

Difference of Solvent Polarity To Phytochemical Content and Antioxidant Activity of *Pluchea indica* Less Leaves Extracts

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ABSTRACT

Pluchea indica Less called local name Beluntas or Luntas, one of herb plants, is usually used as traditional medicine by people in Indonesia. Phytochemical content in this *Pluchea* leaves can reduce odor body and prevent many diseases. Phytochemical polarity in *Pluchea* leaves is various so that can be extracted by different solvents. The phytochemical contents determine their antioxidant capacity. The study was conducted to determine difference of solvent polarity (water, methanol, ethanol, ethyl acetate, and hexanes) to phytochemical contents and antioxidant activity of *Pluchea* leaves extracts. The results showed that major phytochemical in *Pluchea* leaves was polar properties extracted by water, methanol, and ethanol, including flavonoid, saponin, phenol hydroquinone, alkaloid, sterol, tannin, and reducing sugar. A part of them had semi polar properties, such as sterol, flavonoid, phenol hydroquinone, and alkaloid. Another of them had non polar properties, i.e. sterol, flavonoid, and phenol hydroquinone, and alkaloid. Phytochemical content was correlated with total phenolic and total flavonoid contents and antioxidant activity. Methanolic extract had the highest total phenol and total flavonoid, 1185.2 mg GAE/g samples dry base and 911.9 mg CE/g samples dry base, respectively, consequently it had the highest a DPPH free scavenging activity and iron ion reducing power, 794.9 mg GAE/g samples dry base and 2.14 mg GAE/g samples dry base, respectively.

Key words: *Pluchea indica* Less, phytochemical, antioxidant activity.

INTRODUCTION

Pluchea Indica Less usually called as Beluntas or Luntas is a one of herb plants that is used as a traditional medicine to reduce body odor and prevent many diseases. This plant is grouped in *Asteraceae* family. The plant is generally grown as a wild plant in dry land with hard earth texture, many stones, and need enough sunshine (Dalimarta, 2003; Manan, 2002; Raharjo and Horsten, 2008).

Pluchea can use a traditional medicine because it contains many phytochemical compounds. Many researchers have identified that root and leaves of *Pluchea* have many biological activities, such as antiinflammation, antiulcer, antipyretic, hypoglycemic, diuretic, and many pharmacological activities (Biswas et al., 2005; Biswas et al., 2007; Widyawati et al., 2010; Widyawati et al., 2011; Widyawati et al., 2012). This caused *Pluchea* contains many phytochemical compounds, such as lignan, terpene, phenylpropanoid, benzoid, alkanes (Luger, 2000), sterol, 2-(prop-1-unyl)-5-(5,6-dihydroxy hexa-1,3-diunyl)-thiophene, (-)-catechin (Biswas et al. 2005), alkaloid (Ardiansyah et al. 2003), saponin, tannin, phenol hydroquinone, flavonoid (Widyawati et al. 2010; Widyawati et al. 2011), flavonol (quercetin, kaempferol, myricetin) (Andarwulan et al. 2010). Biswas et al. (2005)

said that *Pluchea* root methanolic extract contains stigmasterol (+ β -sitosterol), stigmasterol glycoside (+ β -sitosterol-glycoside), 2-(prop-1-unyl)-5-(5,6-dihydroxy hexa-1,3-diunyl)-thiophene, and (-)-catechin. Traithip (2005) also informed that essential oil of *Pluchea* leaves is composed of boehmeryl acetate, HOP-17 (21)-ene- 3 β -acetate, linaloil glucoside, linaloil apioxyl glucoside, linaloil hidroxy glucoside, plucheoside C, quauhtermone, 3-(2'-3'-diacetoxy-2'-methyl-butiril), plucheol A, plucheol B, plucheoside A, plucheoside B, plucheoside E, and pterocarptriole. Widyawati et al. (2013) said that essential oil of *Pluchea* leaves contains alcohols, aldehydes, aliphatic unsaturated hydrocarbons, esters, ketones, ethers, and sulfoxides. Cyclic unsaturated hydrocarbons is the most numerous and (10S,11S)-Himachala-3-(12)-4-diene (17,13%) is a volatile compound with the highest proportion. Widyawati et al. (2010) and Widyawati et al. (2011) also proved that *Pluchea* leaves methanolic extract and its fractions (ethyl acetate and n-butanol) are arranged tannin, sterol, flavonoid, and phenol hydroquinone, but water fraction doesn't contain sterol.

Many researchers have found antioxidant activity of *Pluchea* Leaves extract, such as ethanolic extract can

Table 1: Yield of various extract of pluchea leaves

No	Extract	Yield (%)
1	Aquadest	40,65 ± 1,47 ^d
2	Methanol	38.07 ± 2.08 ^c
3	Ethyl acetate	32.97 ± 1.24 ^b
4	Ethanol	31.09 ± 1.75 ^{ab}
5	Hexanes	29.46 ± 1.47 ^a

scavenge a DPPH free radical and inhibit β -carotene linoleic acid (Widyawati, 2004; Andarwulan et al., 2010), scavenge ABTS^{2+•}, reduce iron ion, and inhibit malondialdehyde formation (Andarwulan et al., 2010). Widyawati et al. (2012) also explains that methanolic extract has potency to scavenge a DPPH free radical, reduce iron ion, inhibit β -carotene linoleic acid peroxidation, and then ethyl acetate fraction has potency to scavenge a DPPH and superoxide free radicals, reduce and chelate iron ions. Phytochemicals of pluchea leaves have different polarity so that they can be extracted by different solvent polarity. Every phytochemical can contribute antioxidant activity. Until now the study about effect of different polarity solvent to phytochemical content and antioxidant activity of *pluchea indicia less* leaves extracts has not yet been done. The research was conducted to study the different polarity solvent, including water, methanol, ethanol, ethyl acetate, and hexanes to phytochemical content and antioxidant activity of *pluchea indicia less* leaves extracts.

MATERIALS AND METHODS

Plant material: Leaves of *Pluchea indica Less* at 1-6 segment levels were collected from areas at *East Coast*, Bendul Merisi, Keputih, and Wiyung in Surabaya and Kertosono, East Java.

Pluchea india Less leaves Extraction: 1-6 segment level of *Pluchea* leaves from peak was used as sample (Widyawati et al., 2011; Dorman and Hiltunen, 2004). These leaves were dried at ambient temperature and grinded with 45 mesh size. Dried flour of *Pluchea* leaves was measured moisture content. And then this flour was extracted by different polarity solvent (water, methanol, ethanol, ethyl acetate, and hexanes) with soxhlet extractor at a boiling point for three hours. Extract was evaporated by rotary evaporator. The extract was stored at 4°C in black glass bottle until analysis further. Parameters were analyzed including yield, phytochemical content, total phenol, total flavonoid, iron ion reducing power, and DPPH free radical scavenging capacity.

Moisture Content: Moisture content of dried flour of *Pluchea* leaves is determined by gravimetry method (AOAC, 1990). One gram of samples is measured moisture content with vacuum oven at 70°C for 24 hours. Weight difference of sample after heating was moisture content of sample.

Yield Analysis: Yield of pluchea leaves extract was determined by gravimetry method based on Ljubuncic et al. (2005). Yield was measured with comparison between weight of pluchea leaves extract and sample weight stated by weight percentage (% w/w dry base).

Phytochemical Identification: Phytochemical assay was done to determine existence of phytochemical specific in

sample, such as alkaloid, flavonoid, phenolic, sterol, triterpenoid, phenol hydroquinone, saponin, tannin, cyanogenic glycoside, cardiac glycoside, and reducing sugar in pluchea leaves extract (Harborne, 1996).

Total Fenol Analysis: Total fenol of pluchea leaves extract was determined by spectrometry method (Sahreen et al. 2010). Sample was added with potassium carbonate 75 g/L and folin ciocalteus reagent and then sample was shaken. And then sample was homogenous with aquadest. Solution was be incubation in ambient temperature for one hour, and absorbance of sample was measured at λ 760 nm. Total fenol was stated by gallic acid equivalence (GAE)/g sample dry base.

Total Flavonoid Analysis: Total flavonoid was determined by colorimetry method based on aluminium chloride color measurement (Sahreen et al. 2010). *Pluchea* leaves extract was added to aquadest in flask bottle 10 mL. And then this solution was added NaNO₂ 5 % (b/v), AlCl₃ 10 % (b/v), and NaOH 1 M, respectively with shaken and diluted until volume 10 mL. Absorbance of solution was measured at λ 510 nm. Total flavonoid was stated as mg catechin equivalent (CE) /g sample dry base.

Iron Reducing Power Analysis: Iron reducing power was determined based on modified of Oyaizu method (1986). Various concentrations of pluchea leaves extract were mixed with phosphate buffer 200 mM (pH 6,6) and potassium ferricyanide 0,1 %, and then solution was incubated at 50°C for 20 minutes. Chlorogenic acid 10% was added to solution, shaken and filtered. Filtrate was added aquadest and ferric chloride 0,1 %, and then absorbance of sample was measured at λ 700 nm. Increasing absorbance was indicated that iron reducing power was increasing. Reducing power of sample was determined as mg gallic acid equivalent (GAE)/g sample dry base.

2,2-diphenyl-1-picrylhydrazyl radical (DPPH) Scavenging Activity Analysis: Antioxidant activity of pluchea leaves extract was determined based on modified of Sahreen et al. (2010) method. Various concentrations of samples in methanol were added DPPH (60 μ M in methanol). When DPPH free radical reacted with antioxidant compound, capacity of compounds donating hydrogen atom was reduced. Decreasing of DPPH scavenging activity could be known based on absorbance of solution that measured at λ 517 nm after 30 minutes incubation. DPPH free radical scavenging activity was stated as % inhibition = $[(A_0 - A_t) / A_0] \times 100\%$, A₀ was control absorbance at t = 0 seconds and A_t was antioxidant absorbance at t seconds.

RESULTS

Pluchea leaves used to get pluchea leaves extract had moisture content around 14.19 ± 0.17%. This moisture content of pluchea leaves is the same as Widyawati et al. (2011) reported around 14.29%. The yield obtained from solvent extraction with aquadest, ethanol, methanol, ethyl acetate, and hexanes was showed at Table 1. Data showed that aquadest extract had the highest yield (40,65 ± 1,47%). And then methanolic, ethyl acetate, ethanolic and hexanes extracts had yield 38.07 ± 2.08, 32.97 ± 1.24, 31.09 ± 1.75, dan 29.46 ± 1.47 %, respectively. The yield of pluchea

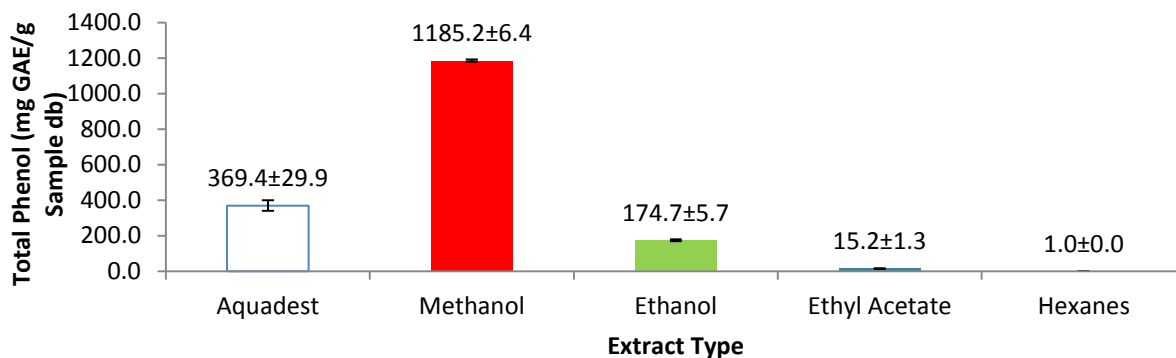


Fig. 1: Total Phenol in Various Pluchea Leaves Extracts

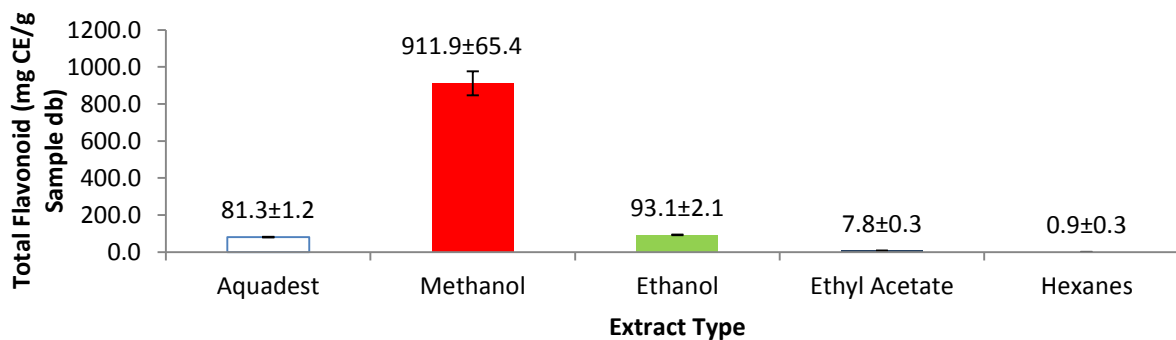


Fig. 2: Total Flavonoid in Various Pluchea Leaves Extracts

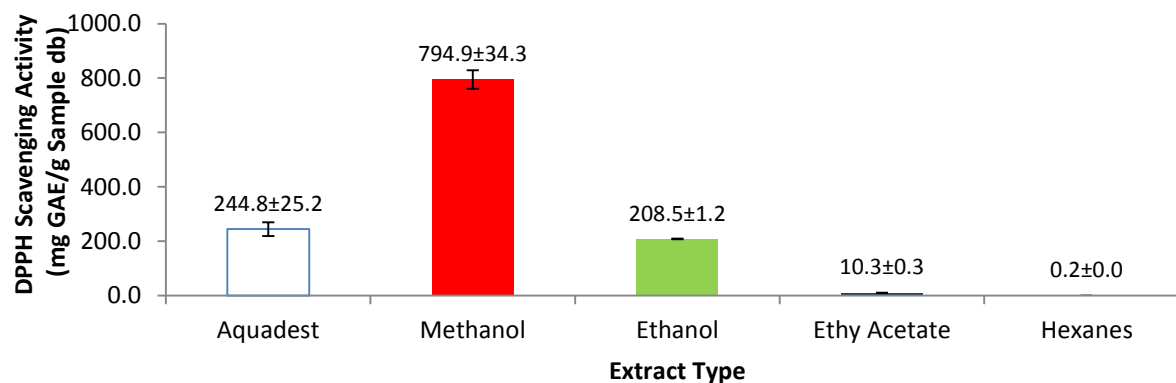


Fig. 3: DPPH Scavenging Activity in Various Pluchea Leaves Extracts

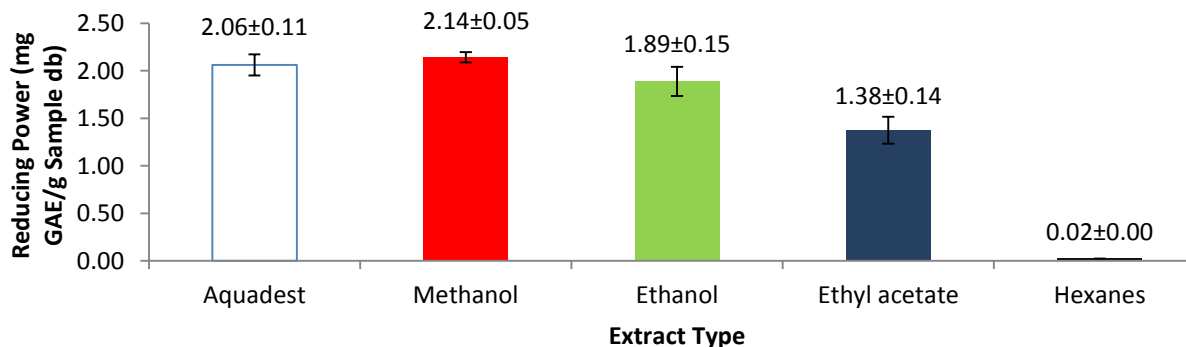


Fig. 4: Reducing Power in Various Pluchea Leaves Extracts

leaves extract contained phytochemical compounds that were showed at Table 2. Data informed that methanol could extract the most chemical compounds of pluchea leaves including sterol, flavonoid, saponint, annin,

phenolic, alkaloid and glycoside compounds compared with aquadest, ethanol, ethyl acetate, and hexanes. Total phenol of methanolic extract (1185.2 mg GAE/g sample

Table 2: Phytochemical compounds in various pluchea leaves extracts

Extract types	Color intensitas of phytochemical compounds							
	Terpenoid	Sterol	Flavonoid	Saponin	Tannin	Phenolic	Alkaloid	Cardiac glycoside
Aquadest	-	-	+++	++	+	+++	+++++++	+++++
Methanol	-	+++	+++++	++++	++++	+++++	+++++	+++
Ethanol	-	++++	++++	+	++	++++	++++	++
Ethyl acetate	-	+++	++	+	-	++	+++	+
Hexanes	-	++	+	-	-	+	+	-

Note: + detected based on color intensity, - not detected based on color intensity

db) was the highest concentration compared with aquadest (369.4 mg GAE/g sample db), ethanolic (174.7 mg GAE/g sample db), ethyl acetate (15.2 mg GAE/g sample db), and hexanes (1.0 mg GAE/g sample db) (Figure 1 and 2). Consequently the methanolic extract of pluchea leaves showed the biggest DPPH free radical scavenging activity (794.9±34.3 mg GAE/g sample db) and reducing power (2.14 ±0.05 mg GAE/g sample db) (Figure 3 and 4).

DISCUSSION

The different moisture content of leaves is determined by environment, climate, earth texture, and weather. The solvent difference used to extract pluchea leaves determined yield of extract. Data also informed that components contained in pluchea leaves were trended polar and non polar components only gave the least composition of pluchea leaves. Many researchers also informed that the components arranged plants are largely polar. Methanol is effective to extract leaves and flower of *Alphinia* species (Wong, 2006), young leaves of *Camelia sinensis* (Chan et al., 2007), Mulberry leaves (Yen et al. 1996), young leaves of *Terminalia catappa* (Chyau et al., 2002). Dehkharghanian et al. (2010) also informed that difference of polarity solvent determines difference of type, composition, and antioxidant activity of phytochemical.

Aquadest could dissolve alkaloid and glycoside compounds, but ethanol was effective to extract sterol, flavonoid, phenolic, and alkaloid. Ethyl acetate was semipolar solvent that could dissolve sterol dan alkaloid. There were less chemical compound of pluchea leaves that had non polar properties. Previous research informed that methanol and ethanol can dissolve polar compounds, such as sugar, amino acid, glycoside compounds (Houghton and Raman, 1998), phenolic compounds with low and medium molecular weights and medium polarity (Yu Lin et al., 2009), aglycon flavonoid (Dehkharghanian et al., 2010), anthocyanin, terpenoid, saponin, tannin, xantoxilin, totarol, quacinoid, lacton, flavone, phenone, and polyphenol (Cowan, 1999). Whereas aquadest is effective to extract glycoside compounds, amino acid, and sugar (Houghton and Raman, 1998), aglycon compounds (Liu et al., 2011; Dehkharghanian et al., 2010), vitamin C (Dalimarta, 2003). Ethyl acetate is effective to extract alkaloid, aglycon, and glycoside compounds (Houghton and Raman, 1998), sterol, terpenoid, and flavonoid (Cowan, 1999). Hexanes can solve non polar compounds,

such as lignin, wax, lipid, and aglycon (Houghton and Raman, 1998), sterol, and terpenoid (Cowan, 1999).

Effectivity of methanol extracted phytochemical compounds in pluchea leaves was supported by total phenol and total flavonoid assays. Data also showed that phenolic compounds in pluchea leaves had polar properties. This was similar to yield assay that pluchea leaves extract was dominant extracted by aquadest, methanol, and ethanol. Phenolic compounds of methanolic extract were effective to donating hydrogen atomic to molybdenum ion in Folin ciocalteus phenol's reagent so that they resulted radical phenoxyl stabilized by resonansi or delocalization. Effectivity of phenolic compounds was depended on type, structure, number, and position of hydroxyl group of benzene ring (Wong et al. 2006; Widyawati et al. 2010; 2011; 2012).

Total flavonoid in methanolic extract also showed the biggest concentration (119.9 ± 65.4 mg CE.g sample db) compared with the other extracts (Figure 2). The phenomena was similar to total phenolic assay because flavonoid was major phenolic compounds in plants with concentration around 80% (Aberoumand and Deokule, 2008). Phytochemical compounds in methanolic extract were potential to donating hydrogen atom so that these compounds could form complex compounds with aluminium ion at total flavonoid assay. Effectivity of flavonoid as radical scavenging and metal chelating activities was determine by hydroxyl group of ortho position in catechol structure (B ring), double bond at C_{2,3} conjugated with carbonyl group at C₄ (C ring), and hydroxyl group at C₅ (A ring) (Tapas et al., 2008; Amic et al., 2003). Based total flavonoid assay could be said that flavonoid compounds dominant arraged of pluchea leaves were polar properties.

Total phenol and total flavonoid of pluchea leaves could be related with antioxidant activity. Capacity of phytochemical compounds scavenging free radical involved donating hydrogen atom or electron (Leopoldini et al., 2011). Nakiboglu et al. (2007) said that capacity of phytochemical compounds donating hydrogen atom/electron could be criteria to measure radical scavenging activity.

DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH

is decolorized which can be quantitatively measured from the changes in absorbance (Ara and Nur, 2009). The reducing power of plants extract is determined on the basis of the ability of antioxidant in extracts to reduce ferric (III) iron to ferrous (II) iron based on decolorization of solution from yellow to green-blue (Liu et al. 2011). Generally, the antioxidant assay is used due to its simplicity and reproducibility (Maizura et al., 2011). Zhang et al. (2009) reported that reductone in extract can separate free radical chain reaction with donating hydrogen atom or electron. Capacity of phytochemical compounds in pluchea leaves, especially phenolic and flavonoid, donating hydrogen atom or electron determined antioxidant activity. Antioxidant properties are determined for the phenolic fractions in the extracts (Amarowicz et al., 2001). Maizura et al. (2011) informed that phenolic compounds in spices and herbs significantly contribute to their antioxidant properties. Total phenolic content is correlated with a DPPH free radical scavenging activity and reducing power. The antioxidant activity of pluchea leaves extract was contributed by phytochemical compounds content. Sterol, flavonoid, saponin, tannin, phenol hydroquinone, alkaloid, and cardiac glycoside have been proven to have antioxidant activity (Nystrom et al., 2007; Li et al., 2007; Tapas et al., 2008; Amic et al., 2003; Hagerman et al., 1998). Phenolic compounds that can donate hydrogen atom or electron depend on structure, number, position, and type of hydroxyl group in benzene ring. Potency of phenolic compounds as antioxidant is determined by stability of phenoxy radical formation (Chludil et al., 2008; Skerget et al., 2006).

CONCLUSION

The results obtained demonstrated that methanol was the most effective solvent to extract phytochemical compounds compared to aquadest, ethanol, ethyl acetate, and hexanes. Phytochemical compounds identified included sterol, flavonoid, saponin, tannin, phenolic, alkaloid and glycoside compounds. Methanolic extract obtained had the highest total phenolic content and antioxidant activity (DPPH scavenging activity and reducing power).

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