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Pharmacognostical Analysis and Preliminary Studies of the Chemical Constituents from the Roots of *Senna alata* Linn.

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ABSTRACT *Senna alata* L. (syn. *Cassia alata* L.) belongs to the family Leguminosae and is locally known as either Gelenggang Besar, Daun Kurap or Ludanggan. The leaves are traditionally used for the treatment of skin diseases such as ringworm and pityriasis versicolor. In this study, a pharmacognostical analysis was performed on the roots of *S. alata* using methods described by the Malaysian Herbal Monograph [1]. Powdered *S. alata* root was dark brown with bitter taste and mild odour; contained microscopical features such as fibres, vessels with spiral and bordered pit thickening, twinned-prism and prism calcium oxalate crystals, parenchymal cells, cork cells and starch cells; and produced distinct colour when reacted with different chemical reagents. A reasonable thin-layer chromatographic profile was obtained using pre-coated silica gel GF_{254}, mobile phase chloroform-methanol (9:1), and then detected under daylight after treatment with Anisaldehyde/H_2SO_4 reagent. The extractive values of powdered root for cold ethanol-soluble, cold water-soluble and hot water-soluble methods were obtained as 1.77%, 1.41% and 1.77% respectively. Determination of moisture content by loss on drying method was 9.37%. In addition, a phytochemical study carried out on the ethyl acetate extract of *S. alata* root resulted in the isolation of two compounds, that is, a steroidal compound, stigmasterol, and emodin (1,6,8-trihydroxy-3-methylanthraquinone), which is a common compound of *Senna* species. The structures were elucidated using spectroscopic techniques (^{1}H-NMR, ^{13}C-NMR and MS) and by comparison with the literature data.

[*Senna alata* L., Leguminosae, pharmacognosy, stigmasterol, 1,6,8-trihydroxy-3-methylanthraquinone (emodin)]

INTRODUCTION

Senna alata L. (syn. *Cassia alata* L.) belongs to the family Leguminosae [2]. The tree is about six feet high and is slightly woody. It is well distributed in the tropical countries, stretching from tropical America to India, Fiji, Indonesia and Malaysia [3]. The plant is known by the Malays as *gelenggang besar, gelenggang gajah, daun kurap, daun kupang* or *ludanggan* [4,5]. In Malaysia, the leaves are used traditionally for the treatment of skin diseases such as ringworm and pityriasis versicolor [4].

More than 5 classes of compounds especially the anthraquinones, flavonoids, quinones, polysaccharides and steroids have been identified from the leaves, barks and seeds of this species. Most of these chemical constituents have shown significant pharmacological activities including anti-microbial, anti-fungal, anti-inflammatory, analgesic and as laxative [6]. Previous studies on the leaves of *S. alata* revealed the presence of aloe emodin, kaempferol, physcione, chrysophanol, deoxycoelulatin, sennosides (A-D), β-sitosterol, isochrysophanol, 4,5-dihydroxy-2-hydroxyanthrone and 4,5-dihydroxy-1-hydroxyanthrone, whereas emodin, galactomannan, chrysoeriol-7-O-(2"-O-β-D-mannopyranosy)-β-D-allopyranoside and rhamnetin-3-O-(2"-O-β-D-mannopyranosy)-β-D-allopyranoside were isolated from the seeds [1].

Previous study carried out by Khan *et al.* [7] showed that the organic (light petroleum,

dichloromethane and ethyl acetate) fractions of root barks of *S. alata* gave broad-spectrum antibacterial activity. Meanwhile, our present work involved the pharmacognostical and phytochemical analyses of the roots of *S. alata*. In the study, pharmacognostical characteristics of the roots were investigated in order to obtain a preliminary data for a systematic and standard parameter for the identification and authentication of the specimen as raw material. On the other hand, the phytochemical work had resulted in the isolation of a steroid, stigmasterol, and an anthraquinone, emodin, that will be discussed in this paper.

EXPERIMENTAL

Preparation of Plant Material

The roots of *Senna alata* Linn were collected from trees found in Taman Pantun, UKM, Bangi. The plant was authenticated at source and was deposited as a voucher specimen at the herbarium of UKM, Bangi, Selangor. The specimen was chopped into small pieces, sufficiently air-dried and then ground to powder. The powdered material was kept in the dark in an airtight container until use.

Pharmacognostical Analysis

Qualitative and semi-quantitative tests such as macroscopical and microscopical examination, colour tests, thin-layer chromatographic identification as well as determination of loss of moisture on drying, ethanol-soluble and water-soluble extractives were performed on the dried powdered roots of *S. alata* based on the methods described by the Malaysian Herbal Monograph [1] and Jamia Azdina *et al.* [10].

Macroscopical and Microscopical Examination

Sensory characters, for instance colour, odour and taste, of the powdered specimen were recorded. The specimen was then examined under a microscope using chloral hydrate, phloroglucinol and HCl, glycerol, N/50 iodine and Sudan Red III mountants.

Colour Test Determination

The specimen (5 mg) was treated individually with concentrated H_2SO_4, concentrated HCl, NaOH (5%), KOH (5%), NH_4OH (25%) and $FeCl_3$ (5%). Any colour change was observed in daylight.

Thin Layer Chromatography

The specimen (500 mg) was extracted with 96% of EtOH (10 ml) by heating over a boiling water bath for 5 minutes. The resultant extract was run on TLC in $CHCl_3$-MeOH (9:1). TLC : silica gel 60 GF_{254}, 0.25 mm (Merck). Spots were detected under daylight and UV light (254 nm), as well as by exposure to iodine vapour and after treatment with anisaldehyde/H_2SO_4 reagent.

Ethanol-Soluble Extractive

The specimen (500 mg) was macerated with 10 ml of EtOH (95 %) for 24 hours, shaking frequently during the first 7 hours and then allowing to stand for 17 hours. The resultant filtrate was evaporated to dryness and later heated at 105°C to constant weight.

Cold Method Water-Soluble Extractive

The specimen (4-5 g) was macerated with 100 ml of distilled H_2O for 24 hours, shaking frequently for the first 6 hours and then allowing to stand for 18 hours. The resultant filtrate (20 ml) was evaporated to dryness and later heated at 105°C for 3 hours.

Hot Method Water-Soluble Extractive

The specimen (4-5 g) was immersed in 100 ml of distilled H_2O, weighed and left for 1 hour. The mixture was refluxed for 1 hour, allowed to cool and weighed. Distilled water was added to obtain original weight as that before reflux. The resultant filtrate (20 ml) was evaporated to dryness and later heated at 105°C for 3 hours.

PHYTOCHEMICAL ANALYSIS

General

The UV spectra was measured with a UV-1601 Shimadzu spectrophotometer using MeOH as solvent while UV shift reagents were prepared based on the methods described by Mabry *et al.* [11] and Markham [12]. ^1H and ^{13}C NMR spectra were recorded on a A-400 (JEOL) spectrometer in $CDCl_3$ and chemical shifts are given on a δ (ppm) scale with TMS as internal standard. MS were recorded at 70 eV using a direct inlet system on a Hewlett Packard S 898 A.

Extraction and Isolation

The dried powdered root (0.880 kg) was successively and exhaustively extracted with *n*-hexane, ethyl acetate and methanol. Evaporation of the solvents gave 1.6 g of *n*-hexane extract, 11.2 g of ethyl acetate extract and 34.8 g

methanol extract. The ethyl acetate extract was purified by flash column chromatography (silica gel, MERCK 230-400 mesh) using *n*-hexane-EtOAc (1:1; 4 L), EtOAc (100%, 2 L), EtOAc : MeOH (1:1; 1 L) and then MeOH (100%, 1 L) as eluents. Several fractions (250 ml each) were collected, analysed by TLC and grouped accordingly. Compounds **1** and **2** were obtained from *n*-hexane-EtOAc (1:1) fractions. The yields (mg) of the compounds isolated from the roots were : **1** (12.0 mg) and **2** (13.9 mg).

Stigmasterol **1**. White needles (12.0 mg). TLC : R_f 0.82 (*n*-hexane-EtOAc, 1:1); UV (MeOH) λ_{max} nm: 188; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ : 5.35 (d, *J*=5.1 Hz; H-6), 5.13 (dd, *J*=8.8, 8.8 Hz; H-22), 5.03 (dd, *J*=8.8, 8.8 Hz; H-23), 3.52 (m, H-3), 2.00 (m, H-4), 1.04 (s, H-21), 1.02 (s, H-19), 0.85 (t, *J*=3.7 Hz; H-29), 0.83 (d, *J*=1.8 Hz, H-26), 0.81 (s, H-27), 0.70 (s, H-18); ^{3}C-NMR ($CDCl_3$, 100 MHz) δ : 140.74(C_1), 138.30 (C_{22}), 129.3 (C_{23}), 121.69 (C_6), 71.78 (C_3), 56.76 (C_{14}), 56.05 (C_{17}), 51.23 (C_9), 50.10 (C_{24}), 42.30 (C_{13}), 39.75 (C_4), 37.78 (C_{12}), 37.25 (C_1), 36.50 (C_{10}), 36.10 (C_{20}), 31.90 (C_7), 31.60 (C_8), 28.90 (C_{25}), 28.20 (C_2), 26.08 (C_{16}), 24.30 (C_{15}), 21.2 ($C_{26,28}$), 21.10 (C_{27}), 21.06 (C_{11}), 19.39 (C_{19}), 19.00 (C_{21}), 12.20 (C_{29}), 12.04 (C_{18}) [8].

1,6,8-Trihydroxy-3-methylantraquinone **2**. Yellow needles (13.9 mg). TLC : R_f 0.25 (*n*-hexane-EtOAc, 8:2); UV (MeOH) λ_{max} nm: 230, 245, 276, 300, 425; UV (MeOH + NaOH) λ_{max} nm: 243, 455 EIMS (70 eV) *m/z* (rel. int.) 270 (100%) 139(5%), 69 (3%). ^{1}H-NMR ($CDCl_3$ with a drop of MeOD, 400 MHz) δ : 12.28 (s, OH), 12.14 (s, OH), 7.54 (br s, H-4), 7.18 (d, *J*=2.2 Hz, H-7), 7.03 (br s, H-2), 6.58 (d, *J*=2.2 Hz, H-5), 2.40 (s, CH_3) [9].

RESULTS AND DISCUSSION

Pharmacognostical Analysis

The powdered root of *S. alata* was dark brown, slightly bitter and had mild odour. Microscopical investigation revealed several characteristic features such as fibres, vessels with spiral and bordered pit thickening, twinned-prism and prism calcium oxalate crystals, parenchymal cells, cork cells and starch cells. The presence of twinned-prism calcium oxalate crystals is rather peculiar for *S. alata*. When the powdered root was treated with several chemical reagents, distinct colours were observed (Table 1). From the analysis, it is suggested that concentrated sulphuric acid and hydrochloric acid could be used to qualitatively identify and determine purity of the raw material of *S. alata* root. Table 2 summarises R_f values and appearance of distinctive bands obtained from the thin-layer chromatogram of ethanolic (96%) extract of chloroform-methanol (9:1). Two major bands were seen at Rf values of 0.78 (light purple) and 0.39 (red) when the TLC plate was detected under daylight after treatment with Anisaldehyde/H_2SO_4 reagent. The bands could indicate the presence of major compounds that could be used as markers for qualitative identification of *S. alata* root. The extractive values of the specimen for cold ethanol-soluble, cold water-soluble and hot water-soluble methods were obtained as 1.77%, 1.41% and 1.77% respectively. The results suggest that the roots of *S. alata* had slightly more organic-soluble phytochemicals than those of the water-soluble. Heat was found to enhance extraction of water-soluble phytochemicals. On the other hand, determination of loss on drying was 9.37%. The value meets the standard limit for moisture content of dried medicinal plant material (must be less than 10%) in order to avoid from deterioration during storage.

Table 1. Colour observations for powdered roots of *Senna alata*.

Test Solution	Colour of Solution Observed
Without reagent	Dark brown
Distilled water	Light yellow
Concentrated sulphuric acid	Dark brown
Concentrated hydrochloric acid	Yellow
Sodium hydroxide (5%)	Light brown
Potassium hydroxide (5%)	Light brown
Ammonium hydroxide (25%)	Light brown
Ferric chloride (5%)	Yellow

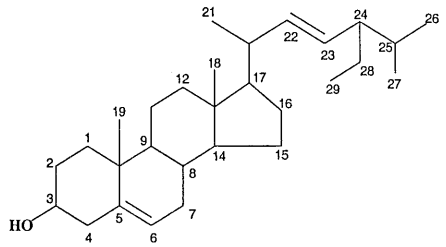
Table 2. R_f values and appearance of distinctive bands observed on TLC plates for ethanolic (96%) extract of *Senna alata* root

Rf Value [a]	Appearance of Major Band			
	Daylight	UV		Anisaldehyde/ H_2SO_4
		λ 254 nm	λ 365 nm	
0.78	Yellow	Dark	Yellow	Light purple
0.75	—	—	—	Yellow
0.70	—	—	—	Light purple
0.60	—	—	—	Light purple
0.39	Light brown	Dark	Yellow	Red
0.30	—	—	—	Grey
0.20	Yellow	Dark	Light orange	Light purple
0.16	—	—	—	Yellow
0.08	Yellow	Dark	—	Light brown
0.00	Brown	Dark	Brown	Dark brown

[a] R_f value of band relative to solvent front.
(—) Band was not seen.

Phytochemical Analysis

The ethyl acetate extract mixture from the roots of *S. alata* was subjected to flash chromatography using silica gel to afford several fractions. Purifications were carried out on selected fractions by repeated column chromatography yielding two pure compounds **1** and **2**. The compounds were identified by spectroscopic methods, i.e. UV, MS and NMR, and as well as by comparison with literature data.



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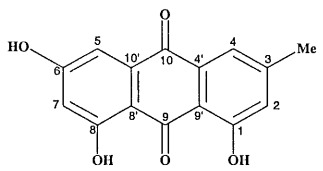

2

Stigmasterol **1**, a steroidal compound, is ubiquitous in most Leguminosae family. The compound was detected on TLC as a dark pink band at R_f 0.82 after spraying with 10 % H_2SO_4 when using hexane- ethyl acetate (1:1) as the mobile phase. ^{1}H-NMR data indicated distinctive peaks at δ 3.52 (m, H-3), δ 5.35 (d, *J*=5.1 Hz, H-6), and two double-doublet peaks at δ 5.13 (*J*= 8.8, 8.8 Hz, H-22) and δ 5.03 (*J*= 8.8, 8.8 Hz, H-23). In addition, the ^{13}C-NMR spectrum showed the presence of six methyl groups at δ 0.70-1.04 (C-18, C-19, C-21, C-26, C-27 and C-29). Thus, compound **1** was concluded to be stigmasterol due to the similarity of its spectroscopic data with the literature [8].

Compound **2** had a molecular ion at *m/z* 270 ($C_{15}H_{10}O_5$). It was thought to be an anthraquinone due to its colour reaction with methanolic NaOH. This is further exemplified by the UV analysis whereby in alkaline methanol the compound exhibited a significant shift from λ_{max} 230 nm to 243 nm and λ_{max} 425 nm to 455 nm, an indication of the presence of 6,8-dihydroxyanthraquinone [9]. On the other hand, ^{1}H-NMR spectrum revealed a methyl group at C-3 (δ 2.40, s, 3H) and two aromatic protons at C-2 (δ 7.54, s, H) and C-4 (δ 7.03, s, H). Whereas two meta-coupled doublets at δ 6.58 and 7.18 (*J*=2.2 Hz) could be attributed to the aromatic protons (H-5 and H-7) of the resorcinol ring. Based on the spectral data of the experiment and literature [9], 1,6,8-trihydroxy-3-methylanthraquinone **2** was assigned to this compound.

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