Phytochemical Screening, Total Phenolic and Flavonoid Content, and Antioxidant Activity of *Leea rubra* Blume

Sarinrat Chattiranan,[1*] Sirimas Niyomthai,[1] and Warangkhana Sabaijai[1]

[1]Division of Chemistry, Faculty of Science and Technology, Phetchaburi Rajabhat University, Phetchaburi, Thailand.

[*]Corresponding author: Sarinrat Chattiranan, Division of Chemistry, Faculty of Science and Technology, Phetchaburi Rajabhat University, Phetchaburi 76000, Thailand.

E-mail address: kae_2028@hotmail.com

Abstract

The objective of this work was to evaluate the preliminary phytochemical screening, antioxidant activity, total phenolic and total flavonoid contents of the ethyl acetate and ethanol extracts from the leaves of *Leea rubra* Blume, which is traditionally used as Thai medicinal plant. Phytochemical screening of the both extracts showed positive results for alkaloids, flavonoids, tannins, steroids and triterpenoids and glycosides, whereas saponins were found in only the ethanol extract. The antioxidant activities were screened by $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays. The ethanol extract showed strong $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radical scavenging activity (IC_{50} = 0.031±0.01 mg/mL and 0.913±0.03 mg/mL, respectively) whereas the ethyl acetate extract showed weak activity (IC_{50} = 1.353±0.09 mg/mL and 33.762±0.76 mg/mL, respectively). Total phenolic contents was measured by using Folin-Ciocalteu reagent at 760 nm, the resultswas expressed in gallic acid equivalent (mg GAE/g extract) and the aluminium chloride colorimetric method was used to determine flavonoid content in terms of quercetin equivalent (mg QE/g extract). The ethanol extract showed value of total phenolic and total flavonoid contents as 257.61 ± 12.97 mg GAE/g extract and 663.59 ± 3.62 mg QE/g extract, respectively whereas the ethyl acetate showed the values of 193.45 ± 2.57 mg GAE/g extract and 347.34 ± 2.09 mg QE/g extract, respectively. The results demonstrated that the ethanol extract of the leaves of *L. rubra* Blume is a potential source of natural antioxidants. Thus, further work should be screened bioactivity, isolated, characterized and elucidated the bioactive compounds of the extract for medicinal value.

Keywords: Phytochemical screening, Phenolic content; Flavonoid content; Antioxidant activity; *Leea rubra.*

Introduction

Medicinal plants contain a number of secondary metabolites including alkaloids, flavonoids, steroids, glycosides, terpenes, tannins and phenolic compounds. These phytochemicals play an important role as source of medicines such as anti-inflammatory, anticancer, antioxidant, and antimicrobial properties [1-2]. The interest in plants derived drugs is due to its less expensive, safe, efficient and rarely side effects [3]. In recent years, numerous researches on screening antioxidant from plants have been reported [4]. The results of studies have shown that many antioxidant compounds from plants such as carotenoids, tocopherols, ascorbic acid, flavonoids and phenolic compounds are the effective nutrients to prevent oxidative stress related disease including diabetes, Alzheimer's, Parkinson's, cardiovascular diseases, cancers and aging process [5-7]. Among the diversity of antioxidant constituents from plants, phenolic and flavonoid compounds have received a great deal of attention as antioxidant to prevent diseases caused as a result of oxidative stress [8]. Phenolic compounds are secondary metabolites which occur in all parts of the plant woods, barks, stems, leaves, fruits, roots, flowers, and seeds. These substances are characterized by one aromatic ring with one or more attached-OH groups, and can be divided into 15 major structural classes such as simple phenols, phenolic acids, flavonoids, lignans and tannins [9]. Flavonoids, the most common groups of phenolic compounds, are divided into eight groups: flavonols, flavanones, flavones, isoflavones, catechins, anthocyanidins, dihydroflavonols and chalcones. The antioxidant activity of phenolic compounds are mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators [10].

Leea rubra Blume, known as Ka-tung-bi-daeng in Thai, belongs to the Vitaceae family. This species is wildly distributed from India, Bangladesh, Myanmar, Cambodia, Laos, Vietnam and Thailand [11]. *L. rubra* is a small shrub growing to about 2.5-3 m tall. It has purplish-red stems and flowers with white stamens. Fruits are dull rusty red, ripening to black. Roots and stems of this plant have been used as traditional medicine for hemorrhoid, anti-pyretics, anti-inflammatory and analgesic effects in the Lanna traditional medicine of Thailand [12]. The extracts of the stems in different solvents like ethyl acetate, ethanol and water were tested for anti-inflammatory and antioxidant activities. All the extracts showed tendency processing anti-inflammatory activity whereas the ethanol and water extracts showed strongest antioxidant activity with $ABTS^{\bullet+}$ and nitric oxide method, respectively [12]. Moreover, ethanolic extract of woods and barks of *L. rubra* Blume showed highest $ABTS^{\bullet+}$ scavenging activity as compared to the other Lanna medicinal plants [13]. The decoction of the leaves was used for wound healing [14]. In addition, the leaves of few species of Leea have been used as traditional medicines by Asians and Africans [15]. However, there is a lack of literature data on phytochemical and biological studies of *L. rubra* leaves. Therefore, the aims of this research were to screen the phytochemicals, evaluate antioxidant activity by $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays, and determine total phenolic and total flavonoid contents of *L. rubra* leaf extracts.

Figure 1. (a) leaves, (b) flowers, and (c) fruits of L. rubra Blume

Materials and Methods

Plant material

Leaves of *L. rubra* were collected in May 2012 from Muang, Kanchanaburi province. The voucher specimens were deposited in Natural Products Chemistry Research Unit, Phetchaburi Rajabhat University.

Chemicals and Reagents

Folin-Ciocalteu reagent, gallic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl radical) and Trolox ((±)-6-hydroxyl-2,5,7,8-tetramethylchromane 2-carboxylic acid) were purchased from Sigma-Aldrich (USA). ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) and quercetin were perchased from Fluka (Switzerland). Sodium carbonate, potassium persulfate, aluminium chloride and potassium hydroxide were purchased from Ajax Finechem (New Zealand). TLC plates (aluminium sheets of silica gel 60 F_{254}) were purchased from Merck (Germany). The other chemicals and solvents used in this experiment were analytical grade and purchased from Merck (Germany).

Preparation of plant extracts

The leaves were washed with tap water, dried at room temperature and powdered. The powdered plant was extracted respectively with ethyl acetate and 95% ethanol by marceration and

concentrated using a rotary evaporator. The dried crude extracts were stored in stock vials and kept in a refrigerator for further use.

Preliminary phytochemical screening

The ethyl acetate and ethanol extracts were tested for the contents of phytochemical constituents like alkaloids, flavonoids, anthraquinone glycosides, tannins, cardiac glycosides, steroids, triterpenoids, saponins, and glycosides using standard procedures.

Alkaloids: A small quantity of each extract was warmed with 2% H_2SO_4 for two minutes, filtered and a few drops of Dragendorff's reagent were added. Brown-orange precipitate indicated the presence of alkaloids [16].

Tannins: A small quantity of each extract was mixed with water, heated on water bath and filtered. A few drops of 1% $FeCl_3$ were added to the filtrate. The formation of bluish black or greenish black solution indicated the presence of tannins [17].

Glycosides: A small quantity of each extract was hydrolyzed with HCl followed by neutralized with NaOH solution. A few drops of Benedict's reagent were added. Brick red precipitate at the bottom of the test tube indicated the presence of reducing sugars [18].

Saponins: A small quantity of each extract was shaken vigorously with 5 mL of distilled water in the test tube. The formation of stable foam was taken as an indicator for the presence of saponins [3].

Flavonoids: The test samples were applied on precoated TLC plates developed in ethyl acetate: methanol: water (100: 13: 10 v/v/v) as a mobile phase, the plates were dried using air dyer and sprayed with 5% $AlCl_3$. Appearance of orange or yellow fluorescence showed the presence of flavonoids [19].

Anthraquinone glycosides: The test samples were applied on precoated TLC plates developed in ethyl acetate: methanol: water (100: 13: 10 v/v/v) as a mobile phase. The plates were then dried using air dyer and sprayed with 10% KOH in alcohol. Anthraquinone glycosides show pink spots in visible light [20].

Cardiac glycosides: For the detection of cardiac glycosides, the extracts were applied on precoated TLC plates developed in ethyl acetate: methanol: water (100:13:10 v/v/v) as a mobile phase, followed by dried and sprayed with Kedde's reagent. Pink spots were observed for cardiac glycosides [21].

Steroids and triterpenoids: For the detection of steroids, the extracts were applied on precoated TLC plates developed in ethyl acetate: methanol: water (100:13:10 v/v/v) as a mobile phase. The plates were dried and sprayed with anisaldehyde-sulfuric acid reagent. Bluish-green spots were observed for steroids and triterpenoids [22].

Determination of total phenolic content

Total phenolic content were determined by Folin-Ciocalteu method with slightly modification [23]. A diluted sample of each plant extract (2 mL of 100 µg/mL) or gallic acid (standard phenolic compound) was mixed with 5 mL of Folin-Ciocalteu reagent and 2 mL of 1 M Na2CO3. The mixtures were allowed to stand for 30 min at room temperature, and centrifuged at 3000 g for 5 min. The absorbance was then measured at 760 nm using a spectrophotometer (UV-mini 1240V Shimadzu, Kyoto, Japan). The standard curve was prepared using

10 to 100 µg/mL solution of gallic acid in ethanol. Total phenolic contents values are expressed in terms of milligrams gallic acid equivalent per gram extract (mg GAE/g extract). Data were reported as a mean ± standard deviation for three replications.

Determination of total flavonoid content

Aluminum chloride colorimetric method was used for flavonoid determimation [24]. Each plant extract (0.5 mL of 500 µg/mL in 95% ethanol) were separately mixed with 0.5 mL of 95% ethanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. The mixtures were allowed to stand for 30 min at room temperature. The absorbance of the reaction mixture was measured at 415 nm using a spectrophotometer (UV-mini 1240V Shimadzu, Kyoto, Japan). The calibration curve was done using quercetin solution at concentration of 10 to 100 µg/mL in ethanol. Total flavonoid content was expressed as milligrams quercetin equivalents per gram extract (mg QE/g extract). Data were reported as a mean ± standard deviation for three replications.

Evaluation of free radical scavenging using DPPH

The stable 2,2′-dipheyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) was used for determination of free radical scavenging activity of the extracts [25]. Briefly, 2.5 mL of $DPPH^{\bullet}$ solution (0.1 mM) was mixed with 0.5 mL of different concentrations of each extract. The mixture was kept in the dark at room temperature for 30 min, and then the absorbance was recorded at 517 nm on a spectrophotometer (UV-mini 1240V Shimadzu, Kyoto, Japan). The experiment was repeated for three times. Trolox was used as a positive control. Percentage of inhibition was calculated using the following equation:

$$(\%)\ \text{inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Evaluation of free radical scavenging using $ABTS^{\bullet}$+

For ABTS assay, the procedure was followed by the method of some modifications [26]. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 mL $ABTS^{\bullet+}$ solution with 60 mL ethanol. Fresh $ABTS^{\bullet+}$ solution was prepared for each assay. The extract (20 µL) was mixed with 2.5 mL of $ABTS^{\bullet+}$ solution for 2 h in a dark condition and the absorbance was taken at 734 nm using a spectrophotometer (UV-mini 1240V Shimadzu, Kyoto, Japan). Percentage of inhibition was calculated and compared with Trolox as follows:

$$(\%)\ \text{inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Results

Preliminary phytochemical screening

The crude extracts of ethyl acetate and ethanol were screened for alkaloids, flavonoids, tannins, saponins, anthraquinone glycosides, cardiac glycosides, steroids and triterpenoids and glycosides using the formation of color or precipitate as analytical response for the tests. Both extracts revealed the

presence of alkaloids, flavonoids, tannins, steroids and triterpenoids and glycosides while saponins were found in only the ethanol extract. The results are shown in Table 1

Table 1 Phytochemical constituents of *L. rubra* leaf extracts.

Constituent	Ethyl acetate	Ethanol
Alkaloids	–	+
Flavonoids		+
Anthraquinone glycosides		
Tannins	–	+
Saponins		+
Steroids and triterpenoids	–	+
Glycosides		+

+ = presence – = absence

Determination of total phenolic content

The amount of total phenolic was measured by Folin-Ciocalteu reagent which increasing the blue color depth is positively related with the total phenolic content. In the assay, gallic acid was used as the standard and the result showed a good linear relationship ($y = 0.0037x – 0.0286$, $R^2 = 0.9933$) (Figure 2). The total phenolic contents of the ethyl acetate and ethanolic extract were 193.45 ± 2.57 and 257.61 ± 12.79 mg GAE/g extract, respectively (Table 2).

Determination total flavonoid content

The aluminium chloride method was used for determination of total flavonoid content and the results are expressed in mg quercetin equivalent (mg QE). The amount of total flavonoid content in the extracts was then calculated using standard curve equation ($y = 0.0021x – 0.0259$, $R^2 = 0.9914$) (Figure 3). Total flavonoid content in the ethyl acetate and ethanolic extract were 347.34±2.09 and 663.59±3.62, mg QE/g extract, respectively (Table 2).

Evaluation of free radical scavenging using DPPH•

$DPPH^{\bullet}$ are free radicals stable at room temperature. These radicals are widely used for radical scavenging assay. The radical can dissolve in methanol or ethanol, and produces a violet solution that shows maximum absorption at 517 nm. The color becomes lighter when the free radicals react with antioxidants. The degree of color change depends on antioxidant concentration. Hence, the $DPPH^{\bullet}$ scavenging activity was determined by decreasing in its absorbance [27]. Trolox was used as positive control for the antioxidant activity measurement. The percent inhibition against the sample concentration was plotted to obtain the amount of antioxidant necessary that caused 50% inhibition of $DPPH^{\bullet}$ radicals (IC50). The values were found at 1.353±0.09, 0.031±0.01 and

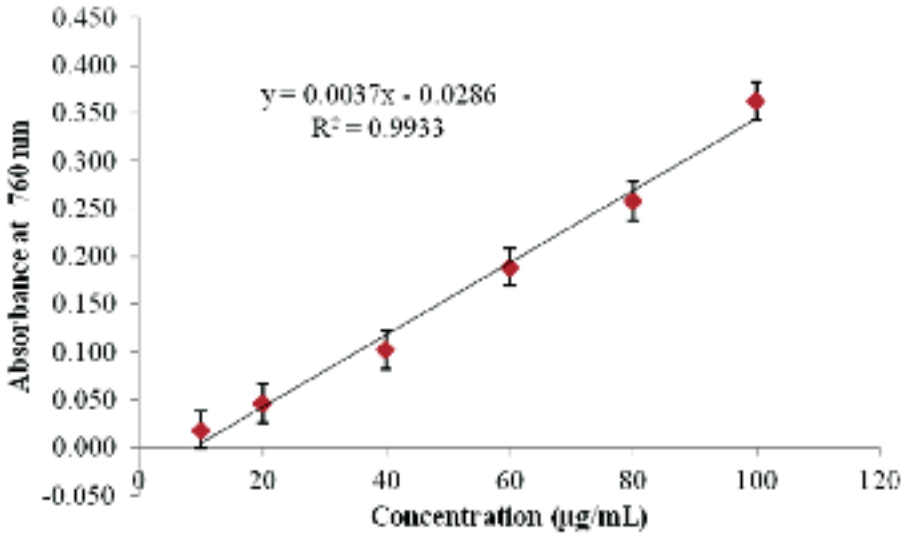

Figure 2 Standard curve of gallic acid

0.015±0.00 mg/mL for ethyl acetate extract, ethanol extract and Trolox, respectively (Table 3).

Evaluation of free radical scavenging using $ABTS^{\bullet+}$

The $ABTS^{\bullet+}$ assay is based on the ability of the antioxidants to scavenge the $ABTS^{\bullet+}$ radical cation by donation of electron or hydrogen atom. Unlike $DPPH^{\bullet}$ radical, $ABTS^{\bullet+}$ radicals must be generated by chemical reaction to give a stable blue-green solution. Like $DPPH^{\bullet}$ assay, $ABTS^{\bullet+}$ scavenging activity was determined by the decrease in its absorbance at 734 nm. The percent inhibition against the sample concentration was plotted to obtain the amount of antioxidant as IC_{50}. The results found that the ethyl acetate, ethanol and Trolox showed IC_{50} values of 33.762±0.76, 0.913±0.03 and 0.032±0.00 mg/mL, respectively (Table 3).

Discussion

In the present study, the ethyl acetate and the ethanol extract showed the presence of alkaloids, flavonoids, tannins, steroids, triterpenoids, and glycosides while saponins were observed only in ethanol extract. Alkaloids were indicated as brown-orange precipitates by using Dragendorff's reagent. Flavonoids showed positive results as orange fluorescence when sprayed with 5% $AlCl_3$. The presenceof saponins was confirmed by foam-producing, tannin showed dark green solution by 1% $FeCl_3$ solution while glycosides showed brick red precipitate with Benedict's reagent. Steroids and triterpenoids were detected by spraying with anisaldehyde-sulfuric acid reagent as blue-green spots. The other secondary metabolites including anthraquinone glycosides and cardiac glycosides were not found by using visualizing agents, 10% KOH and Kedde's reagent, respectively. Besides

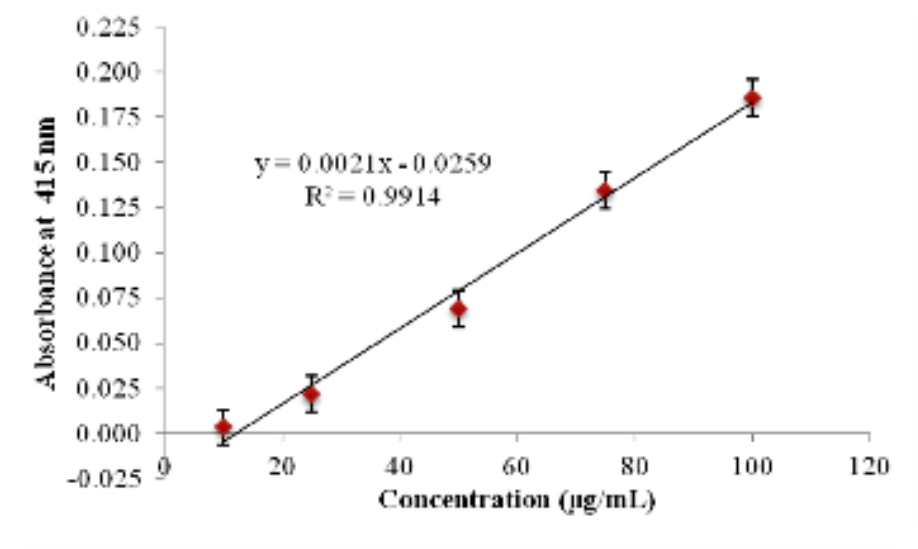

Figure 3. Standard curve of quercetin

Table 2 Total phenolic and total flavonoid content of *L. cubeba* leaf extract.

Extract	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg QE/g extract)
Ethyl acetate	193.45±2.67	347.34±2.39
Ethanol	257.61±12.79	668.59±3.52

blue-green spots of steroids and triterpenoids were observed as major constituents, the plates also showed the reddish spots which did not migrate and remain in the initial spot. According to Sannomiya et al., the red spots seem to be the catechin derivatives [28].

Results of the total phenolic and flavonoid content of the ethyl acetate and the ethanol extracts are shown in Table 2, both of them had similar phenolic content, but higher amount was observed for the ethanol extract. On the contras, we found significantly different in flavonoid contents, the ethanol extract contained almost 2 times much more quantity. The evaluation of total phenolic and total flavonoid content is influenced by a lot of factor such as assay techniques, substrate used, extraction process and polarity of solvent [29][30]. Moure et al. suggested that both methanol and ethanol offered the best results to extract phenolic compounds *from Gevuina* avellana hulls as compared to acetone [31]. In addition, Apak et al. concluded that 70% ethanol and 80% methanol was the most preferred solvent for phenolics extraction from plants [9]. Similarly, the difference of flavonoid content may be due to many factors, such as extracting solvent, analytical method and chemical structure of flavonoids.

Table 3 The DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ inhibition concentration (IC_{50}) of *L. rubra* leaf extract and standard Trolox.

Extract	IC_{50} (mg/mL)	
	DPPH$^{\bullet}$ assay	ABTS$^{\bullet+}$ assay
Ethyl acetate	1.353±0.09	33.782±0.70
Ethanol	0.031±0.01	0.913±0.03
Trolox	0.015±0.00	0.032±0.00

The IC_{50} values, as the concentration of antioxidants required for 50% inhibition free radicals, were evaluated by two methods ABTS$^{\bullet+}$ and DPPH$^{\bullet}$. Both methods showed significantly different in IC_{50} values. In this study, the lower the IC_{50} values of ethanol extract indicates the higher scavenging activity. The IC_{50} value of ethanol extract was 2 times higher than the standard Trolox in case of DPPH$^{\bullet}$ assay. These indicated the potency of ethanolic extract as free radical scavenging resource. Both DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ methods are widely used for the evaluation the scavenging capacities of plants, which obtained similar results when compared to Trolox but different in values because of reaction kinetics [32]. In addition, it is difficult to choose a suitable antioxidant assay method because each antioxidant act by several mechanisms, therefore it is quite unreliable to use solely one method [27]. As the results showed above, it was cleared that total phenolic content, especially flavonoids, were likely to influence scavenging activity of the ethanol extract [8][33][34]. Therefore, the major antioxidant constituents of these plant extracts should be further isolated and identified to search the new, potent and safe compounds for disease prevention.

Conclusion

The leaves of *L. rubra* are rich in natural antioxidants which can be further used for prevention and treatment diseases caused by oxidative stress. Further studies are required on the screening bioactivities, isolation and identification of the phytochemicals which possess strongly antioxidant activity.

Acknowledgements

The authors wish to thanks Faculty of Science and Technology, Phetchaburi Rajabhat University for financial support.

References

1. Singh, P., Shrivastava, R., Saxena, R.C., Sharma, M., Karchuli, M.S., and Tripathi, J. 2011. Phytochemical screening and evaluation of antioxidant activity of *Parkinsonia aculeata* L. (Family-Leguminosae) leaves extract. *Int. J. PharmTech Res*. 3: 1952-1957.
2. Maobe, M.A.G., Gatebe, E., Gitu, L., and Rotich, H. 2013. Preliminary phytochemical screening of eight selected medicinal herbs used for the treatment of diabetes, malaria and pneumonia

in Kisii region, southwest Kenya. *Eur. J. Appl. Sci.* 5: 01-06.

3. Yadav, R.N.S., and Agarwala, M. 2011. Phytochemical analysis of some medicinal plants.*J. Phytol*. 3: 10-14.
4. Gupta, V.K., and Sharma, S.K. 2006. Plants as natural antioxidants. *Nat. Prod. Rad.* 5: 326-334.
5. Halliwell, B. 1999. Establishing the significance and optimal intake of dietary antioxidants: the biomarker concept. *Nutr. Rev.* 57: 104-113.
6. Volko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., and Telser, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. I*nt. J. Biochem. Cell Bio.* 39: 44-84.
7. Guizani, S.V., Essa, M.M., Hakkim, F.L., and Rahman, M.N. 2012. Comparative analysis of total phenolics, flavonoid content and antioxidant profile of different date varieties (*Phoenix dactylifera* L.) from Sultanate of Oman. Int. *Food Res. J.* 19: 1063-1070.
8. Karimi, E., Jaafar, H., and Ahmad, S. 2011. Phenolics and flavonoids profiling and antioxidant activity of three varieties of Malaysian indigenous medicinal herb *Labisia pumila* Benth. *J. Med. Plants Res.* 5: 1200-1206.
9. Apak, R., Guclu, K., Demirata, B., Ozyurek, M., Celik, S.E., Bektasoglu, B., Berker, K.I., and Ozyurt, D. 2007. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with CUPRAC assay. *Molecules.* 12: 1496-1547.
10. Eghdami, A., and Sadeghi, F. 2010. Determination of total phenolic and falvonoid contents in methanolic and aqueous extracts of *Achillea millefolium. Org. Chem. J.* 2: 81-84.
11. Lok, A.F., Ang, W.F., Ng, B.Y.Q., Suen, S.M., Yeo, C.K., and Tan, H. 2011. *Leea* (VITACEAE) of Singapore. *Nature in Singapore*. 4: 55-71.
12. Kadchumsang, S. 2008. *Chemical constituents and biological activity of Leea rubra Blume ex Spreng*. Unpublished master's thesis, Chiang Mai Univesity, Bankok, Thailand.
13. Saenjum, C., Kadchumsang, S., Chansakaow, S., Suttajit, M., and Chaivysut, C. 2007. Screening of Lanna medicinal plants with anti-inflammatory property assessed by free radical scavenging activities. *IJPS.* 3: 65-73.
14. Philippine Medicinal Plants. [online] available: http://www.stuartxchange.com/ Abang-Abang. html. 2012.
15. Kubitzki, K. 2007. *The Families and Genera of Vascular Plants: Voloume IX Flowering Plants Eudicots*. New York: Springer-Verlag Berlin Heidelberg.
16. Wagner, H., and Bladt, S. 1996. *Plant Drug Analysis: A Thin Layer Chromatography Atlas.* (2nd ed.). Berlin: Springer.
17. Evans, W.C. 2002. *Trease and Evans Pharmacognosy. (15th ed.)*. Edinburgh: W.B. Saunders.
18. Nisar, M., Ali, S., and Qaisar, M. 2011. Preliminary phytochemical screening of flowers, leaves, bark stem and roots of *Rhododendron arboretum. Middle East J. Sci. Res.* 10: 472-476.
19. Harborne, J.B. 1984. *Phytchemical Methods: A Guide to Modern Techniques of Plant Analysis. (2nd ed.)*. London: Chapman and Hall.
20. Sakulpanich, A., and Gritsanapan, W. 2009. Determination of anthraquinone glycoside content in *Cassia fistula* leaf extracts for alternative source of laxative drug. *Int. J. Biomed. Pharm. Sci.* 3: 42-45.

21. Grzybowski, A., Tiboni, M., Silva, M.A.N., Chitolina, R.F., Passos, M., and Fontana, J.D. 2012. The combined action of phytolarvicides for the control of dengue fever vector, *Aedes aegypti. Braz. J. Pharmacog.* 22: 549-557.
22. Simpson, B., Claudie, D., Smith, N., Wang, J., Mckinnon, R., and Semple, S. 2010. Evaluation of the anti-inflammatory properties of Dodonaea polyandra, a Kaanju traditional medicine. *J. Ethnopharmacol.* 132: 430-343.
23. Slinkard, K., and Singleton, V.L. 1997. Total phenol analysis: Automation and comparison with manual methods. *Am. J. Enol. Viticult.* 28: 49-55.
24. Chang, C.C, Yang, M.H., Wen, H.M., and Chern, J.C. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.* 10: 178-182.
25. Tan, S.K. Osman, H., Wong, K.C., Boey, P.L., and Ibrahim, P. 2009. Antimicrobial and antioxidant activities of Swietenia macrophylla leaf extracts. *As. J. Food Ag-Ind.* 2: 181-188.
26. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., and Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Bio. Med.* 26: 1231-1237.
27. Badarinath, A.V., Mallikarjuna Rao, K., Madhu Sudhana Chetty, C., Ramkanth, S., Rajan, T.V.S., and Gnanaprakash, K. 2010. A review on *in vitro* antioxidant methods: comparisions, correlations and considerations. *Int. J. PharmTech Res.* 2: 1276-1285.
28. Sannomiya, M., Michelin, D.C., Rodrigues, C.M., Santos, L.C., Salgado., H.R.N., Hiruma-Lima, C.A., Brito, A.R.S.M., and Vilegas, W. 2005. *Byrsonima crassa* Niedenzu (IK): antimicrobial activity and chemical study. *J. Basic App. Pharm. Sci.* 26: 71-75.
29. Meyer, A.S., Heinomen, M., and Frankel, E.N. 1998. Antioxidant interactions of catechin, cyanidin, cafeic acid, quercetin, and ellagic acid on human LDL oxidation. *Food Chem.* 61: 71-75.
30. Azlim Almey, A.A., Ahmed Jalal Khan, C., Syed Zahir, I., Mustapha Suleiman, K., Aisyah, M.R., and Kamaurl Rahim, K. 2010. Total phenolic content and primary antioxidant activity of methanolic and ethanolic extracts of aromatic plants' leaves. *Int. Food Res. J.* 17: 1077-1084.
31. Moure, A., Cruz, J.M., Franco, D., Dominguez, J.M., Sineiro, J., Dominguez, H., Nunez., M.J., and Parajo, J.C. 2001. Natural antioxidants from residual sources. *Food Chem.* 72: 145-171.
32. Razali, N., Razab, R., Junit, S.M., and Aziz, A.A. 2008. Radical scavenging and reducing properties of extracts of cashew shoots *(Anacardium occidentale). Food Chem.* 111: 38-44.
33. Maisuthisakul, P., Suttajit, M., and Pongsawatmanit, R. 2007. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. *Food Chem.* 100: 1409-1418.
34. Terpinc, P., Ceh, B., Ulrih, N.P., and Abramovic, H. 2012. Studies of the correlation between antioxidant properties and the total phenolic content of different oil cake extracts. Ind. *Crop Prod.* 39: 210-217.