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Original Article

AN INITIAL STUDY OF *IMPERATA CYLINDRICA* LEAVES POTENTIAL AS HERBAL MEDICINAL INGREDIENTS

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ABSTRACT

Objective: In traditional medicines, *Imperata cylindrica* (hereinafter referred to as *I. cylindrica*) roots are more frequently used than its leaves. The aim of this study was to determine three parameters, namely the content of total phenolic compounds, total flavonoid content, and antioxidant activity of *I. cylindrica* leaves and roots extracts. Then, root extract parameters were used as a comparison to evaluate the benefits of *I. cylindrica* leaves as herbal medicine.

Methods: The total polyphenol content was measured by using the Folin-Ciocalteu method and total flavonoid content was measured by using aluminium chloride method due to measure the antioxidant activity using 1,1-diphenyl-method 2-pikrilhidrazil (DPPH) method. All methods used visible spectrophotometry.

Results: The percentages of total phenolic content, total flavonoid content, and IC_{50} of antioxidant activity of *I. cylindrica* leaves extract were 8.1 % (GAE), 2.1% (QE) and 80 ppm, respectively. Then, the measurement results of *I. cylindrica* roots extract showed the percentages of total phenolic content, total flavonoid content, and IC_{50} of antioxidant activity were 1.13% (GAE), 0.28% (QE) and 368 ppm, respectively.

Conclusion: The result showed that *I. cylindrica* leaves extract contained phenolic compounds and flavonoids and had antioxidant activity. The three phenolic compounds, flavonoids and antioxidant activity, have been shown to have effects on health. Therefore, the potential of *I. cylindrica* leaves utilization as herbal medicine can be promoted more widely.

Keywords: *Imperata cylindrica*, Total phenolic content, Total flavonoid content, Antioxidant activity

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INTRODUCTION

Imperata cylindrica (hereinafter referred to as *I. cylindrica*) is a weed plant, in Indonesia it grows in over 8.5 million ha areas [1, 2]. In traditional medicines it has positive roles for health, such as to treat wound and to prevent hypertension, tonic drink for health, urinary stones, fever, cough, etc [1-3].

Generally, a part of *I. cylindrica* used as traditional medicines is the root [1, 2]. Its leaves are rarely used and commercially sold as herbal medicine, although there are several other parts used to heal wounds, aches, pains, constipation, etc [1]. This situation is unfavorable because the leaves become a wasted part.

In plants, leaves have a role as a place for the photosynthesis process producing nutrients as a source of energy for their growth and survival. Sunlight is a source of UVA and UVB, which are needed in the photosynthesis process in producing energy. However, the high ultra-violet and visible light exposure cause stress in plants. Plants naturally produce secondary metabolites to protect and defend themselves from various diseases and natural conditions, such as conditions of excessive sun exposure or other environmental hazards. Plants produce, among others, phenolic compound such as flavonoids. These flavonoid's roles depend on their structures which are useful to fight microbial infections, scavenge or quench ROS, etc [4-6].

Nowadays, many *I. cylindrica* compounds had been reported. Among other, its main compounds are saponins, phenols, flavonoids, and glycosides [7]. A previous study proved *I. cylindrica* roots extract had some biologically activities, while *I. cylindrica* roots ethanol extract had nephroprotective activity by ameliorate urea levels, creatinine, and hematological parameters [8]. The acute oral toxicity test on rats proved that its roots methanol extract is safe, and based on oral sub-chronic test, it is also recommended at a lower dose [9]. Rumah Riset Jamu Hortus Medicus, a home for the development and use of herbal medicine and as a service-based research clinic uses *I. cylindrica* roots in the herbal recipe to medicate hypertension, urinary tract infection, urinary tract stones, prostate enlargement, osteoarthritis, stomatitis, and headache [10].

To further introducing the use of *I. cylindrica* leaves, parameters that can support the promotion were measured, namely total phenolic compound, total flavonoid contents, and antioxidant activity.

MATERIALS AND METHODS

Sample collection

The *I. cylindrica* (whole plant) was obtained from a plant nursery in Kediri, East Java, Indonesia and harvested in April 2021.

Chemicals

Materials used in the study were 1.1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, and quercetin from Sigma Aldrich; sodium carbonate, sodium acetate, FolinCiocalteau, methanol, aluminium chloride, all reagents are analysis grade (Merck).

Equipment

The equipment was used to measure the extract as follows.

Eppendorf Biospectrometer Basic AG 22331 Hamburg series: 6135 BJ.

Preparation of *I. cylindrica* leaves and roots extracts [11]

In this procedure, 200 g of *I. cylindrica* leaves powder was macerated with 2 l of methanol and left for 24 h while stirring the solution repeatedly. After 24 h, the methanol extract was filtered and stored in a bottle. Then this procedure was repeated two more times with 1 l of methanol.

All extracts were combined and concentrated.

The same procedure was also carried out on the roots.

Screening of flavonoid in the extract [12]

Flavonoids were identified with magnesium powder and 1 ml hydrochloric acid 2 N. Then, amyl alcohol was added, the presence of flavonoids is indicated by the formation of yellow in the amyl alcohol layer.

Preparation for total phenolic measurement [13, 14]

Reagents were prepared as follows

Solutions of sodium carbonate 7.5%

Aliquot 7.5 g of sodium carbonate was weighed and was put into 100 ml volumetric flask and was dissolved with distilled water to the mark of the flask limit.

Folin-cioucalte 10%

Aliquot 10 ml Folin-Cioucalte was pipetted and was put into 100 ml volumetric flask and was dissolved with distilled water to the mark of the flask limit.

Gallic acid refence standard stock solution in the concentration of 1000 ppm

Aliquot 10 mg of gallic acid reference standard was weighed and was put into 10 ml volumetric flask and was dissolved with methanol to the mark of the flask limit.

***I cylindrica* extract stock solution in the concentration of 1000 ppm**

Aliquot 50 mg extract was weighed and was put into 50 ml volumetric flask and was dissolved with methanol to the mark of the flask limit.

Standard curve of gallic acid reference standard

Gallic acid reference standard stock solution was diluted with methanol to make gallic acid reference standard solution with concentrations as follows: 50 ppm, 100 ppm, 200 ppm, 400 ppm, 800 ppm.

Each gallic acid reference standard was pipetted 0.05 ml, which was put in reaction tubes. Each tube was added 2.5 ml Folin-Cioucalte 10% solution (homogenized) and 2 ml sodium carbonate 7.5% (homogenized). Then, all tubes were incubated at 37 °C for 30 min. The absorbances were measured at a wavelength 750 nm.

The reference standard calibration curve was created by plotting the absorbance against concentration.

Likewise, the same steps were done for 0.05 ml of *I. cylindrica* roots extract.

All works were carried out three repetitions.

The total phenolic content was calculated with the equation bellow.

$$\text{Total phenolic (\%)} = \frac{\text{V(ml).C(mg/ml).Fp}}{\text{weight of sampel (mg)}} \times 100\%$$

With:

Abbreviation:

TPC is the total phenolics content (%)

C is x (equality of gallic acid), calculated from the linier regression of the standard curve of gallic acid reference standard

V is, the volume of the I. cylindrica, extract solution (ml)

F is the dilution factor m is the weight of *I. cylindrica* extract (g)

Preparation for total flavonoid measurement [3]

Reagents were prepared as follows

Solutions of sodium acetate 120 mmol

Aliquot 492.2 mg of sodium acetate was weighed and was put into 50 ml volumetric flasks and was dissolved with distilled water to the mark of the flask limit.

Solutions of $AlCl_3$ 2%

Aliquot 1 g $AlCl_3$ was weighed and was put into 50 ml volumetric flask and was dissolved with distilled water to the mark of the flask limit.

Quercetine reference standard stock solution in the concentration of 1000 ppm

Aliquot 10 mg of quercetin reference standard was put into 10 ml volumetric flask and was dissolved with methanol to the mark of the flask limit.

Standard curve of quercetin reference standard

Quercetin reference standard stock solution was diluted with methanol to make quercetin reference standard solution with concentration as follows: 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm.

Each quercetin reference standard was pipetted 1 ml and added 1 ml $AlCl_3$ 2% solution (homogenized) and 1 ml sodium acetate (homogenized). All were stored in the room (room temperature) for 1 h.

The absorbance's were measured at wavelength 435 nm.

The reference standard calibration curve was created by plotting the absorbance against concentration.

Likewise, the same steps were done for 1 ml of *I. cylindrica* roots extract.

All works were carried out in three repetitions.

The total flavonoid content was calculated with the equation bellow.

$$\text{Total flavonoid (\%)} = \frac{\text{V(ml).C(mg/ml).Fp}}{\text{weight of sampel (mg)}} \times 100\%$$

With:

Abbreviation:

TF is the total flavonoid content (%)

C is x (equality of quercetin), calculated from the linier regression of the standard curve of quercetin reference standard

V is, the volume of the I. cylindrica, extract solution (ml)

F is the dilution factor

m is the weight of *I. cylindrica* extract (g)

Preparation for antioxidant activity measurement [14-15]

Reagents were prepared as follows: **DPPH stock solution 0.5 mmol**

Aliquot 19.5 mg of DPPH was weighed and was put into 100 ml volumetric flask; then it was dissolved with methanol to the mark of the flask limit.

***I cylindrica* extract stock solution in the concentration of 1000 ppm**

Aliquot 50 mg extract was weighed and was put into 50 ml volumetric flask and was dissolved with methanol to the mark of the flask limit. Then, stock solutions were made with the following concentrations: 200 ppm; 160 ppm; 120 ppm; 80 ppm, and 40 ppm.

Measurement of antioxidant activity

Six tubes were prepared, each tube starting from tube number 1 to tube number 6 was filled with 4 ml of the following solution: tube number 1 was filled with 4 ml of methanol, and each of the remaining 5 tubes was filled with 4 ml of *I. cylindrica* stock solution with the following concentrations: 200 ppm; 160 ppm; 120 ppm; 80 ppm, and 40 ppm, respectively. Each tube was added with 1 ml of DPPH stock solution, the mixture was stirred homogeneously, and kept for 30 min.

Each absorbance of all the mixture was measured at wavelength 517 nm.

The antioxidant activity (% IC_{50}) was calculated with the equation below:

$$\%\ IC_{50} = \frac{\text{Absorbance of reference} - \text{absorbance of sample}}{\text{absorbance of reference}} \times 100\%$$

The IC_{50} was calculated with the linear regression equation of DPPH inhibition curve.

The IC_{50} is a parameter of antioxidant activity that shows the ability to reduce 50% of oxidants. Intensity levels of IC_{50} by DPPH method as follow: (1) Highly active, IC_{50} value<50 µg/ml; (2) Active, IC_{50} value 50–100 µg/ml; (3) Moderate, IC_{50} value 101–250 µg/ml; (4)Weak, IC_{50} value 250–500 µg/ml; and (5) Inactive, IC_{50} value>500 µg/ml [15].

This study had been carried out in the period of November 2021–Maret 2022 in Biochemistry and Molecular Biology Laboratory, Faculty of Medicine, Universitas Padjadjaran, Jatinangor, Indonesia.

RESULTS AND DISCUSSION

Flavonoid screening showed a positive result. The extract contained flavonoids

Quantification of total phenolic, total flavonoid and antioxidant activity of *I. cylindrica* leaves extract

Results of the quantification of total phenolic, total flavonoid and antioxidant activity of *I. cylindrica* leaves extract are as follows.

Measurement of total phenolic of *I. cylindrica* leaves extract

The total phenolic content in the leaves extract was measured using a gallic acid reference standard, and the results were equivalent to gallic acid.

Gallic acid reference standard concentrations were measured, and the results were as follows in table 1.

Table 1: Gallic acid reference standard curve

Concentration of gallic acid reference standard (mg/ml)	Absorbance
0.05	0.047
0.1	0.096
0.2	0.213
0.4	0.395
0.8	0.837

The data in table 1 was portrayed in fig. 1

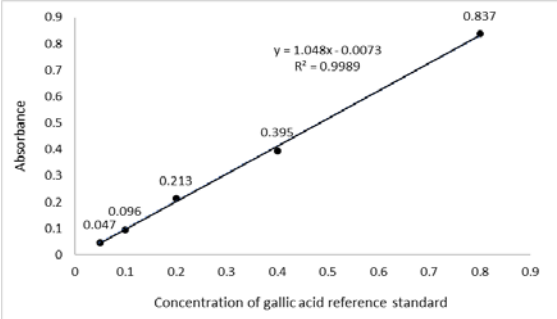

Fig. 1: Gallic acid reference standard curve

The fig. 1 resulted linear regression equation of gallic acid reference standard curve, which was y = 1.048x–0.0073, and the measurement result of *I. cylindrica* leaves extract absorbance was 0.078

x was calculated from the linear regression equation of gallic acid reference standard curve

y = 1.048x–0.0073

x =0.081 mg/ml

Total phenolic content was calculated as follows:

$$\text{Percentage of total phenolic extract} = \frac{\text{V(ml).C(mg/ml).Fp}}{\text{weight of sampel (mg)}} \times 100\% = 8.1\%$$

From the calculation, the percentage of the total phenolic content of *I. cylindrica* leaves extract = 8.1% (GAE) was obtained.

Measurement of total flavonoid of *I. cylindrica* leaves extract

The total flavonoid content in the leaves extract was measured using a quercetin reference standard, and the results were equivalent to quercetin.

Quercetin reference standard concentrations were measured, and the results were presented in table 2.

Table 2: Quercetin reference standard curve

Concentration of quercetin reference standard (mg/ml)	Absorbance
0.005	0.155
0.010	0.263
0.015	0.356
0.020	0.500
0.025	0.614

The data in table 2 was portrayed in fig. 2

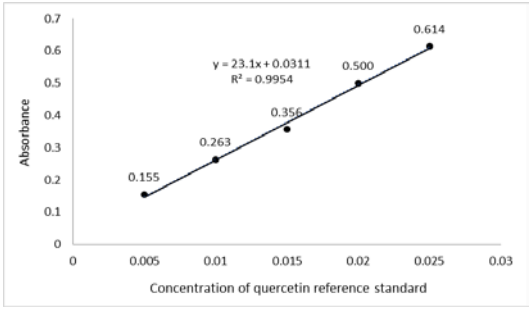

Fig. 2: Quercetin reference standard curve

The fig. 2 resulted linear regression equation of quercetin reference standard curve which isy = 23.1x+0.0311, and the measurement result of the *I. cylindrica* leaves extract absorbance was 0.518.

x was calculated from linear regression equation of quercetin reference standard curve:

y = 23.1x+0.0311

x= 0.0211 mg/ml

Total flavonoid content calculation was as follows:

$$\text{Percentage of total flavonoid extract} = \frac{\text{V(ml).C(mg/ml).Fp}}{\text{weight of sampel (mg)}} \times 100\%$$

= 2.1%

Based on the calculation, the percentage of total flavonoid content of *I. cylindrica leaves* extract was 2.1% (QE).

Measurement of antioxidant activity of *I. cylindrica* leaves extract

The measurement results of the inhibition percentage of *I. cylindrica* leaves extract in the various concentration were described in table 3.

Table 3: Percentage of inhibition of *I. cylindrica* leaves extract

No	Concentration (mg/25 ml)	Absorbance	% Inhibition
Refference		0,876	
1	5	0,060	93.15
2	4	0,085	90.29
3	3	0,220	74.88
4	2	0,422	51.82
5	1	0,645	26.36

The data in table 3 was portrayed in fig. 3

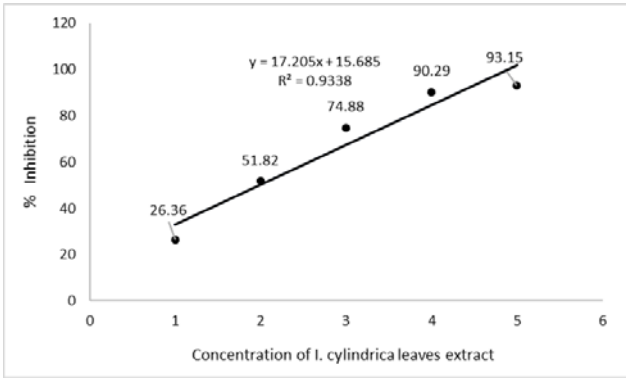

Fig. 3: Percentage inhibition of *I. cylindrica* leaves extract to DPPH

Antioxidant activity (% IC_{50}) was calculated with the linear regression equation from the curve of percentage inhibition of *I. cylindrica* leaves extract to DPPH

y = 17.205x+15.685 and IC_{50} = 80 ppm

Based on the calculation, the IC_{50} of antioxidant activity of *I. cylindrica* leaves extract was 80 ppm.

Quantification of total phenolic, total flavonoid and antioxidant activity of *I. cylindrica* roots extract

The results of the quantification of total phenolic, total flavonoid and antioxidant activity of *I. cylindrica* roots extract are as follows.

Measurement of total phenolic of *I. cylindrica* roots extract

The total phenolic content in the roots extract was measured using a gallic acid reference standard, and the results were equivalent to gallic acid.

Gallic acid reference standard concentrations were measured, and the results were shown in table 4.

Table 4: Gallic acid reference standard curve

Concentration of gallic acid reference standard (mg/ml)	Absorbance
0.05	0.046
0.1	0.090
0.2	0.196
0.4	0.407
0.8	0.880

The data in table 4 was portrayed in fig. 4

The fig. 4 resulted linear regression equation of gallic acid reference standard curve, which is y = 1.1167x-0.0224, and the measurement result of the *I. cylindrica* roots extract absorbance was 0.035.

x was calculated from the linear regression equation of gallic acid reference standard curve

y = 1.1167x-0.0224

x= 0.0113 mg/ml

Total phenolic content calculated is as follows:

Percentage of total phenolic *I. cylindrica* roots extract =

$$\frac{\text{V(ml).C(mg/ml).Fp}}{\text{weight of sampel (mg)}} \times 100\%$$

= 1.13 %

The percentage of total phenol of *I. cylindrica* roots extract based on the calculation was1.13 % (GAE).

Table 5: Quercetin reference standard curve

Concentration of quercetin reference standard (mg/ml)	Absorbance
0.005	0.162
0.010	0.264
0.015	0.349
0.020	0.494
0.025	0.621

The data in table 5 was portrayed in fig. 5

Measurement of total flavonoid of *I. cylindrica* roots extract

The total flavonoid content in the root extract was measured using a quercetin reference standard, and the results were equivalent to quercetin.

Quercetin reference standard curve was measured, and the results are described in table 5.

The fig. 5 resulted linear regression equation of the quercetin reference standard curve, which is y = 22.96x+0.0336, and the measurement result of the *I. cylindrica* roots extract absorbance was 0. 098.

x was calculated from the linear regression equation of quercetin reference standard curve

y = 22.96x+0.0336

x = 0.0028 mg/ml

Total flavonoid content calculated is as follows.

Percentage of total flavonoid of *I. cylindrica* roots extract =

$$\frac{V(ml).C(mg/ml).Fp}{\text{weight of sampel (mg)}} \times 100\%$$

= 0.28%

Based on the calculation, the percentage of total flavonoid of *I. cylindrica* roots extract was 0.28% (QE).

Measurement of antioxidant activity of *I. cylindrica* roots extract

The measurement results of *I. cylindrica* roots extract inhibition percentage in the various concentration are described in table 6.

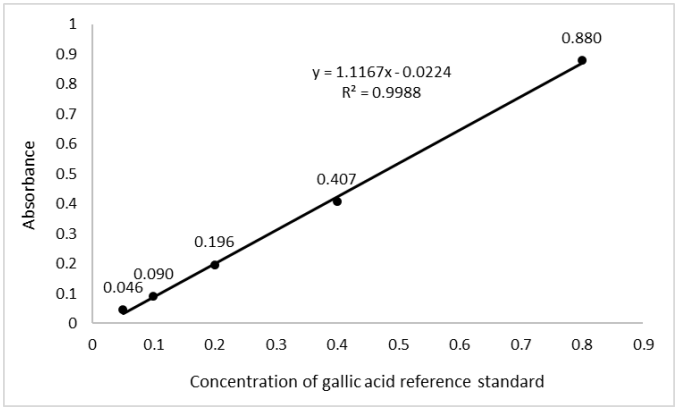

Fig. 4: Gallic acid reference standard curve

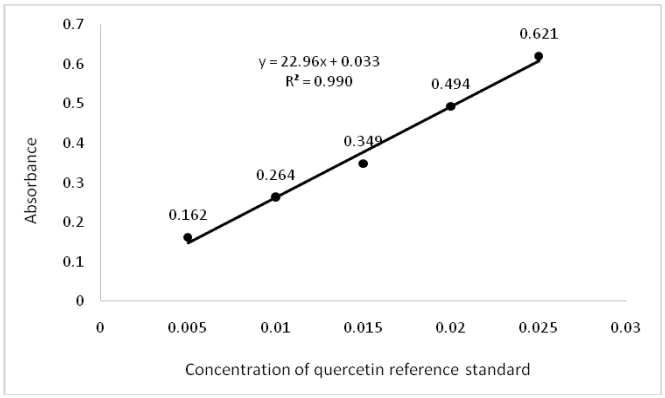

Fig. 5: Quercetin reference standard curve

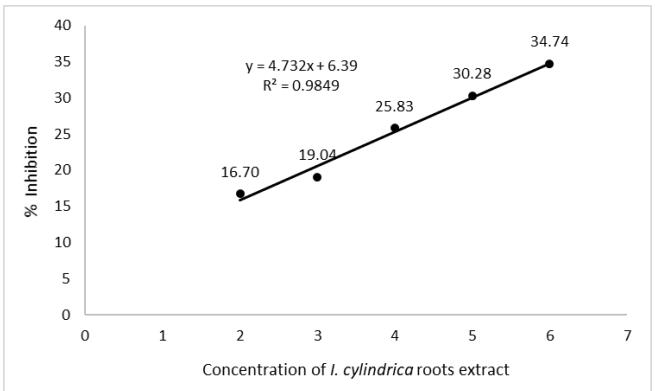

Fig. 6: Percentage inhibition of *I. cylindrica* roots extract to DPPH

Antioxidant activity (% IC_{50}) was calculated with the linear regression equation from curve of *I. cylindrica* roots extract inhibition percentage to DPPH.

y = 4.732x+6.39

And IC_{50} = 368 ppm

From the calculation, it showed that the IC_{50} of antioxidant activity of *I. cylindrica* roots extract was 368 ppm.

Sum of all parameters results, total phenolic, total flavonoid, and antioxidant activity between *I. cylindrica* leaves and roots extracts is as follows, , and add this words: table 7 and fig 7.

Table 6: Percentage of inhibition of *I. cylindrica* roots extract

No	Concentration (mg/25 ml)	Absorbance	% Inhibition
Refference		0.898	
1	6	0,586	34.74
2	5	0,626	30.28
3	4	0,666	25.83
4	3	0,727	19.04
5	2	0,748	16.70

The data in table 6 was portrayed in fig. 6

Table 7: Comparison of three parameters between *I. cylindrica* leaves and roots extracts

No	Sample	Parameters		
		Total phenolic (GAE) %	Total flavonoid (QE) %	Antioxidant activity(ppm)
1	*I. cylindrica* leaves extract	8.1	2.1	80
2	*I. cylindrica* roots extract	1.13	0.28	368

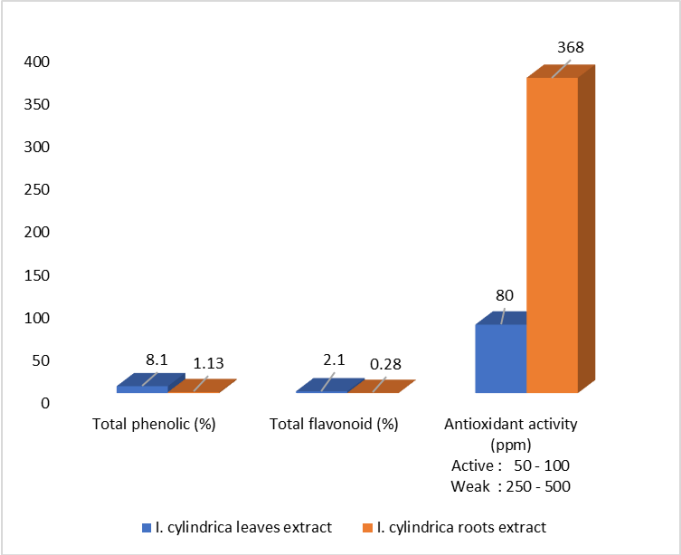

Fig. 7: Sum of total phenolic content, total flavonoid content, and antioxidant activity [18] between *I. cylindrica* leaves and roots extracts

DISCUSSION

The study showed that the *I. cylindrica* leaves extract contained phenolic compounds and flavonoids, both are secondary metabolites.

In the normal cells of living organisms, aerobic metabolism occurs which leads to adenosine tri-phosphate (ATP, the source of energy) production. The process also produces toxic product that is called reactive oxygen and nitrogen species (ROS and RNS) [16]. The ROS may increase due to the influence of environmental conditions or exogenous sources. Excessive ROS production can cause oxidative stress, which can lead to several pathophysiological conditions such as atherosclerosis, neoplasia, destroy bio-molecules structure, etc [17-19].

The ROS and RNS can be deactivated by antioxidants. The living organisms have developed a defense mechanism system to response the ROS/RNS, such as with enzymatic antioxidant, among other enzyme superoxide dismutase, glutathione peroxidase, and catalase, as well as obtained from food intake, among others non enzymatic antioxidant, namely vitamin C, vitamin E, carotenoids, etc [25].

Plants produce secondary metabolites in their leaves to response ROS and RNS [25]. These secondary metabolites are known to have an important role in the adaptation of plants to their environment [16]. One of the secondary metabolites is phenolic compound, it is known that phenolic compounds are secondary metabolite which include several groups (flavonoids, tannins, hydroxy-cinnamate esters, lignin, etc [6, 22, 26]. The phenolic compounds and flavonoids play a role as antioxidants to defend themselves from oxidative stress caused among others by ROS and RNS, which are based on their structure (aromatic ring with-–OCH_3 or OH substituents, degree of hydroxylation, polymerization, conjugations, substitutions and others) [8, 21, 23].

Antioxidant mechanism or radical scavenging of phenolic compounds on ROS occurs *via* hydrogen atom or an electron donation, chelating metal ions such as iron and copper, and prevention of the formation of ROS in the Haber-Weiss reaction [6, 26].

In this study, the *I. cylindrica* leaves and roots extracts contained phenolic compounds and flavonoids and had antioxidant activity.

The *I. cylindrica* leaves extract had the percentage of total phenolic and total flavonoids contents and antioxidant activity (IC50), which were 8.1% (GAE) and 2.1% (QE), and 80 ppm, respectively. The I. cylindrica roots extract had the percentage of total phenolic and total flavonoids contents and antioxidant activity (IC50), which were 1.13% (GAE) and 0.28% (QE), and 368 ppm, respectively.

The previous study of *I. cylindrica* roots methanol extract from Kendari (South East Sulawesi) reported that the extracts had anti-hypertensive activity at doses 60 and 90 mg/kg-BW on hypertension rat models [2].

It is known that phenolic compounds consist of several groups, while each group has its own activity. The role of the phenolic compound included flavonoids on hypertension occurred through various of molecular mechanisms.

Flavonoids are a group of phenolic compounds that has antioxidant activity. It has been reported that its antioxidant properties affect the endothelium; flavonoids have a role as inhibitors of endothelial NAD(P)H oxidase, which continues towards inhibition of superoxide production. Both endothelial NAD(P)H oxidase and superoxide production are involved to the regulation of nitric oxide (NO) levels in the vascular endothelium. Nitric oxide (NO) acts as a vasodilator [27, 28].

This study determined that both leaves and roots of *I cylindrica* extracts contained phenolic compounds and flavonoids and had antioxidant activity. Therefore, the *I. cylindrica* leaves can be promoted like the roots as herbal medicines.

SUMMARY

Both leaves and roots of *I cylindrica* extracts contained phenolic compounds and flavonoids and had antioxidant activity.

The *I. cylindrica* leaves extract contained phenolic compounds and flavonoids which was higher than the roots extract. As well as the antioxidant activity, the leaves extract was stronger than the roots extract.

Based on these facts, the potential utilization of *I. cylindrica* leaves can be promoted like the roots. The leaves have potentials to be used as herbal medicines.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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