

In vitro Antioxidant Properties and α -Glucosidase Inhibition of Combined Leaf Infusions from *Psidium guajava* L., *Syzygium polyanthum* L., and *Annona muricata* L.

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ABSTRACT

Introduction: Guava (*P. guajava*), bay (*S. polyanthum*), and soursop (*A. muricata*) known as natural medicine. Limited report is available on their antioxidant and α -glucosidase inhibitory activities of leaf infusion. The aims of this research were to compare the antioxidant and α -glucosidase inhibitory activities of leaf infusion from guava, bay, and either as individual or combined infusions, and to analyze the chemical composition of the leaf infusion. **Methods:** Air dried leaf powder of guava, bay and soursop were infused separately with boiled aquadest. The infusions were analyzed for their antioxidant activity against DPPH. The α -glucosidase inhibitory assay was conducted against α -glucosidase from *Saccharomyces cerevisiae*. Then the infusions scanned with UV-Vis spectroscopy and analyzed with LC-MS. The synergism activities of the combined infusion were measured. **Results:** Antioxidant activities of leaf infusions of guava and bay showed a comparable result IC_{50} 12.53 ± 0.55 and 10.76 ± 0.20 μ g GAE/mL, but the infusion of soursop showed lower (IC_{50} 19.77 ± 0.35 μ g GAE/mL) than BHT as positive control (11.6 ± 0.31 μ g GAE/mL). If soursop infusion was not added, then the mixture of the guava and bay infusion showed an antioxidative synergistic effect. The α -glucosidase inhibitory activities of the guava, bay and soursop infusion (0.083 ± 0.01 ; 0.025 ± 0.007 ; 0.533 ± 0.039 μ g GAE/mL, respectively) were stronger than acarbose (1285 ± 148 μ g/mL). The α -glucosidase inhibitory activities of the combined infusions showed a synergistic effect. The main constituents of the guava infusion were identified tentatively as chrysin and caffeoylquinic acid, for the bay infusion it was caffeoylquinic, and for the soursop infusion it was luteolin. **Conclusions:** There is a significant synergism of antioxidant activity of Guava and Bay mixture. The combined infusion of Bay and Soursop or Guava and Soursop showed antagonistic effect.

Key words: α -glucosidase, Leaf infusion, *Psidium guajava*, *Annona muricata*, *Syzygium polyanthum*, Synergism.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia, caused by insulin secretion disorder.^{1,2} International Diabetes Federation notes that the number of patients diagnosed with DM reaches 10 million in Indonesia.³ Indonesia has the 7th ranked largest incidence in the world, and it is estimated the prevalence of DM in Indonesia at the year 2030 will reach 21.3 million persons. In 2007, DM was the main cause of death in the 45-50 years age group, ranked second in cities, and sixth in villages.^{4,5} Accordingly, this devastating and preventable disease needs immediate attention and proper treatment. Promotive and preventive efforts should be done simultaneously with health education. As a non-infectious disease, diabetes is also considered a global threat. As a common comorbidity with cardiovascular diseases, DM is the most serious, chronic metabolic disorder and is characterized by high blood glucose levels, contributing to complications including hypertension, end-stage liver disease and renal failure.

Oxidative stress is involved in the pathogenesis and progression of diabetes, and plays a significant role in increasing insulin resistance or impaired insulin secretion, occurring when the formation of Reactive Oxygen Species (ROS)/ Reactive Nitrogen Species (RNS) increases and the ability to eliminate them decreases. This condition occurs because the production and inactivation of ROS is imbalanced (identified as a balance disorder of prooxidants and antioxidants).⁶⁻⁹ Excessive production of ROS and RNS leads to oxidative stress that causes protein, and lipid impairment and DNA damage. These damages develop into permanent cell dysfunction.¹⁰⁻¹² Superoxide radicals in DM cause various damage through multiple pathways, such as Advanced Glycation End Products (AGEs), polyol pathways, hexosamine pathways and Kinase C proteins. This widespread impairment eventually leads to complications of microvascular disease.^{13,14}

According to Glamočlija and Jevrić-Čaušević,¹⁵ there are five main classes of oral pharmacological agents to treat type 2 diabetes: sulfonylureas, meglitinides, metformin (a biguanide), thiazolidinediones, and α -glucosidase inhibitors. α -Glucosidase anchored in

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the mucosal brush border of the small intestine catalyzes the end step of digestion of starch and disaccharides that are abundant in human diet.¹⁶ One therapeutic approach to treat diabetes is to retard the absorption of glucose via inhibition of enzymes, in this case α -glucosidase which is able to catalyze the liberation of α -glucose from the non-reducing end of the substrate. Inhibiting this enzyme slows the elevation of blood sugar following a carbohydrate meal. Inhibitors of α -glucosidase delay the breakdown of carbohydrates in the small intestine and decrease the postprandial blood glucose excursion levels in diabetic patients.¹⁷ This inhibition is a useful and effective strategy to lower the levels of postprandial hyperglycemia. Many researches have been done on the inhibitors of α -glucosidase from various herbal medicines or plant sources, such as leaf infusions, bark extracts, and rhizomes (root medicines). Antidiabetic activities of leaf preparations were reported in a number of articles.

Many α -glucosidase inhibitors have been identified and isolated from plants, including leaves of *Psidium guajava* (guava), *Annona muricata* (soursop) and *Syzygium polyanthum* (bay).¹⁸ Guava, soursop, and bay leaves were reported for their ability of α -glucosidase inhibition.¹⁹⁻²¹ These plants could potentially be rich sources of natural antioxidants as well as natural anti-diabetes medicine.⁷

Guava leaf has been used in the treatment of various diseases, such as diarrhea, gastroenteritis and other digestive complaints. Guava leaves have potential as antimicrobial, antiproliferative activity, antiglycative, anticoagulant in plasma and antiplaque agents.^{22,23} Bay leaves have been used in Indonesian and Malaysian cuisines and traditionally used in the treatment of diabetes in Indonesia.²⁴ Several studies suggest that bay leaves can be used in the treatment of DM. Bay leaves are rich in tannins, flavonoids and terpenoids and also are a significant source of antioxidants.²⁵ Soursop leaf infusion has traditionally been consumed to maintain health, and now is being considered for treating patients with cancer. Soursop extracts have been characterized as antimicrobial, anti-inflammatory, anti-protozoan, antioxidant, insecticide, larvicide, and cytotoxic to tumor cells.²⁶

Several studies showed that there was a relationship among antioxidants with α -glucosidase inhibition and phenolic content properties of the samples. Many researches have been done with non-aqueous solvents, such as methanol, ethanol, ethyl acetate, and hexane. The use of fresh leaves, as tea or infused water is another interesting prospect. A person can just simply mix medicinal leaves with hot water and then drink. For this purpose, a study with vegetables, eatable leaves or other parts of plants is very important. Many herbal medicines use fresh plant materials, and are consumed directly, as food ingredients or as tea. In most previous research activities, non-water solvents were used to prepare the infusions of the individual plant materials. Therefore, there is a need to study the efficacy of the mixture or combination of plant materials as natural medicinal infusions.

The objectives of this study were to compare the antioxidant activity and α -glucosidase inhibition of the leaf infusions of three plants: *P. guajava* (guava), *S. polyanthum* (bay), and *A. muricata* (soursop); and to evaluate the synergetic effect of the infusion from these leaves. An *in vitro* assay of α -glucosidase inhibitory activity was conducted using α -glucosidase from *Saccharomyces cerevisiae*, while an antioxidant activity assay was conducted using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method.

MATERIALS AND METHODS

Plant materials

The leaves of guava, bay and soursop were obtained from various areas in Jakarta, in September 2016, and authenticated by one of the authors, K.H. Timotius. Herbariums of each plant were deposited in the research laboratory for confirming reference.

Preparation of the infusion

The fresh leaves were dried and ground prior to the infusion. 20 mg of guava, 50 mg of soursop, and 30 mg of bay dried leaves were infused with 100 mL hot distilled water.

Determination of DPPH radical scavenging activity

DPPH free radical activity of infusions were measured in terms of hydrogen-donating or radical-scavenging ability using stable radical DPPH following a previously reported method.²⁷ Briefly, 500 μ L of each infusion at different concentrations was mixed with 1500 μ L of 150 μ M DPPH in methanol absolute (acetone free). After 30 min, absorbance was measured at 517 nm using a spectrophotometer (Biochrome Libra S22). DPPH radical scavenging ability was calculated using the following equation in which H and Ho are optical densities of solvent with and without sample, respectively.

$$\text{DPPH radical scavenging activity (\%)} = \frac{1 - H}{H_0} \times 100\%$$

Alpha glucosidase inhibition assay

The α -glucosidase inhibition assay was done according to the previously described methods.²⁸ α -Glucosidase (50 μ L, 0.5 U/mL), in various concentrations of samples (50 μ L), and 50 mM phosphate buffer (pH 6.8, 50 μ L) were mixed at room temperature for 15 min. Reactions were initiated by the addition of 1 mM p-nitrophenol- α -D-glucopyranoside (100 μ L). The reaction mixtures were incubated at 37°C for 30 min in a final volume of 250 μ L, and then 1 M Na₂CO₃ (750 μ L) was added to the incubation solution to stop the reaction. The activities of glucosidase were read in cuvette and the absorbance was read at 405 nm by a spectrophotometer. The negative control was prepared by adding phosphate buffer instead of the sample in the same way as the test. Acarbose was utilized as the positive control. The blank was prepared by adding phosphate buffer instead of α -Glucosidase using the same methods.

The inhibition rates (%) was calculated from the formula below:

$$\% \text{ Inhibition} = \frac{(OD \text{ test} - OD \text{ test blank})}{OD \text{ test blank}} \times 100\%$$

Combination index (CI) analysis

This analysis is used to determine the combination effect to provide a greater benefit. Infusions' combination effect was determined by the Vinholes formula.²⁹

$$\text{Theoretical IC}_{50} = (\text{IC}_{50} \text{ infusion A} \times 0.5) + (\text{IC}_{50} \text{ infusion B} \times 0.5)$$

The classification as additive, synergistic, or antagonistic was performed by comparison of obtained IC₅₀ values with theoretical IC₅₀ value according to the published literature.

Additive: theoretical IC₅₀ and experimental IC₅₀ values show differences lower than 5%.

Synergistic: the experimental IC₅₀ values are more than 5% lower than theoretical values.

Antagonistic effect: experimental IC₅₀ values were more than 5% higher when compared with theoretical values.

UV-VIS Spectroscopic analysis

The water infusion was examined by UV-VIS spectrophotometer for proximate analysis. The infusion was scanned in the wavelength ranging from 200 – 700 nm using a Spectrophotometer Libra S22, which is used to detect the characteristic peaks. Each and every analysis was repeated in triplicate for the spectrum confirmation.

Determination of total phenolic content

Total soluble phenolic contents of infusion were determined using Folin-Ciocalteu's reagent with gallic acid as standard phenolic compounds.³⁰ Briefly, 0.5 mL of each diluted infusion was added to 2.5 mL of 10% Folin-Ciocalteu's reagent. After 10 min, 2.5 mL of 75 g/L aqueous sodium carbonate solution was added to the mixture. The solution was allowed to stand for 2 h at room temperature, and the absorbance of the resulting blue colored mixture was measured at 765 nm against blank containing only infusion solvent (500 μ L). Total phenolic contents were calculated as gallic acid equivalents (GAE) from the calibration curve obtained from the gallic acid standard solution and expressed as GAE/mL.³¹

Total Flavonoid Content (Revised)

Total soluble flavonoid content was determined using the aluminum chloride colorimetric method. Quercetin was used to make the calibration curve. Fifty milligrams of quercetin were dissolved in 95% ethanol and then diluted to make a standard curve. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M sodium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a Biochrom Libra S22 spectrophotometer (United Kingdom). The amount of samples was substituted by the same amount of distilled water in the blanks.³²

LC-MS analysis

LC-MS analysis was performed using a Mariner Biospectrometer, equipped with a binary pump (Hitachi L 6200). The HPLC was interfaced with a Q-tof mass spectrometer fitted with an ESI (Electrospray Ionization) source with positive ion mode. Full-scan mode from m/z 100 to 1200 was performed with a source temperature of 140°C. An HPLC column (Shimp-pack C8, 150 \times 6 mm i.d.) was used. Methanol with 0.3% formic acid was used as solvent and delivered at a total flow rate of 1 ml/min. A 5 μ l sample was injected and eluted isocratically. The compounds were analyzed based on the m/z fragments and then confirmed with the m/z profile in PubChem open source.

Data analysis

All experiments were performed in triplicates, and the data were reported as mean \pm SD. Regression method was used to calculate IC₅₀.

RESULTS

Antioxidant activities

The DPPH scavenging activities of all infusions showed strong radical scavenging activity. The antioxidant activity (IC₅₀) is expressed in Table 2. The values of IC₅₀ of guava and bay leaves infusion showed a comparable result with BHT as a positive control. But the soursop infusion showed lower activity than BHT. The effect of the combined two infusions (Bay and Soursop; Guava and Soursop) and three infusion (Guava and Bay and Soursop) showed that the soursop infusion was antagonist if combined with the other infusions, but combined guava and bay infusion gave a synergistic effect.

Alpha glucosidase inhibitory activities

Each single infusion showed IC₅₀ of AGI activity, stronger than acarbose. Combination of two or three infusions gave a synergistic effect (Table 4).

The total phenolic compounds

The total phenolic compounds of the infusions were 17.84 \pm 2.48; 21.43 \pm 0.13; and 18.48 \pm 2.27 (μ g GAE/mL) of guava, soursop, and

bay leaf, respectively. TPC of the infusion was a determining factor of antioxidant activity. This was indicated by positive correlation between antioxidant capacity and the TPC (Figure 2). TPC was also correlated with the inhibitor of α -glucosidase. Table 1 showed positive correlation of AGI and TPC (r=0.9). The AGI capacity was determined by the value of TPC. It means that there was a strong relationship between TPC, antioxidant activity and α -glucosidase inhibitor effect.

Chemical profiling

The UV VIS spectrophotometer scanning profiles of the infusions (Figure 1) showed that each infusion has a common peak of phenolic, namely 270-280nm. The absorbance value is correlated with the phenolic content of the infusion. The phenolic content is positively correlated with the amount of dried leaves. The pH of the infusion is low acid, 5, 6, and 5, respectively for guava, bay, and soursop leaves.

LC-MS analysis

As listed in Table 6, the infusions of guava, soursop and bay leaf contained different compounds. Two compounds were detected in guava infusions, chrysin and caffeoylquinic acid. Luteolin was detected as the only compound in soursop. The detected constituent of bay leaves was caffeoylquinic acid.

DISCUSSION

It is suggested that oxidative stress is involved in the pathogenesis and progression of diabetes.⁶⁷ Preventive action should be seriously taken, such as the supplemental usage of antioxidants.³³ Previous study has reported a lower antioxidative activity of guava water infusion (> 0.4 mg/mL).³⁴ Sutrisna *et al.* has reported that IC₅₀ of antioxidant activity of bay ethanol extract was 27.60 μ g/mL, while IC₅₀ of bay leaf infusion was 10.76 \pm 0.20 μ g GAE/mL.³⁵ Sayuti *et al.* also conducted a study on the antioxidant capacity of soursop aqueous leaf extract, and the results showed that the IC₅₀ of antioxidant activity of the soursop aqueous leaf extract was 299.92 μ g/mL.³⁶ These three studies have somewhat different results because the solvents used are different. In this study we found that guava, soursop and bay leaf infusions have potential antioxidative activity and high total phenolic compounds, while these infusions have the same IC₅₀ or higher than BHT capacity (Table 2). Among the three individual infusions, soursop has the lowest antioxidative activity compared to guava and bay infusions.

The combination of the guava and bay infusion shows a synergistic effect of antioxidative activity. Synergistic effects occur when the antioxidative activity of the combined infusion increases compared with the single infusion. A lower concentration is obtained by combining infusions. As a result, it will need only a small amount of a single infusion to reduce cell damage because of oxidative stress. Additional soursop infusion gives antagonistic effect. Antagonistic effects occur when the oxidative activity of the combined infusion decreases compared to the oxidative activity of the single infusion.³⁰ Antagonistic effects are caused by the competitive binding of the same compound in the infusion in combination with the same target protein pathway.³⁷

The α -glucosidase enzyme is an enzyme that plays an important role in the breakdown of carbohydrates in human. It works by hydrolyzing carbohydrates, releasing glucose that causes the increasing of post prandial glucose in the blood. When this enzyme is inhibited, it will reduce post prandial blood glucose level effectively. Acarbose is one of the many synthetic drugs available to treat diabetes.³⁸ Synthetic drugs usually cause hepatic disorder and other negative gastrointestinal symptoms.³⁹ The α -glucosidase inhibitors from natural ingredients have better benefits than synthetic drugs to prevent diabetes.

Sukohar *et al.* reported that α -glucosidase inhibitory activity of guava leaf water extract was 2.16 mg/mL.⁴⁰ In this study, the α -glucosidase

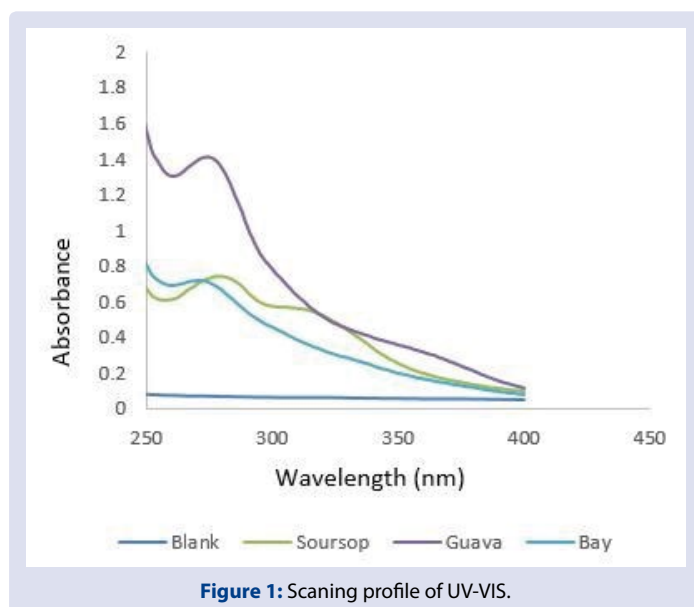


Figure 1: Scanning profile of UV-VIS.

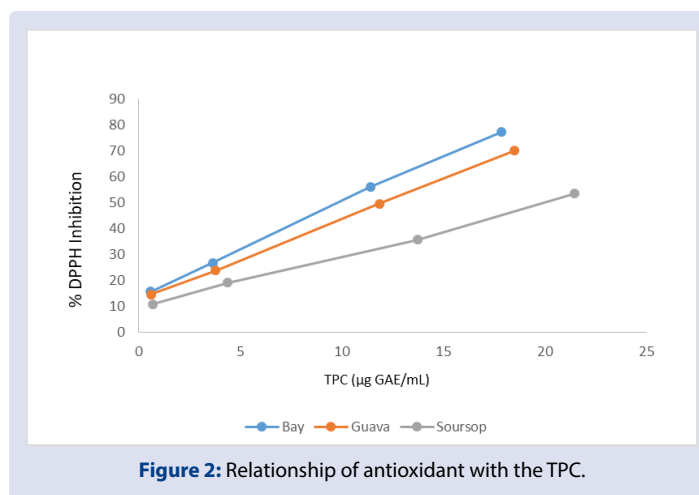


Figure 2: Relationship of antioxidant with the TPC.

inhibitors were evaluated and the results showed that all single samples had much better α -glucosidase inhibitory activity than acarbose. The inhibitory activities of the guava, bay and soursop leaf infusion against yeast α -glucosidase were analyzed and the results are shown in Table 3. There were significant differences in the effects of the three individual infusions. Bay leaf infusion showed the highest activity to inhibit α -glucosidase, followed by guava and soursop (Table 3). This result means that bay leaves have the highest activity to inhibit the number of α -glucosidase enzymes consequently delaying the absorption of sugars from the gut.⁴¹ This is probably related with their total phenolic content.

The combined two and three different infusions showed synergistic effects. Therefore, infusion of guava, soursop and bay leaves are promising natural α -glucosidase inhibitors which is good for preventing or treatment of DM.⁴² So far there is no report on the effect of the constituents of medicinal leaf infusion on the inhibition of α -glucosidase.

Phenolic compound of the infusion was the determining factor of antioxidant activity. The correlation of TPC and antioxidant activity is showed in Figure 2. There is a positive correlation ($r=0.9$) between antioxidant activity and the total phenolic content (TPC). Several studies showed that there was a relationship among antioxidants and

phenolic content properties of the samples. Positive correlation was also showed by AGI and TPC ($r=0.9$). The AGI capacity was determined by the level of TPC. The positive correlations between TPC-antioxidants and TPC-AGI show that there is a positive correlation between antioxidants activity and AGI. The strong inhibition activity of all infusions on α -glucosidase were directly related to the high content of phenolic compounds.³⁸

LC-MS analyses are showed in Figures 3 and Table 6. The peaks showed the content of phenolic acid, and no visible peak was detected in Band 1 of flavonoid. The amount of dried leaves is very important for this analysis. The content of TPC increased with the increase of the infused dried leaves. The tentative substances were chrysin, caffeoylquinic acid, and luteolin. These compounds are known for their antioxidant activity. Caffeoylquinic acid is a natural polyphenolic compound isolated from a variety of plants, such as apple, pear, berries, and aubergine. Caffeoylquinic acid has potential pharmacological properties such as: antioxidants that are synthesized in response to oxidative stresses, and acts as a hepatoprotectant, antibacterial and anticancer agent.^{43,44} Luteolin is a flavonoid compound and is most often found in plants in the form of glycoside. Luteolin has specific characteristics such as anti-inflammatory and anti-carcinogenic, which can be explained by its antioxidant activity.⁴⁵

Table 1: Relationship of AGI with the TPC.

Guava		Soursop		Bay		Coefficient Correlation (r)
Concentration ($\mu\text{g GAE/mL}$)	AGI (%)	Concentration ($\mu\text{g GAE/mL}$)	AGI (%)	Concentration ($\mu\text{g GAE/mL}$)	AGI (%)	
0.018	22.7	0.107	6.8	0.007	6.8	0.9
0.073	48.5	0.214	7.8	0.014	25.1	
0.11	70.4	0.428	31.9	0.028	56.7	
0.166	98.9	0.643	64.8	0.042	87.2	
		0.857	83.7	0.057	97.9	
		0.964	89.8	0.064	98.4	

Table 2: Theoretical versus experimental values of DPPH Scavenging activity EC₅₀ ($\mu\text{gGAE/ml}$) from the mixed herbs.

Samples	Theoretical	Experimental	Effect
Guava		12.53 \pm 0.55	
Soursop		19.77 \pm 0.35	
Bay		10.76 \pm 0.20	
BHT		11.60 \pm 0.31	
Guava-soursop	16.50	18.38 \pm 0.23	AN
Guava-bay	11.85	7.21 \pm 0.09	S
Bay-soursop	15.81	17.19 \pm 0.30	AN
Guava-soursop-bay	14.72	9.95 \pm 0.06	S

The theoretical values were calculated considering additive contributions of the individual herbs

A: additive effect: IC₅₀ of theoretical and experimental values differences lower than 5%.

S: synergistic effect: IC₅₀ of experimental values are more than 5% lower for IC₅₀ when compared with theoretical values.

AN: Antagonistic effect: IC₅₀ of experimental values are more than 5% higher for IC₅₀ when compared with theoretical values.

Table 3: α -glucosidase inhibition of infusions.

Sample	IC ₅₀ ($\mu\text{gGAE/mL}$)	IC ₅₀ theoretical
Guava	0.083 \pm 0.01	
Soursop	0.533 \pm 0.039	
Bay	0.025 \pm 0.007	
Acarbose	1285 \pm 148	
Guava-soursop	0.094 \pm 0.002	0.308
Guava-bay	0.028 \pm 0.0007	0.054
Soursop-bay	0.063 \pm 0.005	0.279
Guava-soursop-bay	0.024 \pm 0.007	0.321

Table 4: Theoretical versus experimental values of α -glucosidase inhibitor activity EC₅₀ (mg GAE/ml) from the mixed herbs.

Samples	Theoretical	Experimental	Effect
Guava-soursop	0.308	0.094 \pm 0.002	S
Guava-bay	0.054	0.028 \pm 0.0007	S
Soursop-bay	0.279	0.063 \pm 0.005	S
Guava-soursop-bay	0.321	0.024 \pm 0.007	S

The theoretical values were calculated considering additive contributions of the individual herbs.²⁹

A: additive effect: theoretical and experimental values differences lower than 5%.

S: synergistic effect: experimental values are more than 5% lower for EC₅₀ when compared with theoretical values.

AN: Antagonistic effect: experimental values are more than 5% higher for EC₅₀ when compared with theoretical values.

Table 5: TPC and flavonoid content.

Leaf	TPC (GAE $\mu\text{g/mL}$)	Flavonoid ($\mu\text{g/mL}$)
Guava	17.84 \pm 2.48	1.3 \pm 0.16
Soursop	21.43 \pm 0.13	2.2 \pm 0.60
Bay	18.48 \pm 2.27	1.6 \pm 0.46

Table 6: LC-MS chromatogram.

Leave	RT	MW	Mass fragments	Tentative identification	Area
Guava leaves	1.34	254	142 (33) 152 (100) 256 (5)	Chrysin	473.73
	1.95	354	132 (5) 198 (3) 203 (7) 219 (100) 381 (18) 445 (3)	Caffeoylquinic acid	653.01
Soursop leaves	2.06	285	130 (11) 138 (53) 146 (8) 174 (100) 219 (55) 231 (8)	Luteolin	1421.08
Bay leaves	1.98	354	166 (7) 198 (4) 203 (18) 219 (100) 221 (9) 381 (14)	Caffeoylquinic acid	929.99

RT: Retention Time

MW: Molecular Weight

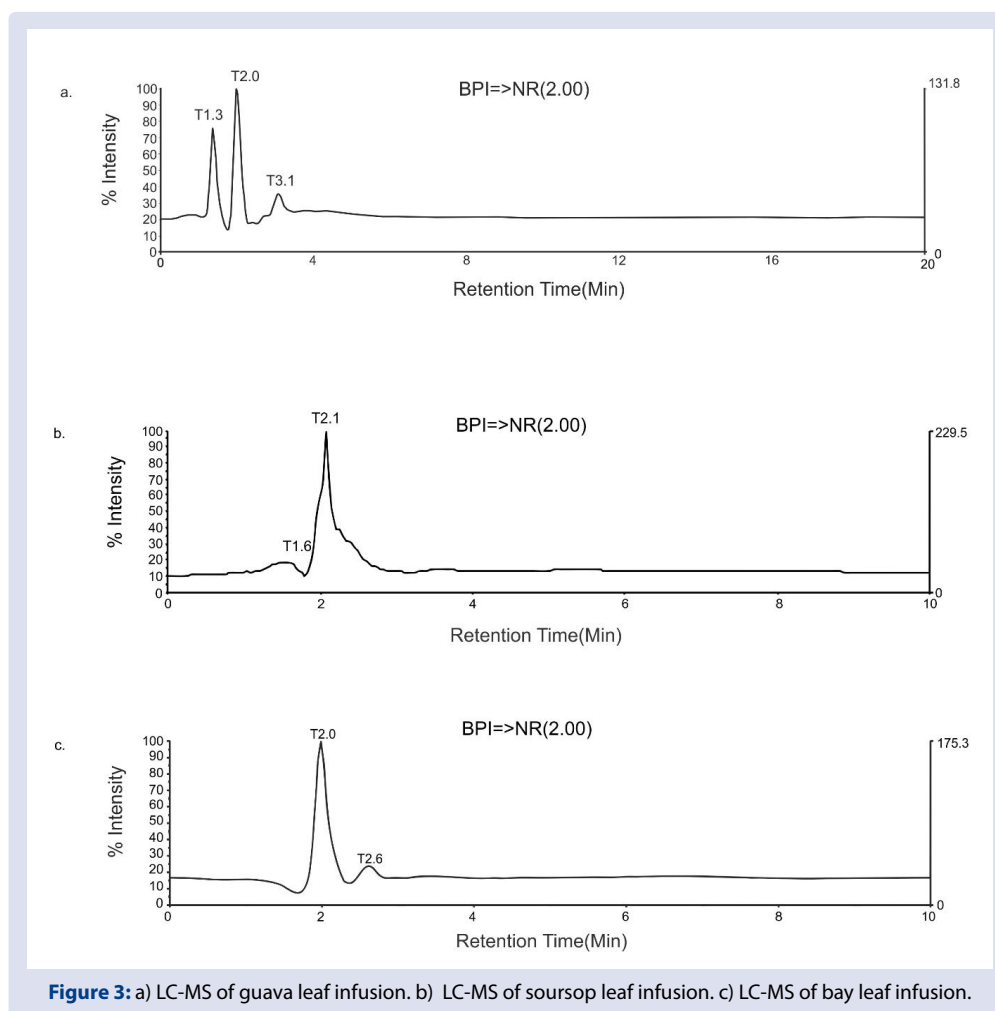


Figure 3: a) LC-MS of guava leaf infusion. b) LC-MS of soursop leaf infusion. c) LC-MS of bay leaf infusion.

CONCLUSIONS

The infusion of guava and bay leaves are better than soursop leaves in terms of their antioxidative activity. Their α -glucosidase inhibition activities were better than acarbose. But their combination is not necessarily better. The addition of soursop infusion may decrease the inhibitory activity of α -glucosidase. Functional herbal drinks can be formulated with combinations of guava, bay and soursop leaf infusions as natural, medicinal sources of health.

ACKNOWLEDGEMENT

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATION

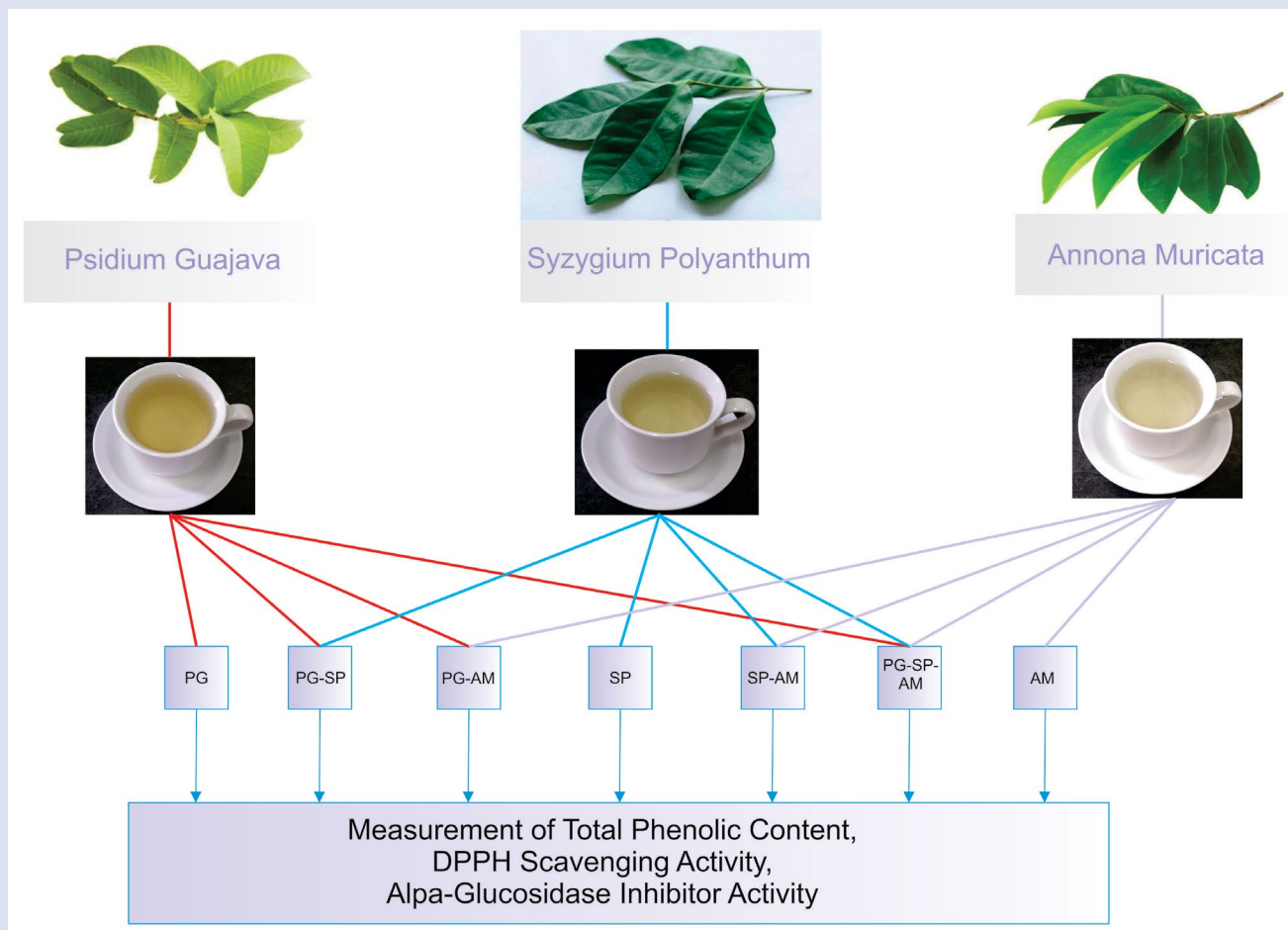
DM: Diabetes Mellitus; **DPPH:** 1,1-Diphenyl-2-picrylhydrazyl; **AGI:** Alpha Glucosidase Inhibitor; **TPC:** Total Phenolic Content; **IC₅₀:** Inhibitor Concentration of 50% inhibition; **GAE:** Gallic Acid Equivalent; **BHT:** Butylated Hydroxytoluene; **LC-MS:** Liquid Chromatography- Mass Spectrometry; **GC-MS:** Gas Chromatography- Mass Spectrometry.

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GRAPHICAL ABSTRACT



SUMMARY

- Antioxidant activities of leaf infusions of guava and bay showed a comparable result, but soursop showed lower than BHT as positive control.
- The α -glucosidase inhibitory activities of the guava, bay and soursop infusion were stronger than acarbose.
- The α -glucosidase inhibitory activities of the combined infusions showed a synergistic effect.
- The major constituent of guava infusion were chrysin and caffeoylquinic acid, for bay infusion was caffeoylquinic, and for the soursop infusion was luteolin.

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