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Prominent Secondary Metabolites from Selected Genus of *Avicennia* Leaves

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Abstract

BACKGROUND: Mangrove plants distributed in the intertidal of the tropical and subtropical region including in North Sumatra, Indonesia. The production of secondary metabolite compounds is well known to mangroves. Characterisation of prominent compounds from mangrove plants such as genus of *Avicennia* is required to explore for their biological and pharmacological properties of these compounds.

AIM: The purpose of this research was to analyse the prominent secondary metabolites through the characterisation of phytochemical, physicochemical, and microscopic of the mangrove genus Avicennia leaves, particularly *Avicennia alba, A. lanata, A. marina,* and *A. officinalis*.

METHODS: Phytochemical screening was carried out on *Avicennia* spp leaves to the established process. Physicochemical characters of mangrove leaves were investigated by simplicial powder consisting of moisture content, water-soluble, ethanol-soluble, ash content and ash soluble acid according to the WHO formula. Microscopic analysis on the simplicial powder was carried out based on the WHO procedure.

RESULTS: The result showed that physicochemical feature displays diversity among the species and important findings on the water concentration was less than 10% as a prerequisite for the drug. The phytochemical search of simplified grain also depicted divergence among the species, only alkaloid, saponin, and triterpenoid or phytosterol were found entirely in *Avicennia* spp leaves. Microscopic search found a similar type of stoma in Avicennia spp leaves, namely diacytic.

CONCLUSION: The prominent secondary metabolites in *Avicennia* spp leaves consisting of alkaloid and saponin in simplicial and triterpenoid/sterol was either in simplicial or hexane extract. The present study may provide significant pharmacological properties from mangrove *Avicennia* genus green foliages, which could accelerate another prospect for non-wood mangrove utilisation.

Introduction

Mangrove plants widespread in the intertidal of the tropical and subtropical region have numerous activities as medicinal plants, and only a few have been exposed [1]. Mangroves are famous for yielding secondary metabolite compounds mainly from isoprenoid compound groups including polyisoprenoids. The polyisoprenoids comprises of two families, i.e., polyprenol and dolichol. Polyprenol is recognised to have some pharmacological property such as anticancer [2], antidyslipidemic [3], anti-influenza, and antiviral activity [4].

The composition and diversity of polyisoprenoids compounds in mangrove forests of Iriomote Island, Japan, and North Sumatra, Indonesia have been reported [5], [6], [7]. Mangrove plants, including mangrove associates or coastal plants, are an excellent source of secondary metabolites largely derived from isoprenoid alcohol [8]. In virtually all living organisms, polyisoprenoid alcohols undeviating five-unit polymers. Secondary metabolites are valued and exploited for their phytochemical and other activities in their interaction with the environment [9].

Isoprenoids are regarded to b necessary as an active biological compound for medicinal properties due to their widespread biological and pharmacological properties [10], and mangrove plants have long been having been in traditional medicine for a long time [11]. Therefore, this study aims to analyse prominent secondary metabolites through the phytochemical, physicochemical, and microscopic of a particular genus of *Avicennia* leaves, namely *Avicennia alba*, *A. lanata, A. marina,* and *A. officinalis.*

Material and Methods

Avicennia alba, A. lanata, A. marina, and *A. officinalis* (Acanthaceae) was compiled from a mangrove community at Lubuk Kertang, Langkat, North Sumatra, Indonesia. The species of plants are determined at the Bogor Research Center for Biology, the Indonesian Institute of Science (LIPI). There the specimen vouchers were deposited. *Avicennia* leaves were compiled, wet sorting was done to separate dirt material, washed under tap water, drained and weighed. The mangrove leaves are then dried in a drying cabinet; dry sorting was performed to separate unwanted parts of plants and other impurities that remain in dry Simplicia, then weighed and stored in tightly closed plastic containers [12], [13].

Each 200 g of Simplicia powder from *Avicennia* leaves was dissolved with 2000 mL of a mixture of chloroform: methanol (2: 1, v / v) for 48 hours. Lipid extract from leaves was saponified at a temperature of 65°C for 24 hours in 86% ethanol comprising KOH 2 M. The non-saponified portion was then dissolved with n-hexane, then the solvent was evaporated. Then re-dissolve in n-hexane, pour or filter. Concentration was carried out using a rotary evaporator at 40°C. Then dried as a concentrated extract was obtained [5], [6], [7]. Avicennia leaves' physicochemical parameters were analysed by simplified powder involving moisture content, water-soluble, ethanol-soluble, ash content, and the essay extract was implemented by the WHO standard [12] and earlier works [13], [14].

On *Avicennia* green foliages to the standard process as described above were performed, a phytochemical method is simplified and hexane extract [12]. As previously reported, phytochemical analysis consisting of secondary metabolites, i.e. alkaloids, flavonoids, glycosides, saponins, tannins, triterpenoids/sterols [13], [14].

Microscopic studies on the simplification of true Avicennia spp leaves mangroves were conducted based on the WHO technique [12]. Specifically, a microscopic examination of the simplified powder was performed by robust chloral hydrate solution on the slide, scattered with simplified powder, then protected by a covering glass and detected under a microscope.

Results

Table 1 recapitulated the physicochemical of *Avicennia* spp photosynthetic tissues. The characterisation in this observation led to simplicial of *A. alba, A. lanata, A. marina,* and *A. officinalis* moisture content was 7.95%, 7.52%, 7.74%, and 7.23% respectively. The lower water content of usually incorporated to the necessity for drug progress was less than 10%. The water concentration enclosed ≥ 10% can occur the process of material degradation and damage, could not be stored for a long time.

Table 1: Physicochemical characteristic of *Avicennia* spp leaves

No	Species	Water content (%)	Water-soluble (%)	Ethanol soluble (%)	Total ash (%)	Ash insoluble acid (%)
1	*A. alba*	7.95	28.16	17.59	10.69	1.93
2	*A. lanata*	7.52	28.57	18.84	9.23	1.54
3	*A. marina*	7.74	25.78	18.45	7.12	1.91
4	*A. officinalis*	7.23	26.35	18.98	8.65	1.78

Phytochemical analysis of simplified powder and n-hexane extracts of preferred *Avicennia* genus mangrove leaves, *A. alba*, *A. lanata*, *A. marina*, and *A. officinalis* performed to collect secondary metabolite group data. Table 2 depicted the results of simplicial grain on the phytochemical screening and *Avicennia* spp n-hexane extract. The results of the grouping are then applied as an advance prominence to classify which types of compounds operate in contradiction of phytopharmacological activities.

Table 2: Phytochemical screening in simplicial and hexane extract *Avicennia* spp

No.	Compound	Solvent	Mangrove species	Simplicial	Hexane
1.	Alkaloids	Dragendorff	*A. alba*	+	–
		Bouchardat	*A. lanata*	+	–
		Mayer	*A. marina*	+	–
			A. officinalis	+	–
2.	Flavonoids	Zn + HCl	*A. alba*	–	–
			A. lanata	+	–
			A. marina	+	–
			A. officinalis	–	–
3.	Glycosides	Molisch	*A. alba*	–	–
			A. lanata	+	–
			A. marina	+	–
			A. officinalis	–	–
4.	Saponin	Hot water + HCL	*A. alba*	+	–
			A. lanata	+	–
			A. marina	+	–
			A. officinalis	+	–
5.	Tannins	$FeCl_3$ 1%	*A. alba*	–	–
			A. lanata	+	–
			A. marina	+	–
			A. officinalis	–	–
6.	Triterpenoid/ Steroid	Liebermann-Burchard	*A. alba*	+	+
			A. lanata	+	+
			A. marina	+	+
			A. officinalis	+	+

Where (+): contain the compound; (–): not contain.

Simplicial powder phytochemical investigation reflects the diversity among *Avicennia* species; for example, alkaloid and saponin were detected in *Avicennia* spp. The phytochemical investigation in hexane extract, on the other hand, did not show any alkaloids, flavonoids, glycosides, saponin, and tannin, but only triterpenoid or phytosterol found in *Avicennia* spp. Phytochemical tests showed that flavonoids, glycosides, and tannins were part of the simplified powder, as summarised in Table 2. Adding Molisch reagent and absorbed sulfuric acid created a purple ring that showed glycoside compounds (Table 2).

Table 3: Isoprenoid and polyisoprenoid pattern in *Avicennia* spp

Species	Tissue	Isoprenoid (%)		Polyisoprenoid (%)		Ref
		Triterpenoid	Phytosterol	Polyprenol	Dolichol	
A. alba	Leaves	70.1	29.9	nd	100	[5], [15]
A. lanata	Leaves	ni	ni	nd	100	[6]
A. marina	Leaves	91.0	9.0	4.2	95.8	[5], [16]
A. officinalis	Leaves	61.5	38.5	nd	100	[6], [15]
A. alba	Roots	47.4	52.6	2.2	97.8	[5], [15]
A. lanata	Roots	ni	ni	nd	100	[6]
A. marina	Roots	57.8	42.2	nd	100	[5], [16]
A. officinalis	Roots	100	nd	7.7	92.3	[6], [14]

Where, nd = not detected; ni = no information.

While Dragendorf, Bouchardat, and Mayer reagent solutions add definite findings as *Avicennia* spp (Table 2), the highest percentage of isoprenoid and polyisoprenoid secondary metabolites for *Avicennia* leaves and roots was shown in Table 3.

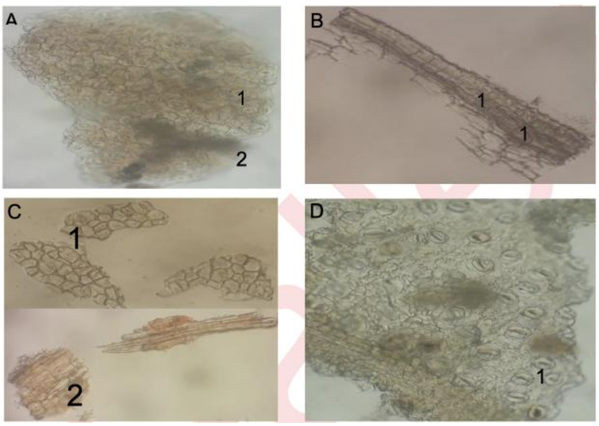

Figure 1: Microscopic observation of the simplicial powder of A. alba leaves; A) which 1. Diacytic type stomata, 2. Prismatic oxalate crystal; B) Simplicial crumb of A. lanata leaves, where 1. Prismatic oxalate crystal; C) Simplicial grain of A. marina leaves, where 1. Diacytic type stomata, 2. Parenchyma tissue; D) Simplicial powder of A. officinalis leaves, where 1. Diacytic type stomata

In the leaves, triterpenoid, and dolichol as well as dominated over phytosterol and polyprenol, respectively (Table 3). By contrast, in the roots, the isoprenoid composition showed variation, only in *A. officinalis* triterpenoid predominated over phytosterol. It is noteworthy that the occurrence of both triterpenoid and phytosterol were detected in *A. alba* and *A. marina* roots (Table 3). Microscopic search detected a similar type of stoma in *Avicennia* spp leaves, namely diacytic type stomata as depicted in Figure 1. In this perspective, a microscopic method is one of the simplest and cheapest ways to study for clarification of the source materials of medicinal plants such as in mangrove plants.

Discussion

Characterisation of Avicennia genera's phytochemical investigation enabled us to analyse the content of ethanol-soluble specifies the constituents existing in ethanol solvents that are considered to be the polar and non-polar signal. Analysis of the water and ethanol soluble content was done to show the number of water and ethanol soluble polar compounds. This result suggested the occurrence of more dissolved constituents in water solvents. To destroy organic compounds and their derivatives, ash content is determined [13], [14]. To define levels of inorganic compounds that are not soluble in acids such as silica gel, the ash content is not acid-soluble. The high total ash concentration describes the presence of inorganic metal elements, for instance, lead, calcium, iron, magnesium, which may be partly caused by contamination [17].

The improvement of solid foam with shaky water and the addition of 2N HCl did not lose an indication that saponin compounds exist [13]. Adding Mg powder and concentrated hydrochloric acid produces a red solution, and with amyl alcohol quantity, the red colour is involved in the amyl alcohol layer indicating the occurrence of flavonoids [13]. FeCl3's addition gave a blackish-blue colour that depicts tannin compounds [18]. Adding LB reagents created blue-green, violet and red-violet colors that specified triterpenoid/steroid compounds [15], [16], [19].

In line with this finding, the polyprenols in the root tissues were also dominated by dolichols [5], [7]. Polyisoprenoids have been tested *in vitro* to be more suitable as an antimicrobial agent and an oxidant in associated mangrove leaves and roots [20], consisting mostly of dolichols. Therefore, these results supported the previous view that polyisoprenoid composition in mangroves was not polyprenols but dolichols.

Microscopic search detected a similar type of stoma in *Avicennia* spp leaves, namely diacytic type stomata. The microscopic study is a vegetation type indicator [21]. In this perspective, one of the simplest and cheapest ways to study a microscopic method is by establishing to clarify the source materials of medicinal plants such as in mangrove plants. Thus, the phytochemical pattern, including microscopic information, will help in standardisation of quality, purity and sample identification.

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