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Content of Phenols and Flavonoids from Distillation of Beluntas (Pluchea indica l.) Leaves with Variables of Distillation Temperature and Pressure

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Abstract

This study focuses on examining the properties of phenolic and flavonoid compounds that result from the distillation of beluntas (Pluchea indica L) leaves, with changes in temperature and distillation pressure as the variables. A randomized block design method used in this reserach to arranged factorially with two factors, namely distillation temperature and distillation pressure. Data is displayed in the form of graphs and tables. The research results show that the variables of pressure and temperature of the distillation have a tendency to influence the bioactive levels of the distillation products analyzed. The pressure factor and increasing distillation temperature have a tendency to influence the levels of polyphenols and vitamin C, namely increasing the levels at a pressure of 14.5 psi, on the other hand reducing the levels of polyphenols and vitamin C at a pressure of 29 psi. Increasing distillation temperature does not tend to increase flavonoid levels at high pressure, while increasing two distillation factors, namely temperature and pressure, appears to increase the antioxidant capacity of distillation products.

Keywords: Pluchea; Distillation; Phenol; Flavonoid; Temperature; Pressure

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INTRODUCTION

Beluntas, or Pluchea indica L (P. indica), is a plant type that is easy to grow and produces throughout the year in Indonesia. It is often considered a wild plant that grows in open areas and is exposed to direct sunlight, such as on empty land (Achmad et al., 2015; Karima et al., 2022). P. indica is a shrub that belongs to the Asteraceae, commonly known as Indian fleabane (King-Jones, 2001; Pelu, 2017; Syaravina et al., 2018). P. indica is important in medicine and is widely used for gastrointestinal diseases, as a diuretic and as an antipyretic (S. R. M. Ibrahim et al., 2022; Nurrohman et al., 2021; Suriyaphan, 2014; Wahyuni et al., 2022).

P. indica traditionally used as both a fresh vegetables and medicines. It is known to have various medicinal properties, including antipyretic, stomachic, diaphoretic, pain relief, anti-inflammatory, and anti-diarrheal effects. P. indica have several active compounds, including alkaloids, flavonoids, tannins, saponins, polyvinyl, and essential oils (Andasari et al., 2021). Due to its bioactive components, P. indica has the potential to be used as a functional food additive and in the chemical industry. However, its use in these areas is not yet optimal. In addition to its medicinal properties, P. indica is also used as a food supplement in Indonesia. One of its traditional uses is to eliminate body odor, and it has been found to have antibacterial activity against Staphylococcus epidermidis, a bacteria that can cause body odor. Roll-on deodorants containing P. indica leaf extract have been tested and found to be effective in eliminating body odor

P. indica contains several chemical compounds, including flavonoids, alkaloids, and phenolics. Many of these compounds have been isolated and identified, including terpenes, sterols, caffeoylquinic acid derivatives, lignans, and thiophenes. Pluchea indica L. has been found to have a high content of total phenolic and total flavonoids(Fitriansyah & Indradi, 2017). Various research reports show the results of evaluations of the chemical content of P. Indica and the most effective extraction methods (Safitri et al., 2018). Many studies have been conducted to investigate the effect of P. Indica and ist components. One study found that the highest levels of polyphenols in beluntas leaves were obtained through rapid steaming at high temperatures (Donowarti & Fidhiani, 2020). Studies have been conducted to investigate the morpho-anatomical characterization of P. indica and other plants. DNA barcoding is a technique that involves analyzing genes from different individuals to identify and classify species (Wahyuni et al., 2022).

No research has been found that specifically measures the levels of phenols and flavonoids from the distillation of P. Indica leaves using variable temperature and distillation pressure. This technique has only been used on Eucalyptus grandis(Setiawan et al., 2022), Patchouli (Asnawi et al., 2018; Muhammad et al., 2022; Muyassaroh et al., 2016), Pimenta racemosa (McGaw et al., 2016), and Psidium guajava (C. G. F. Da Silva et al., 2019). Currently, no research has been found that specifically measures the levels of phenols and flavonoids from the distillation of P. indica leaves using variable temperature and distillation pressure. However, several studies have evaluated the total phenolic and flavonoid contents of P. indica extracts obtained through different extraction methods, including percolation, maceration, Soxhlet, and reflux.

METHOD

Leaves are taken from the P. indica plant which is not cultivated in the yard, then washed clean, drained, and reduced in size. The sample is added with distilled water in a size of 1:1, then put into the distillation apparatus and treated, namely 3 types of distillation temperatures (90˚C, 100˚C, and 110˚C) and pressure (14.5 psi and 29 psi). Data was collected from analysis of distillation results in the form of phenol levels and flavonoid levels.

Measurement of phenol content came from the distillation of 1 ml of P. indica leaves, which was added with Folin-Cioaltou reagent, left for 3 minutes, then 1 ml of saturated Na2CO3 (35%) was added and homogenized until dissolved and left in a dark room for 90 minutes. After that, the absorbance was read using a spectrophotometer at a wavelength of 725 nm. When analyzing the phenolic and flavonoid compounds resulting from the distillation of beluntas leaves, the results were expressed as gallic acid equivalents in μgmL-1 of the extract. To measure the flavonoid levels in P. indica leaves, a distillation process can be conducted using the following steps: (1) Mix 0.2 ml of the leaves with 3.7 ml of 95% ethanol, 0.1 ml of 10% AlCl3, 0.1 ml of 1M potassium acetate, and distilled water up to 5 ml. (2) Leave the mixture for 30 minutes. (3) Measure the absorbance at a wavelength of 437 nm. (4) Create a calibration curve using quercetin with concentration of 100-400 μg/ml. The total flavonoids of the sample were calculated to be equivalent to the amount (g) of quercetin / 100 g of sample.

RESULTS AND DISCUSSION

Phenol content

The phenol content from the distillation of P. indica leaves due to changes in distillation temperature and pressure factors in the distillation process is presented in Table 1, and Figure 1-2.

Table 1. Phenol content from distillation of Pluchea indica L leaves (mg GAE/100g BK)

Distillation Temperature	Total Phenol (mg GAE/100g BK)	
	Pressure 14.5 psi	Pressure 29.0 psi
90 0 C	949.442	963.715
100 0 C	927.223	969.494
110 0 C	938.334	967.296
Total average	938.333	966.815

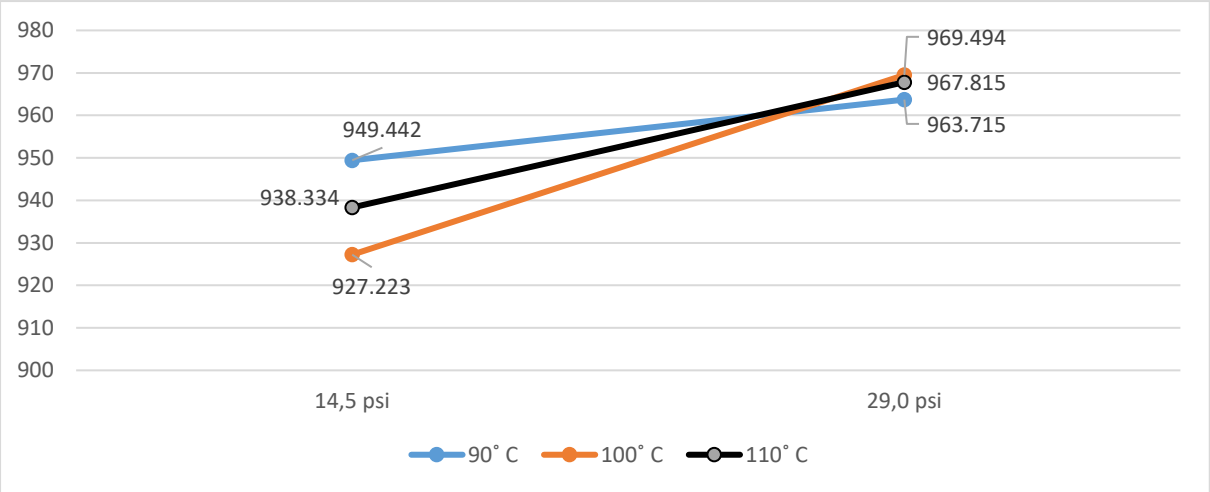

Figure 1. Phenol content from distillation of P. indica leaves (mg GAE/100g DM) from various temperatures due to changes in distillation pressure

It is shown in Figure 1 that the pressure change factor results in changes in the phenol content of the distillation product at each distillation temperature. It can also be seen in Figure 1 that changes in pressure at each temperature in the distillation result in an increase in phenol content in the distilled beluntas leaves. The highest increase in phenol content from distillation of P. indica leaves was obtained when the pressure was increased from 14.5 psi to 29.0 psi at a distillation temperature of 100oC.

It is shown in Figure 2 that the temperature change factor results in changes in the phenol content of the distillation product at each distillation pressure. Figure 2 also shows that at a pressure of 14.5 psi, an increase in temperature from 90oC to 100oC results in a decrease in phenol content in the distillation product. When the temperature is increased to 110 oC there is an increase in phenol content. On the other hand, at a pressure of 29.0 psi, an increase in temperature to 100oC increases the phenol content in the distillation product, but there will be a decrease in the phenol content in the distillation product if the temperature is increased to 110oC.

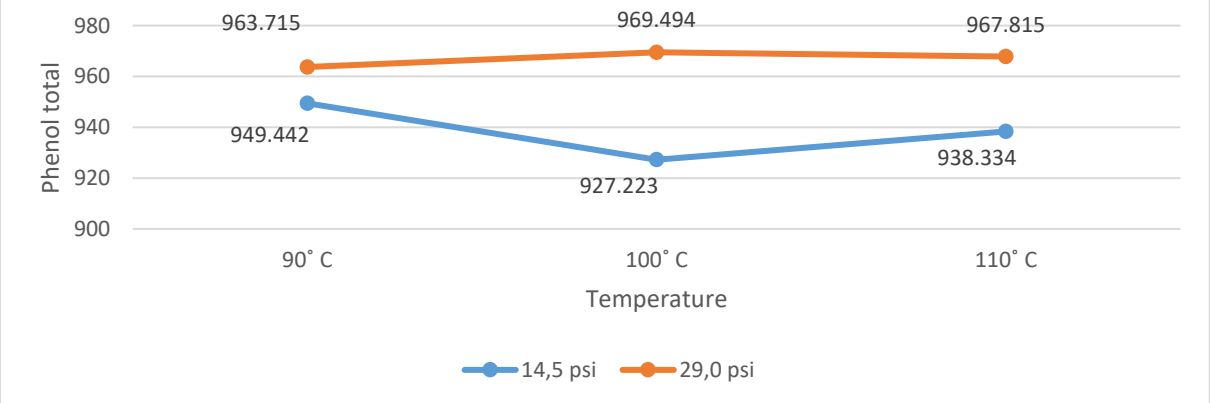

Figure 2. Phenol content from distillation of P. indica leaves (mg GAE/100g DW) from various pressures due to changes in distillation temperature.

The results showed that changes in pressure at each temperature in the distillation resulted in an increase in phenol levels in the distillation of P. indica leaves. The highest increase in phenol content from distillation of P. indica leaves was obtained when the pressure was increased from 14.5 psi to 29.0 psi at a distillation temperature of 100 oC. These results similiar with the results of research on cumin leaf extract that bioactive components such as antioxidants and phenolics in several plants increase with increasing temperature between 45-100°C (Rahim et al., 2010) and temperature have an influence in determining the levels of phenolic substances in galangal extract (Tambun et al., 2017).

Research result of (Qu et al., 2010) shows that temperature in the extraction and distillation process is a factor that has quite an influence on the quality of the extracted compound. Increasing temperature can increase yields because there is an increase in the solubility of secondary metabolite compounds in plant simplicia (Gustavo et al., 2017; Nuri et al., 2020; Sulaiman et al., 2017). Temperature affects the solubility of a compound due to density. The temperature higher, the faster the mass transfer and the greater the yield (Nuri et al., 2020). The use of temperature in the extraction process must be considered, because excessive use of temperature and time causes a decrease in chemical content such as total phenol, antioxidants and water content (Lestari & Juwitaningtyas, 2023).

In detail, the results of the research on the effect of temperature and pressure on phenol content at a pressure of 14.5 psi, an increase in temperature from 90oC to 100oC resulted in a decrease in phenol content in the distillation product, when the temperature was increased to 110oC there was an increase in phenol content. On the other hand, at a pressure of 29.0 psi, an increase in temperature to 100oC increases the phenol content in the distillation product, but there will be a decrease in the phenol content in the distillation product if the temperature is increased to 110oC. These results are in line with research (Yusmita et al., 2023) that the effect of temperature on total phenol explains that total phenol decreases as temperature increases. This can be caused because the heating temperature, although relatively lower, tends to be unstable and is carried out over a longer period of time. It can be seen here that the heating time greatly influences the reduction in total phenol levels (Husni et al., 2014).

Research shows that drying activities will allegedly damage some of the phenol because in dry conditions all the components in the cells are fused so that the phenol extraction process becomes more difficult (Rahim et al., 2010). In addition, using temperatures that are too high results in degradation of cell walls due to damage to carbohydrates and proteins by heat which facilitates the release of phenols from plant tissue (C. C. Silva et al., 2007). The excessive heating process can cause a decrease in the activity of the active compound due to damage to the active component, thereby causing coagulation and reducing free radical scavenging activity (Khatun et al., 2006). High heat temperatures can result in the decomposition of active compounds into other forms (Cheng et al., 2006). A similar study conducted on aloe vera showed that temperatures that were hot enough could change the structure of the components into other substances, resulting in a decrease in antioxidant activity (Miranda et al., 2009).

Flavonoid Levels

Flavonoid levels resulting from distillation of P. indica leaves due to changes in distillation temperature and pressure factors in the distillation process are presented in Table 2, Figure 3 and Figure 4.

Table 2. Flavonoid content from distillation of P. indica leaves (mg QE/100g BK)

Distillation Temperature	Flavonoid Levels (mg QE/100g BK)	
	Pressure (14.5 psi)	Pressure (29.0 psi)
90 ^{0}C	834.36	888.35
100 ^{0}C	835.44	888.88
110 ^{0}C	840.45	888.88
Total average	836.74	888.70

It is shown in Figure 3 that the pressure change factor results in changes in the flavonoid content of the distillation product at each distillation temperature.

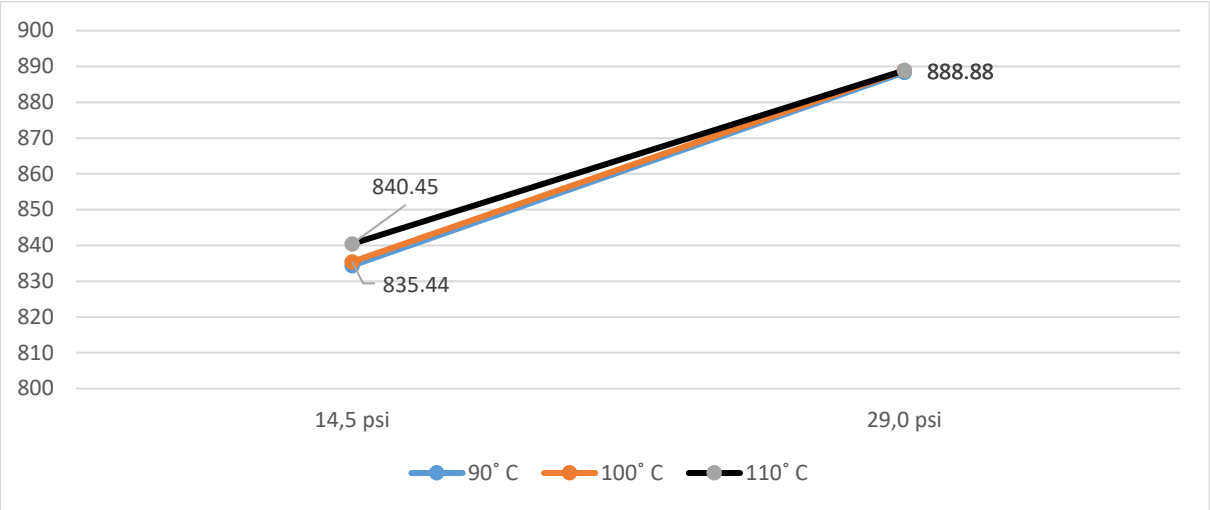

Figure 3. Flavonoid content from distillation of P. indica leaves (mg QE/100g DM) from various temperatures due to changes in distillation pressure.

Flavonoid levels resulting from distillation of P. indica leaves, at a pressure of 14.5 psi and 29.0 psi, showed a tendency to increase when the temperature was increased from 90oC to 100oC and 110oC respectively. It is shown in Figure 4 that the temperature change factor results in changes in the flavonoid content of the distillation product at each distillation pressure.

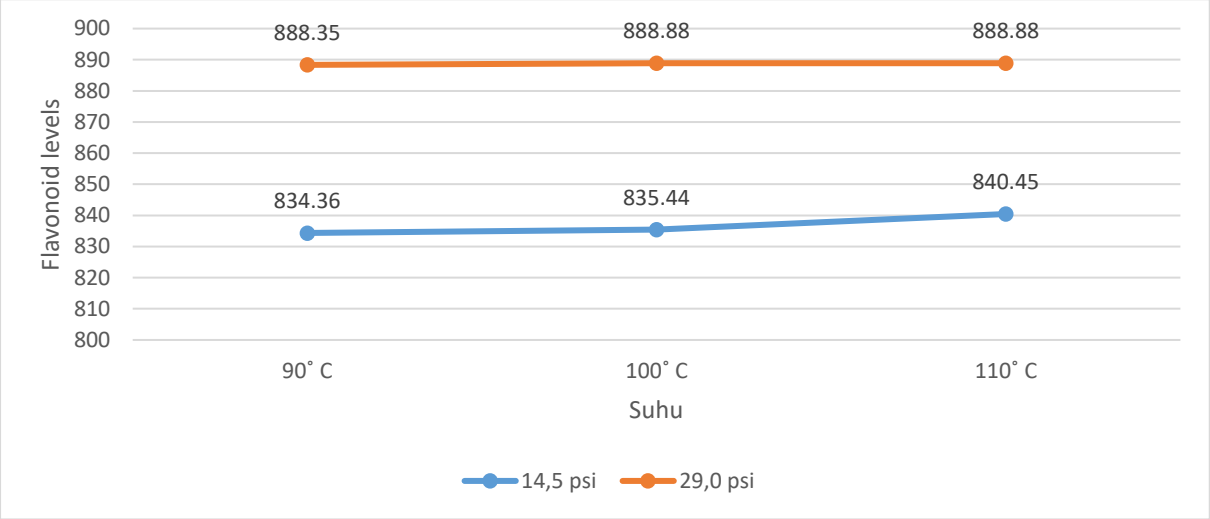

Figure 4. Flavonoid content from distillation of P. indica leaves (mg QE/100g DM) from various temperatures due to changes in distillation pressure.

Flavonoid levels at a pressure of 14.5 psi were seen to increase when the distillation temperature was increased from 90oC to 100oC, and 110oC respectively, while at a pressure of 29.0 psi the flavonoid levels were seen to increase when the distillation temperature was increased from 90oC to 100oC. but tends to have a constant value when the temperature is increased from 100oC to 110oC. The research results are confirmed by the research conducted (Handayani & Sriherfyna, 2016) According to a study on soursop leaves, the total flavonoids produced did not change significantly with higher distillation temperature and longer extraction time. Studies have shown that the total flavonoids in tiwai onion tuber extract decrease with increasing extraction and distillation temperatures (Sa'adah & Nurhasnawati, 2017) and the flavonoids in cat's whisker extract are unstable at too high temperatures (Susiani et al., 2017).

Studies have shown that the drying process used in making nutgrass tuber extract by heating at high temperatures tends to reduce flavonoid levels. This is because flavonoids are thermolabile or sensitive to significant changes in temperature (Syafrida et al., 2018).

The decrease in total flavonoid compounds with increasing temperature occurs because high temperatures can damage the cell structure of the material. This damage can cause the existing components to easily migrate and become easily damaged by various chemical reactions that involve light and oxygen (Zainol et al., 2009). A similar study conducted by (Toripah, 2014) Studies have shown that the flavonoid compounds in Moringa leaves are unstable and easily degraded due to temperature, oxygen content, and light. Degradation of flavonoids occurs due to the breaking of the molecular chain and the occurrence of an oxidation reaction, which causes the oxidation of the hydroxyl group and forms other compounds

Changes in temperature during the distillation process can affect the solubility of a compound due to the influence of density. The higher the temperature, the faster the mass transfer and increase. This is because density is very sensitive to changes in temperature (Bimakr et al., 2011). However, research conducted by (Yuliantari et al., 2017) shows that a distillation temperature that is too low and a short time will result in a low yield. Therefore, the temperature factor needs to be considered (A. M. Ibrahim et al., 2015).

The high and low temperatures in the distillation and extraction process can be controlled by increasing or decreasing the pressure. In the distillation process, pressure and temperature are two important factors that can affect the extraction of bioactive components. The relationship between pressure and temperature is inversely proportional, meaning that the higher the pressure, the lower the temperature (Dewi et al., 2016). Increasing the pressure can reduce the temperature to minimize compound damage due to high temperatures (ElGamal et al., 2023; Jamloki et al., 2021; Moore et al., 2021; Nievola et al., 2017; Schönbeck et al., 2022). The effect of temperature and pressure can be explained if the dissolution process is viewed as an equilibrium state. At equilibrium, an increase in temperature can be beneficial for endothermic reactions. For example, if a compound requires heat to dissolve, then an increase in temperature will increase the solubility of the compound, and vice versa, an increase in temperature can also be detrimental to exothermic reactions. For example, if a compound releases heat when it dissolves, an increase in temperature will reduce the solubility of that particular compound.

CONCLUSION

In conclusion, this study found that the levels of phenolic compounds and alkaloids resulting from the distillation of P. indica leaves are influenced by changes in temperature and pressure. Specifically, increasing or decreasing the temperature and pressure during the distillation process can affect the characteristics of these compounds. The beluntas plant is known to contain various phytochemical compounds, including essential oils, flavonoids, phenolics, tannins, saponins, phenols hydroquinone, and cardiac glycosides compounds. Methanol extract from beluntas leaves is able to produce the highest total phenols and flavonoids compared to water and ethanol as solvents. In addition, P. indica extract is known to have significant antioxidant potential, correlating with the relatively high total phenol and flavonoid content of this plant. Understanding the influence of temperature and pressure factors on phenolic compounds and alkaloids in P. indica leaves can provide an overview of their properties and potential utilization.

RECOMMENDATION

Research related to the properties of phenolic compounds and the properties of alkaloids resulting from the distillation of P. indica leaves needs to be studied further, especially regarding the tolerance of these compounds to the maximum boiling point.

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