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RESEARCH ARTICLE

Free Radical Scavenging Activity of Some Plants Available in Malaysia

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ABSTRACT

The present study was carried out to determine the free radical scavenging properties of some plants found in Malaysia such as, *Muntingia calabura*; *Bauhinia purpurea*; *Dicranopteris linearis*; *Melastoma malabathricum*; *Corchorus capsularis*. The air-dried leaves of each plant (20 g) were soaked in distilled water (1:20; w/v) for 72 h at room temperature. The collected supernatants were tested for the free radical scavenging activity against the DPPH and superoxide anion radical scavenging assays. All extracts were found to show remarkable antioxidant activity in both assays with the percentage of inhibition (%) yielded 94–99% and 83–100%, respectively. Phytochemicals screening of all plants demonstrated the presence of flavonoids, saponins, triterpenes and steroids, but not alkaloids. Tannins was detected only in the leaves of *M. calabura*, *D. linearis*, *M. malabathricum*. The ability to scavenge free radicals indicates these plants could be used as a new source of antioxidant agents, and the activity seen could be attributed to the synergistic effect of various bioactive compounds present in these extracts, particularly of the flavonoids type. Further study has been designed in our laboratory to isolate and to identify the bioactive compounds responsible for the observed antioxidant activity.

Keywords: *Muntingia calabura; Bauhinia purpurea; Dicranopteris linearis; Melastoma malabathricum; Corchorus capsularis; Aqueous extract; Antioxidant activity*

Various forms of activated oxygen, generally known reactive oxygen species (ROS), have been implicated in many diseases, i.e. cancer, diabetes, artherosclerosis and heart disease [1]. ROS, which can be classified into free radicals (i.e. superoxide ions (O^{2-}) and hydroxyl radicals (OHÕ)) and non-free-radicals (hydrogen peroxide (H_2O_2)) [2,3], are produced from endogenous sources within the living organisms *via* various mechanisms (i.e. normal aerobic respiration, stimulated poly-morphonuclear leukocytes and macrophages, and peroxisomes) or from exogenous sources (tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides) [4-6]. Free radicals, generated *in vivo* due to the various biochemical reactions occuring in the living tissues, are chemical species that have tendency to rob electrons from other molecules in the immediate surrounding in order to replace their own losses. This process will lead in damage of crucial bio-molecules including those present in cell membranes, mitochondria, DNA, etc. and thus predisposing various pathophysiological states if not effectively scavenged. Although tissue injury leads to the generation of ROS, the ROS can also cause tissue injury when present in high concentration within the tissues/cells [7].

It is well known that all aerobic organisms possess antioxidant mechanisms to protect against the oxidative damages, and various types of enzymes responsible for the removal or repair of the damaged molecules [8]. Even so, these natural mechanisms can be ineffective sometimes and therefore, dietary intake of antioxidants is essential [9,10]. The fact that synthetic antioxidants (i.e. butylated hydroxytoluene and butylated hydroxyanisole), commonly used in processed foods, possessed some side effects have limited their use as antioxidant agents [11,12]. Studies have demonstrated a converse relationship between the consumption of antioxidant-rich plants/vegetables and the incidence of human diseases [13]. Thus, research to find the new sources of natural antioxidants is important. Also, in recent years, attentions have been directed towards the possible therapeutic potential of antioxidants in controlling degenerative diseases associated with marked oxidative damage. Various plants have been shown to possess significant antioxidant property [14-16] and differ-

Table 1. The Free Radical Scavenging Activity of Aqueous Extracts of Several Plants Found in Malaysia

Aqueous Sample	DPPH Radical Scavenging (%)	Superoxide Scavenging (%)
M. calabura	94.80 ± 1.14	83.70 ± 2.05
D. linearis	96.60 ± 0.60	89.40 ± 0.80
M. malabathricum	98.30 ± 0.75	96.80 ± 1.25
B. purpurea	94.90 ± 1.05	100.00 ± 0.00
C. capsularis	97.50 ± 1.06	94.90 ± 0.50

ent classes of phytochemicals have been demonstrated to be responsible for the plants' antioxidant activity [17-19]. The aim of the present study was to determine the antioxidant activity of the leaves aqueous extracts of five plants available in Malaysia, namely *Muntingia calabura*, *Dicranopteris linearis*, *Melastoma malabathricum*, *Bauhinia purpurea* and *Corchorus capsularis*.

MATERIALS AND METHODS

Plant Material

The leaves of *M. calabura*, *D. linearis*, *M. malabathricum* and *B. purpurea* were collected from its natural habitat in Shah Alam, Selangor, Malaysia whereas the leaves of *C. capsularis* were collected from its natural habitat in Alor Setar, Kedah, Malaysia, between June and September 2005. They have been identified by Mr. Shamsul Khamis, a botanist from the Institute of Bioscience, Universiti Putra Malaysia, Malaysia and voucher specimens have been deposited at the Herbarium of the Laboratory of Natural Products, Institute of Bioscience, UPM, Serdang, Selangor, Malaysia.

Preparation of Aqueous Extract of Plants

The leaves of all plants were washed and rinsed with tap water and then oven-dried for 72 hours at 40°C. The dried leaves were then ground into small particles, weighed (40 g) and soaked for 72 h in distilled water (dH_2O) (1:20; w/v). The supernatant of each plant was collected and filtered using Whatman No. 1 filter paper and then subjected to the free radical scavenging assays.

DPPH Radical Scavenging Activity

Assay for DPPH free radical scavenging potential is based on the scavenging activity of stable DPPH free radicals [15]. Reaction mixtures containing test samples dissolved in methanol and 200 μM DPPH (Sigma) in ethanolic solution in a 96-well microtiter plate were incubated at 37 °C for 30 min. After the reaction, absorbance was then measured at 520 nm, and percent inhibition was calculated.

Superoxide Anion Radical Scavenging Activity

The superoxide anion radical scavenging activity was performed using the method of Okamura et al. [16] with some modification. This assay is based on the removal rate of xanthine/xanthine oxidase-generated superoxide by measuring the reduction of nitro blue tetrazolium (NBT). The sample solution (0.1 mg/mL) in 5% DMSO was added to 1 mL of a mixture of 0.1 mM xanthine and 0.2 mM NBT (Sigma) in 50 mM potassium phosphate buffer (pH 7.5) containing 0.05 mM EDTA. Xanthine oxidase (0.1 mL) (Sigma, USA; 0.8 unit/mL) diluted in 50 mM phosphate buffer (pH 7.5) was added, and the resulting mixture was incubated at 37°C for 20 min. Addition of 2 mL of 2.5 N HCl to the mixtures terminated the reaction, followed by increase of coloration of NBT, which was measured at 540 mM. The percent of removal rate by sample was calculated relative to the control.

Phytochemical Screening of the Leaves of M. calabura, D. linearis, M. malabathricum, B. purpurea and C. capsularis

The phytochemical screening of the selected plants' leaves was carried out according to the standard screening tests and conventional protocols as described by Ikhiri et al. [20].

HPLC Profiling of the Leaves Aqueous Extracts of M. calabura, D. linearis, M. malabathricum, B. purpurea and C. capsularis

HPLC profiling of the plants' leaves aqueous extracts were carried out in the Laboratory of Phytomedicine, Forest Research Institute of Malaysia, Kepong,

Table 2. The Phytochemical Screening of Several Plants Found in Malaysia

Constituents	*M. calabura*	*D. linearis*	*M. malabathricum*	*B. purpurea*	*C. capsularis*
Flavonoids	+++	+	++	+	++
Triterpenes	++	+	++	+	+
Tannins	+	+	++	-	-
Alkaloids	-	-	-	-	-
Saponins	+	+++	+	++	+
Steroids	+++	+++	+++	+++	++

For flavonoids, tannins, triterpenes and steroids – + – weak colour; ++ – mild colour; +++ – strong colour
For saponins – + – 1-2 cm froth; ++ – 2-3 cm froth; +++ – >3 cm froth
For akalioids – + – negligible amount of precipitate; ++ – weak precipitate; +++ – strong precipitate

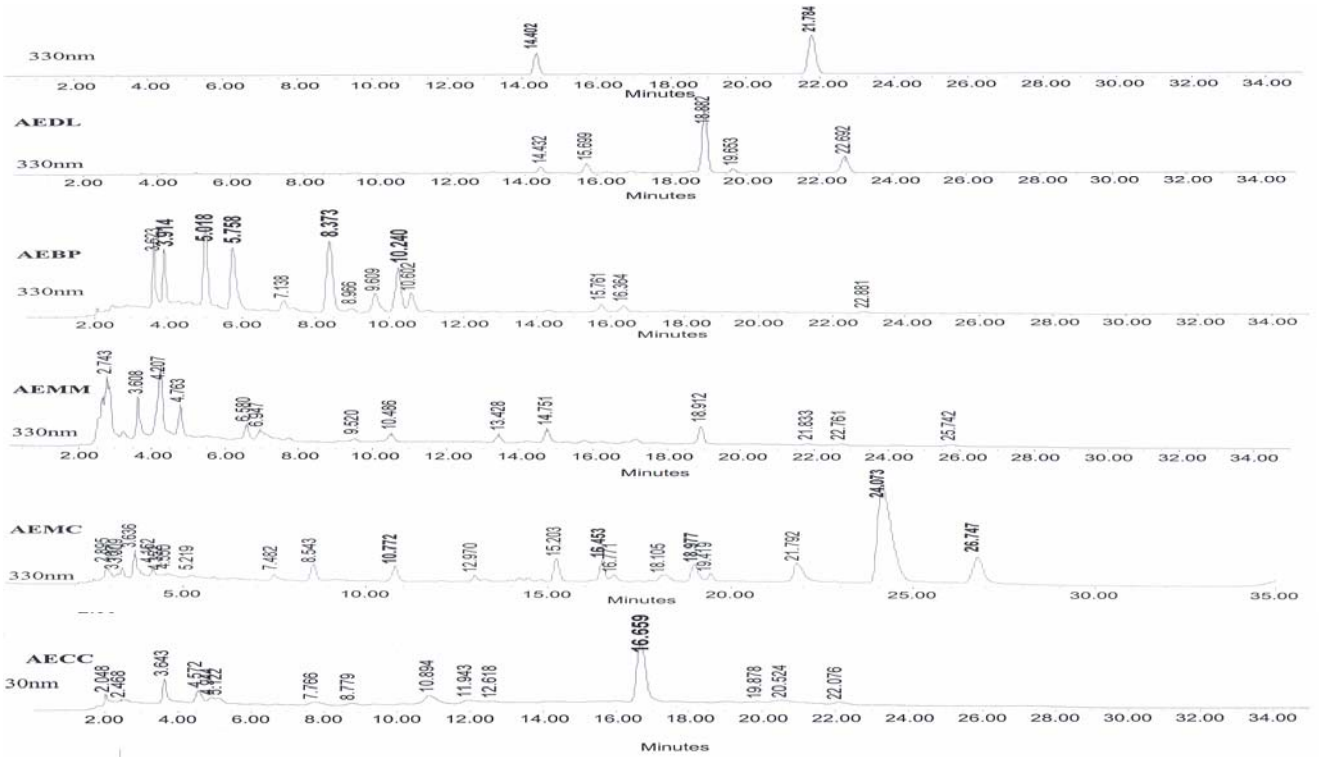

Fig 1. HPLC Profiles of the Selected Plants at 300 nm (AEDL – Aqueous extract of *D.linearis*; AEBP – Aqueous extract of *B. purpurea*; AEMM – Aqueous extract of *M. malabathricum*; AEMC; Aqueous extract of *M. calabura*; AECC – Aqueous extract of *C. capsularis*)

Malaysia. A Waters Delta 600 with 600 Controller and Waters 2996 Photodiode Array (Milford, MA, USA) equipped with an autosampler, online degasser and column heater was used for HPLC analysis. Data were analysed and processed using the installed Millenium 32 Software (Waters Product). The sample were separated at 27°C on a minibore Phenomenex Luna 5µm C_{18} column with dimensions 250 x 4.60 mm using a one step linear gradient. The solvents were (A) 0.1% orthophosphoric acid and (B) acetonitrile. The elution system was as follows: 0-12 min, 0-15% B; 12-22 min, 15-25% B; 22-35 min, 25-15% B with a flow rate of 1000 µL/min. The HPLC was monitored at 330 nm and compared with standards flavonoids, namely rutin and fisetin.

RESULTS

The free radical scavenging effect of aqueous extracts of *M. calabura*, *D. linearis*, *M. malabathricum*, *B. purpurea* and *C. capsularis*, assessed using the DPPH radical scavenging and xanthine/xanthine oxidase superoxide assays, are shown in Table 1. Interestingly all extracts demonstrated remarkable scavenging effects with activity recorded above 80% in both tests.

The phytochemical screenings of the selected plants are demonstrated in Table 2. All plants demonstrated the presence of flavonoids, triterpenes, saponins and high content of steroids, but no traces of alkaloids. Interestingly, only three plants namely, *M. calabura*, *D. linearis* and *M. malabathricum*, showed the presence of tannins.

Fig 1 demonstrated the HPLC profiles of the aqueous extracts of selected plants. Comparison made at the wavelength of 330 nm against standard flavonoids, namely rutin (retention time (R_T = 14.402 min)) and catechin (R_T = 21.786 min), indicate that all extracts possessed R_T that is different from the standards, except for the AEDL (R_T = 14.432 min) and AEMC (R_T = 21.792 min). Although the retention time could not give confirmation on the actual compounds that might be present in the extracts, it can be used as a comparison against standards, which have been known to possess a specific retention time. Further study; however, need to be carried out in order to obtain the actual bioactive compounds responsible for the extracts free radical scavenging activities.

DISCUSSION

The present study demonstrated the radical scavenging property of several plants, namely *M. calabura*, *D. linearis*, *M. malabathricum*, *B. purpurea* and *C. capsularis*, found in Malaysia. The aqueous extracts of the selected plants' leaves, assayed against the DPPH radical scavenging and xanthine/xanthine oxidase superoxide assays, were found to exhibit remarkable radical scavenging activities with the percentage of inhibition recorded for the former and latter assays ranged between 94 – 99% and 83 – 100%, respectively.

It is generally known that the antioxidant activities of putative antioxidants involves various mechanisms, such as radical scavenging, decomposition of peroxides, binding of transition metal ion catalysts, prevention of chain initiation and prevention of continued hydrogen

abstraction [21]. Hence, the free radical scavenging capacity of an extract may serve as a significant indicator of its potential antioxidant activity. Increasing evidences have suggested that many age-related human diseases (i.e. cancer, inflammation and brain dysfunction) are the result of cellular damage caused by free radicals [22,23]. Antioxidants have been shown to play an important role in preventing such diseases. For example, several cancer chemopreventive agents exhibit antioxidant activity through their ability to scavenge oxygen radicals [24,25].

According to Winston [26], the leafy part of the vegetables/plants contains various types of bioactive compounds. In general, all of the plants used in this study demonstrated to contain flavonoids, saponins, triterpenes and steroids, but no alkaloids. In addition, tannins were only detected only in *M. calabura*, *D. linearis*, *M. malabathricum*. The interests in phenolic compounds, particularly flavonoids and tannins, have considerably increased in recent years because of their broad spectrum of chemical and diverse biological properties, which include the antioxidant effects [27] and radical scavenging properties [28]. Flavonoids have been associated with possible role in the prevention of several chronic diseases involving oxidative stress [29], as well as their protective effect against low-density lipoprotein (LDL) oxidation [30]. Thorough phytochemical investigations of the used plants and evaluation for their antioxidant activity by means of other models (i.e. various ex vivo and in vivo biochemical assays) have been planned in our laboratory to characterize the bioactive compounds with potential antioxidant activity.

All of the plants used in this study have been reported to possess anti-inflammatory and antinociceptive activities [31-35]. Studies have demonstrated the link between the anti-inflammatory and antinociceptive, and antioxidant activities of the plants. For example, nitric oxide (NO) is produced/released under the action of inflammatory stimuli (i.e. ROS) [36]. Inhibition of ROS leads to the reduction of NO production, which has been demonstrated to cause anti-inflammatory, antinociceptive and antioxidant activities [37,38]. The free radical scavenging property may be one of the mechanisms by which these plants' are effective in their ethnopharmacological uses against different ailments. In term of the flavonoids, much attention has been given to their antioxidant [39] and anti-inflammatory activities, in vitro and in vivo [40,41]. Flavonoids have been reported to inhibit cytokine (inflammatory stimuli) release from RAW264.7 cells [42] and may modulate the increasing number of cellular processes involving redox reaction, including the regulation of tyrosine phosphatase activity [43].

In conclusion, the present study provides preliminary evidence on the antioxidant property, partly *via* the free radical scavenging activity, of the leaves aqueous extract of several plants found in Malaysia, namely *M. calabura*, *D. linearis*, *M. malabathricum*, *B. purpurea* and *C. capsularis*. Flavonoids, triterpenes, saponins and tannins are believed to act synergistically to produce the observed activity.

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