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Phytochemical Screening and Antioxidant Activity of Ethanolic Extract of *Rhizanthes deceptor* (Rafflesiaceae) and its Host *Tetrastigma papillosum*

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ABSTRACT

Rhizanthes deceptor is a species that found only in Sumatera, Indonesia. Crude ethanolic extract of *Rhizanthes deceptor* and it's host, *T. papillosum papillosum* were examined for their phytochemical properties and antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH). The phytochemical qualitative analysis showed that all the extracts, containing alkaloid, phenolic and flavonoid. Triterpenoid detected in bud extract and root extract, saponin detected in root and stem extract and steroid only detected in stem extract. The result showed high level of total phenolic content from all the extract with their value are 431.52, 323.93 and 271.38 mg GAE/g from bud extract of *R. deceptor*, root and stem of *T. papillosum*, respectively. Presence of detected secondary metabolites thought to contribute to antioxidant activity of this extract. Antioxidant activity of the plants showed that bud extract of *R. deceptor* and root and stem extract of *T. papillosum* had fairly high DPPH antioxidant activity with IC_{50} are 32, 22 and 35 μg mL^{-1}, respectively. The result of antioxidant activity test indicates that *R. deceptor* and *T. papillosum* as, natural sources of antioxidant.

Key words: Antioxidant activity, phytochemical, *Rhizanthes deceptor*, *T. papillosum*

INTRODUCTION

Plants has been used by human for millennia, maybe as old as, humanity itself. Cures as yet undiscovered may exist in plants as yet undescribed. Currently, it is estimated over 50,000 species used worldwide for medicine purposes (Schippmann *et al*., 2002). Demand for traditional medicine is increasing around the world alongside with growing environmental awareness and a desire for natural healing through natural products (Hawkins, 2008).

Rhizanthes deceptor belongs to a parasitic plant, Rafflesiaceae. Unlikely the famous member of Rafflesiaceae, *Rafflesia*, *R. deceptor* found less popular. The knowledge about *R. deceptor* is extremely poor, even traditional knowledge. Taxonomic revision, biology and some ecological information given by Banziger and Hansen (2000). Some books considered *R. deceptor* as, medicinal plant, but no information about what exactly the utility of *R. deceptor*. Traditional people often utilize the plant as, medicinal plant but its not happen to *R. deceptor*. Although, this flower have found only in Sumatera, no information so far about utilization of this flower in Sumatera.

Unlike *R. deceptor*, *Rafflesia* has known in some region as, traditional medicinal plant. This traditional information used by scientist as, starting point for bioprospecting. Research about bioprospecting of *Rafflesia* has been done in some country. Latest research about *Rafflesia kerri*

carried by Puttipan and Okonogi (2014) while research about *Rafflesia cantleyi* and its host *Tetrastigma tuberculatum* carried by Zulkffle *et al*. (2014). Both of this research found high antioxidant ability from their samples.

Host of *R. deceptor* is *T. papillosum* and *T. pedunculare* (Banziger and Hansen, 2000). On *Tetrastigma* genera, there has been some researched about chemical compound and other potential. *Tetrastigma* also well known as traditional medicine in some country. Krishna *et al*. (2013) has examined the content of the chemical compound and test the proximate leaf of *T. leucostaphylum*. *T. hemsleyanum* note useful as, an anti-inflammatory, relieving pain and improve blood circulation (Liu *et al*., 2002), while *T. hypoglaucum* useful as drugs for broken bones and relieving pain (Liu *et al*., 2003) and *T. angustifolia* leaf extract potentially anti diabetes (Junejo *et al*., 2014).

Currently no study about chemical compounds or bioprospecting of *R. deceptor* and its host. If we look at bioprospecting of *Rafflesia*, it is possible that *R. deceptor* have some potential too. This study is proposed to report a preliminary result about potential of *R. deceptor* and its host *T. papillosum* through, the phytochemical screening and antioxidant activity test.

MATERIALS AND METHODS

Plant materials: The fresh bud of *R. deceptor* and root and stem of *T. papillosum* was collected from study site in Biology Research and Educational Forest, Andalas University, Padang, during research on October, 2014. All the plants from this study was taxonomically identified in Herbarium Universitas Andalas (ANDA), Padang.

Extraction of plant materials: Samples in powder form has taken each ±20 g to measure water levels. Then the rest of samples going to extract with maceration method with ethanol for approximately 24 h at room temperature. The filtrate is obtained to 100 ml with a rotary evaporator at temperature 40-50°C and 50 rpm.

Phytochemical testing: Extract of materials has been used on qualitative phytochemical test in g performed to detect the presence of alkaloids, phenolics, flavonoids, saponins, terpenoids and steroids. The phytochemical testing were screened using standard procedures, as described by Harborne (1987). Total phenolic content was determined as Gallic Acid Equivalent (GAE) according to Pourmorad *et al*. (2006).

Brine Shrimp Lethality Test (BSLT) bioassay (Meyer *et al*., 1982): Brine shrimp lethality test is a convenient bioassay for plants constituent (Meyer *et al*., 1982). *Artemia salina* was used to monitor the screening. The eggs of brine shrimp (*Artemia salina* Leach.) were collected from a shop in faculty of marine and fisheries, Bogor Agricultural University and hatched in a tank with seawater and constant oxygen supply for approximately 24-48 h, until the nauplii matured.

The extract dissolved by DMSO and then test solution prepared at a concentration of 1000, 500, 100 and 30 μg mL^{-1}. The BSLT done by inserting 20 *A. salina* shrimp larvae in each test solution by repeating three times at each concentration. Observations were made after ±24 h to see the number of people, who lived and died. The data was obtained and analyzed by probit analysis method to determine the LC_{50} with 95% confidence interval. The LC_{50} is the concentration of the extract that is able to kill 50% of the population tested shrimp larvae. The lower the LC_{50} value means higher toxicity of the extracts were tested.

DPPH (1,1-diphenyl-2-picrylhydrazyl) antioxidant activity test (Sari *et al*., 2011): The extract was dissolved in ethanol, according to the desired concentration. The solution was incubated at 37°C for 30 min and then the absorbance was measured using UV-VIS spectrophotometry at a wavelength of 517 nm. The antioxidant activity of each sample was determined by calculating the percent inhibition, which is calculated by the following equation:

$$\text{DPPH scavening activity (\%)} = \left\{\left(\frac{A-B}{A}\right)\times 100\%\right\}$$

where, A is the absorbance of negative control (DPPH+ethanol) and B is the absorbance of test extracts (DPPH+ethanol+test extract). Value of sample concentration and percent inhibition plotted, respectively on the x-axis and y in the linear regression equation. Linear regression equation obtained in the form of the equation y = a+bx used to find the value of IC_{50} (inhibitor concentration of 50%) of each sample with a stated value of y is 50 and the value of x that will be obtained as IC_{50}. The IC_{50} declare the concentration of the sample solution is needed to reduce DPPH free radicals by 50%.

Data analysis: The results of *in vitro* study are expressed as Mean±Standard Deviation (SD) obtained from triplicate experiments. The data analyzed with Student's t-test and a 'p' value less than 0.05 was considered as significant difference in analysis.

RESULTS AND DISCUSSION

Extraction of buds of *R. deceptor* (RHZ) and part of the root (AKR) and stem (BT1) from *T. papillosum* with ethanol solvent showed varies yield value with different physical form (Table 1). The highest yield resulting from the extraction of *R. deceptor* buds, which is almost 3 times the yield of extract its host. Differences in levels and physical form extracts showed that the content of extractive substances differ between different parts of the network though, the tree is extracted with the same solvent. This is evidenced by research (Alimpic *et al*., 2014) that the ethanol extraction *Salvia amplexicaulis* flowers produce a higher yield than the stalks. Ethanol considered as, a good solvent, because it has been extensively used to extract compounds from various plants and plant-based foods especially to extract antioxidant compounds.

The results of the qualitative phytochemical testing showed that all the samples contain alkaloid, phenolic and flavonoid. Terpenoid detected in bud extract and root extract and Saponin detected in root and stem extract. Steroid detected only in stem extract (Table 2).

Secondary metabolites that detected in all the extract should tell about the bioprospecting potential of *R. deceptor* and its host *T. papillosum*. Alkaloids are known as source to play a defensive role in the plant against herbivores and pathogens. Due to their potent biological activity, many of the approximately 12,000 known alkaloids have been exploited as pharmaceuticals, stimulants, narcotics and poisons (Wink, 1998). Sofiyanti *et al*. (2008) reported that *Rafflesia hasseltii* and its host *T. leucostaphylum* contain nicotine and caffeine as members of alkaloids

Table 1: Extracts yield and extracts physical form of *R. deceptor* (RHZ), root of *T. papillosum* (AKR) and stem of *T. papillosum* (BT1)

Ethanolic extract	Yield (%)	Extracts physical form
RHZ	30.58	Blackish brown, viscous solids, flavorful
AKR	12.19	Dark red-black, solid
BT1	11.51	Blackish brown, solid

Table 2: Result of qualitative phytochemical testing and total phenolics content of *R. deceptor* (RHZ), root of *T. papillosum* (AKR) and stem of *T. papillosum* (BT1)

	Extract		
Chemical group tested	RHZ	AKR	BT1
Alkaloid	+	+	+
Flavonoid	+	+	+
Phenolic	+	+	+
Terpenoid	+	+	-
Saponin	-	+	+
Steroid	-	-	+
TPC values	431.52 mg GAE/g	323.93 mg GAE/g	271.38 mg GAE/g

+: Detected, -: Undetected, TCP: Total phenolics content

Table 3: Mortality and LC_{50} values of ethanolic extract from bud of *R. deceptor* (RHZ), root of *T. papillosum* (AKR) and stem of *T. papillosum* (BT1)

	*Mortality (%)				
Extracts	30 (μg mL^{-1})	100 (μg mL^{-1})	500 (μg mL^{-1})	1000 (μg mL^{-1})	LC_{50} (μg mL^{-1})
RHZ	0	10.0±1.76	95±5	100	283.21±9.68
AKR	0	5.0±2.98	10±1.76	93±11.94	719.95±13.91
BT1	0	1.1±0.99	98±2.98	100	277.65±10.71

*Mean±SD

group. It is possible that *R. deceptor* and its host contain some valuable members of alkaloids but we can't prove it from this research. *Rhizanthes deceptor* and its host also contain phenolics and flavonoids in their ethanolic extract. Phenolics and flavonoids known as, source of natural antioxidant and also are related with antioxidant activity of some plants (Demiray *et al*., 2009; Hossain *et al*., 2011; Patel *et al*., 2012). Although, in some cases the relation is low (Omonhinmin *et al*., 2015). The high values of total phenolic content from *R. deceptor* and its host indicated the probability of high antioxidant ability (Table 2).

The results of toxicity tests on all samples with BSLT method showed that all the samples test edare toxic. An extract said to be toxic, if it is capable of killing *Artemia salina* more than 50% for 24 h (Meyer *et al*., 1982). The highest LC_{50} value is stem extract ethanol of *T. papillosum* with 277.65 μg mL^{-1} followed by bud extract of *R. deceptor* and root extract of *T. papillosum* with 283.21 and 719.95 μg mL^{-1} (Table 3). The BSLT toxicity test method is one of the most convenient bioassay to see the potential for a kind of compound. Meyer *et al*. (1982) states that, if the value LC_{50} extract below 1000 μg mL^{-1}, there is a chance of compound or active extract to fa sample with the potential bioactive, while Carballo *et al*. (2002) states that if the LC_{50} value of a small natural extract slower than 1000 μg mL^{-1}, then its potential as, an anti-cancer. Meyer *et al*. (1982) also stated that the most active compounds in plants are toxic at certain doses, so that, the toxic values obtained are evidence that there is the content of bioactive compounds in the extracts tested. The toxicity results of the test could be potential anti-cancer oranti-pest (Ghisalberti, 1993). The existence of a diverse group of chemical compounds in the extract were tested in the study confirm the possibility of potential antioxidants and other potentials of the samples tested.

The results of radical scavenging activity test by DPPH showed that all tested extracts have high antioxidant activity. Table 4 shows the percentage of DPPH inhibition from each extract and regression equation of the relationship interpolation. The results of the regression equation, this variety will produce for different IC_{50} values for each sample extract. The IC_{50} values were obtained from the regression equation in Table 4 shows that all the extract has the ability of radical scavenging activity. The highest IC_{50} value is the extract of bud of *R. deceptor* with IC_{50} value of

Table 4: IC_{50} values from bud of *R. deceptor* (RHZ), root of *T. papillosum* (AKR) and stem of *T. papillosum* (BT1)

	*Inhibition of DPPH (%)					
Extracts	7.8 (μg mL^{-1})	15.6 (μg mL^{-1})	31.2 (μg mL^{-1})	62.5 (μg mL^{-1})	125 (μg mL^{-1})	IC_{50} (μg mL^{-1})
RHZ	20.31±1.1	28.69±5.14	54.25±1.26	81.83±1.72	90.86±0.66	31.97±4.79
AKR	25.93±0.85	42.57±1.28	64.59±1.05	75.24±0.89	91.54±0.29	21.71±8.01
BT1	17.25±0.67	29.57±1.28	49.36±3.15	60.53±1.05	90.91±0.16	35.23±2.42

*Mean±SD

31.97 μg mL^{-1}, as well as extracts of root sand stems *T. papillosum* with IC_{50} value of 21.71 and 35.23 μg mL^{-1}, respectively. The antioxidant activity which is owned by third extract can be quite high, if it refers to Blois (1958) in Hanani *et al*. (2005) which states IC_{50} of 200 μg mL^{-1} Lasa strong antioxidant activity limits.

Several species of Rafflesia which has been studied previously also represents a good potential as antioxidant. Zulkffle *et al*. (2014) stated that the *Rafflesia cantleyi* and host *T. tuberculatum* absorption of free radical activity is quite high with IC_{50} value of 14.58 and 19.12 μg mL^{-1}, as well as research and Puttipan and Okonogi (2014) on the *Rafflesia kerri*, which concludes the high antioxidant potential in the *Rafflesia*. Hossain *et al*. (2011) stated that the flavonoids and terpenoids are potent antioxidants and the close relationship between the total phenolic content of the extracts with antioxidant activity. From the results obtained in the study and compared with several previous studies of antioxidant activity, it can be said that *R. deceptor* also has potential as a natural source of antioxidants.

CONCLUSION

The study concluded that the qualitative phytochemical testing showed that all the extracts containing alkaloid, phenolic and flavonoid. The toxicity test of BSLT showed the ethanol extract of *R. deceptor* bud sand the roots and stems of *T. papillosum* are toxic with LC_{50} values for 283.21, 719.95 and 277.65 μg mL^{-1}, respectively. The DPPH radical scavenging test showed ethanol extract *R. deceptor* bud and the roots and stems of *T. papillosum* have radical scavenging activity which capable of capturing DPPH with IC_{50} values are 31.97, 21.71 and 35.23 μg mL^{-1}, respectively. All the test results showed chemical compounds contained in all the extracts tested samples are supposed to influence the antioxidant activity and toxicity of the extracts. Overall results of the tests that have been done indicate that *R. deceptor* and *T. papillosum* containing compound has potential as an antioxidant and other potential possibilities indicated by its toxicity. This study certainly not been able to reveal the overall potential intended. Further research needs to bed one to isolate the compounds related to the anti-cancer potential and antioxidant that contained in *R. deceptor* and *T. papillosum*.

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