Original Article

Antidiabetic and Antihypertensive Potential of Selected *Asteraceae* Plant Species

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ABSTRACT

Antidiabetic and Antihypertensive activity of 10 ethnobotanically known plants of *Asteraceae* family was investigated using *in vitro* glucose diffusion, α-amylase, α-glucosidase and Angiotensin I converting enzyme (ACE) inhibition methods. These medicinal plants were able to inhibit α-amylase and α-glucosidase enzymes, which are responsible for the breakdown of oligosaccharides into monosaccharides. Methanolic extracts of all the plants showed differential inhibitory activity against these enzymes. *Artimisia vulgaris, Eclipta alba, Glossocardia bosvallea, Wedelia chinensis* and *Wedelia trilobata* showed inhibition of α-amylase and α-glucosidase enzymes that are responsible for the breakdown of oligosaccharides into monosaccharides. *Wedelia trilobata* strongly inhibited α-amylase enzyme with IC_{50} value of 40 µg/ml. *A. vulgaris, G. bosvallea, S. uliginosa, W. chinensis* and *W. trilobata* showed 84, 70, 67, 88 and 75% inhibition of α-Glucosidase enzyme respectively at a concentration of 50 µg/ml. Glucose movement from sealed dialysis tube to external solution was inhibited by *A. vulgaris* and *W. trilobata* extracts. Methanolic extract of *A. cina, A. vulgaris, G. bosvallea* and *W. trilobata* inhibited the rabbit lung Angiotensin I converting enzyme with IC_{50} of 40, 40, 35 and 30 µg/ml respectively. This is the first report on Angiotensin I converting enzyme inhibitory activity by these plants, suggesting their potential as therapeutic alternative for hypertension.

Keywords: Diabetes, Hypertension, *Asteraceae* plant species.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease caused by inheritance or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin. Such a deficiency results in increased blood glucose level, which in turn can damage many of the body's systems, including blood vessels and nervous

system[1]. Diabetes is one of the most common diseases that affect millions of individuals worldwide. There are two types of diabetes, type 1 (insulin-dependent) and type 2 (insulin-independent). In type 1 diabetes, insulin deficiency originates from allergic reactions in genetically susceptible people eventually destroying the pancreatic β-cells producing insulin. Type 2 diabetes is the most common form of diabetes accounting for 90% of cases worldwide. Patients with type 2 diabetes are not dependent on insulin. Apart from genetic reasons; obesity, age and inactivity are the common factors that expose individuals to type 2 diabetes.

One therapeutic approach for treating diabetes is to decrease the post-prandial hyperglycemia. This is done by retarding the absorption of glucose by inhibiting the carbohydrate-hydrolyzing enzymes α-amylase and α-glucosidase in the digestive tract. Inhibitors of these enzymes delay carbohydrate digestion thus prolonging the overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise[2,3]. Delaying glucose absorption is one of the direct and beneficial types of therapy for non-insulin dependent diabetes. α-amylase and α-glucosidase which are membrane bound enzymes at the epithelium of the small intestine catalyze the cleavage of glucose from disaccharide, impeding digestion and adsorption[4]. This hypothesis was successfully confirmed in animal studies and later clinical studies by the administration of various inhibitors[4-10].

Diabetes mellitus is characterized by hyperglycemia induced by decreased cellular glucose uptake and metabolism. Control of plasma glucose concentration is vital to decrease the incidence and severity of long-term diabetic complications. Currently dietary changes, oral hypoglycemic agents or insulin injections are the methods used to prevent hyperglycemia. According to Dicarli *et al.*, (2003), hyperglycemia has been linked to the onset of type 2 diabetes mellitus and associated cardiovascular complications including hypertension. Hypertension is potentiated by angiotensin II, a potent vasoconstrictor agent, which is formed from angiotensin I, a histidyl-leucine dipeptide, by the action of angiotensin I converting enzyme[12]

At present, drug therapies either alone or in combination cannot restore normal blood glucose homeostasis and many limitations exist in their use[13]. In this regard, plants represent a vast source of potentially useful dietary supplements for improving blood glucose control and preventing long-term complications in type 2 diabetes mellitus[14] and hypertension. Ayurveda, the traditional Indian herbal medicinal system practiced over thousands of years have reports about antidiabetic and antihypertensive plants causing no side effects. Such plants and their secondary metabolites have been widely prescribed for treatment of these diseases all around the world with less known mechanistic basis of their functioning. Several classes of plant derived compounds are known to possess *in vitro* ACE inhibitory activity including some terpenoids and polyphenolic compounds like flavonoids, hydrolysable tannins, xanthones, procyanidins and caffeolyquinic acid derivatives[15,16]. More than 400 plants worldwide have been documented as beneficial in the treatment of diabetes[17]. Majority of traditional antidiabetic and antihypertensive plants need proper medical and scientific evaluation to prove their ability to improve blood glucose control. In recent years, the importance of biologically active substances contained in food and some traditional plants have been noticed and their physiological effects are attributed to α-amylase and α-glucosidase

inhibitors[10,18-20]. One of the most direct and beneficial therapy for non-insulin dependent diabetes is to control blood glucose level after meal by delaying glucose absorption. Testing for glucose diffusion across dialysis membrane is also a convenient model for assessing factors which affect glucose adsorption *in vitro* and ACE inhibition is considered as modern therapeutic principle in the treatment of hypertension.

Based on the traditional ayurvedic medicinal systems, *A. cina, A. vulgaris, E. alba, G. bosvallea, M. micrantha, S. uliginosa, V. cinerea, V. indica, W. chinensis* and *W. trilobata* belonging to *Asteraceae* family were selected to evaluate their antidiabetic and antihypertensive activity based on inhibition of α-amylase, α-glucosidase, angiotensin I converting enzymes and glucose diffusion across dialysis membrane.

MATERIALS AND METHODS

Plant materials and preparation of plant extracts

Whole plants were collected from Srirangapattana, Nanjangud and Mysore, Karnataka and were authenticated by taxonomist, Dr. Sampath Kumar K. K. at University of Mysore (UOM). The Plants were washed and dried under shade. The dried plant samples were crushed to course powder and extracted with hexane, chloroform and methanol using Soxhlet apparatus. The solvents were evaporated to dryness with a rotary evaporator under reduced pressure at 40^0 C. The dried residue was redissolved in 50 % dimethyl sulfoxide (DMSO) and used for the assays.

Chemicals

Pancreatic α-Amylase, yeast α-Glucosidase, Glycyl-glycine (Gly Gly), hippuryl-glycyl-glycine (Hip-Gly-Gly), HEPES, acetone dehydrated rabbit lung, captopril and acarbose were purchased from Sigma Aldrich. All other chemicals and reagents used were of analytical grade.

α-Amylase inhibition test

α-amylase inhibitory activity for each extract was assessed based on colorimetric assay using acarbose as the reference compound[21]. Starch solution (0.5% w/v) was prepared by stirring and boiling 0.25 g of soluble potato starch in 50 ml of deionized water for 15 min. The enzyme solution (0.5 unit/ml) was prepared by mixing 0.001 g of α-amylase in 100 ml of 20 mM sodium phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride. The extracts were dissolved in DMSO to get 50 µg/ml concentration. The color reagent was a solution containing 96 mM 3,5-dinitrosalicylic acid (20 ml), 5.31 M sodium potassium tartrate in 2 M sodium hydroxide (8 ml) and deionized water (12 ml). Plant extract (1 ml) and enzyme solution (1 ml) were mixed in a test tube and incubated at 25°C for 30 min. To 1 ml of this mixture, 1 ml of starch solution was added and incubated at 25°C for 3 min. After incubation, 1 ml of color reagent was added and the closed tube placed in a water bath at 85°C. After 15 min, the reaction mixture was removed from the water bath, cooled and diluted with 9 ml distilled water. The absorbance value was determined at 540 nm. Blanks were prepared for correcting the background absorbance. For blanks, color reagent was added prior to the addition of starch solution and then the tube placed into the water bath. The other procedures were carried out as above. Controls were prepared similarly using 1 ml DMSO instead of plant extracts. Acarbose solution was used as positive control. The inhibition percentage of α-amylase was assessed using the formula:

$$\mathbf{I}_{\text{α-amylase}}\% = 100 \times (\Delta A_{\text{Control}} - \Delta A_{\text{Sample}}) / \Delta A_{\text{Control}}$$

Where $\Delta A_{\text{Control}} = A_{\text{Test}} - A_{\text{Blank}}$

$$\Delta A_{\text{Sample}} = A_{\text{Test}} - A_{\text{Blank}}$$

The $I_{\alpha\text{-amylase}}\%$ was plotted against the sample concentration and IC$_{50}$ value (inhibitory concentration) was calculated. It represents the concentration of sample (mg/ml) necessary to decrease the absorbance of enzyme by 50%.

α-Glucosidase inhibition test

The α-glucosidase inhibitory assay was done by the chromogenic method described by Watanabe *et al*. (1997). Briefly, yeast α-glucosidase (0.7U, sigma) was used as enzyme solution which was dissolved in 100 mM phosphate buffer (pH 7.0) containing 2g/L bovine serum albumin and 0.2 g/L NaN$_3$. Para nitrophenyl-alpha-D-glucopyranoside (5mM) in the same buffer (pH 7.0) was used as a substrate solution. Enzyme solution (50 µl) and test compounds (10 µl) dissolved in DMSO at 5 mg/ml concentration were mixed in a well of a microtitre plate and measured (Absorbance 405 nm) at zero time. After incubation for 5 min, substrate solution (50 µl) was added and incubated for further 5 min at room temperature. The increase in absorbance from zero time was measured. Inhibitory activity was expressed by subtracting relative absorbance difference (%) of test compounds by 100 to absorbance change of the control where test solution was replaced by carrier solvent.

Effect of plant extracts on glucose movement

A simple model system as described by Edwards *et al*. (1988) was used to evaluate effects of plant extracts on glucose movement *in vitro*. The model used consisted of a dialysis tube containing 2 ml of 0.15 M NaCl and 0.22 mM D-glucose. Plant extracts were added into the dialysis tube, sealed at each end and placed in a 50 ml centrifuge tube containing 45 ml of 0.15 M NaCl. The tubes were placed on an orbital shaker. The movement of glucose into the external solution was monitored at set time intervals (5, 10, 15 h). The effect of 50 g/l plant extracts on glucose diffusion to external solution was compared with that of control without plant extract. At the end, the concentration of glucose retained within the dialysis tube was measured. All tests were carried out in triplicate.

ACE inhibition assay

Ten microliters of the rabbit lung extracts (1 g/10 ml) was added to an tube containing 10 µl of the plant extract (100 mg/ml) to be tested, or 10 µl of 50 mmol/l phosphate buffer of pH 8.3 (negative control) or 10 µl of captopril solution (64 nmol/l) (positive control). The mixture was homogenized and preincubated for 5 min at 37^0 C. The enzymatic reaction was started by adding 60 µl of the assay buffer containing HEPES (50 mmol/l), NaCl (300 mmol/l) and Na2SO4 (400 mmol/l) with 10% NaOH solution of pH 8.15 and 30 µl of the substrate solution (200 mg of Hip-Gly-Gly in 4ml of 1 mol/l ammonium hydroxide solution). After homogenization, the mixture was incubated for 35 min at 37^0 C. The reaction was stopped by adding 100 µl of sodium tungstate solution (100 g/l) and 100 µl of sulfuric acid (0.33 mmol/l); the tube was shaken for 10 sec followed by addition of 1 ml of distilled water. The mixture was centrifuged at 2000 rpm for 10 min. An aliquot of the supernatant (75 µl) was placed on a microtitre plate and mixed with 100 µl of phosphate buffer (100 mmol/l, pH 8.5) and 5 µl of TNBS solution (60 mmol/L). The plate was kept in dark at room temperature for 20 min. The absorbance was read in a microtitre plate reader at 415nm against a blank solution prepared in a similar way, except for adding the sodium tungstate and the sulfuric acid solutions before the rabbit lung solution. Assays were performed in triplicates. ACE inhibition was calculated as:

$$\text{Inhibition } \% = 100 - (A_I \times 100)/A_C$$

Where A_I is the measured absorbance at 415nm in the presence of an inhibitor and A_C is the absorbance of the blank solution.

Statistical analysis

All data are expressed as mean ± standard error of three repeated experiment. Statistical software Origin 8.5 was used for analysis of variance. Significance was measured as $p \leq 0.05$.

RESULTS AND DISCUSSION

α-Amylase and α-Glucosidase inhibition test

The *in-vitro* effect of 10 plants on the activity of α-amylase and α-glucosidase was investigated and the results are summarized in the figure 1 and 2. Of the 10 plants, only five showed potent inhibitory activity of the two enzymes. Porcine pancreatic α-amylase was taken as 100% enzyme activity. Incubation of known concentrations of plant extracts with α-amylase and starch showed noticeable decrease in enzyme activity. Methanolic extracts of *E. alba, G. bosvallea, W. chinensis* and *W. trilobata* showed α-amylase inhibition to the extent of 70, 64, 85 and 65 %, respectively at 50 µg/ml concentration, whereas hexane and chloroform extracts showed very negligible inhibition (Fig. 1). The results showed that the methanolic extract contains α-amylase inhibitor compound since less starch was converted to maltose as evident from the lower absorbance value. When compared to hexane and chloroform extracts, methanolic extracts of *A. vulgaris, E. alba, G. bosvallea, W. chinensis* and *W. trilobata* showed α-glucosidase inhibition to the extent of 84, 67, 70, 88 and 75 %, respectively at 50 µg/ml concentration (Fig. 2), whereas *A. cina, S. uliginosa, V. cinerea* and *V. indica,* showed no inhibition against any of the enzymes. Acarbose, which is known as an effective α-amylase and α-glucosidase inhibitor, showed maximum inhibition with IC$_{50}$ value of 30 and 35 µg/ml, respectively. When compared to acarbose, the plant extract is taken as a physiologically functional liquid and its prophylactic effect on diabetes would be considerable. The methanolic extracts of all the five plants exhibited significant effect.

Glucose Diffusion

Concentration of glucose inside the dialysis tubing after 26 h of incubation in the absence or presence of plants was inversely related to the concentration of glucose in the external solution (Gallager *et al*., 2003). Only methanolic extract of *A. vulgaris* and *W. trilobata* demonstrated potential and significant concentration-dependent inhibitory effect on glucose movement into external solution across dialysis membrane. After 15 h of incubation, about 8.2 mM/L of glucose was dialyzed out of dialysis bag in control (without plant extract). In the presence of *A. vulgaris* and *W. trilobata* extracts (50 g/L) glucose diffusion significantly decreased and external glucose concentrations were 5.7 and 4.8mM/L, respectively (Fig 3). When compared to control, 30 and 41% decrease in total glucose diffusion was observed due to *A. vulgaris* and *W. trilobata* extracts, respectively.

ACE inhibition assay

Artimisia cina, A. vulgaris, G. bosvallea and *W. trilobata* exhibited about 50% ACE inhibition against rabbit lung ACE at the concentration of 40, 40, 35 and 30 µg/ml respectively. All four plant extracts exhibited dose dependent inhibition of rabbit lung ACE (Fig 4). When compared to other extracts, *W. trilobata,* an ornamental species of *Asteraceae* family, showed significantly higher ($p \leq 0.05$) ACE inhibition of 96% at 60 µg/ml. The IC50 value of positive control Captopril was 25nmol/l. The ACE inhibitory activity of these species described for the first time seems to substantiate the popular use of these species to treat hypertension.

CONCLUSION

This study showed the potential antidiabetic and antihypertensive activity of some selected plants of *Asteraceae* family. The enzyme inhibitors present in plants act through a variety of mechanisms. It is not possible to indicate the exact mechanism of inhibition of these extracts until the active principles responsible for such effects have been identified. Of the 10 plant extracts tested, only *A. cina, A.vulgaris, G. bosvallea, E. alba, W. chinensis* and *W. trilobata* showed significant inhibition of enzymes. This suggests that these plants contain α-amylase, α-glucosidase and angiotensin I converting enzyme inhibitory compounds. The oral administration of these crude extracts in non-insulin dependent diabetes subjects can moderate the post prandial blood glucose level. The active metabolite which is responsible for inhibition of these enzymes will be taken into further consideration for bioguided fractionation aiming to purify and identify the compounds.

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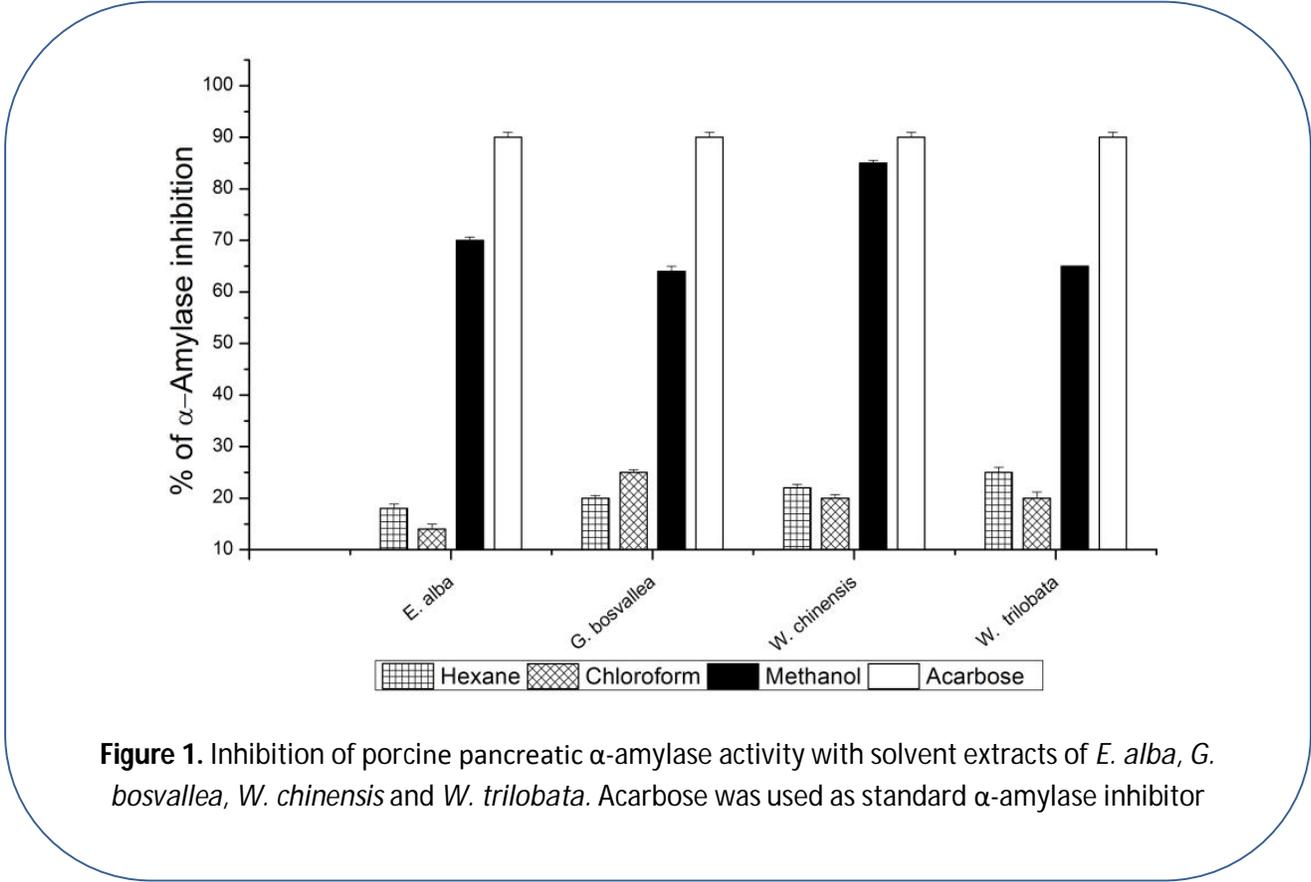
Figure 1. Inhibition of porcine pancreatic α-amylase activity with solvent extracts of *E. alba*, *G. bosvallea*, *W. chinensis* and *W. trilobata.* Acarbose was used as standard α-amylase inhibitor
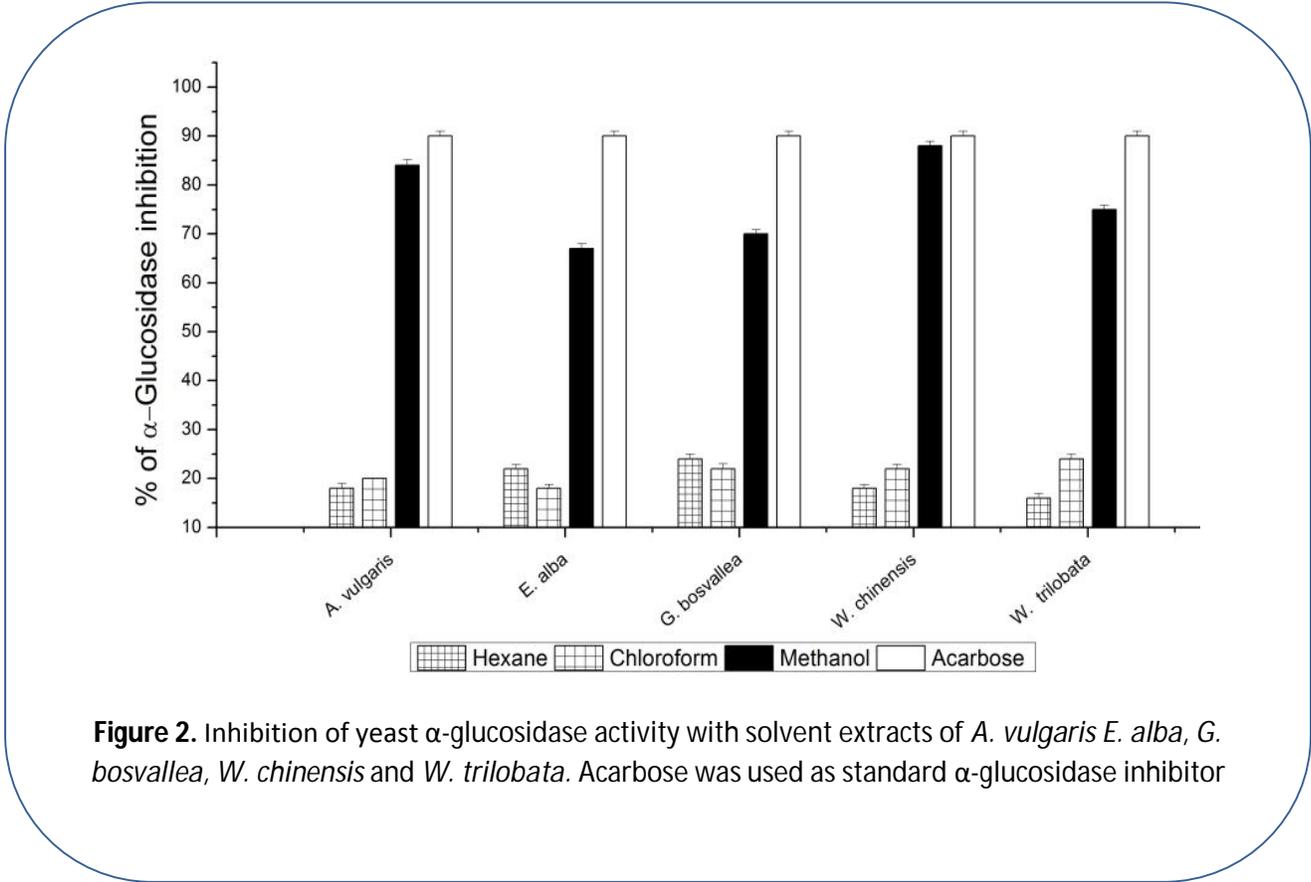

Figure 2. Inhibition of yeast α-glucosidase activity with solvent extracts of *A. vulgaris E. alba*, *G. bosvallea*, *W. chinensis* and *W. trilobata.* Acarbose was used as standard α-glucosidase inhibitor
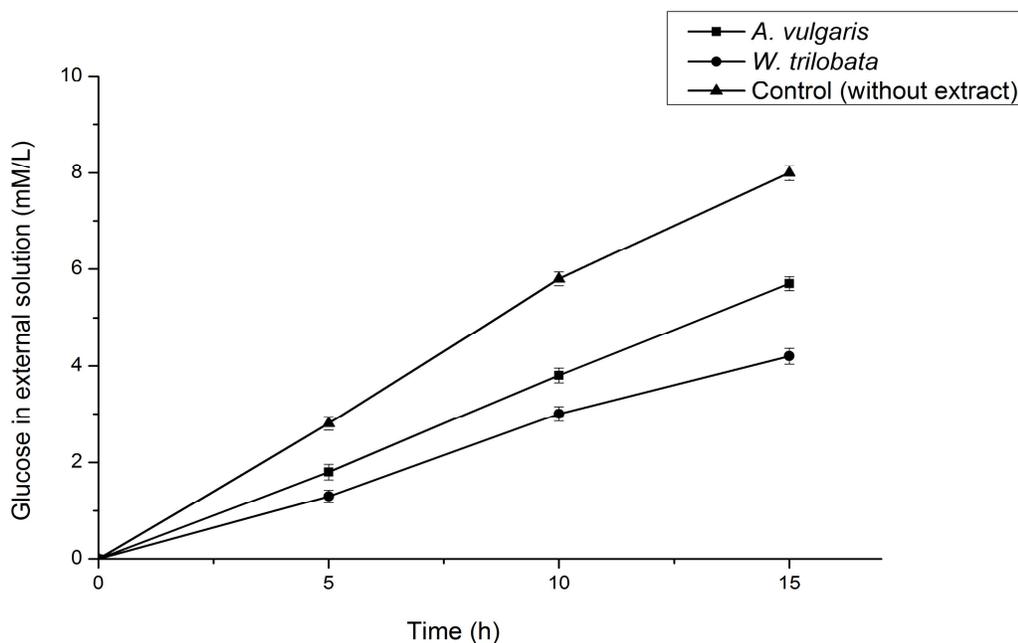
Figure 3. Effect of methanol extract of *A. vulgaris* and *W. trilobata* on the movement of glucose out of dialysis tube. The data indicate ± SEM (n=3, p≤ 0.05)
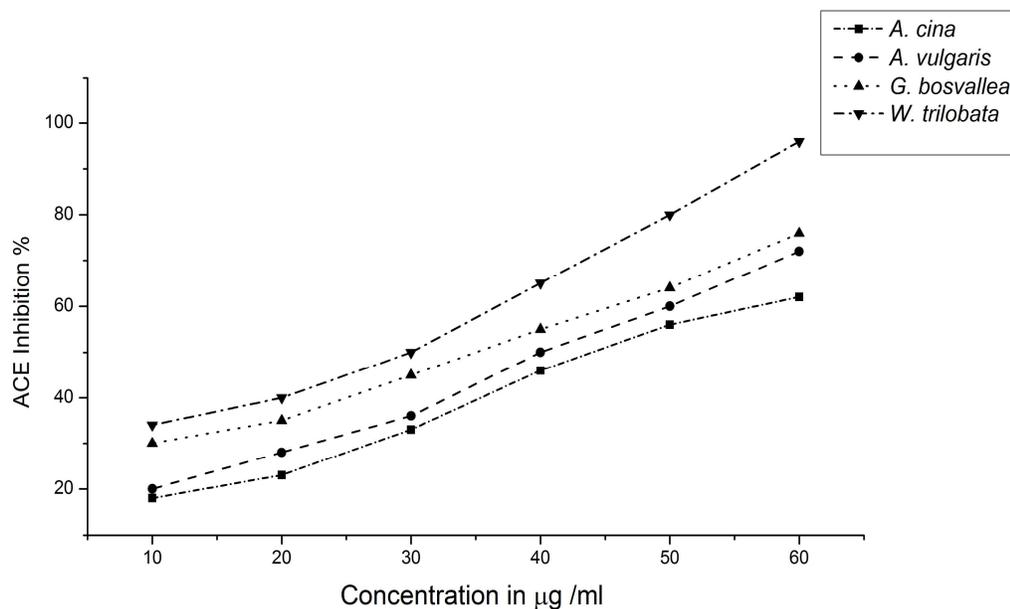

Figure 4. Effect of methanol extract of *A. cina, A. vulgaris, G. bosvallea* and *W. trilobata* on inhibition of angiotensin I converting enzyme. The data indicate ± SEM (n=3, p≤ 0.05)