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ORIGINAL ARTICLE

Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam

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Abstract *Goniothalamus velutinus* Airy Shaw belongs to the family Annonaceae which is known to have anticancer, antitumor and many other bioactivities. Natives of Sabah and Sarawak use root decoction of *G. velutinus* for the treatment of headache and food poisoning while the bark was used as a mosquito repellent. Bark and leaf extracts of this plant, obtained from Brunei Darussalam, were tested for phytochemical and antioxidant activities. Phytochemical screening of plant extracts revealed the presence of alkaloids, steroids, terpenoids and cardiac glycosides. Quantitative determination of total phenolics, total flavonoids, and various in vitro antioxidant activities (DPPH, ABTS and FRAP) of methanolic extract was carried out using colorimetric methods. The total phenolic content, expressed as mg of gallic acid equivalent (GAE) per gram of extract, was found to be 68 mg GAE/g and 78 mg GAE/g for bark and leaves respectively. The radical scavenging activity measurement, expressed in terms of EC_{50} (effective concentration of extract in μg/mL that reduces DPPH absorbance to 50% as compared to negative control), for leaf and bark extracts was found to be 155 μg/mL and 204 μg/mL respectively. Standards trolox and ascorbic acid show EC_{50} value of 5 μg/mL and 4 μg/mL respectively. Trolox equivalent antioxidant capacity (TEAC) was measured using the ABTS and FRAP method. Result for bark and leaf extracts was 79 mg and

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106 mg trolox equivalent (TE)/g respectively for the ABTS method. For FRAP assay, results for bark and leaf extracts were 80 and 89 mg TE/g respectively.

1. Introduction

Plants such as vegetables, fruit, spices medicinal herbs, etc., have been used to cure many diseases since ancient time. Today in this modern world, even though synthetic drugs are readily available and highly effective in curing various diseases, there are people who still prefer using traditional folk medicines because of their less harmful effects. There is a wide diversity of compounds, especially secondary metabolites, found and isolated from plants and studies have shown that these compounds have anticancer, antibacterial, analgesic, anti-inflammatory, antitumor, antiviral and many other activities to a greater or lesser extent (Cai et al., 2004; Miliauskas et al., 2004). Distinguished examples of these phytochemical compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides, stilbenes, tannins, nitrogen compounds (alkaloids, amines, betalains), terpenoids and some other endogenous metabolites (Cai et al., 2004; Abdelwahab et al., 2010).

Antioxidants are significant regarding reducing oxidative stress which could affect and damage biological molecules (Farhat et al., 2013). Oxidative stress is the disproportion between oxidants and antioxidants in favor of oxidants potentially leading to damage. Reactive oxygen species (ROS) are a class of compounds that are formed from oxygen metabolism. These highly reactive molecules such as, hydroxyl radical ($^{\bullet}OH$), peroxide ($ROO^{\bullet}$) and superoxide radicals ($O_2^{\bullet-}$), can cause severe damage to cells and tissues during various diseases which are linked to heart disease, carcinogenesis and many other health issues. Synthetic antioxidants such as butylated hydroxyl anisole (BHA), propyl gallate (PG), butylated hydroxyl toluene (BHT) which have been used to prevent oxidation have been found to cause internal and external bleeding in rats and guinea pigs at high dose (Borneo et al., 2009; Lee et al., 2003). Attention is therefore turned to the use of natural antioxidants such as bioactive flavonoids which are of great importance due to their indigenous origin and strong efficiency to trap/scavenge free radicals. One such example is tea (black & green) which is frequently used as beverage all over the world and is a rich source of polyphenolic compounds (Lee and Shibamoto, 2000; Katalinic et al., 2006; Borneo et al., 2009).

Goniothalamus of the Annonaceae family grows in shady primary rainforest of tropical Asia and approximately 160 species of this genus have been discovered, of which phytochemically 22 species have so far been investigated (Wiart 2007). *Goniothalamus* spp. are widely distributed in the island of Borneo. About 40 species of *Goniothalamus* have been recorded in Borneo. This genus is widely used in traditional medicines by natives for skin diseases, fever, antidotes and especially for abortion and post-partum treatments. It is also known to have antioxidant, antimalarial, anti-inflammatory, anticancer and inhibitory effects on platelets activating factor properties (Abdelwahab et al., 2009a,b). For example, decoctions of *Goniothalamus scortechinii* and *Goniothalamus macrophyllus* are used as a post-partum protective remedy while the roots of *Goniothalamus tapis* and *Goniothalamus giganteus* are used for abortion during early months of pregnancy. *Goniothalamus amuyon* is used to treat scabies. Phytochemical investigations of *Goniothalamus* spp. have resulted in the isolation of acetogenins, styryl lactones and alkaloids with significant cytotoxic, insecticidal and antimicrobial activities (Wiart 2007; Fasihuddin et al., 2010). Some of the alkaloids isolated include goniothalactam, goniopedaline, aristololactam AII, aristololactam BII and velutinam.

This study focuses on *Goniothalamus velutinus*, locally known as 'Limpanas hitam, Kayu hujan panas, talipanas hitam' in Brunei Darussalam, which is one of the interesting *Goniothalamus* spp. found in Borneo. Its specific medicinal uses are not much described but people of Sabah and Sarawak use its root decoction for the treatment of headache and food poisoning while its stem bark, which has a strong smell, has been used as mosquito repellent. Some ethnics in Borneo use *G. velutinus* for treatment of tumors and research also showed that its cytotoxicity on various human cell lines and all the above mentioned alkaloids and styryl lactone goniothalamin have been isolated from this species(Omar et al., 1992; Fassihuddin, 2004; Fasihuddin et al.,2010).

The objective of this study was to carry out preliminary phytochemical screening and to determine the total phenolic, flavonoid, flavonol contents and antioxidant activities (by DPPH, ABTS and FRAP) of *G. velutinus* collected from the rainforest of Brunei Darussalam. To date there has been no study being conducted on *G. velutinus* collected from Brunei Darussalam. Further, to the best of our knowledge, there has been no previous work being published on the phytochemical screening and antioxidant activities of *G. velutinus*.

2. Material and methods

2.1. Plant material and sample preparation

Stems and leaves of *G. velutinus* were collected from Bukit Panjang in Kampung Kulapis Brunei Darussalam in February 2013. They were rinsed with tap water followed by distilled water to remove the dirt on the surface. Barks were removed from stems and cut into small pieces. They were then air dried for 2 days and then freeze dried until a constant mass was obtained. Dried samples were ground into fine powder and kept in desiccators until extracted. The extraction was carried out in a soxhlet apparatus for 10 h using absolute methanol. The solvent was then evaporated using rotary evaporator and the crude extracts were kept in desiccators.

2.2. Chemicals and reagents

All chemicals used were of analytical grade. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchro-