

Removal of Mimosine from *Leucaena leucocephala* (Lam.) de Wit Seeds to Increase Their Benefits as Nutraceuticals

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ABSTRACT

The *Leucaena leucocephala* (Lam.) de Wit tree contains pharmacologically active compounds and can easily be found in abundant quantities. The utilization of this tree as a source of nutraceuticals would increase its value. The extraction yields show the number of compounds that have been extracted by solvent. However, *Leucaena leucocephala* (Lam.) de Wit seeds also contain mimosine, which can cause hair loss. The present study investigated the process of removing mimosine from the seeds. Three treatments were applied to these seeds: treatment without soaking, soaking with distilled water for 24 hours, and splitting the seeds in half and then soaking with distilled water for 24 hours. The seeds were dried, ground, and then extracted with different concentrations of ethanol solvent. The mimosine levels of the extracts were measured with a JascoV-730 spectrophotometer using ferric chloride as a reagent. The results showed that the soaking process and the concentration of ethanol had significant effects on the extract yields and mimosine levels. The unsoaked seeds, extracted with 70% ethanol, produced the highest ratio of extract yield to mimosine level. Therefore, 70% ethanol can be used as a solvent to utilize unsoaked seeds as nutraceuticals.

Keywords: ethanol; extract yield; seeds; mimosine; soaking

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INTRODUCTION

Leucaena leucocephala (Lam.) de Wit is a tree that can be found in tropical and subtropical locations. This plant is a legume belonging to the Fabaceae family (Suryanti et al., 2016; Verma, 2016; Abdelhay & Abdallah, 2016). The common names of *L. leucocephala* include *petai cina* or *lamtoro* (Indonesia), white lead tree (England), and *subabool* (India; Soetjipto et al., 2019; Verma, 2016). The seeds and leaves of this plant contain mimosine/leucaenin/leucenol/leucaenol (Nguyen & Tawata, 2016), terpene, flavonoids, coumarin, sterol, ficaprenol-11, squalene, lupeol, β sitostenone, *trans*-cumarinic acid, *cis*-cumarinic acid, pheophytin-a, pheophorbide a-methyl ester, and aristophyll-C (Chen & Wang, 2010). Furthermore, the seeds of this plant have thiol compound concentrations of $1.5 \text{ mmol} \pm 0.02 \text{ mmol}/100 \text{ g}$ when cooked in boiling water, and when uncooked (Suvachittanont et al., 1996). These seeds also have high levels of sulfhydryl (Wardatun et al., 2020).

The young shoots, leaves, and seeds of *L. leucocephala* (Lam.) de Wit may be used as vegetables for human nutrition (Verma, 2016). The extracts of these seeds have been reported to have anthelmintic (Veerakumari,

2015) and antidiabetic properties (Wan Ahmad et al., 2019). When seeds are extracted with methanol at a ratio of 1:30, these seeds produce antihyperglycemic activity with 71.55% inhibitory activity at a dose of 2.5 mg/ml (Abdelhady & Abdallah, 2016; Syamsudin et al., 2010; Chahyono et al., 2012). The methanol extracts of these seeds have also been known to produce anticancer activity *in vitro* (She et al., 2017) as well as lupeol with an antioxidant activity (inhibition activity/ IC50) level of $102.3 \pm 4.1 \mu\text{M}$ (Li et al., 2012). Seeds that were dried at different times and extracted with ethanol at a ratio of 1:10 resulted in antioxidant activity with an inhibition concentration of 49.74%, and an isolated ethanol extract resulted in seven isolate components with > 80% free radical scavenging activity (Hassan et al., 2014).

The extract yield shows the number of compounds extracted by a solvent (Oreopoulou et al., 2019; Yeop et al., 2019). Polarity plays a significant role in extracting bioactive compounds from plant materials, and it correlates well to yield extract (Xu et al., 2019). Mimosine is contained in *Leucaena leucocephala* (Lam.) de Wit seeds at a dry weight concentration of 6.58% (Soedarjo & Borthakur, 1996). A common side effect of mimosine is hair loss (Halliday et al., 2013).

Therefore, the presence of mimosine in seeds must be considered when using these seeds as a source of nutrition. Additionally, the presence of mimosine in the human body must be considered as well (Rodriguez et al., 2014; Nguyen & Tawata, 2016). A few studies have evaluated the mimosine levels of *Leucaena leucocephala* (Lam.) de Wit. El Haritzh et al. (1986) and Soedarjo & Borthakur (1996) determine levels of mimosine in water from seeds, while Ilham et al. (2015) and Ramli (2019) performed the same determination from leaves. Chanchay & Poosaran (2009) studied the effectiveness of soaking on the reduction of mimosine from leaves and extracted residual mimosine using 0.1 N chloride acid solution. Washing and soaking in water tend to reduce the concentration levels of mimosine from plant material until 94.77% (Koli et al., 2018; Chanchay & Poosaran, 2009). The use of chloride acid solution for extraction is not safe for human health, especially if the extract is purposed for nutraceutical (Ramezanzadeh et al., 2019). However, the level of mimosine in seeds after being soaked in water and extracted with ethanol to obtain the maximum yield of extract has not been studied. Therefore, the aim of the present study is to investigate the remaining mimosine levels after the process of removing from seeds to increase the utilization of these seeds, especially as nutraceuticals. Mimosine was removed from these seeds in one treatment method by soaking them in distilled water for 24 hours (Soedarjo & Borthakur, 1996). In another treatment, these seeds were split in half and then soaked to examine the effect of splitting before soaking. Finally, the seeds were dried and extracted with different concentrations of ethanol solvent. The mimosine content and yield of extract of the soaked seed extract was compared to the unsoaked seed extract.

Ethanol is one of the solvents that was applied in the extraction process and is not toxic (Plotka-Wazyłka et al., 2017). There is no energy demand during evaporation and it is safe for thermolabile compounds (Plaza & Turner, 2015). We used ethanol for the extraction of 30%, 50%, and 70% concentrations. The solvent concentration plays an important role in extracting the metabolites, adjusting the polarity, and considering the cost of the solvent (Azwanida, 2015; Shi et al., 2003).

METHODS

Chemicals and Samples

L-mimosine (Sigma-Aldrich, Singapore), ferric chloride (Merck), and all other chemicals used in this study were of analytical grade and purchased from Merck. Fresh *Leucaena leucocephala* (Lam.) de Wit seeds were collected from Bogor, Indonesia, in December 2018. All the seeds used in this study were mature, green in color, and about 8 mm long and 4 mm wide. The seeds

were characterized at Bogor Botanic Gardens (Center for Plant Conservation) in Indonesia.

Preparation of the Dry Powder

A number of seeds was divided into three parts. In part A, seeds were dried directly in an oven at 40°C for 86 hours. In part B, seeds were soaked in distilled water (1:10) for 24 hours. In part C, seeds were split in length into two parts, and each part was soaked in distilled water (1:10) for 24 hours. Finally, the seeds were dried in an oven at 40°C for 86 hours to produce powdered samples (Soedarjo & Borthakur, 1996; Chanchay & Poosaran, 2009).

Preparation of the Extract

Fifty grams of dried and powdered seeds were macerated with 250 ml of 30% ethanol for 24 hours. Maceration was repeated twice for 24 hours using 150 ml and 100 ml of solvent. The liquid extract was separated from the powdered seeds and evaporated using a rotary evaporator. Finally, the extract yield was calculated. The procedure was also carried out using other concentrations of ethanol (50% and 70%) (Azwanida, 2015).

Analysis of Mimosine in the Extracts

The determination of mimosine content was carried out in accordance with the procedure by Ilham et al. (2015) using a ferric chloride reagent. The liquid was measured at 534 nm (maximum wavelength) using a Jasco V-730 UV-VIS double-beam spectrophotometer. The mimosine content was calculated using a standard calibration curve for the mimosine solution. The results of the determination were expressed in mg/100 g of powdered seeds.

Extract to Mimosine Ratio

The extract to mimosine ratio was calculated by comparing the yield extract to the mimosine levels.

Statistical Analysis

All experiments were carried out in triplicate. The results are presented as the averages \pm standard deviations. An ANOVA test was used to determine statistically significant differences between treatments. $P < 0.05$ was considered as the level of significance.

RESULTS AND DISCUSSION

This study relates to the pretreatment of *Leucaena leucocephala* (Lam.) de Wit seeds before extraction. Mimosine can be removed from the seeds by soaking them in distilled water for 24 hours (Soedarjo & Borthakur, 1996). After soaking, the seeds were dried. The yield of the dried seeds was 27%–28%. Afterwards, the seeds were extracted with different concentrations of ethanol. The extract yields produced by different

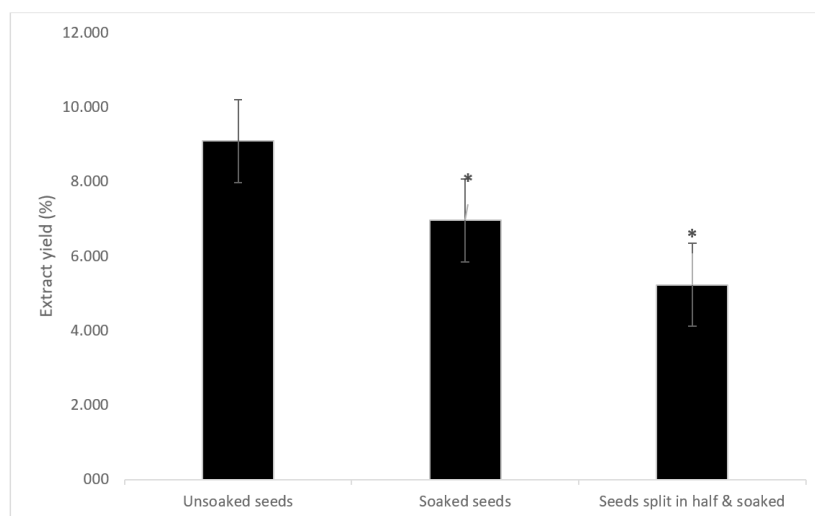


Figure 1. Effect of treatment on the extract yield *Leucaena leucocephala* (Lam.) de Wit seed (mean±SD). *significantly different with unsoaked seeds (P<0.05)

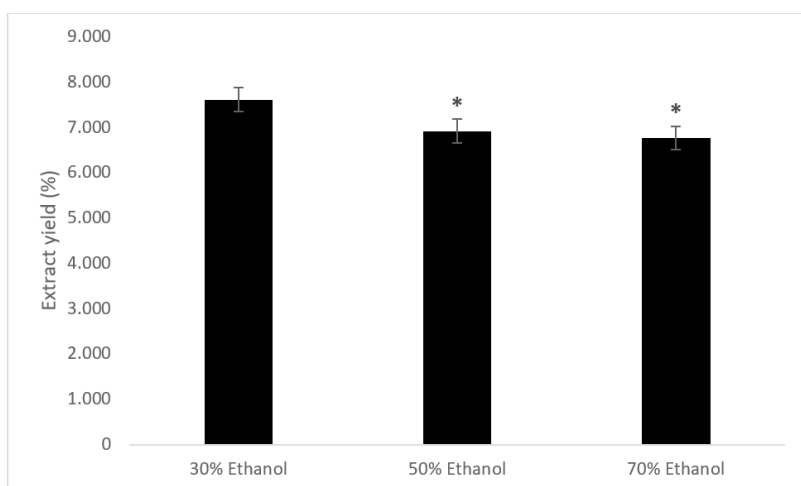


Figure 2. Effect of solvent concentration on the extract yield *Leucaena leucocephala* (Lam.) de Wit seed (mean±SD) *significantly different with 30% ethanol (P<0.05)

treatments and solvent concentrations are presented in Figure 1 and 2.

The process of soaking with distilled water significantly affected the extract yields ($P < 0.05$). For instance, the unsoaked seeds had higher extract yields (9.10%) than the soaked seeds (6.97%) and the seeds that were split in half and then soaked for 24 hours (5.23%). The 30% ethanol concentration had a significant effect on extract yields, while the 50% and 70% ethanol concentrations had no significant effect on the extract yield.

Leucaena leucocephala (Lam.) de Wit seed extracts contain polysaccharides, proteins, fats, polyphenols, flavonoids, mimosine, lignin, sulphhydryl compounds, β -carotene, squalene, α -tocopherol, vitamin C,

phicaprenol, and minerals (Gamal-Eldeen et al., 2007; Chen & Wang, 2010; Suryanti et al., 2016; Hassan et al., 2014; Sahid et al., 2017; Nursiwi et al., 2018; Honda & Borthakur, 2019; Wardatun et al., 2020). A higher extract yield indicates higher extraction efficiency (Yeop, 2019). Soaking the seeds had a significant effect on the extract yield, as soaking in distilled water caused some compounds in the seeds to leach out. Splitting the seeds in half before soaking them increased the surface area of the seeds in contact with the distilled water; thus, the compounds contained in the seeds are more easily leached out when soaked in distilled water. This causes the extract yield to decrease (Yeop, 2019).

The concentrations of the ethanol solvent used during extraction were 30%, 50%, and 70%. These different

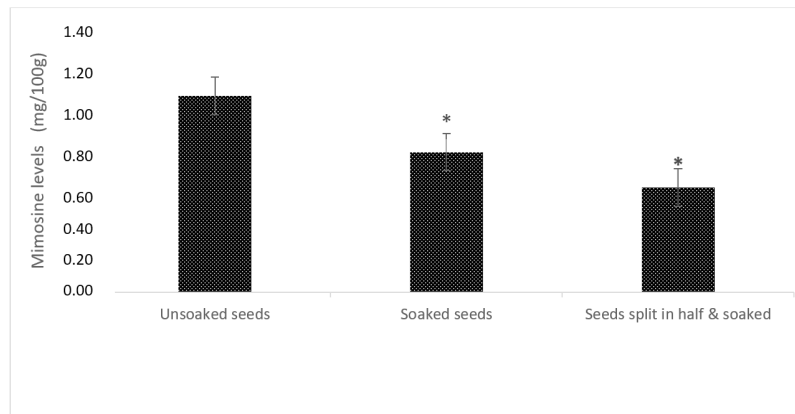


Figure 3. Effect of treatment on the mimosine levels *Leucaena leucocephala* (Lam.) de Wit seed extract (mean±SD). *significantly different with unsoaked seeds (P<0.05)

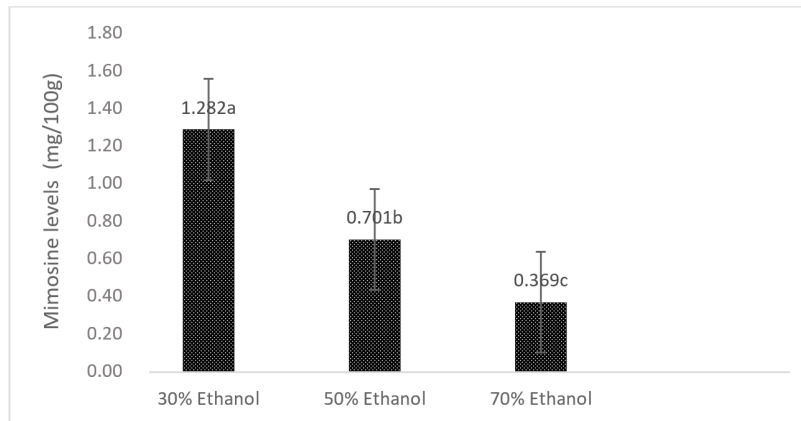


Figure 4. Effect of solvent concentration on the mimosine levels *Leucaena leucocephala* (Lam.) de Wit seed extract (mean±SD).). *significantly different with 30% ethanol (P<0.05)

solvent concentrations cause different solvents polarity and result in different extraction efficiencies (Duarte-Trujillo et al., 2019). Solvents can affect the permeability of cell walls through both chemical changes and physical changes. For example, ethanol can increase the permeability of the cell wall by affecting the phospholipid membrane (Oreopoulou et al., 2019). The 30% ethanol solvent produced higher yield than 50% and 70%, indicating that 30% ethanol extracted the seeds more efficiently than the other solvents.

The amount of mimosine remaining in the extract was measured with a spectrophotometer, as shown in Figure 3 and 4. Soaking had a significant effect on mimosine content ($P < 0.05$). The mimosine content of the unsoaked seeds was higher than the soaked seeds and the split seeds with 1.05 mg/100g, 0.75 mg/100g, and 0.56 mg/100g, respectively. The concentration of the ethanol solvent also had a significant effect on the extracted mimosine content ($P < 0.05$). The amount of mimosine

extracted by 30% ethanol was greater than the extracted seeds by 50% and 70% ethanol, with 1.28 mg/100g, 0.70 mg/100g, and 0.37 mg/100g, respectively.

The split and then soaked seed extract produced the lowest levels of mimosine. The mimosine content for all of the seeds extract in the present study was different from previous studies. This difference can be attributed to the differences between fresh seeds and oven-dried seeds. The present study used oven-dried seeds, which can eliminate mimosine levels by up to 98.50% (from 6.4% in fresh leaves to 0.1% in dry leaves) (Honda & Borthkur, 2019). A decrease in mimosine levels can be caused by mimosinase activity, which degrades mimosine through the release of Carbon-Nitrogen bonds to produce 3,4 dihydroxy pyridine (Nguyen & Tawata, 2016; Ospina-Daza et al., 2017; Koli et al., 2018; Honda & Borthkur, 2019). The extracted seeds that were split in half and then soaked resulted in greater mimosine removal because of the increased surface area of the

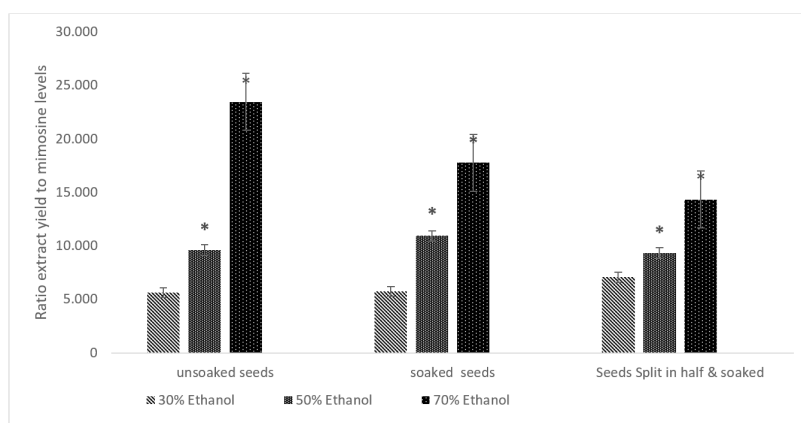


Figure 5. Ratio of extract yield to mimosine level *Leucaena leucocephala* (Lam.) de Wit seed (mean±SD). *significantly different with 30% ethanol in each group (P<0.05)

seeds, which caused more mimosine contact to leach out in water during soaking (Devadasu et al., 2018).

The concentration of the ethanol solvent used for extraction affected the extracted levels of mimosine. The 30% ethanol solvent produced higher mimosine extract levels than the 50% and 70% ethanol solvents.

Mimosine is a polar compound that can dissolve easily in polar solvents (Ilham et al., 2015; Xu et al., 2019). The combination of ethanol with water can change the dielectric constant and polarity of a solvent (Yulianthi et al., 2017). The dielectric constant relates to the solvent polarity (Xu et al., 2019). The solvent polarity may be responsible for the different mimosine extract levels in the different solvents (Aradamola & Onigbinde, 2016). The dielectric constants of water and ethanol were 78.9 and 24.3, respectively (Azwanida, 2015). The high polarity of the ethanol solvent resulted in high levels of mimosine. The polarity of 30% ethanol > 50% ethanol > 70% ethanol explains the fact that mimosine was extracted the most in the concentration of 30% ethanol (Yulianthi et al., 2017).

The ratio of extract yield to mimosine content indicated the effectiveness of the pretreatment to seeds (Figure 5). A higher ratio indicated the higher effectiveness of the pretreatment process and the concentration of ethanol used for extraction. Overall, the soaking process decreased the yield extract and mimosine levels of the seed extracts. Although the solvents used in this study can be used to extract mimosine, the use of 70% ethanol with unsoaked seeds resulted in the highest ratio of extract yield to mimosine level. The unsoaked seeds did not leach out metabolite compounds while soaking to produce a high yield extract (Chanchay & Poosaran, 2009). The lowest polarity, 70% ethanol, causes a lower extraction level of mimosine than 30% ethanol (Yulianthi et al., 2017).

CONCLUSION

As conclusion, removing mimosine can be conducted by soaking in water. Nevertheless, this treatment causes metabolite compound to leach out and yield extract to decrease. However, using a concentration of 70% ethanol for extracted unsoaked seeds produce the high yield extract and minimum levels of mimosine.

CONFLICT OF INTEREST

We declare that we have no conflict of interest

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