Antiobesity, Antidiabetic and Antioxidant Activities of Senna (Senna alexandrina Mill.) and Pomegranate (Punica granatum L.) Leaves Extracts and Its Fractions

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ABSTRACT

Objective: Obesity is a condition characterized by accumulation of excessive and abnormal fat which influence body health. Therapeutic strategies for obesity include antiobesity drugs use and life-style modification. Long-term effect of antiobesity drugs use results in several problems in the gastrointestinal tract. Furthermore, natural products-based medicines promise the best potency. The objective of this research is to evaluate antiobesity, antidiabetic, and antioxidant activities of senna and pomegranate leaves extracts and their fractions through in vitro methods. Methods: Extracts and fractions of senna leaves and pomegranate leaves were tested through in vitro studies such as pancreatic lipase inhibitory activity, alpha-glucosidase inhibitory activity, alpha-amylase inhibitory activity, and antioxidant activity by DPPH method. Results: Results from this study showed that pomegranate leaves extract showed higher activity to inhibit pancreatic lipase (IC_{50} 33.74 µg/ml), alpha-glucosidase (IC_{50} 45.31 µg/ml), and alpha-amylase (IC_{50} 43.24 µg/ml) than senna leaves extracts and their fractions. In addition, pomegranate leaves extract, pomegranate water fraction, pomegranate ethyl-acetate fraction, and senna water fraction showed powerful activity to inhibit DPPH free radical with IC_{50} values 23.39 µg/ml, 45.48 µg/ml, 49.87 µg/ml and 36.36 µg/ml, respectively. Conclusion: It can be concluded that senna and pomegranate leaves extracts and their fractions could be useful as strategies for treatment of obesity and type-2 diabetes mellitus.

Key Words: Antiobesity, Antidiabetic, Antioxidant, Senna, Pomegranate.

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INTRODUCTION

Obesity has sharp increase, becoming a major public health problem in the worldwide. According to the World Health Organization (WHO), obesity is defined as a condition characterized by accumulation of excessive and abnormal fat in the body which has an influence on body health. Obesity condition is associated with several metabolic syndromes involving type 2 diabetes mellitus, hypertension, arthritis, atherosclerotic, dyslipidemia, and cancers [1, 2]. Both obesity and diabetes mellitus have strong correlation. Excessive fat accumulation in the body gives contribution against dysregulation of lipid and glucose metabolism [3]. Furthermore, dysregulation of lipid and glucose metabolism produce oxidative stress mechanism by disseminating to the body. Oxidative stress

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in the next stage affects many organs in the body and decreases antioxidant status of the body. Oxidative stress mechanism plays a key role in the development of diabetes mellitus.

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia (increase of blood glucose level) and abnormality of carbohydrate, lipid, and fat metabolism. Diabetes mellitus were classified into type 1 diabetes mellitus and type 2 diabetes mellitus. This condition is associated with absolute or relative insulin deficiency on the pancreas [4-6]. Various complications of diabetes mellitus involve microvascular and macrovascular such as microangiopathy, cardiovascular disease, cerebrovascular disease, and renal disease [7, 8].

One of the strategies used in the development of antiobesity and antidiabetic agents is exploration of potent lipase inhibitors and digesting enzyme inhibitors (such as alpha-glucosidase and alpha-amylase enzymes) from natural products. For clinical application, synthetic drugs such as orlistat and acarbose are widely used as inhibitor of these enzymes in obesity patient with type 2 diabetes mellitus. However, administration of these drugs was reported to cause several side effects [9, 10]. Therefore, potency of natural products that can inhibit pancreatic lipase enzyme and digesting enzyme must be explored to develop antiobesity and antidiabetic candidates with less side effects. In addition, number of studies showed that antioxidant effect of the natural products had strong correlation with antiobesity and antidiabetic effects. So, natural products which have antioxidant effect, might be effective in treating obesity and diabetes mellitus. Senna (Senna alexandrina Mill.) and pomegranate (Punica granatum L.) are widely used as folk medicine for several purposes. All of parts from these plants have beneficial pharmacological activities. Senna can be found in Africa, India, and Asia. The applicable parts of senna are the leaves, pods, and fruits. Pharmacological activities of senna include purgative, antipyretic, laxative, and diuretic [11]. The other plant, Pomegranate (Punica granatum L.) belongs to the Punicaceae family, which can be found in America, Europa, and Asia. The applicable parts of pomegranate are the roots, barks, fruits, peels, and leaves to treat several diseases including cancer, infections, obesity, and inflammation [12]. Therefore, the aims of this research were to evaluate antiobesity, antidiabetic, and antioxidant activities of senna (Senna alexandrina Mill.) and pomegranate (Punica granatum L.) leaves extracts and their fractions.

MATERIALS AND METHODS

Reagents

All reagents were of analytical grade. Porcine pancreatic lipase enzyme, oleic acid, bovine serum albumin, alpha-glucosidase enzyme from Saccharomyces cerevisiae, p-nitrophenyl-α-D-glucopyranoside (pNPG), alpha-amylase enzyme from Bacillus licheniformis, and the DPPH (1,1-diphenyl-2-picrylhydrazil) were obtained from Sigma-Aldrich. Orlistat and acarbose were received from Bumi Medika Ganesha Pharmacy, Bandung, Indonesia.

Plant material and identification

Senna (Senna alexandrina Mill.) leaves and Pomegranate (Punica granatum L.) leaves were collected from Bandung, West Java, Indonesia. Senna leaves and Pomegranate leaves were identified in Tropical Biopharmaca Research Center, Bogor Agricultural University and Herbarium Bandungense, School of Life Sciences and Technology, Bandung Institute of Technology, West Java, Indonesia, respectively.

Extraction and fractionation

Senna and pomegranate leaves were dried and grinded into powder. The powder of senna leaves and pomegranate leaves were extracted with 96% ethanol by reflux method and evaporated by rotary evaporator at 50^0 C, speed at 50 rpm. Obtained extract was fractionated by liquid-liquid extraction using n-hexane and ethyl acetate. Furthermore, extracts and its fractions were used for pancreatic lipase enzyme, alpha-glucosidase enzyme, alpha-amylase enzyme, and antioxidant assays.

Phytochemical screening of extracts and fractions

Phytochemical screening of extracts and fractions of senna leaves and pomegranate leaves were performed to evaluate the presence of phytochemical constituents such as alkaloids, flavonoids, saponins, tannins, quinones, and steroids/triterpenoids.

Pancreatic lipase enzyme assay

The pancreatic lipase inhibitory activity in this research was determined by measuring the release rate of oleic acid from sesame oil. The method was adopted from Han's et al. method [13] with minor modification. The substrate contained 15 mmol/l sesame oil, 1 mmol/l NaCl, 1 mmol/l $CaCl_2$, 10 mg of bovine serum albumin (BSA)/ml, and phosphate buffer solution (pH 8.0) was prepared by sonication process for 5 min. Furthermore, substrate (100 µl) was added by 50 µl of porcine pancreatic lipase and 100 µl various concentrations of the extracts or fractions of senna leaves and pomegranate leaves (20, 40, 60, 80, 100 µg/ml), incubated for 30 min at 37°C in a total volume of 250 µl. Then, incubation was followed by adding 3 ml of 1:1 (v/v) mixture of chloroform and n-heptane, and extracted by shaking the centrifuge tube for 10 min in a shaker. In addition, the mixture centrifuged at 2000 rpm for 10 min. The upper aqueous phase was removed and the lower phase added with copper reagent (0.5 ml). The tube

was shaken again for 10 min, was centrifuged at 2000 rpm, and 0.5 ml of the upper phase (organic phase) added with 0.5 ml diethyldithiocarbamate-Na solution. The absorbance was then measured at λ 480 nm using UV- Vis spectrophotometer. Lipase inhibitory activity was determined by measuring the effect on the enzyme reaction rate after adding extracts or fractions compared to control. Orlistat was used as positive control. Percentage of pancreatic lipase inhibitory was calculated by the formula:

$$\% \text{ Inhibitory} = (\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}} / \text{Absorbance}_{\text{control}}) \times 100$$

IC_{50} pancreatic lipase inhibitory activity of each extract and fraction was determined using regression linier of its calibration curve.

Alpha-glucosidase enzyme assay

The alpha-glucosidase inhibitory activities of extracts and fractions of senna and pomegranate leaves were determined according to the method which described by Kim et al. [14] with minor modification. In this research, were used alpha-glucosidase enzyme from Bacillus licheniformis and p-nitrophenyl-α-D-glucopyranoside (pNPG) as the substrate. 100 µl alpha-glucosidase enzyme (1.0 unit/ml) was pre-incubated with 50 µl of the various concentrations of the extracts or fractions (n-hexane, ethyl acetate, and water) such as 20, 40, 60, 80, and 100 µg/ml for 10 min. Furthermore, 50 µl of pNPG (3.0 mM) was dissolved in 20 mM phosphate buffer solution (pH 6.9) added to the mixture to initiate the reaction. The reaction mixture was followed by incubation process at 37^0C for 20 min and stopped by using 2 ml of Na_2CO_3 (0.1 M). The alpha-glucosidase inhibitory activity was determined by measuring of the yellow color para-nitrophenol released from pNPG at λ 405 nm. Acarbose was used as positive control. The percentage of the alpha-glucosidase inhibitory activity was determined by the following formula:

$$\% \text{ Inhibitory} = (\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}} / \text{Absorbance}_{\text{control}}) \times 100$$

IC_{50} of alpha-glucosidase inhibitory activity of each sample was observed using regression linier of its calibration curve.

Alpha-amylase enzyme assay

The alpha-amylase inhibitory activity of extracts and fractions of senna leaves and pomegranate leaves were determined according to the method of Dastjerdi et al. [15]. The alpha-amylase solution (0.5 unit/ml) was obtained by mixing between 0.001 g alpha-amylase enzyme in 100 ml of 20 mM sodium phosphate buffer (pH 6.9). The starch solution (0.5% w/v) was prepared by stirring 0.25 g of potato starch in 50 ml of deionized water and boiled for 15 min. Senna leaves extract, pomegranate leaves extract, and its fractions were dissolved in DMSO. 0.5 ml of each extract and fraction of senna leaves and pomegranate leaves mixed with 0.5 ml of the alpha-amylase solution in a test tube. This mixture was followed by incubation process at 37^0C for 30 min. After incubation, 0.5 ml starch solution was added in the mixture and test tube incubated at 37^0C for 10 min. Then, 0.5 ml of the DNS as color reagent was added and the test tube placed into water bath at 85^0C for 15 min. After 15 min, the mixture was removed from water bath and diluted with 9 ml distilled water. Acarbose was used as positive control. The absorbance was read at λ 540 nm. The percentage of alpha-amylase was observed by the following formula:

$$\% \text{ Inhibitory} = (\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}} / \text{Absorbance}_{\text{control}}) \times 100$$

IC_{50} of alpha-amylase inhibitory activity of each sample was calculated using regression linier of its calibration curve.

Antioxidant assay by DPPH method

The antioxidant activity of the extracts and fractions of senna and pomegranate leaves was determined using DPPH which adopted from Fidrianny's et al. method [16]. Radical scavenging activity of extracts and fractions against DPPH was determined by spectrophotometry at λ 516 nm. In this research, ascorbic acid was used as standard. Percentage of DPPH scavenging activity was calculated by the formula:

$$\% \text{ Inhibitory} = A0 - A1/A0 \times 100$$

IC_{50} of DPPH scavenging activity of was determined using regression linier of its calibration curve.

RESULTS

Phytochemical screening of extracts and fractions

Phytochemical screening results of extracts and fractions of senna leaves and pomegranate leaves are exhibited in Table 1 and Table 2. The presence of phytochemical compounds on extracts and fractions might be responsible for its activity on pancreatic lipase enzyme, alpha-glucosidase enzyme, alpha-amylase enzyme, and antioxidant assays.

Pancreatic lipase enzyme assay

In this research, inhibitory activities of senna and pomegranate leaves extracts and its fractions were investigated on pancreatic lipase enzyme. IC_{50} value was calculated for each extract and fraction. Figure 1 revealed the inhibition activity against pancreatic lipase enzyme by senna leaves extract and its fractions. Orlistat was used as

standard. IC_{50} for ethanol extract, n-hexane fraction, ethyl-acetate fraction, and water fraction of senna leaves were 49.79 µg/ml, 57.49 µg/ml, 52.51 µg/ml, and 53.02 µg/ml, respectively. IC_{50} for orlistat was 0.25 µg/ml.

Figure 2 presented the results of inhibition activity of pomegranate leaves extract and its fractions against pancreatic lipase enzyme. IC_{50} values for ethanol extract, n-hexane fraction, ethyl-acetate fraction, and water fraction of pomegranate leaves were 0.25 µg/ml, 33.74 µg/ml, 50.67 µg/ml, 39.43 µg/ml, and 42.88 µg/ml, respectively. IC_{50} value for orlistat was 0.25 µg/ml.

Alpha-glucosidase enzyme assay

Figure 3 demonstrated the inhibition activity of senna leaves extract and its fractions against alpha-glucosidase enzyme. Acarbose was used as positive control. IC_{50} values for acarbose, ethanol extract, n-hexane fraction, ethyl-acetate fraction, and water fraction of senna leaves were 36.17 µg/ml, 49.03 µg/ml, 78.13 µg/ml, 49.46 µg/ml, and 46.93 µg/ml, respectively. Figure 4 expressed the results of inhibition activity against alpha-glucosidase enzyme by pomegranate leaves extract and its fractions. IC_{50} for acarbose, ethanol extract, n-hexane fraction, ethyl-acetate fraction, and water fraction of pomegranate leaves were 36.17 µg/ml, 45.31 µg/ml, 60.00 µg/ml, 58.48 µg/ml and 56.88 µg/ml, respectively.

Alpha-amylase enzyme assay

Figure 5 presented the results of inhibition activity against alpha-amylase enzyme by senna leaves extract and its fractions. Acarbose was used as positive control. IC_{50} values of acarbose, ethanol extract, n-hexane fraction, ethyl-acetate fraction, and water fraction from senna leaves were 27.90 µg/ml, 60.11 µg/ml, 49.98 µg/ml, 46.65 µg/ml and 40.68 µg/ml, respectively.

Figure 6 showed the results of inhibition activity of pomegranate leaves extract and its fractions against alpha-amylase enzyme. IC_{50} for acarbose, ethanol extract, n-hexane fraction, ethyl-acetate fraction, and water fraction of pomegranate leaves were 27.90 µg/ml, 42.71 µg/ml, 62.63 µg/ml, 60.63 µg/ml, and 55.18 µg/ml), respectively.

Antioxidant activity by DPPH method

Table 1 revealed antioxidant activity of senna leaves extract and its fractions by DPPH method. The highest antioxidant activity was given by water fraction of senna leaves with IC_{50} 36.36 µg/ml. While antioxidant activity of pomegranate leaves extract and its fractions by DPPH method were exhibited in Table 2. The lowest IC_{50} was shown by ethanol extract of pomegranate leaves (23.39 µg/ml). IC_{50} value of ascorbic acid standard was 5.26 µg/ml.

DISCUSSION

Obesity was associated with chronic diseases including type II diabetes mellitus, hypertension, musculoskeletal disorders, arthritis, and various cancers. The basic pathophysiology of obesity is an energy imbalance between calorie intake and expenditure. Therefore, inhibition against digestion and absorption process of dietary fat can be used as treatment of obesity [17, 18]. Pancreatic lipase enzyme is important enzyme in the body which has responsibility and contribution against obesity by dietary triacylglycerol hydrolysis mechanism to monoacylglycerols and fatty acids. Pancreatic lipase enzyme is one of the studied targets to establish potential natural products as antiobesity [19]. Pancreatic lipase enzyme could be inhibited by orlistat. Orlistat has mechanism through a covalent bond against site of action of lipase enzyme [20, 21]. Experimental results showed that senna leaves' extract has potency to influence pancreatic lipase enzyme inhibition, although it gave lower activity compared to the pomegranate leaves extract. Senna leaves containing major compound was known as sennoside. Pharmacological observation showed that sennosides A and sennosides B were responsible for stimulation of the auerbach plexus in gastrointestinal tract and did not have strong bond against active site of pancreatic lipase enzyme. Senna leaves were known as laxative agent [11, 22]. Furthermore, pomegranate leaves extract showed powerful activity to inhibit pancreatic lipase enzyme activity. This result is consistent with the previous study using pomegranate leaves extract. Pomegranate leaves extract presented stronger activity to inhibit pancreatic lipase enzyme compared to the senna leaves extract and their fractions [23].

Obesity has relationship with antioxidant status in the body and diabetes mellitus condition. Accumulation of excessive fat in the body produce oxidative stress on several organs such as liver, kidney, heart, and pancreas. If it affects pancreas, then the next problem is dysfunction of IRS (insulin receptor substrates). IRS dysfunction has an important role against diabetes mellitus. Diabetes mellitus is a chronic disorder that influences carbohydrate, protein, and fat metabolism in the body. Diabetes mellitus is classified into type-1 diabetes mellitus and type-2 diabetes mellitus. Both type-1 diabetes mellitus and type-2 diabetes mellitus are caused by problem on the pancreas, especially beta-langerhans islet cells. The problem includes absence of insulin secretion or insulin resistance. The alpha-glucosidase is one of important digesting enzyme, having ability to hydrolyze polysaccharide into glucose. Inhibition on this enzyme is associated with blood glucose control. Alpha-glucosidase enzyme can be found in small intestine [24]. Experimental results showed that both extracts and

fractions of senna leaves and pomegranate leaves have great ability to inhibit alpha-glucosidase enzyme.

Beside alpha-glucosidase enzyme, another enzyme such as alpha-amylase, nowadays has become a target to reduce hyperglycemia. Alpha-amylase enzyme can be found in saliva and pancreas. Alpha-amylase enzyme is one of important digesting enzyme, having ability to hydrolyze polysaccharide into maltose and glucose. Inhibition on this enzyme contributed against blood glucose control [25]. In this research, the inhibition activities of senna and pomegranate leaves extract and its fractions were investigated on alpha-amylase enzyme. Experimental results showed that both extracts and fractions of senna leaves and pomegranate leaves have great ability to inhibit alpha-amylase enzyme.

The in vitro final assay is antioxidant activity measurement using DPPH method. The DPPH method was introduced by Blois [26] and it is widely used to evaluate the ability of compounds to act as free radical scavengers or hydrogen donors. The IC_{50} parameter was used for the results interpretation the DPPH method and defined as the concentration that can scavenge 50% of DPPH free radical. The advantage of DPPH method is fast and simple [27]. Extracts and fractions of senna and pomegranate leaves were examined their IC_{50} values. The highest antioxidant activity was given by water fraction of senna leaves. While, ethanol extract, water fraction, and ethyl-acetate fraction of pomegranate leaves were given powerful activity on antioxidant assay by DPPH method.

Experimental results which were obtained can explain that both extracts and fractions of senna leaves and pomegranate leaves have great activity to inhibit pancreatic lipase, alpha-glucosidase, alpha-amylase, and antioxidant assay using DPPH method.

CONCLUSION

The experimental results reported that pomegranate leaves extract and its fractions exhibited higher activity against pancreatic lipase enzyme inhibitory activity, alpha-glucosidase enzyme inhibitory activity, alpha-amylase enzyme inhibitory activity, and antioxidant activity compared to senna leaves extract and its fractions. Experimental result was obtained given promising prospect for obesity and type II diabetes mellitus treatment.

CONFLICT OF INTEREST

There is no conflict of interest to disclose.

ACKNOWLEDGEMENT

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ILLUSTRATIONS

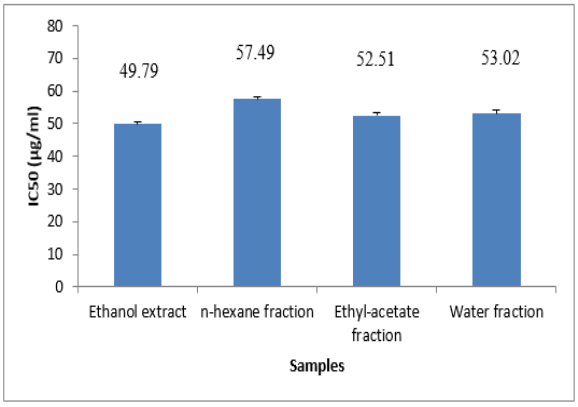

Fig. 1. In vitro assay on pancreatic lipase enzyme inhibition by senna leaves extract and its fractions.

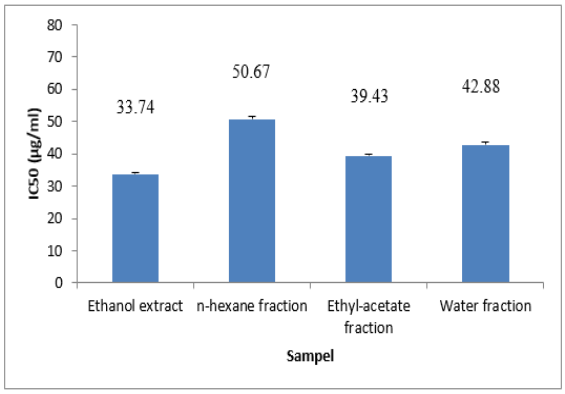

Fig.2 . In vitro assay on pancreatic lipase enzyme inhibition by pomegranate leaves extract and its fractions.

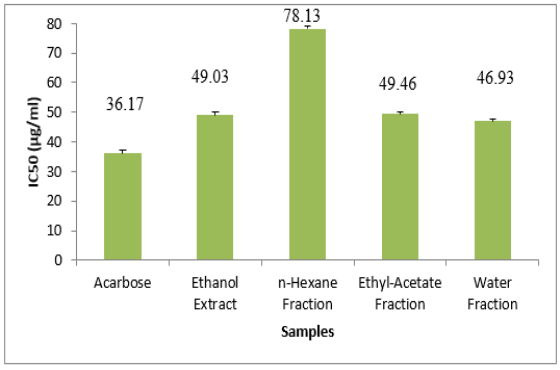

Fig. 3. In vitro assay on alpha-glucosidase enzyme inhibition by senna leaves extract and its fractions.

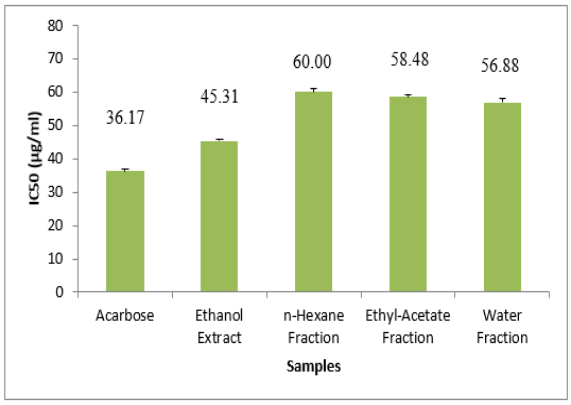

Fig. 4. In vitro assay on alpha-glucosidase enzyme inhibition by pomegranate leaves extract and its fractions.

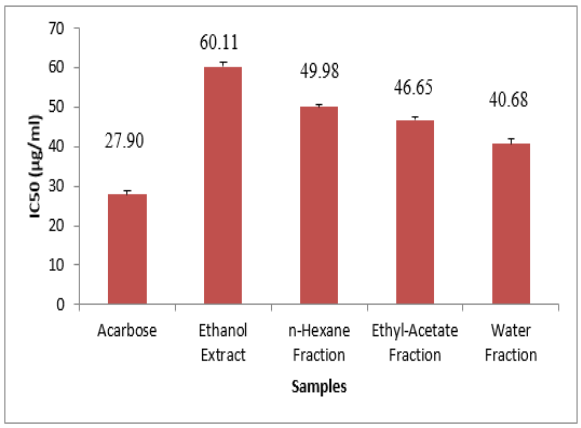

Fig. 5. In vitro assay on alpha-amylase enzyme inhibition by senna leaves extract and its fractions.

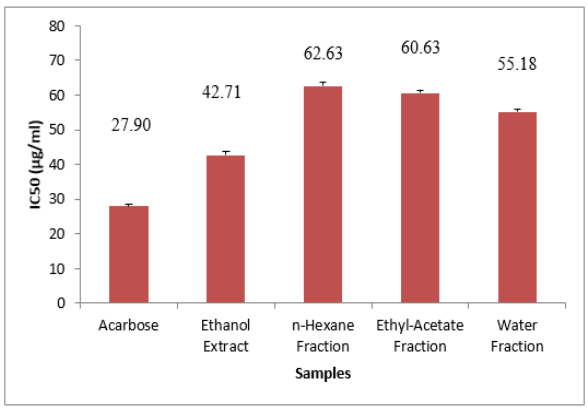

Fig.6. In vitro assay on alpha-amylase enzyme inhibition by pomegranate leaves extract and its fractions.

Table 1. Phytochemical screening of extracts of senna leaves and pomegranate leaves

Phytochemical compounds	Samples	
	Senna	Pomegranate
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Quinones	+	+
Steroids/triterpenoids	+	+

(+): presence of phytochemical compounds; (-): absence of phytochemical compounds

Table 2. Phytochemical screening of fractions of senna leaves and pomegranate leaves

Phytochemical compounds	Samples					
	Senna			Pomegranate		
	Water	Ethyl acetate	n-hexane	Water	Ethyl acetate	n-hexane
Alkaloids	+	+	+	+	+	+
Flavonoids	-	+	-	+	+	-
Tannins	-	+	-	+	+	-
Saponins	+	+	-	+	+	-
Quinones	+	+	+	+	+	+
Steroids/ triterpenoids	-	+	+	+	+	+

(+): presence of phytochemical compounds; (-): absence of phytochemical compounds

Table 3. Antioxidant activity of senna leaves extract and its fractions.

Samples	IC_{50} (μg/ml)
Ethanol extract	54.23±2.68
n-hexane fraction	57.07±3.51
Ethyl-acetate fraction	55.34±4.05
Water fraction	36.36±2.68

Table 4. Antioxidant activity of pomegranate leaves extract and its fractions.

Sample	IC_{50} (μg/ml)
Ethanol extract	23.39±2.79
n-hexane fraction	62.77±2.83
Ethyl-acetate fraction	49.87±2.75
Water fraction	45.48±3.42