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RESEARCH ARTICLE

Phytochemical, cytotoxicity and antioxidant activities of the stem bark of *Piper arborescens*

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Abstract

Crude extract from stem bark of *P. arborescens* was fractionated by using column chromatography to isolate and purify its metabolite content. Six secondary metabolites were successfully isolated and their identification was performed by using Gas Chromatography - Mass Spectrometry (GC-MS), Nuclear Magnetic Resonance (NMR) and Fourier Transform Infrared (FTIR) spectoscopy. The isolated metabolites were identified as caryophyllene oxide **(1)**, *α*-bisabolol **(2)**, benzamide 2-(methylamino) **(3)**, 2-ethylpiperidine **(4)**, piperine **(5)** and methyl eugenol **(6)**. Toxicity test on the four crude extracts of *P. arborescens* shows high cytotoxicity against *Artemia salina* brine shrimp with LC_{50} values ranging from 13.12 to 58.70 µg/mL. Greater cytotoxicity of the crude extracts of *P. arborescens* indicated the presence of potent cytotoxic components in this *Piper* spp. Antioxidant assay of *P. arborescens* against 2-diphenyl-1-picrylhydrazyl (DPPH) indicated moderate antioxidant activities of methanol, dichloromethane, chloroform and hexane crude extracts with EC_{50} values of 21.68, 23.82, 32.88 and 36.88 µg/mL, respectively. It is suggested that the six secondary metabolites identified in *P. arborescens* contribute as an active content for the cytotoxicity and antioxidant activities. This study showed that the crude extracts of *P. arborescens* is definitely having potential to be used as a source of natural product of various application.

Keywords: *Piper arborescens*, phytochemical, cytotoxicity, antioxidant

INTRODUCTION

Most of indigenous *Piper* plants in Borneo are future potential herbs but still underutilized and scientific information on its phytochemical and biological activities are very scarce. *Piper* spp. which is widely distributed over the tropical and subtropical regions of the world is used medicinally in various manners. Beside the well studied of the commercial black pepper (*Piper nigrum*) and the abundance of *Piper aduncum* (Liew *et al.*, 2000; Micheal & Douglas, 2014), studies on other wild *Piper* spp. in the state of Sarawak and Sabah of Borneo region, particularly on the phytochemical and biological activity is a potential and interesting field to be explored. This indigenous *Piper* spp. can be introduced as a potential herb and cultivated as future crops, where further research can be conducted to provide sufficient information on the health beneficial properties of the plants and their specific usage.

This paper discusses phytochemical and biological activities of a wild *Piper* spp. that can be found in the forest mostly throughout Sarawak; namely *P. arborescens* or locally known as *lada hutan*. Few studies on this *Piper* spp. have been conducted previously mostly focusing on its leaves and stems, but there are still potential elements to discover. A study by Lee *et al.* (2004) in Taiwan have discovered cyclobutanoid amides from the stem and leaves of *P. arborescens*, while a study by Tsai *et al.* (2005) also in Taiwan have discovered cytotoxic cyclobutanoid amides and furanoid lignan from the stem of *P. arborescens*. Toxicity of the plants does not always indicated its danger or outright toxicity toward human, but may also suggest the presence of cytotoxic component that may contribute to antitumor or anticancer activities (Moshi *et al.*, 2010). There are various useful method available for assessment of cytotoxicity of the plant extract. The most common and widely used is the Brine Shrimp Lethality assay by using *Artemia salina* (Mentor *et al.*, 2014). *Artemia salina* is an invertebrate inhibiting saline aquatic which suitable to be used in laboratory assay for cytotoxicity screening of the plants by estimation of lethality concentration to kill 50% (LC_{50}) of the test organism.

In this study, the focus is on the stem bark of *P. arborescens*, which the parts are believed associated to medicinal purposes, yet the related scientific literature are very limited. The stem bark of the *P. arborescens* was extracted using several solvents to obtain the crude extracts, followed by various series of chromatographic method such as column chromatography and thin layer chromatography for separation and purification of the secondary metabolites. The isolated metabolite was further analyzed using Gas Chromatography - Mass Spectrometry (GC-MS), Nuclear Magnetic Resonance (NMR) and Fourier Transformed Infrared (FTIR) for identification and confirmation of the compound. The crude extracts of *P. arborescens* were analyzed for their biological activity, which involved cytotoxicity and antioxidant assays.

EXPERIMENTAL

Plant material

The sample of *P. arborescens* was collected from Betong, Sarawak. The stem bark of *P. arborescens* was air-dried, cut into pieces and ground prior to analysis.