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Phytochemical Screening, *in vitro* Hypoglycemic Potential, and Glucose Content of the Extracts of *Allium schoenoprasum*, *Capsicum frutescens*, and *Sechium edule* Leaves

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ABSTRACT
Allium schoenoprasum, Capsicum frutescens, and Sechium edule are plants commonly found in the Philippines. The fresh and decocted extracts of the plant leaves were prepared and tested for presence of secondary metabolites. The results show presence saponins, amino acids, and proteins in all plant extracts. In addition, presence of alkaloids, terpenoids, tannins, flavonoids, cardiac glycosides, reducing sugars, and phenolics are observed. However, none was found for the presence of phytosterols. Hypoglycemic potential of the plant extracts was determined using alpha-amylase inhibition assay. All of the plant extracts show alpha-amylase inhibition activity possibly due to the presence of the phytochemicals such as tannins, phenols and flavonoids. The fresh extracts of Allium schoenoprasum, Capsicum frutescens, and Sechium edule show inhibitions of 79.70-, 72.66-, and 80.77%, respectively and for the decocted extracts, percent inhibitions of 75.15-, 82.10-, and 80.40% are observed, respectively. It is observable that among the extracts, decocted extract of Capsicum frutescens exhibited the highest percent inhibition. Colorimetric determination of glucose concentration in the fresh extracts of Allium schoenoprasum, Capsicum frutescens, and Sechium edule show 43.28-, 58.51-, and 43.85ppm glucose content, respectively, while the decocted extracts it has a glucose content of 44.51-, 42.78-, and 44.51ppm, respectively.
Keywords: *Alpha-amylase assay, Phytochemicals, fresh and decocted extracts, Glucose level*

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INTRODUCTION

Diabetes mellitus is a metabolic disorder. High blood glucose levels are characteristic of diabetes mellitus because of inadequate pancreatic insulin secretion or poor insulin-directed mobilization of glucose by target cells. Diabetes mellitus is aggravated and associated with metabolic complications that can subsequently lead to premature death [1]. It is the fourth leading causes of death in the most developed countries and there is substantial evidence that it is epidemic in many developing and newly industrialized nations. Hence, this is posing a serious threat to be met within 21st century [2].

There is a need to develop strategies for diabetes management in order to decrease its incidence and deaths from diabetes-related complications. One therapeutic approach for management of type 2 diabetes is to reduce glucose absorption by inhibiting alpha-amylase and alpha-glucosidase involved in starch degradation [3]. There were patented medicines for diabetes that are now available in the market. The people around the world are using these. Still there are many who preferred to use herbal medicines and some uses folkloric medications to save money. Many plants have been used for the treatment of diabetes mellitus but only few were evaluated as per modern system of medicine [4]. Herbal medicines have been widely utilized as effective remedies for the prevention and treatment of multiple health conditions for centuries by almost every known culture. The first documented records of herbal medicine use date back 5,000 years in China. Similarly, India's Ayurvedic medicine tradition is thought to be more than 5,000 years old and herbal medicines remain an essential component of its practice [5].

The plants *Allium schoenoprasum*, *Capsicum frutescens*, and *Sechium edule* are abundant in the Philippines. They are commonly used as food and condiments of the Filipino people. As believed by folkloric people,

these plants promote weight loss. Thus, the purpose of this study is to determine the phytochemicals present in fresh and decocted extracts of *Allium schoenoprasum*, *Capsicum frutescens*, and *Sechium edule*, to evaluate the hypoglycemic potential of plant extracts using alpha-amylase inhibition assay and to determine its glucose content using colorimetric method.

MATERIALS AND METHODS

Sampling and preparation

Plant samples were collected in the area of Mindanao State University, Marawi City, Lanao del Sur, Philippines. Fresh plant samples were washed and cleaned using running tap water. Excess water was wiped using a dry cloth. Fresh extracts were prepared using few leaves of the plant samples and small amount of water, it was then pound using a mortar and pestle. It was then filtered using a cheesecloth. For the decocted extracts, 20g of each plant sample was boiled in 100mL distilled water for 30 minutes, allowed to cool and filtered.

Phytochemical screening

Phytochemical screening was carried out for the fresh and decocted extracts to determine the presence of bioactive compounds [6-9].

Detection of alkaloids (Dragendorff's test)

Two milliliters of the fresh and decocted extracts were added individually with 2N hydrochloric acid and filtered. Two milliliters of the filtrates were treated with 6 drops of Dragendorff's reagent. Formation of orange precipitate indicates the presence of alkaloids.

Detection of saponins (Foam test)

Two milliliters of each plant extracts with 2mL distilled water were shaken. Formation of foam that persists for ten minutes indicates the presence of saponins.

Detection of terpenoids (Salkowski's test)

Two milliliters of each plant extracts was treated with chloroform. Few drops of concentrated sulfuric acid were added, shaken and allowed to stand. The appearance of golden yellow color indicates the presence of triterpenes.

Detection of phytosterols (Libermann Burchard's test)

Two milliliters of each plant extracts were added with 2mL chloroform. The extracts were then treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulfuric acid was then added. The formation of brown ring at the junction indicates the presence of phytosterols.

Detection of tannins (Ferric Chloride test)

Two milliliters of each plant extracts were added with 3-5 drops of 5% ferric chloride solution. The formation of brown color indicates the presence of phenols.

Detection of flavonoids (Alkaline reagent test)

Two milliliters of each plant extracts were treated with few drops of 0.1N sodium hydroxide solution. The formation of intense yellow color, which becomes colorless on addition of dilute acid indicates the presence of flavonoids.

Detection of cardiac glycosides (Keller Killiani's test)

Two milliliters of each plant extracts were dissolved in 1mL glacial acetic acid containing 1 drop of 5% ferric chloride solution and was under layered with concentrated sulfuric acid. The brown ring obtained in the interface indicated the presence of cardiac glycosides.

Detection of reducing sugars (Fehling's test)

Two milliliters of each plant extracts were added with 1mL of both Fehling's A, and B and was then shaken and heated in a water bath for ten minutes. The formation of a brick-red precipitate indicates the presence of a reducing compound.

Test for phenolics

Two milliliters of each plant extract was added with 1mL of 1% ferric chloride solution. Blue or green color indicates the presence of phenolics.

Test for proteins and amino acids (Xanthoproteic test)

One milliliter of each plant extract was treated with few drops of conc. nitric acid. Formation of yellow color indicates the presence of proteins.

Hypoglycemic assay

α-amylase inhibition activity

Twenty microliters each of fresh and decocted extracts was mixed with 20μL of 0.02mol/L sodium phosphate buffer (pH 6.9) and 20μL of α-amylase solution (4.5units/ml/minute) and pre-incubated at 25°C for 10 minutes. Then, 20μL of 1% freshly prepared starch solution was added and was incubated again at 37°C for 30 minutes and the reaction was stopped by the addition of 200μL 3,5-dinitrosalicylic reagent. The test tube was then incubated in a boiling water bath for 5 minutes and then cooled to room

temperature. The reaction mixture was diluted with 3mL distilled water and the absorbance was measured at 511nm. The readings were compared with the control and α-amylase inhibition activity (%) was calculated [7, 11].

Colorimetric determination of glucose concentration

Two hundred microliters of the plant sample was added with two hundred microliters of 3,5-dinitrosalicylic acid reagent and was then diluted with 4mL distilled water. The mixture was then heated at 90°C for 5-15 minutes to develop a red-brown color. To stabilize the color, 33μL of 40% potassium sodium tartrate solution was then added and then cooled at room temperature. The absorbance was recorded using a spectrophotometer at 533.6nm. UV-Vis spectroscopy was done at the Chemistry Department, Mindanao State University-Iligan Institute of Technology, Iligan City, Lanao del Norte, Philippines. The glucose concentrations of the samples were determined using the calibration curve obtained from the standard glucose sample [7, 12].

RESULT AND DISCUSSION

Phytochemical screening

As shown in Table 1 the fresh extracts of *Allium schoenoprasum, Capsicum frutescens* and *Sechium edule* have trace of alkaloids. Trace of terpenoids was found in the fresh extracts of *Capsicum frutescens* and *Sechium edule.* Alkaloids and terpenoids were determined to have antifungal, antibacterial and anti-inflammation activities [13]. It was found that all the plant materials used have saponins. Saponins are naturally occurring surface-active glycosides, mainly produced by plants, whose structure consists of a sugar moiety linked to a hydrophobic aglycone (a steroid or a triterpene). Many pharmacological properties have been reported for these compounds, such as anti-inflammatory, immunostimulant, hypocholesterolemic, hypoglycemic, antifungal and cytotoxic activities [14]. However, there is no presence of phytosterols in all extracts. Phytosterols appear to be important in body weight control [14]. Tannins are moderately observed in the fresh and decocted extracts of *Allium schoenoprasum* and slightly evident in *Sechium edule*. In extensive biological tests, representative of tannins were found to have antiviral, antibacterial, and antitumour activity [15]. Flavonoids are evident in all extracts except for the fresh extracts *Capsicum frutescens* and *Sechium edule*. Flavonoids are widely distributed in the plant kingdom and present in considerable quantities in common food products, spices, and beverages, have been used since ancient times by physicians and laymen to treat a great variety of human disease such as diabetes, coronary heart disease, and cancer [16]. Cardiac glycosides are evident in the fresh extract of *Sechium edule*. Some cardiac glycosides are clinically used for the treatment of heart failure. Phenolics were found in all plant extracts except for the decocted *Capsicum frutescens*. Phenolic compounds are well known as antioxidant and scavenging agents against free radicals associated with oxidative damage [17]. Reducing sugars are observed in all extracts except for the decocted extract of *Sechium edule*. And for proteins and amino acids, all plant extracts have shown positive results.

Table 1: Phytochemical constituents present in the fresh and decocted extracts of each plant material.

Phytochemical analysis	*Allium schoenoprasum*		*Capsicum frutescens*		*Sechium edule*	
	FE	DE	FE	DE	FE	DE
Test for alkaloids (formation of yellow precipitate)	+	-	+	-	+	-
Test for saponins (persistent foam)	++	+++	++	+	+++	+
Test for terpenoids (brown ring at the junction)	-	-	+	-	+	-
Test for phytosterols (brown ring at the junction)	-	-	-	-	-	-
Test for tannins (presence of brown precipitate)	++	++	-	-	-	+
Test for flavonoids (appearance of yellow color)	++	++	-	+++	-	++
Test for cardiac glycosides (brown ring at the junction)	-	-	-	-	++	-
Test for reducing sugars (presence of red precipitate)	+	+	++	+	+	-
Test for phenolics (appearance of green color)	+	+	+++	-	+++	+
Test for proteins and amino acids (appearance of yellow color)	+	+	+	++	+	+

Legend: +++ (aplenty present), ++ (moderately present), + (trace), - (absent) FE–Fresh extract, DE–Decocted extract.

Hypoglycemic activity

Pancreatic alpha-amylase catalyzes the hydrolysis of the alpha-1,4-glycosidic linkages of starch, amylose, amylopectin, glycogen and various maltodextrins and is responsible of most of starch digestion in humans [18]. Dietary α-amylase inhibitors that act in the gut by inhibiting the enzymatic breakdown of starch, soluble carbohydrates and their derived and digested products have been identified as a potentially natural and safe approach for controlling hyperglycemia through decreasing of meal-derived glucose absorption [19].

Alpha-amylase inhibition activity

Table 2 summarizes the calculated percent (%) inhibition of each plant extract in the alpha-amylase inhibition assay. Percent inhibition was calculated using the formula,

$$\%\ Inhibition = \frac{\left|Abs_{control} - Abs_{sample}\right|}{Abs_{control}}\ x\ 100$$

The maximum wavelength, λ_{max}, used in the analysis was 511nm and the absorbance range was 0.0 to 6.00. The absorbance obtained from the control was 4.664.

s shown in Table 2, the fresh extracts of *Allium schoenoprasum*, *Capsicum frutescens*, and *Sechium edule* have percent inhibitions of 79.70-, 72.66-, and 80.77%, respectively. For the decocted extracts, their percent inhibitions are 75.15-, 82.10-, and 80.40%, respectively. The extract that shows highest percent inhibition is the decocted extract of *Capsicum frutescens*. Tannins, phenols and flavonoids are alpha-amylase inhibitors and also called as starch blockers since it prevents or slows the absorption of starch into the body mainly by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltose, maltriose and other simple sugar [20]. Reducing sugar can be attributed to lower the activity of amylase and invertase.

Table 2: Percent (%) alpha-amylase inhibition of the fresh and decocted extracts of the plant samples.

Plant sample	Extract	Absorbance	% Inhibition
Allium schoenoprasum	Fresh	0.9470	79.70
	Decocted	1.1590	75.15
Capsicum frutescens	Fresh	1.2750	72.66
	Decocted	0.8350	82.10
Sechium edule	Fresh	0.8970	80.77
	Decocted	0.9140	80.40

Colorimetric determination of glucose concentration

Presence of free hydroxyl group (O-H), so-called reducing sugars, in this method was determined. This involves the oxidation of the hydroxyl group present in glucose and fructose [7]. Simultaneously, 3,5-dinitrosalicylic acid (DNS) is reduced to 3-amino-5-nitrosalicylic acid under alkaline conditions.

Table 3: Glucose concentration present in the fresh and decocted extracts of the plant samples.

Plant sample	Extract	Absorbance	Glucose concentration
Allium schoenoprasum	Fresh	0.0060	43.28
	Decocted	0.0190	44.51
Capsicum frutescens	Fresh	0.1660	58.51
	Decocted	0.0080	42.78
Sechium edule	Fresh	0.0120	43.85
	Decocted	0.0190	44.51

As presented in Table 3, the glucose concentration of the fresh extracts of *Allium schoenoprasum*, *Capsicum frutescens*, and *Sechium edule* are 43.28-, 58.51-, and 43.85ppm, respectively. In addition, for the decocted extracts, the concentrations are 44.51-, 42.78-, and 44.51ppm, respectively. The extract with the highest and the lowest absorbance are from fresh and decocted *Capsicum frutescens*, correspondingly, which means, it also has the highest and lowest concentration of glucose. As observed in the three samples, the relationship between the absorbance and the concentration of glucose is directly proportional, that is, when the absorbance of the sample increases, the glucose concentration in the sample also increases.

CONCLUSION

Presence of secondary metabolites was determined in all plant extracts. The following metabolites are alkaloids, saponins, phytosterols, flavonoids, terpenoids, tannins, cardiac glycosides, reducing sugars, phenols, proteins and amino acids. These bioactive components have corresponding therapeutic properties such as antioxidant, antimicrobial, antiinflammatory and hypoglycemic properties. Some of these metabolites were used as enzyme inhibitors.

The plant extract with the highest percent inhibitory potential is the decocted extract of *Capsicum frutescens* with 82.10%. Colorimetric determination of glucose content shows that fresh plant extract of *Capsicum frutescens* has the highest glucose concentrations in all samples.

CONFLICT OF INTEREST

No conflict of interest.

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