Advances in Bioresearch
Adv. Biores., Vol 9 (3) May 2018: 91-97

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.9.3.9197

Advances in Bioresearch

ORIGINAL ARTICLE

Phytochemical Screening, Glucose Concentration and *in vitro*Hypoglycemic Potential of the Extracts of *Origanum vulgare* L., *Manihotesculenta, Carica papaya* Linn. and *Coffea arabica* Linn.

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ABSTRACT
The fresh leaf extracts of Origanumvulgare L. (Lamiaceae), Manihotesculenta, Carica papaya Linn. andCoffeaarabica Linn. were used as plant samples in this study. Fresh and decocted extracts of the plant samples were subjected to phytochemical screening, colorimetric determination and in vitro α-amylase inhibition assay to determine their bioactive components, glucose concentration and hypoglycemic potential, respectively. The results in phytochemical screening confirmed the presence of saponins and reducing sugars both in fresh and decocted extracts of the four plant samples. In addition to that, alkaloids, flavonoids, terpenoids, tannins, phenolics, cardiac glycosides, proteins and amino acids were also observed but not in all plant samples. No presence of phytosterols were detected in the fresh and decocted extracts of the plant samples. For the colorimetric determination of the glucose concentration, fresh extracts of O. vulgare L. has the highest value of concentration with 71.03ppm. There is an observable increase in glucose concentration of the plant samples as the absorbance of the plant sample extracts increases. Decocted leaf extracts of C. papaya Linn. gave the highest inhibition of alpha-amylase activity with a percent inhibition of 61.02%. Presence of some bioactive components such as flavonoids, cardiac glycosides and reducing sugars can possibly attribute to this effect.Hence, fresh and decocted leaf extracts of O. vulgare L., M esculenta, C. papaya Linn. and C. arabica Linn. possess hypoglycemic potential by inhibiting α-amylase.
Keywords; *Phytochemicals, glucose content, alpha-amylase assay, colorimetric determination*

Received 14.11.2017 Revised 13.01.2018 Accepted 02.03.2018

How to cite this article:
Norkila D. Diangca, Merell P. Billacura. Phytochemical Screening, Glucose Concentration and *in vitro* Hypoglycemic Potential of the Extracts of *Origanum vulgare* L., *Manihotesculenta, Carica papaya* Linn. and *Coffea arabica* Linn.. Adv. Biores. Vol 9 [3] May 2018: 91-97.

INTRODUCTION

Diabetes mellitus is an endocrine disorder due to the inability of the insulin to regulate the homeostasis of the carbohydrates, lipids and proteins. This would result to hyperglycemia. The worldwide prevalence of diabetes has risen to 8.5% among adults, according to statistics [1], which is a staggering rise in prevalence over the last 34 years (4.7% in 1980). As such, the disease now represents a major global health challenge with over 422 million people suffering from diabetes. The efficacies of the synthetic oral hypoglycemic agents are in debate due to the unwanted side effects of these compounds, hence, a search for a new drug for the treatment of diabetes is in demand [3]. One of the seen options of the treatment of post-prandial hyperglycemia is to prevent absorption of glucose by the inhibition of carbohydrate-hydrolyzing enzymes, such as α-glucosidase and α-amylase [2].

Plants contain phytochemicals with medicinal properties. Phytochemicals are not considered essential for normal body function, however, researches revealed that chronic diseases associated with aging are easier to prevent than to treat, and that consumption of phytochemical-rich fruits, vegetables, herbs and spices, can potentially reduce the risk of developing such conditions [4].

The use of traditional medicine remains well-known in developing countries, while use of complementary and alternative medicine is increasing popularly in developed countries. It is sometimes also the only affordable source of health care especially for people relied on cheaper alternative treatment. Using plant based drugs has been known for years as safe and cheaper compare to the synthetic oral hypoglycemic

drugs. The World Health Organization has recommended the evaluation of the traditional plant treatments for they are safe, effective and less to no side effects [5]. Hence, consideration in using plants that are rich in antidiabetic phytochemicals is given much attention.

In this study, phytochemical analysis, determination of glucose content and *in vitro* hypoglycemic assay of the fresh and decocted leaf extracts of *Origanum vulgare*L., *Manihot esculenta, Carica papaya* Linn. and *Coffea arabica* Linn. were conducted to evaluate its potential as a source of hypoglycemic agent.

MATERIAL AND METHODS

Collection of samples

The leaves of *Origanum vulgare* L., *Manihotesculenta and Carica papaya* Linn. were collected within the vicinity of Mindanao State University-Main Campus while the leaves of *Coffea arabica* Linn. were purchased at the downtown market of Marawi City, Lanao del Sur, Philippines. The collected plant materials were washed with running tap water followed by distilled water to remove dust and other unwanted particles. The sample extracts were prepared prior to use.

Preparation of fresh and decocted sample extracts

Fresh sample extracts were obtained by pounding the plant materials using a mortar and pestle in which 5-10mL of distilled water was added. The extracts were filtered using clean cheesecloth. For the decocted sample extracts, 20g of each plant materials were boiled in 100mL of distilled water for 15 minutes. It was then filtered and the residue was discarded.

Phytochemical screening of the fresh and decocted sample extracts

The phytochemical analysis of fresh and decocted plant extracts were carried out to determine the presence of various bioactive components using the procedures set by Billacura and Laciapag [6],and Tiwari *et. al*. [7].

Detection of alkaloids (Dragendorff's test)

4 mL of fresh and decocted sample extracts were dissolved separately with 2mL of 2N HCl and then filtered. The filtrates were then treated with 6 drops Dragendorff's reagent (solution of Potassium Bismuth Iodide). Formation of orange precipitate indicates the presence of alkaloids.

Detection of flavonoids (Alkaline reagent test)

2-3 mL of the extracts were treated with few drops of 0.1N NaOH solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Detection of saponins (Foam test)

2-3 mL of the extracts were shaken with 2mL of distilled water. If foam produced persists for 10-15 minutes it indicates the presence of saponins.

Detection of proteins and amino acids (Xanthoproteic test)

1-2mL of the extracts was treated with few drops of concentrated nitric acid. Formation of yellow color indicates the presence of proteins.

Detection of terpenoids (Salkowski's test)

3 mL of the extracts were treated with chloroform. The extracts were treated with few drops of concentrated sulfuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

Detection of phytosterols (Libermann Burchard's test)

2-3 mL of the extracts were treated with 2mL chloroform. The extracts were treated with few drops of acetic anhydride, boiled, and cooled. Then concentrated sulfuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of tannins (Ferric Chloride test)

2-3 mL of the extracts were treated with 3-4 drops of 5% ferric chloride solution. Formation of brown precipitate indicates the presence of phenols.

Detection of phenolics

2-3 mL of the extracts were treated with 2mL of 1% ferric chloride solution. Formation of green color indicates the presence of phenols.

Detection of cardiac glycosides (Keller Killiani's test)

2-3 mL of the extracts were dissolved in 2 mL glacial acetic acid containing 1 drop of 5% ferric chloride solution and underlayered with concentrated sulfuric acid. A brown ring obtained in the interface indicated the presence of a de-oxy sugar.

Detection of reducing sugars (Fehling's test)

1-2 mL of the extracts were added with 1mL of both Fehlings A and B. The resulting solution was mixed and placed in a boiling water bath for 10 minutes and was allowed to cool for several minutes. Formation of a brick-red precipitate indicates the presence of reducing sugars.

Colorimetric determination of glucose concentration

The determination of glucose concentration in fresh and decocted plant extracts were carried out using the method described by Billacura and Alansado [8] and Miller [9].

Analysis of known sample concentration: Calibration curve

Standard glucose solution of 50, 100, 150, 200 and 350ppm were obtained by serial dilution of the stock solution. The λ_{max} was scanned and their absorbance were then determined to plot a calibration curve.

Analysis of unknown sample concentration

One hundred microliters of the sample extract were added with one hundred microliters of 3,5-dinitrosalicylic acid reagent (DNSA) and it was diluted with 4mL (20-fold times) distilled water. The mixture was then heated at 90^{o}C for 5-15 minutes to develop a red brown color. To stabilize its color, thirty-three microliters of 40% potassium sodium tartrate solution was added. The sample was then cooled to room temperature. The absorbance of the sample extract was determined using a UV-Vis spectrophotometer at 533.6nm. The glucose concentrations of the samples were determined using the calibration curve obtained from the standard glucose samples.

Hypoglycemic assay

The test for *in vitro* hypoglycemic potential of fresh and decocted plant extracts were carried out using an α-amylase solution using the procedures described by Billacura and Alansado [8] and Manual [10].

α-amylase inhibition activity

20-30 μL of each plant extract was mixed with 20μL of 0.02 mol/L sodium phosphate buffer (pH 6.9) and 20-30 μL α-amylase solution (4.5 units/mL/minute) and then pre-incubated at 37°C for 10-15 minutes. 20μL of 1% freshly prepared starch solution was added and was incubated again at 37°C for 25-30 minutes and the reaction was stopped by the addition of 0.2mL DNSA reagent. The test tube was then incubated in a boiling water bath for 5-10 minutes and then cooled to room temperature. The reaction mixture was diluted with 3mL of distilled water and the absorbance was measured at 511nm. The readings were compared with the control and α-amylase inhibition activity (%) was calculated using the formula below:

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

Spectroscopic analysis was done using a double beam UV-Visible Spectrophotometer (Lasany Li 2800) at the Chemistry Department, Mindanao State University-Iligan Institute of Technology, Iligan City, Philippines.

DISCUSSION

Phytochemical screening of the fresh and decocted sample extracts

The systematic screening of the bioactive substances from medicinal plants is a procedural necessity to support their traditional claims in relieving various diseases. Aside from that, it gives an insight on what complex metabolites are intended to be extracted for therapeutic purposes or used as precursors for the synthesis of drugs.

The results presented in Table 1 show the presence of saponins and reducing sugars in the fresh and decocted extracts of *O. vulgare* L.*, M. esculenta, C. papaya* Linn. and *C. arabica* Linn.. Saponins are known to have anticarcinogenic property [11] and elicit antihyperlipidemic action by inhibiting intestinal lipid absorption via resin-like action and inhibition of lipase activity [12]. The presence of reducing sugars in the samples implied that they have hypoglycemic potential[6]. However, the presence of phytosterols, a good cholesterol-lowering agent according to Ostlund[13], was not detected in the four plant samples.

Alkaloids were detected in the fresh extracts except for *M. esculenta*. The presence of the alkaloids showed that the sample has antimicrobial activity by inhibiting DNA topoisomerase [10]. Flavonoids were detected in the decocted extracts of *O. vulgare*L.*, M. esculenta, C. papaya* Linn. and*C. arabica*Linn. and in the fresh extract of *O. vulgare*L. Flavonoids belong to the group of polyphenolic compounds and are typically known for health promoting properties such as antioxidant, antiallergic, antiinflammatory, antimicrobial and anticancer properties [14].Proteins and amino acids are present in the fresh and decocted extracts of the four plant materials except for the decocted extract of *M. esculenta*. The presence of these metabolites indicated that the sample is a good antibiotic and antimicrobial agents[15]. Terpenoids were present in the fresh and decocted extracts of *O. vulgare* L. and *C. arabica* Linn.. It was also detected in the decocted extract of *C. papaya* Linn. but not on its fresh extract. These metabolites can have medicinal properties such as anticarcinogenic, antimalarial, anti-ulcer, hepaticidal, antimicrobial or diuretic activity [16]. For tannins, they were detected in the fresh and decocted extract of both *O. vulgare*

L. and *C. papaya* Linn.. According to Anbuselvi *et. al.* [11], tannins reduces the risk of coronary heart diseases. For phenolics, they were found to be present in all of the sample extracts except for the fresh extract of *M. esculenta*. Phenolics have the ability to protect the plants from microbial infections. They have potential anti-oxidative properties but are also potent anti-infectives [17]. Cardiac glycosides, on the other hand, was only detected in the decocted extract of *C. papaya* Linn. Therapeutic uses of cardiac glycosides primarily involve the treatment of cardiac failure [18].

Table 1. Phytochemical screening of the fresh and decocted extracts of *Origanum vulgare* L., *Manihot esculenta, Carica papaya* Linn. and *Coffea arabica* Linn.

Secondary Metabolites	*O. vulgare*L.		*M. esculenta*		*C. papaya* Linn.		*C. arabica*Linn.		Changes
	FE	DE	FE	DE	FE	DE	FE	DE	
Alkaloids	+	-	-	-	++	-	++	-	Yellow precipitate
Flavonoids	+	+	-	+	-	+	-	++	Intense yellow color
Saponins (Foam test)	+	+++	+	+++	+	+	++	+	Persistent foam
Proteins and amino acids	+	++	+++	-	+++	+	+++	+	Yellow color
Terpenoids	+	+	-	-	-	+	+++	++	Golden yellow color
Phytosterols	-	-	-	-	-	-	-	-	Brown ring at the junction
Tannins	+	+	-	-	+	+	-	-	Brown precipitate
Phenolics	+	+	-	+	++	++	+	+	Green color
Cardiac glycosides	-	-	-	-	-	++	-	-	Brown ring at the junction
Reducing sugars	+	+	+	+	+	+	+++	+++	Brick red precipitate

Legend: (-) absence, (+) trace, (+)(+) present, (+)(+)(+) abundant. FE- fresh extract and DE- decocted extract

Colorimetric determination of glucose concentration

This method is used in order to determine the free hydroxyl group (-OH) called reducing sugars. It involves the oxidation of the free hyroxyl group present in, for example, glucose and the ketone functional group in fructose [8]. Simultaneously, 3,5-dinitrosalicylic acid (DNSA) is reduced to 3-amino-5-nitrosalicylic acid.The λ_{max} for colorimetric determination of glucose concentration was 533.6nm. It was calculated that the slope of the calibration curve for the glucose standard is 0.0105 and the y-intercept is -0.4528 with an R^2= 0.9998.

Table 2 shows the concentration of glucose in the fresh and decocted extracts of the four plant samples. Glucose concentration present in the sample was calculated using the formula below.

$$\text{Glucose concentration (x)} = \frac{\text{Absorbance} - (\text{y} - \text{intercept})}{\text{slope}}$$

Table 2. Glucose concentration of the fresh and decocted extracts of *Origanumvulgare*L., *Manihotesculenta, Carica papaya* Linn. and*Coffeaarabica*Linn..

Plant sample	Plant extracts	Absorbance	Glucose concentration (ppm)
*O. vulgare*L.	Fresh	0.2930	71.03
	Decocted	0.0070	43.79
M. esculenta	Fresh	0.1170	54.27
	Decocted	0.0370	46.65
C. papaya Linn.	Fresh	0.1690	59.22.
	Decocted	0.0280	45.79
*C. arabica*Linn.	Fresh	0.2910	70.84
	Decocted	0.2830	70.08

It can be observed that the fresh extracts of *O. vulgare*L., *M. esculenta, C. papaya* Linn.and *C. arabica*Linn. have higher glucose concentration compared to their decocted counterparts. The fresh extract of *O. vulgare* L. has the highest concentration of 71.03ppm while its decocted extract has the glucose

concentration of 43.79ppm. We can observe that glucose concentration is directly proportional with its absorbance, i.e., as the absorbance increases, the concentration of glucose increases.

Hypoglycemic activity

Hypoglycemic activity of medicinal plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output, inhibiting the intestinal absorption of glucose or facilitating metabolites in insulin dependent processes [19-20].

Alpha- amylase inhibition activity

The λ_{max} for α-amylase inhibition activity was 511nm. Table 3 summarizes the calculated percent inhibition of each extract in the α-amylase inhibition assay. Percent inhibition of each assay was calculated using the formula below.

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

Hyperglycemia is a condition characterized by a rapid increase in blood glucose levels. Postprandial hyperglycemia has been linked to the onset of diabetic complications in Type-2 diabetic patients due to the generation of free radicals leading to damage in the retina, renal glomerulus and peripheral nerves. One of the therapeutic approaches for decreasing postprandial hyperglycemia is to retard the digestion of glucose by the inhibition of these carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase in the digestive tract [21].

Alpha-amylase is a prominent enzyme found in the pancreatic juice and saliva which breaks down large insoluble starch molecules into absorbable molecules. Therefore, inhibition of these carbohydrate-hydrolyzing enzymes can significantly decrease the postprandial hyperglycemia after a mixed carbohydrate diet and can be a key strategy in the control of diabetes mellitus [21].

Table 3. Percent (%) alpha- amylase inhibition of the fresh and decocted extracts of *Origanum vulgare*L., *Manihotesculenta, Carica papaya* Linn. and *Coffea arabica* Linn.

Plant sample	Plant extracts	Absorbance	% Inhibition
*O. vulgare*L.	Fresh	1.0203	58.78
	Decocted	1.0620	58.29
M. esculenta	Fresh	1.2168	58.51
	Decocted	1.2382	57.16
*C. papaya*Linn.	Fresh	1.1885	55.51
	Decocted	0.9625	61.02
*C. arabica*Linn.	Fresh	1.1838	60.89
	Decocted	1.3300	58.38

As shown in Table 3, the decocted leaf extracts of *C. papaya* Linn. have the lowest absorbance among the plant extracts and has the highest inhibition of alpha-amylase activity. According to Kazeem*et. al*. [21], plant extracts that inhibit alpha- amylase competitively, suggests that the active components in the extract compete with the substrate for binding to the active site of the enzyme thereby preventing the breaking down of oligosaccharides to disaccharides. The alpha-amylase inhibition of the decocted *C. papaya* Linn. might have been due to the presence of several phytochemicals such as flavonoids, reducing sugars, cardiac glycosides, saponins, proteins and amino acids, terpenoids, phenolics and tannins.Previous studies on alpha-amylase inhibitors isolated from medicinal plants suggest that several potential inhibitors belong to flavonoid class which has features of inhibiting alpha-amylase activities [22]. The presence also of reducing sugars might have contributed to its inhibitory activity. Reducing sugars has a hypoglycemic effect for the reason that the free hydroxyl group at the anomeric carbon of glucose is oxidized into carbonyl group [8].

The fresh extracts of *O. vulgare* L., *M. esculenta* and *C. arabica* Linn. showed a relatively high percent inhibition compared to their decocted counterpart. Based on the phytochemical screening results, reducing sugars, saponins and proteins and amino acids were detected on the fresh extracts of *O. vulgare* L., *M. esculenta* and *C. arabica* Linn.. Compared to the decocted extract of *C. papaya* Linn., cardiac glycosides was not detected as one of their bioactive components, which suggests that its presence in the decocted *C. papaya* Linn. might have been one of the reasons why it has the highest percent inhibition.

In general, the calculated values for the percent inhibition of α-amylase of each plant extract are very high that we can say that the samples have high hypoglycemic potential. It coincides to the results of the phytochemical screening wherein all of the samples exhibited positive results in reducing sugars.

CONCLUSION

In this study, phytochemical components of the fresh and decocted sample extracts were confirmed. The results indicated that the plant extracts contain reducing sugars, a bioactive component which is said to have hypoglycemic potential. Results in colorimetric determination of glucose showed that fresh extracts of *O. vulgare*L., *M. esculenta, C. papaya* Linn. and *C. arabica* Linn. have relatively high concentration compared to their decocted counterpart. Fresh extract of *O. vulgare* L. has the highest value among them with a concentration of 71.03ppm at 0.2930.

In addition, the hypoglycemic potential of the fresh and decocted sample extracts were assessed using an *in vitro* hypoglycemic activity by the alpha-amylase inhibition. The calculated values for their percent inhibition of α-amylase showed that the decocted extract of the *C. papaya* Linn. has the highest percent inhibition with the value of 61.02%. On the other hand, the fresh extract of the *C. papaya* Linn. showed the lowest percent inhibition of 55.51%.

Therefore, fresh and decocted leaf extracts of *O. vulgare* L., *M esculenta, C. papaya* Linn. and *C. arabica* Linn. possess hypoglycemic potential by inhibiting α-amylase. The present study also suggests that the decocted extract of *C. papaya* Linn. has the highest α-amylase inhibitory activity among the leaf extracts. This can be attributed to the presence of some bioactive components such as flavonoids, cardiac glycosides and reducing sugars. Moreover, this study justifies and supports the claim on the hypoglycemic potential of *O. vulgare* L., *M esculenta, C. papaya* Linn. and *C. arabica* Linn..

However, further work should be carried out to isolate the active compounds responsible for the hypoglycemic potential of the said plant extracts and to understand their mode of action as well.

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