An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

ANTIBACTERIAL ACTIVITY AND FLAVONOID CONTENT OF TORCH GINGER LEAVES (*ETLINGERA ELATIOR)*

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ARTICLE INFO

Key Words

Torch ginger leaves (*Etlingera elatior*), *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, MIC, flavonoid

ABSTRACT

*Staphylococcus epidermidis*and *Pseudomonas aeruginosa*are gram-positive bacterium which is a normal human flora that can turn into a pathogen caused much inflammation. The aim of this reasearch was to investigatedthe candidate of antibacterial compound of *Etlingera elatior* leaves, guided by antibacterial activity with diffusion method and bioautography method.Ethanolic extract of torch ginger leaves (*Etlingera elatior*) from Indonesia were screened for antibacterial activities. Torch ginger leaves was macerated with ethanol 70% as a solvent. Then, dry leaves extract were fractionated by liquid liquid extraction with different solvents. Extract and fractionas were tested its antibacterial activity against *Staphylococcus epidermidis*and *Pseudomonas aeruginosa*with diffusion method. Total flavonoid content assay was done by colorimetric method with quercetin as a standard and measured with uv-vis spectrophotometry at ƛ 420nm. Total flavonoids expressed as % mg/g QE. The determination of the active compounds was carried out by contact bioautography assay. Antibacterial activity against both bacteria was present in ethanol leaves extract (MIC : 400-25 µg/mL) and in its fractions of n-hexane, ethyl acetate and methanol against *Staphylococcus epidermidis*showed MIC respectivelly at 200 , 100 and 200 µg/mL; and against *Pseudomonas aeruginosa*showed MIC respectivelly at 400 , 50, and 100µg/mL. Total flavonoid content in leaves extracts and its fractions of nHexane, ethyl acetate and methanol fraction respectivelly were 0.227±0.01, 0.28 ± 0.02, 0.42 ± 0.05, and 0.40 ± 0.01 % mg/gQE. The highest total flavonoid content was found in the ethyl acetate fraction of torch ginger leaves, and was tested its bioautographic assay on two bacteria. It was showed a clear zone at Rf 0,75 which positively reacted with $FeCl_3$ 10%, $AlCl_3$ 5% and sitroborat spraying reagents. We conclude that antibacterial compound of torch ginger was a phenolic compound of flavonoids.

INTRODUCTION

The unproperly used of antibiotics has led to drug resitance in many bacteria strain, and development of new antibacterial compound is becoming critically important. Increasing the resistance of some antibacterial has many implication for society such as morbidity, mortality and health care cost. The used of natural medicines to treat infection desease has been discussed to be a complementary alternative treatment. The study was expected that herbal medicine can be a first line therapy in treatment of some case of desease (1) Torch ginger (*Etlingera elatior*) is zingiberaceae group (2) which is used in community to treat the infection

desease such as earache, toothache, skin desease and to treat the body odor (3) Several studies have been conducted on the antibacterial and other activities of *E. elatior*. Ethanolic extract of flower of *E. elatior*showed that flavonoid and alcaloid showed the activities on *P. aeruginosa , E. coli, and S. aureus* (4). Tannins and saponin recognized as antimicrobial compound (5), and *E. elatior* showed the inhibition the growth of *K.pneumoniae, S.aureus, Proteus vulgaris, S.thypi,* and *P. Aeruginosa*(6). Plants rich in flavonoids and phenolic are a good source of natural antioxidant and antibacterial activities. (7)

Fig1. *Etlingera elatior* Flower and Leaves

In this work, we report the antibacterial activity of ethanolic extract ofleavesof torch ginger and their fractions against *S. epidermidis* and *P. aeruginosa* by agar diffusion method. In our continuing research on flavonoid compound present in *E. elatior* showed the inhibition on two bacterials with bioautography contact, and also was investigated for the total flavonoid content.

METHOD:

The study was conducted in several stages, including preparation of materials, extraction and fractionation, Total Flavonoid Content, antimicrobial assay and bioautography contact assay.

Plant Collection

Leaves of *E. elatior* commonly used as spices, mouth wash and medicinal purposes, were planted at local garden in Bandung City, West Java, Indonesia. Leaves were harvest after 3 months of growth. This plant were identified and confirm by Herbarium Jatinangor, Biology Departement of Padjadjaran University, Indonesia

Sample Preparation

Leaves of *E. elatior* were washed, cleaned in running tap water, cuts into small pieces and dried to constant weight using oven at 40^0C. The sample were pounded into small size using grinder machine; it was stored in an air tight container till when needed.

Extraction and Fractination

The dried leaves powder of *E. elatior* (1000g) was extracted sequently by maceration method in ethanol 70% (3x 2000 mL) at room temperature. The filtrate was concentrated in vacuo, obtained 199,87 g extract. Leaves extract (20 g) then were fractionated with liquid liquid extraction to obtained nHexane fraction (0,49 g), EtOAc fraction (7,03 g) and water fraction (10,76 g), successively.

Chemicals

Quercetin and $AlCl_3$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ethanol, methanol, ethyl acetate, and n-Hexane as eluan were purchased from CV. Tri Putra Jaya Abadi.All the other chemicals used were of analytical grade

Bacterial Strain

Test was performed against the *S. epidermidis*(ATCC 12228) and *P. aeruginosa* (ATCC 9027)purchase from The American Type Culture Collection (ATCC) obtained from Pharmacy of Institute of Bandung (ITB), Indonesia. The agars used to maintain the microorganism were Muller Hinton Agars medium (Merck).

Preliminary Phytochemical Studies

A preliminary phytochemical analysis wa carried out for qualitative identification of phytoconstituents as a standard procedure (8)

Assay for Antimicrobial Testing

Antimicrobial activity of the samples was assayed separately using an agar diffusion method with paper disc (OXOID CT0998B), aquabidest as negatif control and gentamycine (OXOID CT0024B). Culture of microorganism

were obtained from stocks maintained at Bandung School of Pharmacy Laboratory. The microorganisms were maintained on agar slants and subcultures were freshly prepare before used. Bacteri inocula were made in 5 mL of medium broth (Merck) and grown for 24h at 37^0C. Freshly prepared Muller Hinton Agar (MHA) medium were poured into petri plates. 5 mL portion of medium seeded with the microorganism (0,5%), was poured directly over the surface of the prepared plate. The plate were then allowed to solidify for 5 min and 4 paper disc were applied to the surface of the inoculated plates with steril pinset.Volume of 200 µL of crude extract and fractions (at concentration 400, 200, 100, 50 and 25 µg/mL) were inoculated in each paper disc. Agar plates were incubated overnight at 35^0C. At the end of the incubation periods, inhibition zones were recorded as the diameter of the growth-free zones around disc. Each extract and fractions were tested in triplicate. In all plates, control of disc was used DMSO 2% as negative control and as a standard antibacterial agent was used gentamycin (10 µg/mL, 200 µL) as positive control for bacteria.(9)

Determination of Flavonoid Content

Total flavonoid contents were determined using the method of Ordon ez et al. (10) A volume of 0.5 ml of 2% $AlCl_3$in ethanol solution was added to 0.5 ml of sample solution. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid content were calculated as quercetin (mg/g) using the following equation based on the calibration curve: $y = 0,0609x + 0,2055$, $R^2 = 0,9982$, where x was the absorbance and was the quercetin equivalent (mg/g).

Bioautography Assay: The inocula of representative *S. epidermidis* and *P. aeruginosa*were swabbed onto Mueller-Hinton agar plates for use in contact bioautography technique adopted from the method of Wedge and Nagle (11). The dried TLC plates with corresponding spots were placed aseptically onto the seeded Mueller-Hinton agar plate overlaid with sterile lens paper. The TLC plate was placed face downward with the silica-coated side in contact evenly with the lens paper and was incubated for 12 to 18 hours at 4 ± 2°C. Then, the TLC plate was removed, and the inoculated agar plate was further incubated at 35 ± 2°C for 24 hours in an ambient air incubator. The zone of inhibition was observed and compared with TLC plate value results.

RESULTS AND DISCUSSION

Prelimanary Phytochemical Studies: Phytochemical studies revealed that ethanolic extract of *Etlingera elatior* leaves showed the presence of flavonoid, saponin, tannin, quinone, steroid and terpenoid(Table 1)

Assay for Antibacterial Testing: It was observed the antibacterial activity of extract and fraction of*Etlingera elatior* leaves against *S. epidermidis* and *P. aeruginosa*. Ethyl acetate fraction showed the best activity against both of bacterias. The leave of Etlingera elatior which used for this study may contain the bioactive compound as antibacteria. The present study we have observed promising antibacterial activity of *Etlingera elatior* leaves against*S. epidermidis* and *P. aeruginosa.* In the table 2 shows the inhibition zones observed against both bacteria, indicate of the presence of broad spectrum antibiotic compound in the plant (12). The results given in Table 2 showed that Ethyl acetate fraction showed as the most active as antibacteria. Davis and Stout (13), claimed that if inhibitory zone at 10-20 mm showed a strong as antibacterial. This inhibitory potential towards these bacterias was later confirmed when the ethyl acetate fraction was subjected to bioautography contact.

Determination of Flavonoid Content: The Total Flavonoid Content was obtained from absorbance of the extract and fractions treated with Alumunium chloride (Table 3). The Total Flavonoid Content of extract and fractions of *E. ealatior* was found to be maximum in ethyl acetate fraction than others. The Total Flavonoid Content was obtained from absorbance of the extract and fractions treated with Alumunium chloride reagent (Table 3).

Table 1. Preliminary Phytochemical Analysis of Ethanolic Extract of *E. elatior*

Chemical constituents	Result
Alkaloid	-
Flavonoid	+
Saponin	+
Tannin	+
Quinone	+
Steroid/Triterpenoids	+

+: Presence -: Absence

Table 2. Antibacterial Assay of *Etlingera elatior* leaves

E.elatior Leaves	Conc (µg/mL)	Inhibition zone (mm)	
		P.A	S.E
Extract	400	12,3	20,1
	200	-	18,2
	100	-	14
	50	-	9
	25	-	7
nHexane Fraction	400	10,4	12,3
	200	-	7,6
	100	-	-
	50	-	-
	25	-	-
EtOAc Fraction	400	18,3	17,8
	200	12,7	14,2
	100	9,2	12,2
	50	8,6	-
	25	-	-
Water Fraction	400	16,3	15,8
	200	9,8	7,4
	100	-	-
	50	-	-
	25	-	-
Gentamycine(+)	10	27,5	18,3
DMSO 2% (-)		-	-

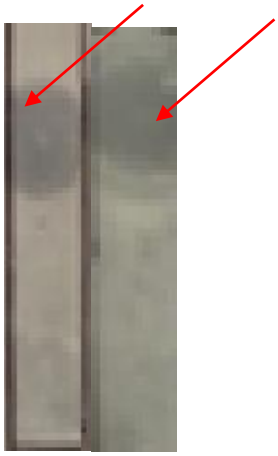
PA; *P. aeruginosa* SA : *S. epidermidis* - : Showed no inhibition

Table 3. Precentage Yield and Total Flavonoid of *Etlingera elatior*

E.elatior Leaves	% yield [a]	Flavonoid (%QE)[b]
Extract	19,98	0,22±0,012
nHexaneFraction	0,25	0,28±0,02
EtOAcFraction	3,52	0,42±0,01
WaterFraction	5,38	0,49±0,005

Note [a] %yield extract or fraction to simplicia

[b]As Quercetin Equivalent (QE) present in correspondingextract/fractions calculated using a standard graph



A B

Fig 2. Bioautography Contact of EtOAc fraction (eluent formic acid: ethyl acetate: water (1: 8: 1))A. In bacteria *Pseudomonas eruginosa* B. In *Staphylococcus epidermidis*

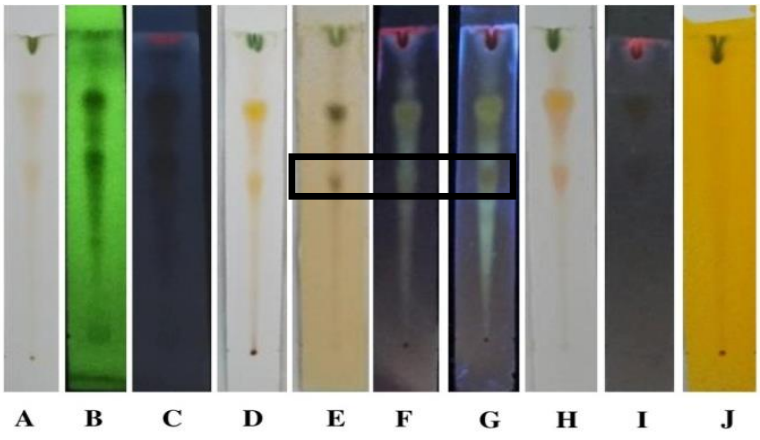


Fig. 3 Results of Compound Detection of On TLC Plate (eluent formic acid: ethyl acetate: water (1: 8: 1)). A. visual B. UV254 C. UV365 D. H2SO4 10% E. FeCl3 10% F. AlCl3 5% (UV365) G. Sitroborat (UV365) H. Vanilin Sulfate I. LB) J. Dragendorf

The Total Flavonoid Content of extract and fractions of *E. ealatior* was found to be maximum in ethyl acetate fraction than others. It was found that ethyl acetate fraction and the antibacterial activity showed a good correlation among sample was observed.

Bioautography Ethyl Acetate Fractions: Ethyl acetate fraction than was chromatographed with selected eluent formic acid: ethyl acetate: water (1: 8: 1). The pieces of TLC plates were then placed aseptically upon the bacterial lawn and were left for a period of two hours, in order to allow the materials from them to diffuse on to the seeded plates. Thereafter, TLC plates were removed from the and the plates were incubated in inverted position for 24 hours. The areas of inhibition were marked and relevant R_f values were recorded. An interesting observation of the ethyl acetate fraction of *Etlingera elatior* leaves was the presence of zones of inhibitionagainst *P. aeruginosa* (at R_f =0, 75) and S. epidermidis (at Rf =0,75) To find out the chemical compounds as an antibacterial agent, the chromatograms were sprayed on the specific spotted reagents of $FeCl_3$ 10%, $AlCl_3$ 5%, Sitroborat, Vanilin Sulfate, Leibermann-Bourchard (LB) and Dragendorf. The presence of clear bands with the same Rf value (0,75) may mean that the same compounds are probably responsible for the antibacterial activity . The R_f values of the antibacteriall compounds present in the same spot after sprayed with $FeCl_3$ 10% (blue black spot at Rf 0,75) showed positive as phenolic compound, with $AlCl_3$ 5% and Sitroborat showed yellow spot as flavonoid compound at Rf 0,75. The result suggested that the flavonoid compound contributed significantly to the antibacterial activity of *E. ealatior*. (7,14). Natural products like spices as antibacterial can further be identified and used as alternative to currently used drug against the pathogenic microbe under study, and may give us a solution to alarming problem about resistance of microbes (15).

CONCLUSION

The antibacterial mediated in vitro studies revealed that flavonoid compound responsible for the antibacterial activity of *Etlingera elatior*against *Pseudomonas eruginosa* and*Staphylococcus epidermidis*bacterias.

ACKNOWLEDGEMENT

The author thanksto Faculty of Pharmacy Bhakti Kencana University for providing financial assistance.

REFERENCES

1. Micozzi, Marc S. Fundamentals of Complementary and Alternative Medicine. St.Louise,Missouri : Saunder Elsevier Inc. 2015
2. Cronquist, Arthur. An Integrated System of Clasification of Flowering Plants. New York: Columbia University Press. 1981.
3. Chan, etal., Phytochemistry and Pharmacological Properties of Etlingera elatior: A Review, Pharmacognosy Journal 2011:3(22), 7
4. Ghasemzadeh A., Secondary metabolites constituents and antioxidant, anticancer and antibacterial activities of Etlingera elatior (Jack) R.M.Sm grown in different locations of Malaysia, International Society for Complementary Medicine Research (ISCMR) 2009;15:335
5. Lim, T. Edible Medicinal and Non Medical Plants volume 8, flowers . London: Springer.2014.
6. T Malar, R. J. Antibacterial Activity of Ethanolic Extract of Selected Medicinal Plants Against Human Pathogens. Asian Pasific Journal of Tropical Biomedicine 2011; 1.
7. Martins, etal., Antimicrobial and Antioxidant Activities of Selected Plants Used by Population from Juruene Valley, Legal Amazon, Brazil, Int J Pharm Pharm Sc 2017: 9(5)
8. Farnsworth, Norman, R. Biological and Phytochemical Screening Of Plants Journal Of Pharmaceutical Sciences. 1966; 55(3)
9. Clinical and Laboratory Standard Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. Twenty -Second Informational Supplement.2012.
10. Ordonez AAL, JD Gomez; MA Vattuone. MI lsla.Food Chem2006; 97: 452–458.
11. Wedge D. E. and Nagle D. G. A new 2D-TLC bioautography method for the discovery of novel antifungal agents to control plant pathogens. Journal of Natural Products, 2000; 63(8): 1050–1054.

12. Doppalapudi, Evaluation of Antibacterial Activity of Seed Extracts of Vigna Unguiculata, Int J Pharm Pharm Sc 2014(6) : Issue 1
13. Davis WW, Stout TR. Disc Plate Methode of Microbiological Antibiotic Assay: I. Factors Influencing Variability and Error. USA National Library Of Medicine Journal Applied Microbiology 1971;(22) 4: 659-665
14. Andersen M, Markham K.R., Flavonoids: Chemistry, Biochemistry and Aplication, CRC Press, 2006.
15. Neeraj Mishra, KK. Behal, Antimicrobial Activity of Some Spieces Against Selected Microbes, Int J Pharm Pharm Sc 2010 ; 2(3)