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Original Article

Quantification of total polyphenols and flavonoids, antioxidant activity, and Sinensetin and Imperatorin contents of *Imperata* cylindrica root ethanol extract

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ABSTRACT

Introduction: *Imperata cylindrica*, commonly known as cogon grass, is currently widely distributed and used as a medicinal plant. The major compounds that have been isolated and identified are polyphenols and flavonoids, which have many biological activities such as antioxidant, and anticancer. Polyphenols and flavonoids are mostly found in the roots and leaves. This study aimed to perform phytochemical screening on *I. cylindrica* root ethanol extract from Sragen, Central Java, Indonesia and determine the total polyphenol, flavonoid, antioxidant activity and quantify Sinensetin and Imperatorin contents of the extract. **Method:** Quantification of all parameters were measured using visible spectrophotometric methods. Total polyphenol, total flavonoid contents, as well as antioxidant activity were measured using Folin-Ciocalteu reagent, aluminum chloride reagent, and 1,1-diphenyl-2-picrylhydrazyl, respectively, and quantification of Sinensetin and Imperatorin were measured using High Performance Liquid Chromatography. **Results:** *I cylindrica* root ethanol extract had a total polyphenol content of 1.109% gallic acid equivalent, total flavonoid content of 0.1% quercetin equivalent, and antioxidant activity IC_{50} 824.30 µg/ml. Sinensetin and Imperatorin were also identified in Fractions 1 to 11 with concentrations of 0.0157 and 0.0178 mg/kg extract, respectively. **Conclusion:** *I. cylindrica* root ethanol extract from Sragen had active phytochemical compounds of polyphenols, flavonoids, and antioxidants as well as Sinensetin and Imperatorin.

Key words: *Imperata cylindrica*, Polyphenol, Flavonoid, Antioxidant.

INTRODUCTION

Imperata cylindrica or cogon grass is a rhizomatous grass native to the Indomalayan and Australasian area.[1] More than 72 active compounds have been isolated and identified from this plant, including flavonoids, phenols, saponins, glycosides, and coumarins. These phytochemical substances exhibit biological activity as an anti-inflammatory, antioxidant, antibacterial, anticancer, immune system booster, and liver protection.[2]

Phenols are widely distributed in *I. cylindrica* and approximately 18 different phenols have been isolated from this plant.[2] Polyphenols exhibit many anticarcinogenic properties, including their inhibitory effects on proliferation of cell cancer, angiogenesis, tumor growth, metastasis, and inflammation as well as inducing apoptosis. Furthermore, they can modulate the immune system response and protection against free radicals damage in normal cells.[3] Nano formulations of natural polyphenols as bioactive agents such as resveratrol, curcumin, quercetin, and coumarin derivatives result in better efficacy for cancer prevention and treatment in dose dependent manner.[4] Polyphenol extracts from Annurca apple showed the ability to inhibit AKt activation and downregulated several oncoproteins including NF-kB, c-myc, and β-catenin that may be an attractive candidate for drug development against triple negative breast cancer.[5] Polyphenols have also shown three major actions as antioxidants, regulation of enzymes in phase I and II, and regulation of cell survival pathways against lung carcinogenesis.[6]

Flavonoids, which are typical compounds present in *I. cylindrica* plants, have been shown to possess an extensive variety of anticancer effects as they modulate reactive oxygen species (ROS)-scavenging enzyme activities, participate in arresting the cell cycle, induce apoptosis, autophagy, and suppress cancer cell proliferation and invasiveness. Flavonoids have dual action regarding ROS homeostasis because they act as antioxidants under normal conditions and are potent pro-oxidants in cancer cells triggering apoptotic pathways and downregulating pro-inflammatory signaling pathways.[7] In many molecular mechanisms of action for prevention against cancer, flavonoids also play a major role by interacting between different types of genes and enzymes. Human clinical trials have shown that flavonoids have important effects on cancer chemoprevention and chemotherapy.[8] These active flavonoids caused cell cycle arrest at G2/M phase and induced apoptosis and autophagy in human breast cancer cells,[9] suppressing oncogenic miRNAs, activating oncosuppressor miRNAs or affecting transcriptional, epigenetic miRNA processing in triple negative breast cancer,[10] and potential as anticancer agent for human colorectal cancer.[11] Natural bioactive compounds and particular flavonoid compounds have proven to have an important role in lung cancer treatment by interfering with the receptor tyrosine kinases cascade, affecting

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cell proliferation, enhancing apoptosis and autophagy, modulating cell cycle and regulating invasion and metastasis in lung cancer.[12]

Several studies have shown that polyphenols not only have anticancer activity, but also good anti-oxidation ability. In *Rhizoma imperatae* extraction, polyphenol IC_{50} for scavenging hydroxyl radicals was 0.0948 mg/mL, while IC_{50} of ascorbic acid was 0.1096 mg/mL.[13] The antioxidant activity of the methanol extracted from *I. cylindrica* root also demonstrated the presence of polyphenol compounds. The extract had a total polyphenol content of 1.53% gallic acid equivalent (GAE) and antioxidant activity IC_{50} of 0.32 mg/mL. The presence of polyphenol compounds supports the antioxidant activity of the extract.[14] The anticancer effect of trihydroxyflavones against carcinoma cell lines A549 and U87 could be related to their antioxidant activity as the anti-proliferative effect directly correlates with 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. On the other hand, 3,3,6-trihydroxyflavone (containing hydroxyl groups attached to the different rings) does not possess antioxidant activity but is a highly active anticancer compound.[15]

Sinensetin and Imperatorin were also found in *I. cylindrica* root methanol extracts from Kendari, with concentrations of 32.348 and 37.014 mg/kg, respectively.[16] Sinensetin proved to have antiproliferation, antiangiogenesis, and antioxidant effect as well as anticancer activity,[17] while Imperatorin showed a significant inducing apoptosis effect both *in vitro* and *in vivo* in various cancer cell lines.[18] However, research on the content of Sinensetin and Imperatorin in *I. cylindrica* and their function is still rare, thus more studies are urgently needed to explore these phytochemical compounds.

MATERIAL AND METHODS

Materials

Ethanol extract of *I. cylindrica* was grown in Sragen, Central Java, Indonesia. All reagents, including gallic acid reference standard (Merck, Darmstadt, German); quercetin standard (MarkHerb, Bandung, Indonesia); Sinensetin and Imperatorin reference standards (ChemFaces, Wuhan, China); ethanol, methanol, and Milli Q-water (HPLC grade, Merck); and Folin Ciocalteau 10%, sodium chloride, sodium acetate, $AlCl_3$ 2%, 1,1-diphenyl-2-picrylhydrazyl (DPPH), sodium carbonate, n-hexane, ethylacetate, acetonitrile, and silica gel 60 H (Merck) were of analytical grade.

Equipment

The equipment used to examine the extract was as follows: Agilent Technology 1220 Infinity LC for High Performance Liquid Chromatography (HPLC); spectrophotometer detector Shimadzu UV-1800, Column: Zorbax SB-C18 4.6×250 mm, 5 µm; Camag Chromato-Uve Cabinet UV Lamp 4; Bransonic Ultrasonic bath CPX2800H-E series; Memmert Waterbath WNB 22; Eppendorf micropippete 10–100 µm.

Ethanol extraction

One hundred grams of *I. cylindrica* dry root powder was extracted by stirring in 750 mL of 96% (v/v) ethanol for 5 days at 20-22°C using a magnetic stirrer, and filtered to separate the insoluble plant material and solvent evaporated at 50 °C under reduced pressure in a rotary evaporator, resulting in 16.320 grams of root ethanol extract.

Phenolic and flavonoid total content

Total phenolic content was measured using gallic acid as reference standard and the result was shown as gallic acid equivalent percentage (GAE). For the measurement, a gallic acid standard curve was determined by diluting various concentrations (100, 150, 250, 300, 400, and 500 ppm) used to calculate the total phenol in the extract. To determine the root extract, the sample was diluted in methanol at 50 mg/ml. From each sample, 0.05 ml was added to 2.5 ml Folin Ciocalteu 10% until homogenized, then 2 ml sodium carbonate (7.5% b/v), and the mixture was incubated at 37 °C for 30 min. The absorbance was measured at 750 nm relative to the blank. By using linear equation from gallic acid standard curve, the sample absorbance were calculated and noted as gallic acid equivalent percentage.[19]

The total flavonoid content was measured using Quercetin as reference standard. A standard curve was produced by diluting quercetin stock solution (1000 ppm) into various concentrations (2.5, 5, 10, 15, 20, and 25 ppm). Each concentration was mixed with 1 ml $AlCl_3$ 2%, stirred, sodium acetate was added until homogenized and kept in room temperature for 60 min. The solution absorbance was measured at 435 nm relative to the blank. The root extract was measured by diluting the sample with methanol at 50 mg/ml. One ml from each sample was added with 1 ml $AlCl_3$ 2%, stirred, mixed with 1 ml sodium acetate and left at room temperature for 60 min. The absorbance was measured at 435 nm. The flavonoid total concentration was calculated by plotting the absorbance against the reference standard curve.[20]

Antioxidant activity

Antioxidant activity was determined using DPPH as reactor. Stock solution was prepared by diluting the extract with ethanol to obtain 1000 ppm. Ethanol extract of *I. cylindrica* root was prepared in various concentrations (62.5; 125; 250; 500; 750; and 1000 ppm). Each extract was mixed with 1 ml of DPPH solution stock, vortexed for 1 min and incubated at room temperature for 30 min. The solution absorbance was measured using a spectrophotometer at 515.5 nm and the content was calculated using the linear equation from the DPPH standard curve. The result was shown as an inhibition concentration percentage (%IC50).[21]

Quantification of Sinensetin and Imperatorin by HPLC

The ethanol extract of *I. cylindrica* roots weighed 150 mg, Sinensetin and Imperatorin in the extract were separated on a silica gel 60H by column chromatography, using gradient eluent system as follows: (1) a mixture of n-hexane-ethylacetate (3:7); (2) a mixture of n-hexane-ethylacetate (2:8); (3) a mixture of ethyl acetate-methanol (8:2); and (4) methanol.[22]

In total, 15 fractions resulted from column chromatography, and each was dissolved into 10 ml methanol and filtered through 0.45 µm Millipore filter. Then, 10 µl of every fraction was injected into the HPLC instrument. Both Sinensetin and Imperatorin reference standard was treated the same way as with the fraction. Various weighed concentrations (10, 20, 30, 40, and 50 mg) of reference standard were dissolved in 10 ml of methanol and then filtered through 0.45 µm Millipore filter, and 10 µl was injected into the HPLC instrument.[23,24]

Samples were treated in a column (Zorbax SB-C18 4), with heating at 40 °C, used as stationary phase and eluted with an isocratic mobile phase of 0.1% formic acid in water-acetonitrile-methanol (40:50:10). The flow rate was 1 ml/min, separation time was 20 min, and the detection was set at 254 nm. The chromatographic data was analyzed using EZChrom Elite Compact software.

Reference standard curves of Sinensetin and Imperatorin were prepared using various concentrations (10, 20, 30, 40, and 50 ppm). Standard calibration curves were established by plotting the peak area against retention time.

Time and place

This study was carried out between January and April, 2022, in the Pharmacy Laboratory, Faculty of Mathematics and Natural Science, Bandung Islamic University.

RESULTS

From the gallic acid reference standard curve, the obtained linear regression equation was y = 0.0018x – 0.0189. With this equation, the total polyphenol in the ethanol extract of *I. cylindrica* root can be calculated by measuring extract sample absorbance as the "y" value, as shown in Table 1 and Figure 1.

The average total polyphenol content in the ethanol extract of *I. cylindrica* root was 1.109 % (w/w) (GAE), as shown in Table 2.

The total flavonoid content in the ethanol root extract was calculated using the obtained linear regression equation y = 0.027x - 0.0141, as shown in Table 3 and Figure 2.

The average total flavonoid content as quercetin equivalent in the ethanol root extract sample of *I. cylindrica* was 0.1% (w/w) (QE), as shown in Table 4.

The antioxidant activities of *I. cylindrica* root ethanol extracts was measured using the curve of % inhibition versus concentration in the sample (62.5, 125, 250, 500, 750, and 1000 ppm). The measurements were conducted in triplicate (Table 5), and the results are shown in Figure 3. The antioxidant activities were calculated using a linear regression equation from the DPPH inhibition curve, y = 0.0547x + 4.911, from which the antioxidant activity of the ethanol extract was IC_{50}= 824.30 ppm (µg/ml).

Quantification of Sinensetin and Imperatorin from ethanol root extract of *Imperata cylindrica*

Sinensetin and Imperatorin in the extract were separated by column chromatography using various gradient eluent, which resulted in 15 fractions, as described in Table 6. Each fraction was analyzed using HPLC and plotted against Sinensetin and Imperatorin reference standards (Figure 4 to Figure 7).

Based on the Sinensetin and Imperatorin reference standard curves, the fractions detected to have similar active content were Fraction 1 until Fraction 11, while very low in Fraction 12 to Fraction 15 (Table 7). The weight in grams for F1-F11 was as follows: 0.01345, 0.01446,

Table 1: Gallic acid reference standard absorbance.

Concentration (ppm)	Absorbance 1	Absorbance 2	Absorbance 3	Average reference standard absorbance
100	0.172	0.168	0.168	0.169
150	0.249	0.249	0.246	0.248
250	0.426	0.441	0.424	0.430
300	0.534	0.527	0.539	0.533
400	0.712	0.709	0.711	0.711
500	0.892	0.892	0.889	0.891

Table 2: Total polyphenol content in ethanol extract of *Imperata cylindrica* root.

Sample concentration	Abs 1	Abs 2	Abs 3	Average Abs	ppm	mg/ml	%
100 mg/ml	0.181	0.181	0.18	0.181	111.05	0.111	1.109

Table 3: Quercetin reference standard absorbance.

Concentration (ppm)	Absorbance 1	Absorbance 2	Absorbance 3	Average reference standard absorbance
2.5	0.051	0.055	0.057	0.054
5	0.119	0.121	0.12	0.120
10	0.265	0.264	0.263	0.264
15	0.376	0.368	0.374	0.373
20	0.544	0.534	0.536	0.538
25	0.657	0.671	0.65	0.659

Table 4: Total flavonoid content in ethanol extract of *Imperata cylindrica* root.

Abs 1	Abs 2	Abs 3	Average absorbance	ppm	mg/ml	%
0.271	0.27	0.264	0.268	10.4481	0.010	0.1

Table 5: Inhibition percentage of *Imperata cylindrica* root ethanol extract.

Concentration (ppm)	Absorbance				% Inhibition
	1	2	3	Average	$\frac{\text{Abs Reference - Abs Sample}}{\text{Abs Reference}} \times 100\%$
1000	0.259	0.258	0.258	0.258	60.84
750	0.374	0.37	0.373	0.372	43.56
500	0.445	0.442	0.441	0.443	32.90
250	0.53	0.524	0.532	0.529	19.86
125	0.579	0.579	0.582	0.580	12.08
62.5	0.61	0.612	0.612	0.611	7.33
DPPH 60 ppm	0.661	0.661	0.657	0.660	

Table 6: Color of the 15 Fractions (F1 to F15) obtained with column chromatography of *Imperata cylindrica* root ethanol extract.

Fraction Name	Separation Result	Eluents (Gradient)				The Result of Separation from column
		Ethyl acetate-n hexane (7:3)	Ethyl acetate-n hexane (8:2))	Ethyl acetate-methanol (8:2)	Methanol	
F1	Greenish yellow	√				
F2	Yellow	√				
F3	Light yellow	√				
F4	Light yellow	√				
F5	Light brown		√			
F6	Brown		√			
F7	Light yellow		√			
F8	Light yellow		√			
F9	Light brown			√		
F10	Brownish			√		
F11	Light yellow			√		
F12	Light yellow			√		
F13	White				√	
F14	Whitish				√	
F15	Brownish				√	

Table 7: Area of reference standard for Sinensetin and Imperatorin in various concentrations.

Standard concentration (ppm)	Area of Reference Standards	
	Sinensetin	Imperatorin
10	6989123	12580744
20	14921477	26655153
30	21673646	38527762
40	29689843	54682182
50	38402230	69020152

Table 8: Content of Sinensetin and Imperatorin in each fraction (F1 to F11) of *I. cylindrica* root ethanol extract.

Fraction	Sinensetin			Imperatorin		
	Retention Time	Area	Concentration (ppm)	Retention Time	Area	Concentration (ppm)
Reference Standard	5.595	y = a + bx	y = -943110 + 775946x	10.992	y = a + bx	y = -1978555 + 1409058x
vial 1	5.57	392904	1.722	11.013	689356	1.893
vial 2	5.577	368876	1.691	11.007	669918	1.880
vial 3	5.58	263969	1.556	11.003	473601	1.740
vial 4	5.587	161458	1.424	11.033	290389	1.610
vial 5	5.593	165920	1.429	11.04	318422	1.630
vial 6	5.593	138316	1.394	11.04	260548	1.589
vial 7	5.593	83036	1.322	11.047	156839	1.515
vial 8	5.593	56447	1.288	11.053	107360	1.480
vial 9	5.6	62605	1.296	11.063	103337	1.478
vial 10	5.557	87660	1.328	10.833	193126	1.541
vial 11	5.593	83195	1.323	10.99	135412	1.500
Total			**15.772**			**17.858**

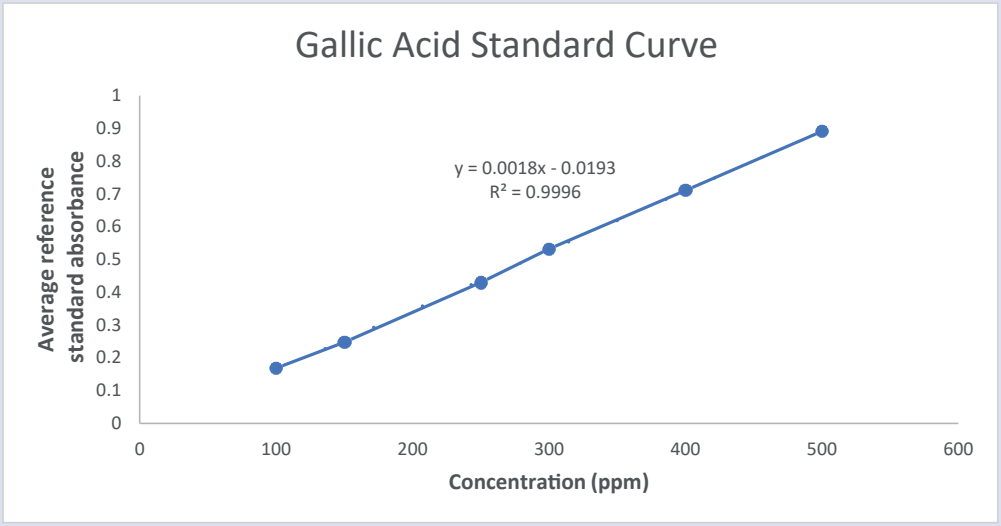

Figure 1: Gallic acid calibration curve.

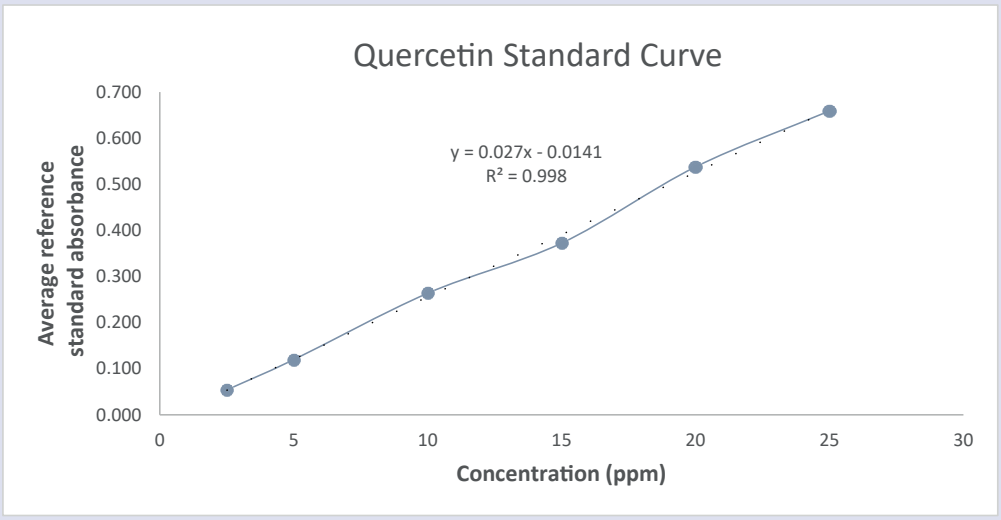

Figure 2: Quercetin calibration curve.

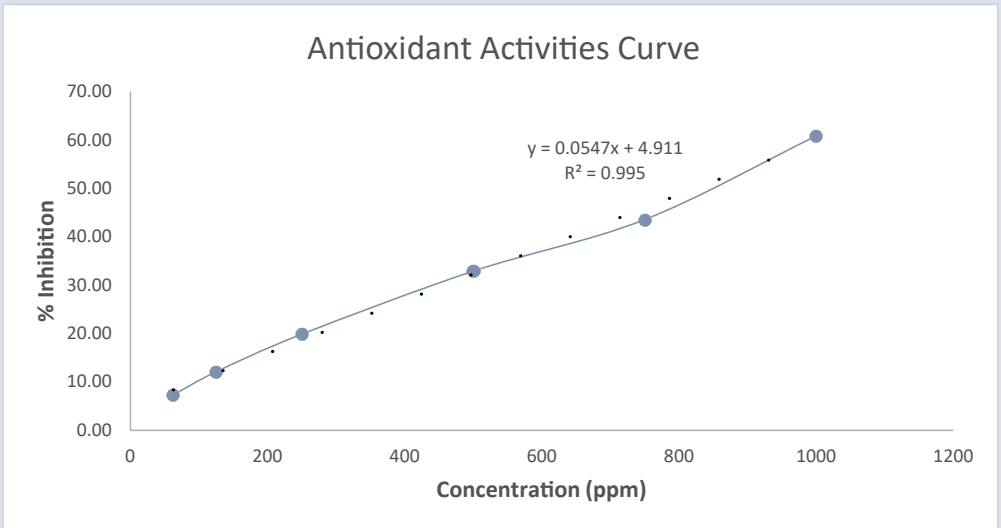

Figure 3: Antioxidant activities of the ethanol extract of *I. cylindrica* root.

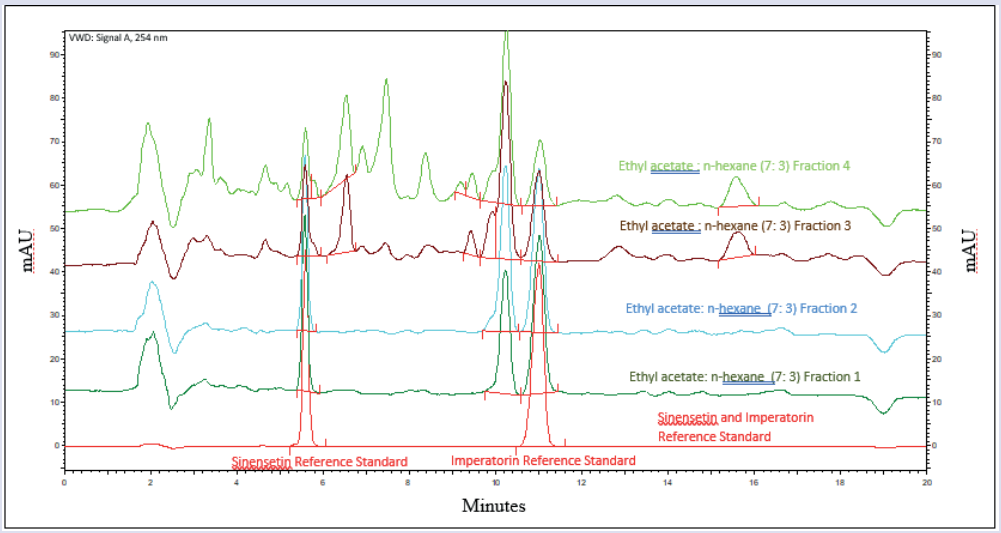

Figure 4: Chromatogram of Sinensetin and Imperatorin reference standard, including Fraction 1-4.

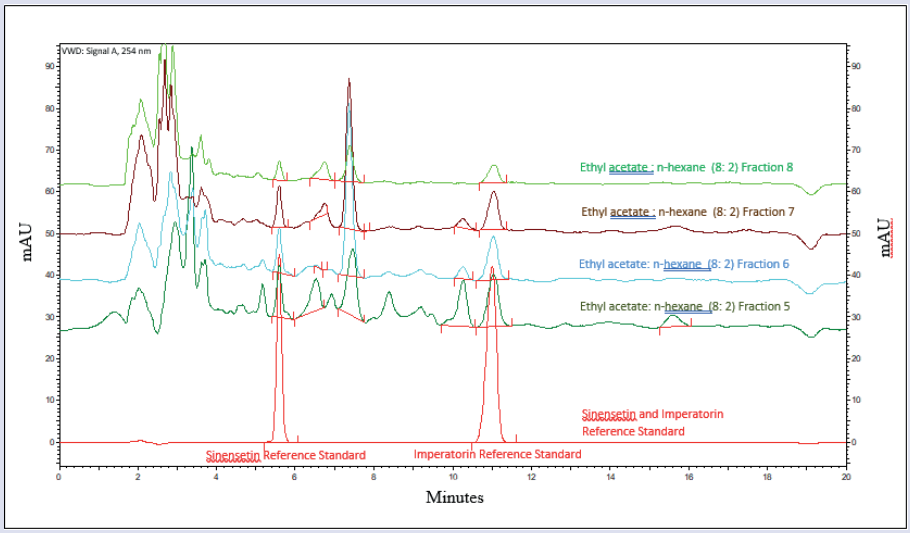

Figure 5: Chromatogram of Sinensetin and Imperatorin reference curves, including Fraction 5-8.

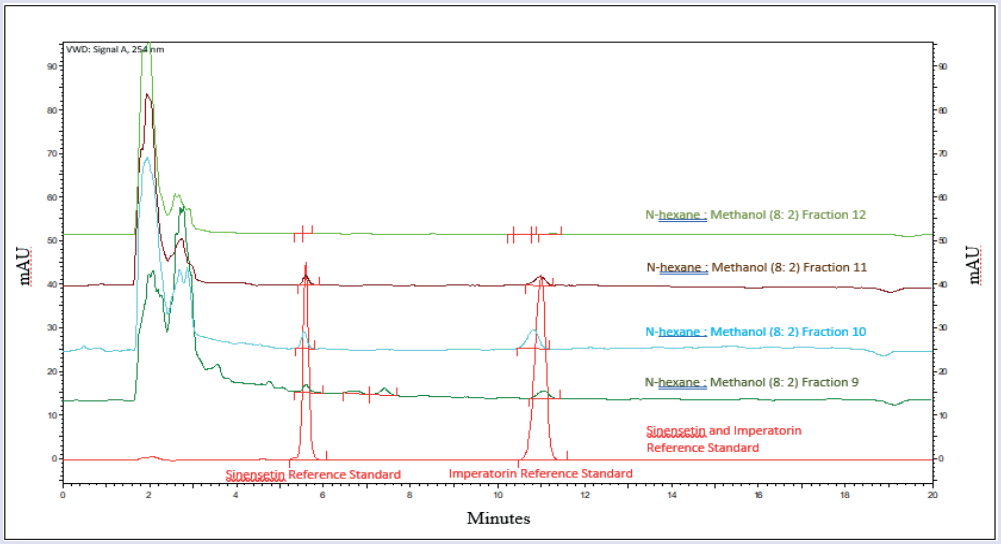

Figure 6: Chromatogram of Sinensetin and Imperatorin reference standard, including Fraction 9-12.

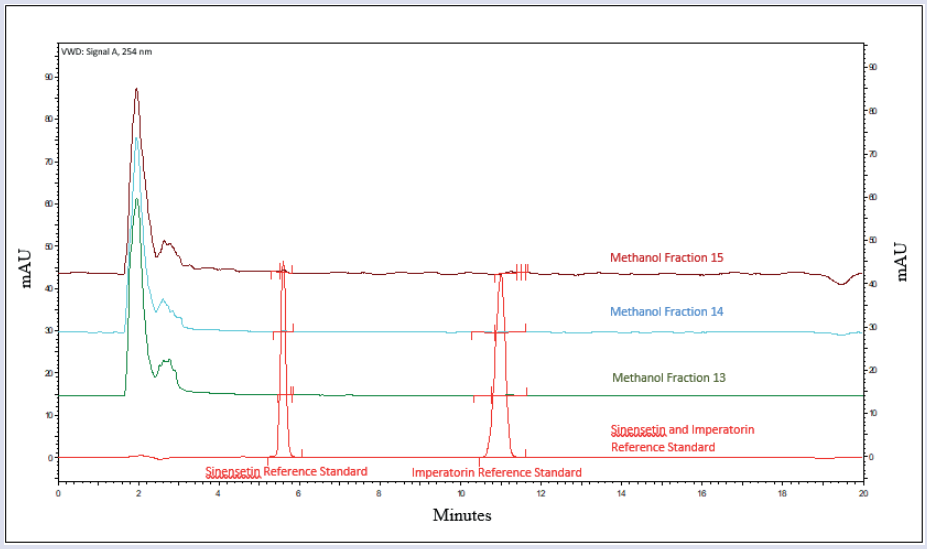

Figure 7: Chromatogram of Sinensetin and Imperatorin reference standard, including Fraction 13-15.

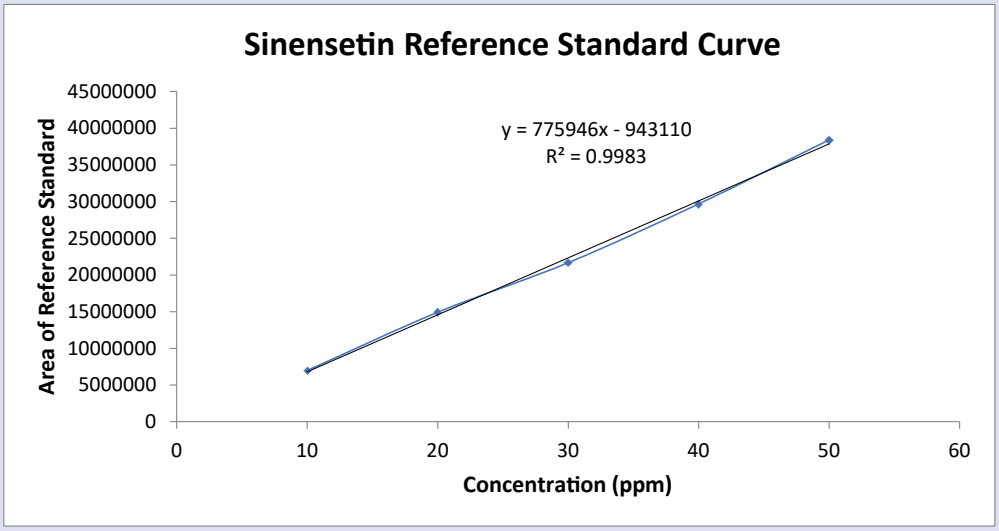

Figure 8: Standard curve of Sinensetin in various concentrations (10, 20, 30, 40 and 50 ppm).

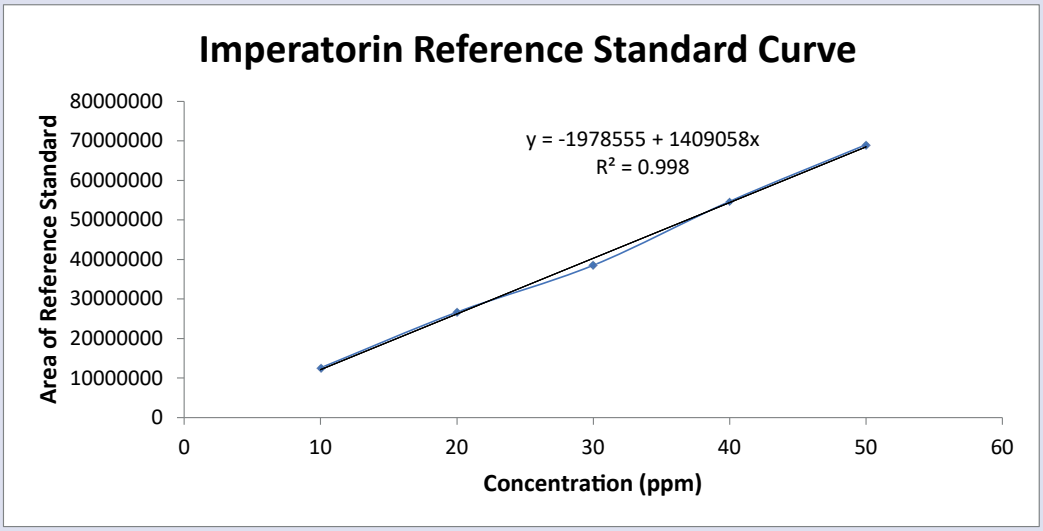

Figure 9: Standard curve of Imperatorin in various concentrations (10, 20, 30, 40 and 50 ppm).

0.01521, 0.01459, 0.01369, 0.0135, 0.00659, 0.00722, 0.00711, 0.0079, and 0.0082.

The content of Sinensetin and Imperatorin from the ethanol extract of *I. cylindrica* root in each fraction (Fraction 1-11) are provided in Table 8. The total concentration of Sinensetin and Imperatorin in their extract (Fraction 1-11) were 0.0157 and 0.0178 mg/kg, respectively.

DISCUSSION

A previous study by Dhianawaty and Ruslin demonstrated that methanol extract of *I. cylindrica* root from Kendari had a total polyphenol content of 1.53% gallic acid equivalent and antioxidant activity IC_{50} 0.32 mg/mL.[25] The use of different solvents for phytochemical constituents screening and antioxidant activity may produce different results, as observed with *Severenia buxifolia,* which showed that methanol extract obtained higher content of phytochemical constituent and antioxidant activity compared to other solvents such as ethanol, chloroform and acetone.[26]

Polyphenols compounds ubiquitously expressed in plants have anti-inflammatory, antimicrobial, antiviral, anticancer, and immunomodulatory properties, all of which are beneficial to human health. Due to their ability to modulate the activity of multiple targets involved in carcinogenesis through direct interaction or modulation of gene expression, polyphenols can be employed to inhibit the growth of cancer cells.[27] Polyphenols target signal transduction pathways involved in carcinogenesis through various mechanism, including the modulation of ErbB receptors signaling pathway, NF-κB pathway, modulation of HH/GLI pathway, cross-talk between ErbB receptors and the HH/GLI and NF-κB signaling pathways, and other signals involved in carcinogenesis such as cell cycle, apoptosis, and angiogenesis.[27]

Prakash *et al.* showed that polyphenol rich sugarcane extract exhibits anti-cancer properties on a range of cancer cells including human (LIM2045), human lung cancer (A549), human ovarian cancer (SKOV-3), promonocytic human leukemia (U937), and mouse (MC38, CT26) colon cancer cells lines, and mouse melanoma (B16) cell lines. Anti-proliferative effects were shown to be mediated *via* alteration in cytokines, VEGF-1 and NF-κB expression.[28] Rubin-Garcia *et al*. showed that a diet high in polyphenol compounds like stilbenes, lignans, hydroxybenzaldehydes, hydroxycoumarins, and tyrosols was associated with a lower gastric cancer risk.[29] Olive oil polyphenols (OOPs), especially hydroxytyrosol (HTyr), tyrosol (Tyr) and their derivatives oleuropein (Ole), express anticancer activity on different cancer models. The results of *in vitro* and *in vivo* studies reported that OOPs have a high potential as chemopreventive and anticancer agents.[30] Other polyphenol-rich component extracted from *Rhus tripartita* has an apoptotic effect on THP-1 cells through the PI3K/AKT/mTOR signaling pathway, and can induce inhibition of cell cycle and induction of apoptosis involved in the mTOR pathway. Therefore, this extract may be a useful candidate as a natural anti-cancer drug to support the treatment of acute monocytic leukemia.[31] Martino demonstrated that the polyphenol extract from Annurca apple selectively kills MDA-MB-231 triple breast cancer cells through RoS generation, sustained JnK activation and cell growth and survival inhibition.[5] Meanwhile, combined polyphenols or mixed with micronutrients have shown to be effective against multiple targets in cancer development and progression.[3]

In lung carcinoma, polyphenols have demonstrated three major actions: antioxidative activity, regulation of phase I and II enzymes, and regulation of cell survival pathways against lung carcinogenesis. In *in vitro* cell culture experimental models, polyphenols can bind with electrophilic metabolites from carcinogens, inactivate cellular oxygen radicals, prevent membrane lipid peroxidation and DNA oxidative damage, and adduct formation. Furthermore, it enhances the activity of detoxifying enzymes such as phase II enzymes, glucuronosyl transferases and glutathione transferases.[6]

Another major compound successfully identified from the *I. cylindrica* root methanol extract was polyphenol. Dhianawaty measured the total flavonoid content by using aluminum chloride method, and the results showed that the extract contained flavonoids, with a total content of 1.17%.[32] Flavonoids consist of a large group of polyphenolic compounds having a benzo-ɤ-pyrone structure with many biological activities such as antioxidant, antibacterial, anti-inflammatory, antiviral, anticancer and hepatoprotection.[33] Several mechanisms have been proposed for the effect of flavonoids on the initiation and promotion stages of carcinogenesis, including the development and hormonal activities through downregulation of mutant p53 protein, cell cycle arrest, tyrosine kinase inhibition, inhibition of heat shock proteins, estrogen receptor binding capacity, and inhibition of expression of Ras proteins.[33] Flavonoids also have anticancer effects as they modulate reactive oxygen species (ROS)-scavenging enzyme activities, play an important role in arresting the cell cycle, induce apoptosis, autophagy, and suppress cancer cell proliferation and invasiveness.[7]

Anticancer activities of flavonoids in various cancer cell lines has been shown in human oral cancer (HSC-2,HSG, SCC-25), breast cancer (MCF-7), thyroid cancer (ARO,NPA, WRO), lung cancer (SK-LU1, SW900, H441, H661, haGo-K-1, A549), prostate cancer (LNCaP, PC3, DU145), colon cancer (Caco-2,HT-29, IEC- 6,HCT-15), and leukemia (HL-60, K526, 4A5, B16 mousemelanoma).[8] In dietary consumption, the flavonoids luteolin and kaempferol are considered as promising candidate agents for the treatment of gastric and ovarian cancers, respectively. Further, apigenin, chrysin, and luteolin have good perspectives as potent antitumor agents for cervical cancer. The anticancer effect of flavonoids toward blood cancer cells depend on their myeloid, lymphoid or erythroid origin, while the cytotoxic effects of flavonoids on breast and prostate cancer cells are highly related to the expression of hormone receptors.[34]

As key gene expression regulators in lung cancer, flavonoids play important preventive/therapeutic actions; flavonoids interfere with the receptor tyrosine kinases cascade, affect cell proliferation, apoptosis and autophagy, are cell cycle modulators, regulate invasion and metastasis, and are miRNA modulators in lung cancer.[12]

Not only flavones were reported to have anticancer activity, but also an antioxidant effect. The antioxidant activity of the methanol extract of *I. cylindrica* root was supported by the presence of polyphenol compounds.[25] Grigalius showed a relationship between antioxidant and anticancer activity from 13 trihydroxyflavones, which possessed anticancer activity against at least one tested cancer cell line (MCF-7). Most compounds inhibited cancer cell growth at IC_{50} values between 10–50 μM. The most active compound was 3',4',5-trihydroxyflavone 7, especially against A549 and MCF-7 cell lines.[15] Antioxidants also decrease the apoptotic effect of 5-Fu in colon cancer by regulating Src-dependent caspase-7 phosphorylation.[35] The flavonoid fraction from the aerial parts of *Cissus quadrangularis Linn*. Was used against human breast carcinoma cell lines and demonstrated antioxidant and anticancer activity.[36] In patients with lung cancer, the total antioxidant capacity status is associated with disease stage and endogenous antioxidant factors rather than with the frequency of consumption of selected food products and smoking.[37]

Sinensetin, a plant-derived polymethoxylated flavonoid, has an anticancer effect through its ability to interact with p-glycoprotein, interaction with breast cancer resistance protein, and has shown antiproliferation and antiangiogenesis effect as well as antioxidant activity. Many *in vivo* and *in vitro* studies have indicated that Sinensetin not only shows good activity against tumor cells, but also exert minimal toxicity to normal cells according to the ratio of IC_{50}

values, and possess high selectivity.[17] While Imperatorin, a naturally occurring furanocoumarin, regulates gene and protein expression, such as IL-6, Bcl-2, Bax, Mcl-1, caspase-3, AChE, PGE2, PPARγ and affects PI3K/Akt, MAPK, NF-κB, Nrf2/HO-1, IKK/IjB/NF-jB, ERK signaling pathways, which may treat a variety of diseases including cancer. Currently, various *in vitro* studies showed that Imperatorin has a significant inducing apoptosis effect on a variety of cancer cell lines.[18]

Based on previous studies, Sinensetin and Imperatorin were identified in fractions 5-9 in *I. cylindrica* root methanol extract from Kendari with concentrations of Sinensetin and Imperatorin of 32.348 and 37.014 mg/kg, respectively, and a total tannin content of 1.06% (w/w)(TAE).[16] As described on the examination, *I. cylindrica* root ethanol extract from Sragen, Central Java, has total polyphenol content of 1.109% GAE, total flavonoid content of 0.1% QE and antioxidant activity IC_{50} 824.30 µg/ml. The phytochemical evaluation of the extract determined a Sinensetin and Imperatorin contents of 0.0157 and 0.0178 mg/kg, respectively. The presence of polyphenols, flavonoids, antioxidant activity, Sinensetin, and Imperatorin in the extract provide the basis to develop *in vitro* experiments of the cytotoxic effect as anticancer agent.

CONCLUSION

This study showed that *Imperata cylindrica* root ethanol extract had polyphenol and flavonoid content with antioxidant activity, with Sinensetin and Imperatorin. These compounds have antioxidant and cytotoxic activity that could support the anticancer effect of the extract. Developing the extract of *Imperata cylindrica* roots in the future, especially Sinensetin and Imperatorin compounds, can thus be used for the cytotoxic effect in cancer studies.

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CONFLICTS OF INTEREST

None

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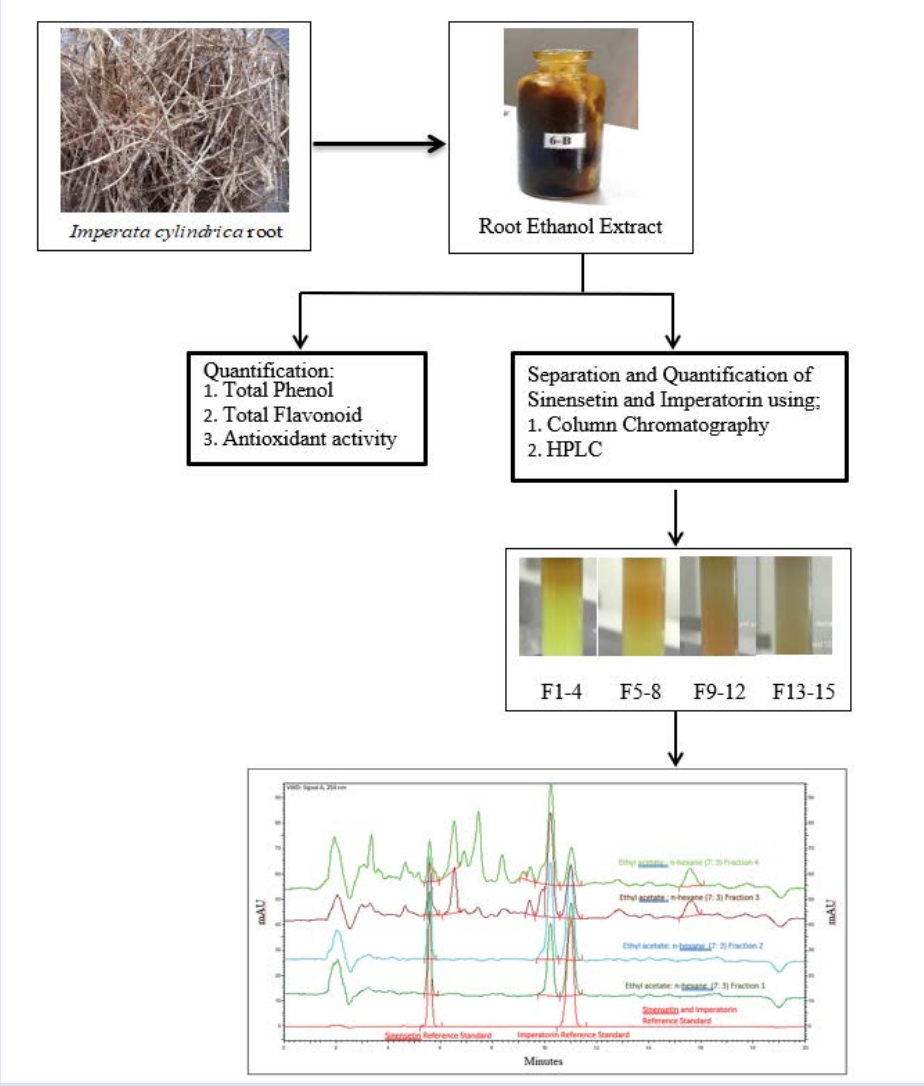
GRAPHICAL ABSTRACT



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