

Research Article

DPPH Scavenging Activities and Phytochemical Content of Four Asteraceae Plants

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ABSTRACT

Free radicals are atoms or molecules that have one or more unpaired electrons on its outer orbital, highly reactive, and could damage cell inside human body. Human body produce antioxidant to neutralize free radicals, but human ageing and stress oxidative conditions would increase the formation of free radicals, therefore an exogenous antioxidant are needed. Asteraceae family is the largest family among the plant kingdom therefore it has great potential as source of exogeneous antioxidants. The objectives of this research were to determine antioxidant activities of aerial part of elephant's foot (*Elephantopus scaber* L.), false daisy (*Eclipta alba* (L.) Hassk.), Indian pluchea (*Pluchea indica* (L.) Less), and dandelion (*Taraxacum officinale* Weber ex F.H Wigg.) using DPPH method, determine total flavonoid and total phenolic content, and analyze correlation between total flavonoid content and total phenolic content with antioxidant activity. Extraction was carried out by reflux with increasing polarity using n-hexane, ethyl acetate, and ethanol respectively. Antioxidant activity was determined by DPPH method. Total flavonoid content was determined using Chang's method and total phenolic content evaluated using Folin—Ciocalteu reagent. Correlation of total flavonoid content and total phenolic content was analyzed by Pearson's method. Ethanolic extract of Indian pluchea showed the highest antioxidant activity with IC₅₀ DPPH 16.66 ± 0.08 µg/mL. The highest total phenolic content (23.49 ± 0.56 g QE (Quercetin Equivalent)/100 g) was given by ethyl acetate extract of Indian pluchea, while the highest flavonoid content (16.48 ± 0.25 g GAE (Gallic Acid Equivalent)/100 g) was showed by ethanolic extract of Indian pluchea. Total phenolic content of elephant's foot, false daisy and Indian pluchea herbs extracts showed significantly negative correlation with their IC₅₀ of DPPH scavenging activities. Indian pluchea herbs extract had the highest antioxidant activity using DPPH method compared to elephant's foot, false daisy and dandelion herbs. Phenolic compounds were the major contributor in antioxidant activities of elephant's foot, false daisy and dandelion herbs extracts by DPPH method.

Keywords: Antioxidant, DPPH, Asteraceae, *Pluchea indica*.

INTRODUCTION

Free radicals are atoms or molecules that have one or more unpaired electrons on its outer orbital, highly reactive, and could damage cell inside human body. Human body produce antioxidant to neutralize free radicals, but human ageing and stress oxidative conditions would increase the formation of free radicals, therefore an exogenous antioxidant are needed¹. Asteraceae is the largest family among the plant kingdom, therefore it has a great potential as source of exogenous antioxidant. Elephant's foot (*Elephantopus scaber* L.), false daisy (*Eclipta alba* (L.) Hassk.), Indian pluchea (*Pluchea indica* (L.) Less.), and dandelion (*Taraxacum officinale* Weber ex F.H Wigg) are some Asteraceae plants that thrives widely in Indonesia. Previous research showed that ethanolic and methanolic extract of *Pluchea indica* gave an antioxidant activity with DPPH scavenging method^{2,3}. Methanolic extract of *Eclipta alba* showed antioxidant activity with DPPH scavenging method^{4,5,6}, nitric oxide radical scavenging assay⁶, lipid peroxidation inhibitory

activity⁶, hydrogen peroxide scavenging assay⁶, and in rat liver⁷. Ethanolic extract of *Eclipta alba* showed antioxidant activity in DPPH scavenging⁸ and other several methods⁹. Ethanolic extract of *Elephantopus scaber* showed antioxidant activity in superoxide scavenging activity, lipid peroxidase inhibition, and hydroxyl radical scavenging methods¹⁰. Methanolic extract of *Taraxacum officinale* showed antioxidant activity with DPPH scavenging¹¹ and FRAP methods^{11,12}. There was no research regarding comparison of antioxidant activity using *Elephantopus scaber*, *Eclipta alba*, *Pluchea indica*, and *Taraxacum officinale* herbs which was extracted by increasing polarity solvent.

MATERIAL AND METHODS

Materials

DPPH (2,2- diphenyl - 1 picrylhydrazyl), gallic acid, quercetin, ascorbic acid were purchased from Sigma-Aldrich (MO, USA), *Elephantopus scaber*, *Eclipta alba*, *Pluchea indica*, *Taraxacum officinale*, methanol,

ethanol, ethyl acetate, n-hexane, and all other analytical materials used in the study.

Sample Preparations

Elephantopus scaber, *Eclipta alba*, *Pluchea indica*, and *Taraxacum officinale* herbs were freshly collected from Lembang, West Java – Indonesia and determined in Herbarium Bandungense, School of Life Sciences and Technology, Bandung Institute of Technology. All samples were sorted, washed, dried at 40⁰-50⁰ C and grinded into powder.

Extraction

All samples were extracted by reflux, using increasing polarity solvents which were n-hexane, ethyl acetate, ethanol respectively. All extraction was performed in triplicate. All extracts were concentrated by rotary evaporator resulted n-hexane, ethyl acetate, and ethanol extracts of *Elephantopus scaber* (ES1), (ES2), (ES3), *Eclipta alba* (EA1), (EA2), (EA3), *Pluchea indica* (PI1), (PI2), (PI3) and *Taraxacum officinale* (TO1), (TO2), (TO3).

Total flavonoid content

Total flavonoid content was conducted by Chang *et al.* method¹³. Quercetin was used as standard. Aquadest 2.8 ml was added with 1.5 ml methanol, 0.1 ml aluminium chloride 10%, 0.1 ml sodium acetate 1 M and 0.5 ml extract in methanol. The mixture was further incubated for 30 minutes in room temperature. Absorbance was measured by UV-visible spectrophotometry at λ 415 nm.

Total phenolic content

Total phenolic content determination was done using Folin-Ciocalteu reagent. Gallic acid was used as standard. Each extract was dissolved in methanol then 0.5 ml extract was added by 5 ml Folin-Ciocalteu reagent and 4 ml sodium carbonate 1 M, then incubated for 15 minutes in room temperature. Absorbance was measured by UV-visible spectrophotometry at λ 765 nm¹⁴.

Antioxidant Activity

Antioxidant activity was done by Molyneux method¹⁵. Methanol was used as blank, DPPH 50 μ g/mL as control and ascorbic acid as standard. Two ml of standard or sample was prepared in various concentration then added into 2 ml DPPH 50 μ g/ml solution and incubated for 30 min before measured by UV-visible spectrophotometry at λ 515 nm and performed triplicate. IC₅₀ of sample or standard was calculated by DPPH scavenging activity calibration curve¹⁶.

Statistical Analysis

Each analysis was performed triplicate and data processing was done by SPSS software. The result presented as mean \pm deviation standard using ANOVA and Tukey post-hoc ($p < 0.05$). Correlation analysis between flavonoid content and phenolic content with antioxidant activity was conducted by Pearson's method¹⁷.

RESULT

Extraction

All dried samples were extracted by reflux. Each sample was extracted with n-hexane then the residue was dried and further extracted with ethyl acetate and the residue was dried and lastly extracted by ethanol. All extraction process was performed in triplicate. Extracts were concentrated by rotary evaporator. Yield of each extract was determined and could be seen in Table 1.

Total flavonoid content

Total flavonoid content of all extract could be seen in Fig 1. The results were varied from 0.83 to 23.49 g QE/100 g. The total flavonoid content of all 12 extract were obtained by using standard calibration curve of quercetin $y = 0.0094x - 0.1573$, $R^2 = 0.9987$.

Total phenolic content

Total phenolic content of all extract could be seen in Fig 2. The results were varied from 3.19 to 16.48 g GAE/100 g. The total phenolic content of all 12 extracts were obtained by using standard calibration curve of gallic acid $y = 0.0051x - 0.1662$, $R^2 = 0.9971$.

Antioxidant Activity

IC₅₀ of DPPH scavenging activities of 12 extracts could be seen in Fig 3. The results were widely varied, ranging from 16.66 to 1109.17 μ g/ml, while IC₅₀ of DPPH ascorbic acid as standard was 1.83 μ g/ml

Correlation of total flavonoid and phenolic content with antioxidant activity

Correlation of total flavonoid and phenolic content with their IC₅₀ of DPPH scavenging activities showed that total phenolic content of *Elephantopus scaber*, *Eclipta alba*, and *Pluchea indica* gave a negative and significant correlation with their IC₅₀ of DPPH scavenging activities, while total flavonoid content showed no significant correlation with their IC₅₀ of DPPH scavenging activities.

DISCUSSION

Extraction with increasing polarity solvent was done to achieve optimum yield of all polarity (non polar, semi polar, and polar) extracts. Firstly extraction was carried out with n-hexane to extract non polar compounds. The second extraction was conducted using ethyl acetate to the residue which extracted semi polar compounds. The last extraction was performed with ethanol to the residue which extracted polar compounds. The yield of the extracts could be seen in Table 1. Ethanolic extract of *Taraxacum officinale* (TO3) showed the highest yield with 13.26%. Ethanolic extract showed the highest yield of all plants compared to n-hexane and ethyl acetate, could be indicate that majority compound of all plants were polar compounds.

Total flavonoid content in ethyl acetate herbs extract of *Pluchea inidica* (PI2) was the highest with 23.49 g QE/100 g followed by n-hexane herbs extract of *Pluchea indica* (PI1), ethyl acetate herbs extract of *Eclipta alba* (EA2), and ethyl acetate herbs extract of *Elephantopus scaber* (ES2) (16.58, 16.02, 12.02 g QE/100 g, respectively). AlCl₃ will form a complex with the OH of flavonoid compounds at C3'-C4', and or OH at C-3 and 4 oxo, and or OH at C-5 and 4 oxo¹⁸. It

Table 1: Yield of various extracts.

Extract	Yield (% w/w)			
	<i>Elephantopus scaber</i>	<i>Eclipta alba</i>	<i>Pluchea indica</i>	<i>Taraxacum officinale</i>
n-Hexane	3.20	1.45	3.26	2.55
Ethyl acetate	3.11	2.80	2.23	2.27
Ethanol	7.70	8.92	11.31	13.26

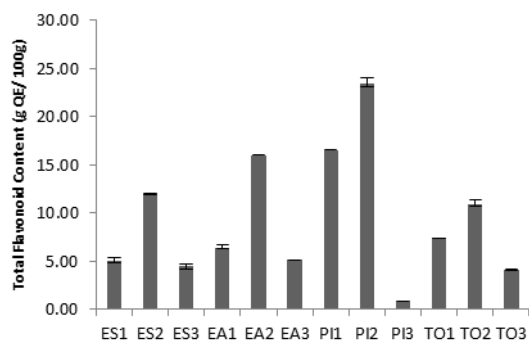


Figure 1: Total flavonoid content of all samples.

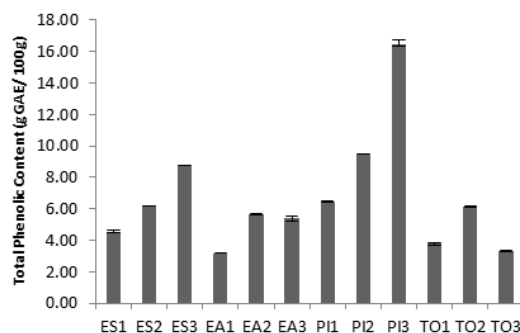


Figure 2: Total phenolic content of all samples.

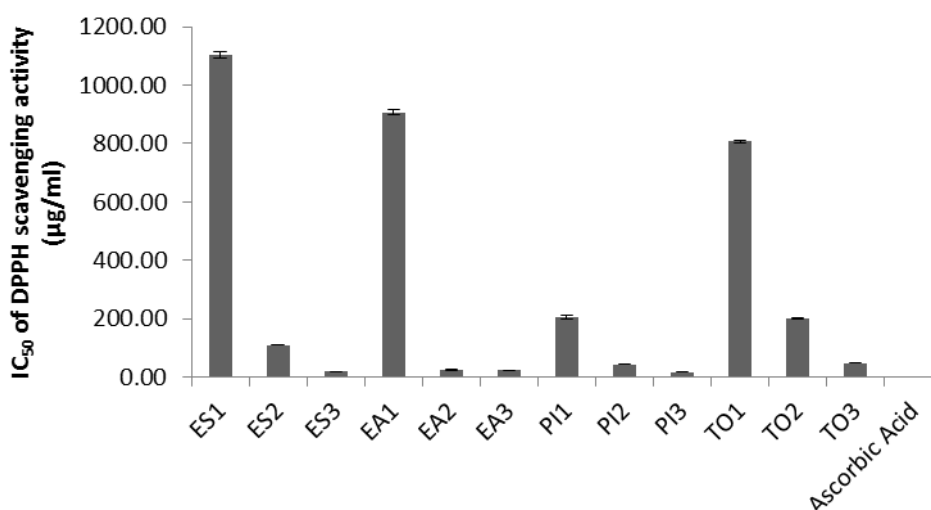


Figure 3: IC₅₀ of DPPH scavenging activity of all samples and standard.

g) compared to the other extracts, followed by PI2, ethanolic herbs extract of *Elephantopus scaber* (ES3)

Table 2: Correlation of total flavonoid and phenolic content with IC₅₀ DPPH scavenging activity.

Antioxidant Parameter	Pearson's Correlation Coefficient (r)	
	Total Flavonoid	Total Phenolic
IC ₅₀ DPPH <i>Elephantopus scaber</i>	-0.079 ^{ns}	-0.952**
IC ₅₀ DPPH <i>Eclipta alba</i>	-0.402 ^{ns}	-0.992**
IC ₅₀ DPPH <i>Pluchea indica</i>	0.353 ^{ns}	-0.819**
IC ₅₀ DPPH <i>Taraxacum officinale</i>	0.166 ^{ns}	-0.180 ^{ns}

ns: not significant, **: significant at p<0.01

can be inferred that many flavonoid compounds was semipolar flavonoid which soluble in ethyl acetate extract and less in ethanolic extract, which indicated the polarity range of flavonoid in these plants. This flavonoid might have some hydroxyl (-OH) and methyl (-CH₃) substitution.

Total phenolic compound in ethanolic herbs extract of *Pluchea indica* (PI3) was the highest (16.48 g GAE/100

and PI1 (9.48, 8.74, 6.48 g GAE/100 g, respectively). Unlike the flavonoids, phenolic compound was highest in ethanolic extracts compared to n-hexane and ethyl acetate except for *Taraxacum officinale* which the ethyl acetate extract contain higher phenolic compound than ethanolic extract. Phenolic compound are well known as polar compound, therefore it's likely to be extracted in polar solvent but not all phenolic are polar

compound. Any compound with hydroxyl substitution at benzene structure is considered as phenolic compound, and in the same structure there might be another substitution such as methyl (-CH₃) that can decrease polarity of a compound. This reason could explain how total phenolic compound of TO2 is higher than TO3.

Antioxidant activity was done by DPPH scavenging method. DPPH is a stable radical, which in its radical form gives a violet color¹⁵. Antioxidant will react with DPPH by electron donate mechanism, which stabilized DPPH was demonstrated by decreasing intensity of DPPH's violet color and slowly turns into yellow and this decrease could be measured by visible spectrophotometry at λ 515nm¹⁹. The highest antioxidant activity was showed by PI3 with IC₅₀ DPPH scavenging activity of 16.66 μ g/ml followed by ethanolic herbs extract of *Elephantopus scaber* (ES3) 18.43 μ g/ml, ethanolic herbs extract of *Eclipta alba* (EA3) 23.79 μ g/ml, and ethyl acetate herbs extract of *Eclipta alba* (EA2) 24.33 μ g/ml. The data in Fig 3 indicated that ethanolic extract of each plant gave the highest antioxidant activities compared to n-hexane and ethyl acetate extracts.

Pearson's correlation was performed to see the correlation of total flavonoid and phenolic content with their antioxidant activities. The result in Table 2 showed that total phenolic content in *Elephantopus scaber*, *Eclipta alba*, and *Pluchea indica* herbs extracts had significantly negative correlation with their IC₅₀ DPPH scavenging activities. Negative correlation means the higher value of total flavonoid/total phenolic content will give lower IC₅₀ DPPH scavenging activity value. The lower IC₅₀ value means stronger antioxidant activity. No significant correlation of total flavonoid with their IC₅₀ DPPH value can be explained as not all flavonoid give a strong antioxidant property. Flavonoid with di -OH substitution at C3' and C4' gives the strongest antioxidant properties, followed by C2-C3 double bond, and -OH substitution at C3. As showed in total flavonoid content, majority of flavonoids were extracted in ethyl acetate which might be lacked of di -OH substitution at C3' and C4'²⁰. The other reason due to methoxylation at C3' and C4' will reduce antioxidant properties of flavonoids. In contrast, total phenolic content in three of four Asteraceae plants had significantly negative correlation with their IC₅₀ DPPH scavenging activities. Phenolic compounds are well known as a good scavenger because of its ability to donate electrons. In case of *Taraxacum officinale* whose not give significant correlation, most likely because the phenolic compounds of this plant might be a weak antioxidant. From the Fig. 2, majority of phenolic compounds of *Taraxacum officinale* were extracted by ethyl acetate which mean majority of phenolic compounds were semipolar and most likely only give a weak antioxidant properties.

This correlation results could explain that the highest antioxidant activity was given by PI3 which had the highest phenolic content and lowest flavonoid content,

therefore phenolic compounds were clearly the major contributors of antioxidant activity. It was similar to EA3 and EA2, thus confirmed phenolic compounds as major contributors of their antioxidant activity.

Overall, *Pluchea indica* showed the greatest antioxidant activity among others. Srimoon and Ngiewthaisong² previously reported that dried stem part of *Pluchea indica* showed the highest antioxidant activity and also had the highest total phenolic content among other parts of plants, while Noridayu *et al.*²¹ exposed that methanolic leaves extract of *Pluchea indica* showed higher antioxidant and total phenolic compound than metanolic stem extract. Widyawati *et al.*³ presented that antioxidant activities of *Pluchea indica* leaves which was extracted by gradient polarity solvent from n-hexane to water exhibited that methanolic extract showed the highest total phenolic content, total flavonoid content, and also the highest antioxidant activity with DPPH scavenging method slightly better than water and ethanolic extracts. It was similar to the present study, ethanolic extract of *Pluchea indica* had the greatest antioxidant activity of all extracts, the ethyl acetate extract was the second after *Eclipta alba* among other ethyl acetate extracts, and n-hexane extract was the greatest among other n-hexane extracts from other plants.

CONCLUSION

Ethanolic extract of *Pluchea indica* herbs (PI3) showed the highest antioxidant activity with IC₅₀ DPPH scavenging activity of 16.66 μ g/ml. The highest total flavonoid content was given by ethyl acetate herbs extract of *Pluchea indica* (PI2) 23.49 g QE/ 100 g and highest total phenolic content was given by ethanolic herbs extract of *Pluchea indica* (PI3) 16.48 g GAE/100 g. Total phenolic content in *Elephantopus scaber*, *Eclipta alba*, and *Pluchea indica* herbs extracts gave significantly negative correlation with their IC₅₀ DPPH scavenging activities.

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