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Determination of Antioxidant Activity and Phytochemical Profile, Sitting Leaf Tea (Des-modium triquetrum), Beluntas Leaf (*Pluchea indica*) and a Combination of Both Leaves

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Abstract

Sitting leaves (*Desmodium triquetrum*) and beluntas leaves (*Pluchea indica*) are herbal plants that are often used as traditional medicine. Medicinal plants are increasingly favored as an alternative therapy. Oxidative stress can occur because the levels of free radicals in the body exceed the levels of antioxidants. Antioxidants are electron donor compounds that can stabilize free radicals. The aim of the study was to determine the antioxidant activity and phytochemical profile of the leaves of the sit (*Desmodium triquetrum*), beluntas leaves (*Pluchea indica*), and a combination of both. Extraction of sit and beluntas leaves was carried out by maceration using ethanol. Then it was fractionated using the liquid-liquid method. The three solvent extracts, n-hexane, ethyl acetate, and water, were measured for their antioxidant activity using DPPH (1,1-Diphenyl-2-pikrihidrazyl). The research results showed that the ethanol extract of sitt leaf tea had antioxidant activity with an IC_{50} value of 1.10 ppm, the ethyl acetate extract of beluntas leaf tea had antioxidant activity with an IC_{50} value of 3.09 ppm. The ethanol extract from the combination of sat leaf and beluntas tea has antioxidant activity with an IC_{50} value of 7.81 ppm. Sitting leaf tea, beluntas leaf tea, and combination leaf tea of both plants are drinks that have very strong antioxidant activity, which can help improve overall health and prevent the development of age-related diseases, as a free radical-fighting compound. The results of the ANOVA test on the extraction of various solvents and samples showed a value ($p<0.01$) which indicated a significant difference.

Keywords: Antioxidants; Sitting Leaves (*Desmodium triquetrum*); Beluntas Leaves (*Pluchea indica*); Combination; DPPH; IC_{50}

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1. Introduction

Indonesia is a country rich in efficacious medicinal plants, so many plants are used as traditional medicines(1,2,3). Medicinal plants are increasingly favored as an alternative therapy. The trend of "back to nature" and the ever-increasing drug prices have made herbal/traditional medicine an option (Rochani). But on the other hand, use which only relies on experience and estimates, is likely to cause adverse effects (4,5). One of the medicinal plants that are spread in several regions in Indonesia and are used as traditional medicine are sitting leaves (*Desmodium triquetrum*) and beluntas leaves (*Pluchea indica L.*).

Sitting leaves (*Desmodium triquetrum* (L.) DC.) is a medicinal plant, which has anti-inflammatory and antipyretic activity which is commonly used by the community to treat inflammation, improve urination, and hemorrhoids. Meanwhile, beluntas leaves (*Pluchea indica* L.) are often used to reduce fever and treat digestive disorders in children (6,7).

Figure 1. Sitting leaves (*Desmodium triquetrum*)

The classification of the sitting leaves (*Desmodium triquetrum*) is as follows: Kingdom: Plantae, Subkingdom: Tracheobionta, Superdivisi: Spermatophyta, Division: Magnoliophyta, Class: Magnoliopsida, Subclass: Rosidae, Nation/Order: Fabales, Family: Fabaceae. Genus/Genus: Desmodium, Type/Species: *Desmodium triquetrum* DC. (8).

Beluntas leaves (*Pluchea indica*) in Figure 2.2 below is an upright shrub with a height of 0.5-2 m and often has many branches. Beluntas leaves (*Pluchea indica*) are light green and hairy. Beluntas leaf blade is elliptical oval or inverted oval with a pointed leaf base and serrated leaf edges. The location of beluntas leaves is alternate and short-stemmed with a leaf length of 2.5-9 cm. The flower of the beluntas plant is a compound flower with a small head shape, gathered in terminal compound flat panicles. Beluntas flowers have a purple anther tube, and a pistil with 2 purple branches that rise far away. The fruit of the beluntas plant is slim, hard and brown in color. The size of the beluntas fruit is very small with a length of 1 mm, has small seeds and is whitish brown in color (9,10,11).

The classification of beluntas leaves (*Pluchea indica*) is as follows: Kingdom: Plantae, Division: Spermatophyta, Sub-division: Angiospermae, Class: Dycotyledonae Nation: Compositales, Tribe: Compositae, Genus: Pluchea, Species: *Pluchea indica* (L.).

Beluntas leaves (*Pluchea indica*) are used by the community to get rid of body and mouth odor, overcome lack of appetite, overcome digestive disorders in children, relieve pain in rheumatism, bone pain and back pain, and overcome vaginal discharge and irregular menstruation (11,12).

Free radicals are defined as atoms or molecules with one or more unpaired electrons and are unstable, short-lived and highly reactive to gain electrons from other molecules in the body to achieve stability causing potential damage to biomolecules by impairing the integrity of lipids, proteins and DNA that leads to increased oxidative stress such as degenerative diseases such as diabetes mellitus, cardiovascular disease, premature aging, and even cancer. Therefore, antioxidants are needed to overcome free radicals (13,14,15).

Figure 2.2 Beluntas leaves (*Pluchea indica*)

Antioxidants reduce free radical molecules by donating one of their electrons to metal binding enzymes which are owned by free radicals, thus protecting cells from the negative effects of oxidant compounds and protecting the body from damage to cells due to unstable molecules owned by free radicals (14,15,16).

Phytochemical compounds are compounds that are naturally found in plants, and their properties can be used as antioxidants. The method of phytochemical screening is mostly a color test reaction with a color reagent. Phytochemical tests on the extracts of the leaves of the leaves (*Desmodium triquetrum*) and beluntas leaves (*Pluchea indica* L.) included examination of flavonoids, polyphenols, alkaloids, tannins and saponins. (17,18). Herbal leaves are medicinal plants whose raw materials are simplicia and have properties or contain certain substances that can be used to treat or cure certain diseases, so that herbal leaves have benefits as traditional medicine or natural medicine, including Sitting leaves (*Desmodium triquetrum*) and beluntas leaves (*Pluchea indica* L)

2.Methodology

The type of research used in this research is qualitative and quantitative. The samples of this research are sit leaves and beluntas leaves. The type of data used in this research is primary data, and the data collection instrument used is Yarsi University Herbal Laboratory tools.

2.1 Research Procedures

Sitting leaves (*Desmodium triquetrum*) used in this study were obtained from gardens in the Cipongkor area, West Java. Meanwhile, beluntas leaves (*Pluchea indica*) used in this study were obtained from gardens in Bekasi, West Java. Sitting leaves (*Desmodium triquetrum*) and beluntas leaves (*Pluchea indica*) are washed using running water for the shortest possible time to remove dirt and microbes attached to the leaves, but not remove the nutritious substances from the leaves. Then the leaves sit (*Desmodium triquetrum*) and beluntas leaves (*Pluchea indica*) are cut into small sizes to increase the surface area so that the extraction process is easier to do, then dry them by aerating or not exposing them to direct sunlight at room temperature. After that,

the leaves sit (*Desmodium triquetrum*) and beluntas leaves (*Pluchea indica*) are blended to become powder. As much as 100 grams of dry sat leaf powder (*Desmodium triquetrum*) and beluntas leaves (*Pluchea indica*) were collected into an Erlenmeyer tube and then mixed with 500 mL of 96% ethanol solvent, stirred in a shaker for 9 hours until homogeneous. Then it was macerated for 36 hours. The filtrate obtained was filtered using Whatman paper no. 41, concentrated with a rotary evaporator at 40°C until a thick extract is formed. The viscous ethanol extract obtained was then fractionated using the liquid-liquid extraction method, using ethyl acetate, n-hexane and water as solvents.

2.2 Data analysis

Data analysis was carried out by testing the antioxidant activity with the DPPH method compared to ascorbic acid. The DPPH radical is an unstable nitrogen-containing organic compound with a strong absorbance at λmax 517 nm and a dark purple color. After reacting with antioxidant compounds, the DPPH will be reduced and the color will change to yellow. These changes can be measured with a spectrophotometer. The decrease in color intensity that occurs is caused by a decrease in conjugated double bonds in DPPH. This can happen if there is an capture of one electron by an antioxidant, causing no chance for the electron to resonate. The discoloration of DPPH occurs due to the presence of compounds that can provide hydrogen radicals to DPPH radicals so that they are reduced to DPPH-H (1,1-diphenyl-2-pikrylhidrazyl). The reduction of DPPH to DPPH-H is due to the presence of hydrogen donors from hydroxyl compounds (19,20).

Antioxidants in the process will donate hydrogen atoms to DPPH radicals and cause DPPH color changes from purple to yellow. The effectiveness of a sample to counteract free radicals from the DPPH method is named IC_{50}. The definition of IC_{50} is the concentration that can reduce 50% of DPPH free radicals. The smaller the IC_{50} value, the greater the antioxidant ability. A compound is said to have very strong group antioxidant activity if the IC_{50} value is less than 50 ppm, the IC_{50} strong group is between 50-100 ppm, the moderate group if the IC_{50} value is 101-150 ppm, and the weak group if the IC_{50} value is between 150-200 ppm (21,22,23).

To determine the effect of different solvents on Sitting Leaf Tea (*Desmodium triquetrum*), Beluntas Leaf (*Pluchea indica*) and a Combination of Both on the value of antioxidant activity, a one-way ANOVA statistical test was carried out.

3. Results

The results of the antioxidant activity test obtained can be seen in Table 1 below:

Table 1. Antioxidant Activity, IC_{50}, Average and Standard Deviation of Sitting Leaf (*Desmodium triquetrum*) Extract

Sample Extract	**Testing**	**IC_{50} (ppm)**	**Mean**	**Standar Deviasion**
1.Ethanol	**1**	**1.08**	**1.10**	**0.13**
	2	1.24		
	3	0.98		
3. Ethyl acetate	1	13.32	12.30	0.88
	2	11.82		
	3	11.75		

Sample Extract	**Testing**	**IC_{50} (ppm)**	**Mean**	**Standar Deviasion**
4. Water	1	13.32	12.30	0.88
	2	11.82		
	3	11.75		
2.N-hexane	1	16.02	15.79	0.90
	2	16.56		
	3	14.80		
5. Ascorbic acid	1	4.50	4.12	0.38
	2	3.75		
	3	4.11		

Table 2. Antioxidant Activity, IC_{50}, Average and Standard Deviation of Beluntas leaf *(Pluchea indica)* Extract

Sample Extract	**Testing**	**IC_{50} (ppm)**	**Mean**	**Standar deviation**
6. Ethanol	1	8.47	8.36	0.40
	2	8.68		
	3	7.91		
7. N-hexane	1	103.95	101.44	5.29
	2	95.36		
	3	105.01		
8. Ethyl acetate	**1**	**3.12**	**3.09**	**0.22**
	2	3.29		
	3	2.86		
9. Water	1	405.92	394.90	10.31
	2	393.28		
	3	385.50		

Table 3. Antioxidant Activity, IC_{50}, Mean and Standard Deviation of **Combination** Sitting Leaf (*Desmodium triquetrum*) Extract and Beluntas Leaf *(Pluchea indica)* Extract

Sample Extract	Testing	IC_{50} (ppm)	Mean	Standar Deviation
10. Ethanol	**1**	**6.83**	**7.81**	**0.94**

	2	7.90		
	3	8.71		
11. N-hexane	1	30.48	29.88	0.52
	2	29.59		
	3	29.58		
	1	9.08	**8.84**	0.41
12. Ethyl acetate	2	8.36		
	3	9.07		
	1	15.02	14.03	0.89
13. Water	2	13.31		
	3	13.75		

Table 4. Antioxidant Activity, IC_{50}, Mean and Standard Deviation of Sitting Leaf (*Desmodium triquetrum*) Extract and Beluntas Leaf *(Pluchea indica)* Extract and Combination of both leaves

Leaf	IC_{50} dan SD			
	Ehtanol	N-Hexcane	Ethyl acetic	Water
Sitting	**1,10 ± 0,13**	15,79 ± 0,90	15,89±2,44	12,30 ± 0,88
Beluntas	8,36 ± 0,40	101,44±5,29	**3.09 ± 0,22**	394,90 ± 10,31
Combination Sitting and Bluntas	**7,81 ± 0,94**	29,88 ± 0,52	8,84 ± 0,41	14,03 ± 0,89

Based on Table 4, above, it can be seen that the antioxidant activity, the IC_{50} value of the leaves Sitting (*Desmodium triquetrum*), are **1.10**, 12.30 , 15.79 and 15.89 ppm in ethanol, water, n-hexane and ethyl acetate solvents respectively.

Antioxidant activity, IC_{50} value of beluntas (*Pluchea indica*) leaves, were **3.09**, 8.36, 101.44 and 394.90 in ethyl acetate, ethanol, n-hexane and water, respectively.

The combined antioxidant activity of Sitting leaves (*Desmodium triquetrum*) and Beluntas leaves (*Pluchea indica*) with IC_{50} values were **7.81**, 8.84, 14.03 and 29.88 ppm in ethanol, ethyl acetate, water and n-hexane solvents respectively participate.

Furthermore, the phytochemical test was determined on the extracts of the leaves sit (*Desmodium triquetrum*) and beluntas leaves (*Pluchea indica* L.) which includes examination of phenolics, flavonoids, tannins and alkaloids. Qualitative test by looking at the color change ((Maryono et al., 2015), in both plants is the method used to identify the chemical content contained in the leaves sit (*Desmodium triquetrum*) and beluntas leaves (*Pluchea indica* L.).

Based on the phytochemical test, the phytochemical results were obtained on the leaves of Sitting (*Desmodium triquetrum*) which showed positive results for the presence of phenolic compounds, flavonoids, tannins and alkaloids, in contrast to beluntas leaves (*Pluchea indica* L.) which showed negative results for

alkaloids, but positive for phenolics. flavonoids, and tannins. So, there is a possibility that the phytochemical substances present in the leaves of sit (*Desmodium triquetrum*) and beluntas leaves (*Pluchea indica* L.) are the same except for the alkaloid content.

4. Discussion

The results of testing the antioxidant activity of the various solvents tested (Tables 1, 2 & 3) show that the ethanol extract of the leaves Sit (*Desmodium triquetrum*) has a very strong antioxidant activity. These results indicate that the ethanol extract of the leaves of the sit (*Desmodium triquetrum*) has the best IC_{50} value with an average of 1.10 ppm. Furthermore, the ethyl acetate extract sample of beluntas leaves (*Pluchea indica*) which has the second best IC_{50} value with an average of 3.09 ppm. The extract samples from the combination of the leaves sit (*Desmodium triquetrum*) and beluntas leaves (*Pluchea indica*) have the third best IC_{50} value with an average of 7.81 ppm.

The antioxidant activity of the ethanol extract of the leaves of the sit (*Desmodium triquetrum*) has an IC_{50} of 1.10 ppm higher than standard ascorbic acid, with an average IC_{50} value of 4.12 ppm. The height where the cultivation grows can affect the growth, production and yield quality of leaf sitting plants. Planting at an altitude of 450 m asl (below sea level) produced higher levels of extracts and flavonoids, while higher leaf production was produced at planting at an altitude of 200 m asl. Planting at an altitude of 1200 m asl only increases plant height growth (24,25). Geyin Zhang's 2020 research on polyphenols from ultrasonic extraction of *Desmodium triquetrum* (L.) DC. strengthens the value of antioxidant activity. The results show that the secondary metabolites contained in black tea beluntas leaves in various proportions are alkaloids, flavonoids, phenolics, saponins, tannins, and cardiac glycosides (26).

In contrast to the results of the study by Defitiana Wanita, *et al* in 2018 using beluntas leaves (*Pluchea indica*) with ethanol extract to get an IC_{50} value of 37.25 ppm (27), but the antioxidant activity of the extract is also relatively strong because of its IC_{50} price less than 50 ppm each. The results of Paini Sri Widyawati's research in 2012 explained that methanol extract from beluntas leaves has the potential as an antioxidant, has the highest total phenolic content (28). The results of Paini Sri Widyawati's research in 2018 were that the secondary metabolites contained in black tea beluntas leaves in various proportions were alkaloids, flavonoids, phenolics, saponins, tannins and cardiac glycosides (2). Moch Hamdan's research results in 2022 show the results of phytochemical screening of ethanol extract of young and old beluntas leaves as follows: alkaloid, flavonoid, tannin and saponin compounds. Antioxidant research using the DPPH method shows that old beluntas leaves has very strong antioxidant activity with an IC_{50} value of 10.14 μg/mL (29). Khowas' research in 2021 showed that the antioxidant activity value (IC_{50}) of the ethanol extract of beluntas leaves was 18.24 ppm, the ethyl acetate extract of beluntas leaves was 44.91 ppm, and the n-hexane extract of beluntas leaves was 35.35 ppm. The results of the phytochemical test of beluntas leaves showed the presence of flavonoids, saponins, tannins, triterpenoids and steroids (30). The results of research by Nur Haryati et al in 2023 showed that the free antiradical activity of ethanolic extract of beluntas leaves originating from the Margoyoso lowlands and the Colo highlands was 127,811 and 71,561 ppm. The difference in results was influenced by altitude, soil pH, sun exposure and habitat. The anti-free radical activity of ethanolic extract of beluntas leaves from Margoyoso is in the medium category and that from Colo is in the strong category (31). Polyphenols are extracted below better conditions, ultrasonic extraction process simple, environmentally friendly and efficient, and obtainable *Desmodium triquetrum* (L.) DC. polyphenols can used as a natural antioxidant in food, which has certain properties development prospects. Susilowati's research results in 2019 showed that the antioxidant activity of the ethyl acetate fraction of bay leaves has the potential to be very strong with an IC_{50} value of 47.7709 ppm and the water fraction of bay leaves has the potential to be strong with an IC_{50} value of 52.3957 ppm (32). This is different from the ethyl acetate extract sample of beluntas leaves (*Pluchea indica*) in this study which had a lower IC_{50} value of 3.09 ppm, which means that the antioxidant activity was stronger.

This difference can be caused by the sampling location. Where is the sampling location for the Defitiana Wanita, Rusmini, Finna Ashfia, and Fidelia Yustisia Adriane 2018 research in Rungkut District, Surabaya City, while in this study the samples were taken in Bekasi, West Java, so that it can affect the content of secondary metabolites, depending on: formulation/composition culture media, physical factors (temperature, light, humidity), genetic factors (cell genotype), and environmental stress factors (heavy metals, UV light).

The results of the ANOVA test on the various solvents used and the samples showed a value ($p<0.01$) which indicated a significant difference between the solvents used and the resulting antioxidant activity values.

Based on the phytochemical test, the phytochemical results were obtained on the leaves of Sitting (*Desmodium triquetrum*) which showed positive results for the presence of phenolic compounds, flavonoids, tannins and alkaloids, in contrast to beluntas leaves (*Pluchea indica L.*) which showed negative results for alkaloids, but positive for phenolics, flavonoids, and tannins. So, there is a possibility that the phytochemical substances present in the leaves of sit (*Desmodium triquetrum*) and beluntas leaves (*Pluchea indica L.*) are the same except for the alkaloid content (33,34). Flavonoid secondary metabolites are polyphenolic compounds that are important for human health because of their diverse pharmacological activities, ranging from free radical scavengers that function as antioxidants, to their potential as agents against various diseases (11,12,13,35,36). The role of flavonoids in humans to fight various diseases is important because many diseases are caused by free radicals that trigger oxidative stress and damage cellular components or interfere with their function. These compounds not only protect cells and cellular components from oxidative damage but also reduce the risk of oxidative stress associated with various degenerative diseases (15,37).

5. Conclusion

The antioxidant activity of tea from the sit leaves, beluntas leaves and the combination of both belongs to a very strong category. All three have the ability as very strong antioxidant compounds that can counteract free radicals. The results of the ANOVA test on the various solvents used and the samples showed a value ($p <0.01$) indicating that there was a significant difference. The phytochemical content of the leaves of sit (*Desmodium triquetrum*) is phenolic, flavonoids and the phytochemical content of beluntas leaves (*Pluchea indica*) is phenolic and flavonoid in all extracts. These secondary metabolites are compounds that have antioxidant capabilities.

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