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Research Article

Quantification of Four Phytochemical Parameters of *Imperata cylindrica* Leaves to Promote Its Use as A Medicinal Plant

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ABSTRACT

Introduction: The benefits of *Imperata cylindrica* (*I. cylindrica*) leaves and their relationship to the content of phytochemical compounds have been widely studied. The aim of this study was to promote *I. cylindrica* leaves from Kertajati Subdistrict in Majalengka Regency in their use as a medicinal plant based on the four parameters, namely the content of total phenolic content, total flavonoid content, total tannin content, and antioxidant activity. The four parameters that become the basis for the benefits of the leaves were compared with the four parameters of the roots. **Methods:** Quantification of total phenolic content, total flavonoid content, total tannin content, and antioxidant activity used Folin-Ciocalteu, aluminium chloride, Fe(III) chloride and 1.10-Phenanthroline, and 1,1-diphenyl-2-pikrilhidrazil (DPPH) methods, respectively. All methods used visible spectrophotometric method. **Results:** The percentages of total phenolic content, total flavonoid content, total tannin content, and IC_{50} of antioxidant activity of *I. cylindrica* leaves extract were 9% (GAE), 2.1% (QE), 5.6% (TAE), and 100.5 ppm, respectively. Then, the percentages of *I. cylindrica* roots extract showed the percentages of total phenolic content, total flavonoid content, total tannin content, and IC_{50} of antioxidant activity were 5.8% (GAE), 0.64% (QE), 3% (TAE), and 241 ppm, respectively. **Conclusion:** The extract of *I. cylindrica* leaves contained the phenolic compound, namely flavonoids, tannins and other phenolic compounds, and had antioxidant activity. All parameters have been reported to have positive effect on health. Therefore, the leaves of *I. cylindrica* from Kertajati Subdistrict are discovered to have effects on health which are suitable to be promoted as a medicinal plant.

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

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INTRODUCTION

The studies on *I. cylindrica* are still ongoing today. There are 2 sides of interest in studying *I. cylindrica*, namely one side is unfavorable factors with their growth on productive land, *I. cylindrica* included in PART 360 – Noxious Weed Regulations - Terrestrial weeds.[1] *I. cylindrica* is in accordance with the classification of weeds which are grouped based on morphology, life cycle, growing habitat, and based on their effect on plants, including in the following order: 1. Classification of Weeds Based on Morphology, including grass class weeds (Grasses); 2. Classification of Weeds Based on Life Cycle, including Perennial Weeds; 3. Classification of Weeds Based on Growing Habitat, including Terrestrial Weeds.[2] On the other hand, there are benefits which have been used as traditional medicine since ancient times, such as to increase urine volumes and urinary stone.[3,4]

The development of *I. cylindrica* benefits has expanded to the other scientific disciplines besides traditional medicine, including the study to prevent the environmental pollution. The study assayed the *I. cylindrica* leaves activity on oil pollution that was caused by spill oil; this pollution has a serious impact on the economy.[5] *I. cylindrica* was reported to have oleophilic and hydrophobic properties which can absorb both water and oil. Leaves of *I. cylindrica* have a capability as an oil sorbent.[6] *I. cylindrica* flower have a good buoyancy property in the water ripple (the gentle wavy motion like in sea) even after shaking in 24 hours.[7] In paper industries *I. cylindrica* can prevent deforestation wherein deforestation causes several environmental problems.[8] *I. cylindrica* can replace wood as a basic material for making paper, since *I. cylindrica* contained 64.9% holocellulose, holocellulose percentage meets paper raw material standards (65% – 75%).[9] The addition of *I. cylindrica* in the concrete formula to shield the body from x-ray radiation still needs to be developed.[10]

The pharmacological activities of *I. cylindrica* on health have been widely studied, especially on its roots, one of which is water extract (decoction) of *I. cylindrica* roots. The extract had a nephroprotective effect on acute kidney injury rat model that was induced gentamicin, mainly in the early stages of acute kidney injury. The nephroprotective effect parameters measurement were the ratio of kidney weight to body weight, histopathological profile (damages in the interstitium and the tubules) and biochemical profile (level of BUN and creatinine). Its nephroprotective effects were based on the dose of extract given and were suggested by the present of flavonoids and reducing substances in the extract.[11]

The studies of *I. cylindrica* leaves activity on health have been done. However, less studies concerning the leaves were conducted when compared to the studies relating to the roots, such as a study which included ethanolic 70% extract on cat (*in vivo*, the anti-hypertensive activity was measured via blood pressure and heart rate of cats) and rabbit (*in vitro*, the anti-hypertensive activity was measured via contractile responses of the rabbit jejunum). The extract showed antihypertensive activity by relaxing and dilating smooth muscles of blood vessels (*in*

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vivo) and reduction in amplitude of contraction of smooth muscle cells of rabbit jejunum (*in vitro*). The antihypertensive activity of the extract was shown by vasodilation activity, this activity is alike to adrenaline mechanism of action.[12] The in vitro study used oral squamous cell carcinoma cell line SCC-9, the *I. cylindrica* leaves methanol extract had cytotoxic activity and induced cell death in a dose-dependent manner. These activities of the extract on SCC-9 cells were measured by the impact on the morphology changed of cell, cytotoxic effect, colony forming ability, cell cycle and DNA fragmentation of the cells. The study determined that the cause of death of SCC-9 cancer cells was due to a decrease in clonogenicity leading to increased DNA fragmentation and induction of apoptosis.[13]

A study of herbal medicine profile by the community in Cicanir and Jatipamor villages in Majalengka Regency reported that 40.79% and 25.86% of people drank herbal medicine, respectively.[14]

Based on the several studies above, the *I. cylindrica* was reported to have many benefits. From these benefits, the *I. cylindrica* as traditional medicine has been well documented by the Indonesian government and some people in Majalengka Regency still use herbal medicines in their daily lives.

The focus of this study is to examine and promote the use of *I. cylindrica* leaves from Kertajati as a candidate medicinal plant based on parameters of their phytochemicals content and antioxidant activity. Then this study results were compared with the same parameters from the roots.

MATERIALS AND METHODS

Materials

Materials are gallic acid, quercetin, tannic acid, 1,10-phenantrolin and 1.1-diphenyl-2-picrylhydrazyl (DPPH) from Sigma Aldrich; sodium carbonate, Folin Ciocalteau, sodium acetate, aluminium chloride, methanol, iron (III) chloride hexahydrate, sodium chloride, gelatine, ethylene diamine tetra acetic acid (EDTA), acetic acid, hydrochloric acid, sulfuric acid, all reagents are analysis grade (Merck).

Equipment

The equipment was used to parameters analysis in the extract as follows.

Eppendorf Biospectrometer Basic AG 22331 Hamburg series: 6135 BJ.

Sample collection

I. cylindrica (whole plant) was obtained from Kertajati Subdistrict in Majalengka Regency, Indonesia and harvested in April 2021.

Sample preparation

The procedures for preparation of extracts, screening phytochemistry, measuring of total phenol content, total flavonoid content, total tannin content and antioxidant activity of both leaves and roots extracts were as follows:

Screening of flavonoid and tannins in the extract[15]

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.

Tannins was identified with 1 % gelatin solution. The presence of tannins is indicated by the formation of white precipitate.

Each preparation of *I. cylindrica* leaves and roots extracts[16]

I. cylindrica powder was macerated by methanol.

Aliquot 200 g of powder was weighted and macerated three times in 24 hours. The 1st time powder was macerated with 2 l of methanol and left for 24 h while stirring the solution repeatedly. After 24 h, the methanol solvent layer was filtered and stored in a bottle, and this is called methanol extract. This procedure was repeated twice with 1 l of methanol.

Then, all methanol extracts from three times macerations were combined and concentrated.

Quantification of total phenolic compounds[17]

The reagents for total phenolic quantification: Folin-Ciocalte 10%, sodium carbonate 7.5% solution.

Standard curve of gallic acid reference standard

Gallic acid refence standard stock solution 1000 ppm

Aliquot 10 mg of gallic acid reference standard was put and dissolved with methanol until 10 ml in 10 ml volumetric flask.

The stock solution of gallic acid reference standard was prepared in the concentrations 50 ppm, 100 ppm, 200 ppm, 400 ppm, and 800 ppm, respectively.

Each gallic acid reference standard above was pipetted 0,05 ml and was put in the reaction tubes, and was followed by the addition of 2.5 ml Folin-Ciocalte 10% solution (homogenized), and 2 ml sodium carbonate 7.5% (homogenized). Then, the mix compounds in each tube were incubated at 37 °C for 30 min. Next, its absorption was measured at a wavelength 750 nm.

The reference standard calibration curve was made by adjusting the concentration against absorbance.

The same steps were done for 0.05 ml of *I. cylindrica* extract for quantification of phenolic compounds content in the extract, as well.

All works were repeated three times

The total phenolic content was calculated with the equation as follow.

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

Abbreviation:

TPC is the total phenolics content in percentage (%)

C is x 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)

Quantification of total flavonoid compounds[17]

The reagents for total flavonoid quantification: sodium acetate 120 mM solution, $AlCl_3$ 2% solution

Standard curve of quercetin reference standard

Quercetin refence standard stock solution in the concentration of 1000 ppm.

Aliquot 10 mg of quercetin reference standard was put and was dissolved with methanol until 10 ml in 10 ml volumetric flask.

The stock solution of quercetin reference standard was prepared in the concentrations 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm, respectively.

Each quercetin reference standard above was pipetted 1 ml and was added 1 ml $AlCl_3$ 2% solution (homogenized), and 1 ml sodium acetate (homogenized). Afterwards, all the mix compounds were stored in the room (at room temperature) for 1 h. Then, its absorption was measured at a wavelength 435 nm.

The reference standard calibration curve was made by adjusting the concentration against absorbance.

As well, the same steps were done for 1 ml of *I. cylindrica* extract.

All works were repeated three times.

The total flavonoid content was calculated with the equation as follow.

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

Abbreviation:

TF is the total flavonoid content (%)

C is x 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)

Quantification of tannin compounds[18]

The reagents for total tannin quantification: 1,10-phenanthroline solution (0.015 mol/l), iron (III) chloride hexahydrate solution (0.01 mol/l), ethylene diamine tetra acetate (EDTA) solution (0.05 mol/l), acetic buffer pH 4.4, acidic sodium chloride solution.

Gelatin solution (0.3% m/V)

The solution was prepared by dissolving 3.00 g gelatin in saturated sodium chloride solution and it was diluted to 1 l with saturated sodium chloride solution.

Standard curve of tannic acid reference standard

Tannic acid reference standard solution 100 ppm

The solution was prepared freshly, aliquot 10 mg of tannic acid reference standard was put and was dissolved with distilled water until 100 ml in 100 ml volumetric flask.

The concentration of each reference standard tannic acid for standard curve was made as follows: 1.0, 2.0, 3.0, 4.0 and 5.0 µg/ml.

Five of 25 ml volumetric flasks were provided, into each flask was put 0.250; 0.500; 0.750; 1 and, 1.250 ml the reference standard tannic acid, respectively. Then, 2.50 ml of iron (III) chloride hexahydrate solution (0.01 mol/l) was added to each flask, was mixed, and was incubated for 20 minutes at 80 ^{0}C in a water bath. Next, 2.50 ml of acetic buffer (pH 4.4), 5.0 ml of 1,10-phenanthroline solution (0.015 mol/l) and 0.50 ml of EDTA (0.05 mol/l) were added to each flask, and let the solution cooled. Finally, distilled water was added to each flask until the solution volume became 25 ml. Then, each solution/mixture absorbance was measured at a wavelength 540 nm relatively to the blank.

The reference standard calibration curve was made by adjusting the concentration against absorbance.

Preparation of blank for I. cylindrica extract

Preparation sample of *I. cylindrica* roots extract

Aliquot of 0.025 g *I. cylindrica* extract was dissolved by distilled water until 100 ml (this solution was called extract A solution). Aliquots of 10 ml of extract A solution was filled to 25.00 ml volumetric flask, and the next step followed the steps of the preparation calibration curve procedure.

Aliquots of 10 ml of extract A solution was put into a 100.00 ml volumetric flask which had previously been added 0.50 ml of gelatine solution (0.3% m/v), 10 ml of acidic NaCl solution. Then, mixture was shaken and filtered (was called Filtrate No.1); Aliquots of 10 ml of Filtrate No.1 was carried out in the same way of the procedure for Filtrate No.1, the result was called Filtrate No.2.

Filtrate No. 2 followed the steps such as tannic acid standards and *I. cylindrica* extract samples.

Preparation of Gelatin blank

The gelatine blank samples were prepared by following the steps of procedure of the blank *I. cylindrica* samples. The sample of gelatin was changed with distilled water.

All works were repeated three times, and the absorbance of the solutions was measured at a wavelength of 540 nm.

The tannin content was calculated with the two equations bellow:

Anbs = Abbs – Agbs

where: Anbs = absorbance of the net blank samples,

Abbs = absorbance of the blank *I. cylindrica* samples

Agbs = absorbance of the gelatine blank sample for the corresponding sample.

At = As - Anbs

where: At = absorbance presents the absorbance of the tannins in the sample

As = absorbance of the *I. cylindrica* sample.

Then total tannin content was calculated from At and equation of calibration curve of standard reference of tannic acid.

Quantification of antioxidant activity[16]

The reagents for antioxidant activity quantification: DPPH stock solution 0.5 mM

Antioxidant activity analysis

I cylindrica extract stock solution

Stock solutions of *I cylindrica* extract for quantification were made/ prepared with the following concentrations: 200 ppm; 160 ppm; 120 ppm; 80 ppm, and 40 ppm.

One tube was prepared (number 1) and was put of 4 ml of methanol.

Five tubes were prepared and given number from number 2 to number 6; each tube was put 4 ml *I. cylindrica* stock solution with the following concentrations: 200 ppm; 160ppm; 120ppm; 80 ppm, and 40 ppm, respectively.

Then, all 6 tubes were added 1 ml of DPPH stock solution, let the solution for 30 minutes.

Next, absorbance of each tube was measured at wave length 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.[17]

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

RESULT AND DISCUSSION

The *I. cylindrica* is a traditional medicine, it is used by several ethnic groups in Indonesia.

It was reported that there are 27 districts that use *I. cylindrica* as traditional medicine. It is used as traditional medicine which is divided in two categories, 1st based on the used plant parts and 2nd based on the types of disease. The details of categories are as follows. The 1st utilization based on plant parts found utilization of roots, leave, flower, and stem were 21,5,2, and 1 time, respectively. The 2nd utilization based on the type of the disease, the results were as follows: fever, blood in urine, scabies, bleeding, toothache, hair conditioner, hepatitis, sore eyes, constipation, aphrodisiac, tonicum, aches, back pain, heat burn, internal disease, kidney disease, hypertension, wound.[19] The using of *I. cylindrica* as traditional medicine has been strengthened by findings from surveys conducted on several ethnic groups in Indonesia [19], books containing its formulation [20], as well as the government regulations.[4]

Data regarding the use of *I. cylindrica* recorded in books published by the government and government regulation were as follows. Reduce blood fat with the following formula: *Imperata cylindrica* L. – roots, *Syzygium polyanthum* (Wight) Walp. –leaves, *Centella asiatica* L. – leaves, *Andrographis paniculata* (Burm. F) Ness. – leaves.[3,20] Help to dissolve kidney stone with the following formula: *Imperata cylindrica* L. – roots, *Orthosiphon aristatus* (Blume) Miq. – leaves, *Centela asiatica* L. – herb, *Helianthus annuus* L. stem, and *Andrographis paniculata* L. Ness – leaves.[3,20] *Increase urine volume* with the following formula: *Imperata cylindrica* L. – roots, and *Orthosiphon stamineus* Benth– leaves.[3,4] Urinary stone with the following formula: *Imperata cylindrica* L. – roots.[3,4]

The result of *Imperata cylindrica* L. development use is based on study as a herbal medicine, for urinary tract stones the formulation is as follows: *Imperata cylindrica* (L.) P.Beauv. – roots, *Sonchus arvensis* L. – herb, *Orthosiphon aristatus* (*Blume*) *Miq*. – leaves, *Strobilanthes crispus* Blume. – leaves, *Curcuma longa* L. – rhizome, *Curcuma xanthorrhiza* Roxb.-rhizome, *Phyllanthus niruri* L. – herb.[3,21]

The use of *Imperata cylindrica* as a traditional medicine and scientific herb has been described.

The pharmacological activities of traditional medicinal herb have been felt by the people for a long time. The pharmacological activity of medicinal plants depends on totally on their phytochemical content. Phytochemicals as secondary metabolites can be divided in three board groups, namely phenolic, terpenoids, and alkaloids compounds. The phenolic compounds cover a large number of plant phytochemicals content, among others phenolic acids, flavonoids and tannins.[22-24]

The next step is determining the parameters of *Imperata cylindrica* that support its role for health.

To promote the use of *I. cylindrica* leaves as herbal medicine, the phytochemical content of *I. cylindrica* leaves was determined and quantitated.

The result of the study regarding identification and quantification of phytochemicals content of *I.cylindrica* leaves and roots is as follows.

Phytochemical screening:

Screening of flavonoid and tannin showed positive result, both of them are phenolic compounds, the extract contained phenolic compounds among other flavonoid and tannin.

Quantification of total phenolic compound content in the extract

The total phenolic content in the extracts (both leaves and roots) were quantified using a gallic acid reference standard, and the results were expressed to be equivalent to gallic acid.

Total phenolic quantification of I. cylindrica leaves extract

The preparation result of gallic acid reference standard is as follows in table 1 and figure 1.

Linear regression equation of gallic acid reference standard curve in the figure 1 was y = 1.0875x - 0.0229, and result of measurement of *I. cylindrica* leaves extract absorbance was 0.075.

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

y = 1.0875x - 0.0229

$$\text{Percentage of total phenolic extract} = \frac{\text{V(ml).C(mg/ml).Fp}}{\text{weight of sampel (mg)}} \times 100\% = 9\%$$

Total phenolic content of *I. cylindrica* leaves extract was 9% (GAE)

Total phenolic quantification of I. cylindrica roots extract

The preparation result of Gallic acid reference standard as follows in table 2 and figure 2

Linear regression equation of gallic acid reference standard curve in the figure 2 was y = 1.0779x – 0.0185, and result of measurement of *I. cylindrica* roots extract absorbance was 0,044.

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

$$\text{Percentage of total phenolic extract} = \frac{\text{V(ml).C(mg/ml).Fp}}{\text{weight of sampel (mg)}} \times 100\% = 5.8\%$$

Total phenolic content of *I. cylindrica* roots extract was 5.8% (GAE)

The quantification result of total flavonoid content in the extract

The total flavonoid content in the extracts (both leaves and roots) were quantified using a quercetin reference standard, and the results were expressed to be equivalent quercetin.

Quantification of total flavonoid content of I. cylindrica leaves extract

The preparation result of quercetin reference standard as follows in table 3 and figure 3

Linear regression equation of quercetin reference standard curve in the figure 3 was y = 23.64x + 0.0188, and result of measurement of *I. cylindrica* leaves extract absorbance was 0.516.

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

y = 23.64x + 0.0188

$$\text{Percentage of total flavonoid extract} = \frac{\text{V(ml).C(mg/ml).Fp}}{\text{weight of sampel (mg)}} \times 100\% = 2.1\%$$

Total flavonoid content of *I. cylindrica* leaves extract was 2.1% (QE)

Quantification of total flavonoid content of I. cylindrica root extract

The preparation result of quercetin reference standard as follows in table 4 and figure 4.

Linear regression equation of quercetin reference standard curve in the fig. 4 was y = 23.4x + 0.0206, and result of measurement of *I. cylindrica* roots extract absorbance was 0.171

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

y = 23.4x + 0.0206

Table 1: Gallic acid reference standard curve absorbances (at wave length 750 nm).

Concentration of gallic acid reference standard (mg/ml)	Absorbance
0.05	0.037
0.1	0.103
0.2	0.169
0.4	0.410
0.8	0.852

The data in table 1 was made in the form of a chart to get the linier regression equation

Table 2: Gallic acid reference standard curve absorbances (at wave length 750 nm).

Concentration of gallic acid reference standard (mg/ml)	Absorbance
0.05	0.045
0.1	0.107
0.2	0.166
0.4	0.410
0.8	0.850

The data in table 2 was made in the form of a chart to get the linier regression equation

Table 3: Quercetin reference standard curve absorbances (at wave length 435 nm).

Concentration of quercetin reference standard (mg/ml)	Absorbance
0.005	0.151
0.010	0.250
0.015	0.348
0.020	0.502
0.025	0.616

The data in table 3 was made in the form of a chart to get the linier regression equation

Table 4: Quercetin reference standard curve absorbances (at wave length 435 nm).

Concentration of quercetin reference standard (mg/ml)	Absorbance
0.005	0.150
0.010	0.251
0.015	0.348
0.020	0.497
0.025	0.612

The data in table 4 was made in the form of a chart to get the linier regression equation

Table 5: Absorbance of extract, blank of extract, and gelatine blank (at wave length 540 nm).

Sample	Absorbance
I. cylindrica extract	1.387
Blank of *I. cylindrica* extract	0.136
Blank of gelatine	0.113

Anbs = Abbs – Agbs
= 0,023
At = As – Anbs
= 1.364

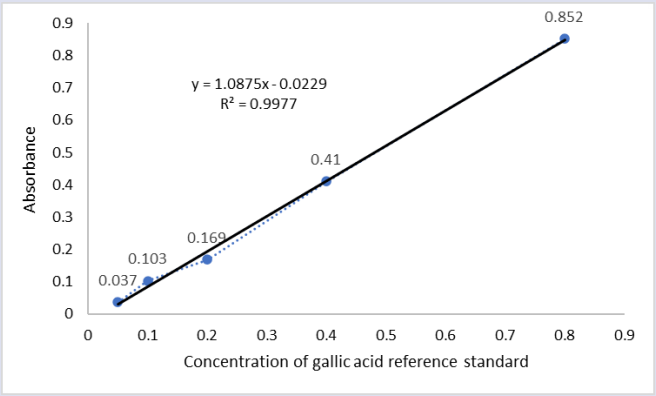

Figure 1: Gallic acid reference standard curve.

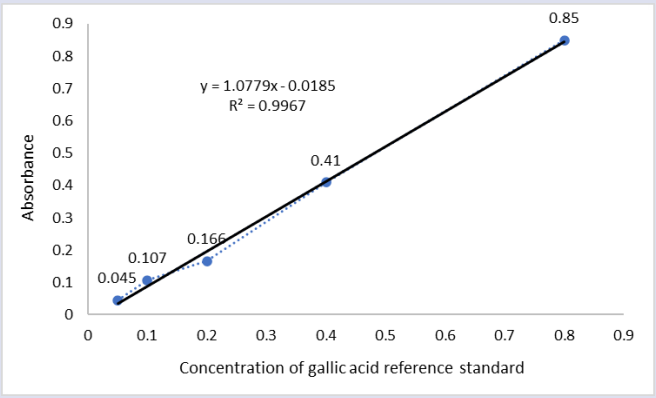

Figure 2: Gallic acid reference standard curve.

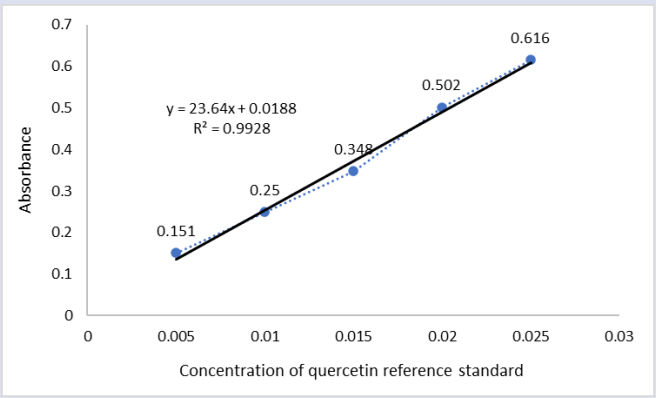

Figure 3: Quercetin reference standard curve.

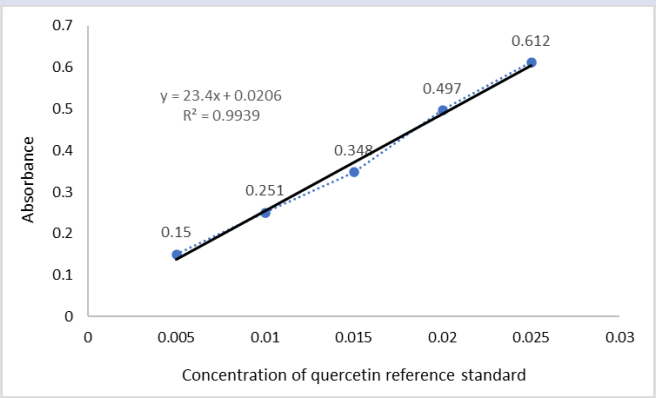

Figure 4: Quercetin reference standard curve.

Percentage of total flavonoid extract = $\frac{V(ml).C(mg/ml).Fp}{\text{weight of sampel (mg)}} \times 100\% = 0.64\%$

Total flavonoid content of *I. cylindrica* roots extract was 0.64% (QE)

Total tannin content quantification of *I. cylindrica* leaves extract

The quantification result of tannin content in the extract as follows:

The tannin content in the extracts (both leaves and roots) were analyzed using a tannic acid standard, and the results were expressed to be equivalent tannic acid.

Tannin content of *I. cylindrica* leaves extract measurement as follow

The preparation results of tannic acid reference standard in table 5, table 6 and figure 5.

Linear regression equation of tannic acid reference standard curve in the figure 5 was y = 0.2485x - 0.0169, and the absorbance of *I. cylindrica* leaves extract (At) was 1.364

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

y = 0.2485x - 0.0169

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

Percentage of tannic acid in extract = $\frac{(100\ ml).0.0056\frac{mg}{ml} \times 2.5}{25\ (mg)} \times 100\% = 5.6\%$

Total tannin content of *I. cylindrica* leaves extract was 5.6% (TAE)

Total Tannin content quantification of I. cylindrica root extract

Linear regression equation of tannic acid reference standard curve in the figure 6 was y = 0.2658x - 0.0174, and the absorbance of *I. cylindrica* roots extract (At) was 1.364

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

y = 0.2658x - 0.0174

x = 3 µg/ml

Percentage of tannic acid in *I. cylindrica* roots extract = $\frac{V(ml).C(mg/ml).Fp}{\text{weight of sampel (mg)}} \times 100\% = 3\%$

Total tannin content of *I. cylindrica* roots extract was 3% (TAE)

Antioxidant activity quantification of *I. cylindrica* extract

The quantification result of antioxidant activity of the extract as follows:

The antioxidant activities of the extracts (both leaves and roots) were quantification using DPPH solution, and the results were expressed to IC_{50}.

Antioxidant activity quantification of I. cylindrica leaves extract

Linear regression equation of DPPH inhibition percentage of *I. cylindrica* leaves extract was y = 16.356x + 8.896, and then this linier regression was used to calculate antioxidant activity (% IC_{50}) from *I. cylindrica* leaves extract.

y = 16.356x + 8.896

x = 2,514 mg/25 ml

IC_{50} = 2,513 mg/25 ml

= 101 ug/ml

Table 6: Tannic acid reference standard curve absorbances (at wave length 540 nm).

Concentration of tannic acid reference standard (µg/ml)	Absorbance
1	0.245
2	0.474
3	0.719
4	0.961
5	1.244

The data in table 6 was made in the form of a chart to get the linier regression equation

Table 7: Absorbance of extract, blank of extract, and gelatine blank (at wave length 540 nm).

Sample	Absorbance
I. cylindrica extract	0.840
Blank of *I. cylindrica* extract	0.157
Blank of gelatine	0.117

Anbs = Abbs - Agbs = 0.040 At = As – Anbs = 0.800

Table 8: Tannic acid reference standard curve absorbances (at wave length 540 nm).

Concentration of tannic acid reference standard (µg/ml)	Absorbance
1	0.255
2	0.466
3	0.808
4	1.108
5	1.263

The data in table 8 was made in the form of a chart to get the linier regression equation

Table 9: Percentage of inhibition of *I. cylindrica* leaves extract.

No	Consentration mg/25 ml	Absorbance	% Inhibition
Reference	-	0.949	
1	5	0.087	85.73
2	4	0.211	77.76
3	3	0.362	61.85
4	2	0.538	43.30
5	1	0.748	21.18

The data in table 9 was made in the form of a chart to get the linier regression equation

Table 10: Percentage of inhibition of I. cylindrica roots extract.

No	Concentration (mg/25 ml)	Absorbance	% Inhibition
Refference		0.934	
1	5	0.543	41.86
2	4	0.626	32.97
3	3	0.705	24.51
4	2	0.754	18.00
5	1	0.856	8.35

Table 11: Comparison of three parameters between *I. cylindrica* leaves and roots extracts.

No	Sample	Parameters			
		Total phenolic (GAE) %	Total flavonoid (QE) %	Total tannin (TAE) %	Antioxidant activity IC_{50}
1	*I. cylindrica* leaves extract	9	2.1	5.6	100.5
2	*I. cylindrica* roots extract	5.8	0.64	3	241

Based on the calculation, the IC_{50} of antioxidant activity of *I. cylindrica* leaves extract was 101 ppm.

Antioxidant activity quantification of *I. cylindrica* roots extract

Linear regression equation of DPPH inhibition percentage of *I. cylindrica* roots extract was y = 8.199x + 0.541, and then this linier regression was used to calculate antioxidant activity (% IC_{50}) from *I. cylindrica* roots extract.

y = 8.199x + 0.541

IC_{50} = 241 ug/ml

Based on the calculation, the IC_{50} of antioxidant activity of *I. cylindrica* roots extract was 241 ppm.

Sum of all parameters result, total phenolic content, total flavonoid content, total tannin content, and antioxidant activity of both *I. cylindrica* leaves and roots extracts is as follows.

All quantification results of phenolic, flavonoids, and tannins compounds which were contained in both *I. cylindrica* leaves and roots extracts were shown in fig. 9 bellow:

The antioxidant activity results which was contained in both *I. cylindrica* leaves and roots extracts were showed in figure 10 bellow:

Table. 11 and figure 9 and 10 were sum of total content of phenolic compound, flavonoids, tannins, and antioxidant activity quantification of both *I. cylindrica* leaves and roots extracts from Kertajati, West Java.

The screening phytochemistry result of *I. cylindrica* leaves and root, both leaves and roots contained flavonoids and tannins. Quantification

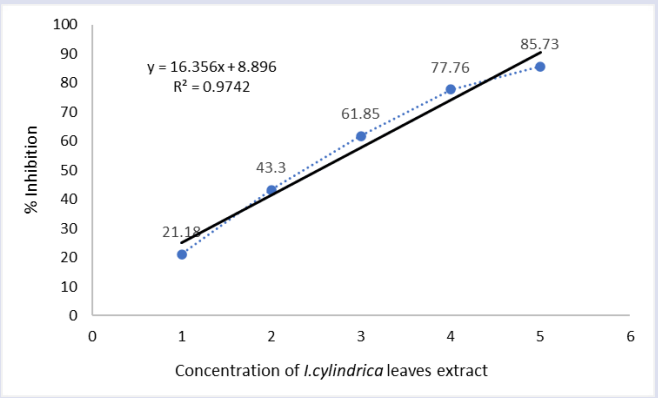

Figure 7: Percentage inhibition curve of *I. cylindrica* leaves extract to DPPH.

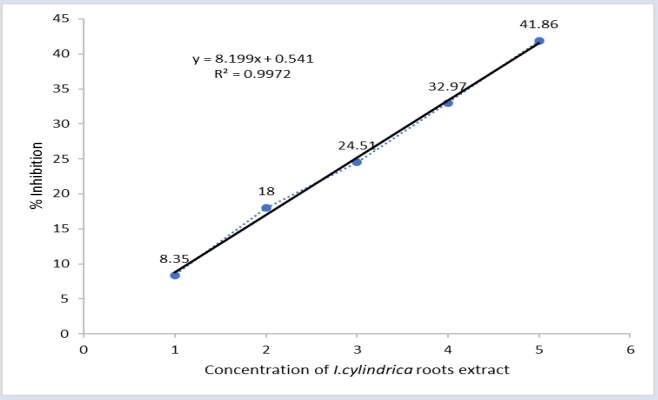

Figure 8: Percentage inhibition curve of *I. cylindrica* roots extract to DPPH.

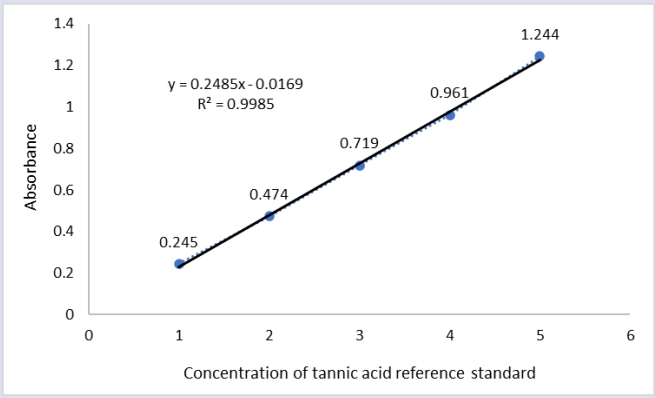

Figure 5: Tannic acid reference standard curve.

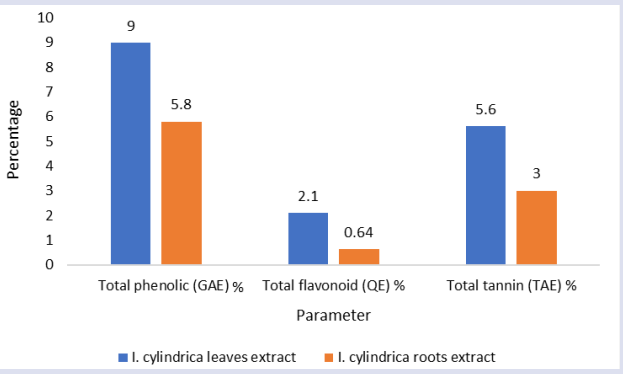

Figure 9: Percentage of total phenolic, total flavonoid and total tannin contents of *I. cylindrica* leaves and roots extract.

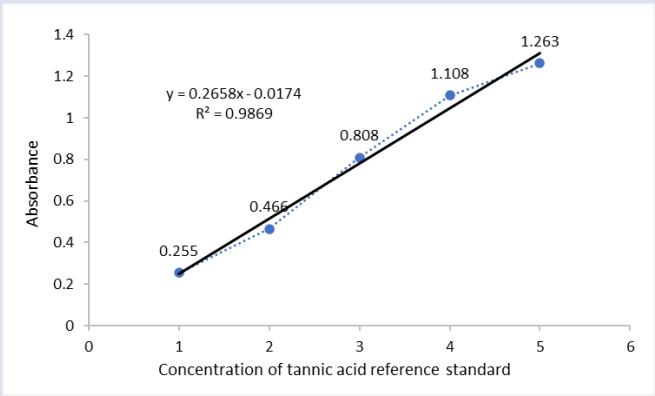

Figure 6: Tannic acid reference standard curve.

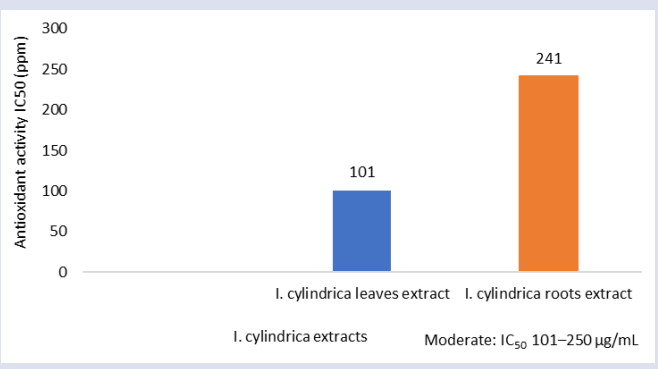

Figure 10: IC_{50} of antioxidant activity of both of *I. cylindrica* leaves and roots extract.

of the total content of phenolics, flavonoids, tannins and antioxidant activity showed that these four parameters are possessed by leaves and roots.

The relationship of four parameters in this study based on several studies reported pharmacological activities of secondary metabolites compounds were caused by its structures.[25] The structure of phenolic compounds had ability to eliminate free radicals (FR), at which the metabolic process increases of the reactive oxygen species (ROS).[26]

Many types of natural antioxidants contain hydroxyl groups, among others phenols; simple phenols are compounds that have a basic skeleton of an aromatic ring with one hydroxyl group attached to the aromatic ring. Their hydroxyl groups have ability to neutralize free radicals by donating an H-atom to deactivate the free radical. The antioxidant activity was determined by the number of hydroxyl group in the ortho position, beside the bond dissociation energy (BDE) of O-H influenced the activity of an antioxidant to neutralize free radicals, also.[27]

Flavonoids have basic structural flavane (2-phenyl-benzo-γ-pyran) as nucleus. The structures have roles in antioxidant activity. The antioxidant activity of flavonoids commonly occurs through the following mechanisms, namely single electron transfer, hydrogen atom transfer, and transition metal chelation.[27]

Antioxidant activity of the phenolic acids was influenced by the number and the position of hydroxyl group in correlation to the carboxyl functional group, esterification, and glycosylation.[28]

Our previous study of *I. cylindrica* roots methanol extract from Kendari (South East Sulawesi) showed the extract had anti-hypertensive activity on hypertension rat models.[29] The phytochemicals content and its activity of this extract were as follows: total polyphenol content was 1.53% (GAE),[30] a total flavonoid content was 1.17% (QE),[31] total tannin content was 1.06% (w/w) (TAE) and antioxidant activity IC_{50} 0.32 mg/ml.[32]

As explained above, the content of phytochemical compounds from a plant provides various pharmacological effects related to health. Therefore, *I. cylindrica* leaves extract from Kertajati Subdistrict which contains phytochemical compounds is certain to have effects on health.

CONCLUSION

The study of *I. cylindrica* leaves extract from Kertajati showed the phytochemical content among others phenolic compound such as flavonoids, tannins, and antioxidant activity. All of these parameters have been reported to have effects on health.

Therefore, *I. cylindrica* leaves extract from Kertajati which contains phytochemical compounds is discovered to have effects on health, so that in the future the use of *I. cylindrica* leaves is valuable to be promoted as a medicinal plant.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTEREST

No conflicts of interest.

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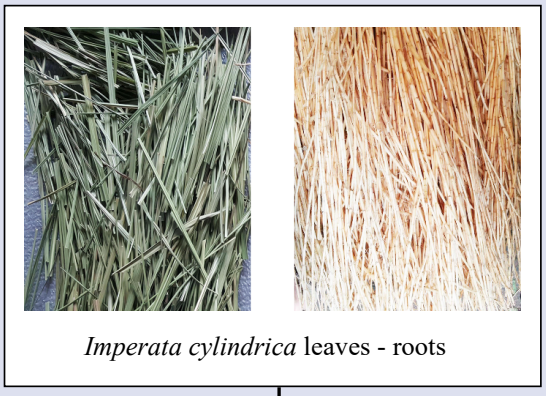
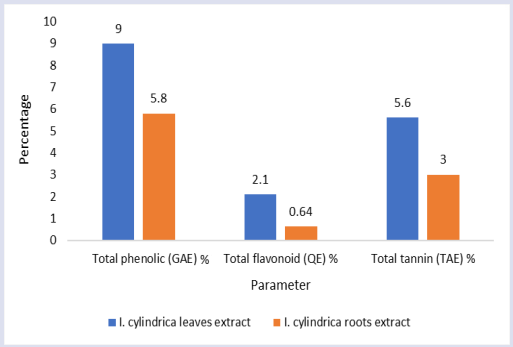
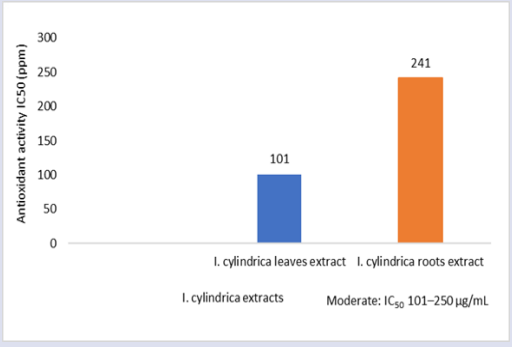
GRAPHICAL ABSTRACT







Sum of all parameters result, total phenolic, total flavonoid, total tannin, and antioxidant activity of both *I. cylindrica* leaves and roots extracts.

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