ANTIOXIDATIVE PROPERTIES OF VARIOUS EXTRACTS OF *LABISIA PUMILA* (KACIP FATIMAH)

Khairul F.K., Nurul Haniza M. & Zhari I.

School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Minden, Penang

A total of ten extracts of *Labisia pumila* were tested for antioxidant properties using xanthine oxidase (XO) inhibitory activity, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and total phenolics content. Water extract of the whole plant was identified as the best extract for XO and DPPH, but these extracts had the lowest total phenolic contents. This showed that there was no relationship between antioxidation activities with total phenolic content.

Key words: *Labisia pumila;* antioxidant; xanthine oxidase; DPPH; total phenolic content

Introduction

Labisia pumila (BI) F. Vill (Myrsinaceae), or locally known as Kacip Fatimah, is one of the most popular and potent ingredient used in traditional herbal preparations or "jamu" for postnatal care. The water decoction of its root or whole plant is given to a pregnant woman between one and two months before she is due to give birth. This plant is also used in treatment of flatulence, dysentery, dysmenorrhoea, gonorrhoea and "sickness in the bones"(Burkill 1966). In this paper, we evaluated the antioxidative activities of ten extracts of *L. pumila*. Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reaction (Grossman *et al.* 2001). Natural antioxidants constitute a broad range of compounds including phenolic and nitrogen compounds and carotenoids (Velioglu *et al.* 1998). Among naturally occurring phenolic compounds, flavonoids have gained a particular interest because of their broad pharmacological activity. Putative therapeutic effects of many traditional medicines may be ascribed to the presence of flavonoids (Braca *et al.* 2003). Recently, the most important reported biological properties of flavonoids are due to their antioxidant abilities, electron transfer capacity, free radical scavenging and chelating abilities,

antioxidant enzyme activation, alpha-tocopherol radicals reduction and oxidase inhibition (Hirano *et al.*, 2001). As plants produce a lot of antioxidants to control oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activities (Scartezzini & Speroni 2000).

Materials and Methods

Plants Extracts

Plants extracts of *L. pumila* was provided by several suppliers, i.e. extract labelled with "KFC" was provided by IMR, extracts labelled with "MMY" was provided by Dr Mashitah, and extracts labelled with "LP" by USM.

Xanthine Oxidase (XO) Inhibition Study

The XO activities with xanthine as the substrate were measured spectrophometrically, based on the procedure reported by Sweeney *et al.* (2001) with modification. The assay mixture consisted of 0.5 ml of extracts (100 μg/ml); 1.3 ml of 0.05 M phosphate buffer (pH 7.5) and 0.2 ml of xanthine oxidase (0.2 unit/ml) solution was pre-incubated for 10 min at room temperature. The reaction was initiated by adding 1.5 ml of 0.15 mM xanthine solution and then incubated at room temperature for 30 minutes. The absorbance was measured at 293nm. A blank was prepared without the plant material. To ensure that there was no absorbance change due to the plant material, a substrate blank (1.5 ml H_20) was added in place of substrate. Allopurinol (100 μg/ml), a known inhibitor of XO, was used as a positive control. A control experiment was performed in the absence of the extracts. All assays were done in triplicates. Xanthine oxidase activity was expressed as percent inhibition of the xanthine oxidase, calculated from the following formula:

Inhibition of xanthine oxidase (%)
= [1-(abs of sample/abs of control)] x 100

1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activities

Experiments were carried out according to the method of Brand-Williams and co-workers (1995) with slight modification. 3.9 ml of recently prepared 60 μM DPPH in methanol was mixed with 0.1 ml of sample solution in methanol. Finally, after 30 min, the absorbance was measured at 515 nm. Decrease in absorbance of DPPH solution indicated

an increase in its radical scavenging activity. This activity was expressed as percentage DPPH radical scavenging that was calculated from the following equation:

Radical scavenging activity (%)
= [100-(abs of sample / abs of control) x 100]

The DPPH solution without sample solution was used as a control. Quercetin was used as the reference standard.

Statistical Analysis

All the analyses were carried out in triplicate. Correlations were obtained by Pearson correlation coefficient in bivariate correlations using statistical software program "The Unscramble® 8.0".

Results and Discussion

XO catalyses the metabolism of hypoxanthine and xanthine to uric acid. The overproduction of this acid leads to the incidence of hyperuricemia such as gout. One of the therapeutic approaches to treat gout is the use of XO inhibitors that block the production of uric acid.

Among the extracts tested, four exhibited high XO inhibitory activity followed by allopurinol (>70%) while others had moderate activity (>50%) (Table 1). Allopurinol, a known inhibitor of XO was used as the positive control. Water extract of whole plant was identified as the best extract with XO inhibitory activity (80.0-90.3%).

DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable dimagnetic molecule. DPPH radicals react with suitable reducing agents and then electrons become paired off. The solution loses colour sitochiometrically with the number of electrons taken up. Such reactivity has been widely used to test either the ability of compounds to act as free radical scavengers, or the antioxidant activity of plant extracts. Reduction of DPPH can be observed by the decrease in absorbance at 515 nm. The free radical scavenging activities of extracts of *L. pumila* are shown in Table 1. Four out of ten extracts showed potent radical scavenging activity (>50%), and only one of them (KFC2) was better than quercetin which was used as the positive control. Considering all the extracts again, water extract of whole plant showed the best radical scavenging activity with an exception of MMY3, where MMY3 had low activity (<50%).

Table 1. Xanthine oxidase inhibition activity, radical scavenging activity and total phenolic content of ten extracts of *Labisia pumila*

	Solvent used/ Plant part	XO inhibition[a]	Radical scavenging activity[a]	Total phenolic content[b]
Allopurinol	-	78.98 ± 0.14	-	-
Quercetin	-	-	87.56 ± 1.64	-
KFC 2	Water / wp	78.20 ± 4.96	89.27 ± 2.07	2.70 ± 0.10
LP01	Methanol / wp	77.71 ± 0.49	32.45 ± 4.65	7.22 ± 0.95
LP02	Water / wp	82.13 ± 1.93	59.00 ± 4.54	6.24 ± 1.12
LP03	50% acetone / root	61.67 ± 0.23	17.80 ± 3.41	4.10 ± 0.02
LP04	Acetone / root	62.80 ± 0.30	19.08 ± 3.33	3.77 ± 0.01
LP05	Ethanol / root	69.41 ± 0.58	21.72 ± 7.18	9.00 ± 0.30
LP06	Ethanol / leaves	56.18 ± 0.69	28.20 ± 6.21	5.70 ± 3.15
MMY 2	Water / wp	88.66 ± 0.88	61.91 ± 5.11	3.86 ± 0.11
MMY 3	Water / wp	90.29 ± 1.79	37.79 ± 4.19	3.62 ± 0.11
MMY 4	Water / wp	68.85 ± 7.37	62.63 ± 4.54	2.27 ± 0.01

wp = whole plants

[a] Value expressed as percentage of inhibition

[b] Value expressed as GAE/mg extract

The total phenolic contents value of the extracts ranged from 2.27 to 9.00 gallic acid equivalent (GAE)/mg of extracts (Table 1). The highest phenolic content was found in ethanol root extract (LP05) and the lowest amount in water extract of whole plant (except LP02).

Flavanoids and phenolic have been implicated as natural antioxidants in plants, fruits and vegetables and have been well documented (Brand-Williams *et al.* 1995; Maillard & Berset, 1995). However, there was no relationship between each antioxidant activity and total phenolic content (r = -0.24, total phenolic vs XO; r = -0.34, total phenol vs. DPPH and r = 0.45, XO vs. DPPH). The same result on relationship between antioxidant activity of plant extracts and phenolic content has been previously reported (Maillard & Berset 1995). Antioxidant activity of plant extracts is not limited to the phenolics; the presence of different antioxidant components in the extracts such as sugars and other compounds that function as hydrogen donors, may erroneously contribute to the concentration of the total phenols determined with Folin-Ciocalteau reagent. Therefore, there is no simple relationship between the concentration of total phenol and the antioxidant activity when comparing plant extracts (Akowuah *et al.* 2004).

Sometimes, it is difficult to decide which of the extracts from natural sources studied can be considered the best when screening for antioxidants. Each of them could exhibit different xanthine oxidase and/or scavenging activities. In our screening, we conclude that water extract of whole Kacip Fatimah plant is the best extract as antioxidants. Further study to standardize Kacip Fatimah extract for antioxidant activity is being conducted in our laboratory.

Acknowledgements

The authors would like to thank the Ministry of Sciences for financing the project and School of Pharmaceutical Sciences, Universiti Sains Malaysia for the laboratory facilities.

References

AKOWUAH, G.A., ZHARI, I., NORHAYATI, I., SADIKUN, A. & KHAMSAH, S.M. 2004. Sinensetin, eupatorin, 3′-hidroxy-5,6,7,4′-tetramethoxyflavone and rosmarinic acid contents and antioxidative effect of *Orthosiphon stamineus* from Malaysia. *Journal of Food Chemistry* (in press).

BRACA, A., FICO, G., MORELLI, I., DE SIMONE, F., TOME, F. & DE TOMMASI, N. 2003. Antioxidannts and free radical scavenging activity of flavonol glycosides from different *Aconitum* species. *Journal of Ethnopharmacology* 86: 63–67.

BRAND-WILLIAMS, W., CURVELIER, M.E. & BERSET, C. 1995. Use of free radical method to evaluate antioxidant activity. *Food, Science & Technology* 28: 25–30.

BURKILL, I.H. 1966. *A Dictionary of the Economic Products of the Malay Peninsula.* Vol.2, Kuala Lumpur: Ministry of Agriculture and Co-operatives. Kuala Lumpur. Pp. 1323–1334.

GROSSMAN, S., BERGMAN, M., VARSHAVSKY, L. & GOTTLIEB, H.E. 2001. The antioxidant activity of aqueous spinach extract: chemical identification of active fractions. *Phytochemistry* 58: 143–152.

HIRANO, I., SASAMOTO, W., MATSUMOTO, A., ITAKURA, H., IGRASHI, O. & KONDO, K. 2001. Antioxidants ability of various flavonoids against DPPH radicals and LDL oxidation. *Journal of Nutritional Science Vitaminol* 47: 357–362.

MAILLARD, M.N., & BERSET, C. 1995. Evolutionary of antioxidant activity during kilning, role of insoluble bound phenolic acids of barley and malt. *Journal of Agricultural and Food Chemistry* 43: 1789–1793.

SCARTEZZINI, P. & SPERONI, E. 2000. Review on some plants of Indian traditional medicine with antioxidant activity. *Journal of Ethnopharmacology* 71: 23–43.

SWEENEY, A.P., WYLLIE, S.G., SHALLIKER, R.A. & MARKHAM, J.L. 2001. Xanthine oxidase inhibitory activity of selected Australian native plants. *Journal of Ethnopharmacology* 75: 273–277.

VELIOGLU, Y.S., MAZZA, G., GAO, Y.L. & OOMAH, B.D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *Journal of Agricultural and Food Chemistry* 46: 4113–4117.