Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 8, 2014

Original Article

SCREENING OF ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT IN *RAPHANUS SATIVUS* POD

TOSSATON CHAROONRATANA[1,*], SUKANYA SETTHARAKSA[2], FAMEERA MADAKA[2], THANAPAT SONGSAK[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Rangsit University, Lak-Hok, Muang, Pathum Thani, 12000, Thailand, [2]Sino-Thai Traditional Medicine Research Center, Faculty of Pharmacy, Rangsit University, Lak-Hok, Muang, Pathum Thani, 12000, Thailand. Email: tossaton.ch@rsu.ac.th

Received: 30 Jun 2014 Revised and Accepted: 05 Aug 2014

ABSTRACT

Objective: The aim of this study was to evaluate the antioxidant activity and total phenolic content of *Raphanus sativus* L. Var. *caudatus* Alef pod extracts.

Methods: In this study, the extract of *R. sativus* pod was prepared in three different types of solvent. The antioxidant activitiy of *R. sativus* extract was determined using a spectrophotometric technique, based on a free radical diphenyl-picryhydrazyl scavenging assay (DPPH assay) and a ferric reducing antioxidant power assay (FRAP assay). Total phenolic content was also observed using the spectrophotometric technique.

Results: The result showed that the antioxidant activity which was expressed by IC_{50} values varied from 1,365 to 4,371 mg/ml and 312 to 6,478 mg/ml, based on DPPH assay and FRAP assay, respectively. Total phenolic content was also evaluated and calculated as gallic acid equivalents which ranged from 0.26 to 34.60 mg gallic acid per 100 g fresh sample.

Conclusion: It was suggested that hexane extract of *R. sativus* pod contained the highest amount of phenolic compounds in comparison with those of dichloromethane part and ethanol part. The result from FRAP assay was positively correlated to total phenolic content which the highest antioxidant value belongs to the hexane extract of *R. sativus* pod. It was concluded that *R. sativus* pod contained phenolic compounds which showed mild antioxidant activity.

Keywords: *Raphanus sativus* L. Var. *caudatus* Alef, DPPH, FRAP, Total phenolic content.

INTRODUCTION

Among the varieties of herbs, vegetables and fruits are increasingly of interest in the food industry. One of a health promoting activity of these edible plants is their ability to act as antioxidants [1]. Antioxidants are significant inhibitors of lipid peroxidation for a defensive mechanism of living cells against oxidative damage [2]. Thus, the potential of the antioxidants in vegetables and fruits for health preservation and protection from cancer is raising attention among the people involving in the food industry as consumers move toward functional foods with explicit health effects [3].

In herbs, it was concluded that the antioxidant activity is mainly from phenolic compounds which its mechanism involving in a redox properties [4-5]. In Thailand, *Raphanus sativus* L. Var. *caudatus* Alef, or Puk-kee-hood, is a culinary plant in Brassicaceae family (Fig. 1). *R. Sativus* pod was suggested to be the functional food since it was reported to have several kinds of minerals, vitamins, and some active pharmaceutical metabolites, such as sulforaphene and sulforaphane [6-7]. Both isothiocyanate compounds were found to possess strong anticancer activity [8].

Fig. 1: *Raphanus sativus* L. Var. *caudatus* Alef

To investigate a possibility of the other exist valuable compounds in *R. Sativus* pod, various kinds of the extract were prepared and the antioxidant activity was observed. Several methods are currently used to assess the antioxidant activity [9-10]. In this study, DPPH and FRAP were selected as the tools to investigate the antioxidant activity of *R. Sativus* pod. Total phenolic content was also observed in parallel.

MATERIALS AND METHODS

Materials

The 2 month-old *R. sativus* pod was collected from a field in Chiang Mai province, Thailand and identified by Dr. Thanapat Songsak. Gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) were purchase from Sigma (USA). All solvents were purchased from Lab scan (Thailand).

Methods

Sample preparation

Fresh *R. sativus* pod (2,300 g) was divided into 2 groups, the first group (1,500 g) was dried in hot air oven at 45 °C for 1 day and ground into small pieces, the another group was ground and remained as it was. Both groups were macerated in hexane (1,000 ml) at 25 °C for 24 h. The mixture was filtrated and a filtrant was separated using separatory funnel to obtain only hexane part, and concentrated under reduced pressure. The steps were repeated once and the extract in the same sample was pooled in one portion. The residue was macerated with dichloromethane and ethanol following with the same steps as described in hexane extract preparation to obtain the dichloromethane extract and ethanol extract portions, respectively.

DPPH assay

The experiment was processed according to Brand-Williams method, which is based on the determination of the concentration of DPPH at steady state in an ethanol solution, after adding the mixture of antioxidants [11]. Each 100 µl of various concentrations of the

extract or gallic acid in 10% DMSO was added to 100 µl of a 200 µM ethanol solution of DPPH. The mixture was incubated in the dark for 30 min at room temperature. The absorbance was determined at 517 nm compared to blank, which was ethanol. Gallic acid was used as a positive control. The percentage of scavenging activity was calculated using an equation below.

$$DPPH\ scavenging\ activity = 100\times(A0-A1)\div A0$$

Where A0 is the absorbance of the control and A1 is the absorbance of the sample. IC_{50} values calculated indicate the concentration of the sample required to decrease the absorbance at 517 nm by 50%.

FRAP assay

The experiment was processed according to Benzie and Strain method, which is based on the determination of the degree of reducing power of antioxidants [12]. Each 30 µl of various concentrations of the extract or gallic acid was added to 270 µl of FRAP reagent (10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3.6H_2O$ in water solution). The mixture was incubated in the dark for 30 min at room temperature. The absorbance was determined at 593 nm compared to blank, which was water. Gallic acid was used as a positive control. The percentage of ferric reducing antioxidant activity was calculated using an equation below.

$$Ferric\ reducing\ antioxidant\ activity = 100\times(A0-A1)\div A0$$

Where A0 is the absorbance of the control and A1 is the absorbance of the sample. The IC_{50} values calculated indicate the concentration of the sample required to decrease the absorbance at 593 nm by 50%.

Total phenolic content

Folin-Ciocalteu reagent was used to determine the phenolic content of the extract [13]. Approximately 12.5 µl of the extract or gallic acid and 50 µl distilled water were mixed in a 96 well plate. Folin-Ciocalteu phenol reagent (12.5 µl) was added to the mixture and shaken vigorously for 6 min. 7% Na_2CO_3 solution (125 µl) was added, mixed, and diluted to 100 µl with water. The reaction mixture was incubated for 90 min before the absorbance was determined at 760 nm compared to blank. Total phenolic content was calculated as gallic acid equivalents mg per 100 g fresh sample.

RESULTS AND DISCUSSION

The antioxidant activity of the extracts is inversely relative to the IC_{50} value, which can be calculated from the linear regression of DPPH scavenging activity or ferric reducing antioxidant activity versus extract concentrations. In the DPPH assay, the IC_{50} values of all extracts were more than 1,000 mg/ml indicating the absence of antioxidant activity. On the other hand, according to the FRAP assay, it was indicated that only hexane extract and dichloromethane extract of dried *R. sativus* pod showed mild antioxidant activity with the values of 312.20 and 599.71 mg/ml, respectively (Table 1).

Table 1: IC_{50} values of *R. sativus* pod extracts by DPPH and FRAP assays

IC_{50} mg/ml	Gallic acid	*FE1	*FD1	*FH1	*DE1	*DD1	*DH1
DPPH assay	5.52 µg/ml	3,788.56	3,626.42	3,501.70	4,371.26	2,389.59	1,365.34
FRAP assay	3.56 µg/ml	6,079.83	1,225.12	2,520.24	6,478.87	599.71	312.20

*Abbreviation: FE1 = fresh *R. sativus* ethanol extract, FD1 = fresh *R. sativus* dichloromethane extract, FH1 = fresh *R. sativus* hexane extract, DE1 = dried *R. sativus* ethanol extract, DD1 = dried *R. sativus* dichloromethane extract, DH1 = dried *R. sativus* hexane extract

The extraction yields from various solvents were reported as FE1 = 0.0057%, FD1 = 0.135%, FH1 = 0.0092%, DE1 = 0.0031%, DD1 = 0.150%, and DH1 = 0.0070% (see abbreviation from Table 1 or Table 2).

Total phenolic content, which expressed as mg gallic acid per 100 g sample, varied extensively among the extracts, and ranged from 0.26 to 34.60 mg gallic acid per 100 g sample (Table 2). Allowing for a wide range of variation, total phenolic content was divided into 2 groups which were moderate (>10 mg gallic acid per 100 g sample) and low (<10 mg gallic acid per 100 g sample) phenolic content. Only hexane extract and dichloromethane extract of dried *R. sativus* pod contained moderate amount of the phenolic content.

Table 2: Total phenolic content in *R. sativus* pod extracts

Phenolic content (mg gallic acid/100 g fresh sample)	*FE1	*FD1	*FH1	*DE1	*DD1	*DH1
	0.34±0.04	0.80±0.12	0.26±0.01	1.79±0.06	23.07±1.73	34.60±3.86

*Abbreviation: FE1 = fresh *R. sativus* ethanol extract, FD1 = fresh *R. sativus* dichloromethane extract, FH1 = fresh *R. sativus* hexane extract, DE1 = dried *R. sativus* ethanol extract, DD1 = dried *R. sativus* dichloromethane extract, DH1 = dried *R. sativus* hexane extract

Positive relationship was found between ferric reducing antioxidant activity and total phenolic content for the extracts of *R. sativus* pod. It was suggested that phenolic compounds, which were found in dried hexane and dichloromethane *R. sativus* extract, may involve in antioxidant activity. The antioxidant effect disappeared in dried ethanol *R. sativus* extract and it was probably because of loss of those phenolic compounds in extraction processes. Interestingly, all fresh *R. Sativus* extracts were not found to possess any antioxidants. The reason was proposed as the fresh *R. sativus* pod contained considerable amount of water, a yield of its dried weight was about 10% of a fresh weight.

As compared to 100 g of sample, total phenolic content of those fresh extracts was found to be lower than those of dried extract drastically. On the other hand, antioxidant activity of *R. sativus* extract cannot be observed using DPPH assay and it is possible since both assay mechanisms are not totally the same. Anyway, trends of the IC_{50} values in both assays were similar in which the values were low in DD1 and DH1 samples. Moreover, sulforaphene and sulforaphane issue was considered and should be discussed in their antioxidant effect. It was reported that these isothiocyanate compounds are vaporous at room temperature. Thus, it is not possible to detect them in dried *R. sativus* extracts, only phenolic compounds might take a role in the antioxidant effect in FRAP assay. In contrast, both sulforaphene and sulforaphane should be presented in fresh *R. Sativus* extracts and exhibit antioxidant activity. Anyway, in both DPPH and FRAP assays, the IC_{50} values of all fresh extracts were more than 1,000 mg/ml, which means no antioxidant activity. The result guided us that both compounds exhibited antioxidant effect through the other mechanism, which was agreed with a previous work [14].

CONCLUSION

There are not many reports on phytochemistry studies of *R. sativus* L. Var. *caudatus* Alef pod. Belonging to a source of isothiocyanate compounds, this plant also possess phenolic compounds which exhibit mild antioxidant activity through FRAP assay. The suggestion for the future works should be concentrated to separate new compounds, based on this activity, or to investigate the other fascinating pharmacological activities of the edible part of this plant.

CONFLICT OF INTERESTS

None

ACKNOWLEDGMENTS

The authors wish to thank the Faculty of Pharmacy and Sino-Thai Traditional Medicine Research Center, Rangsit University, Pathum Thani, Thailand for all instrumental equipments and chemicals.

REFERENCES

1. Shahidi F. Antioxidants in food and food antioxidants. J Nahrung 2000;44:158-63.
2. Halliwell B. Reactive oxygen species in living systems:source, biochemistry, and role in human disease. Am J Med 1991;91:14-22.
3. Loliger J. The use of antioxidants in food. In:Aruoma OI, Halliwell B, editors. Free radicals and food additives. London:Taylor and Francis;1991. p. 129-50.
4. Osawa T, Uritani I, Garcia VV, Mendoza EM, editors. Novel natural antioxidants for utilization in food and biological systems. In:Postharvest biochemistry of plant food-materials in the tropics. Tokyo:Japan Scientific Societies Press;1994. p. 241-51.
5. Pietta PG. Flavonoids in medicinal plants. In:Rice-Evans CA, Packer L, editors. Flavonoids in health and disease. New York:Dekker;1998. p. 61–110.
6. Songsak T, Lockwood GB. Glucosinolates of seven medicinal plants from Thailand. J Fitoterapia 2002;73:209-16.
7. Pocasap P, Weerapreeyakul N, Barusrux S. Cancer preventive effect of Thai rat-tailed radish (*Raphanus sativus* L. var *caudatus* Alef). J Func Foods 2013;5:1372-81.
8. Holst B, Williamson G. A critical review of the bioavailability of glucosinolates and related compounds. J Nat Prod Rep 2004;21:425-47.
9. Settharaksa S, Madaka F, Sueree L, Chankana N, Chakree K, Charoenchai L. Cytotoxic activity and antioxidant potentials of cold pressed rice bran oil. Int J Pharm Tech Res 2014;6:686-91.
10. Oliveira AMF, Pinheiro LS, Pereira CKS, Matias WN, Gomes RA, Chaves OS, *et al*. Total phenolic content and antioxidant activity of some Malvaceae family species. J Antioxidants 2012;1:33-43.
11. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. J LWT-Food Sci Technol 1995;28:25-30.
12. Benzie IFF, Strian JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power":the FRAP assay. J Anal Biochem 1996;239:70-6.
13. Slinkard K, Singleton VL. Total phenol analysis: Automation and comparison with manual methods. Am J Enol Vitic 1997;28:49-55.
14. Hamed A, Soltan M, Fry J, Hammouda F, Zaki A. Antioxidant and cytoprotective properties of three Egyptian Cyperus species using cell-free and cell based assays. J Pharm Crops 2012;3:88-93.