The fractions of phenolic and flavonoid compounds of the leaves of north Sulawesi's bashful plant (mimosa pudica linn)

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The fractions of phenolic and flavonoid compounds of the leaves of north Sulawesi's bashful plant (*mimosa pudica linn*)

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Abstract. The bashful plant or the mimosa plant has a history of use for treatments in various ailments. Various parts of this plant maybe used, but most commonly the leaves, stems, roots and flowers. This research was aimed to investigate the existence of phenolic and flavonoids in the *Mimosa pudica Linn* leaf extracts in solvents of different polarities. During this research dried leaves and wet leaves were respectively macerated in 70% ethanol and then reduced by rotary evaporators. Fractions were obtained by means of HPLC (High Pressure Liquid Chromatography) with 3 different solvents at different polarities (hexane, n-butanol and ethyl-acetate). The results indicated that flavonoid was found mostly in the ethyl-acetate fraction from dry leaves (1.08% w/w). Phenolic was mostly detected in extracts of the dry leaves in the n-butanol (7.27% w/w) and the wet leaf residues (8.68% w/w). Results indicates that with the majority of the detection of phenolics and flavonoids in the mildly polar to strong polar solvents, thus oral intake by means of leaf infusions (such as tea/brew) would be quite effective. Complete extraction process of these compounds and encapsulating them would always be the most effective method of intake while economically the method would raise questions.

1. Introduction

Biodiversity potential of North Sulawesi is considered the largest in Indonesia because bio-geographically North Sulawesi is one of the islands located in the Wallaceae region, an ecological crossover of Asia and Australia (Kinho, Halawane, &Kafiar, 2014) . One of these potentials are seen in a shrub-like plant that grows wild and has become a disturbing weed to local farms known as the bashful plant, in Indonesia is known by the name *Putrimalu*. *Mimosa pudica Linn* is a plant that have captured the attention of many researchers due to their intriguing pharmacological activities, such as anti-diabetic, anti-toxins, anti-hepatotoxins, antioxidants and various wound healing attributes (Azmi, Singh, & Kamal Akhtar, 2011; Muhammad, Hussain, Jantan, &Bukhari, 2016). Phytochemical screening have revealed that Mimosa pudica are rich in alkaloids, flavonoids, terpenoids and saponin, also been reported to enhance antioxidative enzymes such as Superoxide Dismutase (SOD), Catalase and Glutathion Peroxide (Azmi et al.,

2011; Muhammad et al., 2016; Rini, Hairrudin, &Sugiyanta, 2013). Thus there are strong presumptions that *Mimosa pudica Linn* also has chemotheraputic activities.

Phenolic compounds and their derivatives are commonly found in nature and mostly in plants, especially in their leaves while some occur constitutionally and are presumed to have the function of inhibitors (Hossain, AL-Raqmi, AL-Mijizy, Weli, & Al-Riyami, 2013; Nicholson &Hammerschmidt, 1992). Phenolic compounds are known for their activity as cancer chemo-preventive agents and their capacities as inducers of apoptosis in tumor cells, making up their potentials for cancer control (Alves et al., 2016; Fu et al., 2016; Khalatbary, 2013; Senawong et al., 2014). On the other hand flavonoid compounds are also polyphenols that are widely found in fruits, vegetables and most plants in the kingdom(Hidalgo, Sánchez-Moreno, & de Pascual-Teresa, 2010; Xiao, Muzashvili, &Georgiev, 2014) and is known for their diverse bio-activity (Xiao et al., 2014). Flavonoids usually recognized in plants as pigments, and have been intensively studied for their cytotoxic activites and antitumor effects, where these phytochemical compounds have multiple anticancer effects (Kashyap& Singh Tuli, 2018; Sak, 2014; Sak&Everaus, 2015; Tietbohl et al., 2017). Mimosa pudica Linn has a wide array of pharmacological activities that is attributed to various parts of the plant, due to its rich content of metabolites such as phenols and flavonoids (AZIZ, AKTHER, SHAHRIAR, & BHUIYAN, 2014; Baby Joseph, Jency George, 2017; Muhammad et al., 2016).

Extraction process of phenolic compounds and flavonoid compounds in most varieties of plants are very solvent dependent. The polarity of solvents strongly influence the amount and variances of phytochemical extracted, thus would impact the pharmacological activities of the extracts (Alimpić et al., 2017; Dirar et al., 2018; Sepahpour, Selamat, Manap, Khatib, &Razis, 2018). Thus is the importance of investigating the recovery of phenolics and flavonoids in a wide variety of solvent polarity and in a sequential extraction method (Thavamoney et al., 2018). The practical and novel conclusion to this extraction process would be to determine the most effective and economical method of gaining the most of phytochemical properties to a bio-functional product, as such with North Sulawesi's *Putrimalu* (*Mimosa pudica Linn*).

2. Methods

The sample for this investigation was taken from the surrounding forests of Tombatu Village, Southeastern Minahasa Region, North Sulawesi. The collected samples were sorted and separated of the leaves, stems, flowers and various contaminants, where 2kg of the leaves are taken and aired out to drain of the washing water. A set of 1kg of the leave samples are dried in an oven (memert laboratory oven) at 60°C for 90 minutes, while the remaining 1 kg will be set aside as the wet leaves sample. Both the dried and wet samples will each be macerated using 70% ethanol (laboratory grade) at a ratio of 1:2 (sample : ethanol) for 48 hours to extract all the bio-active compounds, this process is indicated with strong green color of the macerate. The ethanolic solvent is then evaporated with a rotary evaporator until a crude extract of the Mimosa pudica Linn is obtained.

The crude extract obtained was sequentially fractionated with solvents as shown in Figure 1. Volume ratio of crude extract and solvent was 1:1. The fractions of hexane, ethyl acetate, and n-butanol were collected individually and dried under vacuum below 45°C, while the residue was kept without any further treatment. The three fractions and residue were subjected for analysis to determine total phenolic content, total flavonoid content, antioxidant activity and identification of phenolic and flavonoid compounds using HLPC (High Performance Liquid Chromatography) according to a method of Wijaya, Widyadinata, Irawaty, &Ayucitra (2017).

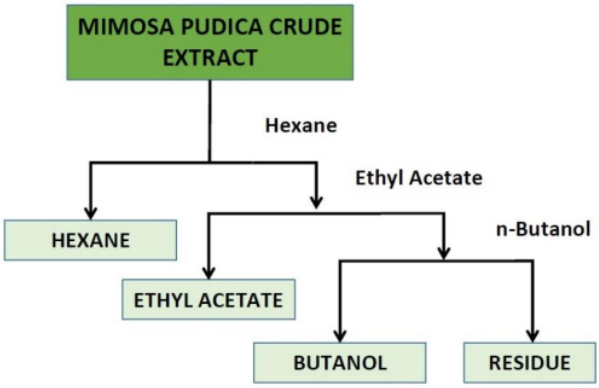

Figure 1. Sequential Fraction Schematics

3. Results & Discussion

As seen in Table 1, Mimosa crude ethanolic extract (Residues) are found to be more rich in phenolic compounds especially in the wet sample (8.68% w/w), on the other hand phenolic compounds are also dominantly extracted by Butanol in the dry samples (7.27% w/w). Comparing effectiveness of solvents in extracting phenolic compounds of wet mimosa it can be observed that Residue > Butanol > Ethyl Acetate > Hexane (Figure 2.), while in dry mimosa Butanol > Ethyl Acetate > Residue > Hexane (Figure 3.). In both wet and dry mimosa Hexane extracts the least phenolic compounds. This indicates that the phenolic compounds of North Sulawesi's Mimosa pudica are mostly polar and are dominated by negatively charged phenolic compounds, since butanol and ethanol are both protic polar solvents. Some Phenolics are also found in ethyl acetate, which is an aprotic polar solvent (4.69% w/w in dry sample and 5.39% in wet sample). This would indicate that some phenolics of Mimosa pudica Linn would be positively charged. The results obtained in this research concurs with some previous work that that has also reported that most phenolics are found to be in strong polar solvent to mildly polar solvents (Alves et al., 2016; Mohammedelnour et al., 2017). Thus would indicate that these phenolic compounds found in Mimosa pudica Linn would be proned to become solute in protic polar solvents as in water.

Table 1. Total Phenolic Compounds and Total Flavonoids In Wet and Dry Extracts With Solvents of Different Polarities

SOLVENTS	Dry Mimosa		Wet Mimosa	
	Phenol (% w/w)	Flavonoid (% w/w)	Phenol (% w/w)	Flavonoid (% w/w)
HEXANE	3.70	0.74	5.27	0.74
BUTHANOL	7.27	0.49	6.65	0.75
ETHYL ASETATE	4.96	1.08	5.39	0.38
RESIDUE	4.48	0.22	8.68	0.21

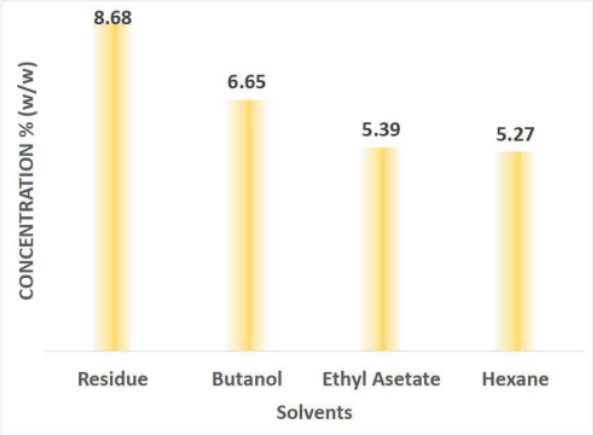

Figure 2. Total Phenolic Contents of *Mimosa pudica Linn* wet leaves in solvents of different polarities.

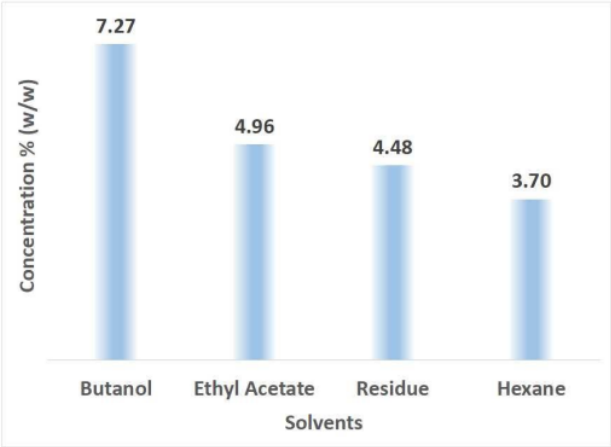

Figure 3. Total Phenolic Contents of *Mimosa pudica Linn* dry leaves in solvents of different polarities.

Although flavonoids aren't dominant compared to Phenolics in Mimosa pudica Linn, some have appeard to be solutes in the solvents and more apparent in the Non Polar Solvents (Hexane; 0.74% in both wet and dry leaf samples) than the Phenolic compounds. Polar flavonoids are also found although in smaller concentrations (solutes in Ethyl Acetate in dry sample and Butanol in wet, 1.08% w/w and 0.75% w/w respectively). Extracting flavonoids of the leaves North Sulawesi's Mimosa pudica Linn it is apparent that in wet mimosa leaves, Ethyl Acetate > Hexane > Butanol > Residue (Figure 4), and in the dry Butanol > Hexane > Ethyl Acetate > Residue (Figure 5.). Compared to Phenolics that are more like to be extracted by strong to mildly polar solvents, flavonoids of Mimosa pudica Linn has the tendencies to be extracted by mildly polar to non-polar

solvents in both wet and dry leaves. Published results of Flavonoid extracts have also shown to be low in various other plant species (Chigayo, Mojapelo, Mnyakeni-Moleele, &Misihairabgwi, 2016).

The polarity of solvents plays an important role in capturing the targeted active components as is expressed by various other studies (Alves et al., 2016; Mohammedelnour et al., 2017; Thavamoney et al., 2018). Water increases the polarity of the solvents in which for compounds such as phenolic compounds extracted by polar solvents would be more effective extracted by water (Chigayo et al., 2016; KOBUS, WILCZYŃSKI, NADULSKI, RYDZAK, & GUZ, 2017; Mohammedelnour et al., 2017).

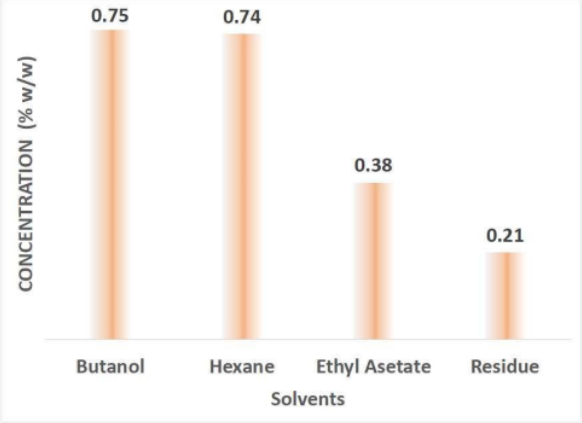

Figure 4. Total Flavonoid Contents of *Mimosa pudica Linn* wet leaves in solvents of different polarities.

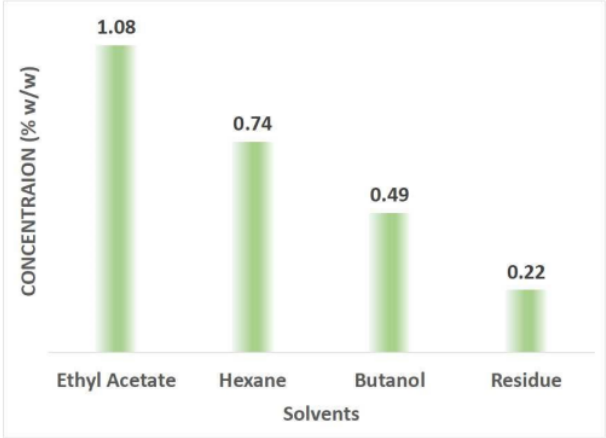

Figure 5. Total Flavonoid Contents of *Mimosa pudica Linn* dry leaves in solvents of different polarities.

4. Conclusion

Evidently polarity of solvent in this study was also crucial in extracting both phenolic compounds and flavonoids. The phenolic compounds of *Mimosa pudica Linn* from North

Sulawesi were mostly extracted by strong protic polar solvents while the flavonoids were less found in North Sulawesi *Mimosa pudica Linn* and are found in mildly polar solvents. Understanding that generally both phenolic compounds and flavonoid compounds are versatile in their diverse bioactivity, also as potential cancer control compounds, it was a positive finding to learn that these bioactive compounds were extracted by strong to mildly polar solvents. As water is also a strong polar solvent and with the results given in this stage of this study it is presumable that water would be an effective solvent for phenolic compounds and some flavonoids. Water should next be be tested as a solvent for their capacity in extracting these bioactive compounds, and thus would affirm that developing the final product in the form of an infusion tea would be the most efficient and economical method in consuming these bioactive compounds. Upon that affirmation the next stage for this research would be a full phytochemical screen and also testing cytotoxicity of the extracts with the most extracted phenolic compounds and flavonoids towards cancer cells to determine their effectiveness as potential cancer control substances.

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