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RESEARCH ARTICLE

Quality of Javanese long pepper (*Piper retrofractum* Vahl) simplicia harvested at different maturity stages and drying temperatures

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ABSTRACT

Javanese long pepper (*Piper retrofractum* Vahl) has become a popular commodity for spice and medicinal usage in Indonesia. Piperine is regarded as the main bioactive compound in the pepper, and the presence is affected by factors such as genetic, chemical classes, hybridization, ecological condition, planting system, post-harvest treatments, and storage. The aim of this research was to evaluate the effects of maturity degree (60, 75, and 90 days after flowering (DAF) and drying temperatures (40, 50, and 60°C) on the quality of pepper simplicia including phytochemical profile, weight loss, total ash content, acid-insoluble ash content, water-soluble extract, methanolic extract, as well as quantity of essential oil and piperine. The results exhibited that the simplicia quality complied with the standard of Indonesian herbal pharmacopeia. In addition, the highest content of essential oil was found in simplicia harvested at 60 DAF and dried at 40 °C. Considering the level of piperine, the highest quantity was obtained at treatments of 90 DAF and 40 °C.

Keywords: Drying; maturity degree; Physicochemical; Piper retrofractum vahl; Piperine

INTRODUCTION

Piper retrofractum Vahl, locally known as Javanese long pepper, originates from Southeast Asia and has been massively cultivated in Indonesia and Thailand (Babu et al., 2006). The plant is a wild climbing shrub often found in Indonesia, Malaysia, Philippine, Thailand, and Vietnam (Lim, 2012; Luca et al., 2021; Razak et al., 2021; Wiart, 2014). It possesses various local names in Indonesia, including Sumatera (*lada panjang, cabai jawa, cabai panjang*), Java (*cabean, cabe alas, cabe jawa, cabe sula*), Madura (*cabbhi jamo, cabbhi ongghu, cabbhi sola*) and Makassar (*cabai*) (Depkes RI, 1977). Among these regions, Province of East Java has been recognized as one of the greatest producers (Choviya et al., 2021).

Javanese long pepper has been traditionally applied in Southeast Asia (Luca et al., 2021; Razak et al., 2021). Unripe and ripe fruits are often used as medicine and spices. Currently, besides fruit, the root of the plant is also explored for further use. In Indonesia, traditional use of Javanese long pepper includes medicinal treatment for anti-flatulent, expectorant, antitussive, antifungal, and appetizer. In addition, the pepper is used as major ingredient in Indonesian traditional drink called as "*jamu*" (Lim, 2012). Many studies reported numerous bioactivities of the pepper, including antioxidant and antidiabetic effects (Mahaldar et al., 2020; Luyen et al., 2014), hepatoprotective and gastroprotective properties (Lim, 2012; Luca et al., 2021), anticancer (Hasan et al., 2016), antiobesity (Kim et al., 2011), antileishmanial (Bodiwala et al., 2007), mosquito larvicidal activity (Chansang et al., 2005) as well as aphrodisiac (Rahardjo, 2010; Muslichah, 2009; Wahjoedi et al., 2004).

Key chemical component in herbs relies highly on genetic factor, chemical classes, hybridization, ecological factor, planting system, post-harvest process, and storage (Kumar, 2016, Khalil et al., 2022, Albadwawi et al., 2022). To gain the highest quality of herbs, post-harvest operations

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shall consider the fruit age and harvesting procedures (Aldhanhani et al., 2022a). The maturity is an essential factor that determines composition of bioactive compounds; in this regard, chemical composition in young herbs can differ with that in mature ones (Yasni, 2013, Aldhanhani et al., 2022b). Takahashi et al., (2018) studied the effects of post-harvest treatments on the content of active compounds in Javanese long pepper, and the results revealed that total organic acid, total free amino acid, piperine, and antioxidant declined as the increase of maturity (Aldhanhani et al., 2022a). Therefore, harvesting should concern the age of herbs in order to produce the optimum quality.

Post-harvest procedure constitutes a crucial phase in preparation of Javanese long pepper. Practically, in Indonesia, Choviya et al., (2021) reported that Javanese long pepper was harvested manually and then sun-dried after blanching. This traditional method is hindered with low consistency and quality, since it depends on numerous external factors. Alternative procedures are reported, including the use of freeze drying (Tambunan, 2001) and combination of oven-sun drying (Takahashi et al., 2017). Tambunan (2001) highlighted the importance of drying procedure for Javanese long pepper, including conservation of product quality; however some bioactive compounds are susceptible to high temperature. Thus, investigating the optimum temperature in the pepper drying is needed. Therefore, the objective of the present study was to investigate the effects of maturity degree (days after flowering/DAF) and drying temperatures on the quality of Javanese long pepper simplicia.

METHOD

Materials

Fresh Javanese long pepper was collected from local farmers in Pamekasan, Indonesia, being one of the main producers (Fig. 1). All chemicals were analytical grade, including piperine (Sigma – Aldrich), ethanol 90% (Merck), HCl, chloroform, ether, bouchardat, mayer, concentrated ammonia, anhydrate natrium sulphate, n-hexane (Merck), dichloromethane, xylene (Merck), ethyl (Merck), TLC silica gel 60 F254 (Merck).

Sample preparation

The fruit was picked from different trees within a species at three levels of maturity as follows: 60, 75 and 90 days after flowering (DAF) (Fig. 2). The samples were then transported inside ice box to the laboratory for drying at 40, 50, and 60°C for 12 h. The drying was carried out in a cabinet dryer.

Phytochemical screening

Alkaloid test

Powdered sample (500 mg) was added with 1 mL of chloride acid 2 N and 9 mL of distilled water. The mixture was heated for 1 min, cooled and filtered. The 3 drops of filtrate were dropped into a watch glass. Subsequently, 2 drops of Bouchardat were added. When brown – black sediment was formed, sample contained alkaloid. Then, 2 drops of Mayer solution were added. Formation of white or yellow floc dissolved in methanol indicated presence of alkaloid (Harborne, 1996).

Flavanoid test

A total of 200 mg of plant samples were extracted with 5 mL of ethanol and heated for 5 minutes in a test tube, and added with a few drops of concentrated HCl and 0.2 g of magnesium powder. Formation of a dark red color (magenta) indicated presence of flavonoid (Harborne, 1996).

Saponin test

A crushed Javanese long pepper (2 g) was added with distilled water in a test tube. The mixture was boiled for 2-3 min, cooled and shaken vigorously. Saponin was confirmed with presence of stable foam (Harborne, 1996).

Tannin test

A crushed Javanese long pepper (20 mg) was dissolved in ethanol. Then, 1 ml of the solution was transferred into a test tube added with 2-3 drops of 1% $FeCl_3$. The formation of a bluish-black or green color indicated presence of tannin. In addition, sample was confirmed to contain tannin with presence of a white precipitate following addition of 1% gelatin solution containing 10% NaCl (Harborne, 1996).

Physicochemical test

Physicochemical test was carried out to evaluate the quality of Javanese long pepper, including weight loss, total ash, acid-insoluble ash (AIA), water soluble extract, and ethanol soluble extract. The procedures followed method of Indonesian Herbal Pharmacopeia (Kementerian Kesehatan RI, 2017).

Quantification of essential oils

Sample simplicial (10 g) was transferred into flask, then added with 300 mL of distilled water. Burette containing 0.2 mL of Xilena P was filled up with distilled water and heated to allow distillation for 6 h. After 15 min of distillation completed, volume of essential oils in burette was recorded, and the yield was expressed as % v/b (Kementerian Kesehatan RI, 2017).

Quantification of piperine

Piperine was quantified using a standard procedure of Indonesian Herbal Pharmacopeia (Kementerian Kesehatan RI, 2017). Simplicia powder (1 g) was transferred into a flask and extracted completely using ethanol 95%. The extract

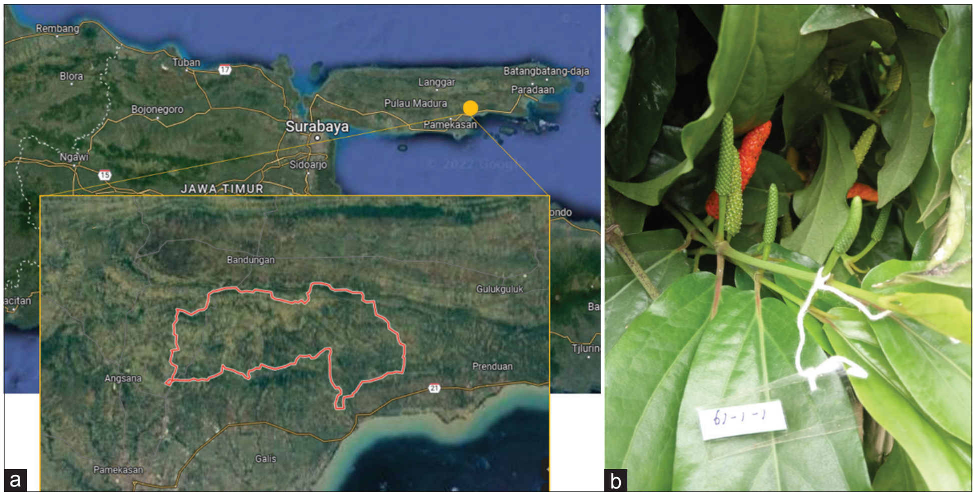

Fig 1. Geographical illustration displaying the location of Javanese long pepper plantation in Pamekasan, Madura Island (a); Javanese long pepper was tagged to observe the maturity stage (b).

Fig 2. Fruit appearances in different levels of maturity.

was filtered and moved into a 100 mL volumetric flask, then filled up with ethanol to the mark. Standard piperine solution was made in 95% methanol at 4 levels. Each level of concentration (5 μL) was injected to silica gel F_{254} using a syringe, then eluted with dichloromethane as a mobile phase. The TLC scanner (CAMAG TLC-Scanner 4) was applied to detect area at wavelength of 254 nm. Piperine was expressed as % w/w using formula as follows:

$$\% = \frac{Au}{Ap} x \frac{Cp}{Cu} x\ f\ x\ 100$$

in which Au is absorption of sample; Ap is absorption of standard solution; Cu is concentration of sample; Cp is concentration of standard; F is dilution factor.

RESULTS AND DISCUSSION

Phytochemical profile

This experiment is designed to identify phytochemical components in Javanese long pepper simplicia, using a qualitative approach. The test detects presence of flavonoid, alkaloid, tannin, and saponin. Tabel 1 presents groups of the chemicals detected in samples: alkaloid, flavonoid, saponin, and tanin. Presence of phytochemicals relies on many factors, such as genetic, geographic origin, climate, ecology, and parts of plant (Jadid et al., 2018). A previous study showed that screening of *Piperaceae* confirmed content of tannin, phenol, coumarin, alkaloid, and anthraquinone (Kadam et al., 2013). Alkaloid becomes a major constituent in Javanese long pepper (Kim et al., 2011). Piperidine, a member of alkaloid, exists in many forms in the pepper, including piperine, pipernonaline, and dehydropipernonaline, which remarkably contribute to biological functions (Hasan et al., 2016; Kim et al., 2011; Mgbeahuruike et al., 2017; Takahashi et al., 2017).

Physicochemical profile

Weight loss is defined as a loss of volatile component mainly water, due to drying process. As presented in Table 2, weight loss of the simplicia ranged 4.66 ± 0.79% - 9.60 ± 1.42%. This range is in accordance with standard of Indonesian Herbal Pharmacopeia at ≤10%. Water is one of the main components evaporated during the process. Moisture content of fresh Javanese long pepper can reach up to 18%

Table 1: Screening results of Javanese long pepper

Drying Temperature (°C)	Maturity degree (DAF)	Alkaloid Test			Flavonoid	Saponin	Tannin Test	
		Bourchardat Reagent	Mayer Reagent	Quinnone Reagent			Polyphenolate	Gelatin
40	60	+	+	+	+	+	+	+
40	75	+	+	+	+	+	+	+
40	90	+	+	+	+	+	+	+
50	60	+	+	+	+	+	+	+
50	75	+	+	+	+	+	+	+
50	90	+	+	+	+	+	+	+
60	60	+	+	+	+	+	+	+
60	75	+	+	+	+	+	+	+
60	90	+	+	+	+	+	+	+

Table 2: Physicochemical profile of Javanese long pepper

No	Drying Temperature (°C)	Maturity degree (DAF)	Parameters				
			Weight loss (%)	Ash content (%)	Acid-insoluble ash (%)	Water-soluble extract (%)	Ethanolic extract (%)
1	40	60	5.64 ± 1.24[a]	5.74 ± 1.69[b]	0.77 ± 0.28[ab]	5.73 ± 0.79[a]	7.82 ± 1.27[a]
2	40	75	6.30 ± 1.24[ab]	4.07 ± 0.48[ab]	0.52 ± 0.17[a]	5.63 ± 0.02[a]	7.66 ± 0.96[a]
3	40	90	9.60 ± 1.42[c]	3.31 ± 0.17[a]	0.81 ± 0.19[ab]	5.98 ± 0.87[ab]	8.32 ± 0.30[a]
4	50	60	5.70 ± 0.19[a]	4.78 ± 0.75[ab]	0.65 ± 0.20[ab]	6.30 ± 1.15[ab]	7.83 ± 0.66[a]
5	50	75	5.03 ± 1.03[a]	4.67 ± 0.62[ab]	0.76 ± 0.28[ab]	6.58 ± 2.08[ab]	9.00 ± 0.40[a]
6	50	90	8.33 ± 0.95[c]	3.88 ± 0.93[ab]	0.65 ± 0.16[ab]	7.50 ± 1.61[ab]	9.89 ± 3.54[a]
7	60	60	4.66 ± 0.79[a]	5.53 ± 0.95[b]	0.65 ± 0.13[ab]	7.26 ± 0.89[ab]	8.45 ± 1.03[a]
8	60	75	5.36 ± 1.46[a]	4.93 ± 0.58[ab]	0.92 ± 0.16[b]	6.94 ± 1.35[ab]	9.29 ± 2.26[a]
9	60	90	7.76 ± 093[bc]	4.18 ± 1.77[ab]	0.84 ± 0.13[ab]	8.72 ± 2.48[b]	9.78 ± 2.46[a]
10	Standard value (2017)		≤ 10%	≤ 6.7%	≤ 1.9%	≥ 5.2%	≥ 8.3%

DAF=Days After Flowering

(db) (Jadid et al., 2018), while dried sample contained water at range of 6% - 9% (wb) (Choviya et al., 2021). Another study reported that moisture content in Piperaceae was 3.43±0.015% (w/w) (Kadam et al., 2013), while it comprised of 0.48±0.44% in P. nigrum L. (Ahmad et al., 2015).

Content of total ash and acid insoluble ash represents inorganic materials which are undesirable in medicinal treatment (Gupta dan Rao 2012). Based on Indonesian Herbal Pharmacopeia, the limit is ≤6.7% (total ash) and ≤1.9% (acid insoluble ash). In this work, content of total ash was 3.31±0.17% - 5.74±1.69% (db), whole acid-insoluble ash reached 0.52±0.17% - 0.92 ± 0.16% db (Table 2). This finding suggests that the levels meet standard of Indonesian Herbal Pharmacopeia. The high content of ash content is linear with the presence of inorganic compounds. Total ash content in Javanese long pepper is approximately 4.29%, representing calcium, cuprum, iron, magnesium, and phosphor, potassium, natrium, and zinc at various concentrations (Jadid et al., 2018). A study reported the concentration of ash and acid-soluble ash was 6.85±0.8% and 3.0±0.1%, respectively (Khan, 2015). Ash content in pipericeae was reported to reach 4.29±0.06%, while acid-soluble ash was 0.44±0.050% (Kadam et al., 2013), meanwhile, the content in *P. nigrum* L. (*Pipericeae*) reached 4.31±0.32% and 0.48±0.44%, respectively (Ahmad et al., 2015).

Water-soluble extract and methanolic extract is a key indicator showing compounds soluble in polar and semi-polar solvents (Thomas et al. 2008, Kumar et al. 2011). In this work, the simplicia contained a water-soluble and methanolic extract of 5.63 ± 0.02 - 8.72 ± 2.48 % db and 7.66 ± 0.96 - 9.78 ± 2.46% db, respectively (Table 2). This indicates that simplicia of Javanese long pepper can be applied for manufacture of *jamu*, prepared by either infusions or ethanolic extract, regarding content of water-soluble extract and methanolic extract reaching ≥ 5.2% db and ≥ 8.3% db. Based on our finding, infusion and ethanolic extract of Javanese long pepper can meet the characteristics for medicinal usages. A previous work reported that water-soluble and ethanolic extract of the pepper reached 6.0±0.2% and 8.6±0.2% (Khan, 2015). In addition, another study showed that their presence was higher in pipericeae, namely 10.03±0.061% and 12.19±0.055%, respectively (Kadam et al., 2013).

Essential oils

Essential oils are characterized as complex and highly volatile compounds mostly terpenoid possessing a strong odor (Bagheri et al., 2014). Content of essential oils in simplicia of Javanese long pepper tends to decline as the increase in drying temperature and maturity stage. In this work, quantity of essential oils ranges between 0.20±0.05

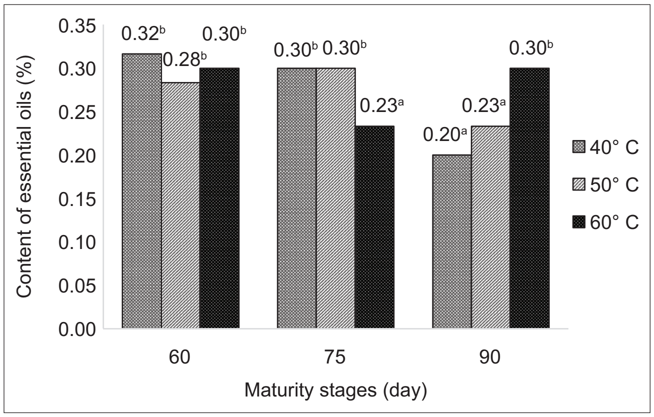

Fig 3. Content of essential oils in Javanese long pepper.

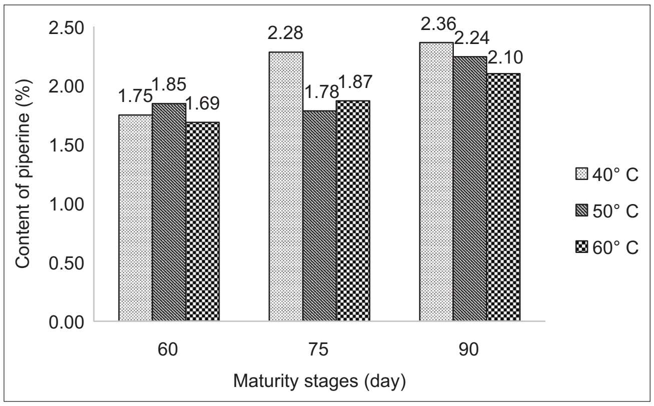

Fig 4. Content of piperine in Javanese long pepper.

and 0.32±0.03% (Fig. 3), which met a standard level of Indonesian herbal pharmacopeia reaching ≥0.15% v/w. This is in accordance with a former work by Conde-Hernnández, Espinosa-Victoria, & Guerrero-Beltrán (2017) finding that essential oil in dried plant materials is <5%. A different result was found, reporting the content of essential oil in Javanese long pepper was 0.8% (Khan, 2015). Dissimilarity of essential oil concentration may occur due to many factors, including climate, type of soil and harvesting time, which significantly dictate the quantity and quality of essential oils. The importance of essential oils in pepper relates to its main role in food processing and health usage, as well as bioactivity since its antioxidant and anti-microbial properties could retard food spoilage (Andrade et al., 2017, Xylia et al., 2022).

Piperine

Piperine belongs to a member of alkaloid with formula $C_{17}H_{19}NO_3$ and molecular weight of 285,34 daltons. The substance is solid, insoluble in water, and weak basicity; even though piperine is tasteless, for a while it can cause burning taste (Chopra et al., 2017). In this work, we quantified piperine in Javanese long pepper harvested at different stages of maturity and dried at various temperatures. The results showed that the highest content of piperine was found in samples of 90 DAF and drying temperature 40ºC, reaching 2.36±0.34% (Fig. 4), which met the standard (>1.05%) of Indonesian Herbal Pharmacopeia. However, in this present work, the amount of piperine was comparable between maturity degrees and drying temperatures (P>0.05), ranging from 1.75% to 2.36%. A lower content piperine, i.e. 0.95%, was reported (Khan, 2015). The present work indicates that a longer harvesting time tends to increase concentration of piperine. Meanwhile, a higher drying temperature seems to decrease piperine concentration of Javanese long pepper simplicia. This is in agreement with Tiwari et al., (2020) reporting that piperine content can be altered by numerous factors such as climate, drying procedures, and origin. They reported a variation of piperine in Piperaceae, ranging 2.0% to 7.4% (Tiwari et al., 2020). Additionally, Dziedziński et al., (2020) confirmed that drying condition markedly affected the physicochemical properties, as reported in Pinus sylvestris L. shoots extracts.

CONCLUSION

The simplicia of Javanese long pepper conformed to standard of Indonesian herbal pharmacopeia. The pepper prepared as infusions and ethanolic extract is applicable for medicinal purposes. Concerning essential oils, the highest content (0.32%) was obtained in samples of harvesting 60 DAF and drying temperature 40 °C. However, the highest concentration of piperine (2.36%) was found in harvesting time 90 DAF and drying temperature 40°C. In conclusion, the temperature has an important role in the contents of bioactive compounds in the pepper, and the recommended 40 °C can be a potential treatment for drying the fruit after harvest and maintained its quality.

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