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Characterization of phytochemical, physicochemical, and microscopic from five selected mangrove associate leaves

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Abstract. This present study reports the analysis of phytochemical, physicochemical, and microscopic of selected mangrove associates leaves, namely *Acacia auriculiformis*, *Hibiscus tiliaceus*, *Pandanus odoratissimus*, *Pometia pinnata*, and *Ricinus communis*. Phytochemical was performed on mangrove leaves based on the developed method. Physicochemical properties of mangrove leaves were determined by simplicial powder that consists of moisture content, water-soluble, ethanol soluble, ash content and the essay extract was carried out to the standard method. Microscopic observation on the simplicial powder was done according to World Health Organization (WHO) method. Phytochemical screening of simplicial powder shows variation among the species, only saponin and triterpenoid/phytosterol were detected in all species. By contrast, the phytochemical analysis in the hexane extract alkaloids, flavonoids, glycosides, saponin, and tannin was not identified, however, only triterpenoid/steroid was observed in all species. Physicochemical properties also depict diversity among the species and significant finding on the water content was under 10% as a condition for the drug. Microscopic screening found a different type of stomata in mangrove associates leaves, *H. tiliaceus* and *P. pinnata* had paracytic stomata-type, *P. odoratissimus* and *R. communis* contained anomocytic, only *A. auriculiformis* was distinct type due to having two kinds of stomata: paracytic and anisocytic. The present study may provide potential biological and medicinal properties from mangrove associates leaves.

1. Introduction

Mangrove plants, including mangrove associates or coastal plants, are an excellent source of secondary metabolites generally derived from isoprenoid alcohol [1-2]. Polyisoprenoid alcohols are undeviating five unit polymers that are in virtually existing all living organisms. Secondary metabolites are useful in their interaction with the environment, in the development molecules valued, and exploited for their phytochemical and other activities [3]. Because of their wide-ranging biological activities, isoprenoids are considered as important as active biologically compound for medicinal properties [4], and mangrove associates have long been practised in traditional medicine [5].

Furthermore selected species, namely *Acacia auriculiformis*, *Hibiscus tiliaceus*, *Pandanus odoratissimus*, *Pometia pinnata*, and *Ricinus communis* are common mangrove associates in North



Sumatra [2]. A number of biological and phytochemical activities of mangrove associates have been described. For example, the antinociceptive and anti-inflammatory activity of phenolic from *H. tiliaceus* and *P. odoratissimus* leaf extracts have been described [6-7]. *R. communis* leaf extracts have been shown to have antimicrobial property [8]. Recently it has been reported the screening of twelve mangrove associate leaves to have significant antibacterial activities that open up the possibility for antimicrobial drug development [9]. In spite of the importance of secondary metabolites from mangrove associates as potential sources of phytochemistry, limited information on the phytochemical and physicochemical of mangrove associate in North Sumatra has not previously been presented. This current study, therefore, aimed to describe the phytochemical, physicochemical, and microscopic analysis of selected mangrove associates leaves to extend our previous works to search isoprenoid and polyisoprenoid compounds from mangrove plants [1-2,10].

2. Materials and method

2.1. Materials

Selected mangrove associates leaves, i.e. *Acacia auriculiformis*, *Hibiscus tiliaceus*, *Pandanus odoratissimus*, *Pometia pinnata*, and *Ricinus communis* were collected from mangrove forest at Lubuk Kertang, Langkat, North Sumatra, Indonesia. The identification of plant species is conducted at Herbarium Medanense, Medan, North Sumatra. The specimen vouchers have been deposited there.

2.2. Phytochemical investigation of simplicial and hexane extract

A phytochemical method in simplicial and hexane extract was performed on mangrove associates leaves to the standard process as earlier described [2]. Phytochemical analysis composed of several primary, secondary metabolites, alkaloids, flavonoids, glycosides, saponins, tannins, triterpenoids/sterols were done as previously reported [11].

2.3. Physicochemical properties

Physicochemical parameters of mangrove associate leaves were analyzed by simplicial powder that involves of moisture content, water-soluble, ethanol soluble, ash content, and the essay extract was implemented to the standard of WHO [12] and the previous study [11].

2.4. Microscopic analysis

Microscopic studies on the simplicial powder of five selected mangrove associate leaves were performed based on WHO method [12]. Concisely, a microscopic investigation of the simplicial powder was conducted by robust the solution of chloral hydrate on the slide, interspersed with simplicial powder, then shielded with a cover glass and observed under a microscope.

3. Results and Discussions

3.1. Phytochemical investigation of selected mangrove associate leaves

The phytochemical analysis of simplicial powder and extracts of n-hexane from chosen mangrove associate leaves, *A. auriculiformis*, *H. tiliaceus*, *P. odoratissimus*, *P. pinnata*, and *R. communis* were performed to get data on the group of secondary metabolites. The results of simplicia powder on the phytochemical investigation and n-hexane extract from five mangroves associate were depicted in Table 1. The classification results are then used as a further emphasis to categorize which groups of compounds have activity against phytopharmacological activities.

Phytochemical investigation of simplicial powder expresses diversity among the species; only saponin and triterpenoid/phytosterol were detected in all species. By contrast, the phytochemical analysis in hexane extract shows no alkaloids, flavonoids, glycosides, saponin, and tannin detected, however, only triterpenoid/phytosterol found in all species. Phytochemical tests displayed that the simplicial powder comprised significant flavonoids, glycosides, saponins, tannins and triterpenoids/steroids with minor of alkaloids (Table 1). The adding of Molisch reagent and focused sulfuric acid formed a purple ring which indicates the occurrence of glycoside compounds (Table 1). The development of stable foam with shaky in water and did not lose with the addition of 2N HCl suggests the existence of saponin compounds [11]. The addition of Mg powder and concentrated

hydrochloric acid yields a red solution, and with the accumulation of amyl alcohol, the red color is attracted to the amyl alcohol layer which indicates the presence of flavonoids [11]. The addition of FeCl₃ gives a blackish blue color that shows the presence of tannin compounds [8]. The addition of LB reagents forms blue-green, purple and red-purple colors which specify the presence of triterpenoid/steroid compounds [11, 13, 14].

While the addition of Dragendorff, Bouchardat, and Mayer reagent solutions give positive results on *A. auriculiformis* and *R. communis* leaves only, by forming turbid solutions. The content of alkaloid compounds in the others samples of simplicia (*H. tiliaceus*, *P. odoratissimus*, and *P. pinnata*) was not traced (Table 1). Polyisoprenoids have tested *in vitro* is more useful as an antimicrobial agent and an oxidant in mangrove associate leaves [9]. Recently it has been reported that polyisoprenoids from *Nypa fruticans* leaves inhibited cyclooxygenase 2 expression of WiDr colon cancer [14]. Dolichols from *Avicennia marina* and *A. lanata* has been shown to inhibit WiDr cells proliferation [15].

Table 1. Phytochemical screening in simplicial and hexane extract

No.	Compound	Solvent	Mangrove species	Simplicial	n-hexane
1.	Alkaloids	Dragendorff Bouchardat Mayer	<i>A. auriculiformis</i>	+	-
			<i>H. tiliaceus</i>	-	-
			<i>P. odoratissimus</i>	-	-
			<i>P. pinnata</i>	-	-
			<i>R. communis</i>	+	-
2.	Flavonoids	Zn + HCl	<i>A. auriculiformis</i>	-	-
			<i>H. tiliaceus</i>	+	-
			<i>P. odoratissimus</i>	+	-
			<i>P. pinnata</i>	+	-
			<i>R. communis</i>	+	-
3.	Glycosides	Molisch	<i>A. auriculiformis</i>	-	-
			<i>H. tiliaceus</i>	-	-
			<i>P. odoratissimus</i>	+	-
			<i>P. pinnata</i>	+	-
			<i>R. communis</i>	+	-
4.	Saponin	Hot water + HCL	<i>A. auriculiformis</i>	+	-
			<i>H. tiliaceus</i>	+	-
			<i>P. odoratissimus</i>	+	-
			<i>P. pinnata</i>	+	-
			<i>R. communis</i>	+	-
5.	Tannins	FeCl ₃ 1%	<i>A. auriculiformis</i>	-	-
			<i>H. tiliaceus</i>	-	-
			<i>P. odoratissimus</i>	+	-
			<i>P. pinnata</i>	+	-
			<i>R. communis</i>	+	-
6.	Triterpenoid/Steroid	Liebermann-Burchard	<i>A. auriculiformis</i>	+	+
			<i>H. tiliaceus</i>	+	+
			<i>P. odoratissimus</i>	+	+
			<i>P. pinnata</i>	+	+
			<i>R. communis</i>	+	+

(+): contain the compound

(-): not contain

3.2. Physical and chemical properties

Table 2 recapitulates the physicochemical of five selected mangrove associate leaves. Description testing leads of simplicial of five mangroves associate *A. auriculiformis*, *H. tiliaceus*, *P. odoratissimus*, *P. pinnata*, and *R. communis* for moisture content 6.92%, 6.46%, 4.73%, 5.76%, and 6.12%, respectively, usually incorporate to the of requirements for drug development $\leq 10\%$. The water content, therefore, contained more than 10% lead the process of decay and damage to the material.

Table 2. Physicochemical characteristic of mangrove associate leaves

No	Species	Water content (%)	Water soluble (%)	Ethanol soluble (%)	Total ash (%)	Ash insoluble acid (%)
1	<i>A. auriculiformis</i>	6.92±0.2	28.63±3.6	17.82±2.8	7.24±	1.83±0.2
2	<i>H. tiliaceus</i>	6.46±0.3	28.37±3.7	18.57±5.7	3.45±	1.79±0.4
3	<i>P. odoratissima</i>	4.73±0.4	22.98±3.8	21.42±4.2	3.71±	0.97±0.1
4	<i>P. pinnata</i>	5.76±0.5	24.17±7.1	20.68±2.5	5.34±	1.73±0.3
5	<i>R. communis</i>	6.12±0.1	17.67±6.7	15.16±5.4	4.26±	2.89±0.1

Data are mean of triplicate analyses ± SD (n= 3).

Determination of the concentration of the mangrove associate leaves which in water-soluble is carried out to search the materials that are present in the water solvent [14]. Analysis of the content of ethanol soluble indicates the substances that are present in ethanol solvents, which are regarded as the polar and non-polar indication. Determination of the content of soluble in water and ethanol was performed to show the number of polar compounds which are soluble in water and ethanol. This result suggests that more dissolved substances in water solvents occurred. Determination of ash content is carried out to destruct organic compounds and their derivatives [11, 16]. The ash content is not acid soluble to define the levels of inorganic compounds which are not soluble in acids such as silica gel.

The high total ash content depicts the existence of inorganic substances of metals, such as Ca, Mg, Fe, Cd and Pb which may partly derive from contamination [17]. High heavy metal content can endanger health, therefore it is necessary to determine the total ash content and acid insoluble ash content to confirm the simplicial leaf does not enclose certain heavy metals surpassing the definite value because it is toxic to health [17]. The simplisia used as raw material for medicines must meet the requirements documents published by the Ministry of Health, Indonesia.

3.3. Microscopic analysis

The microscopic screening established a diverse type of stomata in mangrove associates leaves in line with [18]. *H. tiliaceus* and *P. pinnata* possessed the paracytic stomata-type, while *P. odoratissimus* and *R. communis* contained anomocytic, only *A. auriculiformis* was distinguishable type due to have two type of stomata: paracytic and anisocytic. It has been shown that microscopic study to be an indicator type of vegetation [19].

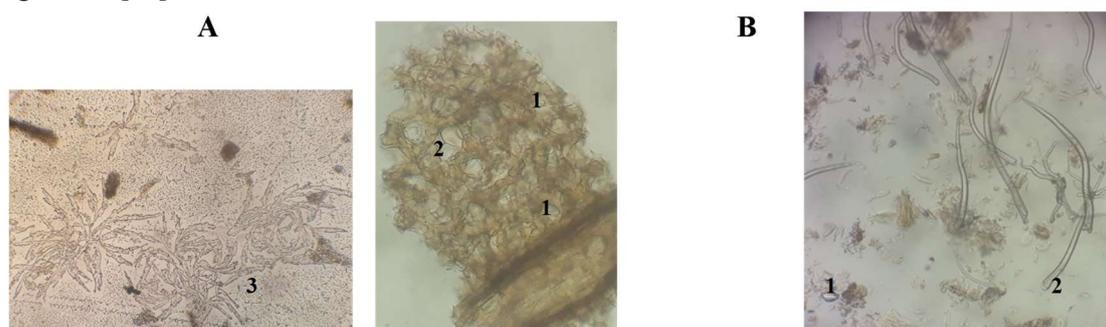


Figure 1. Microscopic analysis on the simplicial powder of *A. auriculiformis* leaves (A), which 1. Paracytic type stomata, 1. Anisocytic type stomata 2. Parenchymal tissue, 3. Fibre. B. Simplicial powder of *H. tiliaceus* leaves, where 1. Paracytic stomata, 2 Hair cover.

Figure 1 represented the microscopic examination of simplicia powder of *A. auriculiformis*, showing the presence of parenchymal tissue and specific fibers. Furthermore, microscopic investigation of simplicia powder of *H. tiliaceus* displayed the presence of specific cover hair.

In this circumstance, microscopic analysis is one of the simplest and cheapest methods to start with for forming the correct identification of the source materials from plants including medicinal plants. Therefore the phytochemical profile along with microscopic data will support the standardisation of clarity and purity of sample proof of identity.

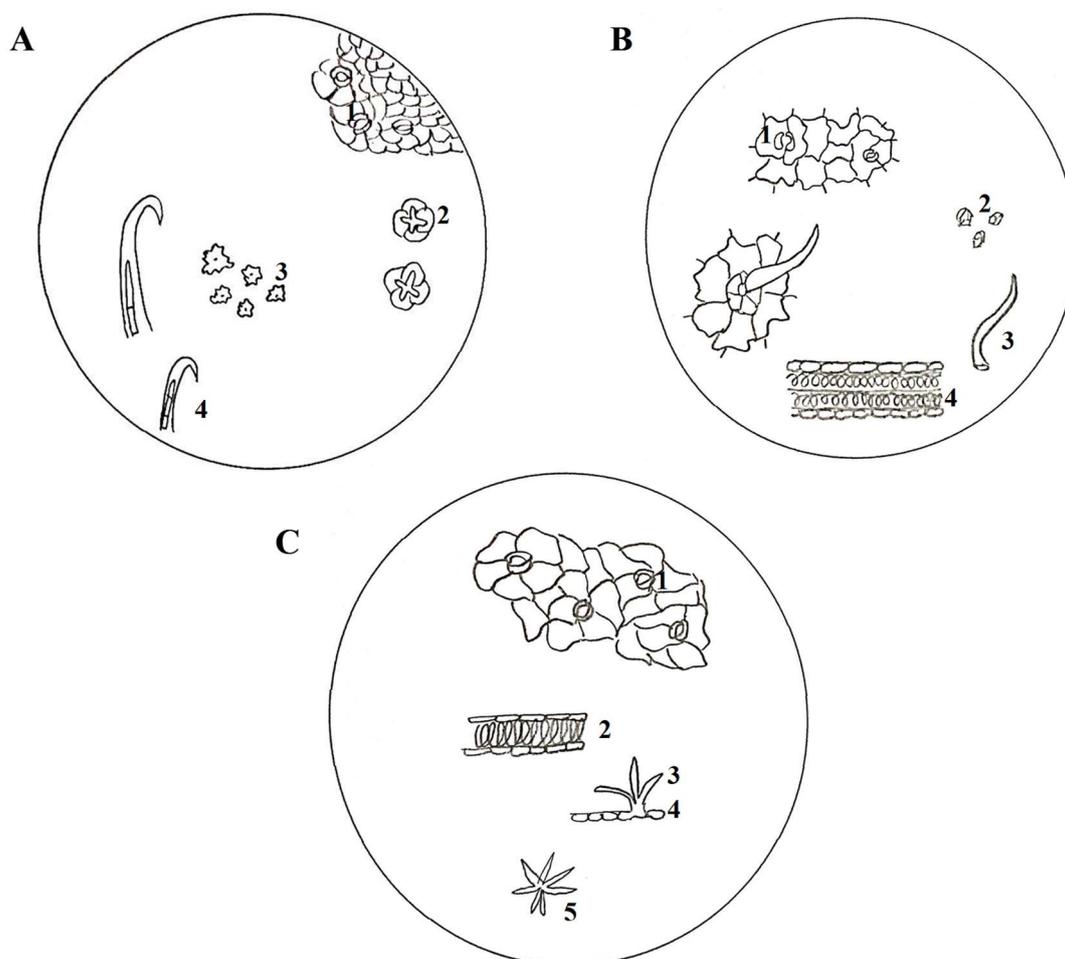


Figure 2. Microscopic analysis of the simplicial powder of *P. odoratissimus* leaves (A), which 1. Anomocytic type stomata, 2. Secretory glands, 3. Druse from oxalate crystal, 4. Hair cover, B. Simplicial powder of *P. pinnata* leaves, where 1. Paracytic type stomata, 2. Calcium oxalate crystals form prisms, 3. Trichoma, 4. Xylem with thickening of the spiral wall. C. Simplicial powder of *R. communis* leaves, where 1. Anomocytic stomata, 2. Transport files with spiral thickening, 3. Hair cover, 4. Epidermis, 5. Hair cover star shape

4. Conclusions

The current report clarified the physicochemical properties also showed the diversity among the species and essential finding on the water content was fewer than 10% as a requirement for drug development. Our study may provide potential phytopharmacological characteristics from mangrove associate leaves, which may open up another possibility of non-wood mangrove potential.

5. Acknowledgment

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