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Effects of Maltodextrin on *Sauropus androgynus* Leaf Extract Characteristics

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ABSTRACT

Katuk (*Sauropus androgynus*) leaves are traditionally used in Indonesia to increase breast milk production in women. Because of the high moisture content, the leaf extract has been formulated into spray-dried extract powder. First, the freeze-dried leaves were extracted using ultrasonic-assisted extraction with 80% ethanol as the solvent. Second, the extract was spray-dried with maltodextrin as the drying aid and mixed with mannitol and crospovidone to improve the characteristics of the resultant powder. Then, the powder was evaluated by comparing its physical properties to those of the spray-dried *S. androgynus* extract, product X containing *S. androgynus*, and spray-dried lactose, including the angle of repose, bulk density, tapped density, true density, Hausner ratio, and moisture content. The results showed that formulating spray-dried *S. androgynus* extract into powder increased the particle size and produced better moisture content and flow, but some other properties still required improvements. After the powder formulation, thin-layer chromatography (TLC)-densitometry was conducted to analyze the samples' metabolic profiles. The dried samples were each dissolved in a suitable solvent and then spotted on the GF_{254} plate with the mobile phase developed from n-butanol:acetic acid:water (60:22:1.2). Based on the TLC profiles (i.e., chromatograms) of *S. androgynus* leaves, freeze-dried leaves, the spray-dried extract, and extract powder, three different spots were observed clearly under the 366 nm UV light. Also, one spot at Rf 0.80 (S3) appearing in all the chromatograms indicated a stable chemical compound, i.e., unaffected by all factors in the entire *S. androgynus* powder formulations: from extraction to drying and formulation.

Keywords: densitometry, metabolic profiles, *S. androgynus*, spray-dried

INTRODUCTION

Sauropus androgynus (L.) Merr., also known as *katuk*, is one of the Indonesian herbs used in folk medicine as anti-obesity, antimicrobial, anti-anemia, analgesic, and galactagogue (Yu et al., 2006; Gothandam et al., 2010; Paul & Anto, 2011; Hasimun et al*.,* 2018; Widiyanti & Heryati, 2018). These activities are potentially due to secondary metabolites or phytochemicals, including α-carotene, β-carotene, vitamin C, vitamin E, phytosterol, lignan and megastigmane glycosides, phenolic compound, quercetin and kaempferol (Kanchanapoom et al., 2003; Yu et al., 2006; Andarwulan et al., 2010; Bose et al., 2018; Yunita et al., 2019). In addition, *S. androgynus* has been proven less toxic based on *in vitro* and *in vivo* toxicity assays (Yunita et al., 2013; Lorensia et al., 2015).

S. androgynus with dark green leaves, 2–6 cm long and 1.5–3 cm wide, is cultivated in various geographies at a wide range of latitudes. Unfortunately, few literature reviews have discussed methods for distinguishing *S. androgynus* cultivars based on their morphological characteristics. However, previous studies revealed that chromatography (Yunita et al., 2019) and several DNA-based methods such as random amplified polymorphic DNA (RAPD) (Yunita & Sulisetiorini, 2013) and sequencing of internal transcribed spacers (ITS) (Yunita et al., 2016) successfully discriminate between geographically sensitive *S. androgynus* cultivars.

S. androgynus and its extract naturally contain high moisture, which may be challenging and problematic for the making of dried *S. androgynus* extract at the industrial scale because

high water content can alter the physicochemical properties of the plant extract (Rocha et al*.,* 2011; Archer et al., 2020). For starters, it forms stronger interparticle liquid bridges, decreasing the flowability of the powder than the one derived from low-moisture plants (Crouter & Briens, 2014). Therefore, some chemicals like maltodextrin are introduced to address the problem. Hardjanti (2008) found that this additive can reduce the hygroscopic characteristic of *S. androgynus* powder. Maltodextrin is a product of starch hydrolysis consisting of D-glucose units linked together mainly by α (1→4) glycosidic bonds. It is primarily used in spray drying because of its physical properties, such as high solubility in water (Cano-Chauca et al., 2005). Also, it has been used in producing Tamarind powder with dispersibility and viscosity significantly depending on both maltodextrin content and drying temperature (Ekpong et al., 2016).

The objective of this study was to formulate dried *S. androgynus* extract by using maltodextrins as the spray drying agent along with mannitol and crospovidone. Mannitol and crospovidone are expected to improve the derived *S. androgynus* powder in Hardjanti (2008), which indicated that using maltodextrin alone in the spray drying does not affect its physical (i.e., color and rehydration) and chemical properties (i.e., water and chlorophyll content). In addition, mannitol and maltodextrin can enhance the compactibility of a tablet formulation, while crospovidone is a disintegrant that helps release active pharmaceutical ingredients from dosage forms. For instance, in Al-Zoubi et al. (2021), paracetamol tablets containing maltodextrin and mannitol has optimal powder flow, mechanical strength, and disintegration.

There has been minimal research on the physicochemical properties of *S. androgynus* powders using the combination of maltodextrin, mannitol, and crospovidone as carriers. In the current study, TLC-densitometry profiling is applied to monitor changes in the metabolic profile during the process. The results are expected to lay a foundation for industrial development and applications of powdered *S. androgynus* extract.

MATERIAL AND METHODS

Sauropus androgynus leaves were collected from several locations, i.e., Kutisari in Surabaya, Seloliman Environmental Education Center in Mojokerto, and Ubaya Training Center in Trawas. All the samples were authenticated by the Center for Information and Development of Traditional Medicine (PIPOT), Faculty of Pharmacy, University of Surabaya, East Java, Indonesia. Product X containing *S. androgynus* was procured from the local market or registered pharmacies. Other materials used in the study were Maltodextrin DE 12 p.g. (Zhucheng Dongxiao Biotechnology, Shandong, China), mannitol p.g. (Pearlitol® 50C), crospovidone p.g. (ISP Technologies INC), spray-dried lactose p.g. (Pharmatose® DCL 14), and HPLC/spectrophotometric-grade ethanol (Merck, Germany).

Preparation of ethanol *S. androgynus* leaf extract

S. androgynus leaves were picked, washed off of dirt, allowed to dry, and quickly stored at -80 ºC in a freeze dryer (Taitec VD-250F) until usage. Then, 225 g of the freeze-dried leaves were grounded and mixed with 80% ethanol (1:10). The mixture was homogenized with a vortex, sonicated for 20 minutes, mixed thoroughly, and then filtered with a Whatman No. 41 filter paper. Finally, the extract was concentrated in *vacuo* in a rotary evaporator before the spray drying.

Spray drying of *S. androgynus* extract

The spray drying was conducted using a laboratory-scale spray dryer (Buchi Mini Spray Dryer -191). Maltodextrin DE-12 was then added to the ethanol leaf extract. The operating parameters during the spray drying were as follows: inlet air temperature of 170–172°C, outlet air temperature of 90–101°C, pump set at 35%, aspirator at 80%, and atomizer pressure of 15 mBar (adapted from the method used by Singh and Singh, 2009). Afterward, the spray-dried leaves extract was stored in a tight container at ± -20°C.

Preparation of *S. androgynus* extract powder

Twenty grams of the spray-dried extract was mixed homogenously with 44 g of mannitol, 31 g of spray-dried lactose, and 5 g of crospovidone for five minutes and then sieved (mesh 40). Finally, the extract powder was ready for physical characterization and evaluation.

Preparation of product X containing *S. androgynus* extract

In addition to the extract powder, product X, a marketed product containing *S. androgynus* leaf powder, was also used in the observation. Its physicochemical properties were compared with the spray-dried extract and the powder. Twenty tablets of *S. androgynus* (Product X) were weighed,

crushed, and homogenized and then collected for the pre-formulation study.

Pre-formulation study

Particle size and distribution

Particle size and distribution were determined using optical microscopy. First, samples were dispersed on the surface of laminas under the microscope. Then, images of the powders were captured at 400x magnification using an Axioskop 40 microscope.

Moisture content

The moisture content (MC) of three samples of the spray-dried *S. Androgynus* extract weighing 500 mg (triplicate) was measured with a moisture analyzer (Mettler Toledo HB-43) and expressed as a percentage. For the wet basis, the water content was calculated as the amount of water per weight of the wet solid, and for the dry basis, the amount of water is divided by the weight of the dry solid. To quantify %MC, the study calculated the dry basis using the formula below (Equation 1).

$$\% \text{ MC} = \frac{\text{weight of water in the sample}}{\text{the dry weight of the sample}} \times 100 \quad \textbf{.....Eq. 1.}$$

Flowability of powder

Various methods are available to evaluate the herbal powder's flowability or flow properties. In the present study, two were used, namely the angle of repose and porosity based on density. Details on the two methods are explained in the following sections.

Angle of repose

To determine whether or not the *S. Androgynus* powder was cohesive, the fixed base cone method was performed (Aghajani et al., 2012). Three grams of the powdered drug were used to measure the angle of repose, which was later calculated using Equation 2 below.

$$\text{Tan } \theta = \frac{h}{r} \quad \textbf{..... Eq. 2.}$$

Where θ = angle of repose (°), h and r = height (cm) and radius (cm) of powder cone

Bulk density, tapped density, and true density

The simple filled cylinder tapping method was used to determine the bulk and tapped densities of the powdered extract (Etti et al., 2016). First, two-thirds of a 100 ml graduated cylinder was filled with the *S. androgynus* powder, then the powder sample on the plane surface was tapped and observed for any changes in the volume (tapped volume). Then, the densities were calculated using Equations 3 & 4.

$$\text{Bulk density} = \frac{m}{V_0} \quad \textbf{..... Eq. 3.}$$

Where m = mass (g), V_0 = bulk volume (cm^3)

$$\text{Tapped density} = \frac{m}{V_f} \quad \textbf{..... Eq. 4.}$$

Where m = mass (g), V_f = final tapped volume (cm^3)

Afterward, the bulk and tapped densities were used to calculate the compressibility indices (Hausner ratio) to measure the flow properties of the powder.

In addition to bulk and tapped densities, the research also determined the true density (ρ_S) of the obtained powder using Equation 5 below.

$$\rho_S = \frac{m_S}{V_S} \quad \textbf{..... Eq. 5.}$$

Where Vs = the volume of measured solid object, i.e., the difference between the volume of water that fills the empty pycnometer (V) and volume V_{H2O}.

Metabolic profiling

Sample preparation

First, 500 mg of each sample, i.e., leaf extract, freeze-dried leaf extract, and spray-dried leaf extract, was weighed and dissolved in 5 mL of ethanol, while 500 mg of the leaf extract powder was weighed and dissolved in 5 ml of aquadest. Next, the samples were homogenized using a vortex for 1 min, filtered with a Whatman No.41 filter paper, and then stored at 4°C until further use.

TLC analysis

Each of the extracts (5 µl band, 8 mm width) was spotted on a pre-coated TLC plate (silica gel 60 F_{254}) using a Nanomat 4 applicator (CAMAG III) with a one-way ascending technique. The plate was developed in n-Butanol:Acetic acid:water (60:22:1.2) and then air-dried and photographed under UV 254 and 366 nm. Afterward, to obtain the TLC profiles, the plate was scanned under UV 254 nm and 366 nm using a densitometer (CAMAG II).

RESULT AND DISCUSSION

Morphology and physical properties identification

The *Sauropus androgynus* extract powder obtained in the study was characterized morphologically and physically. Physically, the powder's particle size and distribution, moisture content, angle of repose, bulk density, tapped density, granular density, and TLC-based metabolic profiles were measured. These properties were compared with spray-dried lactose and product X containing *S. androgynus*.

a b c

Figure 1. Physical appearance of *Sauropus androgynus:* (a) intact plants, (b) freeze-dried leaves, (c) leaf extract powder

The samples were collected and authenticated as *Sauropus androgynus* leaves based on their morphological structures (Figure 1(a)). In the research, mature leaves, characterized by dark green color, were used. The color indicates the presence of more secondary metabolites than young leaves, thus allowing for a more detailed and thorough analysis of *S. androgynus*. After being freeze-dried, the samples became darker in color and drier (Figures 1(b)) and the *S. androgynus* leaf extract powder obtained in the research was light green (Figure 1(c)). The microscopic views of the spray-dried lactose, spray-dried leaf extract, extract powder, and product X (Figure 2).

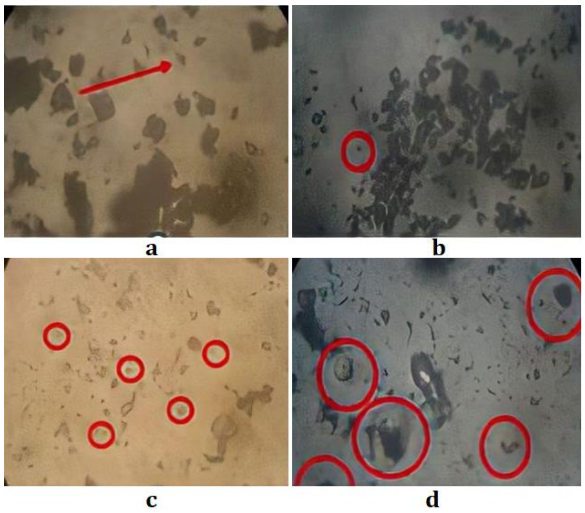

Figure 2. Microscopical images of spray-dried lactose (a), spray-dried *Sauropus androgynus* extract (b), *S. androgynus* extract powder (c), and product X (d) (at 400-fold magnification)

Particle size distribution is related to bioavailability and ensures uniform and constant release of the product. Extract powders with a particle size larger than 200 μm are free-flowing, whereas those with smaller particles increase the risk of cohesion and are non-free-flowing (poor flowability) (Peixoto and Freitas, 2013). Although the particle's mean diameter of the *S. androgynus* extract powder was smaller than 200 μm (60.2 μm), it was the same as that of product X containing *S. androgynus* and ten times higher than the spray-dried *S. androgynus* extract. The spray-dried *S. androgynus* extract had the smallest particle size, 2.5 μm. Khar et al. (2016) stated that the particle size suitable for analysis is > 0.2 μm or within the range of 0.5–150 μm with optical microscopy. Particle size distribution and shape significantly determine the compression and flow of an herbal powder because both properties control the particle's packing geometry and interaction levels (cohesion) (Chaul et al., 2017). Formulating spray-dried *S. androgynus* extract into powder enhances the particle size and physical properties such as moisture content.

In several cases, particle size and moisture content cause a combined effect on flowability. Increasing moisture tends to make powders cohesive; however, it can act as lubricants above a certain level, improving flowability. The derived powder contained half as much as the spray-dried *S. androgynus* extract. Using a powder analyzer, Etti et al. (2016) found that *Andrographis paniculata* with the highest moisture content amongst the pure herbs (9.1 ±0.5 %, dry basis) formed a free-flowing powder base.

The properties are the angle of repose, bulk density, tapped density, true density, Hausner ratio, and moisture content (Table II) . Both the angle of repose and Hausner ratio indicate the flow character of the product. The table shows that the Hausner ratio of the spray-dried *S. androgynus* extract and powder was not significantly different, 1.66 and 1.69, respectively, suggesting similar particle density and dimension. A Hausner ratio of about 1.60 means small-sized and cohesive particles, thus creating an increased probability of friction and resistance to flow (Bunghez et al. 2016). Meanwhile, product X containing *S. androgynus* had a Hausner ratio of 1.16, indicating large particles with low inter-particle friction.

In addition to the Hausner ratio, the angle of repose can also be used to estimate the powder's flowability. The pre-formulation study showed that the angle of repose of the samples varied between 28.61° (product X) and 43.98° (powder), except for the spray-dried *S. androgynus* extract. Because the extract had a high Hausner ratio (cohesive and poor-flowing material), it could not flow through the tunnel, making it not feasible to evaluate the angle of repose.

Table 1. Particle size distribution and mean size diameter* of spray-dried *Sauropus androgynus* extract, *S. androgynus* extract powder, and product X

Sample	Size distribution (%)									Mean size diameter (µm)
Size range (µm)	<11.5	11.5-20.5	20.5-29.5	29.5-38.5	38.5-47.5	47.5-56.5	56.5-65.5	65.5-74.5	>74.5	
Spray-dried lactose	8.20	23.40	13.60	20.00	12.80	11.20	6.40	0.40	4.00	33.2
Spray-dried *S. androgynus* leaf extract	90.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.4
S. androgynus leaf extract powder	10.40	12.80	4.60	12.60	13.40	7.60	7.40	1.60	29.60	60.2
Product X containing *S. androgynus*	7.00	12.20	8.40	12.40	10.40	9.40	6.40	7.00	26.80	62.1

*Number of particles = 500

Table 2. Physical properties of spray-dried lactose, spray-dried *Sauropus androgynus* extract, *S. androgynus* extract powder, and product X containing *S. androgynus*

Physical Properties	Spray-Dried Lactose	Spray-Dried *S. androgynus* Extract	*S. androgynus* Extract Powder	Product X
Angle of repose (°)	35.66 ± 4.972	-	43.98 ± 2.47	28.61 ± 1.504
Bulk density (g/cm^3)	0.64 ± 0.022	0.32 ± 0.028	0.39 ± 0.017	0.76 ± 0.015
Tapped density (g/cm^3)	0.85 ± 0.015	0.53 ± 0.013	0.66 ± 0.011	0.88 ± 0.006
True density (g/cm^3)	1.58 ± 0.107	1.28 ± 0.038	1.22 ± 0.141	1.56 ± 0.068
Hausner ratio	1.33	1.66	1.69	1.16
Moisture content (%)	0.54 ± 0.143	5.48 ± 0.571	2.76 ± 0.188	3.04 ± 0.452

Data are presented in mean ± SD from triplicate analyses

The angle of repose depends on inter-particle frictions and adhesion forces (Peixoto & Freitas, 2013). According to the pharmaceutical requirements in USP 35-NF 30 (2012), the powder's flowability is passable with an angle of repose in the range of 41° to 45°, and adding a lubricant is suggested so to improve it to 36°–40° (fair, no aid is needed), 31°–35° (good), or even up to 25°–30° (excellent).

Although the spray-dried *S. androgynus* extract obtained in this work did not have good flow characters, the moisture content was >10 times much lower than the fresh leaf. This result is not only attributed to the spray drying's temperature but also to the added maltodextrin, which increases total soluble solids and reduces moisture content (Thankitsunthorn et al., 2009), and improves the extract's characteristics, from cohesive to free-flowing powders. Maltodextrin has been commonly used as a drying aid in making free-flowing non-sticky powders because of its physical properties, e.g., high solubility in water (Cano-Chauca et al., 2005). This finding corresponds to several previous study results, where it has been reported to significantly improve the dispersibility of tamarind powders (Ekpong et al., 2016) and optimize the spray-drying process parameters when applied to *Piper betle* leaf extract as a coating (Tee et al., 2012).

The spray-dried *S. androgynus* extract (cohesive and poor-flowing) was transformed into powder by adding spray-dried lactose, mannitol, and crospovidone. Spray-dried lactose is a filler-binder and an agglomerated product that has superior flow compared with regular lactose. Mannitol is widely used in solid dosage forms where rapid and complete solubility is required. When added at low concentrations, super disintegrant such as crospovidone causes faster disintegration of tablets, minimizing softening and flow problems commonly occurring in tablets formulated without one (Khar et al., 2016).

The subsequent process yielded extract powders with half as much moisture as the spray-dried extract, i.e., 2.76±0.188% and 5.48±0.571%, respectively. These results confirm that by adding mannitol and crospovidone, the formulated extract powder contained slightly less moisture and, based

on the angle of repose (28.61±1.504°), had higher flowability than the spray-dried extract. The two additives potentially bring solutions to current issues. For instance, most Indonesian herbal products containing *S. androgynus* leaves are sold in the form of coated tablets and capsules. Among the reasons is the fresh leaf's high moisture content (75.04 ± 0.595 %), making it challenging to create solid dosage forms. As a result, in the preliminary research, Yunita (2012) found several preservatives with high concentrations in *S. androgynus* products.

In addition, the high moisture content of the fresh leaves decreases the quality and, indirectly, quantity (weight) of the leaf extract and its derivatives. Drying the leaves, however, can control the moisture content by either removing or binding it to prevent microbial and chemical degradation. Spray drying, one of the drying methods, is a three-step operation (i.e., atomization, dehydration, and powder collection) widely used in pharmaceutical industries to dehydrate liquid mixture. It involves flash evaporation at a typical inlet air temperature of 100–140^{o}C and product temperature of <30^{o}C. Spray drying produces powders with low water activity and allows easy transportation and storage (Augsburger & Hoag, 2010; Tee et al., 2012). Spray drying has been widely used to significantly reduce moisture or excess moisture in herbal extracts. For example, Peixoto & Freitas (2013) evaluated the physicochemical and biological characteristics of spray-dried extracts of *Syzygium cumini* seeds, and Tee et al. (2012) optimized the parameters of the spray drying process to formulate *Piper betle* L. leaf extract. In addition, this technique was applied to create spray-dried extracts of *Bauhinia forficata* in Souza et al. (2009) and engineered to formulate spray-dried rosemary extracts in Chaul et al. (2017).

Metabolic profiling by TLC-densitometric method

The initial step to control general and batch-to-batch consistency throughout the entire process of herbal medicine production was product profiling at each stage by thin-layer chromatography (TLC)-densitometry, from the extraction process until the pre-formulation stage of the *Sauropus androgynus* extract powder. TLC, also called planar chromatography, is a method of choice to obtain the first characteristic fingerprint profile of a medicinal plant and herbal drug because it is simple, cost-effective, versatile, and usable in all laboratories worldwide. Furthermore, it is not limited to compound identification but can also be used to monitor intermediate stages of the manufacturing process and as the final product's quality control (Braz et al., 2012).

The TLC analysis showed distinctive spots in terms of number, color intensity, Rf value, and diameter. In the analysis, the four samples, i.e., leaf extract, freeze-dried leaf extract, spray-dried leaf extract, and extract powder of *S. androgynus*, produced well-separated spots by the selected solvent at 254 and 366 nm UV light. Moreover, all the spots containing chemical constituents of *S. androgynus* were clearly separated, not blurred, and without any tailing. Srivastava et al. (2018) explained that TLC results are either good or poor based on the number of separated spots and the resolution's intensity, especially after optimizing the system used to separate and visualize (e.g., in different colors) all the components of interest of a particular herbal sample. The method's repeatability was concluded from the intra-day analysis results (data not shown) and the relative standard deviation (R.S.D %), as indicated by Rf values of < 1% for each spot and < 10% for the area; all of which demonstrated good precision for the method.

As shown in TLC-based profiles at 254 and 366 nm (Figure 3), *S. androgynus* leaves and the freeze-dried leaves had similar Rf values, except for S1 (Rf value = 0.42). As marked by the yellow circle under UV 254 nm, S1 emerged the closest to the spotted sample and was exclusively present in freeze-dried leaves. Two spots at Rf = 0.42 (S1) and 0.53 (S2) were not present in spray-dried leaf extract (Figure 3c) and extract powder (Figure 3d).

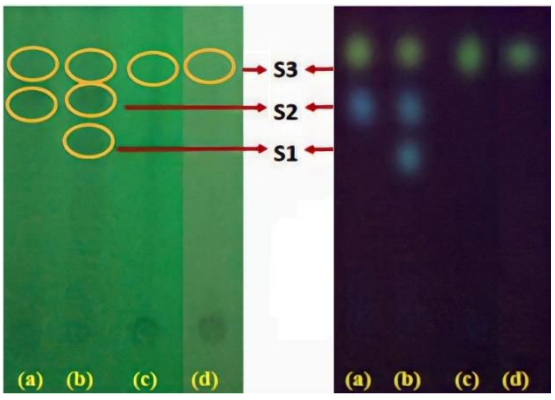

Figure 3. Chromatograms of *Sauropus androgynus* leaf extract (a), freeze-dried leaves (b), spray-dried leaf extract (c), and extract powder (d) in n-butanol:acetic acid:water (60:22:1.2) at 254 nm (I) and 366 nm (II).

The densitogram of *S. androgynus* leaves, freeze-dried leaves, spray-dried leaf extract, and extract powder at 254 nm and 366 nm (Figure 4) showed three peaks, denoted by S1, S2, and S3. S1 only appeared in freeze-dried leaves at Rf 0.42, with an area of 1011.63. S2 appeared in *S. androgynus* leaves and freeze-dried leaves at Rf 0.53, covering an area of 1412.62. Then, S3 consistently appeared in all samples at Rf 0.80, with a total area of 1947.86 for the entire samples.

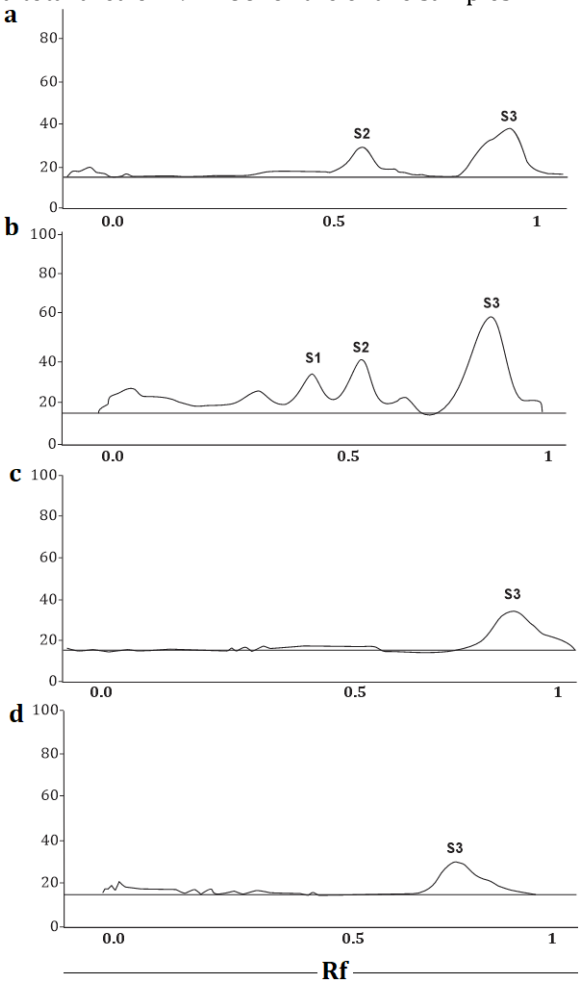

Figure 4. Densitogram showing the metabolic profiles of the ethanol extract of *Sauropus androgynus leaves* (a), freeze-dried leaves (b), spray-dried leaf extract (c), and extract powder (d) in n-butanol:acetic acid:water (60:22:1.2) at 254 nm

Based on TLC-derived profiles, three major spots with distinct Rf values were clearly visible at 366 nm on the chromatogram of *S. androgynus* leaves, freeze-dried leaves, spray-dried leaf extract, and extract powder. Among the spots of the chromatogram, only S3 can be used as a marker or an indicator of stability during the extraction and formulation of *S. androgynus* powder. In the TLC-densitometry analysis, the absorbance value was read at λmax of 254 nm, and the results were similar to the TLC chromatogram of the four samples under the 254 nm UV light in that they showed peak S3. Therefore, it was concluded that S3 is a stable chemical constituent that is not affected by each factor of the herbal formulation process, i.e., extraction, drying, and formulation. Tambunan et al. (2017) proved that markers and chromatographic fingerprinting techniques could standardize batches of *Ageratum conyoides* L. leaf extract and maintain the quality for a long time. Thus, compound markers provide useful information in controlling the making or manufacturing process, minimizing variations in production batches, assuring batch-to-batch consistency, and allowing reproducible results.

After the spray drying and formulation of *S. androgynus* powder, both S1 and S2 did not appear on the chromatogram. Therefore, it was assumed that the two chemical constituents are relatively unstable compared with S3 in the drying process. Papoutsis et al. (2018) also reported that encapsulation of citrus by-product extracts by spray drying could lead to lower polyphenol and antioxidant capacity because the high inlet temperatures generated during the process cause polyphenol degradation/conversion. According to Yunita (2012), metabolic profiling of *S. androgynus* extract powder revealed that the *S. androgynus* fresh leaves generally have higher chemical content than commercial products. Such difference is associated with chemical loss or decomposition during formulation, as confirmed by Meng et al. (2005) that analyzed the chemical content of fresh and dried *Houttuynia cordata*.

The chromatographic pattern of *S. androgynus* leaves displayed a different profile from that of the freeze-dried leaves, as evidenced by the presence of peak S1 only in the latter. This result is attributed to water content reduction, which creates concentrated extract and causes the chemical content represented by S1 to appear on the chromatogram. Roshanak et al. (2016) reported that freeze-drying produced the highest vitamin C (16.36 mg/100gDM) and chlorophyll-a (17.35 mg/l) in green tea (*Camellia sinensis* or *C. assamica*) leaves. Freeze-drying is also suitable for blueberries because the obtained total phenolic concentrations (TPC), total flavonoid contents (TFC), and antioxidant activities are either

increased or unchanged (Vuthijumnok et al., 2013). Also, Antal et al. (2014) suggested that freeze-drying is an effective drying method to prepare samples for chemical analyses because it sublimates water (thus, no liquid state), involves low temperatures—deactivating most microbiological reactions, and produces excellent product quality.

CONCLUSION

Formulating spray-dried *Sauropus androgynus* leaf extract into powder increases particle size and produces better results with minimum moisture content and good flow properties, although some other properties still require improvements. Based on TLC-based profiles, spot S3 at Rf 0.80 has been identified as a stable chemical compound that is unaffected by all factors in the extraction processes through powder formulation. Also, several metabolites that are not visible in the spray-dried extract, possibly attributed to high temperatures during spray drying, have been discovered. Overall, this study can help develop a specific and stable chemical marker to monitor and evaluate of *S. androgynus* products throughout their formulation processes.

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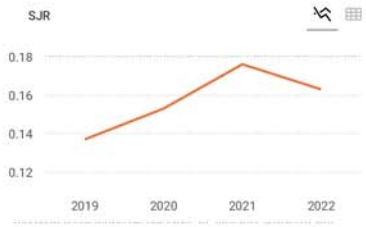

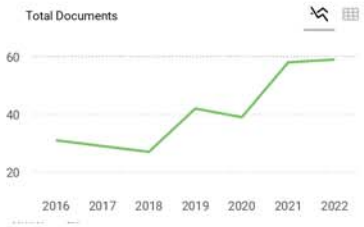

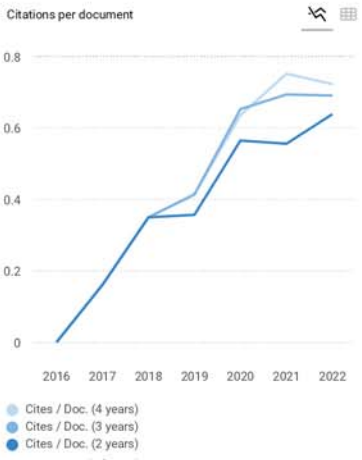

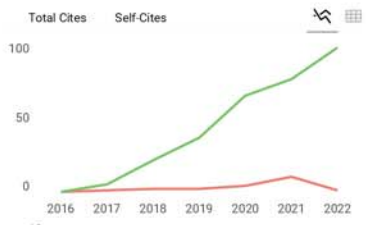

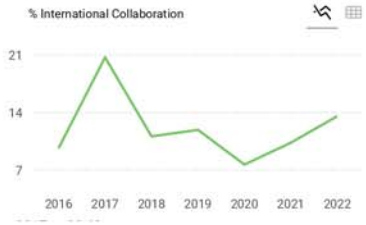

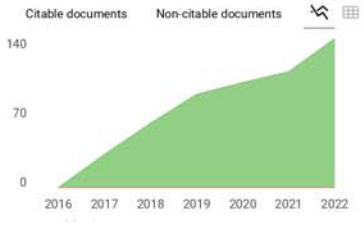

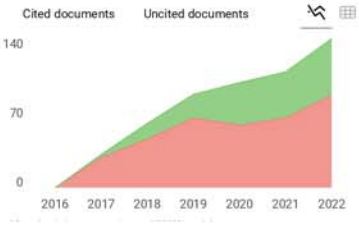

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