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Short Communication: Analysis of the chemical constituents and micromorphology of *Bauhinia scandens* using SEM-EDS techniques

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Abstract. *Lianah L, Idris F, Krisantini K. 2019. Short Communication: Analysis of the chemical constituents and micromorphology of* Bauhinia scandens *using SEM-EDS techniques. Biodiversitas 20: 2055-2060.* Some species of *Bauhinia* had a medicinal efficacy however the specific studies of *Bauhinia scandens* Willd. are relatively limited. Therefore this study analyzed the material and micromorphological characteristics of leaves, stems and roots of *B. scandens* using scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) to quantitatively assess the elements and explore its potential uses. Samples of fresh plant were taken from five locations in Indonesia, and the results showed similar snake-like stem morphology from two locations, while pilot ladder-like stem morphology was observed from the other three locations. The material analysis revealed the presence of carbon, oxygen, silicon, potassium, indium, tellurium, aluminum and iron in the samples. Interestingly, the stems did not contain indium and aluminum whereas iron were only found in the roots. Furthermore, the young leaves did not contain tellurium and silicon. The presence of indium which is potential as a cancer drug in *B. scandens* suggests promising uses of the plant for herbal medicines.

Keywords: *Bauhinia scandens*, morphology, micromorphology, material analysis

INTRODUCTION

Bauhinia is a species of the family Fabaceae and the subfamily Caelsapinioideae (Rudd et al. 1991). Previous research on *Bauhinia* revealed that some species of *Bauhinia* had potential uses in traditional medicines. *B. variegata* is a potential antitumor, antibacterial, antioxidant and anticancer drug (Mishra et al. 2013; Pandey 2017), but there have been relatively limited studies specifically on *Bauhinia scandens* Willd, which is commonly used to produce ropes, but can be a valuable plant for herbal medicines, as it contains antioxidant and antibacterial components (Hossain et al. 2016; Selim et al. 2017).

Bauhinia scandens is a flowering plant that can grow as a woody plant with 30-50 m in height with opposite tendrils, and while its young the branches are brown while its old the stem is wavy and forms a "monkey staircase" and the giant plant, mostly found in limestone areas with altitude up to800 m in lowland Indonesia. *B. scandens* prefers seasonal climates and has not been reported in evergreen forests of the Malay Peninsula, but it is reported to be found in India, Burma, China, Laos, Cambodia, Vietnam and Thailand, as well as in the Indonesian Malesia regions of Sumatra, Java, Madura and Sumba (Larsen and Larsen 1996). In Central Java, Indonesia, *B. scandens* is found in Pagerwunung Darupono Nature Reserve (Kendal District), Silayur Forest (Mijen, Semarang), tomb (Tugurejo, Semarang), Sam Poo Kong (Semarang), Peson Subah II Nature Reserve (Batang District).

Kuswanto (2016) described the morphological characteristic of *B. scandens* from Pagerwunung Darupono Nature Reserve. The young stems of the species are cylindrical, while the old stems have dark color and form ‘monkey ladder' shape. The liana tree has stems with diameter of 15-18 cm and 25 m in height. The unique characteristic of *B. scandens* is its entire leaves have cow’s paws shape. The leaves are ovate lamina with 50-90 mm in length and 40-80 mm in width.

In their study of *B. scandens* in India, Tabakhsh et al. (2008) identified the antibacterial activity due to the presence of indium curcumin and indium diacetyl curcumin extracted from the plant, while its antioxidant properties are as expected due to phenolic components such as flavonoids, phenolic acids and phenolic diterpenes (Shahidi et al. 1992; Osawa, et al. 1994; Pieta, et al. 1998). *B. scandens* has been proposed to treat various diseases, and it is rich in essential organic and inorganic elements with pharmacological functions (Ratna Raju et al. 2016), but it may also contain toxic elements. The elemental contents of medicinal plants depend on soil composition, location of plant specimen or plant parts, and climate (Ratna Raju et al. 2016). Therefore, scientific studies of medicinal plants are required to understand their chemical characteristics and to explore their potential uses.

As highlighted above, studies on *B. scandens* are relatively few, and specifically, studies on material and micromorphology analyses of the plant using scanning electron microcopy-energy dispersive spectroscopy (SEM-

EDS) are essentially non-existent. Yet, such study is required to identify the medicinal characteristics of the plant. SEM-EDS is uniquely suitable to analyze individual elements in a compound and can analyze surfaces, hardness textures, and material reflectance. The technique can analyze the shape, size, and composition of samples qualitatively, but quantitative analyzes are more informative. The information on the concentration of these elements is important for determining the effectiveness of the plant in treating various ailments. SEM-EDS has been applied to obtain microphotograph and elemental concentration of medicinal plants, such as *Eclipta alba* Hassk and *Eclipta prostrata* Linn (Muruganantham *et. al*. 2009). Therefore, the objectives of the study were to quantify the chemical elements and atoms in *B. scandens* and the shape and size of the micromorphology of its leaves, stems, and roots.

MATERIALS AND METHODS

Samples for morphological study were collected from five locations in Central Java, Indonesia which were Sam Poo Kong (Semarang City), Silayur (Mijen, Semarang City), Peson Subah II Nature Reserve (Batang District), Tugurejo tomb (Semarang City), and Pagerwunung Darupono Nature Reserve (Kendal District). Meanwhile, the study of micromorphology and material analysis used samples from Silayur Forest in Mijen, Semarang.

Samples (young and mature leaves, stems and roots) of the fresh plant were taken from Silayur Forest and were preserved with 70% alcohol before analysis. Samples from the upper and lower parts of the epidermis were gently stripped using needles and forceps. The epidermal skin was washed with water two to three times before being dried prior to observation using SEM to record the microphotographs. Elemental identifications were carried out using energy dispersive X-ray spectrometer (EDS) following the procedures in Malesiana (Hou and Larsen 1996), and they were confirmed using the database at the Herbarium Bogoriense (BO), Research Center for Biology, Indonesian Institute of Sciences, Cibinong, Bogor, Indonesia and the Bogor Botanic Gardens, Indonesian Institute of Sciences, Bogor, Indonesia. The analysis of SEM-EDS was carried out in Universitas Islam Negeri Walisongo, Semarang, Indonesia.

RESULTS AND DISCUSSION

Morphology and micromorphology of *Bauhinia scandens*

The morphological observations (Figure 1) of the samples are summarized in Table 1, and there are differences (presence and absence) between the indicated components. Similar morphological form in leaf was found in the samples collected from four of the five locations, monkey staircases form of the stem with 10-25 cm diameter were found in the samples from four locations, while flowers, fruits, and seeds of the plant were collected only from the Sam Poo Kong location.

Most lianas including *B. scandens* have stems with uncommon secondary growth (Beck 2010). A complex developmental process forms stem undulation. The undulations grow along with the stem growth. In monkey ladder species of *Bauhinia*, unique stem shapes are the results of the variant secondary growth and G-fibers combination (Fisher and Blanco 2014).

Figure 2 shows the micromorphology of the samples using the SEM-EDS, and this reveals a network of vessels that is unique compared to the network of ordinary tree vessels. Stomata can be seen in the young and mature leaves with shape variations.

Our result shows that the cross section of the root is unclear or unstructured (Figure 2.A). Whereas in the stem (Figure 2.B), the vascular tissue appears to be unique compared to the normal vascular bundle. According to Fisher and Blanco (2014), the secondary xylem of *B. scandens* is undulated and has two lobes. The xylem structure in this study is spiral (Figure 2.B). Vessel walls are thickening by producing lignified secondary cell walls inside the primary cell wall (Wooding and Northcote 1964). The cell wall thickening is initially formed as annular wall before it becomes helical or spiral with interconnected helices, coils or scalariform thickening (Bierhorst and Zamora 1965).

In Figure 2.C, there are not many clear leaf support tissues and the stomata are still small. Furthermore, in Figure 2.D, the stomata and its network are clearly visible. Stoma density and size of plants in their natural habitat vary significantly. Plants can have either a few big stomata or a group of more small stomata (Frank and Beerling 2009). These stomata enable plants to balance the gas exchange rates and response efficiently to environmental changes (Beerling and Royer 2002). The phloem tissues in this study had a unique variation in shape. Normally, the pattern of vascular bundles forms a collateral structure in which phloem tissues are inside of xylem tissues (Mauseth 1988).

Material analysis

The EDS analysis showed that *B. scandens* contained various elements (Table 2), and Figure 3 shows the EDS spectra of the crude extract of the plant.

Table 1. Morphology data of the *Bauhinia scandens* collected from the five research locations*

Section	Research location				
	Silayur	Darupono	Tugurejo	Sam Poo Kong	Peson Subah II
Leaf shape	+	+	+	-	+
Stem	+	+	+	+	+
Want	+	+	+	+	+
Flower	Pink	-	-	White	-
Fruit	-	-	-	+	-
Seed	-	-	-	+	-

Note: *+ = present,-= absent

Iron was detected in the roots at a concentration of 0.62%. It is a whitish gray metal, which can be forged, and it is the fourth most constituent on earth. In nature, iron is found in various forms and it is highly reactive in damp or high-temperature conditions. In living creatures, iron is an important cell component. The stems contained potassium as much as 0.82%, which is classified as a micronutrient element with magnesium and other elements. Potassium is an alkali element (HAM 2006). In plants, it functions to reduce damaging effects of abiotic stress such as drought, chilling, high light intensity, heat and nutrient limitations (Cakmak 2005).

Carbon, oxygen, silicon, potassium, indium, tellurium, aluminum, iron, and stibium were found in the plant, and the most prominent elements were carbon and oxygen that were found in the samples studied. Aluminum (2.04%) was only found in the roots, and in plants, aluminum enhances plant growth by promoting rhizobacteria (PGPR) and aids interactions between PGPR and plants (Muhammad et al. 2018). Aluminum also has a medical role as it is one of the ingredients in antiperspirants, where it suppresses perspirations by blocking the relevant ducts or chemically inhibiting sweat glands (Quatrale et al. 1985).

Figure 1. *Bauhinia scandens* collected from Central Java, Indonesia: A. Sam Poo Kong, B. Silayur, C. Peson Subah II Nature Reserve, D. Pagerwunung Darupono Nature Reserve, and E. Tugurejo Tomb.

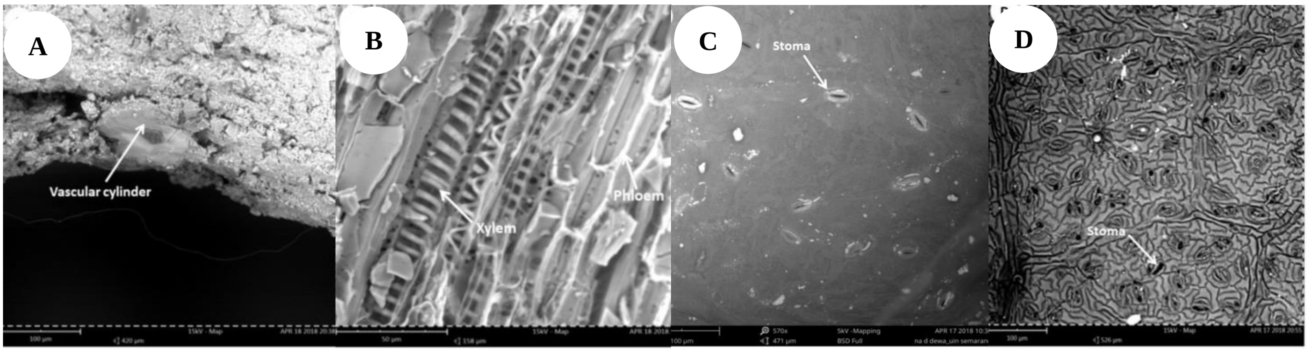

Figure 2. Micromorphology of A. Root, B. Stem, C. Young leaf, D. Mature leaf

Another element, silicon, was found in the roots and the mature leaves of the plant at concentrations of 2.48% and 0.3%, respectively. The absence of silicon in the young leaves could be due to the different time periods between the young and mature leaves in absorbing elements from the soil. Silicon enhances abiotic and biotic stress tolerances in plants (Deshmukh *et. al.* 017), and although silicon functions are not well understood in humans, it has been suggested that it plays a part in the synthesis of collagen and/or its stabilization, and in matrix mineralization of bones (Jugdaohsingh 2007).

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Table 2. The element compositions in *Bauhinia scandens*

Sample	Element			Concentration (%)	
	Number	Symbol	Name	Atomic	Weight
Root	6	C	Carbon	56.36	46.13
	8	O	Oxygen	38.34	41.79
	14	Si	Silicon	2.48	4.75
	13	Al	Aluminum	2.04	3.76
	26	Fe	Iron	0.62	2.35
	49	In	Indium	0.12	0.96
	51	Sb	Stibium	0,03	0,26
Stem	6	C	Carbon	58.36	50.17
	8	O	Oxygen	40.72	46.63
	19	K	Potassium	0.82	2.31
	52	Te	Tellurium	0.10	0.89
Mature leaf	8	O	Oxygen	43.41	49.46
	6	C	Carbon	56.06	47.95
	49	In	Indium	0.17	1.36
	52	Te	Tellurium	0.07	0.62
	14	Si	Silicon	0.30	0.61
Young leaf	8	O	Oxygen	62.73	67.75
	6	C	Carbon	36.98	29.98
	49	In	Indium	0.29	2.27

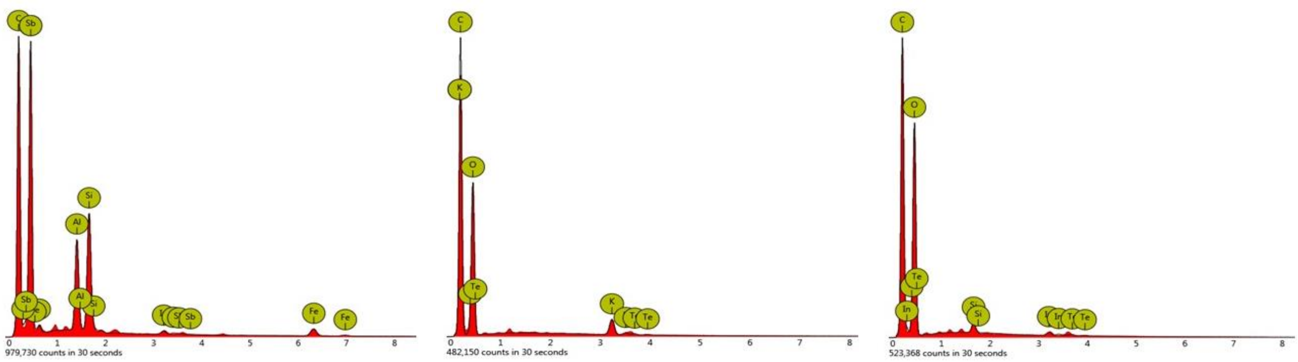

Figure 3. EDS spectra of *Bauhinia scandens*: A. Root, B. Stem, C. Leaf

The stems and mature leaves contained tellurium with concentration of 0.1% and 0.07%, respectively. Tellurium is classified as sulfur (periodic table group VI A), and it can be amorphous and crystalline solids. The crystal is silvery-white, metallic-like, p-type semiconductor, in the burning air with the blue-greenish color of the dioxides; including compounds considered poisonous. In nature, it is found in several minerals especially as telluride from gold (in kalaverit) (HAM 2006). Tellurium is a rare element, it belongs to non-essential trace element group, which are regarded as toxic. Clinical studies have shown that selenium and tellurium are anticancerous (Seng and Tiekink 2008; Sredni 2012).

Moreover, Stibium (Sb) was detected in the root with concentration of 0.03%. Plants are able to take up Sb in a large amount although it does not belong to essential elements (Telford *et. al*. 2009). There are three factors that influence the Sb concentration in plants which are phytoavailability of Sb, Sb speciation, and the ion concentrations that coexist with Sb in soils, for instance, phosphorus and calcium (Feng R 2013). According to Haldar *et. al.* (2011), Sb showed high immunostimulatory effects and increased the capability of normal antigen to stimulate T cell in the Leishmaniasis treatment. Sb also has been used to compose substance as a reliable flame retardant (Dheyaa *et al.* 2018). This ability is possibly useful for plants to reduce the effects of forest fire.

Interestingly, indium was also found in this study and detected in the roots, young leaves and mature leaves of the plant at 0.12%, 0.29%, and 0.17%, respectively. In nature, indium is often found in zinc ore, iron ore, lead and copper ore (HAM 2006). The presence of indium in the roots and leaves could be due to the cooperation between these plant parts in the transportation of minerals from the soil and processing them by photosynthesis in the leaves. The young leaves contained higher concentration of indium compared to the mature leaves possibly due to the remobilization of macronutrient and micronutrient within plant. Remobilization is a strategy to cope with nutrient deficiency by using mature leaves as a source for younger organs including young leaves. Remobilization also contributes to the process of foliar senescence which often occurs in mature leaves (Maillard et al. 2015).

The stems did not contain indium possibly because stems are only a medium or a pathway of the transportation process, and the synergistic work between the roots and leaves is enhanced by three main characteristics: root suction, surface area of plant capillaries and suction power of the leaves. Indium is thought to have antibacterial properties, and Tajbakhsh et al. (2008) reported that indium curcumin is more antibacterially effective than curcumin itself. Furthermore, Srinivas et al. (2015) reported that etoposide phosphate/indium nanoparticles induced tumor cell apoptosis, leading to significant inhibition of tumor growths compared to free drugs when mouse xenograft lung cancer model was used. De Nardo et al. (1997) reported that indium-111/yttrium-90-MX-DTPA BrE-3 can be safely administered to patients with breast cancers.

From the elemental analysis (Table 2), it can be observed that the *B. scandens* samples had macronutrient and micronutrient elements. Macronutrients are necessary elements in relatively large quantities such as the six main ones: carbon, oxygen, hydrogen, nitrogen, sulfur, and phosphorus. The three other macronutrients are potassium, calcium, and magnesium (Campbell 1987). The most widely extracted macronutrient of the soil is nitrogen, which composes nearly 4% of the dry weight of most plants, while potassium (about 5%), calcium (0.5-3.5%) and others such as magnesium, phosphorus, and sulfur are cell constituents that are found in the chlorophyll and various plant enzymes (Fried and Hademenos 2005). According to Fried and Hademenos (2005), micronutrients are minerals found in relatively fewer quantities, but are essential for processes such as activation of enzymes, the development of chloroplasts and the metabolism of other minerals. The eight main micronutrients are iron, chloride, copper, manganese, zinc, molybdenum, boron and nickel, and they serve as cofactors in enzymatic reactions (Campbell 1987).

In conclusion, the morphology of *B. scandens* from the five studied locations showed similar leaves, stems, and roots, with diameter > 65 cm and a snake-like structure that is similar to a monkey ladder. The micromorphology of the stems, roots, and leaves was identical, and the material analysis revealed carbon, oxygen, silicon, potassium, indium, tellurium, aluminum iron and stibium as the main elements. However, the stems did not contain indium, aluminum, iron, and stibium was only found in the roots. The young leaves did not contain tellurium and silicon. Stibium is potential for parasitic diseases treatment while indium is an anticancer drug. Therefore both elements are potential in herbal remedies. Further research will investigate the usefulness of the plant parts and how to extract its useful components for medicinal purposes.

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