Effect of thermal treatment on phenolic content, antioxidant, and α-amylase inhibition activities of *Moringa stenopetala* leaves

Daniel Assefa¹, Engeda Dessalegn² and Chetan Chauhan¹

¹Department of Chemistry, College of Natural and Computational Sciences, Dilla University, P.O Box 149, Dilla, Ethiopia.
²Department of Chemistry, College of Natural Science, Hawassa Teacher Training College, Hawassa, Ethiopia.

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*Moringa stenopetala* is a socioeconomic valued tree that is widely available and cultivated in Southern part of Ethiopia. The leaves have been traditionally used as a food source with high nutritional and medicinal values. The present work was carried out to evaluate the effect of thermal treatment on the total phenolic content, total flavonoid content, antioxidant activities and α-amylase inhibition of aqueous leaf extracts obtained from *M. stenopetala* during maceration and different decoction time interval (5, 10 and 15 min). The total phenolic and flavonoid contents were determined by the Folin-ciocalteu and aluminum chloride methods, respectively whereas antioxidant activities were determined by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging, reducing power, phosphomolybdenum and ferrous ion chelating assays and α-amylase inhibition potential was determined using 3,5-dinitrosalicylic acid method. Total phenolic and total flavonoid contents ranged from 34.35 ± 1.06 to 39.47 ± 1.33 mgGAE/g and 10.44 ± 0.61 to 20.36 ± 0.93 mgQRE/g, respectively. Decoction for 10 min extract showed ferrous ion chelating (92.52 ± 0.17 %), DPPH radical scavenging (91.52 ± 0.59 %), α-amylase inhibition (69.06 ± 0.14 %), ferric reducing power (0.765 ± 0.14) and total antioxidant activity (0.329 ± 0.32), respectively. DPPH, reducing power, total antioxidant and α-amylase inhibition activities showed positive linear correlation ($R^2=0.853$, $R^2=0.857$, $R^2=0.864$ and $R^2=0.930$), respectively with total phenolic content but ferrous ion chelating activity were found to be weakly correlated ($R^2=0.481$). Based on present investigation, it could be concluded that major lose of total phenolic content, antioxidant and α-amylase inhibition activities of the crude leaf extracts of *M. stenopetala* leaves were observed at decoction time for 15 min. Therefore, to maintain the total phenolic content, antioxidant and α-amylase inhibition activities of leaves, cooking practice should be at the optimum decoction time (5-10 min).

Key words: *Moringa stenopetala*, antioxidant, total phenolic content, α-amylase inhibition.

INTRODUCTION

In living systems, free radicals are produced by the normal metabolic processes and quite helpful in many of body’s natural functions. However, uncontrolled production of derived free radicals can initiate the oxidation of biomolecules such as protein, lipid, amino acids and DNA and can induce numerous diseases (Hsu et al., 2003). A diet containing plenty of fruits, vegetables, dietary herbs, spices, whole grains and nuts can supply...
compounds that inhibit or delay the oxidation of such molecules by inhibiting the initiation or propagation of oxidizing chain reactions and such compounds are known as antioxidants (Meena et al., 2013). Several synthetic antioxidant and hypoglycemic compounds are commercially available, but are quite unsafe and of major concern due to their side effects (Vinay et al., 2010, Reddy et al., 2011). Hence, strong restrictions have been placed for such possible use. Nowadays, there is a trend to substitute and discover new and effective antioxidant and α-amylase inhibitors from plants with minimal or no side effects (Shai et al., 2010). Several African medicinal plants were reported to have both antioxidant and hypoglycemnic activities (Atawodi, 2005). Plant foods rich in polyphenols have been reported to cause effects on insulin in the utilization of glucose and act as good inhibitors of key enzymes like α-amylase associated with type II diabetes and decrease the postprandial blood glucose expenditure levels in diabetic patients (Fred-Jaiyesimi et al., 2009).

*Moringa stenopetala* is belonging to the family Moringaceae which believed to be originated from southern Ethiopia, North Kenya, and Eastern Somalia (Mohammed, 2013). *M. stenopetala* is commonly called Shiferaw, shedagha, Halako, Aleko and found in many arid zones of the southern Ethiopia at an altitude range of about 1100 to 1600 m (Edwards et al., 2000). The local people cook the leaves and eat them with their traditional kurkufa (a cereal dish made with maize and surgum) and also use the leaves as a food, tea and medicine (Mekonnen and Gessesse, 1998; Jahn et al., 1986). The diverse medicinal use of the plant by the local people has lead several researchers to carry out investigations to find bioactive constituents that are responsible for various medicinal uses of the plant with ultimate goal of justifying the traditional use of the plant species or discovering drug against different disease (Mutiu et al., 2013).

The leaf extracts were found to lower blood glucose level, cholesterol in mice models, relieving stomach pain, to expel retained placenta by women during giving birth, antidiabetic and for the regulation of thyroid hormone status (Tahlilian and Kar, 2000; Mekonnen, 1999; Jaiswal et al., 2009). The leaves and pods contains high amount of Ca, Mg, K, Mn, P, Zn, Na, Cu, and Fe (Aslam et al., 2005). These results were claimed to be consistent with the traditional use of the leaves of *M. stenopetala*. However, the effect of thermal treatment on antioxidant activities, total phenolic, total flavonoid and α-amylase inhibition has not been reported so far. Therefore, the study aimed to investigate the effect of thermal treatment during decoction process on the total phenolic content, antioxidant activities and α-amylase inhibition of *M. stenopetala* leaf extracts, commonly consumed in south Ethiopia as prepared in boiling water with the hope that the findings would guide future practice on suitable decoction times.

**MATERIALS AND METHODS**

**Sample collection and preparation**

Fresh leaves samples of *M. stenopetala* collected from Hawassa Teacher Training College compound, about 250 km South of Addis Ababa, Ethiopia, in May 2013. The tree was already confirmed and labelled on the tree as per-information at the department of Biology, Hawassa Teacher Training College. After complete cleaning and rinsing with distilled water, the plant materials air-dried in the laboratory at 25°C by continuous turning of the leaves to prevent fungal growth for two weeks to constant weight and then the dried leaf samples were homogenised ground into a fine powder, so as to enhance effective contact of solvent with sites on the plant materials.

**Reagents and chemicals**

In this study, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), acarbose, dinitrosalicylic acid reagent (DNSA), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine) were purchased from Sigma Aldrich, India; and gallic acid, sodium carbonate, methanol, ethylene diamine tetraacetic acid, (EDTA), starch, H₂SO₄ (96%), ascorbic acid (vitamin C), ferric chloride (FeCl₃), Folin-ciocalteu reagent, trichloroacetic acid, butylatedhydroxytoluene (BHT), sodium hydroxide, chloroform, potassium ferricyanide, ammonium molybdate, sodium phosphate, sodium potassium tartarate, quercetin, ferrous chloride( FeCl₂), hydrochloric acid and aluminum chloride were analytical grade.

**Apparatus and instruments**

All the spectrophotometric measurements of antioxidant compounds, antioxidant assay and α-amylase inhibition assay were carried out on UV-visible Spectrophotometer (Spectronic 20, Genesys, USA). Aqueous crude leaf extracts were evaporated to dryness by using rotary evaporator (Buchi, 3000 series, Switzerland).

**Extraction procedure–maceration and decoction**

3 gm of dried powder of *M. stenopetala* leaves were weighed four times into four equal portions. The first portion was macerated into a beaker containing 30 mL of distilled water at 25°C for 24 h with continuous shaking. The other three parts each of 3 gm were poured into three beakers containing 100 mL of distilled water, sealed with an aluminum foil (closed system) and decoction were carried for 5, 10 and 15 min, respectively. Each extract was cooled and filtered by using filter paper (Whatman No 1) and residue was re-extracted with equal volume of water to make final volume. The water was removed under reduced pressure by mechanically rotating the flask containing the combined aqueous extract at controlled temperature (40 to 50°C) to dryness by using a rotary evaporator (Buchi, 3000 series, Switzerland) and re-dissolved

*Corresponding author. E-mail: daniase12@gmail.com. Tel: +251-912135377.

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in distilled water at a concentration of 10 mg/mL and stored in sealed plastic containers and kept in refrigerator at 4°C until further investigation.

**Determination of total phenolic content**

Total phenolic content of cold water and decoction for 5, 10 and 15 min extracts were determined by Folin-Ciocalteu method (Demiray et al., 2009) with little modifications, using gallic acid as a standard phenolic compound. 0.1 mL volume of extracts (1 mg/mL) was mixed with 1 mL Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water). After standing for 5 min at 25°C, 1 mL of Na₂CO₃ (7.5% m/v) was added to the mixture and the reaction was kept at 25°C for 90 min, after which the absorbance were measured at 765 nm by a spectrophotometer. All the experiments were conducted in three replicates and the results expressed as gram of gallic acid equivalent (mgGAE/g) of leaf extract using equation 1:

\[
C = \frac{c \times v}{m}
\]

Where, \( C \) = total phenolic content of compounds, mg/g leaf extract expressed as GAE, \( c \) = the concentration of gallic acid established from the calibration curve (mg/mL), \( v \) = the volume of extract in mL, and \( m \) = the dry weight of leaf extract.

**Determination of total flavonoid content**

The amounts of flavonoid content in the extracts were determined by procedure described by Chang et al. (2002) with some modification. 2 mL volume of *M. stenopetala* leaf extracts (1 gm/mL) were mixed with 2 mL of 2% aluminum chloride. The individual blanks were prepared consisting of 2 mL of sample solution and 2 mL of methanol without aluminum chloride. Then, the absorbance of the resulting color was measured at 415 nm against blank after incubation for 1 h at room temperature. The average of three readings was used and then expressed as milligram of quercetin equivalents (mgQRE/g) of leaf extract using equation 2:

\[
C = \frac{c \times v}{m}
\]

Where, \( C \) is total flavonoid content, mg/g leaf extract expressed as QE, \( c \) = the concentration of quercetin established from the calibration curve in μg/mL, \( v \) is the volume of extract in mL and \( m \) is the weight of crude leaf extract in g.

**Determination of antioxidant activities**

**Free radical scavenging activity (DPPH)**

The hydrogen atoms or electrons donation ability of the corresponding extracts and some pure compounds were measured from the bleaching of purple colored methanol solution of DPPH (Gursoy et al., 2010). The stable DPPH free radical scavenging activity of *M. stenopetala* leaf extracts were carried out as described by Chan et al. (2007) with some modifications. 2 mL (0.06% in methanol) DPPH solution was mixed with 1 mL of various concentrations (50, 100, 250 and 500) μg/mL of leaf extracts. The mixture was shaken vigorously and incubated at 25°C for 30 min in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 520 nm by a spectrophotometer. The solution without any extract and with DPPH and methanol was used as control. The experiment was replicated in three independent assays. Ascorbic acid was used as positive controls and the percentage of DPPH free radical inhibition of leaf extracts were calculated by using equation 3:

\[
\text{DPPH scavenging activity (} \% \text{)} = \frac{A_c - A_s}{A_c} \times 100
\]

Where, \( A_c \) is the absorbance of the control solution (containing all reagents except plant extract); \( A_s \) is the absorbance of the DPPH solution containing leaf extract.

The antioxidant activity of each extract was expressed in terms of IC₅₀ (μg/mL concentration required to inhibit DPPH radical formation by 50%), and was calculated from the graph after plotting inhibition percentage against extract concentration.

**Ferric ion reducing power**

Reducing power of the extracts was determined as described by Oliveira et al. (2007) with slight modification. Different concentrations (50, 100, 250 and 500 μg/mL) of leaf extracts were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid solution was added and the resulting mixture was centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and 0.1% ferric chloride (0.5 mL) and the absorbance of solution was measured at 700 nm using UV-visible spectrophotometer. The extract concentration providing absorbance of 0.5 (IC₅₀) was calculated from the graph of absorbance at 700 nm against extract concentration and ascorbic acid was used as standard.

**Total antioxidant activity using phosphomolybdenum assay**

Phosphomolybdenum assay is a quantitative method to evaluate water-soluble and fat-soluble antioxidant capacity. The total antioxidant capacity of extracts was evaluated using slightly modified phosphomolybdenum method (Prieto et al. 1999). Different concentrations of crude leaf extracts (0.5 to 1 mg/mL) were prepared in methanol from test stock solution. 3 mL of reagent solution (prepared from 10 mL of 0.6 M sulfurous acid, 10 mL of 28 mM sodium phosphate, and 10 mL of 4 mM ammonium molybdate) was added to all the tubes. All the tubes were incubated at 95°C for 90 min. After cooling the sample to room temperature, the absorbance of the solution was measured at 695 nm against blank using UV spectrophotometer. Ascorbic acid was used as standard.

**Ferrous ions chelating activity**

The ferrous ions chelating activity of various *M. stenopetala* leaf extracts and the standards EDTA and BHT were investigated according to the method of Dinis et al. (1994) with slight modification. 3 mL test sample extract solutions with various concentrations (10, 50 and 100 μg/mL) were mixed with 250 μL of ferrous chloride (2 mM) and 0.1 mL of ferrozine (5 mM). The resultant solutions were shaken vigorously and left standing for 10 min at room temperature until solution mixture attain the equilibrium.

Absorbance of the resultant solution was thereafter measured at 562 nm using UV spectrophotometer. The percentage of inhibition of ferrozine-Fe²⁺ complex formation was calculated by using question 4:

\[
\text{Ferrous ions chelating activity (} \% \text{)} = \frac{A_c - A_s}{A_c} \times 100
\]
Figure 1. Total phenolic contents of M. stenopetala leaf extracts. Values expressed as means (n =3) Means with different letters are significantly different (p < 0.05) measured by Duncan multiple range test.

Where, A<sub>c</sub> is the absorbance of the control solution (containing all reagents except extract), A<sub>s</sub> is the absorbance in the presence of the sample extracts. All the tests were carried out in triplicate. IC<sub>50</sub> values of standards and leaf extracts of different solvents were determined from plots of percentage of inhibition of ferrozine-Fe<sup>2+</sup> complex formation versus concentration (μg/mL).

**Determination of α-amylase inhibition activity**

*In vitro* α-amylase inhibitory activity was performed using 3,5-dinitrosalicylic acid (DNSA) described by Jyothi et al. (2011) with slight modification. Briefly, the total assay mixture containing 1 mL of α-amylase and the leaf extracts in the concentration range 1 to 10 mg/mL were incubated at 37°C for 30 min. After incubation 1 mL of 1% starch solution (pH 6.9) was added and the mixture was further incubated at 37°C for 15 min. The reaction was terminated by the addition of 1 mL of DNSA colour reagent then; the test tubes were placed in a boiling water bath for 5 min, cooled at room temperature and diluted with 5 mL of distilled water. Absorbance of all solutions was measured at 540 nm using UV spectrophotometer. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with distilled water. All the tests were carried out in triplicate and acarbose was used as standard. The α-amylase activity was expressed as percentage of inhibition and calculated by using question 5:

\[
\text{α-amylase inhibition activity (\%)} = \frac{A_c - A_s}{A_c} \times 100
\]  

IC<sub>50</sub> values of acarbose and leaf extracts of aqueous solvents were determined from plots of percentage inhibition versus concentration. IC<sub>50</sub> value is defined as the concentration of extract required to inhibit 50% of α-amylase inhibition activity.

**Results and Discussion**

**Total phenolic content**

In the present study, total phenolic content of aqueous extracts of M. stenopetala leaf was shown in (Figure 1). Decoction for 10 min extract of the leaf showed the highest total phenolic content and the lowest total phenolic content was found in decoction for 15 min extract. The total phenolic contents of the extracts increased in the following order: decoction for 15 min (34.86 ± 0.72) < cold water (35.95 ± 0.79) < decoction for 5 min (38.24 ± 0.35) < decoction for 10 min (44.86 ± 0.55) in mgGAE/g. Total phenolic content of decoction for 15 min and cold water extracts were not significantly different (p > 0.05) but these values were significantly different (p < 0.05) from the other extracts. The total phenolic content obtained from decoction for 15 min extract were also in accordance with previous studies that prolonged decocting at high temperature can lead to degradation of phenolic compounds (Perva et al., 2006).

**Total flavonoid content**

The amounts of total flavonoid content obtained from aqueous M. stenopetala leaf were range from 10.44 ± 0.36 to 20.36 ± 0.18 mgQE/g (Figure 2). The highest amounts of total flavonoid were obtained using decoction for 5 min extract (20.36 ± 0.18 mgQE/g) whereas the lowest amounts of total flavonoid contain were obtained in cold water extract (10.44 ± 0.36 mgQE/g). Decoction for 15 min have resulted in significantly (p < 0.05) lower total flavonoid content than that of 10 and 5 min decocting. This may be due to the prolonged decocting time that may result in longer exposure of the leaves to heat.

**Statistical analysis**

All the analysis were carried out in triplicate agents the blank and results were expressed as mean ± standard deviation (SD) for n=3. Statistical analysis was performed with the SPSS 16.0 software (SPSS Inc.) and comparisons were made with one-way ANOVA followed by Duncan’s new multiple range test. The level of significance was set at P < 0.05. Correlations between total phenolic or total flavonoid content and antioxidant activities were determined by linear regression analysis using Microsoft Office Excel 2007. IC<sub>50</sub> was was calculated using Origin 8 software, Northampton, MA 01060 USA.
Figure 2. Total flavonoid contents of *M. stenopetala* leaf extracts (mgQE/g). Values expressed as means (n = 3) Means with different letters are significantly different (p < 0.05) measured by Duncan multiple range test.

Table 1. IC50 values of *Moringa stenopetala* leaf extracts and standards.

| Extracts/standards       | DPPH  | Reducing power | Ferrous ion chelating | α-Amylase inhibition |
|--------------------------|-------|----------------|-----------------------|----------------------|
| Cold water               | 46.51 ± 1.37\(^a\) | 367.5 ± 0.57\(^b\) | 7.46 ± 0.12\(^d\) | >10                  |
| Decoction for 5 min      | 42.7 ± 0.68\(^b\) | 404.4 ± 0.94\(^d\) | 7.19 ± 0.58\(^c\) | 8.86 ± 0.03\(^a\)   |
| Decoction for 10 min     | 41.5 ± 0.71\(^b\) | 235.1 ± 0.60\(^b\) | 6.65 ± 0.16\(^b\) | 8.81 ± 0.13\(^b\)  |
| Decoction for 15 min     | 46.78 ± 0.57\(^d\) | >500            | 7.23 ± 0.15\(^c\) | 8.75 ± 0.09\(^a\)  |
| Ascorbic acid            | 26.5 ± 1.07\(^a\) | 73.26 ± 0.98\(^a\) | -                     | -                    |
| EDTA                     | -     | -              | 5.32 ± 0.18\(^b\) | -                    |
| BHT                      | -     | -              | 38.33 ± 0.26\(^a\) | -                    |
| Acarbose                 | -     | -              | -                     | 3.06 ± 0.15\(^b\)  |

Different superscript letters within the same column indicate significant differences (P < 0.05) within the extracting solvents.

Antioxidant activities

DPPH radical scavenging activity

At 500 µg/mL, the DPPH free radical scavenging activities of the leaf extracts and ascorbic acid increased in the following order: cold water (77.23 ± 1.70%) < decoction for 15 min (82.40 ± 0.74%) < decoction for 5 min (83.51 ± 0.46) < decoction for 10 min (91.52 ± 0.59%) < ascorbic acid (97.81 ± 0.22%). Among water extracts, decoction for 10 min extract showed the strongest DPPH radical scavenging activity. The reason for increase in radical scavenging ability could be due to the higher phenolic content in the decoction for 10 min extracts. This evident that *M. stenopetala* leaf extract contained compound that can donate electron/hydrogen easily and stabilize free radicals. As indicated in Table 1, the IC50 values for DPPH scavenging activity of aqueous *M. stenopetala* leaf extracts were presented. The lowest IC50 indicates the strongest ability of the extracts to act as DPPH scavengers (Azizah et al., 2007). Decoction for 10 min extract exhibited the lowest IC50 value (41.5 ± 0.71 µg/mL) as compared to other extracts but this value was higher than ascorbic acid (26.50 ± 1.07 µg/mL). The IC50 values of decoction extracts (5 and 10 min) were not found to be significantly different (p > 0.05). Similarly, no significant difference (p > 0.05) was observed between decoction for 15 min and cold water extract. However, these values were significantly different (p < 0.05) from the IC50 value of ascorbic acid.

Ferric ion reducing power

Reducing power measures the ability of the extract to donate electron to Fe(III) and it is evaluated by the transformation of Fe3+/ferricyanide complex to ferrous ions (Fe2+) in the presence of the sample extracts (Gulcin et al., 2003). Like the DPPH free radical scavenging activity, the reducing power of all extracts increased with increasing in their concentration. The strongest reducing power was observed in decoction for
10 min extract and the lowest reducing power exhibited by the cold water extract. At 500 µg/mL, the reducing power of extracts and ascorbic acid decreased in the following order: Ascorbic acid (1.53 ± 0.16) > decoction for 10 min (0.765 ± 0.14 nm) > decoction for 5 min (0.556 ± 0.09 nm) > decoction for 15 min (0.449 ± 0.13 nm) > cold water (0.353 ± 0.62 nm). Reducing power increased as decoction extraction time increased from 5 to 10 min but further decocting for 15 min decreased the reducing power and hence decocting for more than 10 min resulted in a significant loss of ferric reducing power which was supported by the results for total phenolic content. IC50 values of the extracts also calculated from the graph of absorbance at 700 nm against extracts concentration and the results have been shown in Table 1. The lower the IC50 value, the higher will be the reducing power. As compare to the standard ascorbic acid, decoction for 10 min extract displayed high IC50 value (235.1 ± 0.60 µg/mL). However, IC50 value of cold water and decoction for 15 min extracts were greater than determined concentration (>500 µg/mL). The obtained results indicate that, an IC50 value of ascorbic acid was significant different (p < 0.05) from all extracts. Moreover, there were also significant different (p < 0.05) between decoction for 10 and 5 min extracts.

Antioxidant activity using phosphomolybdenum method

Decoction for 10 min extract showed the highest total antioxidant activity as compare to other extracts, but this result was lower than the standard ascorbic acid. At 1 mg/mL, the total antioxidant activities of the extracts were found to decrease in the following order: decoction for 10 min extract (0.329 ± 0.32) > decoction for 5 min extract (0.302 ± 0.19) > decoction for 15 min extract (0.254 ± 0.41) > cold water extracts (0.234 ± 0.35). The above results also showed that at there were no significant different (p < 0.05) between cold water and decoction for 15 min extracts. Moreover, no significant difference (p < 0.05) between decoction for 5 min and 10 min extracts but these values were significantly different (p < 0.05) from the standard ascorbic acid.

Ferrous ion chelating ability

The production of the hydroxyl radical is a key initial step to producing other harmful radicals which should be avoided (Prasad et al., 1989). Ferrozine has a tendency to form red-coloured complexes quantitatively with ferrous ion, but in the presence of other iron chelating agents, the complex formation is disrupted resulting in a decrease in the red colour of the complex (Rajauria et al., 2010).

As can be seen from the (Figure 3), decoction for 10 min extract showed the strongest chelating activity, but weakest chelating activity was exhibited by cold water extracts. At the concentration of 0.1 mg/mL, the chelating activity of the leaf extracts and the standards were found to decrease in the following order: EDTA (97.69 ± 0.41) > decoction for 10 min extract (92.52 ± 0.17%) > decoction for 5 min (90.03 ± 0.24%) > decoction for 15 min (86.79 ± 0.47%) > cold water (83.80 ± 0.49%) > BHT (68.19 ± 0.23%). The data presented in this study indicated that aqueous leaf extracts of M. stenopetala have special ability for iron chelating activity, which were even greater than the standard BHT. With regard to IC50 value, boiled water for 10 min extract showed the lowest IC50 value (6.65 ± 0.16 µg/mL) whereas the highest IC50 value were obtain from cold water extract (7.46 ± 0.12 µg/mL) Table
The obtained results indicate that the IC50 values of boiled water for 5 min and 15 min extracts were not significant difference (p < 0.05), but these value were significantly different (p > 0.05) from the other extracts and the standards. The study revealed that there was an increase in the iron chelating ability of the extracts of the leaf with increase in decoction time to 10 min. This can be explained by the fact that decocting caused an increase in the total phenol and flavonoid contents of extracts (Amie et al., 2003).

In vitro α-amylase inhibition activity

Inhibition of α-amylase has been considered to be an effective strategy to control diabetes by suppressing the absorption of glucose composed from starch (Bhandari et al., 2008). In the present study, it was observed that the inhibition activities of the extracts were increased by increasing concentration of the samples. Boiled water for 10 min extract showed the most potent inhibitor of α-amylase activity at all concentrations tested. However, cold water extract showed the lowest α-amylase inhibition (Figure 4). At concentration of 10 mg/mL, α-amylase inhibition activities of the extracts and standard acarbose decreased in the following order: cold water (49.31 ± 0.16%) < decoction for 15 min (52.87 ± 0.52%) < decoction for 5 min extract (54.95 ± 0.27) < decoction for 10 min (59.52 ± 0.33) acarbose (73.86 ± 0.14%). IC50 values of all the extracts were also calculated and the results are shown in Table 1. Cold water extract exhibited the lower IC50 value (> 10 mg/mL) as compared to other extracts. The result also showed that the IC50 values of decoction extracts decreased in the following order: decoction for 5 min extract (8.86 ± 0.03) > decoction for 15 min (8.81± 0.13) > decoction for 10 min extracts (8.75 ± 0.09). The obtained IC50 values indicate that there were no significant difference (p > 0.05) among boiled water extracts but these values were significantly different (p < 0.05) from the standard acarbose.

Correlation analysis

In this study, the dependence of antioxidant activity obtained by different assays and α-amylase inhibition activities in relation to the total phenolic and flavonoid contents were evaluated. DPPH free radical scavenging, reducing power, total antioxidant and α-amylase inhibition activities showed positive linear correlation (R²=0.853, R²= 0.857 , R²= 0.864 and R²=0.930), respectively with total phenolic content but ferrous ion chelating activity were found to be weakly correlated (R²=0.481). Furthermore, strong correlation (R² = 0.967) was also observed between reducing power and DPPH radical scavenging activity. A similar result was found by Sudathip et al. (2006); they suggested that the compounds present in the aqueous extracts capable of reducing DPPH radicals were also able to reduce ferric ions. The present results also revealed that total flavonoid content showed weakly correlation with DPPH radical scavenging (R² = 0.272), reducing power (R² = 0.047) and α-amylase inhibition activities (R² = 0.396) whereas, ferrous ion chelating activity (R² = 0.426) and total antioxidant activities (R² = 0.529) showed moderately weak correlation with total flavonoid content and this weak correlation result is may be due to the total flavonoid content that do not necessarily incorporate all the antioxidants that may be present in extracts.

Conclusions

It is evident from present study that leaf extract of M. stenopetala could be a potential source of natural antioxidant that could have great importance as therapeutic agents in the preventing oxidative stresses-related degenerative diseases and diabetes. Based on
present investigation, it could be concluded that major losses of antioxidant properties and total phenolic content of M. stenopetala leaves were observed at decoction for 15 min extract. Therefore, to prevent the major loss of total phenolic content, antioxidant and α-amylase inhibition activities of M. stenopetala leaf extracts, minimal heat treatment (5 to 10 min) through decoction process is recommended.

Conflict of interests

The authors did not declare any conflict of interest.

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