IN VITRO ANTIOXIDANT CAPACITIES AND ANTIDIABETIC PROPERTIES OF PLUCHEA LEAVES AND GREEN TEA MIXTURES AT VARIOUS PROPORTIONS

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ABSTRACT

Objective: This study was done to determine the effect of pluchea leaves and green tea mixtures at various proportions to antioxidant and antidiabetic properties.

Methods: The research used a single factor randomized block design. Research factors were pluchea leaf and green tea mixture proportions, including 0:100; 75:25; 50:50; 25:75; and 100:0% (w/w), respectively. Each of factors was repeated five times. The parameters observed in this study were the total phenols (TPC) and total flavonoids (TFC) content, free radical DPPH scavenging activity, iron ion reducing power, and α-amylase and α-glucosidase inhibition activities. The data analysis showed a significant analyzed using analysis of variance (ANOVA) at effect then it was continued with duncan’s multiple range test (DMRT).

Results: The phytochemical compounds containing in pluchea leaves and green tea mixtures were alkaloids, flavonoids, phenolics, tannins, triterpenoids, steroids, and cardiac glycosides. The qualitative assay of phytochemical compounds from aqueous extract had a different pattern with total phenolic content (TPC) and total flavonoid content (TFC), especially at 75:25; 50:50 and 25:75 % (w/w) from pluchea leaf and green tea mixture proportions. The 50:50% (w/w) proportions of samples had the highest TPC and TFC. The data showed that there was an interaction between bioactive compounds of pluchea leaves and green tea. TPC and TFC were correlated with free radical DPPH scavenging activity, iron ion reducing power, and α-glucosidase inhibition activity, but the α-amylase inhibition activity had the different graph pattern with the TPC and TFC.

Conclusion: The antioxidant capacity of pluchea leaves and green tea mixtures at various proportions had the graph pattern similar to the α-glucosidase inhibition activity and different with the α-amylase inhibition activity.

Keywords: Pluchea leaves, Green tea, Antioxidant, Anti-diabetic

INTRODUCTION

Pluchea leaves (Pluchea indica Less) and green tea (Camellia sinensis (L) O. Kuntze) are two commodities that are potentially as antioxidant and antidiabetic sources. These are related to phytochemical contents of these plants.

Pluchea indica Less are a herb plant from Asteraceae family that are potentially as antioxidant, especially DPPH free radical scavenging activity, iron reducing power, reactive oxygen species scavenging activity, and β-carotene linoleic acid system inhibition activity [1-3]. Pluchea indica has anti-inflammatory [5], anti-diabetic [6]. Pluchea leaves contain the phytochemical compounds, such as flavonoids, sterols, saponins, tannins, alkaloids, and cardiac glycosides [1, 2, 6, 7].

Green tea contains a major active group of tea leaves’ components (up to 30% of the dry weight of the water-extractable material) [8-10]. They are comprised on epigallocatechin gallate (23.4–112.4 mg/ml) as the major component, followed by epicatechin (184–789 mg/ml), epicatechin gallate (5.6–29.6 mg/ml), epicatechin (4.6–14.5 mg/ml) and catechin (32–8.2 mg/ml) [11]. The catechins effectively scavenge oxi-radicals involved in the pathogenesis of many chronic diseases, including cardiovascular diseases, cancer, neurodegenerative diseases, and ageing [12], antioxidant and prebiotic activities [13], anticancer [9], anti-diabetic [14], anti-inflammatory and analgesic effect [15].

Pluchea leaves and green tea are usually used as a health beverage. Many people generally utilize this commodity as herbal tea that is packed in tea bag packaging. The potency of each commodity as antioxidant and anti-diabetic has been determined [4,5,13,14]. Therefore it is necessary to know the potency of pluchea leaves and green tea mixtures as antioxidant and antidiabetic activities. The pluchea leaves and green tea mixtures can allow the interaction among bioactive compounds that can give synergism or antagonism effects of antioxidant and antidiabetic activities. Until now this interaction is not fully understood. Therefore it is necessary to investigate the effect of a mixture of them at various proportions to antioxidant and antidiabetic activities. This study was done to determine the effect of pluchea leaves and green tea mixtures at various proportions to antioxidant and antidiabetic properties.

MATERIALS AND METHODS

Chemicals and reagents

Pluchea leaves were harvested and collected from a pluchea garden in Manggrove areas, Wonorejo, Rungkut, Surabaya, East Java, a city in the middle of Indonesia in Java Island. The plant was authenticated in the Herbarium of Biology and Food Industry Microbiology Laboratory at the department of Food Technology, Agricultural Technology Faculty, the Widyadarma Catholic University of Surabaya with voucher specimen no FTP-UKWMS-0001 for future reference.

Dried green tea was purchased by PT. Rosas Nusantara Mandiri Surabaya, East Java, Indonesia. Methanol, ferric (II) chloride, potassium ferricyanide, sodium carbonate, ethanol, petroleum ether, copper sulphate, Folin Ciocalteus’s phenol, aluminium chloride, sodium nitrate, sodium hydroxide, amyl alcohol, chloroform, ammonium hydroxide, sulphuric acid, mercury chloride, potassium iodide, iodine, magnesium, acetic acid, chloride acid, ether, chloroacetic acid, sodium dihydrogen phosphate, dihydrogen phosphate, sodium acetate, and starch were purchased from Merck Company (Darmstadt, Germany).
Phytochemical analysis

Phytochemicals are primary and secondary natural constituents occurring in the medical plants, leaves, vegetables and roots that contain the phytochemical constituents can be used to treat diabetes if they have good antioxidant and antiadipic properties. Dastjerdi et al. [25] said that the phytochemical constituents can be used to treat diabetes if they have good antioxidant and antiadipic properties.

**RESULTS AND DISCUSSION**

**Phytochemical analysis**

Phytochemicals are primary and secondary natural constituents occurring in the medical plants, leaves, vegetables and roots that contain the phytochemical constituents can be used to treat diabetes if they have good antioxidant and antiadipic properties. Dastjerdi et al. [25] said that the phytochemical constituents can be used to treat diabetes if they have good antioxidant and antiadipic properties.
[26] and Nanumala et al. [27] said that the capacity to inhibit alpha amylase and alpha glucosidase enzymes are a result of the existence of various phytochemicals like flavonoids, tannins, saponins, anthraquinones, sterols, phlobatannins, terpenoids and polyphenols.

Total phenol content

Total phenol content (TPC) in the water extract from pluchea leaves and green tea mixtures at various proportions was shown in fig. 1. Generally, the TPC of pluchea leaves water extract was significantly (p<0.05) higher compared to the TPC of green tea water extract. This condition was similar to observation of the phytochemical assay, however, there were different phenomena that were observed of samples at 75:25, 50:50, and 25:75 proportions, respectively. These differences may be indicated by the differences in solved phenolic compounds and interaction between phenolic compounds from pluchea leaves and green tea mixtures. These phenomena determined structure of phenolic compounds, such as free or bound phenolics. Many studies have informed that phenolic compounds of fruits and vegetables are mainly inhibit free radicals and block their chain reactions [32-34].

The effectiveness of TPC assay is depended on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenolic compounds [31].

Green tea comprises phenolic compounds, especially catechin [8-11], however, pluchea leaves contain phenolic acids i.e. chlorogenic acid and caffeic acid [4] and flavonoid compounds i.e. kaempherol, myricetin, and quercetin [32]. Natural properties of phenolic compounds have biological and pharmacological properties, such as antioxidant, anticancer, anti-aging, and anti-inflammatory activities [33,34]. Phenolic compounds have been used as antioxidants and usually in free structure higher than in bound structure [29,30]. There was a free form of phenolics of pluchea leaves and green tea in aqueous media showed that there was hydroxyl group free. The functional group can interact to form bound phenolics or hydrogen bond so that interferes with the reaction among hydroxyl group of phenolic compounds, turgent and molybdenum ions of Folin Ciocalteu’s reagent.

Table 1: The phytochemical content of pluchea leaves and green tea mixture at various proportions

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Pluchea leaves and green tea mixtures (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100:0</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++++</td>
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<tr>
<td>Phenolics</td>
<td>++++</td>
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<tr>
<td>Saponins</td>
<td>++++</td>
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<tr>
<td>Tannins</td>
<td>++++</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>++++</td>
</tr>
<tr>
<td>Sterols</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>++++</td>
</tr>
</tbody>
</table>

Note: ++ indicates presence of phytochemicals and = indicates absence of phytochemicals, ++++= shows high concentration, ++= shows low concentration.

Fig. 1: Total phenolic content of aqueous extract of pluchea leaves and green tea mixtures at various proportions (n=5, Values expressed as mean±SD. Data were analyzed by one way analysis of variance (ANOVA) followed by duncan’s multiple range test (DMRT))

Total flavonoid content

Total flavonoid content (TFC) was determined by aluminum chloride method that was based on the formation of a complex between the aluminum ion, Al (III), and the carbonyl and hydroxyl groups of flavones and flavonoids that produce a yellow color [38]. In this study, the flavonoid compounds measured are flavones and flavonoids, the TFC was ranged from 95.73 to 413.73 mg of quercetin equivalent L–1. The results showed that pluchea leaves contained higher TFC than green tea leaves. The pluchea leaves and green tea mixtures at 75:25 % (w/w) proportions had the highest TFC compared with the other proportions. The TFC of samples had a similar pattern to the TPC, because the flavonoid compounds are the major component of phenolic compounds [33]. The existence of a functional group from the carbonyl and hydroxyl groups of flavones and flavonoids in pluchea leaves and green tea in samples can cause synergism effect so that the formation of a complex with aluminium ion increased in samples at 75:25; 50:50; 25:75 proportions, respectively. The flavonoid compounds can act as free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action [39, 40].

Table 2: Total flavonoid content of aqueous extract of pluchea leaves and green tea mixtures at various proportions (n=5, Values expressed as mean±SD. Data were analyzed by one way analysis of variance (ANOVA) followed by duncan’s multiple range test (DMRT))

<table>
<thead>
<tr>
<th>Pluchea leaves and green tea mixtures proportion (% w/w)</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>100:0</td>
<td>538.88±</td>
</tr>
<tr>
<td>75:25</td>
<td>601.13±</td>
</tr>
<tr>
<td>50:50</td>
<td>707.63±</td>
</tr>
<tr>
<td>25:75</td>
<td>587.38±</td>
</tr>
<tr>
<td>0:100</td>
<td>451.13±</td>
</tr>
</tbody>
</table>

Fig. 2: Total flavonoid content of aqueous extract of pluchea leaves and green tea mixtures at various proportions (n=5, Values expressed as mean±SD. Data were analyzed by one way analysis of variance (ANOVA) followed by duncan’s multiple range test (DMRT))

Andarwulan et al. [31] informed that the flavonols contained in pluchea leaves are kaempherol, myricetin, and quercetin. Peres et al. [11] said that flavonols are the major flavonoid compounds in green tea so that they aren’t detected by aluminium chloride method.

DPPH free radical scavenging activity

The method is based on the reduction of the methanolic-DPPH solution because of the presence of antioxidant substances having hydrogen donating groups (RH) such as phenolics and flavonoids.
compounds due to the formation of non radical DPPH-H form [12, 41]. The total antioxidant activities (TAA) determined by DPPH of water extract of pluchea leaves and green tea mixtures at various proportions were expressed as mg of quercetin equivalent L of samples (fig. 3). Total antioxidant activity (DPPH) was highest in 50:50 proportions, followed by 75:25 proportions, followed by 25:75 proportions, followed by 100:0 proportions, and then 0:100 proportions of pluchea leaves and green tea mixtures. There was a trend that hydroxyl groups among phenolic compounds in pluchea leaves and green tea were an anti-oxidant effect so that the TAA of them was lower than the TAA sum of pluchea leaves and green tea. The graph pattern of this TAA was similar to the graph pattern of the TPC. The TAA of pluchea leaves was higher than it of green tea. The differences of TAA among all proportions of pluchea leaves and green tea mixtures were statistically significant. There were significant differences between total antioxidant activities of all proportions of pluchea leaves and green tea mixtures depending on TPC of an extract of samples. Widyawati et al. [2] said that the different antiradical activity of the extract was depend on the polarity of solvent and solute. These determined the intrinsic compounds to be extracted. The different structure of phytochemical constituents determines scavenging activity. Rohman et al. [40] informed that the phytochemicals responsible for the scavenging activity in this species are phenolic and flavonoid constituents. Har and Ismail [41] also explained that phenolic acid and flavonoids are known to be contributing to the antioxidant activity. Chlupicka et al. [42] said that among polyphenols the greatest antioxidant efficacies in pseudocereal breads are showed for quercetin, tannic acid, caffeic acid and gallic acid, while catechin and resveratrol have the lowest ones. This estimated that the hydroxyl group of flavonoid compounds between pluchea leaves and green tea mixtures was interacted to give the sinergism effect of RP. The differences of TAA among all proportions of pluchea leaves and green tea mixtures were statistically significant. There were significant differences between TAA of all proportions of pluchea leaves and green tea mixtures depending on TPC of an extract of samples. Rohman et al. [40] said that TAA of extracts and fractions of red fruit (Pandanus conoideus Lam) determines the total phenolics content and total flavonoid content. He also suggested that phenolic compounds more likely contribute to its reducing activity (RP) than flavonoid compounds. On the contrary, the opposite results were found in this research that the RP was more likely contributed by flavonoid compounds than it of phenolic compounds. Gholivand and Pirvaei [44] studies that the electron donation capacity reflects the reducing power of bioactive compounds which is related to antioxidant activity.

Antioxidants can be explained as reducers, and inactivation of oxidants by reducers can be described as redox reaction in which one reaction species is reduced at the expense of the oxidation of the other. Fe³⁺ reduction is often used as an indicator of electron donating activity, which is an important mechanism of the phenolic antioxidant action.

α-Amylase inhibitory activity

The ability of aqueous extract from pluchea leaves and green tea mixtures at various proportions in inhibiting the α-amylase is presented in fig. 5.
The α-amylase inhibition activity in this study was ranged from 26.15±1.52 to 56.06±3.91%. The 0:100 proportions had the highest inhibitory α-amylase activity compared to the other proportions. The inhibition activity of the green tea extract showed the significantly higher (p<0.05) than it of pluchea leaves extract. In this study, there was no significant correlation found between the TAA (DPPH radical and RP assay) with the α-amylase inhibition activity of pluchea leaves and green tea mixtures at various proportions. This showed that the graph pattern of the TAA and the α-amylase inhibition activity wasn’t similar. The same study also is done by Lim and Loh [45]. However, McCue et al. [46] suggested that the antioxidant activity of phenolic from clonal oregano extracts is affected the five sets of disulphide bridges located on the outer surface of α-amylase. The reduction of these cysteine residues causes inhibition by modifying in the structure of the enzyme. Thus, the α-amylase inhibition activity of the pluchea leaves and green tea mixtures at various proportions may perhaps not due to the TAA but it also involves other mechanisms that worth to be disclosed. This argument was supported by Lim and Loh [45]. Akah et al. [47] informed that the phytochemical compounds, such as terpenoids, saponins, flavonoids, glycosides and carbohydrate have similarly been implicated in the anti-diabetic activities of the plant. Therefore the alpha amylase inhibition activity of aqueous extract from pluchea leaves and green tea mixtures at various proportions may be contributed by phytochemical compounds (table 1).

α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity of the extracts is summarised in fig. 6. Data showed that the 50:50 proportions of pluchea leaves and green tea mixtures had the highest inhibitory α-amylase activity compared to the other proportions. The green tea extract had the higher alpha glucosidase inhibitory activity than it of pluchea leaves extract. However, the TPC and TFC of green tea extract were lower than them of pluchea leaves extract. This phenomenon indicated that the alpha glucosidase inhibitory activity was no be caused by phenolic compounds activity but it was contributed the other phytochemicals. Lim and Loh [45] reported that the α-glucosidase inhibition activity of bound phenolic extract has higher than free soluble phenolic extract because the bound phenolic mostly exists in a β-glucosides form that prefers aqueous phase than the free phenolic which is in the form of aglycones, a non-sugar group. This direct enzyme-inhibitor interaction is predicted to be higher in bound phenolic extracts in the α-glucosidase assay. McCue et al.[48] reported that the mechanisms of inhibition for α-glucosidase may different from α-amylase. There is none disulphide bridges especially not on the surface of the molecule (possible site for interaction with antioxidants) on the structure of Baker’s yeast α-glucosidase. This indicated that the α-glucosidase inhibition activity of phenolic extracts doesn’t involve the interaction of the antioxidant capacity with the disulphide bridges. Therefore, the inhibition is caused by the phenolic extracts through another mechanism. There was a correlation among the TPC, TFC and alpha glucosidase inhibitory activity. Obih et al. [49] said that the higher inhibitory activities also are contributed by the presence of some non-phenolic phytochemicals that probably act as enzyme inhibitors, exhibiting an additive or synergism effect with the phenolics present in the sample.

Fig 6: α-Glycosidase inhibitory activity of aqueous extract of pluchea leaves and green tea mixtures at various proportions (n=5). Values expressed as mean±SD. Data were analyzed by one-way analysis of variance (ANOVA) followed by duncan’s multiple range test (DMRT)

CONCLUSION

The antioxidant activity and alpha glycosidase inhibitory activity of aqueous extract in pluchea leaves and green tea mixtures at various proportions were correlated with TPC and TFC, but the alpha amylase inhibitory activity wasn’t. There was contributed by the other phytochemicals to the alpha amylase inhibitory activity. The inhibition mechanism of alpha amylase and alpha glucosidase was different.

AUTHORS CONTRIBUTION

The first and second authors have participated in the work including participation in the concept, design, analysis, writing, and revision of the manuscript.

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CONFLICTS OF INTERESTS

Authors declare no conflicts of interest

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