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Research Paper ***Open Access***

THE PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES OF PLUCHEA INDICA LESS DRINK IN TEA BAG PACKAGING

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Pluchea indica Less is popular in people as traditional medicine and fresh food. Water, methanolic, and ethyl acetate extracts of Pluchea leaves proved contain tannin, flavonoid, phenol hydroquinone, alkaloid, and cardiac glycoside so that the extracts have antioxidant activity such as DPPH free radical scavenging activity and iron ion reducing power. Methanolic extract is the best potentially as antioxidant source but it has toxic to human body health so that water extract is the most safety as antioxidant source used. Furthermore pluchea leaves are potentially used as functional drink, are packed in tea bag packaging. The research was done to study physicochemical, antioxidant, and organoleptic properties of Pluchea leaves drink in tea bag packaging at various concentrations, i.e., 0.4; 0.8; 1.2; 1.6; and 2% (w/v). The results showed that the increasing of pluchea leaves concentration in tea bag packaging solved possessed physicochemical, antioxidant, and organoleptic properties. pH and chroma values were decreased, but turbidity, total acid titrated and hue values were increased. The identification of phytochemical compounds was known that the highest color intensity of drink was at the biggest pluchea leaves concentration. The compounds were detected including alkaloid, flavonoid, phenolic, saponin, tannin, and cardiac glycoside. The increasing of used pluchea leaves concentration could reduce the solvation of total phenol and total flavonoid. This phenomena happened was predicted that there were interaction among bioactive compounds of solved pluchea leaves, especially hydroxil groups of benzene aromatic ring. They could influence antioxidant activity, including DPPH free radical scavenging activity and iron ion reducing power. The phytochemical compunds solved of functional drink determined panelis score at hedonic test with three parameters, i.e., color, taste, and aroma. Based on the effectiveness test of three hedonic test scores was informed that the best treatment of pluchea leaves drink in the tea bag packaging was 2% (w/v) concentration.

Keywords: *Pluchea indica* Less, Functional drink, Physicochemical, Antioxidant, Organoleptic, Tea bag packaging

INTRODUCTION

Pluchea indica Less is herb plant from *Asteraceae* family, usually is used as traditional medicine and fresh food. Pluchea has been proved having antiinflamation, antiulcer, antipyretic, hypoglicemic, diuretic and many pharmacological activities (Biswas *et al.*, 2005 and 2007).

Water extract of Pluchea leaves has been proved having antioxidant and antidiabetic activities (Widyawati *et al.*, 2014 and 2015). Until now Pluchea leaves still are consumed as fresh food or traditional medicine (Manan, 2002; Dalimartha, 2003; and Raharjo and Horsten, 2008) so that it needs to be developed as functional drink with packing Pluchea leaves

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in tea bag packaging. Srisook *et al.* (2012) has been used pluchea leaves as herbal tea. Pluchea leaves contains phytochemical compounds, such as tannin, flavonoid, phenol hydroquinone, alkaloid, and cardiac glycoside, lignin, terpene, phenyl propanoid, benzoic, alkana (Luger *et al.*, 2000), sterol, 2-(prop-1-unyl))-5-(5,6-dihydroxy hexa-1,3-diunyl)-thiophene, (-)-catechin (Biswas *et al.*, 2005), flavonol (quercetin, kaempherol, myricetin) (Andarwulan *et al.*, 2010).

The research was done to study physicochemical, antioxidant, and organoleptic properties of Pluchea leaves drink in tea bag packaging at various concentrations, i.e., 0.4; 0.8; 1.2; 1.6; and 2% (w/v).

MATERIALS AND METHODS

Material and Reagent

Pluchea leaves at 1-6 level from tip of a leaf, were harvested from many locations, such as Pakuwon City Laguna, East Tenggilis, and Manggrove areas in Surabaya, East Java.

Reagents that were used to analyze were *analytical grade*, except aquadest from PT Aqua Surabaya), mineral water with Aquase Merk.

Preparation of Pluchea Leaves Powder

Pluchea leaves from many locations were graded and picked 1-6 level from tip of a leaf, and then leaves were washed and dried at ambient temperature for 7 days. Dried leaves were powdered with 28 mesh size. And then dried powder was packed by tea bag packaging with 0,4; 0,8; 1,2; 1,6; 2 g weight, respectively.

Drink Preparation of Pluchea Leaves Powder at Tea Bag Packaging

Each tea bag packaging of samples was solved in 100 ml of mineral water with 95 ^{o}C temperature for 5 minute without closed, so that solvent was obtained with 0,4; 0.8; 1,2; 1,6; and 2% (w/v) concentrations, respectively. Solvation was aimed to extract antioxidant and the other compounds that gave color, taste, and aroma organoleptic properties.

Physicochemical Assays

Color Analysis

Color analysis was done by color rider based on Hutchings (1999) method. Color assay was conducted with Hunter system to determine L*, a*, and b* values.

pH Analysis

pH was analyzed by AOAC 973.41 (2005). pH was measured based on activity of compounds to result hydrogen ion by pH meter.

Total Acid Analysis

Total acid was analysis based on acid base titration by AOAC 33.2.06 (2005). Total acid assay was based on netralization reaction between hydrogen ion from acid and hydroxil ion from base so that released water molecule.

Turbidity Analysis

Turbidity was muddy condition or solution transparency decreasing that was caused by suspended particles in liquid. The principle of turbidity assay was based on O'Dell method (1993).

Phytochemical Analysis

Phytochemical identification was done by qualitative analysis based on Harborne method (1996), with color change observation of samples. The phytochemical compounds assay included alkaloid; flavonoid, phenolic, triterpenoid, sterol, saponin, tannin, and cardiac glycoside compounds.

Total Phenol Analysis

Total phenol assay was based on by reaction between phenolic compounds and Folin Ciocalteus phenol reagent (FC) (Muntana and Prasong, 2010). FC can oxidize phenolic (alkali salt) or phenolic group (hydroxil group) reduce poly hydro acid (phospho molybdate-phospho tungstate) in Folin coicalteus phenol reagent to make molybdenum-tungsten complex compounds with blue color (Singleton *et al.*, 1999). Blue color intensity shows total phenol that is measured by spectrophotometer UV-Vis at ë 760 nm. Total phenol is stated by mg gallic acid equivalent.

Total Flavonoid Analysis

Total flavonoid analysis was measured based on stable acid compex compounds formation between $AlCl_3$ and keto group at C-4 and C-3 or hydroxyl group at C-5 of flavone and flavonol (Harborne, 1996). The complex compounds have pink color that can be measured by spectrophotometer at ë 510 nm. Total flavonoid is stated by mg cathecin equivalent.

DPPH Free Radical Scavenging Activity Analysis

This assay was done based on reaction between antioxidant coumpounds and stable DPPH free radical (2,2-diphenyl-1-picrylhydrazyl) (Sompong *et al.*, 2011). This reaction is occured purple color change from DPPH because of

oxidation to make yellow color from DPPH-H. The color change can be measured by spectrophotometer UV-Vis at λ 517 nm. DPPH free radical scavenging activity is stated by mg gallic acid equivalent.

Iron Ion Reducing Power Analysis

Reducing power is potential indicator of antioxidant compounds. Reducing power is measured based on antioxidant capacity to change Fe^{3+} ion to Fe^{2+} ion (Chanda and Dave, 2009). The principle assay is reaction between antioxidant compounds and potasium ferricyanate (Fe^{3+}) to make potasium ferrocyanate (Fe^{2+}). And then the Fe^{2+} ion reacts with ferrichloride (Fe^{3+}) to make complex compounds (ferri-ferrous) that can be measured by spectrophotometer at λ 700 nm. The reducing power is stated by mg gallic acid equivalent (GAE).

Sensories Properties Analysis

Sensoris properties analysis was hedonic test of panelis to aroma, color and taste of pluchea leaves drink. Panelis number used was 80. Sensories assay used scoring test with 1-7 range. 1 score stated very dislike of samples and 7 showed very like of samples (Lawless and Heymann, 1999).

RESULTS AND DISCUSSION

Moisture content of pluchea leaves powder packed in tea bag packaging was 17.92% dry base. The moisture content was determined because it effected physicochemical, antioxidant, and sensories properties. It was effected of phytochemical compounds concentration solved in drink. Tapas *et al.* (2008) informed that phenolic compounds as secondary metabolic of plants having various molecule structures are responsible to sensory properties of food and beverages including color, *flavor*, taste, nutrition, *astringency*, dan *bitterness*. Data analysis of phytochemical compounds was showed at Table 1.

Phytochemical compounds were detected in pluchea leaves drink including alkaloid, flavonoid, phenolic, saponin, tannin, and cardiac glycoside. Previous research also discovers that water extract of pluchea leaves contain alkaloid, flavonoid, phenolic, saponin, tannin, and cardiac glycoside. These compounds can be detected in sample because they have polar properties, this phenomena is appropriate with like dissolve like terminologically (Dey and Harborne, 1997). Its means that the polar compounds are only solved in polar solvent. The metabolic secondary products of plants are usually bonded such as glycoside and ester, or free such as aglycon.

Table 1: Phytochemical Compounds Detected in *Pluchea* Leaves Drink

Phytochemical	Pluchea Leaves Concentration (%b/v)				
	0,4	0,8	1,2	1,6	2,0
Alkaloid	+	2	3	4	5
Flavonoid	+	2	3	4	5
Phenolic	+	2	3	4	5
Triterpenoid	-	-	-	-	-
Sterol	-	-	-	-	-
Saponin	+	2	3	4	5
Tannin	+	2	3	4	5
Cardiac Glycoside	+	2	3	4	5

Note: + color intensity, - + not detection.

Generally, the alkaloid in plants is base properties because it has amino group with one or more nitrogen atoms in cyclic ring. Alkaloid kinds of Pluchea are β-sitosterol, β-sitosterol glycoside, stigmasterol, and stigmasterol glycoside. Phenolic compounds have one or more hydroxyl groups with acid properties. Tannin is phenolic compound groups that has high molecular weight composed with simple phenolic by condensation reaction. Saponin is derivate of triterpenoid glycoside compounds having high polarity properties, so that it can be solved well and stable in water.

Data at Table 1 showed that these compounds values increased appropriate for pluchea leaves concentration that were informed color intensity increasing of samples. Existence of them in drink effected physicochemical properties, such as turbidiy, color, pH, and total acid. Data of physicochemical properties were showed at Figure 1.

Turbidity is capacity of sample to diffuse beam because there are organic and inorganic compounds that suspended and solved in sample. Turbidity is description about transparency decreasing that stated as NTU. Data at Figure 2 showed that turbidity values of drink were decreased along with increasing of pluchea leaves concentration used. Turbidity was effected by suspended particle concentration in drink. The more particles suspended caused the higher turbidity of samples. Phytochemical compounds identified of Pluchea leaves drink giving effect of turbidity that was showed with color intensity change (Table 1). Pluchea also contains vitamin A and C, amino acid (leucine, isoleucine, tryptophan, and treonine), protein (17.78-19.02%), lipid, and

Figure 1: pH Change of *Pluchea* Leaves Drink at Various Concentration

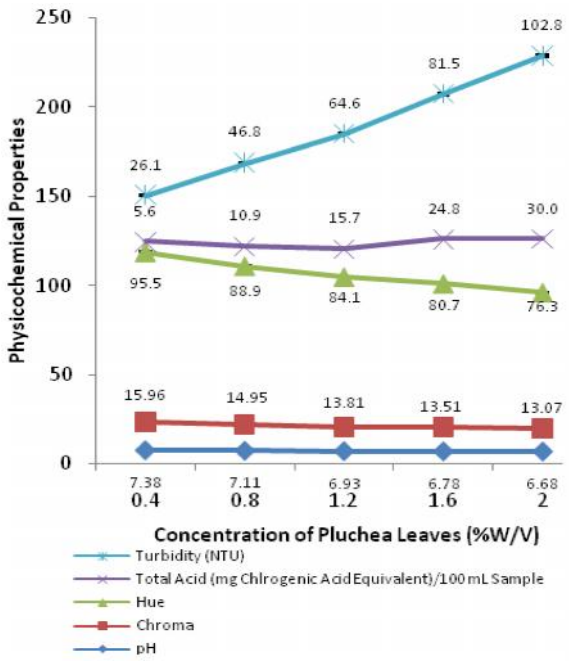
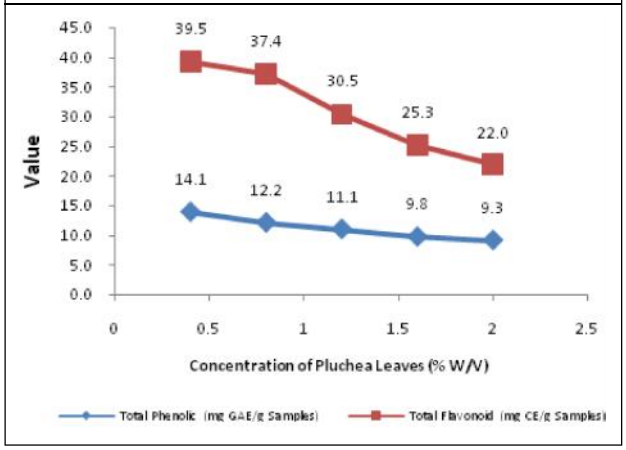


Figure 2: Total Phenolic and Total Flavonoid of *Pluchea* Leaves Drink



mineral (Ca, P, Fe) (Rukmiasih, 2011) that give contribution of turbidity.

Color of Pluchea leaves drink was measured by color reader. Data showed that Hue and Chroma values of drink decreased along with adding of Pluchea leaves concentration steeped. Hue value identified real color of drink, and Chroma value stated intensity of color. Hue value of Pluchea leaves was changed from 95.5 to 76.3. Hutchings (1999) informed that the drink has color from yellow until yellow red and the intensity of yellow color decreased along with Pluchea leaves added. Color drink is effected by solved constituent. Green color was contributed by chlorophyll content, yellow color is given by chalcones and flavones compounds (Ningrum, 2012). Tannin can result yellowness brown (Dey and Harborne, 1997). Tapas *et al.* (2008) informed that phenolic compounds are responsible of physicochemical and sensory properties in food and beverage, such as color, flavor, taste, nutrition, *astringency*, and *bitterness.*

The existence of solved phytochemical compounds in Pluchea drink also effected total acid and pH. The bigger Pluchea leaves concentration used caused the higher total acid and the lower pH of samples. The phenolic acid compositions of Pluchea leaves are chlorogenic acid and caffeic acid with the chrologenic acid as major constituent[4].

The results of phytochemical composition in drink weren't similar to data of total phenolic and total flavonoid (Figure 2). This is predicted because the reaction difference of the principle assay between qualitative and quantitative analysis. Color identification is based on the complex compounds formation, and then total phenolic and total flavonoid are determined using redox reaction between phenolic or flavonoid compounds and reagens. Redox reaction is very depended by hydrogen atom or electron donor from phenolic or flavonoid compounds. If spacing among the phenolic or the flavonoid is very short so that hydrogen atom or electron donor can be inhibited. Finally total phenolic and total flavonoid were measured to be reduced. The other reason, the steric hindrance of hydroxyl groups of aromatic rings influences hydrogen atom or electron donor and reduces total phenolic and total flavonoid content. This phenomena was showed at Figure 2. The higher concentration of Pluchea leaves steeped was the lower total phenolic and total flavonoid.

Phenolic compounds with free structure (aglycon) are potential hydrogen atom or electron donor. Antioxidant capacity of phenolic compounds or flavonoid compounds is depended by molecular structure, position of hydrogen group substituted at aromatic rings, potential reduction, reactivity or time to reach stedy state (Rice-Evans *et al.*, 1997; Sanchez-Moreno *et al.*, 1998; Amic *et al.*, 2003; Meda *et al.*, 2005; and Verzelloni *et al.*, 2007). Space among phenolic compounds or flavonoid compounds determines interaction among hydroxyl group of phenolic and flavonoid

Figure 3: Antioxidant Activity of *Pluchea* Leaves Drink

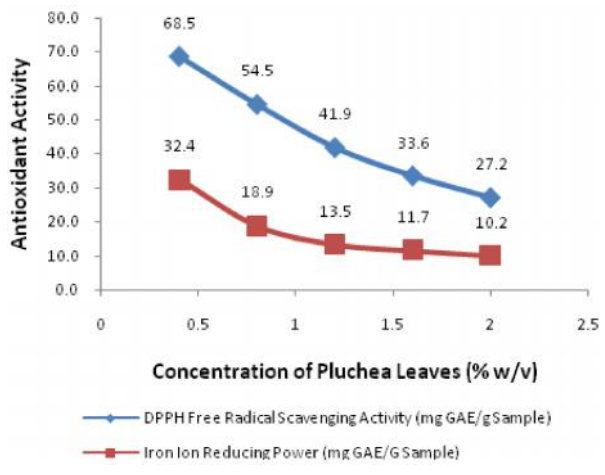
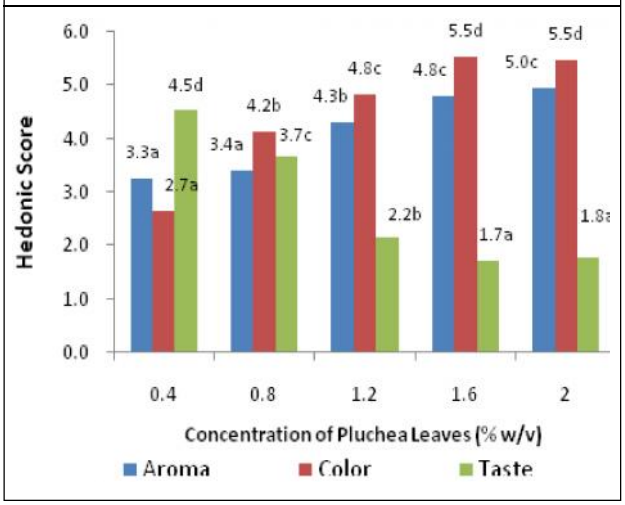


Figure 4: Sensory Test of *Pluchea* Leaves Drink



compounds with ester bond or hydrogen bond so that this interaction can block hydrogen atom or electron donor of phenolic and flavonoid compounds. Tananuwong *et al.* (2010) said that effectivity of phenolic compounds as antioxidant depends chemical structure and reactivity to hydrogen atom or electron donor. Tapas *et al.* (2008) also informed that effectivity of flavonoid as free radical scavenging is determined by resonance stability of flavonoid radical resulted.

Total phenolic and total flavonoid content were influenced of antioxidant activity (DPPH free radical scavenging activity and iron ion reducing power) (Figure 3).

Total phenolic and total flavonoid were directly proportional with antioxidant activity. Based on regression correlation showed that there were strong correlation between total phenolic or total flavonoid and DPPH free radical scavenging activity with R^2 = 0.994 and 0.951, respectively than total phenolic or total flavonoid and iron ion reducing power with R^2 = 0.910 and 0.751, respectively. Thereby phenolic compounds in Pluchea leaves drink were more potential free radical scavenging than iron ion reducing. Phenolic compounds can reduce purple color of DPPH to be yellow color of DPPH-H (Brand-Williams *et al.*, 1995; Vrchovska *et al.*, 2006; and Bortolomeazzi *et al.*, 2010). Phenolic compounds in Pluchea leaves drink were classified as primary antioxidant because they had antioxidant mechanism as radical scavenging (Winarsi, 2007).

Sensory test of this drink showed that the higher Pluchea leaves used caused hedonic score of aroma and color parameters of drink increasing, except of taste parameter (Figure 4). These were influenced by phytochemical solved of Pluchea leaves. The bigger Pluchea leaves steeped caused the higher phytochemical compounds solved that supported by Tapas *et al.* (2008). The Phytochemical compounds are responsible of sensory test. Aroma of Pluchea leaves is contributed by volatile compounds, especially terpene groups such as acetate boehmeryl, HOP-17(21)-en 3β-acetate, linaloil glucoside, linaloil apiocyl glucoside, linaloil hydroxy glucocoside, plucheoside C, cuauhtermone, 3-(2'-3'-diacetoxyl-2'methyl-butyril), plucheol A, plucheol B, plucheoside A, plucheoside B, plucheoside E, pterocarptriol, sesquisterpene, monoterpene, dan triterpene. Widyawati *et al.* (2013) informed that essential oil of Pluchea leaves contains hydrocarbon cyclic unsaturated including alkohol (6.16%), keton (3.49%), hydrocarbon aromatic (2.05%), aldehide (1.79%), hydrocarbon alifatic unsaturated (1.35%), ester (0.08%), sulphoside (0,06%), hydrocarbon heterocyclic (0.05%) and (10S ,11S)-Himachala-3-(12)-4-diene (17.13%).

Color of drink is influenced by tannin compounds of Pluchea leaves. Intensity of tannin detected at phytochemical assay determined color drink. Tannin has red brown color and bitter taste. Sensory test of color parameter was the same as color test by color rider.

The Pluchea leaves drink showed the highest panelis acceptance was determined by effectiveness test (De Garmo *et al.*, 1993). Data showed that Pluchea leaves drink at 2% (W/V) gram/100 ml was the most like by panelis (Table 2).

Table 2: The Effectiveness Test of *Pluchea* Leaves Drink

Concentration of Pluchea Leaves (% W/V)	Value
0.4	0.367
0.8	0.477
1.2	0.548
1.6	0.691
2	0.709

Conflicts of Interest

All contributing authors declare no conflicts of interest.

CONCLUSION

The concentration of Pluchea leaves effected physicochemical, antioxidant activity and sensory test of Pluchea leaves drink. The higher concentration of this drink caused Hue, Choma and pH decreasing, turbidity and total acid increasing. In this case was caused by phytochemical solved increasing but at higher concentration of Pluchea leaves steeped influenced interaction among phenolic compounds or flavonoid constituents so that reduced free phenolic or flavonoid components. Finally this interaction could be decrease total phenolic, total flavonoid, DPPH free radical scavenging activity and iron ion reducing power. The drink from Pluchea leaves at 2% (w/v) was the best treatment because it was the most like by panelis.

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