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Research Article

Total flavonoid and polyphenol content of *Tinospora crispa* cultivated at highland region

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Abstract

Background: *Tinospora crispa* (*T. crispa*) is herbaceous plant which commonly grows wild in tropical regions of South East Asian countries such as Indonesia, Malaysia, and Thailand. In Indonesia this plant is well known to be used as traditional medicine to treat gout, diabetes, hypertension, rheumatic, fever, and appetite stimulant. Researches worldwide indicated that *T. crispa* poses several pharmacological properties. One of those is the antioxidant activity, acting as free radical scavenger. The objective of this study was to determine the antioxidative properties of *T. crispa* by analyzing the total flavonoid and polyphenol content.

Methods: *T. crispa* was cultivated at 850 AMSL field in Materia Medica Batu. The plant was harvested by cutting 5 cm of old stem and dried in 50° C for 7 days. Powder was then made by using milling machine. The amount of 300 g powder was subsequently macerated to get *T. crispa* extract. Total flavonoid and polyphenol contents were determined by using spectrophotometry method.

Result: From 300 g *T. crispa* powder using maceration method gave liquid yield with 56 g weight and 18.66% rendement. Total flavonoid and polyphenol content was 0.04% ± [3.68%] (w/w) and 0.60% ± [0.8%] (w/w) respectively.

Conclusion: Despite low in concentration, flavonoid and polyphenol content was successfully determined from *T. crispa*. In the future free radical scavenging assay need to be conducted to understand better about the antioxidant activity of *T. crispa*.

Keywords: *Tinospora crispa*, Brotowali, Flavonoid, Polyphenol.

INTRODUCTION

The development and progression of several chronic diseases including cancer, neurodegenerative, cardiovascular, diabetes mellitus as well as aging have been suggested due to the involvement of oxidative/nitrosative stress [1, 2]. This condition occurs due to the presence of reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as hydroxyl, superoxide, and nitric oxide radicals in the body [3]. These molecules can cause not only DNA damage but also oxidation of lipid and proteins in the cell [4].

Antioxidant system in human body normally has ability to counterattack these radicals resulting in the balance between oxidation and anti-oxidation. However, the amounts of these radicals are also affected by factors such as life style and environment. The excessive ROS and RNS production can be induced by smoking and alcohol intake as well as radiation and environmental toxins. Hence the balance between oxidation and anti-oxidation was disrupted leading to diseases development [4].

This risk of oxidative stress can be reduced by taking exogenous antioxidants. Medicinal plants are known as one of the valuable sources of exogenous antioxidants [5, 4]. Many medicinal plants which have been used worldwide reported to display antioxidant activity such as Lamiaceae (rosemary, sage, oregano, marjoram, basil, thyme, mints, balm), Apiaceae (cumin, fennel, caraway), and Zingiberaceae (turmeric, ginger) [5]. Antioxidants derived from medicinal plants are mainly polyphenols (phenolic acids, flavonoids, anthocyanins, lignans and stilbenes), carotenoids (xanthophylls and carotenes) and vitamins (vitamin E and C). These natural antioxidants in general pose broad spectrum of biological effects such as antibacterial, antiviral, anticancer, anti-inflammatory, and anti-aging [4].

Tinospora crispa (T. crispa) is one of the traditional medicinal plants which has been reported to have antioxidant activity. *T. crispa* is herbaceous plant which commonly grows wild in tropical regions of South East Asian countries such as Indonesia, Malaysia, and Thailand [6]. *T. crispa* is commonly known as Brotowali, Antawali, and Andawali in Indonesia. The leaves of *T. crispa* have heart shaped 6-12 cm long and 7-12 cm wide. The stems are brownish, fleshy, with protruded blunt tubercles [6]. This plant is well known to be used as traditional medicine to treat gout, diabetes, hypertension, rheumatic, fever, and appetite stimulant.

The habitat of *T. crispa* is in rain forest and deciduous forest with elevation up to 1000 m. the suitable soil for growth is moist or dry with sufficient sunlight exposure [6, 7]. In this study we cultivated *T. crispa* in highland area until 6 months to 1-

year olds. The objective of this study was to determine the antioxidative properties of *T. crispa* cultivated at highland by analyzing the total flavonoid and phenolic content.

MATERIALS AND METHOD

Tinospora crispa cultivation

Cultivation of *T. crispa* was done in using stem cutting in material medika batu field. This area belongs to the highland region with elevation of 875 above mean sea level (AMSL). Watering was done every day until initial growth which was then altered to once a week. Organic fertilizer from cows and goats manure was applied.

Harvesting

Plants were harvested when they reached 6 months to 1 year old. Approximately 5 cm of the old stem was cut and dried in 50° C for 7 days. The dried stem was subjected to milling machine to make powder.

Extraction

The powder was then used for extraction using maceration method. The amount of powder and ethanol 96% as solvent needed was 300 g and 2100 ml. the process of evaporation was 1.5 h.

Tota flavonoid concentration test

The total flavonoid content was determined spectrophotometrically using 1 g of *T. crispa* extract. Solvent consisting of 1 ml 0.5% (W/V) hexamethylenetetramine, 20 ml aceton, and 2 ml HCl 25% (W/V) was added and then refluxed for 2 h after boiling. The sample was filtered using cotton and topped up to 100 ml using aceton and homogenized. Filtrate of 20 ml was taken and added with 20 ml water. Etyl acetate was then added 15 ml and mixed 10 mins. The sample was set aside to get separation and the ethyl actetate phase was then taken. The extraction was repeated in triplicate using 10 ml ethyl acetate. After that, the sample was washed by 50 ml water. Ethyl acetate was then added and homogenized. Ten ml of this sample was prepared and mixed with 1 ml AICl3 solvent and methanol-glacial acetic acid. Solution was then set aside for 30 mins. After that it was scanned between 300 – 500 nm. The absorbance was measured in λ maksimum (± 425 nm). The flavonoid content was calculated using following formula: % Flavonoid = Absorbance x 1.25/g sample.

Total phenolic concentration test

The total phenolic content was determined as galic acid equivalent spectrophotometrically. Sample was prepared by pipetting 0.250 ml into vial and dried using nitrogen gas. For generating standard curve, galic acid solution was diluted in water to get concentration ranging from 5 to 40 ppm. The residue in vial was then dissolved in 1 ml aquadem following the addition of Folin-Ciocalteu 0.5 ml. The sample was set aside for 5 mins before adding 2 ml 10% Sodium Carbonate solution. After 10 mins incubation, absorbance was measured in λ 760 nm. The formula from standard cuve was used to calculate phenolic content.

RESULT

Extraction

From 300 g *T. crispa* powder using maceration method gave liquid yield with 56 g weight and 18.66% (W/W) rendement.

Determination of total flavonoid content

Data on flavonoid content was depicted on table 1. The amount of *T. crispa* extract to perform this analysis was 1 g and done in duplicate. The absorbance was read with 425 nm wavelength giving value about 0.03. these were used to calculate flavonoid content by multiplying with 1.25/g sample. The average of flavonoid content was 0.04 % with 3.68 RPD.

Table 1: Total flavonoid content

Sample	Amount (g)	Absorbance	Flavonoid % (W/W)	Average % (W/W)	ΔX	RPD
1	1.0131	0.032148	0.040	0.04	0.001432	3.68
2	1.042	0.031871	0.038			

Determination of total phenolic content

Total polyphenol was determined as galic acid equivalent spectrophotometrically in duplicate. The detail data was given in table 2. The extract of *T. crispa* was prepared and dissolved in 1 ml aquadem. The absorbance measurement revealed about 0.4 values which was then converted into concentration giving approximately 11 ppm. By using formula derived from the standards, it was found that average of total polyphenol content in the sample was 0.60% with RPD 0.80.

Table 2: Total polyphenolic content

Sample	Volume (ml)	Absorbance	ppm	Polyphenol % (W/W)	Average % (W/W)	ΔX	RPD
1	1	0.42233	11.86	0.59	0.60	0.005	0.80
2	1	0.42581	11.97	0.60			

DISCUSSION

The extraction from 300 g *T. crispa* powder yielded 18.66 % (W/W) rendement. The result of this maceration method was relatively high compared to several literatures. Study conducted by Irianti et al in 2015 and 2011 indicated yield of 3.1 % (W/W) and 12.02 % (W/W) respectively using ethanol 96% as solvent [8,9]. The use of ethanol 80% was also observed giving lower yield of 16.4 % (W/W) [10]. Likewise, Mutiah et al who performed 15 maceration using ethanol 80% indicated the highest yield was 15.916 % (W/W) [11]. The use of ethanol 99.9% showed also relatively lower yield (15.08 % (W/W)) [12]. Interestingly one of the study using ethanol 70% indicated relatively higher yield which was 19.34 % (W/W) [13]. Comparison to the yield from further fractionation process showed that our result was relatively lower than n-hexane and water solvent. The yield from n-hexane fractionation by Irianti and warsinah indicated 24.1 % (W/W) [9] and 22.06 % (W/W)

[14] respectively. While water solvent showed high yield up to 31.22 % (W/W) [9] and 57.9 % (W/W) [14]. The success of extraction method depended on the use of solvent and its polarity. Comparing our result with the others ethanol solvents with different percentage, ethanol 96% could be ideal solvent for *T. crispa* extraction. In addition, ethanol 96% was considered to have low toxicity. For future fractionation, water and n-hexane are the good candidates since they could give high yield of extract.

Table 3: Comparison of flavonoid and phenol content with the literatures.

Part of *T. crispa*	Solvent	Flavonoid (%W/W)	Phenol (%W/W)	Ref
Stem	**Ethanol 96%**	**0.04**	**0.6**	**Sample**
Stem	Ethanol 95%	6.207	21.3	(15)
Stem	Methanol	NA	6.467	(16)
Stem	Ethanol 70%	3.265	NA	(13)
Stem	Ethanol 99.9%	5.1	9.147	(12)
Stem	Water	0.267	7.9	(17)
	Methanol	0.953	25.533	
	Chloroform	0.538	17.233	
NA	Ethanol 80%	0.452	2.983	(18)
Leaves	Ethanol 80%	1.021	0.698	(19)
Stem	Methanol	55.58	6.12	(20)

The total flavonoid (TFC) and phenolic (TPC) content were determined and expressed in % (W/W) unit. TFC and TPC was 0.04 % and 0.6 % respectively. Several reports showing flavonoid and phenol content were displayed for comparison with our result (table 3). To make appropriate comparison and avoid ambiguity due to the various units in literatures, all the values were converted into % (W/W) unit. The data indicated that both flavonoid and phenol content of sample was low.

Several results using same solvent (ethanol) despite the distinct in percentage gave higher flavonoid content. The lowest value of flavonoid was 0.452 % (W/W) which was extracted using ethanol 80%. In general, non ethanol solvents yielded flavonoid less than 1 % (W/W). In exception there was one report indicated really high flavonoid content (55.58 % (W/W)) which was extracted using methanol. In case of phenol, the lowest concentration was 0.698 % (W/W) using ethanol 80% as solvent. However, this case was reported from the leaves of *T. crispa* while in this study stem was the source of extraction.

This study appears to be the first to validate the total phenolic and flavonoid content of *T. crispa* which was cultivated at highland (850 AMSL). Our result indicated that the polyphenolic content was higher compared to flavonoid. It was expected since flavonoid belongs to phenolic group. Although our result indicated high yield of *T. crispa* extract nevertheless total phenolic and flavonoid content were low. This might be due to the growth condition and the age of *T. crispa*. It was known that plant maturity and the growth condition affect the phenolic content [21,22].

The cause of this low yield of phenol and flavonoid might be also due to inappropriate handling during sample preparation and extraction. It was reported that methods including sample preparation, hydrolysis, and extraction give impact in phenolic content. Pretreatment procedure cannot be equated since phenol consists of molecules with diverse polarity, acidity, concentration level, and matrix complexity [21]. It was also reported that reaction times and acidity level affected the yield [21.23.24]. One of the study revealed that acidic condition decreased the different forms of phenolic acids, ranging from 15 to 95% for ocoumaric acid and sinapic acid, respectively [25]. In addition, the content of both phenol and flavonoid molecules was examined two times only. This lack of repeatability became the limitation of our study.

Despite low in concentration, flavonoid and polyphenol content was successfully determined from *T. crispa* which was grown in highland area. It is suggested that the cultivation condition of *T. crispa* at 850 AMSL need to be improved. Different fertilizers and time-points of harvest can be examined to observe the results of phenolic and flavonoid content. More data need to be generated to validate the low yield of phenolic and flavonoid level. In the future free radical scavenging assay need to be conducted to understand better about the antioxidant activity.

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