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Full Length Research Paper

Flavonoid compounds and their antioxidant activity in extract of some tropical plants

Ali Ghasemzadeh[1]*, Maryam Azarifar[2], Omid Soroodi[3] and Hawa Z. E. Jaafar[4]

[1]Department of Agronomy, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[2]Department of Food Sciences, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran.
[3]Department of Agronomy, Jiroft Branch, Islamic Azad University, Jiroft, Iran.
[4]Department of Crop Science, Faculty of Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

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The study was counducted to investigate flavonoids content and antioxidant activities in some tropical plants namely cabbage (*Brassica oleracea*), green chilli (*Capsicum annum*), red chilli (*Capsicum annum*), carrot (*Daucus carota*), red spinach (*Amaranthus gangeticus*), white radish (*Raphanus sativus*), lemon grass (*Cymbopogon citratus*) and turmeric (*Curcuma longa*). Total flavonoid (TF) content was observed to be highest in red chili (0.939 mg/g DW) and low content of TF was observed in red spinach (0.066 mg/g DW). High performance liquid chromatography (HPLC) was employed to identify and quantify the five important flavonoid components (quercetin, catechin, kaempferol, apigenin and rutin). High content of quercetin (0.639 mg/g DW), catechin (0.14 mg/g DW), apigenin (0.047 mg/g DW) and rutin (0.069 mg/g DW) was detected from red chilli and high content of kaempferol (0.13 mg/g DW) was detected from carrot. Red chilli with high flavonoid compounds showed highest antioxidant activity (78.66%) determined by the 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay. Results suggested a good potential of flavonoid compounds and antioxidant activity in red chilli and this plant could be useful for both food flavourings and in traditional medicine.

Key words: Total flavonoid, high performance liquid chromatography (HPLC), red chilli, antioxidant activity.

INTRODUCTION

Medicinal plants and herbs have always been used by human as sources of medicines. Information on ancient uses of herbs and plants as medicines can be found in history books, old literature and pharmacopoeias. Of the 250,000 higher plant species which exist on the earth, more than 80,000 possess therapeutic properties (Satyabrata, 2007). Among these species only 7000 to 7500 species worldwide are used for their medicinal values by traditional communities. Phenolic compounds confer unique taste, flavour and health-promoting properties found in vegetables and fruits (Tomas-Barberan and Espin, 2001). Therefore, increasing the phenolic content in these plants can enhance their quality. Phenolic compounds are crucial for plants growth and reproduction, and are produced as a response to environmental factors (light, chilling, pollution, etc) and to defend injured plants (Valentine et al., 2003). Like as phenolic acids flavonoids are secondary metabolites of plants with polyphenolic structure. Flavonoids are well known for their antioxidant activity (Ghasemzadeh and Ghasemzadeh, 2011). Antioxidants are specific compounds that protect human, animal and plant cells against the damaging effects of free radicals reactive oxygen species (ROS). An imbalance between antioxidants and free radicals results in oxidative stress, will/may lead to cellular damage (Kukic et al., 2006). At present, most antioxidants are manufactured synthetically, belonging to the class of synthetic antioxidants.

The main disadvantage of synthetic antioxidants is the side effects when consumed *in vivo* (Chen et al., 1992). Flavonoids from tea, cocoa, chocolate, fruits, vegetables and wine, are highly potent antioxidant compounds that help to reduce incidence of stroke, heart failure, diabetes

*Corresponding author. E-mail: upmali@yahoo.com.

and cancer. Their anticancer effects have been thoroughly investigated. Apart from antioxidant positive effects on improving health, antioxidants are also added in food to prevent or delay the oxidation of food, initiated by free radicals formed during their exposure to environmental factors such as air, light and temperature (Hras et al., 2000). Isolated polyphenols from different plants have been considered in a number of cancer cell lines at different stages of cancer growth. For example, the isolated polyphenols from strawberry including kaempferol, quercetin, anthocyanins, coumaric acid and ellagic acid, were shown to inhibit the growth of human breast (MCF-7), oral (KB, CAL-27), colon (HT-29, HCT-116), and prostate (LNCaP, DU-145) tumor cell lines (Zhang et al., 2008; Damianaki et al., 2000). Similar results have also been reported in previous studies with wine extracts and isolated polyphenols (resveratrol, quercetin, catechin, and epicatechin) (Kampa et al., 2000), as well as green tea polyphenols (epigallocatechin, epicatechin) (Weisburg et al., 2004). The objectives of the present study were to determine total flavonoids, five major flavonoids content (quercetin, catechin, kaempferol, apigenin and rutin) and antioxidant activity of the extracts from some tropical plants.

MATERIALS AND METHODS

Plants were collected from herbal farm and TPU, University Putra Malaysia. Selected plants were: cabbage (*B. oleracea*), green chilli (*C. annum*), red chilli (*C. annum*), carrot (*D. carota*), red spinach (*A. gangeticus*), white radish (*R. sativus*), lemon grass (*C. citratus*) and turmeric (*C. longa*). Selected parts were freeze dried to constant weights before been used in the extraction. For antioxidant analysis, plant parts were made in to powder and one gram of the powder was used in the extraction using 50 ml methanol, with continuously. The solution was then swirled for 1h at room temperature using an orbital shaker. Extracts were filtered (0.45 µm) under suction and stored at -20°C for further use.

Determination of total flavonoid contents (TF)

The TF were measured following a previously reported spectrophotometric method (Bushra et al., 2009). Extracts of each plant material were diluted with water in a 10 ml volumetric flask. Initially, 5% $NaNO_2$ solution was added to each volumetric flask; at 5 min, 10% $AlCl_3$ was added; and at 6 min, 1.0 M NaOH was added. Water was then added to the reaction flask and mixed well. Absorbance of the reaction mixture was read at 430 nm. The results were expressed in mg quercetin/g DW by comparison with the quercetin standard curve, which was made in the same condition.

Analysis of flavonoid compounds

Reversed-phase HPLC was used to assay compositions of flavonoids. Agilent HPLC system consisted of a Model 1100 pump equipped with a multi-solvent delivery system and a L-7400 ultraviolet (UV) detector was used. The column type was Agilent C18 (5 µm, 4.6 × 250 mm) and the mobile phase was composed of acetic acid (aqueous) and acetic acid (aqueous) and acetonitrile (50:50 v/v). The mobile phase was filtered under vacuum through a 0.45 µm membrane filter before use. The flow rate was 1 ml/min. UV absorbance was measured at 280 to 365 nm (Wang et al., 2007). The operating temperature was maintained at room temperature. Identification of the flavonoids was achieved by comparison with retention times of standards, UV spectra and calculation of UV absorbance ratios after co-injection of samples and standards.

Antioxidant activities

1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) assay

Free radical scavenging activity was done according to the technique described by Mensor et al. (2001). Alcohol solution of DPPH (3 ml) was added to 1 ml samples containing different concentrations originating from different plants extracts. The samples were first kept in a dark place at room temperature and their absorbance was read at 518 nm after 30 min. The antiradical activity (AA) was determined using the below formula:

AA% = ((Absorbance sample−Absorbance empty sample) ×100)/Absorbance control

Blank samples contained 1 ml ethanol + 2.5 ml from various concentrations of plant extract. Control sample containing 1 ml of 0.3 mM DPPH + 2.5 ml ethanol. The optic density of the samples, the control and the empty samples were measured in comparison with ethanol. BHT (butylhydroxytoluene) and α-tocopherol were used as positive controls.

Statistic analysis

The experimental results were expressed as mean ± standard deviation of three replicates. Data were analyzed using analysis of variance by Statistical Analysis System (SAS, system 9.0, 2002). Mean separation test between treatments was performed using Duncan multiple range test and P-value of $\leq$ 0.05 was regarded as significant.

RESULTS AND DISCUSSION

Total flavonoid and flavonoid analysis by HPLC

The results obtained from the preliminary analysis of flavonoids are shown in Table 1. The total flavonoid (TF) content in the red chilli (0.939 mg/g DW) was found to be highest compared to other plants. The lowest content of TF was observed in red spinach (0.066 mg/g DW). According to the data obtained, significant differences were observed among plants extract for flavonoids content. It was shown that red chilli had more flavonoid compounds compared to other plants. It can be seen from the data in this table that quercetin content in red chili (0.639 mg/g DW), when compared with cabbage (0.033 mg/g DW), green chilli (0.058 mg/g DW), carrot (0.047 mg/g DW) and white radish (0.016 mg/g DW). Quercetin was not detected from red spinach, turmeric and lemon grass. Quercetin belongs to the flavonoids group with powerful antioxidant activity (Davis et al., 2000). It is also a natural anti-histamine and anti-inflammatory. Previous studies showed that quercetin may help to prevent cancer, especially prostate cancer (Verschoyle et al., 2007; Rietjens et al., 2005). Scambia

Table 1. Flavonoid content in selected tropical plants extract

Tropical plants	TF	Quercetin	Catechin	Kaempferol	Apigenin	Rutin
Cabbage	0.174±0.013^{cd}	0.033±0.07^{b}	ND	ND	0.042±0.032^{a}	0.037±0.021^{b}
Green chilli	0.14±0.023^{de}	0.058±0.031^{b}	0.0085±0.0028^{c}	0.027±0.003^{bcd}	ND	0.022±0.008^{bc}
Red chilli	0.939±0.018^{a}	0.639±0.044^{a}	0.14±0.036^{a}	ND	0.047±0.005^{a}	0.069±0.033^{a}
Carrot	0.362±0.031^{b}	0.047±0.022^{b}	0.058±0.002^{b}	0.13±0.121^{a}	0.018±0.011^{bc}	ND
Red spinach	0.066±0.027^{e}	ND	0.011±0.079^{c}	ND	0.021±0.009^{b}	0.019±0.042^{bc}
White radish	0.098±0.012^{de}	0.016±0.003^{b}	0.0057±0.004^{c}	0.0385±0.05^{bc}	0.014±0.006^{bc}	0.012±0.004^{bc}
Lemon grass	0.229±0.039^{c}	ND	0.037±0.009^{c}	0.054±0.004^{b}	ND	ND
Turmeric	0.094±0.019^{de}	ND	ND	0.016±0.0052^{cd}	ND	0.02±0.051^{bc}

All analyses are mean of triplicate measurements ± standard deviation. Means not sharing a common letter in columns were significantly different at P≤0.05. Results expressed in mg/g DW; ND: non detected.

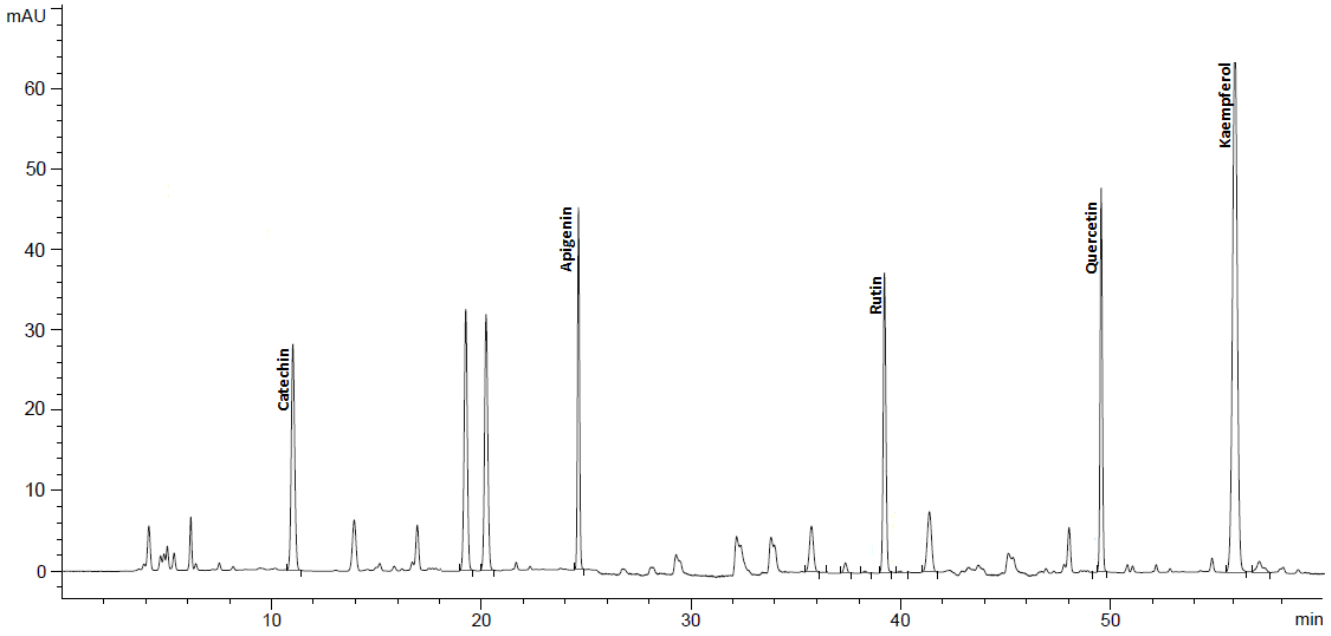

Figure 1. HPLC chromatogram of flavonoids isolated from white radish extract.

et al. (1994) reported quercetin inhibited human breast cancer cells (MCF–7 and MDA–MB–231) significantly. Regarding to the obtained results, quercetin was found as abundant flavonoid in studied plant extract. In this study, high content of catechin was detected from red chilli with concentration of 0.14 mg/g DW while, this flavonoid was not observed in cabbage and turmeric extract. Arts et al. (2002) reported catechin ability to control postmenopausal cancer in woman. He found that catechin intake may protect against rectal cancer. Kaempferol is a rare flavonoid component in plants, but it was detected in green chilli (0.027 mg/g DW), carrot (0.13 mg/g DW), white radish (0.0385 mg/g DW), lemon grass (0.054 mg/g DW) and turmeric (0.016 mg/g DW). Luo et al. (2009) showed that kaempferol inhibited the growth of ovarian cancer cell lines (91%), and A2780/CP70 (94%) by concentration of 20 and 40 µM, respectively. Apigenin belongs to the isoflavonoids group and in current study high concentration of this isoflavonoid compound was recorded in red chilli (0.047 mg/g DW). In addition, red chilli showed the highest concentration of rutin (0.069 mg/g DW). The HPLC chromatogram (Figure 1) show some of the flavonoid compounds found in white radish extract.

Antioxidant activity (DPPH assay)

The results obtained from the antioxidant activity are shown in Table 2. Free radical scavenging activity of red chilli extract represented stronger activity (78.66%) than carrot (69.04%) followed by lemon grass (62.47%). However, these values were lower than the BHT and

Table 2. Antioxidant activity (DPPH assay) and IC_{50} value in eight tropical plants extract

Plants	DPPH (%)	IC_{50}
Cabbage	58.05±0.81^{cd}	480.4±12.4^{b}
Green chilli	52.4±1.42^{de}	575.7±10.32^{a}
Red chilli	78.66±0.91^{a}	376.3±14.72^{c}
Carrot	69.04±1.15^{b}	405.3±17.63^{bc}
Red spinach	40.38±0.82^{f}	ND
White radish	47.33±0.93^{ef}	ND
Lemon grass	62.47±0.88^{bc}	448.4±16.74^{bc}
Turmeric	50.66±0.51^{de}	600.7±15.38^{a}
α-Tocopherol	88.59±1.27	112.2±7.47
BHT	81.37±0.96	139.5±11.38

All analyses are mean of triplicate measurements ± standard deviation. Means not sharing a common letter in columns were significantly different at $P \leq 0.05$. ND: non detected.

α-tocopherol as antioxidant standards. The IC_{50} (required concentration to inhibit 50% of DPPH radicals) of α-tocopherol, BHT, red chilli, carrot and lemon grass were found as 112.2, 139.5, 376.3, 405.3 and 448.4 µg/ml respectively.

In addition, a significant difference was observed between extracts for antioxidant activity. Flavonoid compounds are important due to their ability to serve as power antioxidants, which are extensively found as secondary metabolites in plants (Ghasemzadeh et al., 2010). Many phenolic compounds have been reported to possess effective antioxidant activity and anti-carcinogenic, anticancer, anti-inflammatory, anti-bacterial and anti-viral activities in a greater or lesser extent. Plant flavonoids are an important part of the diet because of their effects on human nutrition. The most important function of flavonoids is the antioxidants properties. Flavonoids have been shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and various free radicals (Ghasemzadeh and Jaafar, 2011). Red chilli with high level of TF (0.939 mg/g DW) showed highest antioxidant activity (78.66%). As the levels of TF increased from 0.066 mg/g DW to 0.939 mg/g DW the antioxidant activity increased from 40.38% to 78.66%. In present study, a positive relationship between antioxidant activities and TF contents was observed. Many researchers had shown that high total flavonoids content increases antioxidant activity and there was a linear correlation between flavonoids content and antioxidant activity (Karimi et al., 2012; Praven et al., 2007; Jung et al., 2007).

Conclusion

The antioxidant activities of the selected tropical plants extracts are still less effective than the commercial available synthetic like α-tocopherol and BHT. Quercetin was abundant flavonoid in red chilli and this plant contained more flavonoid compounds with highest antioxidant activity compared to other studied plants. This result revealed that quercetin and other flavonoid compounds may be responsible for the antioxidant activity in this plant and suggested that there seemed to be a good correlation among these compounds and antioxidant activity and other biological activity in this plant. Red chilli was showed a resource with higher flavonoid compounds, that has a wide source and low price and therefore it can serve as a cheap and important material in food. As the plant extracts are quite safe and their toxicity is a not a problem of concern unlike BHT and α-tocopherol, they could be used as antioxidant additives or as nutritional supplements.

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