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Original Article

Chemical and pharmacognostical characterization of two Malaysian plants both known as Ajisamat

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ABSTRACT

Ajisamat, an herb commonly used as an aphrodisiac in the Malaysian traditional medicine, corresponds to two different species from different families - *Salacia macrophylla* Blume, Celastraceae, and *Prismatomeris glabra* (Korth.) Valetton, Rubiaceae. Macromorphological inspection of the vegetative parts both plants reveals only a slight difference in the arrangement of the petioles. Microscopic investigation of the plants roots used as crude drugs revealed however distinctive anatomical features. Prismatic calcium oxalate crystals and banded paratracheal parenchyma are characteristics of *S. macrophylla* while *P. glabra* displays an abundance as crystals. Other features such as vessels diameters and arrangements are also of diagnostic importance. Some of these characters were also identified in the powder of these plant materials and proposed for diagnostic purpose. The values for extraction of ethanol and water as well as total ash, acid-insoluble ash, water-soluble ash and sulfated ash were determined for both plants. Phytochemical studies were carried out on hexane and chloroform extracts of *S. macrophylla* and methanolic extract of *P. glabra*. *S. macrophylla* was shown to contain highly oxidized pentacyclic triterpenes while *P. glabra* contains anthraquinones. The pharmacognostical and phytochemical information can be utilised as the identification tools for *Salacia macrophylla* and *Prismatomeris glabra*.

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Introduction

Ajisamat, Haji Samat or Tongkat Haji Samat is a commonly used plant in Malaysian traditional plant medicines. The aqueous extract of the dried roots of the plant have been traditionally used by the aborigines and certain rural Malays for wellness,

enhancing stamina and for its ergogenic effect. This plant has been used for generations to an aphrodisiac in Kelantan and Terengganu (Azmi et al., 2011). The names Ajisamat, Haji Samat or Tongkat Haji Samat were initially believed to correspond to *Jackia ornata* (syn. *Jackiopsis ornata*) of family Rubiaceae (Department of Forestry Malaysia, 2005). Later it appeared

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that the name referred to other two plants of different species and family i.e. *Prismatomeris glabra* (Korth.) Valetton, Rubiaceae, and *Salacia macrophylla* Blume, Celastraceae. The appearance of the vegetative parts including roots of both plants are nearly similar. Therefore, this study was carried out in an attempt to characterize the morphological, microscopic and chemical profiles of both types of *Ajisamat* plants.

Salacia macrophylla is a woody liana or climber found in swamps, lowland forest, on the mountain slopes as well as in old rubber estates. Usually it climbs up trees to several meters in height (Metcalf and Chalk, 1965). Plants of this genus are known to possess pentacyclic triterpenes with highly oxidized A/B rings, as well as thiosugars, salacinol and kotalanol (Dhanabalasingham et al., 1996; Muraoka et al., 2008). These compounds have been reported to show a variety of biological activities such as cytotoxicity, antimicrobial, antifertility, anti-inflammatory, antimalarial and α -glucosidase inhibitory properties (Jeller et al., 2004; Maregesi et al., 2010).

Prismatomeris glabra is a slender tree and usually found in keranga and undisturbed mixed dipterocarp forests up to 700 m altitude in Peninsular Malaysia, Sumatra and Borneo (Slik, 2006). Usually it is found on hillsides and ridges, along rivers and streams. It can grow on sandy to clay soils. The genus *Prismatomeris* usually contains anthraquinones and iridoids, compounds having cytotoxic (Hao et al., 2011) and antitumor activities (Krohn et al., 2007).

Materials and methods

Plant materials

Salacia macrophylla Blume, Celastraceae, was collected from a swamp forest near Taiping, Perak, Malaysia, while *Prismatomeris glabra* (Korth.) Valetton, Rubiaceae, was collected in Puchong, the dry lowland rain forest reserve, Selangor, Malaysia.

Life specimens of both plants were brought to Forest Research Institute Malaysia, Kepong, Selangor, Malaysia, and were identified by a botanist of specimens of both plants were deposited in the Herbarium of Faculty of Pharmacy, UiTM, Puncak Alam Campus under accession number UiTM191/HAB28 (*P. glabra*) and UiTM 278/TAS003 (*S. macrophylla*).

Macromorphological characterization

The macromorphological examination includes visual observation of the size, shape, color and was carried out following the method outlined by World Health Organization (WHO, 1998).

Micromorphological characterization

Micromorphological inspection was carried out on samples of the dried roots of the plants following the botanical microtechnique procedures described by Teh (1996). The dried roots were boiled and sectioned (25 μ m in thickness) with a Reichert Jung sliding microtome slider. The obtained sections were bleached, stained with safranin, alcian green/blue and phloroglucinol, dehydrated, mounted with Canada balsam and

then studied under microscope. Microscopical examinations of the powdered roots of both plants were carried out by using the method modified from WHO (1998). The root powders of both plants were thoroughly mixed with two drops of glycerol on a glass slide and observed under the microscope.

Chemical characterization

Chemical characterization of both plant material included determination of solvent extractive values, ash values and color reaction tests.

Ethanol and water extraction amounts were determined following the methods described in WHO (1998) and Malaysian Herbal Monograph (2009). Briefly, the root powder *S. macrophylla* was used to calculate the values of the hot and cold extraction in water and ethanol, while the powder of root of *P. glabra* was extracted with only cold water and cold ethanol due to insufficient sample.

Measurements of total, acid-insoluble and water-soluble ashes were made according to the procedures described in Wiart and Kumar (2001) and in WHO (1998), while determination of sulfated ash was carried out following the method described in British Pharmacopoeia (2010).

Color reaction tests were done following the method prescribed by the Malaysian Herbal Monograph (2009). One gram sample of powdered root was treated with each of the reagents from a specific list at room temperature and the observation on the colors produced was done under normal light. The reagents were concentrated sulfuric acid, concentrated hydrochloric acid, sodium hydroxide 5%, potassium hydroxide 5%, ammonium hydroxide 25% and ferric chloride 5%.

Phytochemical analysis

The powder of the roots of *S. macrophylla* (170 g) was successively extracted with n-hexane, chloroform and ethyl acetate, while the powder roots of *P. glabra* (400 g) using hexane, ethyl acetate and methanol.

TLC analyses were performed on silica gel 60 F₂₅₄ plates (Merck, Germany). Spots were detected at 254 and 366 nm; ceric sulfate was used as a spray reagent for the detection of triterpenes, while the characterization of anthraquinones was achieved with Bornträger reaction (5% KOH in ethanol). Column chromatography was carried out using silica gel (40-63 μ m, Merck, Germany). The ¹H-NMR, ¹³C-NMR, ¹H-¹H-COSY, HMQC and HMBC spectra were recorded on Bruker Avance II 400 and Bruker Avance III 500 MHz NMR spectrometers; chemical shifts are in ppm (δ) relative to the SiMe₄ as internal standard and coupling constants are in Hz. MS were measured on Jeol HX 110 mass spectrometers (*m/z*) and Agilent 6220 LC/MS TOF.

Results and discussion

Macromorphological characterization

Salacia macrophylla plant is slender with few branches (Fig. 1A). The petioles are grooved and the stipule is present. The leaves

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