

## Evaluation of In vitro Antidiabetic Potential of *Thymus schimperi* R. and *Thymus vulgaris* L.

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### Abstract

Diabetes has become the most common metabolic disease worldwide. In particular, type 2 diabetes is the most commonly encountered type of diabetes, which is characterized by impaired insulin secretion and/or action. One of the effective methods to control diabetes is to inhibit the activity of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes which are responsible for the breakdown of starch to more simple sugars, using plant products. This study evaluated the total phenolic (TPC), total flavonoid (TFC), and antidiabetic potential of *Thymus schimperi* and *Thymus vulgaris* via *in vitro* inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase, using the hot water and aqueous: methanol (20:80, v/v) extracts. The  $\alpha$ -amylase inhibitory potentials of the extracts were investigated through reducing sugars analysis using 3,5-dinitrosalicylic acid color reagent (DNSA) using starch solution as substrate. The  $\alpha$ -glucosidase inhibition was determined by pre-incubating  $\alpha$ -glucosidase with different concentrations of the extracts followed by the addition of p-nitrophenylglucopyranoside (pNPG). Aqueous: methanol (20:80, v/v) extract of *T. schimperi* contained highest TPC ( $46.01 \pm 4.54$  mg GAE/g dw) and TFC ( $14.72 \pm 1.14$  mg QE/g dw) also showed stronger  $\alpha$ -amylase inhibition activity ( $IC_{50} = 0.33 \pm 0.05$  mg/mL) and the hot water extract exhibited stronger  $\alpha$ -glucosidase inhibition ( $IC_{50} = 0.05 \pm 0.01$  mg/mL) capacity than that of *T. vulgaris*. The TPC and TFC were positively related ( $p < 0.05$ ) with  $\alpha$ -amylase inhibition activity but negatively correlated ( $p > 0.05$ ) with  $\alpha$ -glucosidase inhibitory activity. These results indicated that the inhibition of these enzymes can lead to lower postprandial blood glucose.

**Keywords:**  $\alpha$ -Amylase, Antidiabetic, Diabetes mellitus,  $\alpha$ -Glucosidase, Thymus, Total phenolics

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### 1. Introduction

Diabetes mellitus is a metabolic disease characterized by hyperglycemia and disturbances in fat and protein metabolism that results from defects in both insulin secretion and/or insulin action. In particular, type 2 diabetes mellitus is the most encountered form of diabetes, accounting for more than 80% of the total cases of diabetes (Chan *et al.*, 2010). Various pharmacological approaches have been used to improve diabetes via different modes of action including stimulation of insulin release, inhibition of gluconeogenesis, increasing the number of glucose transporters and reduction of glucose absorption from the intestine (Ahmed *et al.*, 2010). One of the most effective ways of controlling postprandial hyperglycaemia is to suppress starch digestion as it is the main contributor of glucose in the human body from diet. Suppression of starch hydrolysis is conducted through the inhibition of carbohydrate hydrolyzing enzymes such as  $\alpha$ -amylase and  $\alpha$ -glucosidase in the digestive organs (Mustafa *et al.*, 2010). Inhibitors of these enzymes delay carbohydrate digestion and prolong the overall time for carbohydrate digestion, resulting in a decrease in the rate of glucose absorption (Yang *et al.*, 2012).

Traditional medicinal plants have been used for many years by different cultures around the world for the management of diabetes. In recent years, investigation on herbal medicines has become progressively important in the search for a new, effective and safe therapeutic agent for the treatment of diabetes. Many pure bioactive compounds isolated from plants have been demonstrated to have blood glucose-lowering effect, several of which are flavonoids (Kati *et al.*, 2010), triterpenoids (Wenli *et al.*, 2009), carotenoids (Miaad and Saeed, 2017), and alkaloids (Soon *et al.*, 2013). Studies indicated that some of the dietary plants possessed inhibitory effect against  $\alpha$ -glycosidase and or  $\alpha$ -amylase, such as sorghum, foxtail millet and proso millet, (John *et al.*, 2014) guava leaves (Shakeera *et al.*, 2013) and eggplant (Esther *et al.*, 2013). In addition, *in vitro* inhibitory activities have been reported for phenolic extracts of foods, including fruits (Jayaprakasam *et al.*, 2005; Castañeda-Ovando *et al.*, 2009; Misbah, 2013), vegetables (Obboh *et al.*, 2012), medicinal herbs (Abdullah and Izabela, 2013), green and black tea (Kati *et al.*, 2010), and berries (McDougall *et al.*, 2005).

Even nowadays some spices and culinary herbs play an important role in primary health care and in the treatment of diabetes, especially in developing countries (Wongsa, 2012). Natural hypoglycemic compounds may be attractive alternatives to synthetic drugs or reinforcements to currently used treatments. Their huge advantage is that they can be ingested in everyday diet. *T. schimperi* Ronniger is a wild endemic herb to Ethiopia and is traditionally used for food flavoring as well as medicinal ingredient. The dried leaves are used to flavor tea, coffee,

food and also boiled as a tea substitute and are believed to be good for diabetic patients (Nigist & Sebsebe, 2009). *T. vulgaris* L. is an important medicinal plant (Golmakani and Rezaei, 2008; Al-Bayati, 2008) which has been used for centuries as spice, home remedy, drug, perfume and insecticide, and also reported to have antidiabetic activity (Rime *et al.*, 2014). So far, there are few studies on  $\alpha$ -amylase inhibition activities of the dried leaf *T. schimperi* (Engeda *et al.*, 2015). Therefore, the objective of the present study was to compare TPC, TFC and *in vitro* antidiabetic potentials of hot water and aqueous: methanol (20:80, v/v) extracts of the dried leaves of *T. schimperi* and *T. vulgaris*. Also correlation between total phenolic contents and  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition capacity of these herbs was evaluated.

## 2. Materials and Methods

### 2.1. Chemicals

Gallic acid, Folin–Ciocalteu reagent, quercetin, acarbose, 3, 5-dinitrosalicylic acid (DNSA), potato starch, phosphate buffer, sodium chloride, sodium carbonate, aluminum chloride, sodium potassium tartarate,  $\alpha$ -glucosidase,  $\alpha$ -amylase, and p-nitrophenyl- $\alpha$ -D-glucopyranoside were purchased from Sigma-Aldrich. The other chemicals and solvents used in this experiment were of analytical reagent grades.

### 2.2. Sample preparation and extraction

Fresh leaves of *T. schimperi* Ronniger were collected from Dinsho, Bale Mountain National Park, South East Ethiopia and fresh leaf of *T. vulgaris* was collected from garden in Dalhousie Agricultural College, Canada. The leaves were air dried for 10 days and then ground to fine powder using electric grinder (FM100 model, China). The hot water and aqueous: methanol (20:80, v/v) extracts were prepared by dissolving 1 g of the leaf fine powder separately in 10 mL each solvent. The hot water extract was heated for 5 min using water bath. The mixtures were then subjected to sonication (model 750D, VWR Intl. Ltd., Montreal, QC, Canada) for 15 min x 3 times, with 10 min intervals in between sonication cycles to keep the temperature below 30°C during the extraction. After centrifugation (model Durafuge 300, Precision Scientific, Richmond, VA, USA) at 5000 rpm for 10 min, the supernatant was filtered using Whatman number 1 filter paper. The methanol was evaporated from aqueous: methanol (20:80, v/v) extract under N<sub>2</sub> and the remaining water (in aqueous: methanol (20:80, v/v) extract) and hot water extract were freeze dried for 10h using freeze drier (model 2085C0000, Kinetics Thermal Systems, Stone Ridge, NY, USA). Samples of each treatment were extracted and analyzed in triplicate and immediately stored in amber vials at -20°C until used for analysis.

### 2.3. Determination of total phenolic content (TPC)

The TPC was estimated by Folin-Ciocalteu method as described in Shan *et al.* (2005) with slight modification using gallic acid as the standard. To 0.1 mL of the extract, 1 mL Folin- Ciocalteu reagent (diluted ten times) was added and the mixture was left for 5 min and then 1 mL (75 g/L) of sodium carbonate was added. The absorbance of the resulting blue color was measured at 765 nm with a UV-Visible spectrophotometer (JENWAY, 96500, UK) after incubation for 90 min at room temperature. The TPC was estimated from gallic acid (1–100  $\mu$ g/mL) calibration curve ( $y = 0.015x + 0.09$ ,  $R^2 = 0.99$ ) and the results were expressed as milligram gallic acid equivalent/gram of dried plant material (mg GAE/g dw).

### 2.4. Determination of total flavonoid content (TFC)

The TFC was determined as described in Ayoola *et al.* (2008) with minor modifications. The analysis was based on the formation of yellow color of flavonoid-aluminum complex. Aluminum chloride (2 mL, 2%) was mixed with the same volume of the leaf extract (1 mg/mL). Individual blanks were prepared consisting of 2 mL of sample solution and 2 mL of methanol without aluminum chloride. Then absorbance readings at 415 nm were taken after 1 h of incubation at room temperature against a blank sample. The TFC was determined using a standard curve ( $y = 0.24x + 0.11$ ,  $R^2 = 0.98$ ) of quercetin (1- 40  $\mu$ g/mL) and values were calculated as milligram quercetin equivalents/gram of dried plant material (mg QRE/g dw).

### 2.5. Porcine pancreatic $\alpha$ -amylase inhibition assay (DNSA method)

The DNSA assay for reducing sugar was conducted using various crude extracts of the leaves and starch as a substrate for amylase enzyme as described in Kwon *et al.* (2008) with minor modification. Test samples 200  $\mu$ L (0.01- 2.5 mg/mL) in a 0.02 M sodium phosphate buffer solution (pH 6.9 with 0.006 M sodium chloride) containing 200  $\mu$ L Porcine pancreatic  $\alpha$ -amylase were incubated at 25°C for 10 min, after which, 200  $\mu$ L of 1% boiled potato starch solution in 0.02 M sodium phosphate buffer solution (pH 6.9 with 0.006 M sodium chloride) was added. After incubation of the reaction mixture at 25°C for 10 min, the reaction was stopped by adding 400  $\mu$ L of DNSA reagent (1.0 g of 3, 5- dinitrosalicylic acid, 20 mL of 2 M NaOH and 30 g of sodium potassium tartarate in 100 mL distilled water). The sample test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 4 mL distilled water and absorbance of 200

μL of brown solution of 3-amino-5-nitrosalicylic acid was measured at 540 nm using micro plate reader (FLUO star Optima, BMG Labtech, Durham, NC, USA). Distilled water (without amylase inhibitor) (200 μL) was used as a control.

To remove matrix sugar interference, the absorbance of the mixture consisted of 200 μL of sample (may contain reducing sugars), 200 μL of phosphate buffer (no amylase), 200 μL of starch, 400 μL 3, 5- dinitrosalicylic acid, and 4 mL of distilled water was recorded at 540 nm as blank. Acarbose was used as reference. The α-amylase inhibitory activity was expressed as % inhibition and was calculated as shown below:

$$\% \text{ inhibition} = [A_{\text{control}} - (A_{\text{sample}} - A_{\text{blank}})/A_{\text{control}}] \times 100$$

## 2.6. α-Glucosidase inhibition assay

α-Glucosidase inhibitory activities were evaluated according to the chromogenic method described by Ivan *et al.* (2012), with some modifications. The enzyme solution contained 20 μL α-glucosidase (0.5 unit/mL), 20 μL of sample (at various concentrations) or drug (acarbose) and 60 μL 0.01 M phosphate buffer (pH 6.9). The mixture was incubated at 37 °C for 15 min. After 15 min, 20 μL of p-nitrophenyl-α-D-glucopyranoside (pNP-G) (5 mM) in the same buffer (pH 6.9) was used as a substrate solution and again incubated at 37°C for 15 min. The reaction (Figure 6.3) was terminated by adding 80 μL of 0.2 M sodium carbonate solution. Each experiment was conducted in triplicate. The change in the absorption observed at 405 nm due to the hydrolysis of p-nitrophenyl-α-D-glucopyranoside (pNP-G) was monitored in a 96-well plate with micro plate reader (FLUO star Optima, BMG Labtech, Durham, NC, USA). Increase in absorption at 405 nm was due to enzyme activity as the enzyme hydrolyzes the pNP-G to release p-nitrophenolate ion. The temperature was maintained at 37°C during the experiment. The positive control sample was the mixture of the enzyme (20 μL) and substrate (20 μL) without inhibitors. Instead 20 μL of working buffer was added. The sample controls and blanks were the mixtures of sample and control, respectively, except α-glucosidase which was replaced instead with buffer. The IC<sub>50</sub> values of samples were calculated and reported as the mean ± SD of the three experiments. The enzyme inhibitory rates of samples were calculated as follows:

$$\text{Inhibition}\% = [(A_S - A_{SB}) / (A_C - A_{CB})] \times 100$$

Where, A<sub>S</sub>, A<sub>SB</sub>, A<sub>C</sub>, A<sub>CB</sub> are the absorbance of sample, sample blank, control, and control blank, respectively.

## 2.7. Statistical analysis

The data were subjected to analysis of variance (ANOVA) and Duncan's multiple range tests were used for mean separation at  $p < 0.05$ . Linear regression analysis was used to calculate IC<sub>50</sub> value. Pearson correlations among antioxidant activities, total phenolic and flavonoid contents were considered at  $p < 0.05$ .

## 3. Result and Discussions

### 3.1. Total phenolic and flavonoid contents

The TPC in various solvent extracts from the leave of *T. schimperi* and *T. vulgaris* varied widely, ranging from 15.65 ± 4.01 to 46.0 ± 4.5 mg GAE/g dw (Table 1). The TPC content followed the order: aqueous: methanol (20:80, v/v) extract of *T. schimperi* > aqueous: methanol (20:80, v/v) extract of *T. vulgaris* > hot water extract of *T. schimperi* > hot water extract of *T. vulgaris*. There was no significant difference ( $p > 0.05$ ) in TPC between aqueous: methanol (20:80, v/v) extracts of *T. schimperi* and *T. vulgaris* but these values were significantly higher ( $p < 0.05$ ) than the TPC of hot water extracts of *T. schimperi* and *T. vulgaris*. According to the study conducted by Hasya *et al.* (2019), *Thymus zygioides* var. *lycaonicus* showed stronger TPC (193.47 ± 4.45 mgGAE/g) than that of both *T. schimperi* and *T. vulgaris*.

**Table 1.** Total phenolic (mgGAE/ g dw) and total flavonoid (mgQRE/g dw) contents of *T. schimperi* and *T. vulgaris*

Extract	TPC (mgGAE/g dw)* ± SD	TFC (mgQRE/g dw)** ± SD
TS. ( aqueous: methanol:20:80, v/v)	46.0 ± 4.5 <sup>c</sup>	14.7 ± 1.1 <sup>c</sup>
TS (hot water)	21.55 ± 3.80 <sup>b</sup>	3.69 ± 1.42 <sup>a</sup>
TV ( aqueous: methanol:20:80, v/v)	45.23 ± 13.02 <sup>c</sup>	10.65 ± 2.15 <sup>b</sup>
TV (hot water)	15.65 ± 4.01 <sup>a</sup>	1.13 ± 0.20 <sup>a</sup>

TS: *Thymus schimperi*; TV: *Thymus vulgaris*; dw: dried weight of plant material. Where \* and \*\* are total phenolic and total flavonoids expressed as gallic acid and quercetin equivalents per gram of dried weight, respectively. Values are expressed as mean ± SEM (n = 3) from triplicate experiments. Means with different letters in a column were significantly different at the level of  $p < 0.05$ .

The TFC (mg QRE/g dw) varied from 1.13 ± 0.2 to 14.7 ± 1.1 and decreased in the order of aqueous: methanol (20:80, v/v) extract of *T. schimperi* > aqueous: methanol (20:80, v/v) extract of *T. vulgaris* > hot water extract of *T. schimperi* > hot water of *T. vulgaris* (Table 1). TFC in aqueous: methanol (20:80, v/v) extracts of *T. schimperi* and *T. vulgaris* were significantly different ( $p < 0.05$ ), but in the hot water extracts were not significantly different

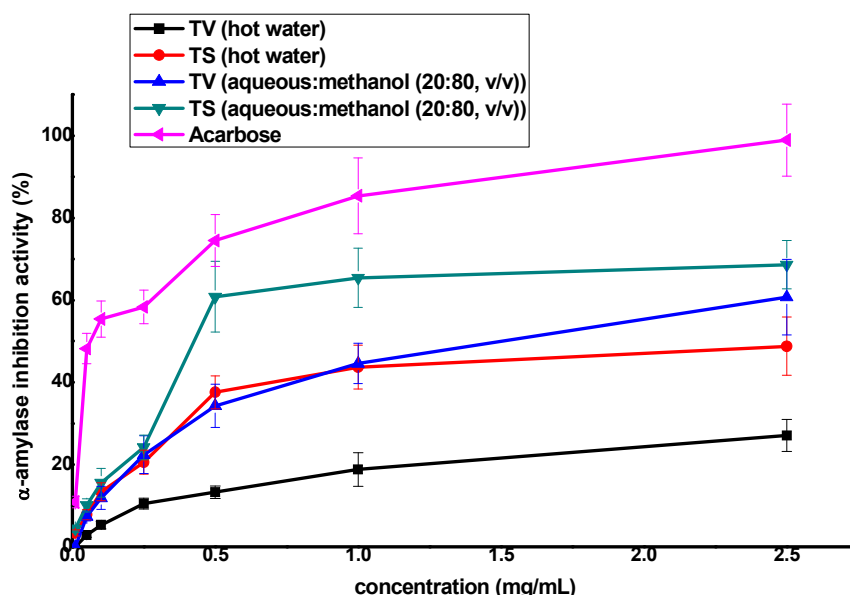
( $p > 0.05$ ).

### 3.2. Porcine $\alpha$ -amylase inhibitory activity (DNSA method)

One of the effective methods to control diabetes is to inhibit the activity of  $\alpha$  - amylase enzyme which is responsible for the breakdown of starch to more simple sugars (Probhakar and Doble, 2011). This is contributed by  $\alpha$ - amylase inhibitors, which delays the glucose absorption rate thereby maintaining the serum blood glucose in hyperglycemic individuals (Cazzola *et al.*, 2011; Wadkar *et al.*, 2008). Different studies have shown that phenolic compounds play a role in mediating  $\alpha$ -amylase inhibition and therefore have potential to contribute to the management of type 2 diabetes (Cheplick *et al.*, 2010; Ranilla *et al.*, 2010).

The extracts from the crude *T. schimperii* and *T. vulgaris* leaf extracts screened for *in-vitro*  $\alpha$ -amylase enzymes inhibitory activity. The results were shown in Figure 1. The  $\alpha$ -amylase enzymes inhibitory activity was concentration dependent. At 2.5 mg/mL, the porcine  $\alpha$ -amylase inhibitory activity of aqueous: methanol (20:80, v/v) extract from *T. schimperii* was  $68.6 \pm 5.9\%$ , and the inhibitory activity of its boiling water extract was  $48.7 \pm 7.1\%$ . The inhibitory activity of aqueous: methanol (20:80, v/v) extract from *T. vulgaris* was  $60.7 \pm 9.2\%$ , and the inhibitory activity of its boiling water extract reached  $27.1 \pm 3.9\%$ . Aqueous: methanol (20:80, v/v) extracts of *T. schimperii* demonstrated stronger percentage of  $\alpha$ -amylase enzyme inhibitory activities than that of *T. vulgaris* extracts. These values are lower than citronella grass, lemongrass oils (Jumepaeng *et al.*, 2013) and finger millet (Shobana *et al.*, 2009 ), but higher than cereal grains such as wheat, buckwheat, corn and oats (Randhir *et al.*, 2008) and foxtail millet (Kim *et al.*, 2011). As positive control, at the concentration of 2.5 mg/mL, acarbose showed the strongest  $\alpha$ -amylase inhibition activity ( $98.9 \pm 8.8\%$ ).

The inhibitory activity was determined as the mean of triplicate measurements and expressed as the 50% inhibitory concentrations ( $IC_{50}$ ) values (Table 2). The aqueous: methanol (20:80, v/v) extract of *T. schimperii* demonstrated stronger percentage of  $\alpha$ -amylase enzyme inhibitory activity than the rest of the extracts. As positive control, acarbose showed the strongest  $\alpha$ -amylase inhibition activity, five times stronger than the inhibition potential of aqueous: methanol (20: 80, v/v) extract of *T. schimperii* .Whereas, hot extract of *T. vulgaris* showed the lowest  $\alpha$ -amylase inhibition activity ( $IC_{50} > 2.5$  mg/ml). There were significant differences ( $p < 0.05$ ) in the  $IC_{50}$  values among the extracts. But  $\alpha$ -amylase enzyme inhibitory activities of these extracts were significantly lowers ( $p < 0.05$ ) than the  $\alpha$ -amylase enzyme inhibitory activity of acarbose. The  $\alpha$ -amylase inhibition capacity of aqueous: methanol (20: 80, v/v) extract of *T. schimperii* of our previous study (Engeda *et al.*, 2015) was weaker ( $IC_{50} = 0.44$  mg/mL) than that of the present study ( $IC_{50} = 0.33$  mg/mL). This variation may be because of the geographical location and growing conditions of *T. schimperii*, such as soil and climate (Yang *et al.*, 2018).



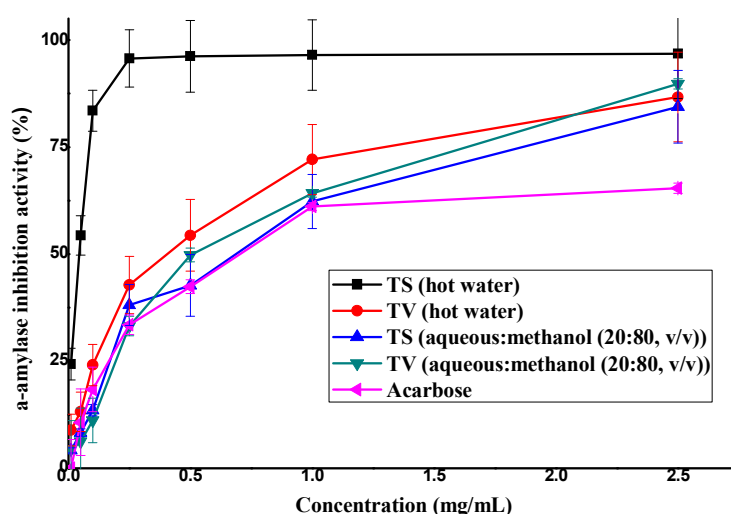
**Figure 1.** The porcine  $\alpha$ -amylase inhibitory activities of aqueous: methanol (20: 80, v/v) and boiling water extracts of *T. schimperii* and *T. vulgaris*. Results were expressed as mean  $\pm$  SD ( $n = 3$ ).

### 3.3. *In vitro* $\alpha$ - glycosidase inhibition activity

The extracts were also tested through the  $\alpha$ -glucosidase inhibitory assay and the results were shown in Figure 2. At the concentration of 2.5 mg/mL, hot water extract of *T. schimperii* showed the highest  $\alpha$ -glucosidase inhibition activity ( $96.8 \pm 10.5\%$ ) followed by hot water extract of *T. vulgaris* ( $86.7 \pm 8.3\%$ ), aqueous: methanol (20:80, v/v)

extract of *T. schimperi* ( $84.4 \pm 8.5\%$ ), aqueous: methanol (20:80, v/v) extract of *T. vulgaris* ( $60.7 \pm 9.2\%$ ) and acarbose ( $65.4 \pm 5.8\%$ ).

The  $IC_{50}$  values were shown in Table.2. Hot water extract of *T. schimperi* showed strongest  $\alpha$ -glucosidase inhibition activity ten times stronger than that of aqueous: methanol (20:80, v/v) extract of *T. vulgaris* and more than four times stronger than the  $\alpha$ -glucosidase inhibition activity of hot water extract of *T. vulgaris* and more than thirteen times stronger than that of  $\alpha$ -glucosidase inhibition activity and acarbose. There was no significant difference in  $IC_{50}$  values ( $p > 0.05$ ) between aqueous: methanol (20:80, v/v) extract of *T. schimperi* and acarbose in their  $\alpha$ -glucosidase inhibitory effects. But these values were significantly different from that of hot water extracts and aqueous: methanol (20:80, v/v) extract of *T. vulgaris*. Similar result was reported by Toshiyuki and Miyazawa (2012) on safflower (*Carthamus tinctorius* L). According to this study the different extracts exhibited stronger  $\alpha$ -glucosidase inhibition activity than acarbose. Similarly, according to the study conducted by Abid *et al.* (2014), ethyl acetate fraction of *Thymelaea hirsute* showed stronger  $\alpha$ -glucosidase inhibition potential than that of acarbose. The hot water extracts of *T. schimperi* and *T. vulgaris* as natural sources thus can be potentially used to suppress glycemic load by reducing  $\alpha$ -glucosidase activity.



**Figure 2.**  $\alpha$ -glucosidase inhibitory activity of aqueous; methanol (20:80, v/v) and boiling water extracts of *T. schimperi* and *T. vulgaris*. Results were expressed as mean  $\pm$  SD ( $n = 3$ ).

**Table 2.**  $IC_{50}$  (mg/mL) of  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition activity of *T. schimperi* and *T. vulgaris* leaf extracts.

Extract	$\alpha$ – amylase	$\alpha$ -glucosidase
TS (aqueous: methanol:20:80, v/v)	$0.33 \pm 0.05^b$	$0.69 \pm 0.04^d$
TS (hot water)	$2.24 \pm 0.53^d$	$0.05 \pm 0.01^a$
TV (aqueous: methanol:20:80, v/v)	$1.56 \pm 0.09^c$	$0.51 \pm 0.02^c$
TV (hot water)	$> 2.50$	$0.24 \pm 0.09^b$
Acarbose	$0.07 \pm 0.01^a$	$0.71 \pm 0.12^d$

TS: *Thymus schimperi*; TV: *Thymus vulgaris*. Values are expressed as mean  $\pm$  SD ( $n = 3$ ) from triplicate experiments. Means with different letters in a column were significantly different at the level of  $p < 0.05$ .

### 3.4 Correlation Analysis

The analyses of linear correlation between enzymes' inhibitory activities and TPC and TFC (Table 3) showed that the inhibitory effects of samples against the activity of  $\alpha$ -amylase could be due to the levels of phenolic compounds existing in the extracts with the correlation coefficients +0.78, +0.67, at  $p < 0.05$ , respectively. These results suggest that higher phenolic content does confer higher  $\alpha$ -amylase inhibitory activity. Inhibitory activities of the extracts against  $\alpha$ -glucosidase were negatively proportional to both TPC and TFC, and the correlation coefficients were  $R^2 = -0.22, -0.24$ , at  $p > 0.05$ , respectively. Several studies have found a direct correlation between the amount of phenolic compounds in plant extracts and their capacity to inhibit  $\alpha$ -enzymes (Patrick *et al.*, 2005; Chen & Kang, 2014). However, not always plant extracts with the high phenolic content have been demonstrated to exert the inhibitory activity on  $\alpha$ -amylase (Hairong and Baojun, 2014), which points out the importance of the

nature of the different molecules and the interactions among them. Furthermore, different studies confirmed the negative correlation between phenolic contents and  $\alpha$ -glucosidase inhibition activity. According to the result reported by Jeonga *et al.* (2013),  $\alpha$ -glucosidase inhibition activity of *Rehmannia glutinosa* tuberous root extracts was negatively correlated with TPC. To the contrary the study conducted by Hairong and Baojun (2014) on onion, reported that TPC values of samples were positively correlated to  $\alpha$ -glucosidase and negatively correlated with  $\alpha$ -amylase inhibitory activities. Silva Pinto *et al.* (2009), have also shown a positive correlation between  $\alpha$ -glucosidase activity and TPC of *Gingko bilibo* L. leaves extracts.

**Table 3.** Correlations between enzyme inhibition activity and total phenolic and flavonoid contents.

Inhibition Activity	TPC (mgGAE/g dw)*	TFC (mgQRE/g dw)**
$\alpha$ -amylase	+0.78	-0.22
$\alpha$ -glucosidase	+0.67	-0.24

Where \* and \*\* are total phenolic and total flavonoids expressed as gallic acid and quercetin equivalents, respectively; dw: dried weight of plant material.

### Conclusion

The present investigation showed that the *in vitro* antidiabetic properties of *T. schimperi* and *T. vulgaris* were related with their  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory effects. Hot water extract of *T. schimperi* had stronger  $\alpha$ -glucosidase inhibitor and aqueous: methanol (20:80, v/v) extract showed stronger  $\alpha$ -amylase inhibitory potential in comparison with *T. vulgaris*. These results also indicated that there was positive linear correlation between TPC and  $\alpha$ -amylase inhibition activity but negatively correlated with  $\alpha$ -glucosidase inhibitory activity. However, phenolic compounds may not be the only class of active compounds to contribute to antidiabetic effects of these two herbs. The  $\alpha$ -glucosidase inhibition activity of hot water extract of *T. schimperi* was much stronger than that of the standard drug acarbose and thus this extract might help in the identification of new lead molecules for natural  $\alpha$ -glucosidase inhibitors. However isolation and characterization of the active compounds associated with  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition have to be carried out to confirm these observations. It can be therefore concluded from this study that the presence of the phytochemicals in these plants might be the reason for these inhibitions and that the plants may essentially contain herbal bioactive compounds which require further structural elucidation and characterization to identify the specific bioactive constituents. Also further *in vivo* and clinical investigations should be done for confirming the antidiabetic activity of these dietary herbs.

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### Conflicts of interest

The authors declare that they have no conflicts of interest.

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