ISSN: 0128-7680
Pertanika J. Sci. & Technol. **19 (1)**: 19 – 24 (2011)

Quantification of Total Phenolics in Different Parts of *Pluchea indica* (Less) Ethanolic and Water Extracts

Normala H.[1*] and Suhaimi M.I.[2]
[1]Department of Environmental Sciences,
Faculty of Environmental Studies, Universiti Putra Malaysia,
43400 UPM, Serdang, Selangor, Malaysia
[2]Faculty of Biotechnology and Life Sciences,
Universiti Industri Selangor,
40000 Shah Alam, Selangor, Malaysia
[*]E-mail: mala_upm@putra.upm.edu.my

ABSTRACT

One of the compounds present in *Pluchea indica* extracts is antioxidant which plays an important role in inhibiting free radicals and thus protects humans against infections and degenerative diseases, such as cancer, arthritis, and ageing process. The main objective of this study was to investigate and determine the total phenolic compounds of *Pluchea indica* in different concentrations of ethanolic extracts. This species was chosen because of its high phytonutrient compounds with potential medicinal properties. There was a significant difference ($P \leq 0.05$) in the total phenolic among the different parts of the tested plant. 50% of the ethanolic extract produced the highest total phenolic compounds (1775.00±86.00 to 658.95±5.00 µmol/g), followed by water extract (759.79±1.53 µmol/g) and 100% ethanol extract (352.72±22.30 to 249.29±5.37 µmol/g), respectively. In terms of the plant parts, the leaves contained the highest phenolic compounds (1775.00±86.00 µmol/g in 50% ethanol extract, 759.79±1.53 µmol/g in 100% aqueous extract and 352.72±22.30 µmol/g in 100% ethanol extract), followed by the stems (990.22±24.00 µmol/g in 50% ethanol extract, 990.22±24.59 µmol/g in 100% aqueous extract and 293.48±0.00 µmol/g in 100% ethanol extract). Meanwhile, lower total phenolic compounds were detected in the flowers (727.71±11.00 µmol/g in 50% ethanol extract, 603.81±8.46 µmol/g in 100% aqueous extract and 249.29±5.37 µmol/g in 100% ethanol extract) and roots (658.95±5.00 µmol/g in 50% ethanol extract, 450.00±10.76 µmol/g in 100% aqueous extract and 272.28±0.53 µmol/g in 100% ethanol extract). Based on these findings, *Pluchea indica* has potential medicinal properties that can be further developed to produce neutraceutical products, diet supplements or cosmetic products. However, further research should first be conducted on the effects of these compounds on laboratory animals.

Keywords: Antioxidant, phenolics, *Pluchea indica,* herbal medicines

INTRODUCTION

Food provides energy needed to perform daily functions and maintain normal metabolic processes. It contains neutraceutical compounds that are essential to prevent diseases. For example, phenolics are one of the groups of antioxidative compounds that can scavenge free radicals in human body and are continually needed in human diet. Bleary of eye problems can occur where diets are deficient in β-carotene (Beecher, 1999). The essential nutrients, such as antioxidants other than vitamins that are needed to prevent specific diseases, have been a major focus of human nutrition research for the past century. Through research, scientists have determined the amount of each essential

Received: 13 March 2009
Accepted: 25 March 2010
[*]Corresponding Author

nutrient required to prevent illnesses. The interesting part of food is the linkage between diet and illnesses. It cannot be entirely explained by the absence or presence of the various essential nutrients in our diets. A multitude of components found in food have been investigated to determine the role they play in maintaining health and reducing the risk of diseases. Numerous phytochemicals (plant chemicals) occurring in fruits and vegetables are taking centre stage in this research, as more evidence accumulates regarding their health-promoting properties (Beecher, 1999).

The importance of plants in nutrition research is the re-evaluation of the medicinal practices of past and present cultures (Zeisel, 1999). The traditional medicines are based largely on the use of plant extracts. For example, Chinese medicines predate modern medicine by thousands of years. It employs a vast array of botanical extracts for the treatment of diseases and maintenance of health. Meanwhile, East Indian Ayurvedic medicine, early European folk medicines, and native North American medicines are based largely on the use of plant extracts (Huang *et al.*, 2005).

Pluchea indica is a common herbal plant found in Malaysian coastal areas. It grows as an erect shrub. In Malaysia, Indonesia and Thailand, it is consumed as vegetable and post-natal treatment (Smith *et al.*, 1996). Other potential uses of this plant include reduction of muscle pain, haemorrhoids, dissolving kidney stones, lowering blood sugar, diuretic, promoting digestion, and elixir of longevity. Many researchers have identified that herbs have high contents of nutraceutical compounds. Meanwhile, antioxidant in herbs of plant origin is the substance that reduces oxidative damage caused by free radicals (Huang *et al.*, 2005). Free radicals are highly reactive chemicals that attack molecules by capturing electrons and thus modifying chemical structures. Antioxidants include a number of enzymes and other substances, such as vitamins C and E and beta carotene that are capable of counteracting the damaging effects of oxidation (Wright and Carpenter, 2001). Antioxidants are commonly added to food products, such as vegetable oils and prepared food, to prevent or delay their deterioration. Free radical generation is directly related with oxidation in food and biological systems.

The objectives of this study were to extract and determine the total phenolics content in different solvent extracts of *Pluchea indica.* This plant is believed to contain high phenolic compounds but limited research on nutraceutical compounds of this plant has been reported. This research may lead to further investigation on the potential of this particular plant as neutraceutical products, diet supplements or cosmetics.

MATERIALS AND METHODS

The leaves, stems, flowers, and roots of *Pluchea indica* were harvested from Kampung Kuala Kangkong, Simpang Empat, which is located in Alor Setar, Kedah, Malaysia. The samples were washed with water to remove all debris, as well as damaged and diseased portions. The leaves, stems, flowers and roots were dried in an oven at 45°C for 48 hours until a constant weight was obtained. After that, 0.2g of the dried leaves, stems, flowers and roots were separately ground using a warring blender. Each of the different plant parts of fine powder was extracted using 20 ml of distilled water, 50% ethanol, and 100% ethanol. The samples were centrifuged using Eppendorf Centrifuge at 4500 rpm for 30 minutes, and they were later stored in -20°C freezer for determination of the total phenolic compounds.

The total phenolic compounds were determined using the Folin Ciocalteu (Fluca Biochemical) reagent using the procedure proposed by Gao *et al*. (2000). 100µl extract of each different vegetative part was mixed with 200µl of Folin Ciocalteu reagent. 2000µl distilled water was then added to the mixture. Each sample was mixed with 1000µl sodium carbonate (Systerm ChemAr) and vortexes. The sample mixture was then incubated for two hours at the room temperature. All the samples were diluted into two series of dilution, 1:5 dilution and 1:10 dilution. This was to ensure the reading within the standard curve range of between 0.0 and 350mg/L gallic acid equivalent. The

determination was done in three replications. Gallic acid was used as a standard. The concentration of the total phenolics in the blue mixture was then measured at wavelength 765 nm using UV-spectrophotometer.

A stock solution of gallic acid was prepared by dissolving 2.5 mg gallic acid (Sigma) in 1.0 ml of distilled water. The test tubes were labelled according to the concentrations of solution. Standards of varying concentrations were prepared by serial dilution, as shown in Tables 1 and 2. The analysis of variance was used to detect the differences in the total phenolic compounds in the different parts of *Pluchea indica* at significant difference of $P \leq 0.05$.

TABLE 1
Dilution for gallic acid standards

Final concentration (ppm)	Volume stock (µL)	Volume of dH_2O (µL)
0	-	1000
5	2	998
10	4	996
50	20	980
75	20	970
100	40	960
250	100	900
300	20	880
375	150	850
500	200	800

TABLE 2
Sample solution dilution

Dilution factor	Volume of sample (µL)	Volume of solvent (µL)
5	200	800
10	100	900

RESULTS AND DISCUSSION

Total Phenolic Compounds

All the different solvent extraction systems used showed a wide range of the total phenolic compounds, i.e. from 249 to 1775 µmol/g, in the different vegetative parts depending on the solvent used (see *Fig. 1*).

The 50% ethanol extract contained significantly ($P \leq 0.05$) higher amounts of the total phenolic compounds compared with 100% ethanol extract and aqueous extract. It was found that the leaves contained 1775.00 ± 86.00 µmol/g, while the stems contained 990.22 ± 24.00 µmol/g of the total phenolic compounds. The total phenolic compounds in the flowers were 727.71 ± 11.00 µmol/g. There was a significantly ($P \leq 0.05$) lower phenolic compound in 50% ethanol solvents in the roots.

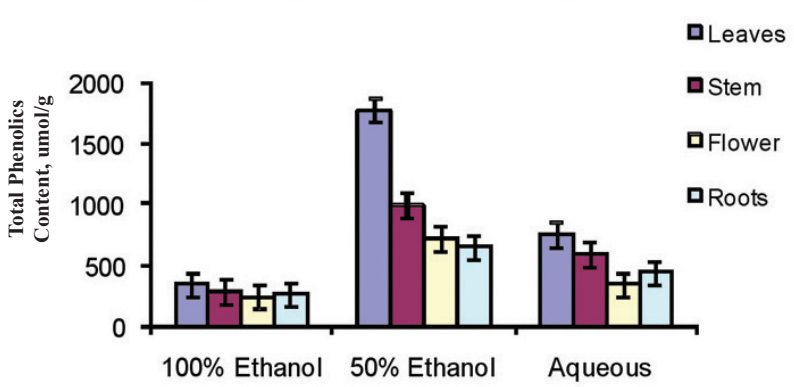

Fig. 1: Total phenolics in different solvent extractions of Pluchea indica*. The values were expressed as mean ± standard deviation (n = 3), significantly different at P ≤ 0.05*

The total phenolic compounds in the roots were 658.95 ± 5.00 µmol/g. This was due to the fact that the solvent in all different vegetative parts of *Pluchea indica* could be dissolved in 50% ethanol extract. The 50% ethanol extract allowed more extracts to form hydrogen bonds with phenolic compounds and thus, extracted more phenolic compounds than the 100% ethanol and water extract. Adding water to ethanol allows compounds that are able to bind with the oxygen molecule in the water to be extracted (Carey, 2003). From the statistical test, it could be concluded that the total phenolic compounds content was significantly influenced by the solvent and different vegetative parts. Meanwhile, water is able to form the hydrogen bonds with phenolic compounds that may not have numerous hydroxyl groups and therefore can extract more phenolic compounds than methanol alone (Carey, 2003). Extracting aqueous methanol increased the phenolics content as compared to pure methanol. These results are consistent with the previous findings of several researchers (e.g. Yen *et al*., 1996; Fukumoto and Mazza, 2000; Siddhuraju and Beecker, 2003).

The concentration of the total phenolic compounds in the leaves of water extract was 759.79 ± 1.53 µmol/g higher than that of the stems, while the concentration of total phenolic compounds was of 603.81 ± 8.46 µmol/g. The phenolic compounds in the flowers were 350.55 ± 34.58 µmol/g and this was 450.00 ± 10.76 µmol/g in the roots. The lowest phenolic compounds in aqueous was in the flowers. The aqueous extract allows the compounds to bond with the oxygen molecules in the water. This is because of the ability of the aqueous extract to form the hydrogen bonds with the phenolic compounds. From the statistical test, it was found that the total phenolic compounds content was significantly influenced by solvent and different vegetative parts. Water allowed the compounds to bind with the oxygen molecules in the water. In addition, water is also able to form hydrogen bonds with phenolic compounds that may not have numerous hydroxyl groups, and therefore, extracting more phenolic compounds than methanol alone (Carey, 2003). Water can also extract compounds such as sugar. As such, phenolic compounds that are bound to sugar may be extracted with the addition of water.

The lowest total phenolic compounds were found in 100% ethanol solvents. The leaves showed 352.72 ± 22.30 µmol/g. In the stems, the phenolic compounds were 293.48 ± 0.00 µmol/g. The phenolic compounds in the roots were 272.28 ± 0.53 µmol/g. Meanwhile, the lowest phenolic compounds in *Pluchea indica* was detected in the flowers (249.29 ± 5.37 µmol/g). The 100% ethanol extracts allowed less compounds bonding with the oxygen molecules as compared to water extract and 50% ethanol extract. Therefore, 100% ethanol solvent may cause inability to the

compounds to bond with phenolic compounds that may have numerous hydroxyl groups. It could be concluded that the total phenolic compounds content was significantly influenced by solvent and different vegetative parts. Tanaka *et al*. (1998) suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested up to 1.0 g daily from a diet rich in fruits and vegetables.

This result shows that the polarity of the phenolics in aqueous extracts of *Pluchea indica* was higher compared to 100% ethanolic solvent which has redox properties that make it act as reducing agents. Meanwhile, the phenolic compounds in plants are the largest group of compounds with antioxidative properties. Nagai *et al*. (2003) and Yang *et al*. (2002) reported that the phenolic compounds have the redox properties which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers. The redox potential of phenolic compounds plays an important role in determining the antioxidant capacity (Rice-Evans *et al.*, 1997). The plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators. Therefore, it is important to determine the total amount of phenolics in selected plant extracts. As one of the most diverse and widespread group of natural compounds, polyphenol is probably the most important natural phenolics (Agrawal, 1989). These compounds possess a broad spectrum of chemicals and biological activities including radical scavenging properties, and such properties are especially distinct for flavonols.

This study showed that the variation in the total phenolic compounds of various extracts varied widely, depending on the polarities of the solvent used. The aqueous solvents were more efficient in extracting the total phenolics as compared to their corresponding absolute ones. Similar observations were also reported in a previous study by Wang *et al.* (2004), in which various concentrations of ethanol (ranging from 15% to 96%) were investigated for the extraction of phenolic compounds.

CONCLUSIONS

The study showed that *Pluchea indica* vegetative parts have high total phenolic compounds. The highest total phenolic compounds were in 50% ethanol, followed by aqueous and the least in 100% ethanol. In the 50% ethanolic extracts with different vegetative parts, the leaves showed the highest phenolic compounds, followed by the stems, flowers, and roots. In 100% water extracts, the highest content of the total phenolics was found in the stems, and this was followed by the leaves, flowers, and roots. Finally, in 100% ethanolic extracts, the leaves had the highest total phenolics content, followed by the stems, and roots, while the lowest phenolic compound was found in the flowers.

Extraction by ethanol solvents is more efficient for phenolic compounds, and this is probably correlated to their antioxidative activity. The antioxidant of phenolics in *Pluchea indica* could act as a substance that significantly decreases the adverse effects of the reactive species, such as reactive oxygen and nitrogen species, on normal physiological functions in humans. This antioxidant can scavenge reactive oxygen species to stop radical chain reactions or inhibit the radical. The extract can be used as the production of food supplements and cosmetic products.

ACKNOWLEDGEMENT

This research project was supported through a grant from University Industri Selangor (UNISEL), Malaysia.

REFERENCES

Agrawal, P.K. (1989). Carbon-13 NMR of flavonoids. *Elsevier*. New York. 37-52.

Beecher, G.R. (1999). "Phytonutrients" role in metabolism: Effect on resistance to degenerative processes. *Nutrition Reviews*, *57*(9), S3–S6.

Carey, F. (2003). *Organic Chemistry* (5th Edn.). New York. McGraw-Hill Co.

Fukumoto, L. and Mazza, G. (2000). Assessing antioxidant and prooxidant activities of phenolic compounds. *Journal of Agricultural and Food Chemistry*, *48*, 3597-3604.

Gao, X., Bjork, L., Trajkovski, V. and Uggla, M. (2000). Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. *Journal of the Science of Food and Agriculture*, *80*, 2021–2027.

Huang, D., Ou, B. and Prior, R. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, *53*, 1841-185.

Nagai, T., Reiji, I., Hachiro, I. and Nobutaka, S. (2003). Preparation and antioxidant properties of water extract of propolis. *Food Chemistry*, *80*, 29–33.

Rice-Evans, C.A., Miller, N.J. and Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science*, *2*, 152-159.

Siddhuraju, P. and Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of Drumstick tree (*Moringa oleifera* Lam.) leaves. *Journal of Agricultural and Food Chemistry*, *57*, 2144-2155.

Smith, M.A., Perry, G., Richey, P.L., Sayre, L.M., Anderson, V.E., Beal, M.F. and Kowal, N. (1996). Oxidative damage in Alzheimer's. *Nature*, *382*, 120-121.

Tanaka, M., Kuei, C.W., Nagashima, Y. and Taguchi, T. (1998). Application of antioxidative milland reaction products from histidine and glucose to sardine products. *Nippon Suisan Gakkaishil*, *54*, 1409-1414.

Wang, S.Y. and Lin, H.S. (2004). Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *Journal of Agricultural and Food Chemistry*, *48*, 140–146.

Wright, J., Johnson, E. and Carpenter, D. (2001). Predicting the activity of phenolic antioxidants: Theoretical method, analysis of constituent effects, and application to major families of antioxidants. *JACS*, *123*, 1173-1183.

Yang, J.H., Lin, H.C. and Mau, J.L. (2002). Antioxidant properties of several commercial mushrooms. *Food Chemistry*, *77*, 229–235.

Yen, G., Wu, S. and Duh, P. (1996). Extraction and identification of antioxidant components from the leaves of mulberry (*Morus alba* L.). *Journal of Agricultural and Food Chemistry*, *44*, 1678-1690.

Zeisel, S.H. (1999). Regulation of "Nutraceuticals". *Science*, *285*, 185-186.