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Full Length Research Paper

Phenolics and flavonoids profiling and antioxidant activity of three varieties of Malaysian indigenous medicinal herb *Labisia pumila* Benth.

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A research was carried out to investigate total phenolics (TP) and total flavonoids (TF) profiling of three varieties of *Labisia pumila* (alata, pumila, lanceolata), and their antioxidant activities in different plant parts as determined by DPPH and FRAP assays. Leaves of all varieties exhibited higher antioxidative activities compared to other parts indicating high foliar antioxidant in all varieties, especially that of alata followed by pumila and lanceolata (at 500 µg/ml) but lower values than the standards (BHT; α-tocopherol). Similarly, leaf methanolic extract showed highest TP and TF values compared to roots and stems with higher leaf TF in pumila (1.539 mg rutin equivalent (E)/g dry weight (DW)) than alata (1.323) and lanceolata (1.286). Conversely, var *alata* registered higher TP (2.65 mg galic acid equivalent (GAE)/g DW) than pumila (2.561) and lanceolata (2.435). Results showed the potential of this plant as a source of natural antioxidants, especially from the leaf.

Key words: *Labisia pumila* Benth., 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, ferric reducing/antioxidant potential (FRAP), total flavonoids content, total phenolics content, methanolic extracts, (*Labisia pumila).*

INTRODUCTION

Antioxidant research is an important topic in the medical field as well as in the food industry. Studies on the free radical-scavenging properties of flavonoids have allowed characterization of the major phenolic components of naturally named phytochemicals as antioxidants (Halliwell and Gutteridge, 1989). Furthermore, the commercial development of plants as sources of antioxidants that can be used to enhance the properties of foods, for both nutritional purposes and for preservation. Antioxidants are substances that delays or inhibits oxidative damage when present in small quantities compared to an oxidizable substrate. Hence, antioxidants can help in disease prevention by effectively neutralizing the free radicals or inhibiting damages that are created by them. Free radical-induced oxidative damage is involved with various human diseases like cardiovascular diseases, diabetes and cancer (Halliwell and Gutteridge, 1989; Sies, 1996). Numerous researches on the antioxidant activities of herbal plant properties have been reported (Djeridane et al., 2006; Chanwitheesuk et al., 2005). Halliwel and Gutteridge (1989) indicated that, the biological systems against the harmful effects of oxidative processes on macromolecules like proteins, lipids, carbohydrates and DNA can be protected by the antioxidants such as vitamin A, vitamin C, vitamin E, carotenoids, polyphenolics and flavonoid compounds. These antioxidants constitute a range of substances that contribute to the prevention and treatment of diseases in

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which reactive species of oxygen are involved. This protection can be explained by the capacity of the antioxidants of plants to scavenge free radical (Argolo et al., 2004; Tepe et al., 2005).

The importance of antioxidants in maintaining health and protection from coronary heart disease and cancer is raising great interest among scientists, food manufacturers and consumers (Robards et al., 1999). Kim and Lee (2003) suggested that, the total anti-oxidant activity of plant foods is the result of the combination of individual activities of the individual anti-oxidants present, such as ascorbic acid, tocopherols, carotenoids, and (poly) phenolics compounds; however, polyphenols seem to have the greatest anti-oxidant potential and could be the most beneficial anti-oxidants. Many of these common anti-oxidant compounds were found in fruits and vegetables, and as confirmed by Sun et al. (2002) these plants possess polyphenolic compounds such as flavonoids, and other phytochemicals such as carotenoids. Vinson et al. (2001) proposed that, fruits have been found to contain a variety of (poly) phenolics and (poly) phenolic derivative compounds, and that many of these compounds could be potential anti-oxidant sources.

Labisia pumila Benth. (*Myrsinaceae* family), commonly known as Kacip Fatimah in Malaysia, is a member of a small genus of slightly woody plant. It is a popular herb that has long been recognized to contain high bioactive compounds (Jamal et al., 1998; Jaafar et al., 2008) and demanded for its medicinal value as female tonics and health products (Jamal et al., 1998). Demand for *L. pumila* is expected to increase substantially with the recent discovery of its estrogenic activity (Jamal et al., 1998; Jaafar et al., 2008; Ayida et al., 2007). Furthermore, *L. pumila* has also been used in other medicinal treatments such as for dysentery, flatulence, dysmenorrhoea and gonorrhoea (Burkill, 1966; Rozihawati et al., 2003). There are three varieties of *L. pumila* namely, *L. pumila* var. *pumila*, *L. pumila* var. *alata* and *L. pumila* var. *lanceolata* (Stone, 1988), and each has its own use. These varieties are distinguished from each other by their petiole and leaf characteristic. *L. pumila* var. *pumila* has a marginate petiole and ovate leaf blade shape while *L. pumila* var. *alata* has a winged petiole and red vein and *L. pumila* var. *lanceolata* has a long and non-winged petiole (Stone, 1988). Information on the antioxidative capacities, and phenolics and flavonoids contents in all parts of *L. pumila* are very limited, especially that of var. *lanceolata*. In order to hasten the development of biopharmaceutical aspect of *L. pumila*, it is pertinent to establish the technical knowledge of its medicinal properties as little is known about the chemical constituents of this plant. Therefore, the aims of the present research were to determine the phenolics and flavonoids contents, and to evaluate the antioxidant activities in the leaves, stems, and roots of three varieties of *L. pumila* Benth.

MATERIALS AND METHODS

Plant material and maintainence

Seedlings of *L. pumila* varieties *alata* (Stone 6030 (KLU)), *pumila* ((Stone 7233 (KLU)), and *lanceolata* ((Stone 8385 (KLU)) were, respectively, collected from places of origin at Hulu Langat, Selangor; Sungkai, Perak; and Kota Tinggi, Johore, and raised under glasshouse for 18 months before used in the study. Healthy and uniform seedlings in terms of leaf numbers were selected from the three varieties. The leaf, stem and root of *L. pumila* Benth were cleaned, separated, and freeze dried for further analysis.

Preparation of extracts

Samples were extracted using methanol as a solvent based on Crozier et al. (1997) 2 g freeze-dried of leaf, stem and root were weighed and placed into a 100 ml conical flask, and added with 40 ml of 80% (v/v) methanol. It was followed by an addition of 10 ml of 6 M HCl. The mixture was refluxed for 2 h at 90°C and filtered by using Whatman No. 1 filter paper (Whatman, England) continued by evaporation of filtrate using a vacuumed Rotary Evaporator (Buchii, Switzerland). The dried crude extract was weighed and dissolved in methanol and stored at -20°C for further experiments.

Total phenolics content

The amount of total phenolic compounds in all samples extract was determined with the Folin-Ciocalteu's reagent according to Halici et al. (2005). Results of total phenolic contents were expressed as milligrams of gallic acid equivalents (GAE) per gram dry weight (DW).

Total flavonoid content

Total flavonoids compound was measured by the aluminum chloride colorimetric assay based on Zhishen et al. (1999). Total flavonoid compound of extracts were expressed as mg rutin equivalent/g dry weight (DW).

Antioxidant activity (DPPH free radical scavenging activity)

The free radical scavenging activities of the extracts were determined as reported by Gulcin et al. (2004). All determinations were conducted in three replications. Lower absorbance values of the reaction mixture indicated higher free radical scavenging activity. The free radical scavenging activities of the tested samples were expressed as percentage of inhibition and were calculated according to the following equation (Yen and Chen, 1994).

$$\text{Percent (\%) inhibition of DPPH activity} = [(A_0 - A_1) / A_0)] \times 100\ \%$$

Where A_0 was the absorbance value of the blank sample or control reaction and A_1 was the absorbance value of the test sample. A curve of percent inhibition or percent scavenging effect against samples concentrations was plotted and the concentration of the

Table 1. Total phenolics and total flavonoids contents in different parts of three varieties of *L. pumila*. *Similar alphabet across the columns of different varieties either for total phenolics or total flavonoids indicates a non-significant difference. N=3.

	Variety	Leaf	Stem	Root
Total phenolics[1]	Alata	2.65±0.04[a*]	0.97±0.01[f]	1.29±0.03[d]
	Pumila	2.56±0.01[b]	1.15±0.05[e]	1.30±0.02[d]
	Lanceolata	2.44±0.02[c]	0.88±0.07[g]	1.18±0.01[e]
Total flavonoids[2]	Alata	1.32±0.02[b]	0.51±0.08[ed]	0.61±0.01[c]
	Pumila	1.54±0.05[a]	0.31±0.02[f]	0.55±0.04[d]
	Lanceolata	1.29±0.01[b]	0.48±0.06[e]	0.56±0.05[cd]

[1]mg gallic acid equivalent/g dry weight (DW); [2]mg rutin equivalent/g DW; means with the different alphabets within the columns are significantly different. Values are means of three replications.

sample required for 50% inhibition was determined. The value for each of the test sample was presented as inhibition curve at 50% or IC_{50}.

Ferric reducing antioxidant power (FRAP)

The ferric reducing property of the extracts was determined using an assay described by Yen and Chen (1995). The assay was carried out in triplicate. BHT, α-tocoferol and vitamin C were used as standard antioxidant.

Statistical analysis

The antioxidant activities, total phenolic and flavonoid contents were analyzed using analysis of variance (ANOVA) with Statistical Analysis System (SAS) Version 9 (SAS Institute, Cary, NC). Significant differences among means from triplicate analyses ($p<0.05$) were determined by Duncan's Multiple Range Test.

RESULTS

Total phenolics and flavonoids contents

Results on the phenolics and flavonoids contents in the leaves, stems and roots of three varieties of *L. pumila* showed significant difference ($p<0.05$). In all three varieties, the leaf part contained higher phenolics and flavonoids compared to the root and stem (Table 1). *L. pumila* var. *pumila* had higher total flavonoids content (1.54 mg rutin equivalent /g DW) in the leaves than var. *alata (*1.32 mg rutin equivalent /g DW) and var. *lanceolata* (1.29 mg rutin equivalent /g DW). But the leaf of *L. pumila* var. *alata* contained higher total phenolics (2.65 mg galic acid equivalent/g DW) than var. *pumila* (2.56 mg galic acid equivalent/g DW) and var. *lanceolata* (2.44 mg galic acid equivalent/g DW). According to Table 1, total phenolic content of leaf in three varieties of *L. pumila* indicated siginificant difference at $p<0.05$. On the other hand, the total flavonoid content of leaf was only siginificant among *L. pumila* var. *pumila* and *L. pumila* var. *alata* and *lanceolata* but these two varieties were not significant with each other. Results of the present study indicated consistently lowest values for both total phenolics and total flavonoids contents of variety lanceolata when compared to the other two varieties, which are popularly researched on. Findings also established the main source of total phenolics and total flavonoids in *L. pumila* to be the leaves, which was about 122.6 to 176.1% difference, when compared to total phenolics in the stem, and about 96.9 to 105.9% difference when compared to the root. Regardless of varieties, the differences in total flavonoids contents in the leaves to those of the stems and the roots were in the range of 158.8 and 393.5%, and 116.39 to 178.18%, respectively.

Antioxidant activities

The antioxidant activities of *L. pumila* determined by free radical scavenging activity (DPPH) and FRAP assay methods indicated a steady increase in the scavenging activity of free radicals in all the extracts and the standards in the range between 0 and 500 µg/ml (Figures 1 and 2, respectively). As observed in total phenolics and flavonoids contents, the leaf part of all the three varieties of *L. pumila* also possessed higher DPPH (1,1-diphenyl-2-picrylhydrazyl radicals) free scavenging activities compared to the stem and root, as indicated by value of inhibition percentage at 50% or IC_{50} (Table 2). The results obtained showed that *L. pumila* var. *alata* contained higher antioxidative activities compared to var. *pumila* and var. *lanceolata*. However, these values were lower than the tested antioxidant standards, BHT (butylated hydroxittoluene) and α-tocopherol. There was no notable antioxidant activity observed in the stem and root of all three varieties.

The IC_{50} (required concentration to inhibit 50% of DPPH radicals) of α-tocopherol, BHT, and leaves of varieties *alata*, *pumila* and *lanceolata* were found to be

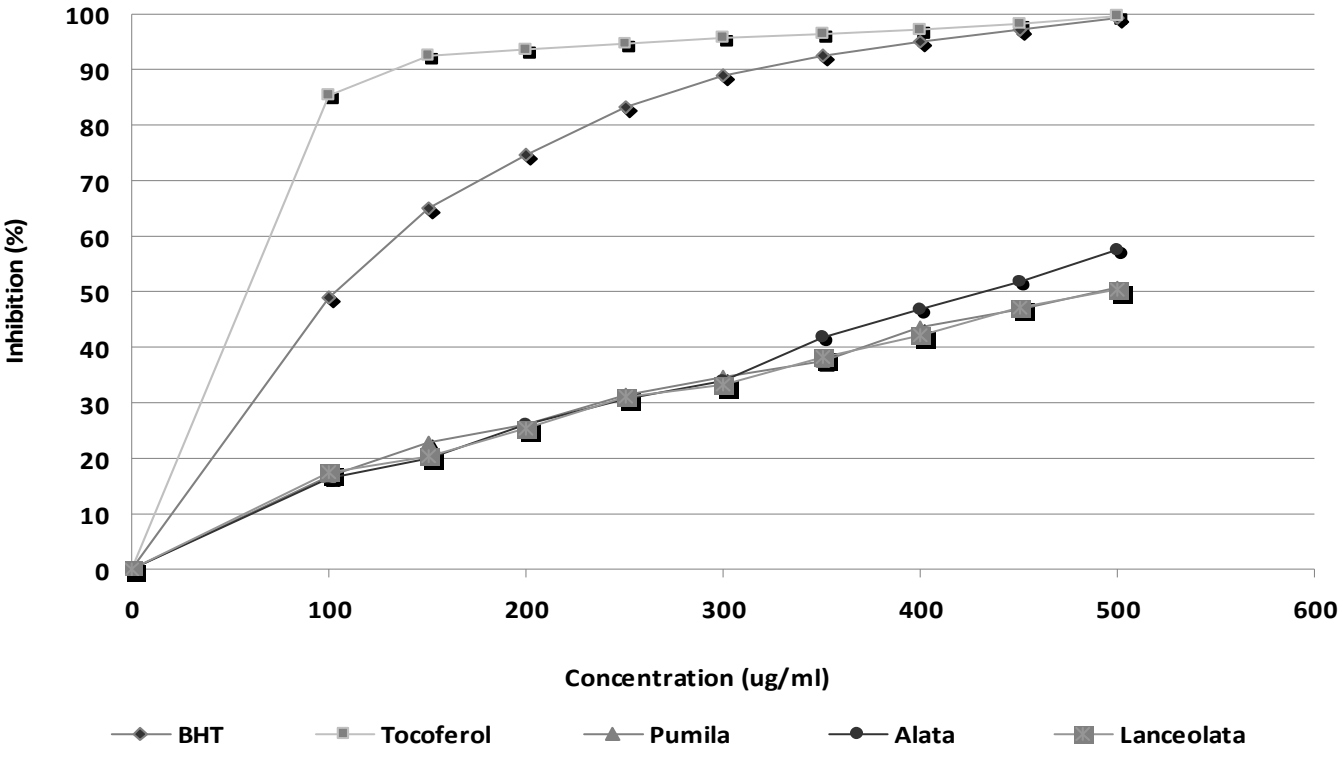

Figure 1. Free radical scvenging activity of three varieties of *L. pumila* leave extract (Pumila, –▲–; Alata, –●–; Lanceolata, –*–), BHT (butylated hydroxytoluene; –♦–) and α-tocopherol (–■–) by 1,1-diphenyl-2-picrylhydrazyl radicals. n=3.

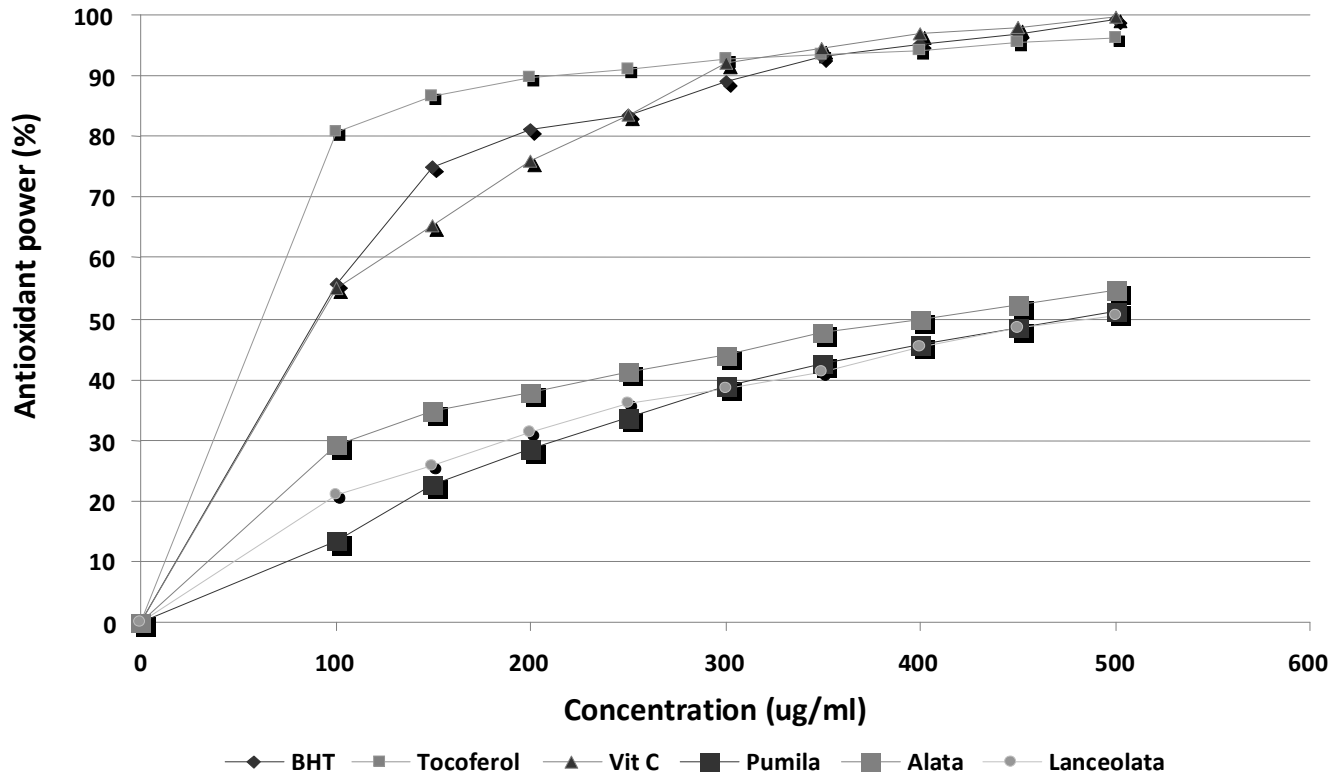

Figure 2. Ferric reducing activity power of three varieties of *L. pumila* leaves extract (Pumila, -×-; Alata,–*–; Lanceolata, –●–), BHT (butylated hydroxytoluene; –♦–) and α-tocopherol (–■–) by 1,1-diphenyl-2-picrylhydrazyl radicals and vitamin C (–▲–). n=3.

Table 2. DPPH free scavenging activities of the methanolic extracts in different parts of three varieties of *L. pumila.* BHT and α-tocopherol were used as controls. All analyses were the mean of triplicate measurements.

Variety	Extract source	IC_{50} (µg/ml)
Alata	Leaf	435.99
	Stem	>500
	Root	>500
Pumila	Leaf	492.53
	Stem	>500
	Root	>500
Lanceolata	Leaf	493.38
	Stem	>500
	Root	>500
Standard (controls)	BHT	78.752
	α-tocopherol	36.031

Table 3. FRAP activities of the methanolic extracts in different parts of three varieties of *L. pumila.* BHT, α-tocopherol and vitamin C were used as controls. All analyses were the mean of triplicate measurements.

Variety	Extract source	IC_{50} (µg/ml)
Alata	Leaf	456.07
	Stem	>500
	Root	>500
Pumila	leaf	482.55
	Stem	>500
	Root	>500
Lanceolata	leaf	487.65
	Stem	>500
	Root	>500
Controls	BHT	89.76
	α-tocopherol	61.86
	Vitamin C	90.90

36.031, 78.752, 435.99, 492.53, and 493.38 µg/ml respectively (Table 2). FRAP assay was similar to the DPPH results, indicated that the reductive potential of leaf extracts in all 3 varieties and standards increased with increasing sample concentration (Figures 1 and 2). Leaf parts compared with stem and root in all 3 varieties showed antioxidant activities; however, they were lower than that of the standards. As comparison, the reductive potential of leaf extracts in all 3 varieties and standards at concentration of 500 µg/ml (Figure 2) were as follows vitamin C > BHT > α-tocopherol > alata leaf > pumila leaf > lanceolata leaf with respective values of 99.58, 99.18, 96.17, 54.80, 51.20 and 50.68%. The FRAP activities of the reference substances and the extracts studied in this study are shown in Table 3. Leaf extracts in three varieties of *L. pumila* Benth have been revealed to possess antioxidative potential. As shown in Table 1, the lower content of total flavonoid and phenolic in root and stem extracts, might be the reason of lower antioxidant activity. Therefore, the results were well correlated with the amount of phenolic and particularly flavonoid contents.

DISCUSSION

Flavonoids are a class of polyphenolics that can be synthesized from the amino acid phenylalanine. They are the most common and widely distributed group of plant phenolics compounds, occurring in virtually all plant parts. Phenolic compounds, on the other hand, are a large group of anti-oxidant compounds found in many food systems and are very common in fruits and vegetables (Sun et al., 2002). According to Rice-Evans et al. (1997) the number of hydroxyl groups and the amount and types of conjugation are two important factors in the anti-oxidant potential of phenolics compounds. The better anti-oxidants are generally more conjugated and have numerous hydroxyl groups present (n=2 to 5), which enables the anti-oxidant to scavenge several radicals at once. As shown in Table 1, the flavonoids content of *L. pumila* var. *pumila* was higher as compared to *L. pumila* var. *alata and lanceolata.* In contrast, *L. pumila* var. *alata* showed higher phenolic content as compared to *L. pumila* var. *pumila and lanceolata.* This finding is supported by Norhaiza et al. (2009) who reported that, *L. pumila* var. *pumila* showed significantly higher total flavonoids content than *L. pumila* var. *alata* with 1.281 mg rutin equivalent/g FW. While the total phenolics content in the leaves of these two varieties did not differ dramatically, and they ranged between 2.53 and 2.55 mg/g FW. These observations are in agreement with our results.

Natural antioxidants constitute a broad range of compounds including phenolics, nitrogen compounds and carotenoids (Velioglu, 1998). Among bioactive compounds naturally occurring phenolic flavonoids have gained a particular interest because of their broad pharmacological activity. Putative therapeutic effects of much traditional medicine may be ascribed to the presence of flavonoid (Braca, 2003). The results presented here suggested that the three varieties of *L. pumila* Benth. are a potential source of natural phenolics, flavonoids and anti-oxidants. These findings are an important first step towards the development of value added products from this plant. The leaf is the main source of anti-oxidants, which have shown radical scavenging activities and reducing potential. Total phenolics and flavonoids contents were positively correlated with reducing potential and radical scavenging. These results were compatible with those obtained by Norhaiza et al. (2009) who concluded that, the antioxidant activity of leaf occurred in two varieties of *L. pumila, alata* and *pumila*. Similar to the result obtained in this research, the leaf of *L. pumila* var*. alata* indicated higher antioxidant activities as compared to *L. pumila* var*. pumila.* In the overall, the synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been widely used for many years to retard lipid oxidation. However, the safety of using these synthetic antioxidants in food industry has become a concern among scientists and leading to current interest in uncovering natural antioxidants (Karimi et al., 2010).

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REFERENCES

Argolo ACC, Sant-Ana AEG, Pletsch M, Coelho LCBB (2004). Antioxidant acitivity of leaf extracts from Bauhinia monandra. Biosour. Technol., 95: 229-233.

Ayida AW, Wan Nazaimoon WM, Farihah HS, Azian AL (2007). Effect of ovariectomy, *Labisia pumila* var. *alata* treatment and estrogen replacement therapy on the morphology of adipose tissue in ovariectomized Sprague dawley rats. J. Med. Bio. Sc. 1: Online J. available at http://www.scientificjournals.org.

Braca A, Fico G, Morelli I, De Simone, FTome F, Detommasi N (2003). Antioxidants and free radical scavenging activity of flavonol glycosides from different Aconitum species. J. Ethanpharmacol., 86: 63-67.

Burkill IH (1966). A dictionary of the economic products of the Malay Peninsula. Vol. II (I-Z) Government of Malaysia and Singapore by the Ministry of Agriculture and Cooperative, Kuala Lumpur.

Chanwitheesuk A, Teerawutgulrag A, Rakariyatham N (2005). Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. Food Chem., 92: 491-497.

Crozier A, Lean MEJ, Mc Donald MS, Black C (1997). Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce and celery. J. Agric.Food Chem., 45: 590-595.

Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N (2006). Antioxidant activity of some Algerian medicinal plant extracts containing phenolic compounds. Food Chem. 97: 654-660.

Gulcin I, Sat IG, Beydemir S, Elmastar M, Kufrevioglu OI (2004). Comparisan of antioxidant activity of clove (Eugenia caryophylata Thunb) buds and lavender (Lavandula stoechs L.). Food Chem., 87: 393-400.

Halicia M, Odabasoglua F, Suleymanb H, Cakirc A, Asland A, Bayir Y (2005). Effects of water extract of Usnea longissima on antioxidant enzyme activity and mucosal damage caused by indomethacin in rats. Phytomed., 12: 656-662.

Halliwell B, Gutteridge JMC (1989). Free Radicals in Biology and Medicine, 2nd ed.; Clarendon Press: Oxford, UK.

Halliwell B, Gutteridge JMC (1989). Lipid peroxidation: a radical chain reaction. In: Free Radical in Biology and medicine, 2nd edition. Oxford: Clarendon Press: Oxford, UK, pp. 189-267.

Jaafar HZE, Mohamad Haris NB, Rahmat A (2008). Accumulation and Partitioning of Total Phenols in Two Varieties of *Labisia pumila* Benth under Manipulation of Greenhouse Irradiance .

Jamal AJ, Houghton PJ, Milligan SR (1998). Testing the *Labisia pumila* for aestrogenic activity using a recombinant yeast scene. J. Pharm., 50: 79.

Karimi E, Oskoueian E, Hendra R, Jaafar H ZE (2010). Evaluation of Crocus sativus L. Stigma Phenolic and Flavonoid Compounds and Its Antioxidant Activity. Mole. J., 15: 6244-6256.
Kim DO, Jeong SW, Lee CY (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem., 81: 321-326.
Norhaiza M, Maziah M, Hakiman M (2009). Antioxidative properties of leaf extracts of a popular Malaysian herb, Labisia pumila. J. Med. Plants Res., 3 (4): 217-223
Rice-Evans CA, Miller NJ, Pagangaa G (1997). Antioxidant properties of phenolic compounds. Trends Plant Sci., 2: 152-159.
Robards K, Prenzler PD, Tucker G, Swatsitang P, Glower W (1999). Phenolic compounds and their role in oxidative processes in fruits. Food Chem., 66: 401-436.
Rozihawati Z, Aminah H, Lokman N (2003). Preliminary trial on rooting ability of *Labisia pumila* cuttings. In: Malaysian Science and Technology Congress, Agric. Sci., Cititel, Midvalley, Kuala Lumpur.
Sies H (1996). Antioxidants in disease, mechanisms, and therapy. Academic Press, New York.
Stone BC (1988). Notes on the Genus Labisia Lindl. (Myrsinaceae). Malayan Nat. J., 42: 43- 51.
Sun J, Chu YF, Wu X, Liu R (2002). Antioxidant and antiproliferative activities of common fruits. J. Agric. Food Chem., 50: 7449-7454.
Tepe B, Sokmen M, Akpulat HA, Sokmen A (2005). In vitro antioxidant activities of the methanol extracts of four Helichrysum species from Turkey. Food Chem., 90: 685-689.
Velioglu YS, Mazza G, Gao YL, Oomah BD (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J. Agric. Food chem., 46: 4113-4117.
Vinson JA, Su X, Zubik L, Bose P (2001). Phenol antioxidant quality and quantity in foods: fruits. J. Agric. Food Chem., 49: 5315-5321.
Yen GC, Chen HY (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agric. Food Chem., 43: 27-32.
Yen GC, Duh PD (1994). Scavenging effect methanolic extracts of peanut hulls on free-radical and active oxygen species. J. Agric. Food Chem., 42: 629-632.
Zhishen J, Mengcheng T, Jinming W (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., 64: 555-559.