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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.) Valeton, 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.) Valeton, 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

are simple, broadly elliptic in shape and glabrous. The leaf base is symmetrical (Fig. 1A) and arrangement of the petioles is subopposite (Fig. 1B). The roots of *S. macrophylla* are hard taproots, yellowish-brown in color and some with lateral roots. The root bark exfoliates easily. The outer layer is brown and the internal layer appears orange (Fig. 1C-D).

The second type of *Ajisamat* plant, *Prismatomeris glabra*, is a small plant, similar in appearance to the coffee plant. The leaves are simple, broadly elliptical in shape with an entire margin, and are oppositely arranged (Fig. 2A). Their scars or stipules are present and interpetiolar (Fig. 2B). Flowers are white with five petals, are black drupes when mature (Fig. 2C). The roots are hard woody taproots, yellowish-orange color, with lateral roots (Fig. 2D). The roots of this species also show the unusual feature of an orange inner bark.

Generally both plants show a similar physical appearance. The leaf shape is similar with acute to acuminate apices. They are truly opposite for *P. glabra*, while slightly subopposite for *S. macrophylla*. However the two species can be distinguished by the presence of stipules or stipule scars and the raised midvein in *P. glabra* leaves.

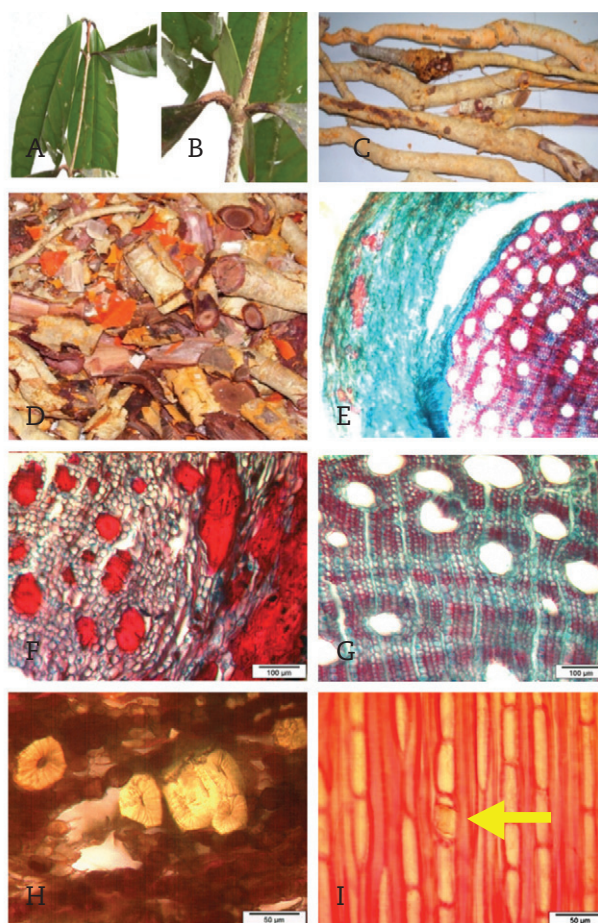


Fig. 1 - Photograph of *Salacia macrophylla*. A, leaf blades; B, arrangement of the petioles; C, whole roots; D, root pieces with exfoliated orange barks; E, TS of root; F, TLS of root bark showing phelloderm region; G, TS of root secondary xylem showing banded paratracheal axial parenchyma; H, brachysclereids (unstained); I, TLS root of secondary xylem showing an inclusion of prismatic calcium oxalate crystal in a ray cell (arrow).

Micromorphological characterization

The micromorphological inspection of both types of *Ajisamat* plant revealed distinctive differences in the anatomical structures between the plants.

The vessel elements of *S. macrophylla* are mostly solitary, while some are in pairs, lack tyloses (Fig. 1E). Following the category of vessel characteristics by Menon (1967), the vessel size of *S. macrophylla* is moderately small (43-84 μm) and the vessels are numerous (21 to 40 vessels per mm^2). The axial parenchyma of *S. macrophylla* appears banded paratracheal of the 2- to 4-cells wide as well as confluent (Fig. 1G). In the tangential sections, (Muraoka et al., 2008), of root bark, brachysclereids are abundant within the phelloderm layer of the young and old roots (Fig. 1F). These are thick-walled with narrow pit canals isolated or arranged in groups (Fig. 1H). Uniseriate rays are of different heights, some with solitary prismatic crystals of calcium oxalate (Fig. 1I).

In *P. glabra*, the vessels elements are solitary, few are in pairs and lacking tyloses (Figure 2E). The vessel size for *P. glabra* can be categorized as extremely small (12-25 μm) and the number

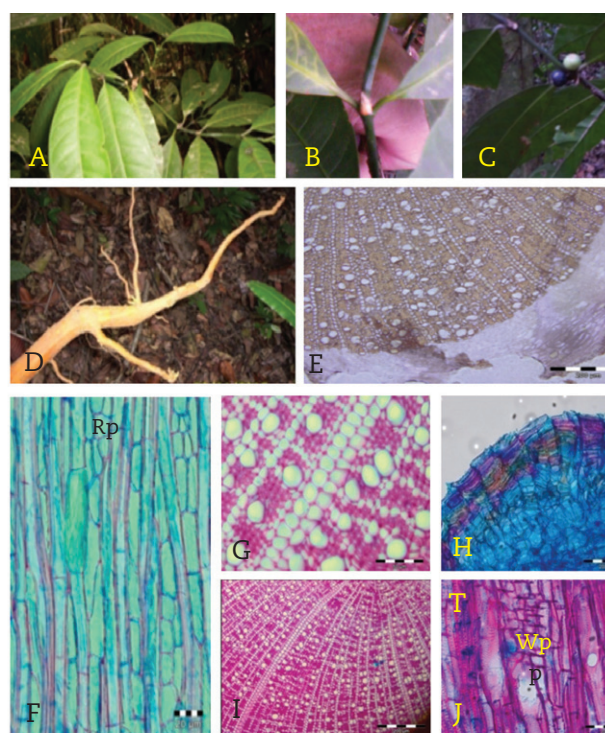


Fig. 2 - Photograph of *Prismatomeris glabra*. A, leaf blades; B, stipule; C, drupes; D, whole root; E, TS of root (stained with phloroglucinol); F, TLS of root showing a bundle of raphides of calcium oxalate crystals (Rp) included in an axial parenchyma cell; G, TS of root showing uniseriate rays, multiseriate rays thick-walled fibres; H, TS of root bark and cork showing phelloderm (double-stained with safranin and alcian blue); I, TS of root showing distribution of vessels, fibers, axial parenchyma paratracheal and apotracheal (double-stained with safranin and alcian blue); J, TLS root of showing imperforated tracheids (T), vessels with alternate pitting wall (Wp) and simple perforation plate (P).

of vessels as very numerous (more than 40 vessels per mm²). The parenchyma of *P. glabra* appears as both paratracheal and apotracheal axial. The paratracheal type is mostly of 2-cells wide, forming short tangential lines, while the apotracheal type includes uniseriate short tangential lines extending from rays to rays (Fig. 2 G-I). No brachysclereids were observed in *P. glabra* root bark (Fig. 2H).

Tangential longitudinal section (TLS) of *P. glabra* root reveals tracheids, vessels with alternate wall pitting and simple perforation plates (Fig. 2J). The parenchyma is irregularly distributed among fibers, some with inclusion of calcium oxalate crystals in the form of bundles of raphides (Fig. 2F).

The anatomical structure of tangential sections of the roots of both types of *Ajisamat* plants are presented in drawings in Fig. 3 and Fig. 4. The summary of the distinctive anatomical features between the two plants is presented in Chart 1.

Microscopical studies of root powder of both plants were carried out. The diagnostic features of powdered roots of *S. macrophylla* and *P. glabra* are presented in Figs. 5 and 6 respectively. Their differential identification is therefore relatively easy. *S. macrophylla* is characterized by abundant brachysclereids and solitary calcium oxalate crystals, while *P. glabra* contains numerous raphides and no sclereids.

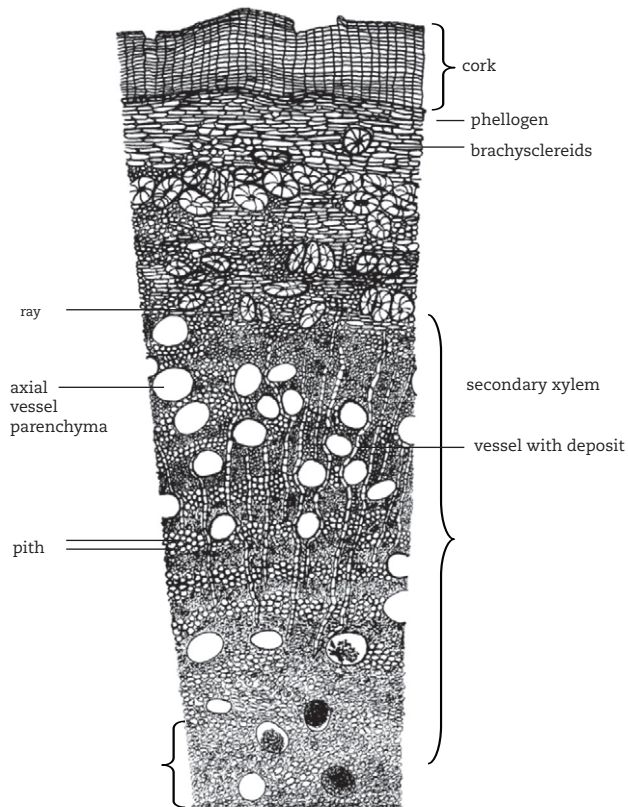


Fig. 3 - Anatomical characters of *Salacia macrophylla* root (TS).

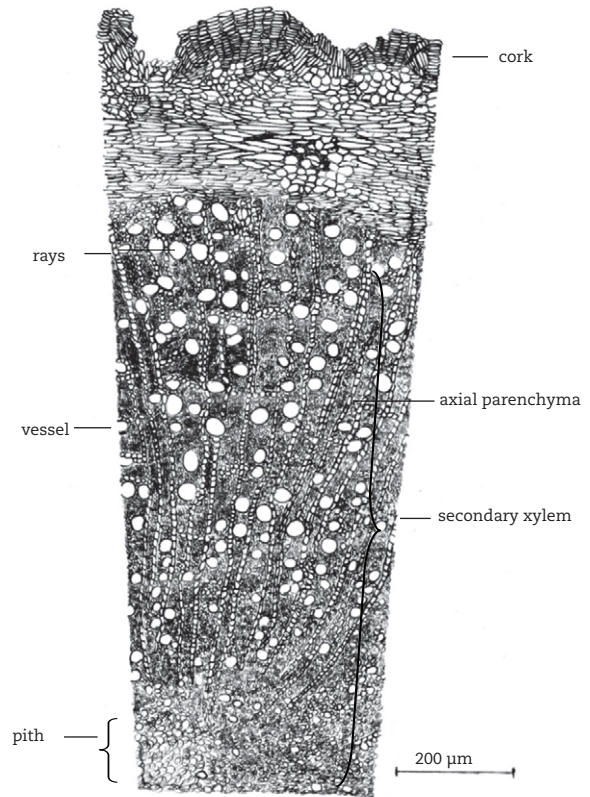


Fig. 4 - Anatomical characters of *Pristomeris glabra* root (TS).

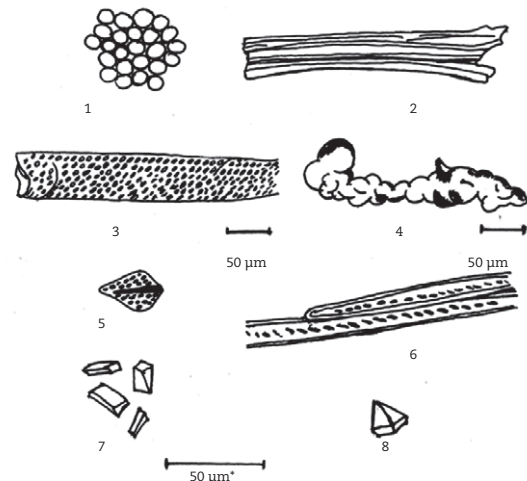


Fig. 5 - Diagnostic features *Salacia macrophylla* powdered root; 1, corks cells; 2, fiber Bundle ; 3, vessel element; 4, group brachysclereids; 5, fragment of vessel element; 6, tracheary elements; 7, unknown orange crystals; 8, prismatic crystals of calcium oxalate. (The scale marked * is for feature 7 and 8).

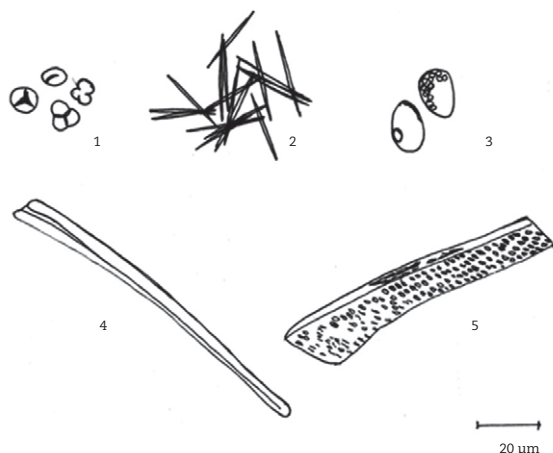


Fig. 6 - Diagnostic features *Prismatic glabra* root powder. 1, starch grains; 2, raphide crystals; 3, oil globules; 4, fiber; 5, fragment of vessel element.

Chart 1

Summary of anatomical characteristics of the roots for *S. macrophylla* and *P. glabra*.

	<i>S. macrophylla</i>	<i>P. glabra</i>
Cork	Brick shaped with orange content	Brick shaped with content becoming blue or pink after staining with safranin-alcian blue
Parenchyma	Banded paratracheal parenchyma	Both apotracheal and paratracheal parenchyma
Xylem vessels	Mainly solitary and some in pairs, moderately small in size and numerous, lack tyloses, with content that stains red with safranin	Solitary, extremely small in size and very numerous, lack tyloses, without vessel content
Sclereids	Brachysclereids, mostly in groups	None
Calcium oxalate	Prismatic crystals in ray cells, abundant	Raphide crystals in bundles, in axial parenchyma, abundant
Rays	Uniseriate	Uniseriate to multiseriate

Chemical characterization

For *S. macrophylla*, both ethanol and water extractives were of similar value when produced by the cold method. With the hot method, the extractives are about four times greater than those produced by cold method (Table 1). In contrast, by cold method *P. glabra* produces a water extractive almost six times more abundant than the ethanol extractive.

The ash values obtained for *S. macrophylla* and *P. glabra* are presented in Table 2. Regardless of the type of the ashes, the values for *P. glabra* appear to be consistently higher than that of *S. macrophylla*. Prabhu et al. (2009) reported that the presence of abundant calcium oxalate might contribute to high amounts of

Table 1

Extractive values for *Salacia macrophylla* and *Prismatic glabra*.

Method and solvent	Mean extractive values (%)		
	<i>S. macrophylla</i>	<i>P. glabra</i>	
Cold method	ethanol	1.4	1.1
	water	1.4	6.2
Hot method	ethanol	5.6	n.d. ^a
	water	5.3	n.d. ^a

^aNot determined due to lack of sample.

Table 2

Ash values for *Salacia macrophylla* and *Prismatic glabra*.

Determination	Mean ash values (%)	
	<i>S. macrophylla</i>	<i>P. glabra</i>
Total ash	1.9	3.1 or 3.2
Acid-insoluble ash	0.14	1.2 or 1.3
Water-soluble ash	0.77	2.5
Sulfated ash	14	47

ash, especially for the sulfated ash values. Similarly, Shinkar (2007) reported that high values of acid-insoluble ash are related to the high content of calcium oxalate in plants. For *P. glabra*, calcium oxalate crystals were found to be abundantly present in the form of raphide needles. Therefore, the high ash values for this plant are likely due to the high content of calcium oxalate.

Chart 2 summarizes the color changes resulting from the reactions of root powders with reagents prescribed by the Malaysian Herbal Monograph (2009) for color reaction tests. Some distinctive differences in colors were produced were observed between the two types of *Ajisamat* plants that indeed could be used for fast discrimination between them.

Phytochemical analysis

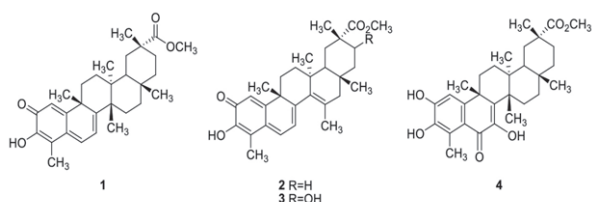
Chart 2

Color reaction tests for powdered roots of both *Ajisamat* plants.

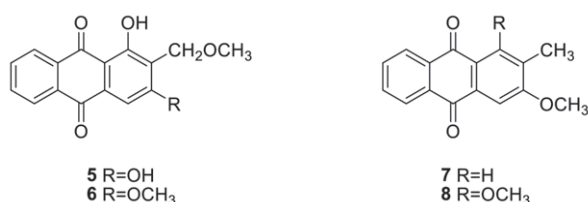
Reagent	Color	
	<i>S. macrophylla</i>	<i>P. glabra</i>
Distilled water	yellowish orange	yellowish orange
Concentrated sulfuric acid	Black	Green
Concentrated hydrochloric acid	No color changes	Dark green
Sodium hydroxide (5%)	Brown	Dark red
Potassium hydroxide (5%)	Brown	Dark red
Ammonium hydroxide (25%)	Light brown	Red
Ferric chloride	Light green	Brown

Repeated column chromatography (silica gel) of the *n*-hexane extract afforded *S. macrophylla* afforded β -sitosterol Patra et al., 2010 and four pentacyclic triterpenes namely, pristimerin (**1**) (Khalid et al., 2007), netzahualocoyene (**2**) (Dhanabalasingham et al., 1996) and netzahualocoyonol (**3**) (Jeller et al., 2004). The chloroform extract, when subjected to a similar fractionation process, produced 2,3,7-trihydroxy-6-oxo-1,3,5,10,7-tetraene-24-nor-friedelane-29-oic acid methyl ester (**4**) (Ankli et al., 2000). Structure elucidations of compounds (**1-4**) were carried out by comparison of spectral data with literature. Triterpenes **1-4** belong to the D:A-friedo-nor-oleanane group and show the rare quinone-methide moiety. Such derivatives are significant chemotaxonomic characteristic of the family Celastraceae (Gamlath et al., 1990).

Chemical studies on the methanolic *Prismatomeris glabra* extract led to the isolation of four anthraquinones. These compounds were isolated by MPLC (Medium Pressure Liquid



Chromatography) and preparative thin layer chromatographic techniques. They were identified as 1,3-dihydroxy-2-methoxymethyl-9,10-anthraquinone (**5**) (Reanmongkol et al., 2003), 1-hydroxy-3-methoxy-2-(methoxymethyl)-9,10-anthraquinone (**6**) (Joshi et al., 1955), 2-methyl-3-methoxy-9,10-anthraquinone (**7**) (Burnett and Thomson, 1968; Sartori et al., 1995) and 1,3-dimethoxy-2-methyl-9,10-anthraquinone (**8**) (Fraga et al., 2009).



This study further illustrates another case in which a plant vernacular name corresponds to two or more different species. Only the eyes of an expert botanist can differentiate the plants in their original habitat. It is not surprising that traditional healers are unable to differentiate them fully. The implementation of simple tests recommended notably by WHO would enable the differentiation of the two species clearly. It is proposed that the information gathered above be incorporated into future monographs for *Salacia macrophylla* and *Prismatomeris glabra* in the forthcoming volume of Malaysian Herbal Monograph. The chemical compositions of these plants are very different. The traditional claims can not be supported by any information from literature so far. Azmi et al. (2011) have earlier reported that *P. glabra* aqueous extract negatively affected the cognitive functions in mice. This combined with the known cytotoxicity

of anthraquinones warrant caution on the use of this plant. To the best of our knowledge, no pharmacological/toxicological study was carried out on *S. macrophylla*. The above mentioned triterpenes are also known for significant cytotoxicity (Ankli et al., 2000; Setzer et al., 1998). Yet, due to their low water solubility, it cannot be ascertained that a water extract of this plant is toxic without further tests. *S. macrophylla* therefore needs to be fully investigated. This is an endeavour undertaken by our group and results will be reported in due time.

Authorship

TASTM (MSc student) contributed in collection and identification of plant samples, confection of herbarium specimens, running laboratory work, analysis of date and drafted the paper. RSJ (MSc student) contributed to the phytochemical studies. MRAR (MSc student) and KH contributed in microscopic analysis. HN and JFFW designed the study, supervised the laboratory work. HN, JFFW and KH contributed to critical reading of the manuscript. AA conceptualized the research problem and facilitated administrative procedures. All the authors have read the final manuscript and approved the submission.

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