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HYPOGLYCEMIC EFFECT OF SOME ETHNOMEDICINAL PLANTS AND ITS APPLICATIONS ON PANCREAS IMPROVEMENT

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ABSTRACT

Diabetes is a disease that causes the blood glucose level to rise and if left untreated, could yield in many complications such as amputations of body parts, muscle disorders and fatigue. The alarming increase in the number of patients suffering from diabetes requests for more studies to be conducted to find alternatives in producing a cure to this disease. Many chemical methods are adapted towards the treatment of diabetes, so it is crucial that studies on medicinal plants are carried out to produce a solution that does not rely on chemicals alone, and can inhibit the diabetic activity as much as the existing medications. This study was conducted to collect and extract ethnomedicinal plants from Malaysia using different solvents, to select the most potent ethnomedicinal plant extract using anti-diabetic screening assays and to screen for combination effects of the plant extracts on anti-diabetic activity. The plant extracts are then subjected to α-amylase and assays whereby detection of potent plants that result in more than 50% inhibition

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is selected. Selected plants are combined to one another in dual, triple and quadruple plant extract combinations and anti-diabetic assays are performed once again to find the most effective combination that gives the highest inhibition value. Once the combined inhibition values are obtained, these values determined and classified into synergistic and antagonistic groups depending on its relationship with one another, whether it enhances inhibition value or not. For alpha-amylase inhibition, the chloroform extract of *E. longifolia* gave the highest inhibition of α-amylase enzyme at 82.42%.

***Keywords**:* Antidiabetic, Ethnomedicinal, α-Amylase, Combination

INTRODUCTION

Diabetes mellitus is a categorized as a metabolic disease that is represented by hyperglycaemia that occurs due to defects in insulin secretion, insulin action, or both (Letchuman et al., 2010). The protracted hyperglycaemic effect of diabetes is contributes to malfunctioning of certain organs such as the kidney, eyes, heart and other blood vessels as well as causing a long-term damage and dysfunction of the organs (Patel, Kumar, Laloo, and Hemalatha, 2012). Diabetes mellitus is a very common disease, mainly due intake of excess sugary or starchy food that contains high content of carbohydrate. It has been increasing relatively since the 1960s (Letchuman et al., 2010). Symptoms of this wildly escalating disease includes the wounds and sores that take very long to heal, proneness toward infections, feeling thirsty often, having dry skin, feeling very distressed, urination very often, feeling intensely hungry, sudden weight loss, feeling numbness and tingling in the hands and feet and also sudden changes in vision. These symptoms could also be accompanied by nausea, vomiting, or stomach pains in the abrupt onset of insulin-dependent diabetes, now called Type 1 diabetes (Beagley et al., 2014).

Ethnomedicinal plants have variety of uses in treating diseases and illness such as kidney stones, muscle cramps, warts and diabetes. Even though the usage of traditional or ethnomedicine have been eroding rapidly due to the rise in modern technology medicine and treatment, it is important that documentation of such medicines is done for knowledge, in order to produce much efficient medicines (Malik, Bhat, Ballabha, Bussmann, and Bhatt, 2015). According to (Samuel et al., 2010), in Malaysia particularly, these ethnomedicinal plants are commonly and widely used by the 'Orang Asli' and can be found in many of its rural area such as the Kampung Bawong, Perak of West Malaysia. Despite the fact that ethnomedicinal plant refers to plants used by indeginious people from rural area to treat disease, the presence of variety of chemical identity possessed by a plant is what gives it a good pharmacological value and the anti-diabetic effect(Gharti Kul, Buddhi et al. 2015).

In an *in vitro* method performed by (Bhutkar and Bhise, 2012), it was found that the inhibition of alpha-amylase activity was possible using some aqueous indigenous plant extracts, namely *Tamarindus indica*, *Catharanthus roseus*, and *Caesalpinia bonducella*, whereby *C.bonducella* yielded in an inhibition as high as 87.26%. Starch was used as the substrate to the enzyme, alpha-amylase in order to yield in glucose production. However, presence of plants extract was meant to inhibit the activity of the alpha-amylase, therefore reducing the production of glucose. DNS reagent was used in this research paper to end the activity and readings were obtained at an absorbance of 540nm before inhibition is calculated.

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MATERIALS AND METHODS

Materials

For the collection and preparation of plant extracts, chemicals used includes the solvents, methanol, ethanol, chloroform and water. For the α-amylase inhibition assay based on the methods of (Kazeem et al., 2013), the chemicals used include the α-amylase enzyme (EC 3.2.1.1) from Saccharomyces cerevisiae, plant extracts, 0.02M PBS, 1% starch, DNS reagent and distilled water.

Collection and Extraction of Plant Extracts

In order to study the effects of ethnomedicinal plants on the anti-diabetic effect, the method of (Kazeem et al., 2013) is referred with modified procedure. About 7 different types of ethnomedicinal plants were picked from Kuantan, Pahang, Malaysia and as shown in table 1. The plant parts were cleaned and dried under shade at room temperature for approximately one week. Dried parts were ground to a powder using a grinder. The powder (10g) of the specific plant is then divided and extracted using different solvents, chloroform, ethanol, methanol and water (200ml). The extracts were left for 5 days in conical flasks wrapped in silver foil in order to steep. The infusion formed was then decanted, filtered, centrifuged (8000rpm/10 minutes) and evaporated by rotary evaporator. The concentrated extracts were then stored in bio-bottles, where the cap of the bottle was wrapped with silver foil and stored in the refrigerator.

Table 1: Plant parts used

NO	Plants name	Family	Part used
1	Averrhoa bilimbi	Oxalidaceae	Leaves
2	Andrographis paniculata	Acanthaceae	Whole plant
3	Orthosiphon stamineus	Lamiaceae	Leaves
4	Gynura procumbens	Asteraceae	Leaves
5	Eurycoma longifolia	Simaroubaceae	Roots
6	Punica granatum	Lythraceae	Peels
7	Swietenia macrophylla	Meliaceae	Seeds

Alpha-amylase Inhibition Assay

After the preparation and extraction of plant is done, α-amylase inhibitory assay was performed using the modified procedure of (Kazeem et al., 2013). About 50 µL of the plant extract was added in a micro-centrifuge tube together with 50 µL of 0.02M sodium phosphate buffer of pH 6.9 containing α-amylase solution (0.5mg/ml). Pre-incubation was done at 25°C for about 10 minutes. After that, 50µL of 1% starch solution in 0.02M sodium phosphate buffer of pH 6.9 was added at timed intervals. Then, the solution was incubated at 25°C for 10 minutes. In order to end the reaction, 100µL of the dinitrosalicyclic acid (DNS) reagent was added. Incubation of the tubes in boiling water for 5 minutes, followed by cooling down of the solution at room temperature was performed following that. An ml of distilled water was added to the reaction mixture prior to measuring the absorbance using the spectrophotometer at 540nm. The control was prepared by following the same procedure, except that the plants extract was substituted with distilled water. The blank was just the distilled water.

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Combination of Plant Extracts on Alpha-amylase Inhibition Assays

From the percentage value obtained, the most potent plant extract was taken and screened again for combination effects by combining two and more of the plant extracts that showed inhibition more than 50%, using the same procedure as (Kazeem et al., 2013) and (Kim et al., 2005). Then, the data analysis was performed and the most suitable and potent combined plant extract was identified.

Determination of Antagonistic and Synergistic Combined Plant Extracts and Identification of Most Potent Ratio of Plant Extracts

After the determination of inhibition percentage of the combined plant extracts, it was analyzed and grouped as antagonistic and synergistic by comparing the inhibition value from the single potent plant and combined potent plant.

RESULTS AND DISCUSSION

Alpha-amylase Inhibition Assay

The α-amylase inhibition assay yielded in positive results where the inhibition was more than 50% for only four plant extracts out of the 27 extracts as shown in table 2. The chloroform E.longifolia extract yielded in the highest inhibition for α-amylase inhibition assay, which is 82.42%, followed by chloroform P.granatum extract, 69.73%, chloroform A.paniculata extract, 68.24% and chloroform A.bilimbi extract, 50.55% as shown in figure 1. The positive results of more than 50% inhibition were only obtained for the four plants with chloroform as the solvent. This means that chloroform is an ideal solvent when it comes to extracting the compound of interest that inhibits the enzyme action compared to ethanol, methanol and water. This also indicates a good inhibition against the α-amylase enzyme, which can contribute to the reduction and normalization of blood glucose levels in diabetic patients.

Table 2: α-amylase enzyme inhibition (+/-) of plant extracts

NO	Plant name	Water	Chloroform	Ethanol	Methanol
1	A. bilimbi	-	+	-	-
2	A. paniculata	-	+	-	-
3	O. stamineus	-	-	-	-
4	G. procumbens	-	-	-	-
5	E. longifolia	-	+	-	-
6	P. granatum	-	+	-	-
7	S. mycrophylla	-	-	-	-

(+): present; (-): not detected.

Comparison the Result with Others Research Papers

According to (Pushparaj et al., 2000), based on studies performed using ethanolic extract of *A. bilimbi* on hypoglycemic effect and hypolipidimic effect, it was found that about 50% of the hypoglycemic effect was managed to be reduced in just two weeks. However, when the plant extract was tested against the enzyme alpha amylase in a research by (Ali et al., 2006a) in Malaysia, it did not yield in a positive results. According to (Premanath and Nanjaiah, 2015),

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when rats that were streptozotocin induced was treated with *A. paniculata* leaf extract, it has been recorded that the fasting blood glucose level decreased. Based on studies conducted by (Verma et al., 2013), STZ-induced rats showed normalization of pancreas activity when treated with the ethanolic extract of *A.paniculata*. When tested for normoglycaemic and hyperglycaemic effects on rats, aqueous extracts showed positive and high effects for hyperglycaemic whereas no significant reduction for normaglycaemic effect (Bhat and Karim, 2010). According to (Ayesha, 2016), rats with induced diabetes that has been treated with pomegranate peel extracts showed hypoglycaemic effects, as well as hypolipidemic effects. Pomegranate aqueous extract also showed positive results when tested with the STZ-induced rats (Bagri, et al., 2009). Based on the above studies, when we compared between all these results and the result of this study. According to α-amylase inhibition assay for 7 plants, yielded to positive results with chloroform solvent. While the others solvents water, methanol and ethanol were yielded to negative results.

Combination Effect of Plant Extracts on Alpha-amylase Inhibitory Assay

Table 3 shows the highest inhibition percentage obtained in the combination of two plants was between the chloroform extract of *E. longifolia* and the chloroform extract of *A. paniculata*, which yielded in 77.04% inhibition of α-amylase. Lowest inhibition was seen for the combination of chloroform extract of *E. longifolia* with chloroform extract of *A. bilimbi*, which is only 9.30% inhibition of α-amylase enzyme. As for the triple combination of the plant extracts, the combination of chloroform extract of *E. longifolia*, chloroform extract of *A. bilimbi* and chloroform extract of *P. granatum* yielded in the highest percentage of inhibition which was 51.37% inhibition of α-*amylase* enzyme. The lowest percentage of inhibition was recorder for the combination of chloroform extract of *E. longifolia* with chloroform extract of *A. bilimbi* and chloroform extract of *A. paniculata*, which was 12.22% inhibition of α-amylase as shown in table 3. As for the quadruple combination of all four potent plants, the chloroform extract of *E. longifolia*, chloroform extract of *A. bilimbi*, chloroform extract of *A. paniculata* and chloroform extract of *P. granatum*, the inhibition percentage that was obtained was only 3.54%, lesser than any of the inhibition percentages of the four plants as in table 3 bellow. Overall, all of the plants tested for combination effect under α-*amylase* inhibition assay yielded in antagonistic relationship, whereby they did not cause any increase in the inhibition percentage as compared to the single plant extract. That's mean, the single plant yields in higher inhibition percentage as compared to the plants combined therefore, the combination of the potent plants are not suitable for the inhibition activity. The plant might as well be used as a single plant extract with solvent to reduce and normalize the blood glucose levels for better result.

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Table 3: Combination of two, triple and quadruple plant extracts on α-amylase inhibition (%).

Plant extract	Inhibition%
ElE : OsC	9.30
ElE : ApC	77.03
ElE :PgC	64.91
OsC : Apc	16.17
OsC : PgC	31.80
ApC : PgC	55.64
ElC : AbC : ApC	12.22
ElC : AbC : PgC	51.37
ElC : ApC : PgC	46.52
AbC : ApC : PgC	35.71
ElC : AbC : ApC : PgC	3.53

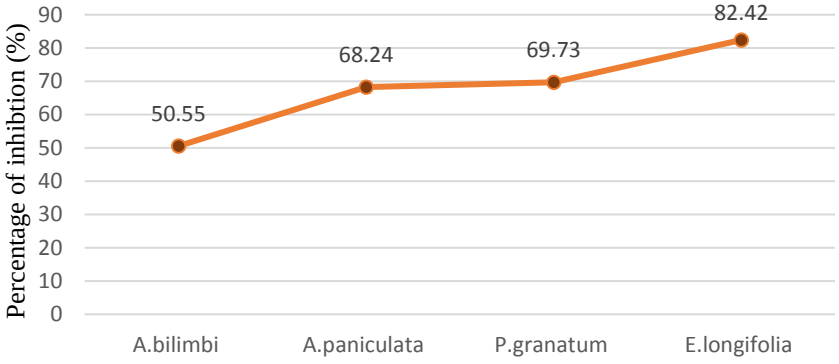

Figure 1. The inhibition percentage for positive results

CONCLUCTION

In the current study, seven plants used for the treatment of diabetes mellitus, about twenty-seven of plant extracts were tested in α-amylase assay and the best results were obtained for the chloroform extract of *E. longifolia* at percentage of 82.42% inhibition. Selection of most potent ethnomedicinal plants were performed successfully by choosing the plant extracts that yielded in a percentage of inhibition of more than 50%. The potent plants was used to perform combination action whereby highest value of α-amylase inhibition was recorded at 77.03% for the combination of two plants, chloroform extract of *E. longifolia*, chloroform extract of *A. paniculata.* However, none of the combined plant extracts portrayed synergistic relationship for α-amylase inhibition. All the plants showed antagonistic relationship.

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