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Distribution of Phenolics in Various Malaysian Medicinal Plants

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Abstract: Phenolics, which are widely distributed in plant kingdom, appear to have desirable medicinal properties and play a major role in both plant and animal health. Some have been reported to be antitumor agents and to exhibit antiviral and antimicrobial activities, hypotensive effects and antioxidant properties. These compounds, either as isolates or in conjunction with other compounds, may be used for various health benefits. In this study, forty types of Malaysian medicinal plants were examined to develop an understanding of the distribution of phenolics and to give an assessment of the diversity present in the selected plants. The selection of plants was based on their frequent usages by local folks for their medicinal benefits. Total phenolic content was analyzed by Folin and Ciocalteau's phenol reagent and their concentration was expressed as Gallic Acid Equivalent (GAE). All plant samples gave positive result with varying concentrations. Highest amount was obtained from *Piper betle* L. which had phenolic content of 8986.67 mg L^{-1} GAE, while the lowest concentration of 133.33 mg L^{-1} GAE was obtained from *Canna indica* Linn. This information can be used to assess taxonomic classifications, evaluate potential sources of phenolic compounds for agricultural and pharmaceutical uses and evaluate breeding program results.

Key words: Phenolics, *Piper betle* L., *Canna indica* Linn., gallic acid equivalent

INTRODUCTION

Malaysia has about 12,000 species of flowering plants of which about 1,300 species were said to be medicinal and only about a hundred so far, have been investigated fully for their medicinal potential. The huge diversity of the Malaysian flora means it can be expected that they have well diverse chemical structures from their secondary metabolite and chemical diversity, which is one of the plus factors that makes natural products excellent venture for screening programs (Ismail, 2001). Herbal and traditional medicines have already been used to treat various diseases and improved health since thousand years ago (Cordell *et al*., 1991). It has been proved that herbs have both medicinal and nutritional values, such as garlic is used for infection and hypertension.

Herbs continue to play an important role in treatment of various diseases, particularly in developing countries, where most of the people have limited resources and do not have an access to modern treatment. Beside, with the emergence of new diseases and failure of treatment by using modern drugs people were trying to find other alternatives for their health care. The increase in demand in industrially developed countries to use alternative approaches to treat disease such as plant-based medicine was also due to the side effects associated with the used of synthetic drugs.

The extraction and characterization of active compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic value. Examples of drugs, which have been utilized for many years and still have importance, are aspirin, which was initially discovered as salicylic acid in willow bark and leaves for relieving pain and inflammation; and taxol which is recently proven to be effective against breast and ovarian cancers, was initially discovered from bark of yew trees (Huie, 2002).

Recent studies showed that various medicinal plants have been identified, studied and evaluated using modern scientific approaches. The focus of such studies has been on plant research all over the world (Tapsell *et al*., 2006; Triggiani *et al*., 2006). This area, therefore, is the most promising site for discovery of novel biologically-active substances (Lee and Houghton, 2005) and has, thus,

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played a dominant role as a source of highly effective conventional drugs for the treatment of many forms of diseases (Cordell *et al*., 1991).

Phenolic acids are plant metabolites widely spread throughout the plant kingdom. Recent interest in phenolic acids stems from their potential protective role, through ingestion of fruits and vegetables, against oxidative related diseases such as coronary heart disease, stroke and cancers (Yang *et al*., 2004). Phenolic compounds were essential for the growth and reproduction of plants and are produced as a response for defending injured plants against pathogens (Sahelian, 2005). The importance of antioxidant activities of phenolic compounds and their possible usage in processed foods as a natural antioxidant requires more research in this area.

These compounds have demonstrated promising health benefits for humans. Therefore, the aim of this study was to examine the distribution of phenolic compounds in Malaysian traditional herbs so that it could become as platform for further investigation to evaluate a potential source of phenolic compounds to be used for agriculture and pharmaceutical purposes.

MATERIALS AND METHODS

Sample collection: Forty species of Malaysian medicinal plants were collected in the states of Perlis and Perak, Malaysia. Some of the plants were bought from local market in the Selangor state and Forest Research Institute of Malaysia (FRIM).

Pre-treatment of plant samples: All the plants leaves were washed using tap water three times and one time with distilled water to clean it completely from contaminants. Then, it was dried in the drying oven (60°C) for several days. The dried samples were grounded into powder using warring blender.

Preparation of plant extracts: The dried powdered material of all plants (1 g for each plant)) was successively extracted with methanol as a solvent (10 mL for 1 g) at room temperature for 24 h with 50 rpm agitation speed. All extracts were kept at room temperature prior to the analysis of total phenolic content. The total phenolic content in the samples were determined by Folin-Ciocalteu reagent and expressed as gallic acid equivalents (mg gallic acid/L sample). Three replicates were done for each experiment. The best plant sample was chosen according to the maximum total phenolic content.

Screening of extraction media: The solvents used for screening of suitable media were methanol, distilled water, hexane and dichloromethane. All the conditions and procedures were the same for sample preparation except, the extraction time and volume of solvent used. Sampling was done just after 12, 15, 18 and 24 h. Three replicates were analyzed for each set of the experiment. The best solvent was selected on the basis of maximum total phenolic content.

Determination of total phenolic amount by Folin-Ciocalteau method: The total phenolic contents of the extracted samples were determined by Folin-Ciocalteau method (Singleton *et al*., 1999). In a 100 mL volumetric flask, 0.5 g of dry gallic acid was dissolved in 10 mL of ethanol and diluted to volume with water. For the preparation of standard curve 0, 1, 2, 3, 5 and 10 mL of the phenol stock solution was taken into 100 mL volumetric flasks and diluted to required volume with water. These solutions had concentrations of 0, 50, 100, 150, 250 and 500 mg L^{-1} gallic acid.

In 15 mL falcon tube, 2370 μL of distilled water, 30 μL of sample and 150 μL Folin-Ciocalteu reagent were added and vortexes. After 1 min, 450 μL of aqueous sodium carbonate (20%) was added and then the mixture was vortexes and allowed to stand at 40°C for 30 min before reading the absorbance. The absorbance was taken at 750 nm. All measurements were carried out in triplicate. The total phenolic acids concentration was calculated from the calibration curve, using Gallic acid as the standard and the results were expressed as mg L^{-1} of Gallic acid equivalents (GAE mg L^{-1}).

RESULTS

Screening of plant samples: Screening of plant samples was required to determine the best plant herb that is capable of producing the maximum amount of total phenolic content under controlled process conditions. Forty types of Malaysian plant herbs were screened for their bioactivity. All plant samples with a ratio of 1:10 solid to solvent were shaken for 24 h at room temperature with 50 rpm agitation speed. Methanol was used as a solvent for this experiment. Folin-Ciocalteau test was carried out to determine the total phenolic content in those samples since this reagent was sensitive to reduce compounds including polyphenols, thereby producing blue color upon reaction, which was measured by spectrophotometer (Singleton *et al*., 1999). The total phenolic concentration was calculated from the calibration curve, using Gallic acid as a standard (Fig. 1) and the total phenolic content were expressed as mg L^{-1} Gallic Acid Equivalents (GAE).

The distribution of phenolic content of all forty medicinal plant leaves is shown in Table 1. The results indicated that all plant samples gave positive results with

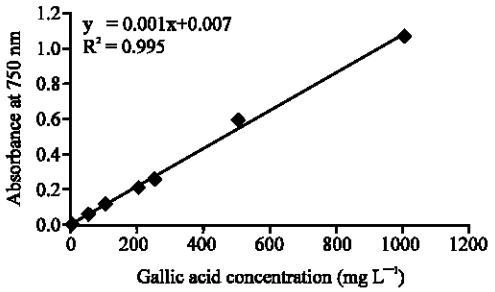

Fig. 1: Folin-Ciocalteau gallic acid standard curve

Table 1: Total phenolic content distribution in forty Malaysian medicinal plants

	Total phenolic concentration (GAE mg L^{-1})			
	Sample			
Plant species	1	2	3	Average
Leucaena leucocephala	3620	3900	4160	3893.33
Bletilla striata (Thunb) Reichb.f	850	720	430	666.67
Piper betle L.	8320	9170	9470	8986.67
Pedilanthus tithmoloidesc (L.) poit	920	530	720	723.33
Ervatamia divarcata (L.) Burk	2150	770	400	1106.67
Costus speciosus	2930	910	1110	1650.00
Cosmos caudatus	4940	4460	4040	4480.00
Orthosiphon aristatus	1280	1140	1150	1190.00
Canna indica Linn.	-10	60	350	133.33
Asystasia gangetica	-70	-60	-70	-66.67
Averrhoa carambola	1750	1750	2020	1840.00
Crinum asiaticum L.	20	110	50	60.00
Pluchea indica (L.) Less	610	590	620	606.67
Averrhoa bilimbi	1590	1530	1530	1550.00
Lantana camara Linn.	2180	1880	2120	2060.00
Peperomia pellucid	490	680	530	566.67
Euphorbia tircalli L.	970	940	9000	3636.67
Centella asiatica	560	530	660	583.33
Eugenia polyantha	3520	3510	3590	3540.00
Anacardium occidentale	4480	5780	5610	5290.00
Morinda citrifolia	300	360	310	323.33
Ixora stricta Roxb.	4530	4660	4900	4696.67
Strobilanthes crispus	870	960	840	890.00
Catharanthus roseus (L) G.Don	1010	1070	1050	1043.33
Solanum tarvum	670	490	580	580.00
Gynura procumbens	180	140	170	163.33
Piper sarmentosum Roxb.	1320	1460	1320	1366.67
Azadirachta excelsa	580	710	480	590.00
Rhoeo discolor (L.Her) Hance	260	260	3130	1216.67
Cucumis sativus	660	610	550	606.67
Azadirachta indiana A. juss	690	910	740	780.00
Pereskia saecnarosa	500	590	640	576.67
Piper Nigrum L.	290	270	360	306.67
Murraya koenigi	1240	1300	1210	1250.00
Labisa pumila	1900	1850	1850	1866.67
Curcuma xanthorrhiza	910	930	990	943.33
Gendarusa vulgaris	1010	1040	1040	1030.00
Caesalpinia pulcherrima (L.) Sw	3050	2900	3210	3053.33
Acorus calamus	340	360	480	393.33
Micromelum pubescens	3310	2930	2840	3026.67

varying concentrations except for *Asystasia gangetica*. Highest amount was obtained from *Piper betle* L., which has phenolic content of 8986.67 mg L^{-1} GAE, while the lowest concentration of 133.33 mg L^{-1} GAE was obtained from *Canna indica* Linn. Second best plant which gave higher phenolic content of 5290.0 mg L^{-1} GAE was *Anacardium occidentale*.

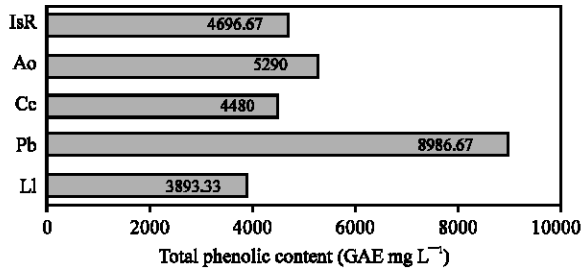

Fig. 2: Total phenolic content (GAE mg L^{-1}) of five plants sample which gave higher result in methanol extract. There are *Ixora atricta* Roxb (IsR), *Anacardium occidentale* (Ao), *Cosmos caudatus* (Cc), *Piper betle* L. (Pb) and *Leucaena leucocephala* (Ll)

Figure 2 shows the best five plant herbs which have higher amount of total phenolic content in plant leaves. Further screening of various solvent was demanded because this result was obtained only for methanol extract using fixed process conditions. This study has included the result of screening for the best plant only.

Screening of extraction media: Screening of the extraction media was done to determine the best solvent that can extract the maximum amount of total phenolic content using fixed process conditions. *Piper betle* (L.) was selected as the potential plant herb for the screening of four solvents: methanol, dichloromethane, hexane and distilled water. The process conditions (time and amount of plant material to solvent) for this extraction were slightly different from screening of potential plant herb. The samples were analyzed for total phenolic content just after 12, 15, 18 and 24 h.

Methanol showed the highest amount of total phenolic content right after 15 h that is 2883.33 GAE mg L^{-1}, followed by dichloromethane and distilled water while hexane had the lowest values (Fig. 3). The amount of total phenolic content increased with time for all solvents but slowly decreased at the end of extraction time. Extraction by using dichloromethane, distilled water and hexane showed the highest amount of total phenolic content just after 18 h extraction but extraction with methanol showed a decreasing trend over increased period of time. Highest amount of TPC obtained was 2706.67 GAE mg L^{-1} by using the solvent dichloromethane and 1423.33 GAE mg L^{-1} with distilled water. Hexane gave the lowest value of all that is

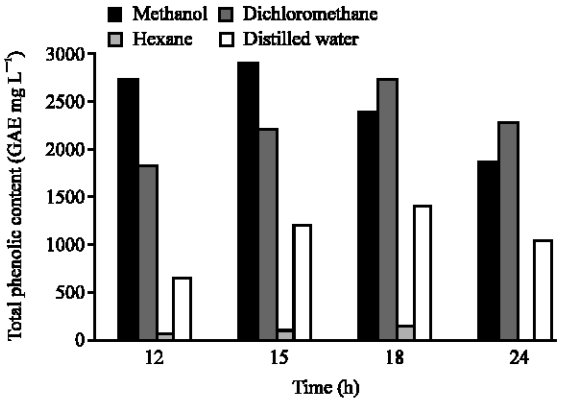

Fig. 3: Total phenolic content (GAE mg L^{-1}) obtained by extracting with four solvents: methanol, dichloromethane, hexane and distilled water

150 GAE mg L^{-1}. From the results obtained, five plants were selected as potential sources (Fig. 2). Methanol was selected as potential solvent for further optimization studies.

DISCUSSION

Medicinal plants constitute an effective source of both traditional and modern medicines; herbal medicine has been shown to have genuine utility. About 80% of rural population depends on it as primary health care (Akinyemi *et al*., 2005). Over the years, the World Health Organization advocated that countries should have interact in traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non microbial and other diseases such diabetes, as well (World Health Organization, 1978).

The results of the study showed that the entire forty plants sample gave positive result for total phenolic content except *Asystasia gangetica.* The plant extracts showed negative value which indicated no phenolic content present in the extract using conditions applied in the experiment. *Piper betle* (L.) gave the highest result in methanolic extract. This plant was known among Malaysian traditional folks to treat headaches, arthritis and joint pain. It also can be used to heal wounds. Second best plant which gave higher phenolic content was *Anacardium occidentale.* The bark and leaves of this plant were believed to have medicinal applications such as treating inflammation, diarrhea treatment and reduction of blood pressure. The fruit of this plant can be eaten fresh or as a juice and nuts can be roasted.

All selected solvents; used for solid liquid extraction of TPC from *Piper betle* (L.) showed positive results with Folin-Ciocalteau reagent but showed varying degree of total phenolic content. Hexane was not a suitable solvent for the potential plant because the result was very low. It might be because the process conditions applied for this extraction was not suitable for total phenolic content extraction. Perhaps it requires further optimization to improve the result. However, the main purpose of this research was to establish the distribution of phenolic content in Malaysian traditional herbs and was successfully achieved.

Earlier studies showed that phenolic compounds have high potential for improving human health. Furthermore, this component plays an important role in plant as a defender against plant's predator and pathogens. Extra virgin olive oil, which contains abundance of phenolic antioxidants including simple phenols, aldehydic secoiridoids, flavonoids and lignin, was believed to have potential as anticancer compound (Owen *et al*., 2000). All of these phenolic substances were potent inhibitors of reactive oxygen species attack on, e.g. salicylic acid, 2-deoxyguanosine. Currently, there was growing evidence that reactive oxygen species were involved in the aetiology of fat-related neoplasms such as cancer of the breast and colorectum (Owen *et al*., 2000).

Phenolic compounds in olive oil, which also have antioxidant, anti-inflammatory and anti-clotting properties, may explain cardiovascular health benefits associated with the so-called Mediterranean Diet (Ruano *et al*., 2005). The study shows the importance of phenolic compounds towards human health as well as to plant defenses.

CONCLUSION

The used materials and adopted methods have fulfilled the objective of this project to discover the distribution of phenolic compounds in Malaysian plant herbs. Malaysia can be a key global player in the herbal medicine industry with its rich biological heritage, cultural background and trade links. Nowadays, medicinal plant was a source of great economic value in Malaysia. Results obtained in this research show that Malaysian plants herbs contain valuable components which have a great potential towards improving human health. *Piper betle* L. gave the highest amount of phenolic content compared to other herbs. Methanol was chosen as a potential solvent, as it has the ability to extract the highest amount of total phenolic content from a plant sample. It can be concluded that most of Malaysian traditional herbs used in this study have potential to be used as an alternative

treatment source for various diseases as long as the benefit is scientifically proven. This information hopefully will trigger a new interest among modern scientists to investigate further in this area.

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