ANTIOXIDANT PROPERTIES OF FLAVONOIDS EXTRACTED FROM SOME VIETNAMESE MEDICINAL PLANTS

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ABSTRACT

This study aims extracting of flavonoids, determinating total flavonoids contents and antioxidant properties due to DPPH radical scavenging reaction of four medicinal plants (*Hedyotis diffusa*, *Carica papaya*, *Pseuderanthemum bracteatum* and *Scutellaria barbata*). The results showed that extracting by using ultrasonic method at 50^{O}C with methanol solvent was chosen. Total flavonoids contents and IC_{50} values of *Hedyotis diffusa*, *Carica papaya*, *Pseuderanthemum bracteatum*, *Scutellaria barbata* were determinated, 5.83 mg/g dw and 60.50 µg/ml, 12.15 mg/g dw and 62.17 µg/ml, 3.99 mg/g dw and 90.36 µg/ml, 4,01 mg/g dw and 69.68 µg/ml, respectivity.

Keywords: flavonoid, antioxidant, *Hedyotis diffusa*, *Carica papaya*, *Pseuderanthemum bracteatum*, *Scutellaria barbata.*

INTRODUCTION

Free-radicals in body result from endogenous or exogenous sources. Although the body has antioxidant mechanism, cell damage caused by oxidation are popular. The oxidative damages, which does not cause cell death, can stimulate development of cancer at different phases. Besides, they can cause many incurable diseases such as diabetes, heart disease, Parkinson's, Alzheimer's, immune deficiency, aging faster desease ... and more than 200 other health problems (Dreher and Junod, 1996).

The important role of oxidation in the development of clonal cell lines of tumor and malignant transformation, so there are many evidences that demonstrate the need of anti-free radicals. The oxidation could be considered as an important class of carcinogenic substances. In cancer treatment process, the antioxidation need to be added into treatment regimen, no matter what kinds of methodology in which therapy of modern medicine or alternative cancer treatment is applied. In the near future, new knowledges about actions of the tumor suppressor genes and DNA repair mechanisms can bring forward many solutions for cancer treatment by increasing antioxidants (Dreher and Junod, 1996).

The flavonoids, which are primarily benzo-γ-pyrone (phenylchromone) derivatives, comprise a massive group of polyphenolic compounds (Harborne, 1986; Harborne and Williams, 2000). These natural antioxidants constitute more than 4000 chemically unique and distinct moieties and enjoy almost ubiquitous distribution in the plant kingdom. The immensely diverse group broadly comprises distinct classes such as flavonols, flavans, proanthocyanidins, anthocyanidins, flavanones, flavones, isoflavones and neoflavonoids (Kanadaswwami et al., 2005). Because of having antioxidant capacity, flavonoids play significant role in anti-tumor (benign, melanoma) by free-radicals quenching mechanism

such as OH, ROO (mutagenic, cell damage, carcinogenic, accelerated ageing factors…) (Packer et al., 1999; Ali et al., 2008).

Located in the tropical monsoon climate with hot and humid climate characteristics, so there are abundant and diversified resources of medicinal plants in Vietnam. Many researches all over the world have showed that four Vietnamese medicine plants (*Hedyotis diffusa*, *Carica papaya* leaves, *Pseuderanthemum bracteatum* leaves and *Scutellaria barbata*) had high anti-liver cancer activity (Shi et al., 2008; Chen et al., 2012; Otsuki et al., 2010; Tran Cong Khanh et al., 2007; Dunkhunthod, 2014; Tang et al., 2006; Xu and Zhang, 2012; Dai et al., 2013). Therefore, in this study, to premise for later research about anti-liver cancer cells, we tested *in vitro* antioxidant activities due to DPPH radical scavenging reaction of flavonoids extracted from four medicinal plants.

MATERIALS AND METHODS

Materials and chemicals

H. diffusa, *C. papaya* leaves, *P. bracteatum* leaves and *S. barbata* were taken from the nature. Standard substances: ascorbic acid, catechin, DPPH were supplied by Merck company (Germany).

Methods

Preparation of sample

Samples were washed cleanly, then dried at $50^{O}C$, after that milled into powder for using in later tests.

Testing flavonoids extraction conditions

0.5 g sample powder and 15 ml solvent were added into falcon tube. Ultrasonic method was used to extract sample in 2 hours. Extracted solution was obtained, then filtered. Flavonoid content in supernatant was determinated. Changing solvent types, solvent concentration and extraction temperature following Table 1. Each treatment was repeated 3 times. After choosing accordant flavonoids extraction conditions, we extracted 15 g sample powder at chosen conditions to prepare extract. Extracted solution was removed of solvent by rotary evaporator and dried at $40^{O}C$. Extract was weight, then dissolved in methanol and kept at $-20^{O}C$.

Table 1. Treatments of flavonoids extraction conditions test

Treatment	Solvent type	Solvent concentration	Temperature
1	H_2O	100%	$30^{O}C$
2	MeOH	90%	$50^{O}C$
3	EtOH	80%	$70^{O}C$
4		70%	
5		60%	

Determination of total flavonoids content (Dewanto et al., 2002)

0.25 ml of the extract solution or catechin standard solution was mixed with 1.25 ml of distilled water in a test tube followed by addition of 75 µl of a 5% $NaNO_2$ solution. After 6 min, 0.15 ml of a 10% $AlCl_3$ solution was added and allowed to stand for another 5 min before 0.5 ml of 1 M NaOH was added. The absorbance was measured immediately against the blank at 510 nm. Results showed as mg/g dw. Standard substance was catechin.

Determination of antioxidant activity by DPPH radical scavenging method (Jahan et al., 2010)

The reaction mixture included 100 µl extracted solution and 100 µl 0.1 mM DPPH solution (in 80% ethanol). This mixture was then allowed to stand at dark place at 37°C for 30 min. The absorbance of samples was read against a blank at 517 nm. Percentage of DPPH radical scavenging of sample was calculated by the following formula: % DPPH radical scavenging = [1- (OD_{sample} / $OD_{control}$)] × 100. The IC_{50} value was the concentration at which DPPH radicals were scavenged 50%, this value was determinated by interpolating from a linear regression analysis. The lower the IC_{50} values, the higher the antioxidant activities.

Data analysis: Each treatment was repeated 3 times. Data were analyzed by Microsoft Excel 2013 and SAS 9.1 softwares.

RESULTS AND DISCUSSIONS

Effects of solvent types on flavonoids extraction

In extraction, choosing accordant solvent type was very important, that not only ensured for extracting desired subtances the most, but also saved time and responsed economic requirements. The organic solvent types cause a major effect on yield of natural compounds extraction that depended on the polarization of the solvents and the extracted compounds. The results in Table 2 showed that methanol was the best solvent to extract flavonoids in three tested ones. Flavonoid contents in the methanol extracted solution of *H. diffusa*, *C. papaya* leaves, *P. bracteatum* leaves and *S. barbata* were 2.79 mg/g dw, 8.32 mg/g dw, 1.08 mg/g dw and 1.79 mg/g dw, respectively.

Table 2. Effects of solvent types on extracted flavonoids content

Solvent type	Extracted flavonoids content (mg/g dw)			
	H. diffusa	*C. papaya* leaves	*P. bracteatum* leaves	*S. barbata*
H_2O	$2.32^{b} \pm 0.02$	$3.35^{c} \pm 0.22$	$0.58^{b} \pm 0.04$	$1.34^{b} \pm 0.05$
MeOH	$2.79^{a} \pm 0.16$	$8.32^{a} \pm 0.30$	$1.08^{a} \pm 0.03$	$1.79^{a} \pm 0.14$
EtOH	$2.02^{b} \pm 0.14$	$7.60^{b} \pm 0.15$	$0.63^{b} \pm 0.04$	$0.81^{c} \pm 0.05$
CV (%)	5.20	3.60	5.22	6.73

Each value in the table was the average of 3 repetitions ± standard deviation (Mean ± SD). The different letters (a, b, c...) showed significant difference at p = 0.01 according to Duncan's method.

Effects of solvent concentrations on flavonoids extraction

The results in Table 3 showed that 100% methanol was the best solvent concentration to extract flavonoids from *H. diffusa*, *C. papaya* leaves and *S. barbata*. Extracted flavonoid contents were proportional to the concentration of extraction solvent. In other hand, 90% methanol was the best solvent concentration to extract flavonoids from *P. bracteatum* leaves.

Table 3. Effects of solvent concentrations on extracted flavonoids content

Solvent concentration	Extracted flavonoids content (mg/g dw)			
	H. diffusa	*C. papaya* leaves	*P. bracteatum* leaves	*S. barbata*
100% MeOH	$3.51^{a} \pm 0.02$	$8.01^{a} \pm 0.18$	$1.02^{c} \pm 0.07$	$2.42^{a} \pm 0.09$
90% MeOH	$2.25^{b} \pm 0.05$	$3.95^{b} \pm 0.06$	$2.39^{a} \pm 0.07$	$1.89^{b} \pm 0.04$
80% MeOH	$1.90^{c} \pm 0.05$	$3.03^{c} \pm 0.06$	$1.24^{b} \pm 0.04$	$1.27^{c} \pm 0.05$
70% MeOH	$1.52^{d} \pm 0.02$	$2.23^{d} \pm 0.02$	$1.14^{bc} \pm 0.03$	$1.04^{d} \pm 0.06$
60% MeOH	$1.35^{e} \pm 0.02$	$2.04^{d} \pm 0.14$	$0.47^{d} \pm 0.05$	$0.87^{e} \pm 0.01$
CV (%)	1.58	2.85	4.27	3.94

Each value in the table was the average of 3 repetitions ± standard deviation (Mean ± SD). The different letters (a, b, c...) showed significant difference at p = 0.01 according to Duncan's method.

Effects of extraction temperature on flavonoids extraction

Extraction temperature was one of the factors that greatly influenced on the extraction. If temperature increases, the materials are bloated and compounds will become more flexibly. That stimulated the extraction of substances. Otherwise, if the temperature rises too high, that will cause the chemical changes of the components in the materials and affect the quality of the compounds. Thus, suitable temperature was a very important factor. The results in Table 4 showed that if extraction temperature increases from 30^{O}C to 50^{O}C, flavonoids contents in the extracted solutions also increase. However, if the temperature rises to 70^{O}C, we can observe the reduction of extracted flavonoids contents. So, 50^{O}C was the accordant temperature for extraction of flavonoids from *H. diffusa*, *C. papaya* leaves, *P. bracteatum* leaves and *S. barbata*.

Table 4. Effects of extraction temperature on extracted flavonoids content

Temperature	Extracted flavonoids content (mg/g dw)			
	H. diffusa	*C. papaya* leaves	*P. bracteatum* leaves	*S. barbata*
30^{O}C	$2.89^{b} \pm 0.03$	$8.29^{b} \pm 0.05$	$2.18^{b} \pm 0.26$	$1.48^{c} \pm 0.11$
50^{O}C	$3.59^{a} \pm 0.06$	$9.44^{a} \pm 0.08$	$2.65^{a} \pm 0.06$	$2.56^{a} \pm 0.09$
70^{O}C	$2.74^{b} \pm 0.10$	$7.26^{c} \pm 0.11$	$2.00^{c} \pm 0.01$	$1.96^{b} \pm 0.11$
CV (%)	2.32	1.01	1.90	5.28

Each value in the table was the average of 3 repetitions ± standard deviation (Mean ± SD). The different letters (a, b, c...) showed significant difference at p = 0.01 according to Duncan's method.

The chosen extraction conditions: extraction temperature was 50^{O}C, extraction solvent was 100% methanol for *H. diffusa*, *C. papaya* leaves and *S. barbata*, 90% methanol for *P. bracteatum* leaves. Extracts prepared following chose process were used in later tests.

Extraction yields

Table 5. Extraction yields

Sample	Yield (%w/dw)
H. diffusa	19.13
C. papaya leaves	30.20
P. bracteatum leaves	12.47
S. barbata	16.47

The extraction yields of the four tested medicinal plants are presented in Table 5. The yields ranged from 12.47% to 30.20%. Among the extracts, *C. papaya* leaves produced the highest yield (30.20%), followed by *H. diffusa* (19.13%), *S. barbata* (16,47%) and *P. bracteatum* leaves (12.47%). The yields depended on extraction solvents, polarization of extracted compounds and characteristics of samples. So that, being extracted at same time and conditions, the different samples produced the different extraction yields. 100% methanol extractions yielded more components than 90% methanol extraction.

Total flavonoid contents

The total flavonoid content was expressed as mg of catechin equivalent per gram of dry weight. The total flavonoid contents in these medicinal plants ranged from 3.99 to 12.15 mg/g dw. *C. papaya* leaves contained the highest flavonoid content (12.15 mg/g dw), followed by *H. diffusa* (5.83 mg/g dw), *S. barbata* (4.01 mg/g dw) and *P. bracteatum* leaves (3.99 mg/g dw). The total flavonoid contents in these extracts ranged from 23.91 to 37.70 mg/g. *P. bracteatum* leaves contained the lowest flavonoid content in four herbs but this sample produced the lowest yield, so the flavonoid content in this extract still achieved

decent performance (30.39 mg/g), higher than the one in *H. diffusa* extract. While the total flavonoid content in *S. barbata* was equivalent to the one in *P. bracteatum* leaves, the flavonoid content in extract of this sample was only 23.91 mg/g because *S. barbata* produced higher yield than *P. bracteatum* leaves (16.47 % > 12.47%). In addition, the extract yields couldn't achieve 100%, which caused the loss và the difference among the tested samples.

Table 6. Flavonoid contents in herbs and extracts

Sample	Total flavonoid content in herb (mg/g dw)	Total flavonoid content in extract (mg/g sample)
H. diffusa	5.83 ± 0.28	28.90 ± 0.49
C. papaya leaves	12.15 ± 0.23	37.70 ± 0.73
P. bracteatum leaves	3.99 ± 0.12	30.39 ± 0.65
S. barbata	4.01 ± 0.14	23.91 ± 0.47

Scavenging activity against DPPH

The relatively stable organic radical DPPH is widely used in modeling systems to investigate the scavenging activities of several natural compounds, such as phenolics and anthocyanins, as well as crude mixtures, such as methanol or water extracts from plants. The DPPH radical is scavenged by antioxidants through the donation of electrons forming the reduced DPPH. The color changes from purple to yellow after reduction, and the accompanying decrease in absorbance can be quantified at wavelength 517 nm (Ho et al., 2012).

IC_{50} values of standard substance and extracts were presented in Tables 7 and 8. To have a basic for the comparison of the activities of tested samples, we used ascorbic acid as positive control substance due to its stronge activity against free radicals. Besides, it is also mentioned as a standard in many literature references (Khor and Wong, 2014; Dai Thi Xuan Trang et al., 2015). The results in Table 8 showed that the IC_{50} values of *H. diffusa* and *C. papaya* leaves extracts were rather low, 60.50 µg/ml and 62.17 µg/ml (~2 folds higher than IC_{50} of ascorbic acid), respectively, followed by *S. barbata* extract 69.68 µg/ml (~2.5 folds higher than IC_{50} of ascorbic acid). *P. bracteatum* leaves extract had the highest IC_{50} value, achieved 90.36 µg/ml (~3 folds higher than IC_{50} of ascorbic acid). These were similar results of many precious researches in the world about IC_{50} values of the tested herbs distributed in different sites and the plants within the same species (Ahmad et al., 2005; Indran et al., 2008; Mamadalieva et al., 2016). Because the lower the IC_{50} values, the higher the scavenging DPPH activities. So, among four tested extracts, the extracts of *H. diffusa* and *C. papaya* leaves had the best antioxidant capabilities due to DPPH radicals scavenging abilities.

Table 7. DPPH radicals scavenging activities of ascorbic acid.

Sample	Concentration (µg/ml)						IC_{50} (µg/ml)
	5	10	20	30	40	50	
Ascorbic acid	26.92 ± 0.90	33.31 ± 0.30	41.89 ± 1.00	53.28 ± 0.70	62.39 ± 0.64	66.56 ± 0.69	28.71

Table 8. DPPH radicals scavenging activities of *H. diffusa*, *C. papaya* leaves, *P. bracteatum* leaves and *S. barbata* extracts.

Sample	Concentration of extract (µg/ml)				IC_{50} (µg/ml)
	25	50	75	100	
H. diffusa	32.42 ± 0.64	47.90 ± 0.76	55.43 ± 0.86	67.58 ± 0.33	60.50
C. papaya leaves	34.35 ± 0.62	46.80 ± 0.86	52.71 ± 1.04	66.69 ± 0.60	62.17
P. bracteatum leaves	35.04 ± 0.27	39.28 ± 0.63	46.69 ± 0.19	54.43 ± 0.51	90.36
S. barbata	28.74 ± 0.23	43.74 ± 0.66	50.88 ± 0.74	63.76 ± 0.99	69.68

In addition, the antioxidants were natural substances which could avoid or reduce the damages caused by oxidant reactions. That also help human body to inhibit the damages of free radicals to cells. Several randomized controlled clinical trials which were tested in cancer patients who used the antioxidants during cancer treatment process, showed decreasing the toxic of modern medicine treatments. So, the use of antioxidants to fight free radicals in cancer treatments has given better results (Dreher and Junod, 1996). Our results showed that four tested extracts had good DPPH radical scavenging capabilities when they were compared to the positive control. Besides, there were many completed researches which is about medicinal plants distributed in different sites such as Taiwan (Shyur et al., 2005; Ho et al., 2012), Iran (Pourmorad et al., 2006) and India (Kumar et al., 2008). By comparing our results and their results, the extracts mentioned above are also great potential antioxidant.

Thus, all of four tested medicinal plants (*Hedyotis diffusa*, *Carica papaya* leaves, *Pseuderanthemum bracteatum* leaves and *Scutellaria barbata*) showed their antioxidant abilities, which contribute to bring positive results if they are used in cancer treatments. These were also the advantages for us to continue study of anti-liver cancer cells later.

CONCLUSIONS

At corresponding extraction conditions (extraction temperature: $50^{O}C$, extraction solvent: 100% methanol (*Hedyotis diffusa*, *Carica papaya* leaves and *Scutellaria barbata*), 90% methanol (*Pseuderanthemum bracteatum* leaves)), the total flavonoid contents in samples and extracts were determined. *Carica papaya* leaves and its extract contained the highest total flavonoids in the tested herbs (12.15 mg/g dw) and extracts (30.70 mg/g sample).

Basing on antioxidant activities (DPPH radical scavenging capabilities), the extracts of *H. diffusa*, *C. papaya* leaves, *P. bracteatum* leaves and *S. barbata* proved their high competence. Their antioxidant activities were ranked as follows: *P. bracteatum* leaves < *S. barbata* < *C. papaya* leaves ≤ *H. diffusa* < ascorbic acid.

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