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Short Communications

The Potential of *Trichosanthes tricuspidata* Lour. from Bangli, Baturiti, Bali for Free Radicals Scavenging

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ABSTRACT

In addition to the studies on potential medicinal uses of *Trichosanthes*, a screening on phytochemical compounds and antioxidants activity of *Trichosanthes tricuspidata* from Bangli, Baturiti, Bali, Indonesia, was conducted on its leaves, fruits, peels, and seeds. Qualitative phytochemical tests were conducted to find out the chemical constituents of *T. tricuspidata*, while its antioxidant activity was tested by applying DPPH (1,1-diphenyl-2-picrylhydrazyl radical) method. As a result, flavonoid, alkaloid, terpenoid, tannin, and saponin were present in all methanolic extracts of *T. tricuspidata*. Furthermore, the best antioxidant activity was exhibited by peel extract. After all, *T. tricuspidata* contains a prospective compound agent for medicinal use.

Trichosanthes, belongs to Cucurbitaceae, is characterized by its climbing habit, mostly branched tendrils, distinct fringed petals, and bright-colored fruits (Duyfjes & Pruesapan 2004). Its natural distribution is widely spread from India eastwards to Japan, Malaysia regions, and downwards to Australia. Among 103 species included in this genus, around 39 species grow in Malaysia regions, and 8 species are reported to be found in Indonesia (Rugayah & Darnaedi 2004). One of the notable species of this genus is *Trichosanthes tricuspidata* Lour. which is potentially believed to treat a broad spectrum of diseases, is spreaded from Asia to tropical Australia (Bhandari et al. 2008; Ahuja et al. 2019).

Trichosanthes is used traditionally for food and medical purposes. Kumar et al. (2012) reported that *T. dioica* is used as a vegetable and an ailment of numerous illnesses including asthma, ulcer, and diabetic for traditional uses. It is also used as a traditional medicine in Ayurvedic, Unani, and Thai medical systems to treat fever, inflammation, worm infection, migraine, and is used as laxative agent (Bhandari et al. 2008; Ahuja et al. 2019). Meanwhile, in Indonesia, Windadri et al. (2006) reported the use of *T. tricuspidata* for scabies treatment by Muna tribe people in Southeast Sulawesi.

In addition to the research on potential medicinal uses, phytochemical screening studies have also been conducted. Cycloartane glycosides, cucurbitane, hexanorcucurbitane, octanorcucurbitane glycosides, and cucurbitacins were isolated from *T. tricuspidata* (Kasai et al. 1999; Kanchanapoom et al. 2002; Mai et al. 2002). Phenol and flavonoid were also isolated from *T. anguina* (Marsetya et al. 2009). Meanwhile, *T. kirilowii* extract contains numerous chemical compounds including flavonoid (Xu et al. 2012). Additionally, the antioxidant potential of *Trichosanthes* has been widely reported. Marsetya et al. (2009) documented antioxidant activity of *T. anguina* extracts. Significant antioxidant activity was also shown by water extracts of *T. cucumeriana* aerial parts (Arawwawala et al. 2011).

Although extended research of *Trichosanthes* phytochemical and antioxidant screening studies was presented, a study in this subject for Indonesian *Trichosanthes* is still limited. Known as *anggur memedi* by the locals and considered as weeds, *T. tricuspidata* is also found in Tabanan, Bali. The present study is aimed to examine the phytochemical compounds and antioxidant activity of *T. tricuspidata* methanolic extract. It is expected that this study will enhance our understanding of phytochemistry of *T. tricuspidata* and support its future domestication and conservation efforts.

This research was conducted from November 2020 to January 2021. Specimens of *T. tricuspidata* were collected from a paddy field at *Banjar* Apid Yeh (691 asl), of Bangli Village, Baturiti, Tabanan, Bali, in November 2020 (Figure 1A). Plant extraction was prepared in Bali Botanic Garden Seed Bank and Applied Botany Laboratories. *T. tricuspidata* leaves and fruits were separated (Figure 1B). The fruits were peeled to separate its epicarp (outermost skin/peels), mesocarp (fleshy part/fruits), and seeds. Leaves, fruits, peels, and seeds were washed under running tap water, chopped, and air-dried at room temperature for 3 to 5 days (Figure 1C). The simplicia was then be extracted using methanol as a solvent. Methanolic extract from each simplicia was subjected to be tested for its phytochemical screening and antioxidant activity at Herbal Materia Medica Laboratory, Batu, East Java.

Qualitative chemical tests were conducted to find out the chemical constituents of *T. tricuspidata* following the methanolic extracts, while its antioxidant activity was conducted by applying DPPH (1,1-diphenyl-2-picrylhydrazyl radical) method. The extracts concentrations were used as follow: leaf extract 125, 250, 500, 1000 ppm; peels extract 31.5, 62.5, 125, 250 ppm; fruit extract 500, 600, 700, 800, 900, 1000 ppm; and seed extract 31.5, 62.5, 250, 500, 1000 ppm.

To examine the antioxidant activity, a total amount of 100 mL DPPH solution by 0.1 mM was added to each extract. A spectrophotometer was used to measure the solution absorbance at 515 nm wavelength. The percentage of inhibition obtained was then be arranged in a regression graph to determine the 50% inhibition concentration (IC_{50}) of the extract.

The percentage of *T. tricuspidata* methanol extract production ranged from 1.248 to 10.649 percent. The highest extract was yielded from the fruit,

Figure 1. *Trichosanthes tricuspidata* A: in its natural habitat at *Banjar* Apid Yeh, Bangli, Baturiti, Tabanan, Bali; B: leaves and fruits were separated; C: seeds were extracted from the fruits and air-dried for 3-5 days.

while the seed produced the lowest extract percentage. The complete yield of *T. tricuspidata* methanolic extract is presented in Table 1.

The phytochemical screening revealed the presence of flavonoid, alkaloid, terpenoid, tannin, and saponin in all *T. tricuspidata* methanolic extracts. Terpenoid was present in the form of triterpenoid while steroid was absent in all extracts. The result of *T. tricuspidata* extracts screening conducted in this study is presented in Table 2.

All of phytochemical compounds detected in *T. tricuspidata* methanol extract are valuable in medicinal sciences. Previously, flavonoid was isolated from *T. anguina* fruit extracts (Marsetya et al. 2009; Tripathy et al. 2014; Aseervatham et al. 2019) to treat cancer and cardiovascular diseases, which act as antioxidants, anti-inflammatory, anti-diabetic, and anti-bacteria (Arifin & Ibrahim 2018). In line with that finding, we found flavonoids in all parts of *T. tricuspidata*. Among natural products, flavonoids are one of the important secondary metabolites in plants. Furthermore, *T. tricuspidata* roots extract showed the presence of biologically active compounds that contribute to its antibacterial activities such as phenol, tannin, alkaloid, triterpenoid, and saponin (Bhardwaj & Rashmi 2015).

Table 1. The yield of *Trichosanthes tricuspidata* crude extract.

Plant material	Weight of simplicia (g)	Amount of extract (mL)	Yield (%)
Leaves	200	16	8.000
Fruits	185	19.7	10.649
Peels	245	24.7	10.082
Seeds	882	11	1.248

Table 2. Phytochemical analysis of *Trichosanthes tricuspidata* extracts.

Plant materials	Compounds							
	Flavonoid	Alkaloids			Tannin	Terpenoids		Saponin
		Mayer test	Dragendorff test	Bouchardat test		Steroid	Triterpenoid	
Leaves	+	+	-	+	+	-	+	+
Peels	+	+	+	+	+	-	+	+
Fruits	+	+	-	+	+	-	+	+
Seeds	+	+	-	+	+	-	+	+

Note: + = Present, - = Absent

Kavitha (2017) reported the presence of alkaloids in *T. dioica* leaves and fruits methanolic extracts, but in *T. tricuspidata*, this compound found in leaves methanolic extract (Yuvarajan et al. 2015), while Tripathy et al. (2014) found alkaloid in fruit extract. Out of three tests conducted to examine the presence of alkaloids in this study, Mayer and Bouchardat tests showed a positive result in all *T. tricuspidata* extracts, while the Dragendrof test only showed a positive result in the peels extract. The positive results of alkaloid from Mayer's test for all extracts shows that *T. tricuspidata* might contain alkaloids with quaternary nitrogen structure. Alkaloids are best known for their physiological action on the animal organism which placed them as a high valued compound for medicine (Maldoni 1991). All extracts showed a positive result for at least two alkaloid tests except for peels extract that showed positive results in all three alkaloid tests.

Tannin was found in *T. lobata* and *T. dioica* extracts (Kumar et al. 2012; Rajasekaran & Periyasamy 2012). In this study, tannins were detected in all methanolic extracts of *T. tricuspidata*. However, Tripathy et al. (2014) reported that tannin was only found in *T. tricuspidata* fruit aqueous extract, but it was not found in fruit methanol extract. Tannins have been known as a compound that acts against infections and cell abnormalities, as well as possess antibacterial and antifungal potencies (Gupta & Pandey 2020).

Tripathy et al. (2014) reported the presence of terpenoid in *T. tricuspidata* fruit n-butanol extract, but it was absence in the methanol extract. On the contrary, we found the presence of terpenoid in the methanolic fruit extract. Similarly, cucurbitacins, a triterpenoid compound from the Cucurbitaceae, were also extracted from *T. tricuspidata* fruit (Attard & Martinoli 2015). Triterpenoid was also presented in *T. tricuspidata* roots extracts (Bhardwaj & Rashmi 2015). Meanwhile, the steroid was not found in all extracts tested in this study. In contrast, Tripathy et al. (2014) reported the presence of steroids in *T. tricuspidata* fruit methanolic extract. Moreover, triterpenoids and saponin were detected on each extract from *T. tricuspidata,* which potentially obtain antimicrobial activity (Barre et al. 1997; Amaral et al. 1998; Gupta & Pandey 2020). A previous phytochemical study showed that the presence of various phytochemical compounds including terpenoids of *T. tricuspidata* extract exhibited a strong anti-diabetic activity (Kulandaivel et al. 2013).

Leaves and roots extracts of *T. tricuspidata* also contain saponin (Bhardwaj & Rashmi 2015; Yuvarajan et al. 2015). Identically, we found that saponin was present in all extracts of *T. tricuspidata*. However, Tripathy et al. (2014) found that saponin only presented in aqueous fruit extract but was absent from the fruit methanol extract. Saponin is a chemical compound with numerous therapeutic benefits such as anti-inflammatory, antifungal, hypoglycemic, hypocholesterolemic, immunostimulant, cytotoxic, and obesity treatment potential (Marrelli et al. 2016).

To analyze the potential of *T. tricuspidata* as an antioxidant agent, a DPPH scavenging activity test was conducted to each extract. Delocalisation of the spare electron from the DPPH molecule had been measured to have an inhibitory percentage. The inhibitory percentage indicates the sample's ability to bind free radical electrons. DPPH assay is used widely to assess free radical scavenging activity due to its convenience, stability at room temperature, and its production of violet solution in a solvent. The scavenging activity of plant extracts were indicated by the discoloration in the solvent (from violet to yellow) (Singh et al. 2016). The current study results showed different DPPH scavenging activities from different parts of the plant (Table 3).

Leaves, fruits, peels, and seeds extract of *T. tricuspidata* showed the ability of scavenging free radicals. The increase of extract concentration is directly proportional to the percentage of plants that ward off free radicals. This result was supported by Ghasemi et al. (2009) which revealed the radical-scavenging activities of all extracts of peels and tissues of citrus species increased with increasing concentration. In this study, concentrations of leaves, fruits, and seeds extracts of 100 ppm inhibited 67.84, 69.06, and 70.34% free radicals, respectively. Unlike the peels, to inhibit up to 67.26%, free radicals

Table 3. Inhibition percentage of *Trichosanthes tricuspidata* extracts toward free radical.

Extract	Extract concentration (ppm)	Absorbance value	Inhibition	Inhibition percentage (%)
Leaves	125	0.461	0.453	45.302
	250	0.436	0.483	48.270
	500	0.384	0.544	54.442
	1000	0.271	0.678	67.844
Fruits	500	0.331	0.573	57.267
	600	0.305	0.606	60.615
	700	0.291	0.624	62.424
	800	0.273	0.647	64.725
	900	0.250	0.677	67.724
	1000	0.240	0.691	69.055
Peels	31.5	0.466	0.447	44.697
	62.5	0.416	0.507	50.668
	125	0.399	0.527	52.650
	250	0.276	0.673	67.262
Seeds	31.5	0.471	0.441	44.139
	62.5	0.449	0.467	46.655
	250	0.417	0.505	50.513
	500	0.339	0.598	59.760
	1000	0.250	0.703	70.336

required a concentration of 250 ppm. Correspondingly, Orak et al. (2012) reported that *Punica granatum* peels extract showed higher scavenging activity than its juice and its seeds extracts. Similarly, Singh et al. (2016) found that the antioxidant activity of four Cucurbitaceae species were significantly higher in peels than pulps.

A higher concentration at 500-1000 ppm on leaves, fruits, and seeds extracts showed a non-significant increase in inhibitory activity. The scavenging activity is considered effective if the requirements of low extract concentration with high inhibition percentage. Therefore, the scavenging activity given below (Table 4) is calculated of the half maximum (50%).

Table 4. The IC_{50} value of *T. tricuspidata* extracts on DPPH radical.

Extract	IC_{50} (ppm)
Leaves	315.970
Fruits	171.992
Peels	78.029
Seeds	208.582

The IC_{50} value is obtained through a regression analysis that describes the concentration of a test solution that can reduce 50% free radicals and represents antioxidant activity (Widyasanti et al. 2016). Antioxidant activity or IC_{50} values in leaves, fruits, peels, and seeds extracts were 315.970, 171.992, 78.029, and 208.582 ppm, respectively. Compared to other extracts, *T. tricuspidata* peels extract has the strongest antioxidant activity. According to Molyneux (2004), antioxidant activity is grouped into very strong (IC_{50} < 50 ppm), strong (IC_{50} 50–100 ppm), moderate (IC_{50} 100–150 ppm), and weak (IC_{50} 150–200 ppm).

The peels exhibited the highest ability in the DPPH scavenging activity test with IC_{50} 78.029 ppm, the lowest value compared to the other parts. Our result may suggest that peels have a high concentration of phenolic compounds that contribute to antioxidant activity. This result is similar to the previous study in *T. tricuspidata* that showed antioxidant activity was cumulated in the pericarp region (Mai et al. 2002). Similarly, Singh & Prakash (2013) were also reported that the best IC_{50} of *T. cucumerina* extracts was found in fruits rather than in leaves, stem, and roots. Moreover, our finding in the highest antioxidant activity is specific and narrowed to the epicarp or peels.

Phenols and polyphenolic compounds, such as flavonoids, anthocyanins, and tannins, have proven to exhibit antioxidant properties (van Acker et al. 1996; Hosu et al. 2014). Anthocyanins are flower and fruit dominant pigments that produce their distinctive reddish, bluish, and purple hues (Rice-Evans et al. 1997). As reported by Febrianti et al. (2016), the dark rind contains higher polyphenol. Therefore, the strong antioxidant activity of the peels is thought to be due to their red color. Polyphenols in their chemical activity are reduced as hydrogen or electron donor agents predicting their potential action as free radical scavengers (antioxidants) (Rice-Evans et al. 1997). Moreover, Wangensteen et al. (2004), Marsetya et al. (2009), Ardekani et al. (2010), Kumar et al. (2014), and Febrianti et al. (2016) stated that total phenols in extracts are positively correlated with the antioxidant activity. Thus, further research is needed to investigate the quantitative phytochemical compounds that act as active antioxidants. However, a strong antioxidant activity of *T. tricuspidata* that resulted in this study could be important scientific evidence supporting the use of this species in traditional medicine for centuries.

To conclude, phytochemical screening on *T. tricuspidata* showed the presence of flavonoid, alkaloid, terpenoid, tannin, and saponin. Leaves, fruits, peels, and seeds showed antioxidant activity. Above all, peel extract showed the highest antioxidant activity. Subsequently, we conclude that this species contains a prospective compound agent for medicinal use.

AUTHORS CONTRIBUTION

ASL collected the materials, prepared and extracted the plant, composed the manuscript, and evaluated the manuscript; FK collected the materials, composed the manuscript, and evaluated the manuscript; ARUW collected the materials and composed the manuscript; CIMS extracted the plant and composed the manuscript; while PKW collected the materials and extracted the plant. Thus, we declare that ASL, FK, and ARUW are the main contributors, while CIMS and PKW are the member contributors.

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CONFLICT OF INTEREST

The authors state that there is no conflict of interest regarding the research substance and funding.

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