Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.77, 2020

Effect of Extraction Solvents on Antioxidant and α-amylase Inhibition Activities of Spiced Green Chili Paste: An In Vitro Study

Engeda Dessalegn
Department of Chemistry, Hawassa College of Education, P.O. Box 115, Hawassa, Ethiopia

Abstract
Chili pepper (*Capsicum frutescens* L.) is widely produced and consumed as raw or processed products. Spiced green chili paste, locally known as Datta, is hot spicy paste consumed in South Ethiopia. Under this study, total phenolic contents (TPC), total flavonoid contents (TFC), *in vitro* antioxidant, and α-amylase inhibition activities of different extracts of green Datta paste were investigated. The TPC and TFC of the extracts were determined by the Folin ciocalteu and aluminum chloride methods, respectively. The antioxidant activities were determined by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging, ferric reducing power, and total antioxidant using phosphomolybdenum methods. *In vitro* porcine pancreatic α-amylase inhibition activity was evaluated using the dinitrosalicylic acid (DNSA) assay. The acetone extract contained the highest TPC (24.92 ± 2.88 milligram gallic acid equivalent/ gram of dried extract) and TFC (28.05 ± 8.36 milligram catechin equivalent/gram of dried extract). Similarly this extract showed stronger antioxidant capacity, 6.34 ± 1.21 milligram of ascorbic acid equivalent/gram of dried extract, 1.46 ± 0.22 milligram butylated hydroxytoluene equivalent/ gram of dried extract, and 46.99 ± 2.60 µg/mL as determined by ferric reducing power, total antioxidant activity, and DPPH scavenging (IC_{50}) activity, respectively. The same extract also exhibited the strongest α-amylase inhibition activity (IC_{50} = 0.45 mg/mL). TPC and TFC were strongly correlated with DPPH (R^2 = 0.99, R^2 = 0.91), reducing power (R^2 = 0.86, R^2 = 0.97), and total antioxidant activity (R^2 = 0.79, R^2 = 0.97), respectively. The α-amylase inhibition activity was strongly correlated with TPC (R^2 = 0.90) but weakly correlated with TFC (R^2 = 0.18). Thus the result indicated promising perspectives for the development and usage of acetone extract of Ethiopian spiced green chili pepper with considerable levels of natural antioxidants which can be used as functional food for preventing oxidative stress mediated human disorders.
Keywords: Antioxidant, chili pastes Datta, α-amylase, Phenolic content.
DOI: 10.7176/ALST/77-02
Publication date: January 31st 2020

Introduction

Most foods of plant origin contain components which are active as inhibitors of undesirable oxidative processes in food also in the human body as well. They are mainly compounds of natural origin, which are formed by regulated biosynthesis in plants. Dietary herbs and spices have been added to foods since ancient times as flavoring and preservatives; also show potential health benefits (Iris *et al*., 2006; Hui-Yin *et al*., 2007; Abdullah *et al*., 2013; Kai *et al*., 2013). Ethiopia is among the largest consumer of these products in Africa. People use these products to flavor and preserve different cultural foods and also as folk medicine (Nigist and Sebsebe, 2009).

In Ethiopia, the spiced red pepper locally known as Berbere, is widely used in daily meal of the people. Similarly, people use the spiced green and red chili pepper to prepare paste locally known as Datta. It is a traditional food in the southern part of Ethiopia, consumed mainly with raw meat and known to improve appetite (Nigist and Sebsebe, 2009). In general, the ingredients used in the preparation of the paste are ginger, garlic, Basil (leaf), seed of Ethiopian cardamom locally known as korarima, coriander (leaf and fruit), chili pepper (red or green), and salt.

Few scientific data has been reported on biofunctional activity of spiced chili pepper such as spiced red chili paste (Engeda, 2015), Keang-hleung paste made of red chili pepper, ginger, garlic, galangal rhizomes (*Alpinia galanga*), and turmeric rhizomes (Seah *et al*., 2010), and spiced green chili made of green chili onion and garlic (Ruanma *et al*., 2010). The objective of this study was to investigate effect of the extracting solvents on TPC, TFC, *in vitro* antioxidant, and α-amylase inhibition activities of spiced green chili paste. In addition, the correlation between total phenolic contents and antioxidant assays was also evaluated.

Materials and methods

Reagents

Catechin, gallic acid, Folin-Ciocalteu reagent, butylated hydroxytoluene (BHT), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), L-ascorbic acid, 3, 5-dintrosalicylic acid (DNSA), acarbose, and porcine pancreatic α-amylase were purchased from Sigma-Aldrich. Petroleum ether, acetone, methanol, sodium carbonate, ammonium molybdate, sulfuric acid, sodium phosphate, potassium ferricyanide, ferric chloride, trichloroacetic acid, disodium hydrogen phosphate, and sodium dihydrogen phosphate were of analytical grade reagents purchased from FLUKA.

Collection of ingredients and preparation of paste

The ingredients and the adapted procedure were representative of household preparation of Datta paste in the Southern region of the country in which the chili pepper is the major component of the paste. Fresh leaf and fruit of coriander, leaf of basil, Garlic, Ginger root, seed of Korarima (*Aframomum corrorima*), and green chili were purchased from local market in Hawassa town, South Ethiopia, in October 2017. All samples were sorted and washed thoroughly with distilled water to remove dust and dirt. Based on household preparations, 200 g of green chili pepper, 20 g portion of each spice and herbs, and 12 g of salt were mixed and pounded together to make green Datta paste. The prepared Datta paste sample for analysis was freeze-dried (Model 2085C0000, Kinetics Thermal Systems, Stone Ridge, NY, USA) and then ground to fine powder using electric grinder (FM100 model, China). Sample was stored at −20°C until used for the *in vitro* assays.

Extraction of the paste

The petroleum ether, water, acetone, methanol, and aqueous: methanol (20:80, v/v) extracts were prepared by dissolving 5 g of the powder separately in 50 mL of each solvent. The contents were kept in orbital shaker for 8 h at room temperature. Thereafter, each extract was filtered using Whatman no.1 filter paper and the filtrate evaporated to dryness under vacuum at 40°C by using a rotary evaporator (Buchi, 3000 series, Switzerland). After completing the extraction the residues of each extract was discarded. For each solvent, the extraction was done in triplicate and the resulting evaporated extracts were kept in a sealed dark glass bottle and stored at -20°C. Then each extract was dissolved in methanol to prepare stock solution with concentration of 5 mg/mL, for conducting further investigation.

Determination of extraction yield

The percentage yield on dry weight basis of green Datta paste was calculated from the equation: extraction yield (g/100 g) = (W1 × 100)/W2 where W1 is the weight of the extract residue obtained after solvent removal and W2 is the weight of the freeze dried green Datta paste.

Total phenolic content (TPC)

This assay evaluates the TPC based on the change in color from a yellow Folin-Ciocalteu reagent color to dark blue in the presence of antioxidants and measures the absorbance at 750-765 nm. TPC was estimated as described by Shan *et al.* (2005) with slight modification using gallic acid as standard. To 0.2 mL of the extract (1 mg/mL), 2 mL Folin-Ciocalteu reagent (diluted ten times) was added and the mixture was left for 5 min and then 2mL (7.5% w/w) of sodium carbonate was added. The absorbance of the resulting blue color was measured at 765 nm with a UV-visible spectrophotometer (Spectronic 20, UK) after incubation for 90 min at room temperature. The TPC was estimated from gallic acid calibration curve ($y = 0.006x + 0.07$, $R^2 = 0.99$) and results were expressed as milligram gallic acid equivalent/gram of dried extract (mgGAE/g).

Total flavonoid content (TFC)

The TFC was determined as described by Ayoola *et al.* (2008). The extracts (1mL, 1mg/mL) were diluted with 1.25mL distilled water and 75µL 5% $NaNO_2$ was added to the mixture. After 6 min, 150µL 10% $AlCl_3$ was added and after another 5min, 1mL Na OH was added to the mixture. Immediately, the absorbance of the mixture, pink in color, is determined at 510 nm versus prepared water blank. All the calculations were done using standard equation catechin ($y = 0.011x + 0.132$, $R^2 = 0.99$) obtained from standard calibration curves. Results were expressed as milligram of catechin equivalents per gram of dry extract (mgCE/g).

Determination of antioxidant activity

DPPH method

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the extracts from green spiced chili paste was determined as described by Katerere and Eloff (2005) with slight modification. Different concentrations (50 to1000 µg/mL) of the extracts were taken in different test tubes. Freshly prepared DPPH solution (2 mL, 0.06%, 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 taken at 520 nm. Methanol was used as blank. The ability to scavenge the DPPH radical was calculated using the equation:

$$\text{DPPH scavenging (\%)} = [(1 - As / Ac)] \times 100$$

Where Ac is the absorbance of the control and As is absorbance in presence of sample extracts. The antioxidant activity of the extract was expressed as EC_{50}. The EC_{50} value was defined as the concentration (in µg/mL) of extracts that scavenges the DPPH radical by 50%.

Ferric ion reducing power

The reducing power was determined by assessing the ability of the extracts to reduce ferric ion as described by Oyaizu (1987). One milliliter of the paste extract with concentration of 1 mg/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 50ºC 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 estimated from ascorbic acid calibration curve (($y = 0.06x + 0.15$, $R^2 = 0.99$) and results were expressed as milligram ascorbic acid equivalent/g of dry extract (mgAAE/g).

Total antioxidant activity using phosphomolybdenum assay

The total antioxidant capacity of spiced green chili paste extracts was spectrophotometrically determined by the phosphomolybdenum assay using the method described by Prieto *et al.* (1999). Briefly, 0.3 mL of a 1 mg/mL extract solution in methanol was mixed with 3 mL phosphomolybdenum reagent (28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulphuric acid) in capped test tubes. Incubation was then carried out for 90 min in a water bath at 95°C. After cooling to room temperature, the absorbance of the solutions was measured 695 nm against a blank (3 mL methanol without plant extract). The total antioxidant activity was expressed as milligram butylated hydroxytoluene equivalent/gram of dried extract (mgBHTE/g) based on the calibration curve; $y = 0.24x + 0.11$, $R^2 = 0.98$.

Porcine pancreatic α-amylase inhibition assay (DNSA method)

The DNSA (dinitrosalicylic acid) 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 µL (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 µL porcine pancreatic α-amylase were incubated at 25ºC for 10 min, after which, 200 µL 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 µL of DNSA reagent (1.0 g of 3, 5- dinitrosalicyclic 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).

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- dinitrosalicyclic acid, and 4 mL of distilled water was recorded at 540 nm as blank. Acarbose and catechin were used as reference. The *α*-amylase inhibitory activity was expressed as % inhibition and was calculated as shown below:

$$\% \text{ inhibition} = [Ac - (As - Ab)/Ac] \times 100$$

Where Ac is control absorbance (100% enzyme activity), As is test sample absorbance (with enzyme) and Ab absorbance of the blank (a test sample without enzyme). The inhibition activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in mg/mL) of extracts that inhibits the enzyme activity by 50%.

Statistical analysis

A triplicate data were analyzed by one way analysis of variance (ANOVA) using SPSS 20.0 statistical software. Mean separation was conducted using Duncan's multiple range tests at $p < 0.05$. The inhibitory concentration 50% (IC_{50}) was calculated from the dose response curves obtained by plotting the percentage of inhibition versus the concentrations and correlations between total phenolic content and antioxidant activities were determined by linear regression analysis using Origin 8 software.

Results and Discussions

Extraction yield

The amount of crude extracts obtained from green spiced chili paste, using different extraction solvents are presented in Table 1. The yields of the five extracts varied from 4.12 ± 0.51% to 147.10 ± 9.33g/100 g of dried paste. The water extract gave the highest yield (147.10 ± 9.33 mg/g), while the petroleum ether extract gave the least yield (4.12 ± 0.51 mg/g). These findings are in agreement with previous investigation by Engeda (2015)] who reported that maximum extract yield from paste of spiced red chili pepper was obtained when water solvent was used. The variations in extract yield using different solvents might be explained by the polarity of extracted components and solvents applied (Engeda 2015; Engeda *et al*., 2015). According to the report by Elsorady and Ali (2015) on peanut skin extracts, the presence of water increased permeability of seed tissue and thus enabled a

better mass transport. Similarly, increasing the water concentration in the solvent enhanced extraction yield of rice bran (Chata *et al*., 2006), some medicinal plants (Sultana *et al*., 2009), and *Limnophila aromatica* (Diem *et al*., 2014).

Total phenolic and flavonoid contents

Phenolic compounds present in foods have received considerable attention because of their potential antioxidant activity (Bakchiche *et al*., 2013). Several studies showed that TPC determined differed with polarity of solvent used in extraction. High solubility of phenols in polar solvents provides high concentration of these compounds in the extracts. For instance, absolute methanol, acetone ,and methanol 80% used for the extraction of *Thymus schimperi* were found to be more effective than the water and petroleum ether extracts (Engeda *et al*., 2015). In addition, Diem *et al* (2014) reported that mixture of water and organic solvents used such as water/methanol, water/ethanol, and water/acetone affected significantly the TPC and TFC of the *Limnophila aromatica* extracts. Results of the present study (Table 1) showed that among all the solvent extracts of green Datta paste, acetone extract had the highest TPC and TFC which was similar to the study conducted by Engeda (2015). The TPC followed the order: acetone > aqueous: methanol (20:80, v/v) > petroleum ether > methanol > water. There was no significant difference ($p > 0.05$) in TPC between aqueous: methanol (20:80, v/v) and petroleum ether extracts but these values were significantly higher ($p < 0.05$) than that of water, and methanol extracts, lower ($p < 0.05$) than the TPC of acetone extract. TPC of green Datta paste obtained from this study was higher than that of red chili (Neelam *et al*., 2016), garlic (Otunola *et al*., 2013), *Aframomum corrorima* (Eyob *et al*., 2008), and ginger (Ghasemzadeh *et al*., 2010) but lower than the TPC of *Ocimum basilicum* accessions (Javanmardi *et al*., 2003). Also this paste had greater TPC than that of chili pepper sauce (Pamela *et al*., 2006), spiced red chili paste (Engeda 2015), green spiced chili paste made of green chili, onion, and garlic (Ruanma *et al*., 2010) and Turmeric-chili paste or yellow curry paste made of garlic, turmeric, Galangal, and red chili pepper Seah *et al*., 2010). Similarly the acetone extract was the richest source of TFC ($p < 0.05$) and decreased in the order of acetone > petroleum ether > methanol > aqueous: methanol (20:80, v/v) > water (Table 1). There was no significant difference ($p > 0.05$) in TPC between aqueous: methanol (20:80, v/v), methanol and water extracts but these values were significantly lower ($p < 0.05$) than that of petroleum ether and acetone extracts.

Table 1 Extraction yield (mg/g of dw), TPC (mgGAE/g of dried extract) and TFC (mgCE/g of dried extract) contents of green Datta paste.

Extract	Extraction yield ((g/100 g dw)	TPC (mgGAE/g)*1	TFC (mgCE/g)*2
Petroleum ether	4.12 ± 0.51^{a}	13.77 ± 2.07^{c}	14.64 ± 1.92^{b}
Water	147.10 ± 9.33^{e}	1.83 ± 0.34^{a}	1.74 ± 0.22^{a}
Acetone	25.80 ± 3.96^{b}	24.92 ± 2.88^{d}	28.05 ± 8.36^{c}
Methanol	51.70 ± 15.41^{c}	8.09 ± 0.67^{b}	6.30 ± 0.40^{a}
Aqueous: methanol (20:80, v/v)	120.80 ± 3.40^{d}	16.47 ± 1.77^{c}	5.95 ± 0.16^{a}

*dw: Dried weight of the green Datta paste; *1: Total phenolic content expressed as gallic acid equivalent per gram of dried extract; *2: Total flavonoid content expressed as catechin equivalent per gram of dried extract; values are expressed as mean ± SD (n = 3). Means with different letters in a column were significantly different at the level of $p < 0.05$.

DPPH scavenging activity

The DPPH is stable free radical, which 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, can be quantified by its absorbance reduction at wavelength 520 nm (Von Gadow *et al*., 1997). As the concentration of the sample increased, the percent inhibition of DPPH radical also increased (Fig. 1). At the concentration of 1 mg/mL used, the scavenging effect of L- ascorbic acid, BHT, and green Datta paste extracts (aqueous: methanol (20:80, v/v), acetone, methanol, petroleum ether and water), on the DPPH radical scavenging decreased in the order of L- ascorbic acid > BHT > acetone > aqueous: methanol (20:80, v/v) > petroleum ether > methanol > water, which were 97.32 ± 5.14%, 94.70 ± 4.23%, 89.69 ± 7.70%, 72.55 ± 6.71%, 71.20 ± 6.4%, 61.94 ± 4.45 %, and 47 ± 2.00%, respectively.

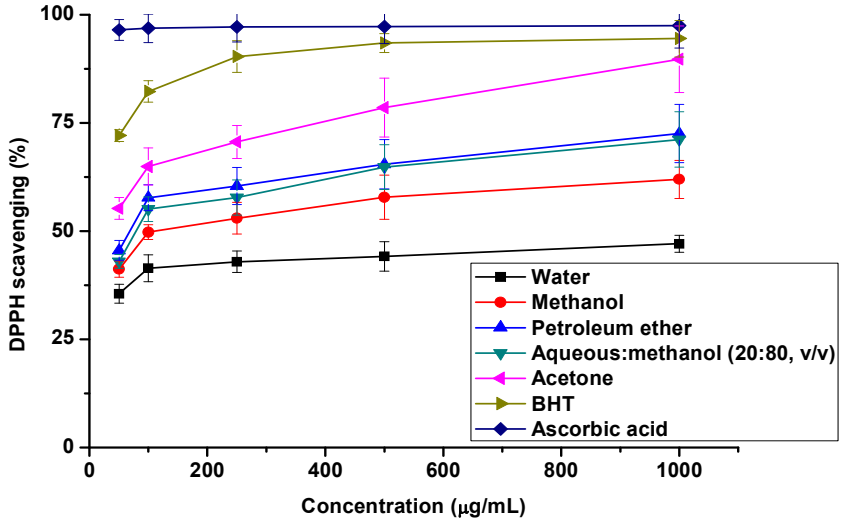

Figure 1 DPPH radical scavenging activity (%) of petroleum ether, water, acetone, methanol, and aqueous: methanol (20:80, v/v) extracts from dried green Datta paste and references (L-ascorbic acid and BHT). Values are average of triplicate measurements (mean ± SD).

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 stronger is the scavenging potential. The IC_{50} values ranged from 46.99 ± 2.60 µg/mL to 119.28 ± 27.92 µg/mL. The strongest scavenging activity was recorded for acetone extract ($p < 0.05$) which appeared more than one and half times stronger than that of petroleum ether and aqueous: methanol (20:80, v/v) extracts and more than two folds stronger than that of methanol extract. The water extract showed the weakest DPPH scavenging ($IC_{50} > 1x10^3$ µg/mL). The IC_{50} values of petroleum ether, and aqueous: methanol (20:80, v/v) extracts were not significantly different ($p > 0.05$), but these values were significantly stronger than ($p < 0.05$) the DPPH scavenging activity of methanol extract.

Table 2 IC_{50} values of DPPH radical scavenging and α-amylase inhibition activities in various solvent extracts from green Datta paste.

Extract	DPPH scavenging (µg/mL ± SD)	α-amylase inhibition activity (mg/mL ± SD)
Petroleum ether	71.95 ± 2.41^{c}	2.40 ± 0.44^{f}
Water	$> 1x10^3$	0.81 ± 0.12^{c}
Acetone	46.99 ± 2.60^{ab}	0.45 ± 0.06^{b}
Methanol	119.28 ± 27.92^{d}	$1.44 \pm 0.0.9^{e}$
Aqueous: methanol (20:80, v/v)	82.29 ± 3.90^{c}	1.00 ± 0.10^{d}
BHT	36.56 ± 2.90^{ab}	-
Ascorbic acid	23.38 ± 2.43^{a}	-
Catechin	-	2.47 ± 0.33^{f}
Acarbose	-	0.14 ± 0.04^{a}

Values are expressed as mean ± SD (n = 3). Means with different letters in a column were significantly different at the level of $p < 0.05$.

The DPPH scavenging potential of water, petroleum ether, aqueous: methanol (20:80, v/v), and methanol extracts were found to be significantly weaker ($p < 0.05$) than the that of BHT and L-ascorbic acid, while that of the acetone extract was found to be similar ($p > 0.05$) to the scavenging activity of BHT and L-ascorbic acid. The DPPH scavenging activity of green Datta paste was stronger than the scavenging activity of green chili (Ruanma *et al*., 2010), A*framomum corrorima* (Eyob *et al.,* 2008) and ginger (Ghasemzadeh *et al*., 2010). This may be due to the synergistic antioxidant activity which is similar to the findings of Seah *et al.* (2010). According to these authors, a Keang-hleung spice mix (red chili pepper, ginger, garlic, galangal rhizomes, and turmeric rhizomes) showed accumulative antioxidant activity, which was stronger than that of individual components. Also the combination of green tea with some herbs showed stronger antioxidant activity than that of all individual extracts (Dheeraj *et al*., 2011). The DPPH scavenging activity of the present study was stronger than that of red Datta paste (Engeda, 2015) and spiced green chili pepper made of green chili, onion, and garlic (Ruanma *et al*., 2010) but weaker than commercial spiced Ethiopian blend red pepper purchased from local market (Loizzo *et al*., 2011).

Ferric reducing power

The ferric reducing method is based on the reduction of the ferricyanide complex to the intensely Perl's Prussian blue colored ferrocyanide complex by the antioxidants in the acidic medium. The concentration of Fe^{2+} was monitored by measuring the absorbance at 700 nm and the results (Table 3) showed the reducing potential of different extracts of green Datta paste expressed intems of mg AAE/g of dried extract. Similar to DPPH scavenging activity the acetone extract of spiced green Datta in this assay also, showed the strongest iron reducing power activity. The reducing power of extracts was found to decrease in order: acetone > petroleum ether > methanol > aqueous: methanol (20:80, v/v) > and water extracts. Methanol and petroleum ether extracts showed no significant difference ($p > 0.05$) in their iron reducing power. But these values were significantly stronger than ($p < 0.05$) the iron reducing power of aqueous: methanol, 20:80, v/v) and water extracts.

Table 3 The ferric reducing, and total antioxidant activities in various solvent extracts from green Datta paste.

Extract	Ferric reducing power (mgAAE/g)*	Total antioxidant (mgBHTE/g)**
Petroleum ether	2.90 ± 0.92^{c}	1.00 ± 0.17^{b}
Water	0.41 ± 0.38^{a}	0.07 ± 0.03^{a}
Acetone	6.34 ± 1.21^{d}	1.46 ± 0.22^{c}
Methanol	2.43 ± 0.46^{c}	0.18 ± 0.08^{a}
Aqueous: methanol (20:80, v/v)	1.65 ± 0.14^{b}	0.17 ± 0.04^{a}

*: Ferric reducing power expressed as ascorbic acid equivalent per gram of dried extract; **: Total antioxidant expressed as butylated hydroxytoluene equivalent per gram of dried extract; values are expressed as mean ± SD (n = 3). Means with different letters in a column were significantly different at the level of $p < 0.05$.

Total antioxidant activity using phosphomolybdenum assay

Table 3 presents the total antioxidant capacity obtained through the phosphomolybdenum assay. This result is consistent with the strongest total antioxidant effect of acetone extract as determined by the DPPH assay and the weakest total antioxidant activity was found in the water extract. No significant difference ($p > 0.05$) was found in the total antioxidant activity of water, aqueous: methanol, 20:80, v/v), and methanol extracts ($p > 0.05$). However, these values were significantly lower ($p < 0.05$) than the total antioxidant activity of acetone and petroleum ether extracts.

Porcine pancreatic α-amylase inhibition assay (DNSA method)

The α-amylase inhibitory activity was concentration dependent (Figure 2). At a concentration of 2.5mg/mL, the acetone extract containing the highest TPC and TFC demonstrated stronger percentage of porcine pancreatic α-amylase inhibitory activity (80.10 ± 4.37%) followed by aqueous: methanol (20:80, v/v) (63.70 ± 2.53%), water (62.78 ± 4.00%), methanol (58..66 ± 3.50%), and petroleum ether (51.34 ± 4.85%) extracts. As positive control, at the concentration of 2.5 mg/mL, acarbose showed the strongest α-amylase inhibition activity (98.9 ± 8.8%).

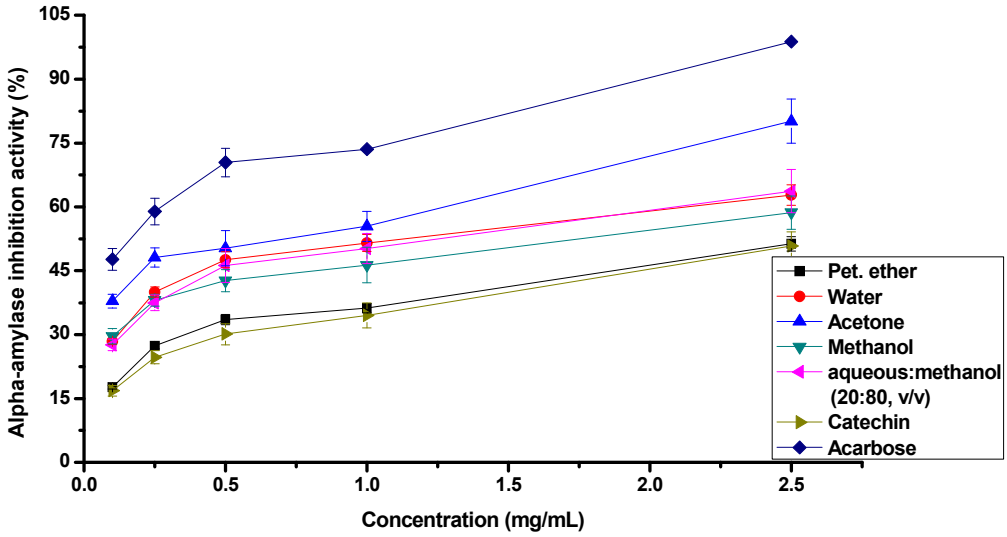

Figure 2 α- amylase inhibition activity (%) of various solvent extracts from green Datta paste and references (acarbose and catechin). Values are expressed as mean ± SD (n = 3).

The inhibitory activity was expressed as the 50% inhibitory concentrations (IC_{50}) values (Table 2). The acetone extracts demonstrated stronger percentage of α-amylase enzyme inhibitory activity ($p < 0.05$) than that of the rest extracts. It showed more than five times stronger than the inhibition potential of petroleum ether extract and catechin, more than threefold stronger than that of methanol extract and two fold and 1.8 times stronger than that of aqueous: methanol (20:80, v/v) and water extracts respectively. There were significant differences ($p < 0.05$) in the IC_{50} values among the extracts. But α-amylase inhibitory activity of these extracts was significantly weaker ($p < 0.05$) than that of acarbose. The α-amylase inhibition potential of the present result was stronger than leaf extract of coriander (Kamaran *et al*., 2012), Garlic (Eidia *et al*., 2006), and *Ocumum basilicum* (El-Beshibishy and Bahashwan 2012), but weaker than α-amylase inhibitory activity of the purchased Ethiopian spiced red pepper blend (Loizzo *et al*., 2011).

Correlation analysis

Different studies reported the relationship between total phenolic content and antioxidant activity (Mohammad et al., 2008; Petra *et al*., 2012; Bakchiche *et al*., 2013). As shown in Table 4, the TPC was strongly correlated with DPPH scavenging ($R^2 = 0.99$, $p < 0.001$), ferric reducing power ($R^2 = 86$, $p < 0.05$) and total antioxidant activity ($R^2 = 0.79$, $p > 0.05$). Similarly, a strong positive correlation was also found between DPPH scavenging ($R^2 = 0.91$, $p < 0.01$), reducing power ($R^2 = 0.97$, $p < 0.01$), total antioxidant activity ($R^2 = 0.96$, $p < 0.01$) and the TFC in green Datta paste extracts. In this study, it seemed that, the higher TPC of the extracts resulted in higher antioxidant activity as similarly reported by Engeda (2015) and Ruanma., *et al.* (2010).

Table 4: Correlations between antioxidant activities of the various solvent extracts of Datta and total phenolic and flavonoid contents ($p < 0.05$).

Antioxidant activities	Total phenolic (mgGAE/g)	Total flavonoid (mgCAE/g)
DPPH scavenging (%)	0.99**	0.91*
Ferric reducing power	0.86*	0.96**
Total antioxidant activity	0.79*	0.97**
α-amylase inhibition	0.90*	0.18

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

Different studies have shown that phenolic compounds play a role in mediating α-amylase inhibition and therefore have a potential to contribute to the management of type 2 diabetes (Cheplick *et al*., 2010; Ranilla *et al*., 2010). Under this study TPC was strongly correlated but TFC weakly correlated (Table 3) with α-amylase inhibition activity with coefficient of correlations, $R^2 = 0.90$, $p < 0.05$ and 0.18, $p > 0.05$, respectively. Similar study was reported by Engeda (2015) showing strong correlation between TPC and α-amylase inhibition activity of spiced red chili paste. Strong correlation between TPC and α-amylase was reported by Prinya *et al.* (2012), they also reported that caffeic acid content and inhibition against α-amylase were strongly correlated.

Conclusions

The observed antioxidant and α-amylase inhibition activities of the extracts of green Datta paste followed a dose-dependent pattern, with the highest activity observed at the highest concentration. The acetone extract of green data paste containing the highest TPC and TFC, also showed strongest DPPH radical scavenging, ferric reducing power, total antioxidant capacity, and α-amylase inhibition activity. This suggested that the antioxidant and α-amylase inhibition activities of the tested extracts were closely associated with their TPC. This is an indication that green Datta paste is a potential source of dietary antioxidant that can be used in the prevention and management of various oxidative stress-related ailments such as suppressing glycemic load by reducing activities of starch hydrolyzing enzymes.

Acknowledgment

The author would like to acknowledge the Hawassa College of Education, Hawassa, South Ethiopia for financing this project

References

Abdullah D. & Izabela K. (2013). Phenolic contents, antioxidant capacities and inhibitory activities against key metabolic syndrome relevant enzymes of herbal teas from Eastern Anatolia. *Industrial Crops and Products,* 44: 383–390.

Ayoola G., Ipav S., Solidiya M., Adepoju-Bello A., Coker H. & Odugbemi, T. (2008) Phytochemical screening and free radical scavenging activities of the fruits and leaves of *Allanblackia floribunda* oliv. (Guttiferae). *International Journal of Health Research.* 1**:** 81–93.

Bakchiche B., Gherib A., Smail A., Custodia G. & Grac M. (2013). Antioxidant activities of eight Algerian plant extracts and two essential oils. Industrial. *Crop and Products.* 46*:* 85– 96**.**

Chatha S. A. S., Anwar F. & Manzoor M. (2006). Evaluation of the antioxidant activity of rice bran extracts using

different antioxidant assays. *Grasas Y Aceites.* 57: 328–35.
Cheplick S., Kwon Y., Bhowmik P. & Shetty K. (2010). Phenolic-linked variation in strawberry cultivars for potential dietary management of hyperglycemia and related complications of hypertension. *Bioresourse Technology.* 101: 404–413.
Dheeraj P. J., Shyam S. & Pancholi R. P. (2011). Synergistic antioxidant activity of green tea with some herbs. *Journal of Advanced Pharmaceutical Technology and Research.* 2(3): 177–183.
Diem Q. D., Artik E. A., Phuong L. T. N., Lien H. H., Felycia E. S., Suryadi I. & Yi-Hsu Ju. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic. Journal of Food and Drug Analysis.* 22: 296 –302.
Eidia A., Eidib M. & Esmaeili E. (2006). Antidiabetic effect of garlic (Allium sativum L.) in normal and streptozotocin-induced diabetic rats. *Phytomedcine.* 13: 624–629.
El-Beshibishy H. & Bahashwan S. A. (2012). Hypoglycemic effect of basil (*Ocimum basilicum*) aqueous extract is mediated through inhibition of α-glucosidase and α-amylase activities: An *in vitro* study. *Toxicology and Industrial Health.* 28(1): 42–50.
Elsorady N. E. I. & Ali S. E. (2015). Antioxidant activity of roasted and unroasted peanut skin extracts. *International Food Research Journal.* 25(1); 43-50.
Engeda, D. (2015). *In Vitro* Antioxidant and α-Amylase Inhibition Activities of Spiced Red Chili Paste (Datta) from South Ethiopia. *Ethiopian Pharmaceutical journal, 31,* 93–106.
Engeda D., Geremew B., Gulelat D. H. & Rupasinghe V. H. P. (2015). Antioxidant and α-amylase inhibition activities *in vitro* of various solvent extracts of *Thymus schimperi* Ronniger. *Journal of Medicinal Plants Research.* 9(15): 515-524.
Eyob S., Martinsen B. K., Tsegaye A., Appelgren M. & Skrede G. (2008). Antioxidant and antimicrobial activities of extract and essential oil of Korarima (*Aframomum corrorima* (Braun) PCM. Jansen). *African journal of Biotechnology.* 7: 2585–2592.
Ghasemzadeh A., Jaafar H. Z. E. & Rahmat A. (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). *Molecules.* 15: 4324–4333.
Hui-Yin C., Yuh-Charn L. & Chiu-Lan H. (2007). Evaluation of antioxidant activity of aqueous extract of some selected nutraceutical herbs. *Food Chemistry. 104:* 1418–1424.
Iris H., Damien, D. & Raimo H. (2006). Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chemistry.* 97*:*122–129.
Javanmardi J., Stushnoff C., Locke E. & Vivanco J. M. (2003). Antioxidant activity and total phenolic content of Iranian Ocimum accessions. *Food Chemistry.* 83: 547–550.
Kai TK., Marilyn K., Li Xuan O., & Ken, N. (2013). Screening culinary herbs for antioxidant and a-glucosidase inhibitory activities. *International journal of Food Science and Technology.* 48:1884–1891.
Kamaran J. N., Mohd A. & Javed A. (2012). Antidiabetic Activity of Aqueous of Extract of *Coriandrum sativum* L. Fruits in Streptozotocin Induced Rats. *International journal of Pharmacy and Pharmaceutical Science.* 4(1): 254–258.
Katerere D. & Eloff J. (2005). Antibacterial and antioxidant activity of *Sutherlandia frutescens* (Fabaceae), a reputed Anti-HIV/AIDS. *Phytotherapy Research.* 19: 779–781.
Kwon Y. I., Apostolidis E. & Shetty K. (2008). *In vitro* studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. *Bioresoure Technology.* 99: 2981–2988.
Loizzo M. R., Lecee G., Boselli E., Bonesi, E., Bonesi M., Menichini F., Menichini F. & Frega N. G. (2011). *In vitro* antioxidant and Hypoglycemic activities of Ethiopian spice blend Berbere. *International journal of Food Science Nutrition.* 62(7): 740–9.
Mohammad A. E., Fereshteh P. & Ahmad R. B. (2008). Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. *African Journal of Biotechnology,* 7: 3188–3192.
Neelam G., Madhu G., Darshana M. & Bhupendra K. M. (2016). Chemical composition, total phenolic and flavonoid contents, and in vitro antimicrobial and antioxidant activities of crude extracts from red chilli seeds (Capsicum frutescens L.). *Journal of Taibah University for Science.* 10: 462–470.
Nigist A. & Sebsebe D. Aromatic Plants of Ethiopia, Shama books, Addis ababa, Ethiopia, (2009), pp: 137–138.
Otunola G. A. & Afolayan A. J. (2013). Evaluation of the polyphenolic contents and some antioxidant properties of aqueous extracts of Garlic, Ginger, Cayenne Pepper and their mixture. *Journal of Applied Botany and Food Quality.* 86: 66 –70.
Oyaizu M. (1986). Studies on product of browning reaction prepared from glucose amine. *Japanese Journal of Nutrition.* 44: 307–315.
Petra T., Barbara C., Nataˇsa P. & Helena A. (2012). Studies of the correlation between antioxidant properties and the total phenolic content of different oil cake extracts. *Industrial Crops and Products.* 39: 210–217.
Prieto P., Pineda M. & Aguilar M. (1999). Spectrophotometric quantitation of antioxidant capacity through the

formation of a phosphomolybdenum complex: specific application to the determination of Vitamin E. *Analytical Biochemistry.* 269: 337–341.

Prinya P. W., Jiranun C. & Anis Z. (2012). *In vitro* screening of phenolic compounds, potential inhibition against α-amylase and α-glucosidase of culinary herbs in Thailand. *Food Chemistry.* 131(3): 964-971.

Ranilla LG., Kwon Y. & Shetty K. (2010). Phenolic compounds, antioxidant activity and *in vitro* inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. *Bioresourse Technology.* 101: 4676–4689.

Ruanma K., Shank L. & Chairote G. (2010). Phenolic content and antioxidant properties of green chilli paste and its ingredients. *Maejo International Journal of Science and Technology.* 4: 193–200.

Seah R., Siripongvutikorn S. & Usawakesmanee W. (2013). Antioxidant and antibacterial properties in Keang-hleung paste and its ingredients. *Asian Journal of Food and Agro-Industry.* 3: 213–220.

Shan B., Cai Y. Z., Sun M & Corke H. (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of agricultural and Food Chemistry.* 53: 7749–7759.

Von Gadow A., Joubert E. & Hansmann C. (1997). Comparison of the antioxidant aactivity of aspalathin with that of other plant phenols of Rooibos tea (*Aspalathus linearis*), α-Tocopherol, BHT, and BHA. *Journal of Agricultural and Food Chemistry.* 45: 632–638.