# Phytochemical Compounds and Antihyperglycemic Activities from Beverage Composed Pluchea indica Less-Black Tea-Honey

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# Phytochemical Compounds and Antihyperglycemic Activities from Beverage Composed *Pluchea indica* Less-Black Tea-Honey

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***ABSTRACT***

*Pluchea indica* Less *has been developed into tea bag because it contains a number of phytochemical compounds, such as lignin, terpenes, benzoids, phenylpropanoids, alkanes, saponins, catechins, alkaloids, tannins, sterols, phenolhydroquinones, and flavonoids, which have been shown to have several biological activities, such as antioxidants, anti-inflammatory, anticholesterol, and antihyperglycemic. In its development, pluchea tea can be combined with black tea to produce certain functional values. Consumption of pluchea-black tea in daily life can be done with the addition of lemon or honey to increase antihyperglycemic activity. However, this study focused on determining the profile of phytochemical compounds and antihyperglycemic activity of pluchea-black tea honey drink. The research design used was a Randomized Block Design (RAK) with one factor including honey concentration consisting of six treatment levels: 0, 1, 2, 3, 4 and 5% (v/v). Each treatment was repeated four times. Parameters carried out are the profile of phytochemical compounds and antihyperglycemic activity through in vitro analysis, namely the activity of inhibiting $\alpha$-amylase and $\alpha$-glucosidase enzymes. The results showed that the use of honey in the manufacture of pluchea-black tea had an effect on the profile of phytochemical compounds and antihyperglycemic activity. The use of honey with a concentration of 5% produced the highest antihyperglycemic activity, 73.21±3.57% for the ability to inhibit the alpha-amylase enzyme and 77.27±5.25% for the ability to inhibit the alpha-glucosidase enzyme, respectively.*

**Keywords***: antihyperglycemic, black tea, honey, pluchea tea, phytochemicals*

## INTRODUCTION

*Pluchea indica* Less is an herb plant included *Asteraceae* or *Compositae* family (GBIF 3132728). This plant is known by people as traditional medicine, such as antistress (Setiaji and Sudarman, 2005), the woung healing process (Handayani, 2019), toothache (Pargaputri et al., 2015), inflammation (Ningtyas, 2014), facial skin health (Nadhira. 2020), reduced odour and prevent many diseases (Widyawati et al., 2014). The potency of pluchea leaves as traditional medicine related to phytochemical composition. Widyawati et al. (2014) said that pluchea leaves contain phytochemical polar compounds, i.e., flavonoid, saponin, phenol hydroquinone, alkaloid, sterol, tannin, and reducing sugar. Silalahi (2019) informed that pluchea leaves are composed by phytochemical compounds, namely, thiophenes, chlorogenic acid, 3,4-O-dicaffeoylquinic acid and 3,5-O-dicaffeoylquinic acid. Widyawati et al. (2022; 2023) also confirmed that pluchea leaves compose β-carotene, vitamin C, phenolic acids (chlorogenic acids, caffeic acids), flavonoids (quercetins, kaempferols, myricetins), anthocyanins, carotenoids, 3-O-caffeoylquinic acid, 4-O-caffeoylquinic acid, 5-O-caffeoylquinic acid, 3,4-O-dicaffeoylquinic acid, 3,5-O-dicaffeoylquinic acid, and 4,5-O-dicaffeoylquinic acid. The active compounds of pluchea leaves have many biology activities, Ibrahim et al. (2022) informed that they have anti-Inflammatory, antiulcer, antioxidant, cytotoxic, analgesic, cytotoxity, lipase inhibitory, antihyperlipidemic, anti-dyslipidaemia,

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fibroblast hyperproliferation inhibition, antidiabetic, milky production, and growth hormone promotion activities.

The process of pluchea leaves to be pluchea tea has been carried out and shown to have antioxidant (Widyawati et al. 2016) and antidiabetic activities (Werdani and Widyawati, 2018). The consumption of pluchea tea can be done by combining it with various other ingredients or components with the aim of increasing the level of consumer acceptance and antioxidant activity. Widyawati et al. (2020) have added lemon to pluchea drink can increase antioxidant and antidiabetic activity. Widyawati et al. (2017) have added green tea to pluchea drink with a ratio of 50:50% is able to increase antioxidant and antidiabetic activity. Widyawati et al. (2018) have added black tea to pluchea drink which can reduce antioxidant activity. Erejuwa et al. (2012) reported that honey is potential to improve glycemic control in body and metabolic derangements. Bioactive compounds in honey include phenolics, flavonoids, organic acids, carotenoid-derived compounds, nitric oxide metabolites, ascorbic acids, Maillard reaction products, aromatic compounds, trace elements, vitamins, amino acids, proteins. Vallianou et al. (2014) also claimed that honey contains flavonoids, phenolics, amino acids, carotenoid derivatives, nitric oxide metabolites, and enzymes such as glucose oxidase, invertase, diastase, catalase, phosphatase, and peroxidase. Therefore, in this study, honey was added with the aim of increasing antidiabetic activity. Erajuwa et al. (2012) claimed that honey is potential as antidiabetic agent because it can improve glucose level in blood. Anggraeni et al (2016) have used pluchea tea and black tea in a ratio of 25:75% with the addition of 5% honey to obtain the most preferred beverage by the panelists. Dharmamihardjo (2016) has found that the addition of 5% honey of pluchea-black tea beverage resulted in the highest antioxidant activity. So far, the effect of honey addition to increase antihyperglycemic ability of pluchea-black tea drink on has not been studied further. The purpose of this study was to determine the profile of phytochemical compounds and their ability as antihyperglycemic agents of pluchea-black tea-honey beverage.

## METHODS

### *Materials*

Pluchea leaves with leaf segments numbered one to six from the shoots of plants leaves (Widyawati et al., 2014) obtained from plantations in the Surabaya area. Then washed and dried at room temperature for 7 days. The dried leaves were powdered with a size of 45 mesh, then oven-dried at 120$^o$C for 10 minutes. Premium quality black tea is weighed with pluchea tea, then put in a tea bag with a ratio of 25:75% (w/w), then steeped by 100 mL water at 95$^o$C for 15 min and added with various concentrations of honey, including 0, 1, 2, 3, 4, and 5% (v/v).

The chemicals were needed in this analysis such as methanol ($CH_3OH$) (Fulltime), distilled water and aquabides (PT. Akua Surabaya), chloroform ($CHCl_3$) (Merck), ammonia ($NH_3$) (Mallinckrodt), sulfuric acid ($H_2SO_4$) (Merck), mercury (II) chloride ($HgCl_2$) (Merck), potassium iodide (KI) (Merck), iodine ($I_2$) (Merck), sodium hydroxide (NaOH) (Merck), hydrochloric acid (HCl) (Merck), n-amyl alcohol, magnesium (Mg) (Merck), iron (III) chloride ($FeCl_3$) (Merck), potassium sodium tartate tetrahydrate (Sigma-Aldrich), copper (II) sulfate ($CuSO_4$) (Merck), gallic acid (Riedel-deHaen), Folin-Ciocalteau (Merck), sodium carbonate ($Na_2CO_3$) (Riedel-deHaen), (+)-catechins (Sigma), sodium nitrite ($NaNO_2$) (Merck), aluminum chloride ($AlCl_3$) (Schuchardt OHG), sodium dihydrogen phosphate ($NaH_2PO_4$) (Merck), sodium acetate (Riedel-deHaen), glacial acetic acid (Merck), α-amylase (Sigma-Aldrich), starch (Merck), monobasic sodium phosphate ($Na_3PO_4$), sodium phosphate based ($Na_2HPO_4$ $7H_2O$), α-glucosidase (Sigma-Aldrich), and P-nitrophenyl-α-D-glucoside (PNP).

***Tool***

The tools used for analysis were digital balance (Mettler Toledo), vacuum oven (Binder), waterbath (Buchi Waterbath B480), hot plate (Cimarec 3 Thermolyne), UV-Vis Spectrophotometer (Shimadzu UV VIS Spectrophotometer 1700 Pharmaspec).

***Phytochemical screening***

Profile phytochemical screening of the pluchea-black tea-honey and honey beverage was carried out using standard procedures (Kujur et al., 2010; Elgailani, 2015), to check for the presence or absence of bioactive compounds from secondary metabolites such as alkaloids, phenolics, flavonoids, saponins, tannins, and cardiac glycosides.

***Total phenol content analysis***

The principle of total phenol content analysis is the reaction between phenolic compounds and Folin Ciocalteu/FC reagent (in which phosphomolybdic acid and phosphotungstic acid are present). This Folin-Ciocalteau reagent oxidizes phenolics (alkali salts) or phenolic-hydroxy groups reducing heteropoly acids (phosphomolybdate-phosphotungstic acid) present in the Folin-Ciocalteau reagent to a blue molybdenum-tungsten complex. Furthermore, the intensity of this blue color was measured using a UV-Vis Spectrophotometer at $\lambda$=760 nm with gallic acid as a standard (Muntana and Prasong, 2010).

***Total flavonoid content analysis***

Analysis of total flavonoid content using aluminum chloride colorimetric method. The principle of analysis of total flavonoid content is that the reaction between $AlCl_3$ compounds and flavonoid compounds contained in the sample can form a stable acid complex between $AlCl_3$ and C-4 keto groups, C-3 or C-5 hydroxyl groups of flavones and flavonols, in addition to $AlCl_3$ also form labile acid complexes with ortho-dihydroxyl groups on the A- or B- rings of yellow flavonoids, which will then turn pink when NaOH is added, which can be measured spectrophotometrically at $\lambda$= 510 nm. The standard solution used is (+)-catechin (Al-Temimi and Choundary, 2013).

***Analysis of α-amylase enzyme inhibitory activity***

Analysis of the inhibitory activity of the α-amylase enzyme in the sample was based on the spectrophotometric method. The principle of this test is the inhibition of the activity of the α-amylase enzyme in hydrolyzing starch. The α-amylase enzyme can hydrolyze starch into simple sugars such as glucose and maltose. The addition of acetate buffer at pH=5.0 and the α-amylase enzyme was measured spectrophotometrically at $\lambda$= 540 nm. The inhibitory activity of the α-amylase enzyme was expressed in percentage (%) (Odhav et al., 2010 with modification).

***Analysis of α-glucosidase enzyme inhibitory activity***

Analysis of the inhibitory activity of the α-glucosidase enzyme was carried out based on the enzymatic reaction using P-nitrophenyl-α-D-glucopyranose (PNGP) as a substrate. The α-glucosidase enzyme hydrolyzes P-nitrophenyl-α-D-glucopyranose (PNGP) into yellow D-

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glucopyranoside and P-nitrophenol. The intensity of the yellow color formed from p-nitrophenol was determined by its absorbance using a spectrophotometer at $\lambda$= 410 nm. Enzyme activity was determined based on the absorbance of P-nitrophenyl formed. The higher the ability of pluchea leaf extract to inhibit α-glucosidase activity, the less P-nitrophenyl formed (Mayur et al., 2010 with modification).

***Design of experiment and statistical analysis***

Design of experiment used a randomized block design with one factor, i.e., concentration of honey, including 0, 1, 2, 3, 4, and 5% (v/v). Each treatment was repeated four times in order to gain 24 experiment units. The homogenous data that analyzed triplicate was expressed as the mean ± SD and determined using ANOVA at $p \leq 5\%$. The results of the ANOVA test showed significantly difference, that followed by DMRT (Duncan Multiple Range Test) at $p \leq 5\%$ to determine significantly affect among the treatment levels. The statistical analysis applied SPSS 17.0 software (SPSS Inc., Chicago, IL, USA).

**RESULT AND DISCUSSIONS**

Analysis of phytochemical profiles and antihyperglycemic activity of pluchea-black tea-honey was carried out on samples that steeped by water at 95°C for 15 min, with the appearance of the samples was showed at Figure 1. This steeping water was obtained from pluchea tea and black tea with a proportion of 25:75% (w/w), having water content of 16.70 ± 0.46% (w/w) and 8.65 ± 0.25% (w/w), respectively.

(a) (b)

Figure 1. The appearance of pluchea - black tea - honey beverage
(a) side view (b) top view

***Identification of phytochemical compounds profile in pluchea-black tea-honey beverage***

Identification of the profile of phytochemical compounds in black tea-honey pluchea drink was carried out to determine the profile of phytochemical compounds qualitatively. The test was done before quantitative assay of phytochemical compounds, which included total phenolic content (TPC) and total flavonoid content (TFC). Brewing of samples with hot water at temperature of 95°C for 15 min was a suitable solvent for extracting phytochemical components. This is supported by Naczk and Shahidi (2003), that phytochemical compounds in plants usually form free aglycone structures or bind to form glycosides or esters with other phytochemicals, carbohydrates or proteins. The brewing process at temperature of 95°C for 15 min can cause phytochemical compounds that are bound to break and dissolve in water, according to Nawaz et al. (2018), that the level of solvent polarity determines the level of polarity of the extracted components. Polarity of solvent was influenced bioactive content and

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biological activities. Water is a polar solvent that can extract polar phytochemical compounds.

Pluchea black tea drinks are detected composing the polar bioactive compounds, namely alkaloids, phenolics, flavonoids, saponins, tannins, and cardiac glycosides (Dharmamihardjo, 2016). However, the pluchea leaves also comprise caffeoylquinic acids, phenolic acids, flavonoids, thiophenes, carotenoids, saponins, anthocyanins, and vitamin C (Widyawati et al., 2014; Silalahi, 2019; Widyawati et al., 2022). Steeping water of pluchea leaf tea (2 g/100 mL) is proven total phenolic content of 9.3 mg EAG/g, total flavonoid content 22.0 mg EC/g, DPPH free radical scavenging activity DPPH 27.2 mg EAG/g, and reduced iron ion 10.2 mg EAG/g. Vallianou et al. (2014) also supported that honey contains polar phytochemical compounds, such as flavonoids, phenolics, amino acids, carotenoid derivatives, nitric oxide metabolites, and enzymes such as glucose oxidase, invertase, diastase, catalase, phosphatase, and peroxidase. Morever, Hariyati (2010) informed that honey has a pH range of 3.2-4.5, this is related to opinion of Saputra (2012) that organic acids in honey include gluconic acid, acetic acid, formic acid, butyric acid, lactic acid, oxalic acid, tartaric acid, succinic acid, pyroglutamic acid, malic acid, pyruvic acid, and α-ketoglutaric acid.

The data in Table 1 showed that the pluchea-black tea-honey drink contained alkaloids, phenolic compounds, flavonoids, saponins, tannins, and cardiac glycosides, while in Table 2. showed that honey contained phenolic, flavonoid, alkaloid, and cardiac glycosides. Based on these data, it could be informed that the increase in alkaloid compounds in the pluchea-black tea-honey drink and the control drink were caused by increasing of honey concentration. According to Gilbert and Senyuva (2008), honey contains pyrrolizidine alkaloids. Erejuwa et al. (2012) and Vallianou et al. (2014) also claimed that honey is composed phytochemical compounds, such as phenolics and flavonoids. Andarwulan (2010) also emphasized that alkaloids are amino acid derivatives as secondary metabolites that are alkaline and polar so they can dissolve in water. Therefore, the addition of honey could increase the color intensity of the alkaloid compounds detected in the pluchea-black tea-honey drink.

Table 1. Profile of phytochemical compounds in pluchea-black tea-honey at various concentration of honey

| Phytochemical Compounds | Honey Concentration (% v/v) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| Alkaloids | +3 | +3 | +3 | +4 | +4 | +4 |
| Phenolics | +2 | +2 | +2 | +3 | +3 | +3 |
| Flavanoids | +1 | +2 | +2 | +3 | +4 | +4 |
| Saponins | +1 | +2 | +2 | +3 | +3 | +4 |
| Tannins | +2 | +2 | +3 | +3 | +4 | +4 |
| Cardiac Glycosides | +1 | +2 | +2 | +3 | +4 | +5 |

Note: + sign showed color intensity of beverage, and - sign showed not detection.

Meanwhile, the color intensity of phenolic and flavonoid compounds identified in pluchea-black tea-honey increased in intensity with the addition of honey by 4 and 5% (v/v). Honey has phenolic components including phenolic acids and flavonoids. Phenolic acids identified in honey are ellagic acid, caffeic acid, p-coumaric acid, ferulic acid, gallic acid, chlorogenic acid, coniferyl acid, benzoic acid, trans-cinnamic acid (Moniruzzaman et al., 2014). While the flavonoids of honey include apigenin, kaempferol, quercetin, galangin, chrysin, pinocembrin,

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hesperidin, catechin, naringin, myricetin, naringenin (Vallianou et al., 2014). In honey, no phenolic compounds were detected in treatments 0, 1, 2, and 3% (v/v) because the addition of honey concentration was very low, consequence the color intensity of pluchea-black tea-honey beverage didn't show different intensity because the honey contribution was not very small in the drink.

Table 2. Profile of phytochemical compounds in honey at various concentration

| **Phytochemical Compounds** | **Honey Concentration (% v/v)** | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| Alkaloids | - | +1 | +2 | +2 | +3 | +3 |
| Phenolics | - | - | - | - | +1 | +1 |
| Flavanoids | - | - | - | - | +1 | +1 |
| Saponins | - | - | - | - | - | - |
| Tannins | - | - | - | - | - | - |
| Cardiac Glycosides | - | +1 | +2 | +3 | +4 | +5 |

Note: + sign showed color intensity of beverage, and - sign showed not detection.

Phytochemical tests also showed that the pluchea-black tea-honey and honey drinks contained saponins, this was indicated by the presence of foam that appeared along with the increase in the concentration of honey, while the control formed a little foam. The appearance of foam indicates the presence of glycosides that can form foam in water which is hydrolyzed into glucose and other compounds (Batubara et al., 2020).

The tannin test in the pluchea-black tea-honey drink is indicated by a dark blue color that gets darker as the concentration of honey is added. In contrast to the control, the results of the tannin test could not be detected because the honey was degraded due to the interaction of flavonoid compounds in the honey so that the color of the tannin test did not appear.

Cardiac glycosides can be tested using Fehling's solution so that it can detect reducing sugars. In general, phytochemical compounds present in plants are glycoside bound (Widyawati et al., 2011). Cardiac glycoside test results showed that the more concentration of honey added, the greater the intensity. This is indicated by the formation of a brick red precipitate that is formed. The control showed that the intensity was getting bigger as the concentration of honey was added because most of the sugar content in honey contained fructose and glucose.

***Total phenol content analysis***

Total phenol content (TPC) in pluchea-black tea-honey was determined by the Folin-Ciocalteau method, which is based on the oxidation reaction of phenolic compounds with reagents containing phosphomolybdic acid and phosphotungstic acid to form a blue molybdenum-tungsten quinone complex (Samaiyah et al. al., 2018). The results of the TPC test on the pluchea-black tea-honey drink could be seen in Figure 1. The results showed that there was a significant increase in TPC along with the addition of honey concentration in the pluchea-black tea drink. The highest TPC was obtained in the sample with the addition of 5% honey concentration, which was 447.25 ± 4.35 mg GAE/L. This is due to the interaction between the OH functional group in the phenol compound and the molybdenite ion in the Folin Ciocalteus reagent which increases with the addition of honey concentration. The higher the interaction between the two compounds is largely determined by the position of

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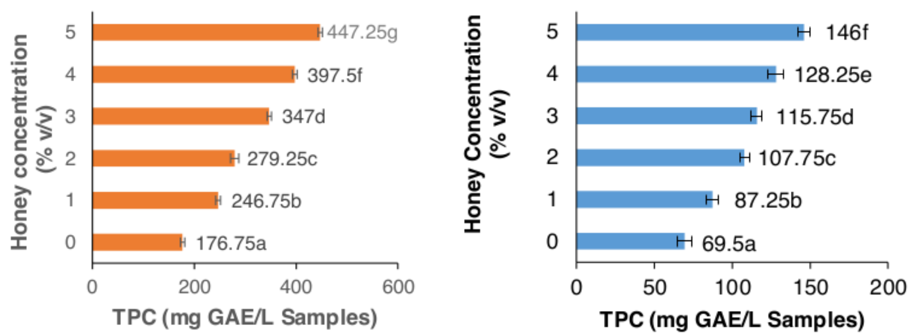
the OH group, the number of OH groups and the structure of the phenolic compound (Phuyal et al., 2020). The phenolic compounds in each of the pluchea-black tea-honey drinks were free or bound structure, as confirmed by Naczk and Shahidi (2003) and Hatamia et al. (2014). Differences in the structure of phenolic compounds can determine the solubility of these phenolic compounds in steeping water. The heterogeneity of phenolic compounds determines the biological activity

The data in Figure 1. showed that there was a synergistic effect between honey and pluchea-black tea drinks. Based on research by Anggraeni (2016), that the addition of honey at various concentrations can decrease the pH value of pluchea-black tea drinks, which ranges from 6.98-6.65. The increase in total phenol is thought to have a synergistic effect which is influenced by the decreasing pH of honey. The decrease in pH is due to the contribution of organic acid compounds contained in honey, as confirmed by Hariyati (2010) and Saputra (2012). It is also suspected that the decrease in the acidity of the drink can influence the breaking of glycoside and ester bonds that occur between phenolic compounds and carbohydrates, proteins or other phenolic compounds, thereby increasing free phenolic compounds were impacted of TPC values and the ability to inhibit the increase in blood sugar. Phenolic compounds are good electron donors because the number and position of their hydroxyl groups can directly contribute to inhibit free radicals, decompose peroxide, inactive metal, and scavenge oxygen (Aryal et al., 2019), that support antihyperglycemic activity (Hitami et al., 2014).




(a) (b)

Figure 1. Total phenol content (TPC) of pluchea-black tea-honey beverage at various concentration of honey (a) and honey beverage at various concentration (b)

***Total flavonoid content analysis***

The total flavonoid content (TFC) of pluchea-black tea-honey drink was determined by the aluminum chloride method. The power of flavonoids as antioxidants is by chelating metals and scavenging oxygen radicals and free radicals or as scavengers, and inhibiting the pro-oxidant enzymes including lipoxygenase, myeloperoxidase (Rukmiasih et al., 2011; Doughari, 2012). The results of total flavonoid content analysis in pluchea-black tea-honey could be showed in Figures 2.

The data reported that the addition of honey at various concentrations increased the TFC of pluchea-black tea-honey drink significantly. This was due to the contribution of flavonoid compounds, especially flavanols and flavonols from honey. As described by Liu et al. (2017)

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that the $AlCl_3$-$NaNO_2$-NaOH method is not precise in determining total flavonoids, it still allows interactions with non-flavonoid compounds such as aso-dihydric phenols. According to Chayati and Miladiyah (2014) that the total flavonoids in honey ranged from 60-500 mg/100 g, which consisted of pinobanksin (1.43 mg/100 g), quercetin (0.26 mg/100 g), luteolin (0.07 mg/100 g), pinocembrin (0.34 mg/100 g), chrysin (0.31 mg/100 g), and flavonols, while Suriyaphan (2014) stated that the total flavonoids in pluchea were 6.39 mg/100 g, consisting of quercetin (5.21 mg/100 g), kaempferol (0.28 mg/100 g), myricetin (0.09 mg/100 g), luteolin, and apigenin. There were several types of flavonols in honey that were the same as black tea and pluchea, such as quercetin, kaempferol, and myricetin thereby increasing the flavonols in the sample. Aryal et al. (2019) stated that phenolic and flavonoid compounds have proven potential as antioxidant activity are associated with a lower incidence of cardiovascular diseases, cancer, diabetes and neurodegenerative diseases. Zhao et al. (2019) stated that polyphenolic compounds have strong antioxidant activity in vitro and in vivo, depending on their solubility, polarity and molecular size.

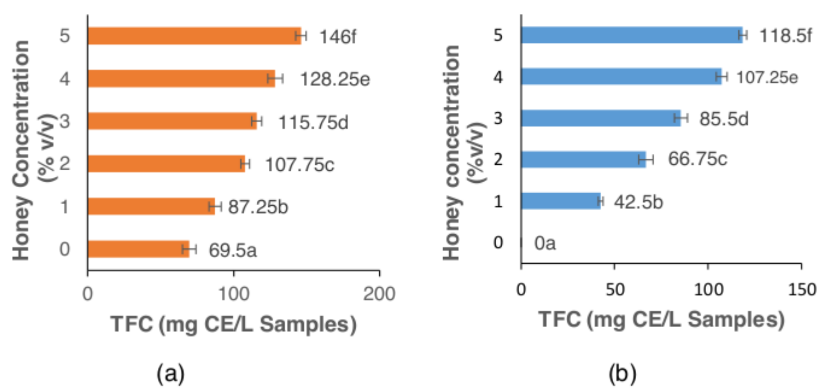


Figure 2. Total flavonoid content (TFC) of pluchea-black tea-honey beverage at various concentration of honey(a) and honey beverage at various concentration (b)

***Alpha amylase enzyme inhibition capacity***

The activity of inhibiting the alpha amylase enzyme is one indication of the ability of phytochemical compounds to prevent an increase in blood sugar (antihyperglycemic). This enzyme functions catalyze the endohydrolytic of long α-1,4-glucan chains such as starch and glycogen. Therefore alpha amylase inhibitors are starch blockers that prevent or slow the absorption of starch into the body mainly by blocking the hydrolysis of α-1,4-glycosidic linkages of starch and other oligosaccharides (Wickramaratne et al., 2016). Data analysis of the inhibitory ability of alpha amylase is showed in Figures 3. The data showed that the addition of honey concentration had a significant effect on the ability to inhibit the alpha amylase enzyme from pluchea-black tea-honey drink and honey drink (control). Pluchea-black tea-honey drink has the activity of inhibiting the alpha amylase enzyme by 52.27±2.9%-77.27±5.25%. while the ability to inhibit honey drink (control) against the alpha amylase enzyme was 69.7±4.29%-75.76±4.29%. The highest alpha amylase enzyme inhibitory activity of black-honey tea was the addition of 5% honey concentration, i.e., 77,27±5.25%, while the honey drink with 5% honey concentration was 75,76±4.29%. Different capability of this beverage to inhibit alpha amylase enzyme was caused by different composition and

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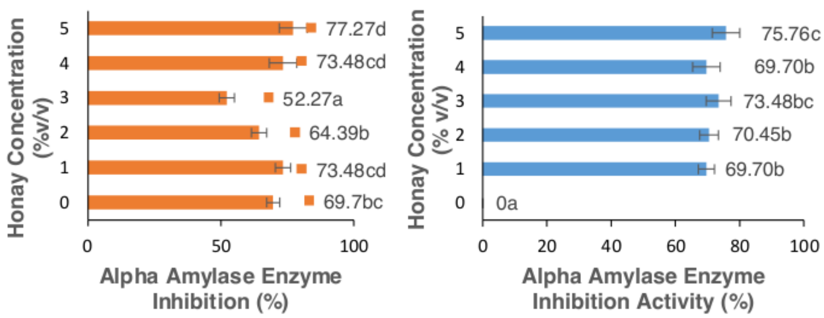
interaction of phytochemical compounds of them that influenced number and position of free hydroxyl group from phytochemical compounds, especially phenolic compounds.




(a) (b)

Figure 3. Alpha amylase enzyme inhibition activity of various concentration of pluchea-black tea-honey beverage(a) and honey beverage at various concentration (b)

Based on inhibition activity, this means that both the pluchea-black tea-honey drink and the honey drink were able to block the activity of the alpha amylase enzyme to convert carbohydrates into simple sugars that were easily absorbed by the small intestine. Pluchea-black tea-honey drink had almost the same activity as honey drink (control), it was suspected that there was an interaction between phytochemical compounds and sugars contained in honey. Sudha et al. (2011) said that alpha amylase inhibitor activity of *O. tenuiflorum* extract is caused by alkaloids, tannins and flavonoids.

Data in Table 1 and 2 showed that pluchea-black tea-honey and honey beverages composed phenolics, alkaloids and flavonoids. The existence of these compounds could be inhibited alpha amylase enzyme activity, the capability to block enzyme activity depends on hydroxyl group to donor hydrogen atom. Flavonoid and phenolic compounds have potential as antioxidant and antidiabetic agents. The antidiabetic activity has the ability to chelate with metals from the active site of the enzyme. The ability of flavonoids to inhibit antidiabetic activity by donating hydrogen atoms through metal chelators. In addition, the presence of tannin compounds also contributes to inhibit of the alpha amylase enzyme (Shah et al., 2018).

***Alpha glucosidase enzyme inhibition capacity***

The action of glucosidases is the breakdown of complex carbohydrates (starch and oligosaccharides) into simple sugars such as maltose and glucose that the fast uptake of glucose in the intestine (Nurcholis et al., 2014; Bhatia et a., 2019). Data analysis showed that the alpha-glucosidase inhibitory activity of black tea-honey pluchea and honey was significantly different at the addition of different concentrations of honey (Figure 4). The ability to inhibit alpha glucosidase enzymes from pluchea-black tea-honey drinks has an uncertain pattern, it is suspected that there is a similar phenomenon in the ability to inhibit alpha amylase enzymes, namely there are interactions between phytochemical compounds. The highest glycoside enzyme inhibitory activity of black-honey tea was the addition of 5% honey concentration, i.e., 73.21±3.57%, while the honey drink with 2% honey concentration was 64.29±5.83%.

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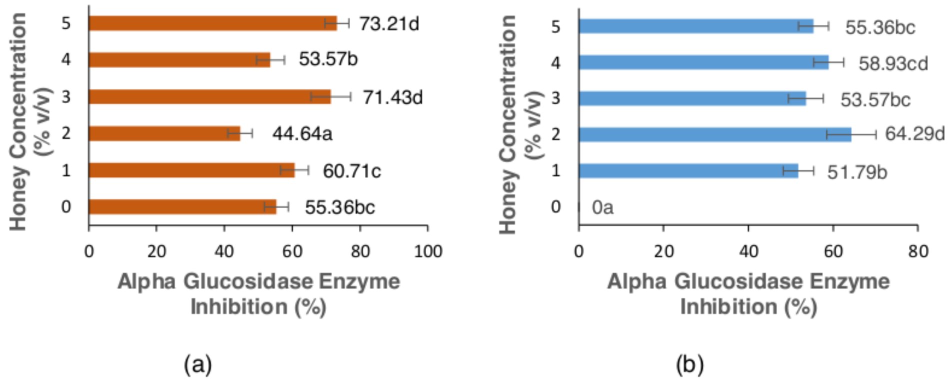


Figure 4. Alpha glucosidase enzyme inhibition activity of various concentration of pluchea-black tea-honey beverage (a) and honey beverage at various concentration (b)

Nurcholis et al. (2014) informed that the alpha glucosidase inhibition activity of ethyl acetate- and hexane extracts of *G. pictum* is contributed by the presence of several bioactive compounds such as alkaloids, tannins and steroids. Bathia et al. (2019) said that the inhibition activity of these plant extracts can be attributed to phytoconstituents present in them, such as flavonoids, alkaloids, terpenoids, anthocyanins, glycosides, phenolic compounds. Based on the ability to inhibit the alpha glucosidase enzyme from the pluchea-black tea-honey drink and honey drink, it can be said that both drinks have the ability to block the activity of the glucosidase enzyme which functions to break down complex carbohydrates into simple sugars. Fluctuate values of enzyme inhibition activity was caused by different interaction of number and position of hydroxyl group from phenolic compounds that determined enzyme inhibition capability. The other hand, the phenolic compounds could bind with sugar from honey that influence the ability to reduce enzyme activity.

## CONCLUSION

The addition of honey at various concentrations affects the profile of phytochemical compounds, especially the presence of alkaloids, phenolic compounds, flavonoids and cardiac glycosides. the higher the concentration of added honey, the effect on the TPC and TFC levels of the pluchea-black tea-honey drink. TPC and TFC levels did not have the same pattern as antihyperglycemic activity, namely the ability to inhibit the activity of alpha amylase and alpha glucosidase enzymes. It is suspected that there is an interaction between phytochemical compounds and the sugars found in honey. The addition of 5% honey had the highest antihyperglycemic activity, 73.21±3.57% for the ability to inhibit the alpha-amylase enzyme and 77.27±5.25% for the ability to inhibit the alpha-glucosidase enzyme, respectively. The addition of honey at various concentrations affects the profile of phytochemical compounds, especially the presence of alkaloids, phenolic compounds, flavonoids and cardiac glycosides. the higher the concentration of added honey, the effect on the TPC and TFC levels of the pluchea-black tea-honey drink. TPC and TFC levels did not have the same pattern as antihyperglycemic activity, namely the ability to inhibit the activity of alpha amylase and alpha glucosidase enzymes. It is suspected that there is an interaction between phytochemical compounds and the sugars found in honey. The addition of 5% honey had the highest antihyperglycemic activity, 73.21±3.57% for the ability to inhibit the

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alpha-amylase enzyme and 77.27±5.25% for the ability to inhibit the alpha-glucosidase enzyme, respectively.

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