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Vol. 9(15), pp. 515-524, 17 April, 2015
DOI: 10.5897/JMPR2014.5431
Article Number: 779975D52769
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Antioxidant and α-amylase inhibition activities *in vitro* of various solvent extracts of *Thymus schimperi* Ronniger

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Received 25 March, 2014; Accepted 8 April, 2015

***Thymus schimperi* Ronniger is a wild endemic herb to Ethiopia, and is traditionally used as food flavoring, preservative as well as medicinal ingredient. This paper reports the total phenolic and flavonoid contents, antioxidant capacity and α-amylase inhibition activity of various solvent extracts of the dried leaves. The acetone extracts contained the highest total phenolic content (122.0±11.6 mg GAE/g). Total flavonoid content was the highest in methanol extract (45.1±2.9 mg QRE/g). The aqueous methanol extract showed the highest 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging ability (IC_{50} =11.0±1.0 µg/ml), iron reducing power (60.1±1.0 mg AAE/g), and total antioxidant capacity (1.1±0.1 mg BHTE/g). The water extract exhibited the highest iron chelating activity (IC_{50} = 65.4±1.1 µg/ml) while the methanol extract exhibited the highest percentage of α-amylase inhibition activity (IC_{50} = 335. 6±90.4 µg/ml). Except for iron chelating activity, all antioxidant activities were positively correlated with total phenolic and flavonoid contents. The study revealed that antioxidant and α-amylase inhibitory activities of the crude extract were variable when extracted by different solvents indicating a high potential to be used as natural antioxidants in food preservation as well as for preventing oxidative stress mediated human disorders.**

Key words: Antioxidant activity, α-amylase, BHT, phenolic, *Thymus schimperi* Ronniger, thyme.

INTRODUCTION

Dietary herbs have been added to foods since ancient times as flavoring agents and preservatives. They also show potential health benefits as they possess antioxidant activities (Iris et al., 2006; Hui-Yin et al., 2007). In addition to imparting a characteristic flavors, certain herbs are also known to prolong the storage life of

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foods by preventing rancidity (Kathirvel and Rupasinghe, 2012; Gebrehana and Shimelis, 2013) and the growth of microorganisms (Bin et al., 2011; Gian et al., 2011) due to the presence of specific phytochemicals. In recent decades, many studies have been conducted on bioactive phytochemicals and their effects on human health. In particular, researches have focused on antioxidants, hypoglycemic agents (Adeneye et al., 2008), and anticancer agents (Miloˇs et al., 2014) from natural products.

The genus *Thymus* (Lamiaceae) includes about 350 species worldwide, and is widely distributed in temperate zones (Sebsebe, 1993). The essential oil known as thyme oil is used in the food flavoring and preservatives (Ehivet et al., 2011), perfumery and pharmaceutical industries (Ballester-Costa et al., 2013). Analysis of the essential oils from different species indicated the presence of different components mainly the high phenolic monoterpenes such as carvacrol, thymol and α-terpineol (Ehivet et al., 2011; Zouari et al., 2011) and solvent extracts contain many phenolic acids such as rosmarinic, ferulic, caffeic, chlorogenic and p-coumaric acids and also different flavonoids (Iness et al., 2012; Zeghad and Merghem, 2013). The essential oils exhibited strong antioxidant (Iness et al., 2012), antifungal (Eugénia et al., 2013) and antibacterial (Fethi et al., 2013) activities.

Thymus schimperi Ronniger, locally known as 'Tosign' in Ethiopia, is a wild endemic aromatic herb to Ethiopia, occurring in open grassland between bare rocks on slopes and tops of mountains, sometimes growing near ditches. The essential oil is a pale yellow liquid with a rich aromatic, warming, herbaceous odor. The main constituents of the essential oils are p-cymene, gamma-terpinene, thymol and carvacrol (Nigist et al., 2000). 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 and Sebsebe, 2009). A tea made by the herb in water is also recommended as a local medicinal remedy for respiratory problems (cough, bronchitis, sore throat), gastrointestinal disorders, (colic, dyspepsia gastritis, flatulence, and diarrhea) and liver disease (Abebe and Ayehu, 1993). Essential oil obtained from steam distillation of the freshly collected leaf also exhibited an antihelmentic effects (Jemal et al., 2011).

Though *T. schimperi* Ronniger is widely used as food flavoring and traditional medicine, scarce information is available on the antioxidant activities of this herb (Gebrehana and Shimelis**,** 2013). To our knowledge, there is no report on total phenolic and flavonoid contents, antioxidant, and α-amylase inhibition activities of various solvent extracts of the dried leaf. Therefore, the objective of the present study was to estimate total phenolic and flavonoid contents, *in vitro* antioxidant and α-amylase inhibition activities of petroleum ether, water, acetone, methanol and aqueous: methanol (20:80), v/v) extracts of the dried leaf. In addition, the correlation between phenolic contents and antioxidant assays was also evaluated.

MATERIALS AND METHODS

Chemicals

Gallic acid, butylated hydroxytoluene (BHT), Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, catechin, ferrozine, L-ascorbic acid, 3,5-dintrosalicylic acid (DNSA), and α-amylase were purchased from Sigma-Aldrich. The other chemicals and solvents used in this experiment were of analytical grade.

Plant material

Fresh leaves of *T. schimperi* Ronniger were collected from 50 km north of Addis Ababa, on the road side to Fiche town, North Shoa, Ethiopia in October, 2011 and identified at the Department of Biology, College of Natural Sciences of Addis Ababa University, Addis Ababa, Ethiopia. The voucher specimen was deposited at the center of Food Science and Nutrition.

Preparation of plant extracts

Fresh leaves of *T. schimperi* Ronniger were air dried for fifteen days and then ground to fine powder using electric grinder (FM100 model, China). The petroleum ether, water, acetone, methanol, and aqueous:methanol (20:80, v/v) extracts of all were prepared by dissolving 10 g of the leaves fine powder separately in 100 ml each solvent. The contents were kept in orbital shaker for 6 h at room temperature. Thereafter, each extract was filtered using Whatman no.1 filter paper and evaporated to dryness under vacuum at 40°C by using a rotary evaporator (Buchi, 3000 series, Switzerland). The extraction was done in triplicate for each solvent and the resulting extracts were stored in a sealed plastic container at 4°C until further investigation. Unless specifically mentioned, all analysis were conducted on triplicate analysis.

Determination of phenolic contents

Total phenolic content was estimated by Folin-Ciocalteu method as described in Shan et al. (2005) with slight modification using gallic acid as standard. 0.1 ml of the extract (1 mg/mL), 1 ml Folin-Ciocalteu reagent (diluted ten times) were 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 total phenolic content was estimated from gallic acid (1-100 µg/mL) calibration curve ($y = 0.015 x + 0.023$, $R^2 = 0.994$) and results were expressed as mg gallic acid equivalent/g of dry extract (mg GAE/g).

Determination of total flavonoid content

The total flavonoid content 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 total flavonoid content was determined using a standard curve of quercetin at (1- 40 µg/ml), and values were calculated as mg quercetin equivalents/g of dried extract (mg QRE/g) using the following equation based on the quercetin calibration curve: $y = 0.024 x + 0.112$, $R^2 = 0.99$.

Determination of antioxidant activity by DPPH radical scavenging assay

The DPPH radical scavenging activity of the extracts from *T. schimperi* leaves was determined as described by Katerere and Eloff (2005) with slight modification. Different concentrations (10 to1000 µg/ml) of the extracts were taken in different test tubes. Freshly prepared DPPH solution (2 ml, 0.006%, w/v) in methanol was added in each of the test tubes containing 1 ml of the extract. The reaction mixture and the reference standards (ascorbic acid and BHT) were vortexed and left to stand at room temperature in the dark for 30 min. The absorbance of the resulting solution was then taken at 520 nm. Methanol was used as blank. The ability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH\ scavenged\ (\%) = \frac{(Ac - As)}{Ac} x100$$

Where *A*c is the absorbance of the control and *A*s is the absorbance in presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/ml) of extracts that scavenges the DPPH radical by 50%.

Antioxidant activity using ferric ion reducing power

The presence of antioxidants in the extract causes the reduction of the yellow ferric/ferricyanide complex to the ferrous form which can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Amarowicza et al., 2004). Plant extract (1 ml) solution (final concentration 250-1000 µg/ml) was mixed with 2.5 ml sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. Then the mixture was incubated at 50°C for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added to the mixture, which was then centrifuged at 3000 rpm (Centurion, 1000 series, UK) for 5 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (0.1%) and absorbance was measured at 700 nm. The reducing power was expressed as milligram ascorbic acid equivalents/gram of dried extract (mg AAE/g) using the following equation based on L-ascorbic acid calibration curve: $y = 0.027x + 0.159$, $R^2 = 0.99$).

Total antioxidant capacity by phosphomolybdenum method

The total antioxidant capacity of the crude extracts was evaluated by the phosphomolybdenum method (Prieto et al., 1999) with slight modification. The method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds or crude extract and subsequent formation of green Mo (V) complexes with a maximal absorption at 695 nm at acidic medium (Mohamed et al., 2011). Plant extract (0.3 ml, 0.5 and 1 mg/ml) was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The samples were incubated at 95°C for 90 min, cooled to room temperature and absorbance was measured at 695 nm and methanol (3 ml) was used as blank. The total antioxidant activity was expressed as milligram butylated hydroxyltoluene equivalent/gram of dried extract (mg BHTE/g) based on the calibration curve; $y = 0.432x + 0.076$, $R^2 = 0.99$.

Chelating effects on ferrous ions

The ferrous chelating activity was determined according to the method of Dinis et al. (1994). Various concentrations (100-800 µg /ml) of the extracts (3 ml) in methanol were added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.1 ml). Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the solution was measured at 562 nm. Ethylenediamine tetraacetic acid (EDTA), L-ascorbic acid, quercetin and butylated hydroxytoluene (BHT) were used as a control. The inhibition percentage of ferrozine–Fe^{2+} complex formation was calculated by using the formula given below:

$$Metal\ chelating\ effect\ (\%) = \frac{(Ac - As)}{Ac} x100$$

Where Ac is control absorbance (the control contains $FeCl_2$ and ferrozine, complex formation molecules) and As is test sample absorbance.

***In vitro* α-amylase inhibition activity**

This assay was conducted using various crude extracts of the leaves as described in Bahman et al. (2008) with minor modification. Test samples of 1 ml (1 mg/mL) in a 0.02 M sodium phosphate buffer solution (pH 6.9 with 0.006 M sodium chloride) containing 1 ml of α-amylase (from *Aspergillus oryzae*) were incubated at 25°C for 30 min, after which, 1 ml 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 1 ml of 3, 5-dinitrosalicylic acid color reagent (1.0 g of 3, 5-dinitrosalicyclic acid, 20 ml of 2M 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 5 ml distilled water and absorbance was measured at 540 nm. Control representing 100% enzyme activity was conducted in similar way by replacing extract with buffer. The generation of reducing sugar was quantified by the reduction of 3, 5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid. L-ascorbic acid, BHT, Acarbose and catechin were used as a positive control. The α-amylase inhibition was calculated as follows, and then expressed as percentage of inhibition:

$$Inhibition\ (\%) = \frac{[(Ac+) - (Ac-)] - [As - Ab]}{(Ac+) - (Ac-)} x100$$

Where, Ac+ is absorbance of 100% enzyme activity, Ac- is 0% enzyme activity (only solvent without enzyme), As, test sample (with enzyme) and Ab is blank (a test sample without enzyme) respectively (Hansawasdi et al., 2006).

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_{50} value. Pearson correlations among antioxidant activities, total phenolic and flavonoid contents were considered at $p < 0.05$.

Table 1. Total phenolic and flavonoid contents from *T. schimperi* leaf, *extracted* with different solvents.

Extract	Total phenolics (mg GAE/g)*1 ± SEM	Total flavonoids (mg QRE/g)*2 ± SEM
Petroleum ether	11.3±0.0[a]	20.6±2.6[b]
Water	29.0±1.3[b]	10.9±0.2[a]
Methanol	92.4±2.7[c]	45.1±2.9[d]
Acetone	122.0±11.6[d]	21.4±0.4[bc]
Aqueous: methanol (20:80, v/v)	96.0±4.5[c]	26.7±1.1[c]

Where *1 and *2 are total phenolic and total flavonoids expressed as gallic acid and quercetin equivalents, respectively. Concentration of sample was 1 mg/ml. 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 $p < 0.05$.

RESULTS AND DISCUSSION

Determination of total phenolic and flavonoid contents

The total phenolic contents in various solvent extracts from the leaf of *T. schimperi* varied widely, ranging from 11.3±0.0 to 122.0±11.6 mg GAE/g (Table 1). The total phenolic content followed the order: acetone > aqueous: methanol (20:80, v/v) > methanol > water > petroleum ether extracts. There was no significant difference ($p > 0.05$) in total phenolic content between aqueous: methanol (20:80, v/v) and methanol extracts but these values were significantly different ($p < 0.05$) from acetone, petroleum ether and water. The total flavonoid contents (mg QRE/g) varied from 10.9±0.2 to 45.1±2.9 and decreased in the order of methanol > aqueous: methanol (20:80, v/v) > acetone > petroleum ether > water extracts (Table 1). The total flavonoid contents in petroleum ether, water, methanol and aqueous: methanol (20:80, v/v) extracts were significantly different ($p < 0.05$), but in the acetone extract was not significantly different ($p > 0.05$) from petroleum ether and aqueous: methanol (20:80, v/v) extracts.

Antioxidant activity based on DPPH scavenging

The DPPH radical scavenging effects of *T. schimperi* leaf extracts are shown in Figure 1. DPPH radical is scavenged by antioxidants through the donation of hydrogen forming the reduced DPPH. The color changes from purple 2, 2-diphenyl-1-picrylhydrazyl radical to reduced yellow diamagnetic 2, 2-diphenyl-1-picrylhydrazine molecule, which can be quantified by its absorbance reduction at wavelength 520 nm. At the concentration of 1 mg/ml used, the DPPH radical scavenging effects were decreased in the order of L-ascorbic acid (97.7±0.3%) > aqueous: methanol (20:80, v/v) extract (96.2 ± 1.2%) > acetone extract (95.4 ± 2.0%) > BHT (94.5 ± 0.2%) > methanol extract (92.2 ± 0.4%), > (71.4±1.9%). As the concentration of sample increased, petroleum ether extract (74.7±3.7%) > water extract the percent inhibition of DPPH radical also increased (Huang et al., 2005). Therefore, the percentage of DPPH radical scavenging capacity of acetone, methanol, and aqueous: methanol (20:80, v/v) extracts are comparable with commercial antioxidants, L-ascorbic acid and BHT at the concentration of 1 mg/ml. This suggested that dried leaf extract of *T. schimperi* contain compounds that can donate electron/hydrogen easily and stabilizes free radicals. The IC_{50} values of all the extracts were calculated from plotted graph of percentage scavenging activity against concentration of the extracts (Table 2). The lower the IC_{50} value, the higher is the scavenging potential. The IC_{50} values ranged from 11.0±1.0 µg/ml for aqueous: methanol (20:80, v/v) extract to 89.5±1.6 µg/ml for water extract. All extracts were significantly different ($p < 0.05$) and strongest scavenging activity (lower IC_{50} values) was recorded for aqueous: methanol (20:80, v/v) extract which appeared more than eight times stronger than that of water extract and four times stronger than that of methanol extract. The IC_{50} values of water, petroleum ether, acetone and methanol extracts were found to be significantly different ($p < 0.05$) from BHT and L-ascorbic acid IC_{50} values, while that of the aqueous:methanol (20:80, v/v) extract was found to be similar ($p > 0.05$).

Ferric ion reducing power

All results (Figure 2) revealed iron reducing power in dose dependent manner at concentration of 0.25 to 1 mg/ml. At 1 mg/ml, the reducing power of *T. schimperi* leaf extracts was found to decrease in this order: aqueous:methanol (60.1±1.0 mg AAE/g) > acetone (56.2±0.8 mg AAE/g) > methanol (39.1±3.4 mg AAE/g) > water (15.3±0.6 mg AAE/g) > petroleum ether (3.6±0.6 mg AAE/g). At various concentrations, the methanol, water, and petroleum ether extracts showed significant difference ($p < 0.05$) in their iron reducing power. At a concentration of 1 mg/mL, iron reducing power of acetone and aqueous methanol extracts were not significantly different ($p > 0.05$).

Table 2. IC_{50} (µg/ml) values of DPPH radical scavenging, ferrous chelating, and α-amylase inhibition activities in various solvent extracts from leaf of *T. schimperi.*

Extract	DPPH assay (µg/ml) ± SEM	IC_{50} of iron chelating activity (µg/ml) ± SEM	IC_{50} of α-amylase inhibition activity (µg/ml) ± SEM
Petroleum ether	73.9 ± 2.4^{d}	> 8x10^{2}	> 2.5x10^{2}
Water	89.5 ± 1.5^{e}	65.4 ± 1.1^{a}	2160. 8 ± 155.1^{d}
Methanol	45.8 ± 3.0^{c}	655.5 ± 13.6^{c}	335. 6 ± 90.4^{b}
Acetone	19.8 ± 1.27^{b}	> 8x10^{2}	462. 1 ± 87.6^{c}
Aqueous:Methanol (20:80, v/v)	11.0 ± 1.0^{a}	136.6 ± 14.0^{b}	447. 3 ± 121.2^{c}
BHT	8.8 ± 1.6^{a}	> 8x10^{2}	-
L-Ascorbic acid	5.9 ± 0.1^{a}	> 8x10^{2}	-
Quercetin	-	> 8x10^{2}	-
EDTA	-	50.2 ± 0.6^{a}	-
Catechin	-	-	> 2.5x10^{2}
Acarbose	-	-	71.2 ± 9.2^{a}

Values within a column with different letters are significantly different at $p < 0.05$.

Determination of total antioxidant activity by phosphomolybdenum assay

The results (Figure 3) revealed that aqueous:methanol (20:80, v/v) extract of *T. schimperi* leaf had the highest total antioxidant activity (1.1±0.1 mg BHTE/g) and the lowest total antioxidant activity (0.3±0.1 mg BHTE/g) was found in the water extract. No significant difference ($p > 0.05$) was found between the total antioxidant activity of petroleum ether (0.5±0.1 mg BHTE/g) and water extracts (0.3±0.1 mg BHTE/g) and also between acetone (0.7 ± 0.1 mg BHTE/g) and methanol (0.7±0.2 mg BHTE/g) extracts. However, these values were significantly lower ($p < 0.05$) than that of aqueous: methanol (20:80, v/v) extract.

Ferrous chelating activity

Metal chelating agents may have a dramatic effect on increasing the oxidation stability through blocking the pro-oxidant metal ions, and thus limiting the formation of chain initiators by preventing metal-assisted homolysis of hydroperoxides in lipid peroxidation (Praveen et al., 2012). Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted resulting in a decrease in the red color of the complex. Measurement of the color intensity reduction at 562 nm wavelength allows estimation of the metal chelating activity of the chelators. In this assay, both the extracts and standard compounds were assessed for their ability to compete with ferrozine for Fe^{2+} in the solution. The percentage of iron chelating activities of all extracts and references were concentration-dependent (from 100 to 800 µg/ml) (Figure 4). At 800 µg/ml the percentages of iron chelating capacity of the extracts and references decreased in the order of: EDTA (99.7±0.1%) > water (95.8 ± 0.5%) > aqueous: methanol (80:20, v/v) (86.4±0.4%) > methanol (60.8±0.3%) > BHT (49.5±1.2%) > ascorbic acid (41.3±0.3%) > petroleum ether (32.8±0.6%) > acetone 28.1±0.2% > quercetin (24.5±3.6%). Water extract was better chelator than the other tested extracts. Table 2 shows distinctive difference ($p < 0.05$) between the IC_{50} values of methanol (655.5±13.6 µg/ml) and aqueous: methanol (136.6±14.0 µg/ml) extracts, but these values were significantly lower ($p < 0.05$) than that of acetone and petroleum ether. However, the IC_{50} value of water (65.4±1.1 µg/mL) extract and EDTA (one of the most powerful metal chelator ever known, IC_{50} = 50.2±0.6 µg/ml) were not significantly different ($p > 0.05$). Nevertheless, in this assay ascorbic acid and BHT showed weaker chelating activity of iron (II) ions than water, methanol, and aqueous: methanol (80:20, v/v) extracts, which is consistent with the findings of Yen et al. (2002), but greater than that of acetone, petroleum ether and quercetin.

Correlation analyses

A relationship between phenolic content and antioxidant activity was extensively investigated, and both positive and negative correlations were reported. Bakchiche et al. (2013) and Petra et al. (2012) together with many other research groups stated that there was a positive correlation. Contrary to the above, insignificant correlation was also reported (Mohammed et al., 2008). In the present study, the dependence of antioxidant activity, obtained by different assays, in relation to the total phenolic and flavonoid content, was evaluated (Table 3). The total phenolic content correlated well with iron reduction (R^2 = 0.95), DPPH radical scavenging (R^2 = = 0.97) and total antioxidant assay (R^2 = 0.87). Similarly, a positive correlation was also found between DPPH radicals (R^2 = 0.96), reducing power (R^2 = 0.80) and total antioxidant (R^2 = 0.99) with the flavonoid contents in *T. schimperi* leaf extracts. But both total phenolic and

Table 3. Correlations between antioxidant activities of the various solvent extracts of *T. schimperi* leaf, total phenolic and flavonoid contents in the extracts ($p < 0.05$).

Antioxidant activities	Total phenolic	Total flavonoid
DPPH scavenging	0.97**	0.96**
Ferric ion reducing power	0.95**	0.80**
Total antioxidant capacity	0.87*	0.99**
Iron chelating activity	-0.31	-0.32

* indicates significance at $p < 0.05$, ** indicates significance at $p < 0.01$.

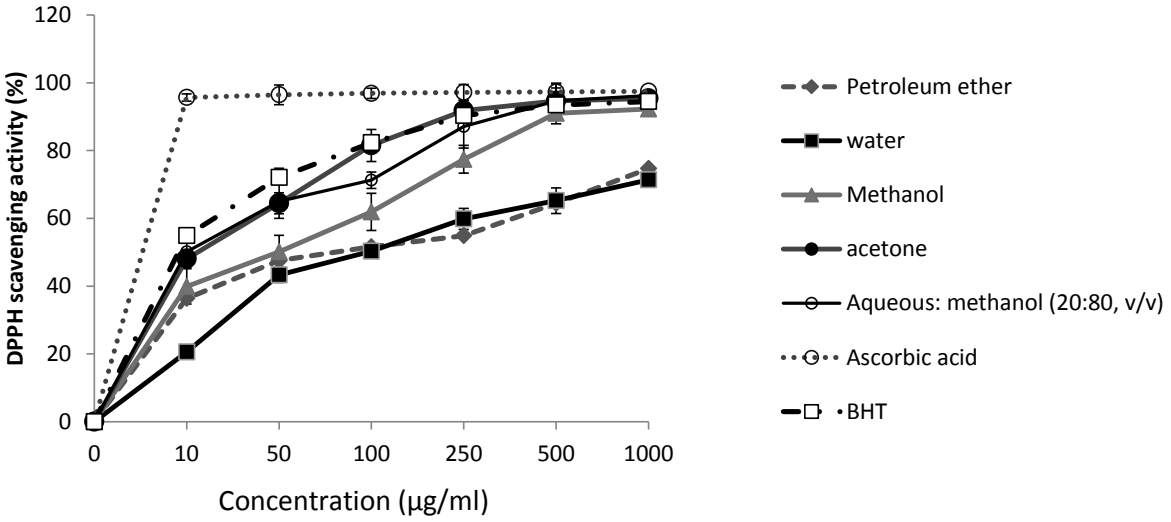

Figure 1. DPPH radical scavenging activity (%) of petroleum ether, water, acetone, methanol, and aqueous: methanol (20:80, v/v) extracts from dried leaf of *T. schimperi* and controls (L-ascorbic acid and BHT). Values are average of triplicate measurements (mean ± SEM).

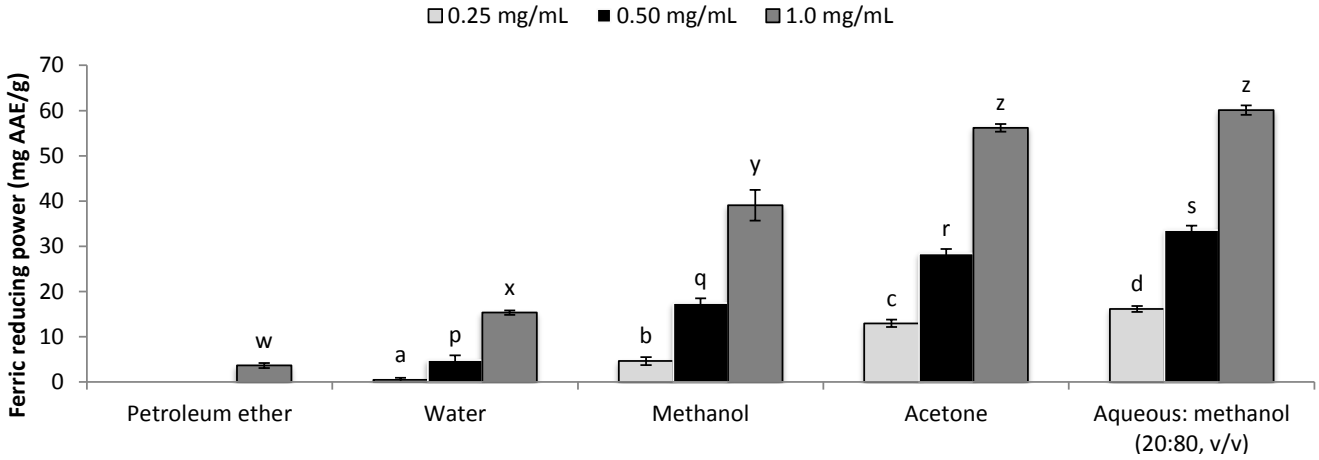

Figure 2. Ferric ion reducing power capacity (mg AAE/g) of petroleum ether, water, acetone, methanol, and aqueous: methanol (20:80, v/v) extracts from dried leaf of *T. schimperi* at different concentrations (mg/mL). Values are average of triplicate measurements (mean ± SEM). Values within the same concentration with different letters in the histogram bar are significantly different at $p < 0.05$.

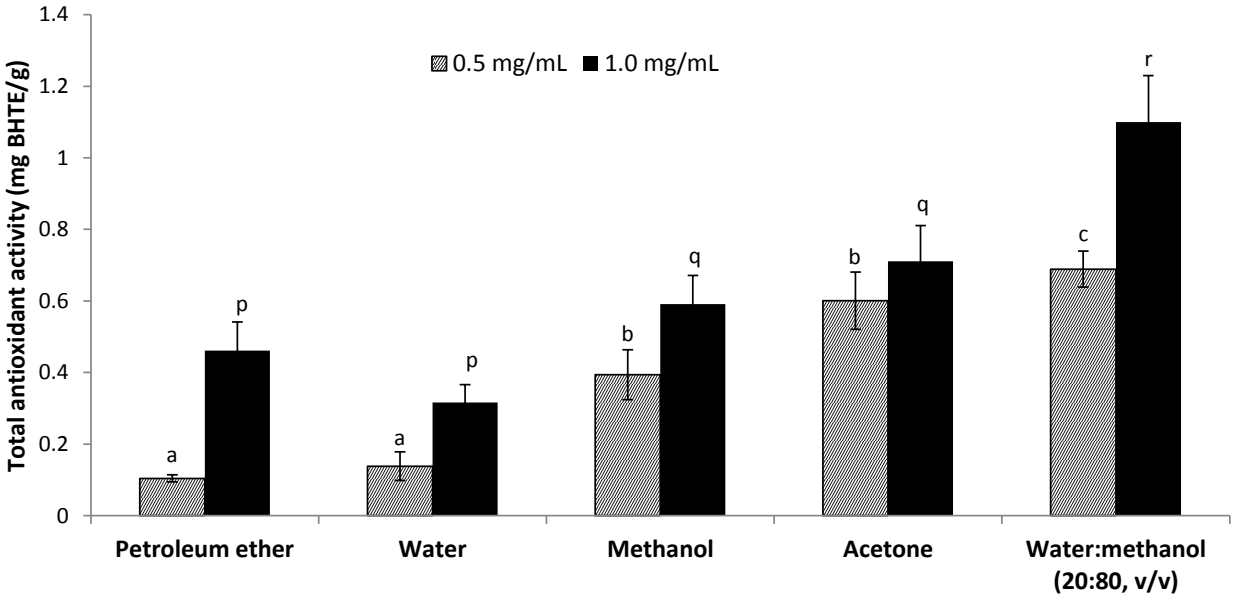

Figure 3. Total antioxidant capacity (mg BHTE/g) of petroleum ether, water, acetone, methanol, and aqueous: methanol (20:80, v/v) extracts from dried leaf of *T. schimperi* at different concentrations (mg/mL). Values are average of triplicate measurements (mean ± SEM). Values within the same concentration with different letters in the histogram bar are significantly different at $p < 0.05$.

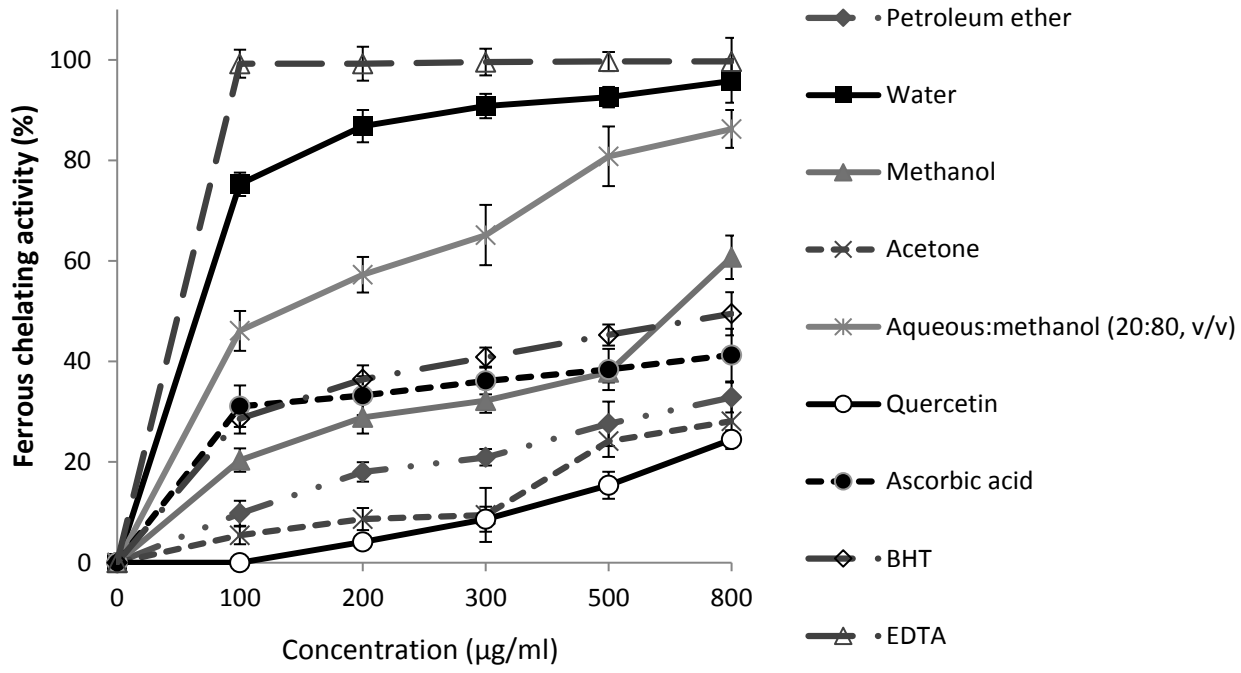

Figure 4. Ferrous ion chelating activity (%) of petroleum ether, water, acetone, methanol, and aqueous: methanol (20:80, v/v) extracts from dried leaf of *T. schimperi* and controls (L-ascorbic acid, BHT, EDTA, and quercetin). Values are average of triplicate measurements (mean ± SEM).

flavonoid contents showed negative correlation with chelating activity. Thus, the phenolics from the leaf extract of *T. schimperi* showed a high hydrogen-donating capacity, as well as high reactivity to free radicals, leading to the stabilization and termination of the radical chain reactions.

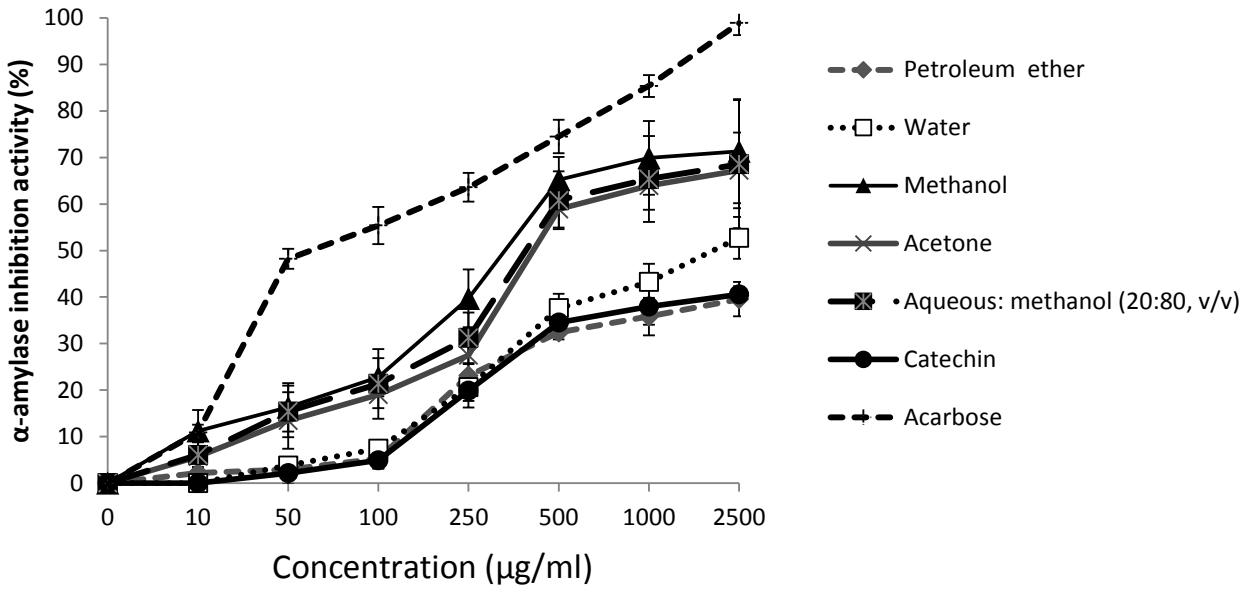

Figure 5. α-Amylase inhibition activity (%) of various solvent extracts from leaf of *T. schimperi* and references *(*acarbose and catechin). Values are average of triplicate measurements (mean ± SEM).

α–Amylase inhibition activity

One of the effective methods to control diabetes is to inhibit the activity of α-amylase enzyme which is responsible for the breakdown of starch to more simple sugars (Probhakar and Doble, 2011). This is contributed by α-amylase inhibitors, which delays the glucose absorption rate thereby maintaining the serum blood glucose in hyperglycemic individuals (Wadkar et al., 2008). Different studies have shown that phenolic compounds play a role in mediating α-amylase inhibition, and therefore have potential to contribute to the management of type 2 diabetes (Ranilla et al., 2010). The ability of the crude *T. scimperi* leaf extracts to inhibit α-amylase activity *in vitro* was investigated and the result was presented in Figure 5. The results revealed that *T. schimperi* leaf extracts inhibited α-amylase in a dose-dependent manner (10–2.5x10^3 µg/ml). However, as revealed by the IC_{50} (extract concentration causing 50% enzyme inhibition) values (Table 2), methanol (IC_{50} = 335.6 ± 90.4 µg/mL), aqueous:methanol (20;80, v/v) (IC_{50} = 447.3 ± 121.2 µg/mL), and acetone (IC_{50} = 462.1 ± 87.6 µg/mL) extracts demonstrated a significantly ($P < 0.05$) higher α-amylase inhibitory activity than water (IC_{50} = 2160.8 ± 155.1 µg/mL) and petroleum ether (IC_{50} > 2.5x10^3 µg/mL) extracts. These values are lower than citronella grass and lemongrass oils (Jumepaeng et al., 2013), 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, acarbose showed the highest α-amylase inhibition activity (IC_{50} = 71.2 ± 9.2 µg/ml), whereas catechin demonstrated lowest α-amylase inhibition activity, comparable to that of petroleum ether extract (IC_{50} > 2.5x10^3 µg/ml). There was no significant difference ($p > 0.05$) between acetone and aqueous:methanol (20:80, v/v) extracts in IC_{50} values. But these values were significantly different from the IC_{50} values of methanol, petroleum ether and water extracts. The *T. schimperi* leaf extracts as natural sources thus can be potentially used to suppress glycemic load by reducing α-amylase enzymes activities.

Conclusion

The study showed that total phenolic and flavonoid contents of *T. Shimperi* were highest in the acetone and methanol extracts, respectively. Aqueous: methanol (80:20, v/v) extract showed the highest value of DPPH radical scavenging, total antioxidant capacity and iron reducing power. On the other hand, methanol extract exhibited the highest percentage of α-amylase inhibitory activity. This suggests that the antioxidant activities of the tested extracts were closely associated with their phenolic constituents. The study revealed that *T. schimperi* contain a considerable amount of phenolic compounds, and has significant antioxidant activity, which can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical applications. However, the specific phenolic compounds or other components responsible for the antioxidant activity in various solvent extracts of *T. schimperi* leaves are unknown and require further investigations. Furthermore, research revealed the bioactive compounds present in the leaves of *T. schimperi* have the potential to be used as possible

natural substitutes for controversial synthetic antioxidants currently used in food preservation.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial assistance provided by the Addis Ababa University, and also extend thanks to Hawassa College of Education for the use of laboratory facilities. Professor Sebsebe Demisew of Biology Department, Addis Ababa University is acknowledged for identifying the plant material.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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