive)and *E. coli* (gram negative) bacteria using diffusion disk method by measuring clear zone diameter. Observed antibacterial activity is described in Table 1.

Analitic thin layer chromatography (ATLC)

Table 2 and Figure 1 described that concentration (13:4:1) without UV light had one stain with R_f = 0.837, on λ 254 showed 2 two stains with R_f = 0.837 and 0.975, λ 366 gave 2 stains with Rf = 0.837 and 0.862.
Concentration (65:50:10) without UV light had one stain with R_f = 0.85. On λ 254 gave stain with R_f = 0.9 whilst λ 366 showed 3 stains with R_f = 0.85, λ 254 had one stain with R_f = 0.9 showed three stains with R_f = 0.85, 0.9 and 0.987.

Concentration (20:60:4) without UV light showed one stain with R_f = 0.75, on λ 254 had two stains with R_f = 0.126 and R_f = 0.75, λ 366 showed one stain with R_f = 0.812. Concentration (20:60:10) without UV light had one stain with R_f = 0.975, λ 254 had 2 stains with R_f = 0.875 and 0.975

Eluent chlorofom:methanol:distilled water of concentration (20:60:4) was the best to separate triterpenoid-saponin on coarse extract of *S. trifasciata* roots. This concentration is suitable with eluent polarity, well separated the components inside coarse extract of *S. trifasciata* roots, therefore; it can be applied as eluentfor preparative TLC.

Sheela (2012) described that saponins isolation can be conducted by fractional extraction using methanol, diethyl ether and n-buthanol, separation of isolated product by TLC methods. On plate *silica gel* F_{254} with mixed eluent chlorofom-methanol-distilled water (20:60:20) showed three stains.

Preparative thin layer chromatography (PTLC)

Except the extract quantity being used, preparation results of PTLC were similar to qualitative TLC. Silica gel TLC plate (10 × 20 cm) was used for preparative TLC. The best ATLC eluents, chloroform:methanol:distilled water (20:60:4), was also applied for PTLC.

Following Philip et al. (2011), saponins isolation was conducted by fractional extraction using methanol, diethyl ether and n-buthanol. The resulting fractions were then separated by PTLC, using silica gel F254 and eluent mixture of chlorofom-methanol-distilled water (20:60:20) which showed three stains. Each isolated stains was then further characterized by ^{1}H-NMR.

Sethi (2013) analyzed saponins-triterpenoid from ginseng roots by eluent chlorofom:methanol:distilled water (20:60:4) gave ten stains with R_f 0.35-0.75. PTLC with eluent chlorofom:methanol:distilled water (20:60:4) had 3 stains: Isolated compounds I Rf=0.162; II Rf=0.75; III R_f =0.812. It was confirmed isolated compound II is saponin-triterpenoid.

Saponins extract showed inhibition zone against E. *coli* started with the concentration of 100 mg/mL. Inhibition zone diameter increased 18.67 mm with the concentration of 200 ppm, 17.0 mm and 17.7 mm with the concentration of 300 and 700 ppm. Inhibition zone of 400 ppm was similar to concentration 100 ppm, that was 11.67 with concentration of 500 and 600 ppm had 16.33 and 16 mm.

It is assumed that bacteria grew using this concentration had resistance mechanism against saponins antimicrobial coarse extract, which caused a formation of several resistant bacteria populations. As the results, inhibition zone around the disk was smaller.

Saponins exhibit polar character which facilitated the penetration through the gram positive bacteria cell wall.

Table 1. Inhibition zone caused by saponins extract at various concentrationsagainst *S. aureus* and *E. coli*.

Concentration (ppm)	Inhibition zone (mm)							
	S. aureus			Average	*E. coli*			Average
100	18	18	18	18	10	9	12	10.33
200	23	23	23	23	19	19	20	19.33
300	19	19	20	19.33	18	18	20	18.66
400	20	20	20	20	13	13	10	12
500	9	7	8	8	17	17	16	16.66
600	14	11	15	13.33	15	14	14	14.33
700	14	13	14	13.66	17	17	17	17
800	12	14	12	12.66	15	16	15	15.33

Table 2. Analytical TLC with eluent chloroform extract:methanol:distilled water.

No.	Chloroform eluent:methanol:water	Rf		
		No UV	UV λ254	UV λ366
1	13:4:1	0,837	0,837	0,837
		-	-	0,862
		-	0,975	-
2	65:50:10	0.85	-	0,88
		-	0,9	0,987
		-	-	0,987
3	20:60:4	-	0,162	-
		0,85	0,85	-
		-	-	0,812
4	20:60:10	0,812	0,875	-
		-	0,875	0,875

Hence, experimental material on inhibition zone had higher value against *S. aureus,* with the concentration of 200 ppm. Saponins can easier penetrated to *S. aureus* cell wall due to its higher composition of peptidoglycan (protein and sugar) compared to lipid (fat). The cell wall is thinner, therefore; it is easier to be penetrated by saponins which have polar character.

The solvent choice for extraction should fulfill some requirements such as: Affordability, common availability, physical and chemical stability, neutral reaction towards extracted compound, high boiling point, high flash point, selective and neutral towards the bioactive compound (Mai, 2013). Glycoside compounds like saponins and cardiac glycosides are insoluble in nonpolar solvents. This compound is most suitable to be extracted from the plant using ethanol or methanol 70 to 95% (Robinson, 1995). In their research, Kingsley (2014) used methanol to extract *Sansevieria roxhburghiana*, as saponins are polar. It is easier to dissolve and have more extracts. In this research saponins compound was characterized by infrared (IR) and Proton Nuclear Magnetic Resonance (1H-NMR). Moreover, 6 dark purple stains were obtained by TLC on R_f 0.68 with eluent mixture of chloroform - methanol - distilled water.

Anti-microbe effectiveness test

Antibacterial effectiveness test on PTLC isolated compound was similar as antibacterial test on coarse extract of *S. trifasciata* roots. The isolated compounds used were from PTLC with optimum concentration (200 mg/L).

It was observed that the Isolated compounds I and II were effective as antibacterial. This was observed from the inhibition zones, produced by the compounds on E.*coli and S. aureus.* Compound I showed zone of inhibitionof 5.52 mm on *E. coli* while compound II showed zone of inhibition of growth of on 2.50 mm on *E. coli*. Compound I showed zone of inhibition of growth of 1.54 mm on *S.*

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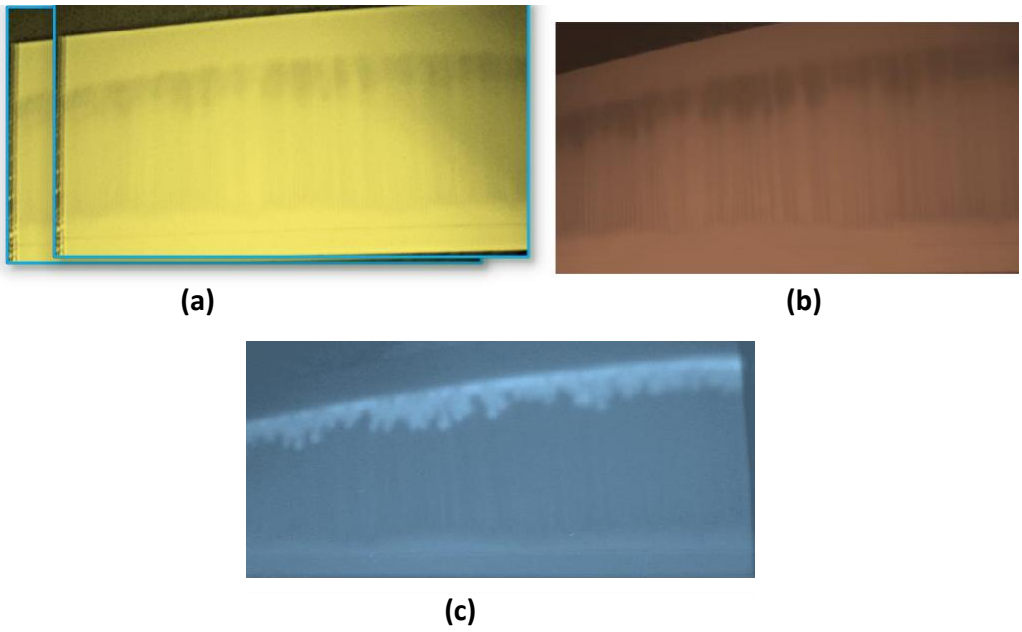

Figure 1. TLC analytical:a. No UV; b. UV λ 366 nm; c. UV λ 254 nm.

aureus- while compound showed zone of inhibition ofgrowth of 0.68 mm on *E. coli*. While compound III did no show activity isolated compound III was ineffective. There was no inhibition zone around the disk. The extract had larger inhibition zone of inhibition than the isolated compound. It was assumed that the antibacterial mechanism from the extract is synergic. The components that exhibited potential antibacterial activity were mutually reinforcing. If the isolated compound I or II was separated from the extract, the antibacterial activity decreases.

It was found from PTLC results that the inhibition zones of isolated compound I and II were smaller than on the extract.

The isolated compound III had no inhibition zone around the disk. On certain concentration, the presence of isolated compound III in the extract tend to influence antibacterial inhibition ability (isolated compound III was antagonist) towards antibacterial compounds in the extract. Therefore; it would decrease its antibacterial activity.

Saponins effectiveness from *S. trifasciata* var. Golden Hahnii roots towards bacterial species tested had good potency, compared to other plant that has high saponins such as star fruits. The star fruits had inhibition towards *E. coli* of 15 mm and *S. aureus* of 13 mm (Faradisa,2008), on Mimosa roots it had inhibition zone of inhibition towards *E. coli* (18 mm) and *S. aureus* (23 mm) (Jaya, 2010). Sheela (2012) described saponins as antimicrobial agents. Saponins extract from wheat (*Sorghum bicolor* - linn), fractionated by column chromatography and TLC, were inhibitive for gram positive bacteria. On gram positive bacteria growth, which is *S. aureus,* the minimum inhibition level was 25 mg/mL, whilst on gram negative bacteria *E. coli* and fungi *C. albican,* it was not inhibitive.

Saponins-are polar, facilating the-penetration of gram positive bacteria cell wall. The experimental material value on inhibition zone showed lower number on the test towards *S. aureus,* with a concentration of 200 mg/mL. Saponins were easier to penetrate *S. aureus cell wall* as it was thin and contained more peptidoglycan (protein and sugar) than lipid (fat). *E. coli* cell wall that contained more lipid (fat) and thicker, was more difficult to be penetrated and more resistant towards saponins. It was showed by the presence of inhibition zone for the first time needs coarse extract of saponins with the concentration of 300 mg/mL, higher than the treatment for bacteria *S. aureus.*

Saponins can be dissolved in fat and water will be concentrated in cell membrane, the important and fine part (Sheela, 2012; Dey, 2014). Sethi (2013) described that saponins-are strong compounds to decrease the surface tension, working as antimicrobial activity by disturbing bacteria cell membrane stability, and therefore; making the bacteria prompt for lysis.

Saponins are polyphenol compounds which can inhibit bacteria by destroying its cytoplasma membrane that consists of 60% protein and 40% lipid, commonly phospholipids. This destruction caused metabolite leakage which inactivated bacteria enzyme system. The damage on cytoplasma membrane can prevent nutrition input needed by the bacteria to produce energy. As the result, the bacteria will experience limited growth and mortality. Every bacteria cell is surrounded by cytoplasma membrane predominantly consisting of selective permeable ergosterol. Phospholipid is also an important

compound in bacteria cytoplasma membrane formation. On cytoplasma membrane destruction, saponins (polyphenol) released ion H^+, attacked hydrophilic head (hydroxy and phosphate groups) on cell membrane surface. It caused hydroxy group on ergosterol molecule bonding with broken hydrogen, and the cell membrane was not able to hold the internal tension. As the results, cytoplasma of the cell will be expelled. On phospholipid molecule, ion H^+ of saponins will attack polar head (phosphate head) so that this molecule will be degraded as glycerol, carboxylic acid, and acid phosphate. Phospholipid will not hold the cytoplasma-membrane form. The substances for bacteria cell metabolism will be expelled resulting in the bacteria mortality.

Conclusion

S. trifasciata-roots have potency as an antibacterial agentand had ability to inhibit the growth of *E. coli* (18.67 mm) and *S. aureus (*24 mm*)* at 200 ppm concentration level. The best eluent that separated the triterpenoid-saponins was chloroform: methanol:aquades (20:60:4) with 3 stains showed separately with R_f 0.125; 0.75 and 0.812.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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