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ORIGINAL ARTICLE

Total flavonoids content and biochemical screening of the leaves of tropical endemic medicinal plant *Merremia borneensis*

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Abstract The developing and under developed countries mostly rely on traditional medicines. This herbal or traditional medicine involves the use of different types of organic extracts or the bioactive chemical constituents. This type of biochemical investigation provides health care at an affordable cost. This survey such as ethnomedicine keenly represents one of the best avenues in searching new economic plants for medicines. Keeping this view in mind, the present study is carried out in *Merremia borneensis* leaves of University Malaysia Sabah, Sabah, Malaysia. The plant has several beneficial properties, such as antioxidant activity. The dry powder of the leaves of *M. borneensis* was extracted with hexane, ethyl acetate, chloroform, butanol and aqueous ethanol. The flavonoids content of the extracts was determined by Willet method. The flavonoids content of the extracts as quercetin equivalents was found to be highest in aqueous ethanol (53.28%) followed by chloroform (38.83%), ethyl acetate (24.51%), butanol (12.54%) and hexane extract (3.44%). The results suggest the presence of phytochemical properties in the leaves, which are used in curing the ailments.

1. Introduction

Phyto is the Greek word for plant. There are so many families of phytochemicals and they help the human body in a variety of ways. Phytochemicals may be protecting the human body from a host of diseases. Phytochemicals are non-nutritive plant bioactive chemicals that have protective or disease preventive properties. A plant produces these bioactive chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are so many phytochemicals in fruits, vegetables and herbs and each works differently.

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In recent years, consumers desire to reduce the risk of or manage a specific health condition through improved diet. Plants have evolved different phytochemicals, ingredients and enzymes as an antioxidant defense to maintain growth and metabolism (Pandhair and Sekhon, 2006). Concern about improving health, involving agricultural products with high potential benefits, has enhanced advance research on antioxidants (Moore et al., 2005). Many degenerative human diseases including cancer, diabetics, cardio, and cerebro-vascular diseases have been recognized as being a possible consequence of free radical damage to lipids, proteins, and nucleic acids (Choi and Lee, 2009; Don et al., 1992; Gruber et al. 1999; Hoffman, 1975).

Various possible ways to fight these diseases is to improve our body's antioxidant defenses. Comparatively high consumption of vegetables and fruits has been associated with a lowered incidence of such degenerative and incurable diseases (Bajpai et al., 2009; Kalveram and Forck, 1978; Kirtikar and Basu, 1975; Kusumoto et al., 1995; Muruganandan et al., 2001). Fruits also help to improve health in other ways. For example, fruit juice, can also be taken to alleviate sore throat and seasickness. The functional bioactivity of a plant organic extract, in general, depends upon the presence of compounds, such as polyphenols, carotenoids, terpenoids, and chlorophyll (Negi et al., 2002). Plants can contribute in this area primarily due to the antioxidant activity of phenolic and flavonoids compounds (Higdon and Frei, 2003; Terao et al., 1994; Gardner et al., 2000; Hancock and Sahl, 2006; Li et al., 2001; Allan et al., 2004; O'Callaghan et al., 2004; Mhatre et al., 2009).

Several studies have revealed that the antioxidant activity may be from compounds, such as flavonoids, isoflavones, flavones, anthocyanins, catechins, and other phenolics (Kahkonen et al., 1999; Alothman et al., 2009; Isabelle et al., 2010). Oxidative stress has been linked to various curable and incurable diseases (Alothman et al., 2009; Isabelle et al., 2010), while food industry has long been concerned with issues, such as rancidity and oxidative spoilage of foodstuffs (Shahidi and Wanasundara, 1992). The enzymatic oxidation as well as auto oxidation of amino acid or lipids during storage and processing is the major reaction responsible for the deterioration in food quality affecting the colour, flavour, texture, and nutritive value of the foods. Antioxidants are often added to the foods to prevent the radical chain reactions of oxidation by inhibiting the initiation and propagation step leading to the termination of the reaction and a delay in the oxidation process.

Flavonoids, present as colouring pigments in plants also function as protective antioxidants at various levels. Some studies showed that flavonoids could protect membrane lipids from oxidation (Shahidi and Wanasundara, 1992). *Merremia borneensis* is a shrub widely distributed in the South East Asia especially in Malaysia. The leaves are suitable to be used as a wrapper for the famous fermented rice or fermented tapioca known in Malaysia as 'Tapai.' The plant creeps well and is very productive in shady areas as well as in open areas and is known to blanket a whole tree or any object that it chooses to make its habitat. The stem contains latex that is higly sticky and the flowers are white in colour. This plant has been shown to have a wide range of biological activities. The leaves, according to natives in Sarawak, Malaysia, are used to relieve breast cancer (Prieto et al., 1999). *M. borneensis* is an important medicinal plant that is consumed in many parts of the world as herbal medicine. It has a high nutritive and oxidative value and is a rich source of vitamins A, B, and C besides several minerals such as calcium, phosphorus and iron. Though there are some reports on the antioxidant activities of apple fruits in relation to other fruits (Higdon and Frei, 2003; Terao et al., 1994; Gardner et al., 2000; Hancock and Sahl, 2006; Li et al., 2001), they only deal with one or two parameters and not in detail or do not suggest any possible components/mechanisms. During the course of our study on the biologically active constituents of this plant, we examined the constituents of the leaves of *M. borneensis* widely used in Sabah community, Malaysia. Hence, the aim of this present study has been made to investigate the phytochemical and biochemical screening of the powder leaves crude extract of *M. borneensis*.

2. Materials and methods

2.1. Materials

Aluminum chloride, quercetin, potassium acetate, hydrochloric acid, sulfuric acid were obtained from Sigma–Aldrich. Solvents for extraction were ethanol, hexane, butanol, chloroform (reagent grade) obtained from Merck (Darmstadt, Germany). The water was purified from water distillation plants in our laboratory. All other chemicals were of analytical grade or GC grade. UV spectra UV–Vis spectra measurements were done using a Spectro (Thermo Fisher Scientific, model 4001/4) spectrophotometer.

2.2. Sample collection

The fresh green leaves of *M. borneensis* were collected from the campus of University Malaysia Sabah, Malaysia. The leaves of this plant were harvested during the month of September, 2010. The leaves were collected from 2:00–3:00 pm on September 2, 2010 and packed in polyethylene bags and stored at 4 °C until required. The plant was initially identified by the morphological features and from the database present in the library, School of Biology, University Malaysia Sabah, Malaysia. Approximately 50 g of leaves were ground using a grinder (Blender 80115) for 20 s. The unfermented *M. borneensis* leaves were kept in the oven at 40 °C and put in a desiccator for at least 24 h prior to analysis.

2.3. Extraction

The small pieces of leaves were homogenised in a grinder for 3 min to 30–40 mesh size. The air-dried leaves were pulverized into a powdered form. The dried leaves powder (50 g) was extracted three times with 70% ethanol (3×200 ml) at room temperature and combined. The combined extracts were evaporated by a vacuum rotary evaporator (Buchi Labortech AG, model 1, R-215). The ethanol extract was (7.3 g) diluted by water and extracted successively with hexane, chloroform, ethyl acetate, and butanol to give hexane (1.97 g), chloroform (0.93 g), ethyl acetate (0.78 g), and butanol (0.391 g) and residual ethanol fractions (0.58 g), respectively. The extract was filtered using Whatman No. 41 filter paper to obtain a particle free extract. The residue was re-extracted twice by solvent and filtered. The extracts were pooled and then concentrated and dried under vacuum pressure. The same extraction procedure was followed for the other solvents, such as hexane, ethyl