

PHYTOCHEMICALS SCREENING AND ANTIBACTERIAL ACTIVITY OF *Curcuma Longa* Linn, *Ziziphus Mauritiana* AND *Centella Asiatica* L.Urban EXTRACT

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ABSTRACT

Phytochemical screening and antibacterial activity have been carried out on several plants that have a potency to heal the wounds, namely *Curcuma Longa* Linn, *Ziziphus Mauritiana* and *Centella Asiatica* L.Urban. This aim of this study to see the potency of *Curcuma Longa* Linn, *Ziziphus Mauritiana* and *Centella Asiatica* L.Urban as a wound healing. The phytochemical screening was performed to identify the secondary metabolites that presence in those extracts. In the other hand, antibacterial activity was performed against *E. coli*, *S. aureus*, and *P.aureginosa*. Phytochemical screening showed the presence of phenolic, saponins and terpenoids in *Curcuma Longa* Linn and *Ziziphus Mauritiana*. While in the *Centella Asiatica* L.Urban extract positively contained terpenoids. Antibacterial activity of *Curcuma Longa* Linn extract showed the highest inhibition zone against *S.aereus*, and *P.aureginosa* at 1000 ppm. *Ziziphus Mauritiana* And *Centella Asiatica* L.Urban extract showed the highest antibacterial activity at 1000 ppm against *S.aureus* bacteria.

Keywords: Phytochemicals Screening, Antibacterial Activity, *Curcuma Longa* Linn, *Ziziphus Mauritiana*, *CentellaAsiatica*L.Urban.

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INTRODUCTION

Indonesia is a tropical country that has a lot of medicinal plants. This kind of plant has been widely studied and utilized in traditional and modern medicine.¹Many plants can be used as a medicine, i.e. *Curcuma Longa* Linn, *Ziziphus Mauritiana*, and *Centella Asiatica* L.Urban. The presence of secondary metabolites in such plants can be determined using the qualitative method, name as a phytochemical test.

Phytochemicals consist of two words, python meaning 'plant' and chemistry. So, phytochemicals are used to examine important nutrient compounds in plants and their effects as essential nutrients for health. Phytochemical standardization guidelines on food products or nutritional labels have been issued by Regulatory body that regulates food labeling in Europe and the United States.²

For centuries, curcumin has been used as traditional medicine and as a cooking spice. Over time, curcumin is also used to treat various diseases, treat type II diabetes, and various cancers, including gastrointestinal, melanoma, genitourinary, breast, lung, hematology, head and neck, neurological and sarcoma,³ potential therapeutic, against neurodegenerative.⁴

Curcumin, a phenolic compound, can be found in *Curcuma Longa* Linn has been used as a natural antioxidant, anti-microbial, anti-Alzheimer,⁵a potent immunomodulatory agent,⁶anti-inflammation, anti-cancer.^{7,8} *Zizipus Mauritiana*. is traditional Arabic medicine, also as a Chinese date. The leaves are used as poultices and are helpful in liver troubles, asthma, fever,⁹ Antibiotic or antiseptic,¹⁰ cancer treatment,^{11,12} employed for treating toothaches, mouth sores, wound healing and as blood tonic.¹³ *Centella Asiatica* L.Urban is traditional herbal medicine. In Indonesia, *Centella Asiatica* L.Urban leaves are eaten fresh as salad, cooked as a vegetable, and blended as a drink.^{14,15} *Centella Asiatica* L.Urban is a tropical plant and used as traditional and modern medicine. It is used for the management of the central nervous system, skin and gastrointestinal disorders,^{16,17} as an antidepressant, even though its primary application is for promoting wound healing.^{1,18,19}

EXPERIMENTAL

Materials

The materials were obtained from the Biochemistry laboratory of Universitas Sumatera Utara, methanol, aquadest, HCl, Mayer, Wagner and Dragendorff reagents, chloroform (pa Sigma), acetic acid (pa Sigma), NaOH (pa Sigma), sodium hypochlorite (pa Sigma), oxalic acid (pa Sigma). *Curcuma Longa Linn* and *Ziziphus Mauritiana*, *Centella asiatica L. Urban*. DMSO (pa Aldrich)

Preparation and Extraction of Sample

The sample was peeled, washed and then dried. Then the methanol extract of the sample was made using the maceration method. The dried sample was mashed into powder and soaked in methanol for 24 hours. After 24 hours, the solution obtained was collected and evaporated using a rotary evaporator. The evaporation process was continued in a water bath to obtain a concentrated extract. Then the phytochemical screening tests and anti-bacterial tests were carried out.

Phytochemical Screening Test

Alkaloid Identification

About 0,5 gram of extract was added with 1 mL of HCl 2 M and 9 mL aquadest. The solution was heated for 2 minutes. The solution then filtered and the filtrate was divided into three and each part was added with Mayer, Wagner and Dragendorff reagent.

Flavonoid Identification

The concentrated extract was dissolved in hot methanol, then 0.1 Mg powder was added into the solution and followed by 5 drops of concentrated HCl.

Terpenoid and Steroid Identification

Some amount of extract was dissolved in 0.5 mL chloroform, 0.5 mL acetic acid glacial, and 2 mL H₂SO₄.

Tannin Identification

The extract was dissolved in 10 mL of aquadest and filtered. The filtrate was added with FeCl₃ 1%.

Saponin Identification

The extract was dissolved in 10 mL of hot water, the solution was shaken for 10 s.

Antibacterial Activity Test

The antibacterial test was performed using Kirby-Bauer method.

Pre-sterilization

The workplace was sterilized using a disinfectant, and hand was washed using alcohol 70%.

Preparation of the Bacterial Suspension

The bacterial suspension was prepared from stock and dilutes using 10 mL of aquadest. The turbidity was equated to McFarland solution, equal to 10⁸ CFU.

Extract Bioactivity

The MHA medium was poured into petri dish and formed solid agar. Sterilized cotton bud was dipped into the bacterial suspension and swiped on the MHA medium. The marker was placed to identify which concentration of extract was used (10, 100, 1000 ppm). A similar thing was performed for control, but the extract solution was substituted using amoxycillin. After 24 h of incubation, the clear zone was measured using caliper.

RESULTS AND DISCUSSION

Phytochemical Screening Test

The phytochemical screening's result of *Curcuma Longa Linn*, *Ziziphus Mauritiana* and *Centella Asiatica L. Urban* is displayed in Table-1. The result in Table-1 shown there were no alkaloids and flavonoids in all sample. *Curcuma Longa Linn* extract contains high phenolic (++++), saponin (++++), and triterpenoid/steroid (++++). *Ziziphus Mauritiana* extract contains high phenolic (++++), low saponin

(++), and high triterpenoid/steroid (++++). *Centella Asiatica* extract contains no phenolic, no saponin, and high triterpenoid/steroid (+++). *Curcuma Longa Linn* extract contains the highest phenolic, saponin and triterpenoid/steroid from the others.

Table-1: Phytochemical Screening

	Reagent	Result		
		<i>Curcuma Longa Linn</i>	<i>Ziziphus Mauritiana</i>	<i>Centella Asiatica L. Urban</i>
Alkaloid	Mayer	-	-	-
	Bouchardat	-	-	-
	Dragendorff/ Wagner	-	-	-
Flavonoid	Mg + conc. HCl	-	-	-
Phenolic	Aquadest	++++	++++	-
Saponin	Air + HCl	++++	++	-
Triterpenoid /Steroid	Liebermann-burchard	++++	+++	+++

Note: (+) = positive, (-) = negative

Phenolic, triterpenoid/steroid display antibacterial, antifungal and antitumor activities²⁰. *Curcuma Longa Linn* extract contains curcumin, a phenolic compound. *Ziziphus Mauritiana* and *Centella Asiatica* extract contain ziziphin and asiaticoside, a triterpene compound.^{15,18}

The results of this positive terpenoid test are evidenced by the appearance of strong red color in the test sample. Hydroxyl groups from Terpenoids when reacting with free radicals will give hydrogen atoms so that the reaction of acetylation of hydroxyl groups form double bonds,²¹ then the release of hydrogen groups causes conjugated double bonds, then a conjugation prolongation reaction occurs due to the release of electrons, and absorb the strong red spectrum.

Antibacterial Activity

The antibacterial test was carried out by an in vitro process using gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aureginosa*)²² and Amoxycillin as a positive control. The result is shown in Table-2.

The antibacterial activity of these extracts was observed by the presence of clear zone in around of wells.²³ The result of antibacterial activity against *Escherichia coli* bacteria, *Curcuma Longa Linn*, *Ziziphus Mauritiana*, and *Centella Asiatica L. Urban* have good antibacterial activity.

On the same concentration, *Curcuma Longa Linn* is the best than the other, and the max antibacterial activity is the highest concentration (1000 ppm). The clear zone can be seen in Fig.-1.

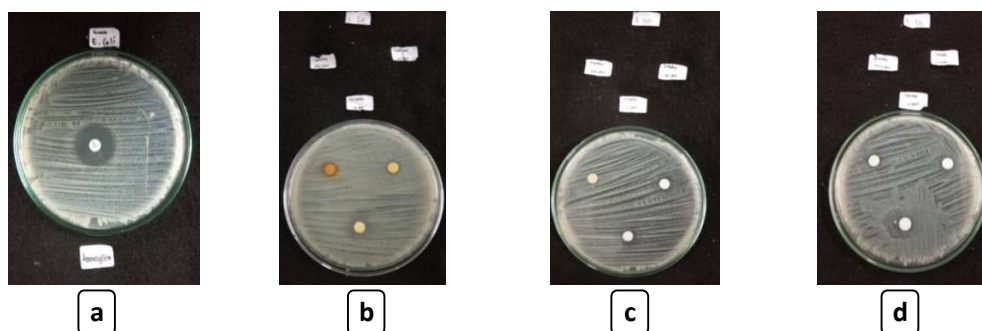


Fig.-1: Antibacterial Activity of (a) Amoxycillin, (b) *Curcuma Longa Linn*, (c) *Ziziphus Mauritiana* and (d) *Centella Asiatica L. Urban* against *E. coli*

Tabel-2: The clear zone's diameter of *Curcuma Longa Linn*, *Ziziphus Mauritiana* and *Centella Asiatica L. Urban*

Bacteria	Concentration	<i>Curcuma Longa Linn</i>	<i>Ziziphus Mauritiana</i>	<i>Centella Asiatica L. Urban</i>
<i>Escherichia coli</i>	10 ppm	7.61 ± 0.15	7.05 ± 0.07	7.06 ± 0.06
	100 ppm	7.83 ± 0.11	7.06 ± 0.06	7.10 ± 0.14
	1000 ppm	9.08 ± 0.18	7.77 ± 0.10	7.22 ± 0.16
	Amoxycillin	20.28 ± 0.04	20.28 ± 0.03	20.28 ± 0.04

<i>Staphylococcus aureus</i>	10 ppm	7.58 ± 0.11	9.04 ± 0.06	8.56 ± 0.08
	100 ppm	8.74 ± 0.09	9.83 ± 0.11	8.63 ± 0.11
	1000 ppm	10.43 ± 0.11	11.28 ± 0.04	9.11 ± 0.13
	Amoxycillin	7.53 ± 0.04	7.48 ± 0.03	7.41 ± 0.13
<i>Pseudomonas aureginosa</i>	10 ppm	8.22 ± 0.12	8.61 ± 0.13	-
	100 ppm	8.70 ± 0.14	9.21 ± 0.16	-
	1000 ppm	10.37 ± 0.18	9.44 ± 0.08	-
	Amoxycillin	12.16 ± 0.08	12.14 ± 0.05	12.12 ± 0.03

The result of antibacterial activity against *Staphylococcus aureus* bacteria, *Curcuma Longa* Linn, *Ziziphus Mauritiana*, and *Centella Asiatica* L. Urban have good antibacterial activity.

On the same concentration, *Ziziphus Mauritiana* is the best than the other, and the max antibacterial activity is the highest concentration (1000 ppm). The clear zone can be seen in Fig.-2.

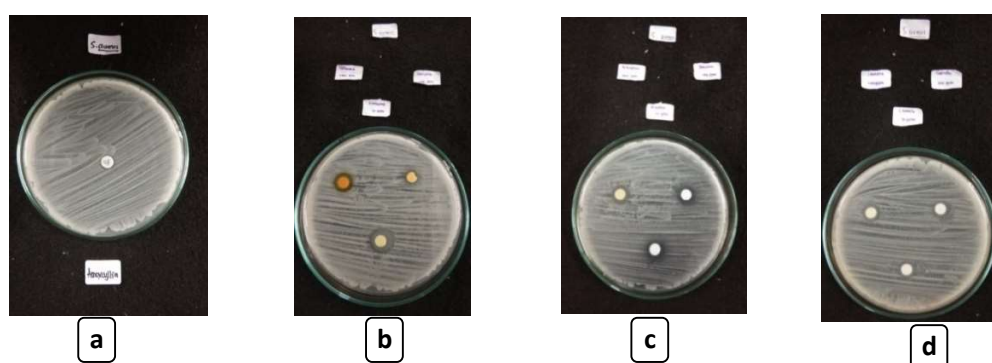


Fig.-2: Antibacterial activity of (a) Amoxicyllin, (b) *Curcuma Longa* Linn, (c) *Ziziphus Mauritiana* and (d) *Centella Asiatica* L. Urban against *S. aureus*

The result of antibacterial activity against *Pseudomonas aureginosa* bacteria, *Curcuma Longa* Linn, and *Ziziphus Mauritiana*, have good antibacterial activity.

On the same concentration, *Ziziphus Mauritiana* is the better than *Curcuma Longa* Linn, but the max antibacterial activity is the highest concentration of *Curcuma Longa* Linn (1000 ppm). The clear zone can be seen in Fig.-3.

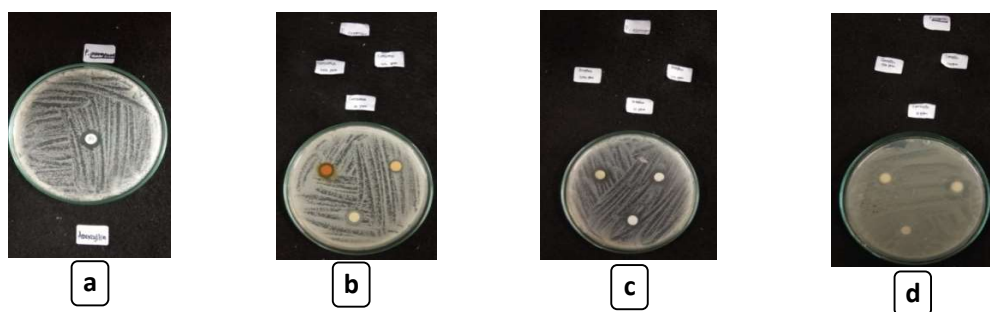


Fig.-3: Antibacterial Activity of (a) Amoxicyllin, (b) *Curcuma Longa* Linn, (c) *Ziziphus Mauritiana* and (d) *Centella Asiatica* L. Urban against *P. aeruginosa*

The result of the antibacterial activity test shown *Curcuma Longa* Linn and *Ziziphus Mauritiana* have antibacterial activities against *E.coli*, *S.aereus* and *P.aureginosa*. *Centella Asiatica* has antibacterial activities against *E.coli* and *S.aereus*, however have no antibacterial activity against *P.aureginosa*. enhancement concentration of extract can increase the inhibitory effect on bacterial growth. The highest inhibition zone against *E.coli*, *S.aereus* and *P.aureginosa* at 1000 ppm.

Research results in *Curcuma Longa* Linn and *Ziziphus Mauritiana* that contain phenolic, saponin and triterpenoid/steroids have higher antibacterial activity than *Centella Asiatica* that contains no phenolic and saponin.

CONCLUSION

Phytochemical screening showed the presence of phenolic, saponins and terpenoids in *Curcuma Longa* Linn and *Ziziphus Mauritiana*. While in the *Centella Asiatica* extract positively contained terpenoids. Antibacterial activity of *Curcuma Longa* Linn extract showed the highest inhibition zone against *S.aereus*, and *P.aureginosa* at 1000 ppm. *Ziziphus Mauritiana* And *Centella Asiatica* L.Urban extract showed the highest antibacterial activity at 1000 ppm against *S.aureus* bacteria.

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