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Potential bioactivity evaluation of *Arytera littoralis* Blume (Sapindaceae)

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Abstract. *Praptiwi Windadri FI, Sulistiarini D, Ersaliany NPQ. 2022. Potential bioactivity evaluation of* Arytera littoralis *Blume (Sapindaceae). Biodiversitas 23: 4977-4983.* Bioactivity of *Arytera littoralis* (Sapindaceae) and its chemical compounds from different parts of the plants (flower, leaves, and stem) using the different polarities of solvents was carried out in this study. Phytochemical analysis was performed using the standard method. Total phenolic content (TPC) and total flavonoid contents (TFC) were analyzed using the spectrophotometry method. A qualitative assay for antioxidant and antibacterial activity against *Streptococcus aureus* and *Escherichia coli* was carried out by Bioautography-Thin Layer Chromatography (TLC). The minimum inhibitory concentration (MIC) value and IC_{50} value for antioxidant was carried out by microdilution methods on a 96-well microtiter plate. The results showed that different parts of the plant contained different phytochemical compounds. The highest TPC and TFC were obtained from stem extract, followed by leaves and flowers. The antibacterial activity of *A. littoralis* extracts against *S. aureus* and *E. coli* were categorized as weak antibacterial, while their antioxidant activity was categorized as a strong antioxidant. The antioxidant activity index (AAI) strongly correlates with TPC, TFC, and IC_{50} values.

Keywords: *Arytera littoralis*, antioxidant, antibacterial, TPC, TFC

INTRODUCTION

Plants produce various bioactive compounds, such as primary or secondary metabolites. The use of plant species in daily life, such as vegetables, housing, staple food, and medicine, has been known for a long time. Plants and microbes are the main sources of natural bioactive compounds (Haruna and Yahaya 2021). Plants are considered the sources of natural bioactive compounds due to their ability to synthesize various bioactive compounds (Farias et al. 2013). Furthermore, the plant growth site affects the synthesis and accumulation of secondary metabolites (Cirak and Radusiene 2019). More than 400,000 plant species contain bioactive compounds worldwide, but only a few have been explored (Shoemaker et al. 2005). The use of medicinal plants has increased in the last three decades, and about 80% of the world still relies on herbal medicine for their primary healthcare (Ekor 2013). The use of the medicinal plant is related to the bioactive compound content. Hence, the increasing demand and use of herbal medicines are due to relatively low prices, low adverse side effects, or low toxicity (Jigna and Sumitra 2006).

The Sapindaceae (soapberry family) is a flowering family consisting of approximately 2000 species that grow from temperate to tropical regions worldwide (Diaz and Rossini 2014). Sapindaceae is a family of plants that are widely used as medicinal plants. The bioactivity of several plants belonging to the Sapindaceae have been studied, among others, are antioxidant, anti-inflammatory, and antidiabetic (Simpson et al. 2010; Muthukumran et al. 2011) and anti-malarial (Waako et al. 2005). Various bioactive compounds from Sapindaceae that have bioactivity have also been isolated (Ito et al. 2004; Gaillard et al. 2011).

Arytera littoralis (Sapindaceae) has the local name as bidara emping or Ki lalayu (Sukarya et al 2013; Lailati and Ekasari 2015). It is a small evergreen tree with a height of 40 m and a diameter of 7-91 cm with smooth bark, greyish-green to reddish black. Twigs are hairy when they are young. Leaves are 1-3 jugate with ovate to elliptic leaflets, 4.2-31.3 cm long; 4-12 cm wide. Inflorescences axillary. Flower 1-3.5 mm diam. Fruit 0.7-3.6 cm long, 0.5-2.3 cm wide. Fruit are ellipsoid to orbicular. This species usually grows in primary and secondary forests on all soil types with an altitude of 0-1500 m and is native in South China to tropical Asia and Carolina Island. In Indonesia, it is found in Kalimantan, Java, Lesser Sunda Islands, Sulawesi, and Sumatra (Asiaplant 2022). *Arytera littoralis* is listed as the least concern in the IUCN Red List of Threatened Species (Oldfield 2020)

The plants are widely grown as ornamental plants in public parks and recreation areas and are distributed in primary and secondary forests in Malaysia and Singapore (FRIM, 2013). Young leaves of *A. littoralis* are purplish gray; the young fruits are orange and turn dark red when mature (Naimah 2022). *Arytera littoralis* was planted in the Bogor Botanical Gardens in 1816 (Fitriyati 2020). However, the bioactivity potential and chemical constituents of *A. littoralis* have not been widely studied. On the other hand, antibiotic resistance to pathogenic bacteria is increasing, which poses a serious global health concern. Evaluating the plant's potential bioactivity may

lead to new effective compounds and drug discoveries (Jigna and Sumitra 2006). The chemical compounds of plants could also be good sources of natural antioxidants. Recently, natural antioxidants are also in great demand compared to synthetic ones. It is due to the possible adverse effect related to synthetic antioxidant consumption (Lourenço et al. 2019). Therefore, the increasing safety concern of synthetic antioxidant consumption also increases the need for safer sources of natural antioxidants of interest nowadays (Stanković et al. 2016).

The plant chemical compounds generally having antioxidant activity are polyphenols, related to their redox properties (Zheng and Wang 2001), namely, absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, and peroxide decomposition. On the other hand, the common and widespread groups of phenolic compounds in plants are flavonoids, which can prevent free radical damage by directly scavenging free radicals and inhibiting enzymes involved in free radical production (Shandar et al. 2011). Therefore, the present study was conducted to determine the antibacterial and antioxidant potential of different plant parts using solvents of different polarities.

MATERIALS AND METHODS

Collection and preparation of *Arytera littoralis*

Different parts of the plant of *A. littoralis* (stems, leaves, and flowers) were collected from Tinangkung village, Tinangkung District, Banggai Island. The sample was identified and authenticated in Herbarium Bogoriense, Research Center for Biosystematics and Evolution, National Research and Innovation Agency. Samples were separated from impurities and cut into small pieces to dry easily. Samples were dried under sunlight and then grounded, followed by storage for further use

Extraction

Each sample (stem, leaves, and flower) was macerated in hexane as a solvent for 24 hours, shaken with a magnetic stirrer for 15 min, and left at room temperature for 24 hrs. Then, it was filtered. It was done thrice. The filtrate was composited and then concentrated with a rotary. The concentrated extract was dried with liquid nitrogen to remove residual solvent. After extracting with hexane, the sample was macerated with dichloromethane. The procedure of extraction was the same as that of hexane. The 3rd and the 4th solvent were ethyl acetate and methanol, respectively. The concentrated extract was weighed and stored in the refrigerator.

Qualitative analysis of phytochemical compounds of the extract

The chemical compounds of extracts, i.e., saponin, tannins, alkaloids, flavonoids, phenols, triterpenoids, and steroids, were analyzed qualitatively using the standard method by Harborne (1996). The extract was mixed with distilled water in a test tube and shaken vigorously. The formation of frothing more than 1 cm and stable after 15 min indicated the presence of saponin. The extract was added with ferric (III) chloride. The presence of blackish-blue indicated the presence of gallic tannin, while blackish-green indicated catechol tannin. The extract was mixed with 2% HCl, divided into 3 parts, and treated separately with Mayer reagent, Dragendroff reagent, and comparison solution. The existence of turbidity or yellowish-white precipitate with Mayer reagent or orange precipitate with Dragendroff indicated the presence of alkaloids. The extract was mixed with a few pieces of magnesium ribbon and concentrated HCl. Color change to red or pink indicated the presence of flavonoids. One milliliter of the extract was added with 5% iron (III) chloride. A positive result will indicate a color change to brown-orange (Harborne 1996). The extract was added with 0.5 mL of chloroform and 0.5 mL of anhydrous acetic acid, homogenized and allowed to stand for a while. Then, 6 drops of the solution were transferred into a test tube, and 2 to 3 drops of concentrated sulfuric acid were added. The presence of triterpenoids was indicated by the color changes to red, orange, or purple, while the blue or green color indicates the extract contains steroids.

Estimation of Total Phenolic Content (TPC) by spectrophotometric method

Folin-Ciolcalteu reagent (50%) was added to 68 µL of the extract (1mg/mL) and then homogenized using vortex for 1 min. After homogenizing, the mixture was added with 1,364 µL of Sodium carbonate (Na_2CO_3) 2% and incubated for 30 minutes at room temperature. Sixty-eight µL gallic acid was standard with the concentration range of 0.031-0.250 mg/mL. The absorbance of the standard solution (gallic acid) and the extract was measured at 750 nm using UV-Vis spectrophotometry. The total phenolic content of the extract was expressed as gallic acid equivalent (mg/h extract).

Estimation of Total Flavonoid Content (TFC) by UV-Vis Spectrophotometry

One hundred and fifty microliters of extract (1 mg/mL) and standard quercetin at the concentration of 0.03-0.50 mg/mL in ethanol pro analysis, p.a) was added with aqua dest (600 µL), $NaNO_2$ 5% (45 µL), and allowed to stand at room temperature for 6 min. Then, the mixture was added with 10% $AlCl_3$ (45 µL) and left for 6 min, followed by the addition of 4% NaOH solution (600 µL) and adjusted to the final volume of 1.5 mL with aqua dest, vortexed, and incubated under dark conditions at room temperature for 15 min. The absorbance of the extract and standard quercetin was measured at 510 nm. The total flavonoid content was expressed in quercetin equivalent (mg/g extract)

Isolation of secondary metabolites by Thin Layer Chromatography

Each extract (leaves, stems, and flowers) with a different solvent stock solution was made with 10 mg/mL. On the silica plate F_{254} (Merck), 10 µL of the extract was transferred. After transferring the extract, the plates were air-dried. The hexane extract was eluted with a mobile phase hexane: ethyl acetate 3:2. Dichloromethane and ethyl

acetate extracts were eluted with a mobile phase of dichloromethane: methanol 10:1. In contrast, methanol extract was developed with a mobile phase of chloroform: methanol: water (6:4:1). After completing elution, the results were observed under UV light at 254 nm and 366 nm. The spots were marked. The same procedures were applied to the other TLC plates, and the plates were sprayed with visualizing reagents (vanillin sulfate and cerium sulfate) (Figure 1).

Screening of antibacterial and antioxidant activity

Antibacterial activity

The antibacterial activity of extracts was evaluated by the Dot-Blot method and developing the extract with the mobile phase. It was carried out against *Escherichia coli* Ina-CC B4 and *Staphylococcus aureus* Ina-CC B5 (Legerská et al. 2020). Ten microliters of the extract were transferred onto a silica plate. After transferring, the plate was air-dried and dipped in the bacterial suspension with a density equivalent to 0.5 McFarland. The plates were then incubated in a humid condition at 37°C for 24 hrs. After incubation, the plates were sprayed with iodonitrotetrazolium (INT) solution. The extract with antibacterial activity was indicated by white area formation around the extract on purple background. Ten microliters of the extract were transferred onto a silica plate. After transferring, the plate was air-dried and developed with a mobile phase. After development was complete, the same procedure as the Dot-Blot method was applied. The antibacterial activity was indicated by the formation of white bands on purple background.

Antioxidative activity

The antioxidative activity was performed on a TLC plate using DPPH free radical scavenging. It was also performed by the Dot-Blot method and elution using a development solvent (Ismail et al. 2012). Ten microliters of the extract were transferred onto a silica plate. After transferring, the plate was air-dried and sprayed with DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in methanol. The formation of a white area around the extract indicated that the extract has antioxidative activity as a free radical scavenger. Ten microliters of the extract were transferred onto a silica plate. After transferring, the plate was air-dried and developed with a mobile phase. After development was complete, the plate was sprayed with DPPH solution in methanol. The white band on the TLC plate indicated the active compounds in the extract.

Measuring the value of Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) value of the extract was measured by the microdilution method on a 96-well microplate against *S. aureus* and *E. coli*. First, wells of row A were transferred with 100 µL of double strength Mueller Hinton broth (MHB), and 90 µL sterilized distilled water and then added with 10 µL of extract (10.240 µg/mL) in dimethyl sulfoxide (DMSO). Next, row B downward was filled with 100 µL of MHB. Next, row A was homogenized, and 100 µL was taken and transferred to row B. The same procedure was applied to row B downward. After homogenizing, one hundred µL of the solution was discarded from the last row. Finally, 100 µL of the bacterial solution was added to each well, comparable to 0.5 McFarland turbidity. The microplate was incubated in the incubator at 37°C for 18 hours. When the incubation process was complete, each well was added with iodonitrotetrazolium solution (10 µL) and incubated for 10-15 minutes. The Minimum Inhibitory Concentration (MIC) was determined at the well with the lowest concentration, which did not change color.

Measuring the value of IC_{50} for antioxidative activity, and Antioxidant Activity Index

The IC50 value for antioxidative activity was also measured by serial microdilution concentration on the 96-well microplate. Wells in row A were filled with 195 µL of methanol p.a.and 5 µL of extract (10.240 µg/mL) in DMSO. Wells in row B downward were filled with 100 µL of methanol p.a. Row A was homogenized, and 100 µL was transferred to row B. The same procedure was applied to row B downward. After homogenizing, one hundred µL of the solution was discarded from the last row. After dilution, each well was filled with 100 µL of diphenyl picrylhydrazyl solution (61.5 µg/mL) in methanol and incubated at room temperature for 90 min under dark conditions. After incubation, the absorbances of the extracts were determined using a microplate reader (*Varioscan Flash, Thermo Scientific*) at a wavelength of 517 nm. The inhibitory percentage was calculated using the formula as follows:

$$IC\ (\%) = (A_{DPPH}\ 100\% - A_{extract}) * 100 / A_{extract}$$

IC: Inhibitory percentage
A_{DPPH}: Absorbance of DPPH
$A_{extract}$: Absorbance of extract

A linear curve was constructed based on the inhibitory concentration, and the IC50 value was calculated using the linear regression equation (El-Abbasy et al. 2012).

Antioxidant Activity Index (AAI) was determined based on the equation as follows:

$$AAI = \text{concentration of DPPH} / IC50 \text{ value}$$

Data analysis

The total phenolic and flavonoid levels test results were analyzed using SPSS 16.0 with Duncan's method. The experiment was carried out with three replications. The results of the analysis were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Screening of phytochemical compounds of the extracts

Screening of phytochemical content in different plant parts on methanol extract was performed. It is due to methanol being a solvent for holistic extraction (Truong et al. 2019). Phytochemical screening of the extracts showed

differences in the content of chemical compounds in different plant parts. In all plant parts, chemical compounds include saponins, tannins, flavonoids, phenols, and alkaloids. However, triterpenoids and steroids are not present in all plant parts (Table 1).

Isolation of secondary metabolites and Screening of antibacterial and antioxidative

Isolation of secondary metabolites in the extract and bioactivity as antibacterial and antioxidative was performed using thin-layer chromatography (TLC). TLC was an effective, fast, and inexpensive method for determining the inhibitory effect of compounds in the extract (Legerská et al. 2020). The results of antibacterial and antioxidant assays by TLC-Bioautography showed that several active antibacterial and antioxidant compounds were present in the extracts. White spots or bands indicated antioxidant activity (Figures 2 and 3).

Total phenolic content and total flavonoid content

Total phenolic content (TPC) and total flavonoid content (TFC) were analyzed (Table 2). The TPC was calculated based on the calibration curve of gallic acid. Table 2. showed that the highest TPC and TFC were obtained in the methanol stem extract. The TPC and TFC of flowers, leaves, and stems differed significantly ($p<0.05$).

Minimum Inhibitory Concentration value

Minimum inhibitory concentrations (MIC) of *A. littoralis* against *S. aureus* and *E. coli* were categorized as a weak antibacterial activity with a MIC value of >256 µg/mL (Table 3).

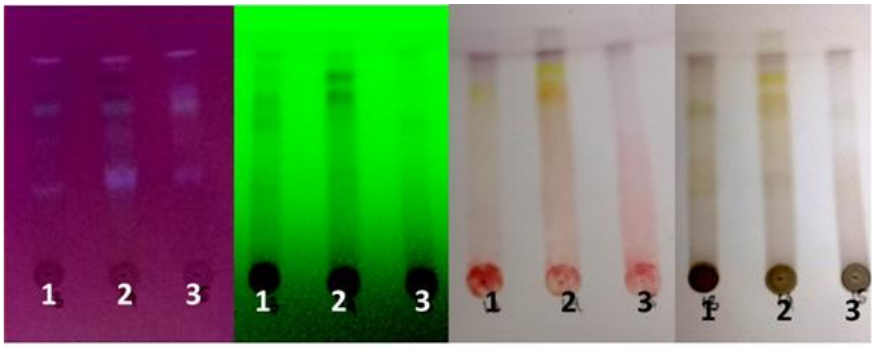
Measuring the IC50 value and AAI for antioxidant activity

All parts (flowers, leaves, and stems) of *A. littoralis* have very strong antioxidant activity as DPPH free radical scavengers (Table 4). Furthermore, the Pearson Correlation Coefficient indicated that TPC and TFC strongly correlate with AAI (Table 5). Therefore, it might show that increasing TPC and TFC values increase the antioxidant activity of the extract of *A. littoralis*. However, the value of IC50 was inversely proportional to the AAI value.

Table 1. Phytochemical analysis of the methanolic extract of *Arytera littoralis*

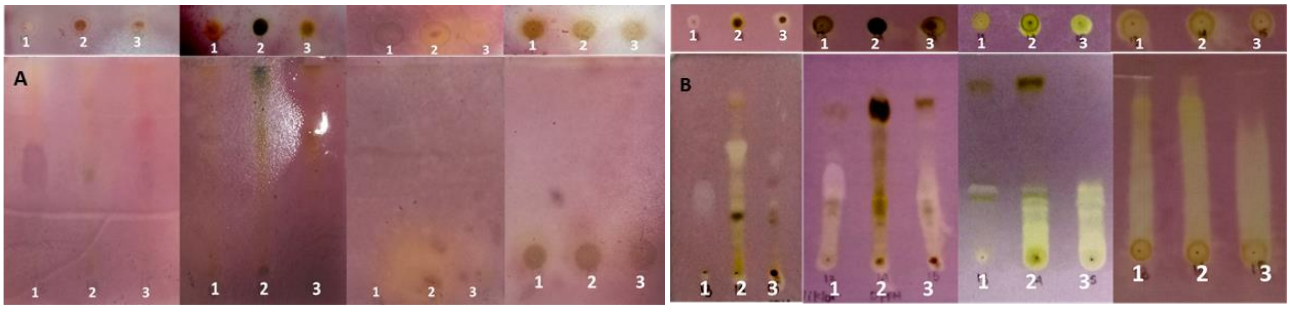
Phytochemical compounds	Reagent	Plant parts		
		Flowers	Leaves	Stem
Saponins	Aquades	+	+	+
Tannins	$FeCl_3$ 1%	+	+	+
Flavonoids	HCl + Mg	+	+	+
Phenols	$FeCl_3$5 %	+	+	+
Alkaloids	Dragendorff	+	+	+
	Mayer	+	-	+
Triterpenoid	$Ac_2O + H_2SO_4$	+	-	+
Steroids	$Ac_2O + H_2SO_4$	-	+	-

Note: +: present; -: absent



A B C D

Figure 1. Chromatogram of *Arytera littoralis* methanol extract: 1. Flower, 2. Leaves, 3. Stem developed with chloroform: methanol: water (6:4:1). Observed under A. UV-Vis 366nm; B. UV-Vis 254nm; C. sprayed with vanillin sulfate; D. sprayed with cerium sulfate



A B

Figure 2. Bioautogram of antibacterial activity of 1. Flower, 2. Leaves, 3. Stem of *Arytera littoralis* extract against: A. *Escherichia coli* and B. *Staphylococcus aureus*. Dot-Blot method (*above*), Eluted with chloroform: methanol: water (6:4:1) (*below*). Halo area indicated bacterial growth inhibition

Table 2. Total phenolic and total flavonoids content of *Arytera littoralis* extract

Sample	TPC (mg GAE/g extract)	TFC (mg QE/g extract)
Arytera littoralis flowers hexane extract	$0.00^{i} \pm 0.00$	$97^{g} \pm 1.77$
Arytera littoralis leaves hexane extract	$0.00^{i} \pm 0.00$	$2.80^{j} \pm 1.67$
Arytera littoralis stem hexane extract	$8.71^{g} \pm 0.02$	$156^{f} \pm 1.22$
Arytera littoralis flowers dichloromethane extract	$37.67^{f} \pm 0.00$	$9.77^{i} \pm 1.84$
Arytera littoralis leaves dichloromethane extract	$0.00^{i} \pm 0.00$	$0.00^{k} \pm 0.00$
Arytera littoralis stem dichloromethane extract	$0.00^{i} \pm 0.00$	$17.25^{h} \pm 1.3$
Arytera littoralis flowers ethyl acetate extract	$3.85^{h} \pm 0.10$	$0.00^{k} \pm 0.00$
Arytera littoralis leaves ethyl acetate extract	$123^{d} \pm 2.29$	$223^{d} \pm 1.98$
Arytera littoralis stem ethyl acetate extract	$71.46^{e} \pm 0.04$	$170^{e} \pm 2.84$
Arytera littoralis flowers methanol extract	$186^{c} \pm 0.06$	$3.48^{c} \pm 0.39$
Arytera littoralis leaves methanol extract	$280^{b} \pm 0.30$	$5.11^{b} \pm 0.36$
Arytera littoralis stem methanol extract	$366^{a} \pm 0.78$	$841^{a} \pm 1.16$

Notes: TPC: Total phenolic content; TFC: Total flavonoids content; GAE: gallic acid equivalent; QE: quercetin equivalent

Table 3. Minimum Inhibitory Concentration (MIC) of different plant parts of *Arytera littoralis*

Solvent	Sample	MIC			
		Staphylococcus aureus	**Category**	***Escherichia coli***	**Category**
Hexan	*Arytera littoralis* flowers extract	>256	Weak	>256	Weak
	Arytera littoralis leaves extract	>256	Weak	>256	Weak
	Arytera littoralis stem extract	>256	Weak	>256	Weak
Dichloromethane	*Arytera littoralis* flowers extract	>256	Weak	>256	Weak
	Arytera littoralis leaves extract	>256	Weak	>256	Weak
	Arytera littoralis stem extract	>256	Weak	>256	Weak
Ethyl acetate	*Arytera littoralis* flowers extract	>256	Weak	>256	Weak
	Arytera littoralis leaves extract	>256	Weak	>256	Weak
	Arytera littoralis stem extract	>256	Weak	>256	Weak
Methanol	*Arytera littoralis* flowers extract	>256	Weak	>256	Weak
	Arytera littoralis leaves extract	>256	Weak	>256	Weak
	Arytera littoralis stem extract	>256	Weak	>256	Weak

Table 4. IC_{50} value and AAI of methanol extract of *Arytera littoralis* methanol extract

Sample	IC50 (µg/mL)	AAI	Category of antioxidant
Arytera littoralis flowers hexane extract	128d ± 0.00	0.24d ± 0.00	Weak
Arytera littoralis leaves hexane extract	128d ± 0.00	0.24d ± 0.00	Weak
Arytera littoralis stem hexane extract	128d ± 0.00	0.24d ± 0.00	Weak
Arytera littoralis flowers dichloromethane extract	128d ± 0.00	0.24d ± 0.00	Weak
Arytera littoralis leaves dichloromethane extract	128d ± 0.00	0.24d ± 0.00	Weak
Arytera littoralis stem dichloromethane extract	115d ± 21.16	0.24d ± 0.00	Weak
Arytera littoralis flowers ethyl acetate extract	128d ± 0.00	0.24d ± 0.00	Weak
Arytera littoralis leaves ethyl acetate extract	17.6b ± 3.8	1.82c ± 0.4	Strong
Arytera littoralis stem ethyl acetate extract	70c ± 5.3	0.43d ± 0.03	Weak
Arytera littoralis flowers methanol extract	13.08ab ± 0.32	2.35b ± 0.05	Very strong
Arytera littoralis leaves methanol extract	12.41ab ± 1.86	2.51b ± 0.38	Very strong
Arytera littoralis stem methanol extract	3.7a ± 0.03	8.32a ± 0.07	Very strong

Note: Values in each column with the different letters are significantly different ($P<0.05$). Statistical criteria of AAI values for extract follows : weak < 0.5 < Moderate < 1 < Strong < 2 < very strong (Scherer and Godoy 2009)

Table 5. Pearson correlations coefficient (r) between TPC, TFC values, and Antioxidant Activity Index (AAI)

Variables	Pearson correlation coefficient®	Category
	AAI	
TPC value	0.902**	Very strong
TFC value	0.938**	Very strong
IC50 value	-0.738**	Strong

Note : (**) Correlation is significant at the 0.01 level (2-tailed)

Discussion

The results of the phytochemical analysis showed that different parts of the plant contained different phytochemical content. These results follow the study by Khare et al. (2012), which reported that the TPC and antioxidant activity of *Urtica dioica* L. were different in different plant parts. Differences in chemical content are also affected by different stages of growth and the environment (Li et al. 2020).

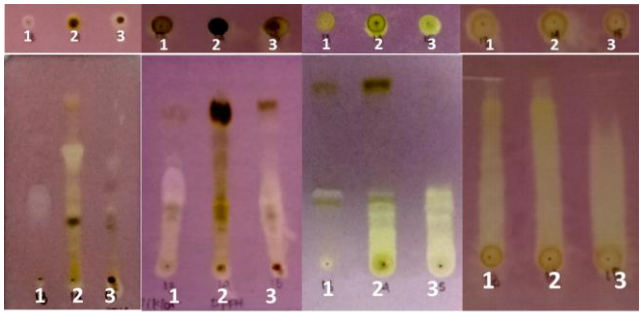


Figure 3. Bioautogram of antioxidative activity of 1. Flower, 2. Leaves, 3. The stem of *Arytera littoralis* extract was observed 30 min after spraying with 2% DPPH solution in methanol. Yellowish-white bands indicated antioxidant activity. Dot-Blot method (*above*), developed with chloroform: methanol: water (6:4:1) (*below*)

Different chemical compounds in different parts of the plant may affect the phytopharmacological activity of each part of the plant (Sembiring et al., 2018). Chemical compounds such as saponins, tannins, phenols, and flavonoids in flowers, leaves, and stems might affect the bioactivity of the extract. Phenols have been known to have many bioactivities, such as anti-inflammatory, antibacterial, cardiovascular disease, anti-allergic, anti-analgesic, and anti-Alzheimer (Shahidi and Yeo 2018). The biological activity of saponins includes membrane-permeabilizing, anti-fungal, antiviral, immunostimulant, and hypoglycemia (Francis et al. 2002). Previous studies showed that tannins have a lot of biological effects, such as antioxidants, radical scavenging, antimutagenic, antimicrobial, and antiviral (Sieniawska 2015).

The active antibacterial compounds were indicated by the formation of white spots or white bands. The formation of white spots or white bands is caused by converting tetrazolium salts to colored formazan in the presence of dehydrogenase enzymes of living microbes (Choma and Grzelak 2011). The TLC-Bioautography for antibacterial activity showed that *A. littoralis* were more susceptible to *S. aureus* than *E. coli*. The differences in sensitivity are due to the cell wall structure differences between Gram-positive and Gram-negative bacteria. Gram-negative bacteria are more resistant to antibacterial compounds due to an outer membrane consisting of three layers (Breijyeh 2020). However, the MIC value shows that the extract of *A. littoralis* is a weak antibacterial against *S. aureus* and *E. coli*.

The free radical scavenging antioxidant activity was performed using free radical DPPH. This method is widely accepted to predict the antioxidant activity of plant extract (Rahman et al. 2015). DPPH is a very stable free radical that reacts with compounds that can donate hydrogen. The degree of color change or absorbance is proportional to antioxidant potency (Reddy et al. 2012). The absorbance decrease in the reaction mixture indicated potent free radical scavenging activity (Krishnaiah et al. 2011). The antioxidant assay showed several compounds in the extracts that were active as DPPH free radical scavengers. They were indicated by yellowish-white spots or bands on the purple background. In the presence of antioxidant compounds in the extract, the violet color of the DPPH solution was reduced to yellow-colored diphenylpicryl hydrazine (Rahman et al. 2015; Afsar et al. 2018).

Based on the IC_{50} value and AAI, the ethyl acetate extract of leaves and methanol extract have the potential as the antioxidant source. The DPPH free radical scavenging antioxidant activity strongly correlates with TPC and TFC. In addition, phenolics and flavonoids can scavenge DPPH by their ability to donate hydrogen (Huang et al. 2005).

In conclusion, the methanol extract of different plant parts of *A. littoralis* had very strong antioxidants; it also contains the highest total phenolic content. The study indicated that methanol extract *A. littoralis* methanol extract could be a potent source of antioxidants. However, the chemical compounds responsible for antioxidant activity have not been known yet. Therefore, further study is needed to isolate and identify the antioxidant compounds of *A. littoralis.*

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All authors contributed equally as the main contributor.

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