Comparative study of Anti-oxidant and Alpha amylase Inhibitory Activity of Six herbs used in traditional medicine

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Abstract

The inhibition of α-amylase enzyme can be an important stratery in the postpradial blood glucose level in type II diabetes. In this study six traditional herbs were tested for α-amylase inhibition. Various concentrations of extracts were incubated with enzyme substrate solution and the activity of enzyme was measured. In addition, total phenolic content, total flavonoid content and *in vitro* antioxidant activity (reducing power, $ABTS^+$ and DPPH radical scavenging assay) were evaluated. The results show that Methanol and Ethanol are effective solvents to extract phenolic and flavonoid content. The reducing power was ranked: *A. altilis*> *P. vulgaris* > *S. barbata* > *C. fruticosa*> *C. nutans* > *M. charantia* (2.32, 2.165 and 1.397, 0.944, 0.833 and 0.503, respectively). Relatively high levels of DPPH and $ABTS^+$ radical scavenging activity were detected in extracts of *P. vulgaris, A. altilis and S. barbata* (IC_{50} value <5, 10.048±0.83, 6.656±0.3 for $ABTS^+$ and IC_{50} value 23.97±2.69, 35.49±2.34, 37.37±2.65 for DPPH, respectively). α-amylase inhibitory activity was found to be highest in extract of *C. fruticosa* (IC_{50} value 9.07±0.16) followed by *A. altilis, A. barbata, M. charantia, P. vulgaris and C. nutans* with IC_{50} value (10.72±0.304, 15.15±0.59, 22.53±0.36, 29.02±1.09 and 32.53±0.94, respectively). Further detailed investigations of *C.fruticosa, A. altilis, A. barbata* to identify the compounds responsible for the α-amylase inhibitory activity is in progress.

Keywords: Antioxidant, α- amylase, fenugreek, flavonoids, phenolics.

1. Introduction

Antioxidant results have a prominent role in many human diseases comprising cancer diabetic complications, heart disease, liver damage, autism, and Alzheimer's disease (Liang et al., 2010). Oxidants such as reactive oxygen species (ROS) created from activated neutrophils and macrophages have been reported to play an substantial function in the pathogenesis of diverse pain-related diseases, containing neurodegenerative disorders and cancer (Winrow et al., 1993; Confortia et al., 2007). The use of conventional medicine is pervasive and plants still present a vast source of natural antioxidants that might serve as leads for the evolution of novel drugs. Several antiinflammatory digestive, antinecrotic, neuroprotective, and hepatoprotective drugs have recently been shown to have an antioxidant and/or antiradical scavenging mechanism as part of their activity (Lin and Huang, 2002; Repetto and Llesuy, 2002). In the recent decates some herbs have been commodiously studied for their antioxidant activity and radical scavenging activity (De las Heras et al., 1998; Desmarchelier et al., 2000; Schinella et al., 2002; VanderJagt et al., 2002).

One of the causes of very high morbidity and mortality in the world had been reported to being relative with type II diabetes mellitus (T2DM) as an epidemic disease associated with increased considerable social and fiscal burden. (Stumvoll M et al., 2005). The control of hyperglycemia is very significant in the treatment of all forms of diabetes by reason that in the long term, acute and chronic complications can happen when the blood glucose concentration is at unbalance level (Funke and Melzig, 2006). A widely held notion is that postprandial hyperglycemia (PPHG) is a original risk factor in the development of T2DM and its complications via multi-factorial mechanisms (Labonte ED et al., 2006). In spite of greatly efforting to normalize blood glucose level in clinic practice, it is still a dreadful challenge. Even more difficult is the control of PPHG (Mooradian AD and Thurman JE, 1999). Nowadays, the more difficult is the control PPHG based on the α-glucosidase, α-amylase, amylin analogues as targets (Bischoff H, 1994). Also, α-amylase inhibitors are one of the anti-diabetic drug families, of which Acarbose is the most well-known. These drugs have a very strong advantage and are suitable for healing noninsulin-dependent diabates mellitus (type 2 diabetes) (Upadhyay and Ahmad, 2011). However, the continuous use of those synthetic agents should be restrained because those agents may cause side effects such as flatulence, abdominal cramp, vomiting, and diarrhea (Henefeld M, 1998; Chakrabarti R and Rajagopalan R, 2002). Accordingly, several researchers are

investigating and developing nutritional strategies to perfectly control postprandial glycemia, without inducing negative occasions in the digestive system, medicinal herbs due to easy accessibility and also lower nagative effects have a special place in medicine to treat various diseases (O'Keefe et al., 2008). Otherwise, overwhelming researches suggest that natural antioxidants may be used to reduce oxidative damage and decrease the occurrence of diabetic complications (Rahimi et al., 2005; Jonhansen et al., 2005). Therefore, it is a prospective strategy that PPHG and ROS are used as dual-target to screen the natural drugs to combat the multiple disorders of T2DM.

It has been reported that there is an inverse ralationship between the antioxidative status occurence of human diseases (Rice-Evans et al., 1997). In addition, Active compounds derived from medicinal herbs may be a source of new α- amylase inhibitors. Furthermore, some plants show a great amount of phenolic substances, therefore accordingly, they have antioxidant activity (Cicero et al., 2013). The choice of our investigated plants is based on a reason that nowadays mistakable usage among natural herbs because of informative confusion being more and more increasing. In addition, there are a lot of researches for individual herb with antioxidant activity and diabetic inhibitor while seldom combined and comparative studies from herbal medicines are. These led to a result that so herbal consumers have in effectively used herbs in folk therapies to cure various deseases, such as abdominal diseases, various cancerous lesions of the stomach, colon, lung and liver. In rural and backward area of Vietnam and some asia countries, several plants are commonly used as herbal medicine for the treatment of cancer and diabetic diseases. The purpose of this study was to evaluate a variety of six medicinal plants consisting of *Scutellaria barbata* (Lamiaceae), *Prunella vulgaris* L (Labiatae), *Momordica charantia* L (Cucurbitaceae), *Cordyline fruticosa* (Liliaceae), *Clinacanthus nutans* (Acanthaceae), *Artocarpus altilis* (Moraceae) that are of the same location and have grown in the same conditions. This study is related to the flavonoid and phenolic content and their α-amylase inhibition activity and antioxidative activities.

2. Materials and Methods

2.1. Chemicals and reagents

Folin-Ciocalteu reagent, Sodium carbonate (Na_2CO_3), Gallic acid, Aluminium Chloride ($AlCl_3$), Potassium acetate (CH_3COOK), Quercetin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), Potassium persulphate, Ascorbic acid (Vitamin C),

Potassium ferricyanide ($K_3[Fe(CN)_5]$, α-amylase, Sodium chloride, Phosphate buffer, $FeCl_2.H_2O$, Ferrozine-1,2,4-triazine, Trichloroacetic, Buthylated foluene (BHT), and some other chemicals and reagents.

2.2. Extraction

Samples of medicinal plants were harvested at herbal farm in department of plant industry, National Pingtung University of Science and Technology, Taiwan (Table 1).

Table1: Botanical species

Scientific name	Family name	Organ
Scutellaria barbata	Lamiaceae	Whole plant
Prunella vulgaris. L.	Labiatae	Mature flower
Momordica charantia L.	Cucurbitaceae	Whole plant
Cordyline fruticosa	Liliaceae	Mature leaf
Clinacanthus nutans	Acanthaceae	Whole plant
Artocarpus altilis	Moraceae	Mature leaf

The fresh parts of these herbs were washed by distilled water and dried in oven at 44 ^{o}C for 24 hours. The dry materials were crushed into 50-mesh sieve powder. Extracts were obtained as follows: 5 grams of each herb was separately extracted twice with Methanol, Ethanol, Acetone at various concentrations: 90, 70 and 50% (v/v) and hot water in circulator bath for 2 hours at 80 ^{o}C and then was filtered. The filtrates were combined and concentrated in a vacuum evaporator at 45 ^{o}C. The dehydrated fractionation were weighted to calculate yield, then dissolved in dimethyl suforide (DMSO) to a regular concentration and the activity was measured.

2.3. Determination of total phenolics (TP)

The total phenolic content (TPC) was determined according to the Folin-Ciocalteu method (Singleton and Rossi, 1965). The reaction mixture was composed of 0.1 mL of extract, 7.9 mL of distilled water, 0.5 mL of the Folin-Ciocalteu's reagent, and 1.5 mL of 20% sodium carbonate. The absorbance was measured at 765 nm in a UV-3000 Spectrophotometer (The same equipment was used in all the assays). The total phenolic content were calculated using a calibration curve for gallic acid (0-10 microgram/mL) with $y = 0.014x + 0.026$; $R^2 = 0.9670$, curve standardized in the lab for the calculation of Gallic acid equivalent per gam of extracts. All samples were analyzed thrice and results averaged.

2.4. Determination of total flavonoids (TF)

Total flavonoids content was determined by using the aluminium chloride colorimetric method. A mixture of 0.5 mL sample, 100 µL 10% aluminum chloride, 100 µL 1 M potassium acetate and 2.3 mL distilled water were incubated at room temperature for 30 min. The absorbance was measured at 415 nm. Quercetrin (0.2-1 mg/mL) as a standard was used to make the calibration curve with $y = 0.494 x + 0.028$; $R^2 = 0.997$. The estimation of total flavonoids in the extracts was carried out in triplicate and the results were averaged.

2.5. Determination of reducing power

The extracts with highest total phenolics were used for antioxidant and alpha amylase inhibition assay**.** The reducing power of the extracts was determined according to the method of Oyaizu (1986) with some modifications. First, 1mL of extract solution (0.2, 0.5, 0.8 and 1.0 mg/mL) in 95% ethanol was mixed with phosphate buffer (2.5 mL, 0.2 mol/L, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). This mixture then was incubated at 50 ^{o}C for 20 min. At the end of the incubation, trichloroacetic acid (2.5 mL, 10%) was added to the mixture, which was centrifuged at 3,000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%), and the absorbance was measured at 700nm against a blank.

The blank sample was prepared under the same conditions without the addition of any extract. Increased absorbance of the reaction mixture indicated increased reducing power. The reducing power of BHT (0.2, 0.5, 0.8, and 1.0 mg/mL) also was assayed for comparison. All the tests were performed in triplicate, and the results were averaged.

2.6. $ABTS^+$ radical scavenging assay

$ABTS^+$ radical-scaveging activity was measured by modifying the method described by Re et al., 1999. $ABTS^+$ was dissolved in de-ionized water to 7 mM concentration, and potassium persulphate was added to a concentration of 2.45 mM (final concentration). After well mixed, the reaction mixture was left to stand at room temperature overnight (12-16 h) in the dark before usage (Fresh stocks of $ABTS^+$ solution were prepared every five days due to self-degradation of the radical). 200 µL sample in methanol at different concentrations of 0, 5, 10, 20, 40, 80, 160 µg/mL respectively was mixed with 0.3 mL $ABTS^+$ solution and 0.5 mL distill water. The mixture was allowed to stand at room

temperature for 2 min, and the absorbance at 745 nm was immediately recorded. Vitamin C served as a positive control. All the tests were performed in triplicate and graph was plotted with the mean values. The assay was first carried out on the percentage of inhibition which calculated by the following formula: $ABTS^+$ radical – scavenging activity (%) = $(1-A_s/A_c)$ x100 . Where A_c was the absorbance of the blank (without sample) and A_s was the absorbance of the sample.

2.7. DPPH radical scavenging activity

Assay for the scavenging of stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was carried out as reported earlier with some modification (Blois, 1958). Briefly, 100 µL sample in different solvents was mixed with 1.9 mL of 0.1 mM DPPH in ethanol. The concentration of the tested samples in the mixture was 0, 5, 10, 20, 40, 80, 160, 320 µg/mL, respectively. The reaction mixture was shaken well and incubated in dark at 37 ^oC for 30 min and the absorbance was measured at 517 nm. The blanks contained all the reaction reagents except the extract of positive control substances. BHT was used as positive control with IC_{50} 10.08µg/mL. The percentage scavenging values were calculated from the absorbance of the blank (A_0) and of the sample (A_s) using the following equation: DPPH radicals scavenging activity (%) = $(1-A_s/A_0)$x100. Where A_s was the absorbance of the sample and A_0 was the absorbance of the blank (without sample).

2.8. *In vitro* inhibitory alpha amylase assay

The 500 µL of plant extract was incubated with 500 µL of α-amylase solution (enzyme solution (2 units/ml) was obtained by dissolving 0.001 g of α-amylase in 100 ml of 0.02 M sodium phosphate buffer pH 6.9 with 6.7mM sodium chloride) at room temperature (32^oC) for about 10 minutes. After incubation, 500 µL of 1% starch solution (dissolving 1 g of potato starch in 100 ml of distilled water with boiling and stirring for 15 minutes) was added and was incubated at room temperature (32 ^oC) for about 10 minutes. To the above, 1ml of DNSA reagent was added to stop the reaction and was incubated in hot water bath (85 ^oC) for 5 minutes. After 5 minutes, reaction mixture color changed to orange-red and was removed from water bath and cooled to room temperature.

It was dilute up to 5 ml of distilled water. Extracts at different concentrations (2, 5, 10, 15, 20 mg/mL) were performed in triplicates. Individual blank was performed by replacing extract with solvent. Fenugreek seed powder (2, 5, 10, 15, 20 mg/mL) was used as positive control. Absorbance was measured at 540 nm. Enzyme unit is defined as one unit enzyme will liberate 1 mg of maltose from 1

% starch in 5 minutes under defined condition i.e. room temperature. Logarithmic regression curve was established by plotting percentage of alpha amylase inhibition against sample concentration in order to calculate IC_{50} (inhibitory concentration) value. This represents sample concentration (mg/ml) required to decrease the absorbance by 50% of alpha amylase (Paul and Banerjee, 2013). The inhibition percentage of α-amylase was assessed by the following formula: The α-amylase inhibitory activity = $(Ac^{+}) – (Ac^{-}) – (As-Ab) / (Ac^{+}) – (Ac^{-})$ x 100. Where Ac^{+} that absorbance of 100% enzyme activity (only solvent with enzyme); Ac^{-} that absorbance of 0% enzyme activity (only solvent without enzyme); As that absorbance of test sample with enzyme; Ab that absorbance of test sample without enzyme. All the tests were run in triplicate. The IC_{50} values (concentration required to inhibit 50% enzyme activity) were calculated by applying logarithmic regression analysis from the mean inhibitory values.

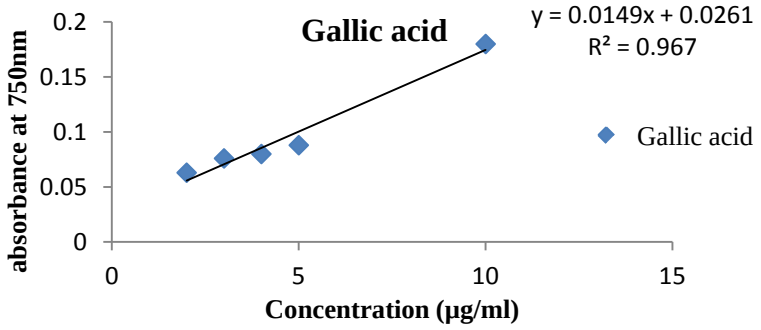
2.9. Statistical analysis

The data were analyzed using one-way ANOVA, and the level of Least Significance Difference (LSD) was determined using Duncan's multiple range test at $p<0.05$ for comparing means of the treatments. All analyses were performed using the SAS statistical package (SAS Institute 1990).

3. Results and discussion

3.1.Total phenolics and flavonoid content

Phenols and polyphenolic compounds, such as flavonoids, are widely found products derived from plant sources, and they have been shown to possess significant anti-oxidant properties. The high amount of phenols and flavonoids in extracts may explain their high anti-oxidant activities (Ebrahimzadeh et al., 2010). In this study, the total phenolic content for Methanol, Ethanol, Acetone and hot water extracts were estimated by Folin Ciocalteu's method using gallic acid as standard. The gallic acid solution of concentration (0-10 ppm) conformed to Beer's Law at 765 nm with a regression co-efficient (R^2) = 0.967. The plot has a slope (m) = 0.014 and intercept =0.026. The equation of standard curve is y = 0.014x + 0.026 (Fig.1).



R^2 values represented mean data set of n=3

Fig.1: Total phenolic content for standard gallic acid

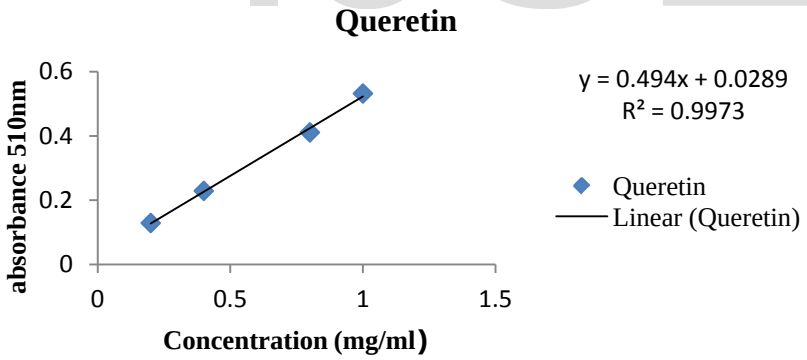
The yield of studied herb medicine extracts, content of total phenolics in Table 2 is shown. The amount of components extracted from different herbs using different solvents varied widely. The highest total phenolics were extracted with 50 % methanol from *S. barbata, M. charantia*, *C. nutans*. Whereas, 50 % ethanol etracted more components from *C. fruticosa*, 50% Acetone from *P. vulgaris* and *A. altilis*. The lowest total phenolics were extracted with hot water from almost of herbs, but the extraction from *M. charantia* showed highest value for total phenolics in this solvent. The data indicated that each of different solvents is effective to extract phenolic compounds for a kind of herbs. However, some former works show that methanol and ethanol has been proven as effective solvent to extract phenolic compounds (Siddhuraju and Becker, 2003). Ethanol is preferred for the extraction of antioxidant compounds mainly because it lowers toxicity (Karadeniz et al., 2005). The total phenolics of herb were able to be ranked as follows: *A. altilis* (30.72 mg) > *S. barbata* (29.93 mg) > *P. vulgaris* (20.76 mg) > *C. fruticosa* (20.62 mg) > *M. charantia* (7.35 mg) > *C. nutans* (6.3 mg GAE per gram of dry material). Previous studies found that a hightly positive linear relationship exists between antioxidant activity and total phenolic content in many spices and herbs (Cai et al., 2004; Shan Eet al., 2005). Consistent with this, our results showed that *A. altilis* and *S.barbata*, *P. vulgaris, C. fruticosa* contain a considerable amount of phenolic compound, implying that these herbs may be a resource of efficient natural antioxidant and could be useful in relation to diseases involving free radical reactions.

Table 2: Yield, contents of phenolics expressed as mg of gallic acid equivalent weight (GAE)

Method, Herb	Methanol concentration(%v/v)			Ethanol concentration (%v/v)			Acetone concentration (%v/v)			Hot water(%v/v)
	90	70	50	90	70	50	90	70	50	100
S.barbata	11.69b	13.75b	29.93a	11.77ab	13.86a	24.57a	11.9b	14.91b	22.97b	2.83b
P.vulgaris	9.34c	9.43d	17.11b	9.04c	10.49c	17.14c	8.47d	12.66c	20.76c	1.42c
M.charantia	3.49d	3.97f	7.35c	3.62d	5.2e	6.94d	2.06e	2.17e	5.14e	21.05a
C.fruticosa	11.85b	11.86c	19.35b	12.36a	9.56d	20.62b	9.11c	9.77d	13.65d	2.49c
C.nutans	3.43d	4.82e	6.3c	3.34d	3.86f	5.43e	1.82e	2.65e	4.65e	1.5c
A.altilis	14.71a	15.03a	7.65c	10.99b	11.41b	21.26b	12.59a	18.17a	30.72a	1.3a
LSD	0.68	0.65	3.01	1.14	0.63	0.98	0.54	0.72	1.62	3.58

Notes: LSD, least significantly difference. Means followed by the same letters do not significantly differ (P<0.05).n

The total flavonoid content for methanol, ethanol, acetone and hot water extracts were measured with the aluminium chloride colorimetric assay using quercetin as standard. The quercetin solution of concentration (200-1000 ppm) conformed to Beer's Law at 510 nm with a regression co-efficient (R^2) = 0.997. The plot has a slope (m) = 0.494 and intercept = 0.028. The equation of standard curve is y = 0.494 x + 0.028 (Fig.2).



R^2 values represented mean data set of n =3.

Fig.2: Total flavonoid content for standard quercetin

Data represented in Table 3 showed that extracts with 90% ethanol from *P. vulgaris* and *C. fruticosa*, 70 % ethanol from *M. chanrantia* had the highest content of total flavonoids (P<0.05). Whereas, 50% methanol extracted more components from *S. barbata*, 70% methanol for *A. altilis* and

90% acetone for *C. nutans*. The total flavonoids of herbs were able to be ranked as follows: *M. charantia* > *C. fruticosa* > *A. altilis* > *S. barbata* > *C. nutans* > *P. vulgaris.* In this survey, the data also pointed out that Ethanol is preferred as effective solvent to extract flavonoid compounds.

Table 3: Yield, contents of flavonoids expressed as mg of quercetin equivalent weight (QE)

Method, Herb	Methanol concentration(%v/v)			Ethanol concentration (%v/v)			Acetone concentration (%v/v)			Hot water(%v/v)
	90	70	50	90	70	50	90	70	50	100
S.barbata	5.86c	5.67c	8.88a	6.17cd	7.58c	6.58b	7.44b	6.75b	4.87b	2.92ab
P.vulgaris	3.56d	3.34d	3.46c	7.05c	5.27d	4.39c	5.52c	4.49c	4.11bc	1.48b
M.charantia	7.61b	5.05c	6.41b	10.98b	16.02a	10.26a	3.63d	7.06b	3.24c	3.56a
C.fruticosa	5.88c	9.27b	6.75b	14.99a	12.67b	10.34a	9.37a	9.67a	4.62b	2.12ab
C.nutans	5.1c	4.63cd	2.82c	5.87d	5.55d	4.51c	7.23b	1.85d	3.97bc	2.19ab
A.altilis	9.36a	11.52a	4.38c	3.69e	8.08c	9.27a	5.27c	10.16a	10.89a	3.26a
LSD	1.23	1.38	1.64	1.1	1.44	1.5	0.8	0.81	1.14	1.74

Notes: LSD, least significantly difference. Means followed by the same letters do not significantly differ ($P<0.05$)

Difference in the amounts of total Phenolics and Flavonoids in different extractions from various herbs could be explained by the fact that presence of phenolics is affected by plant species, maturity at harvest, growing conditions and post-harvest treatment (Jaffery et al., 2003). At the same solvent with different concentrations, there were significant differences in TP and TF from the same herb. These could be because of sub-cellular level and structure within tissues.

3.2. Reducing power of the extracts

The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron (Yildirim et al., 2000). In this assay, the ability of extracts to reduce Fe^{3+} to Fe^{2+} was determined. The presence of antioxidants in the extracts resulted into reduction of the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}). In reducing power assay, antioxidants cause the reduction of the Fe^{3+} to Fe^{2+}, thereby changing the solution into various shades from green to blue, depending on the reducing power of the compounds (Ferreira et al., 2007). Strong reducing agents, however, formed Perl's Prussian blue colour and absorbed at 700nm. The data for the reduction of the ducing potential of the samples and BHT were assayed, and the comparative results are shown in Fig. 3

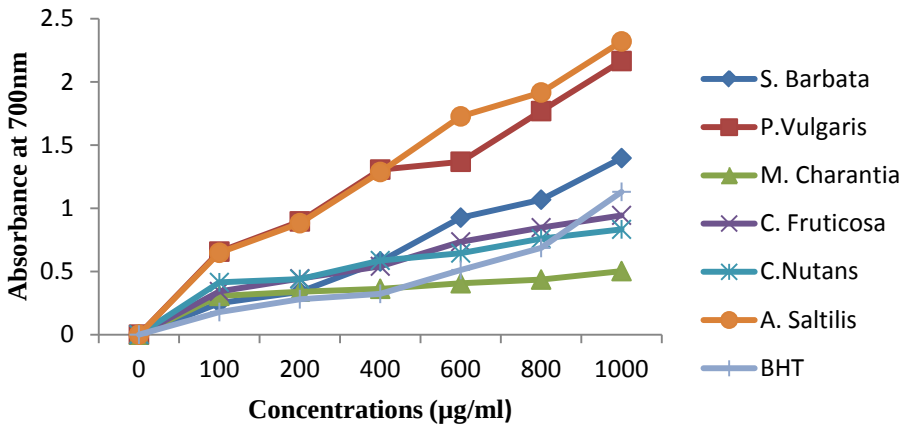


Figure 3. Reducing power of herbs

The reducing potential of selected herb extracts measured for the concentration up to 1000 µg/mL, showed general significant increases in activity at $P<0.05$ when concentration increased. The results obtained indicated that the activity, which varied at 1000 µg/mL from 0.503 to 2.32, significantly ($P<0.05$). The antioxidant activity has been reported to be concomitant with the reducing power (Tanaka et al., 1988). Thus, the reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron which is an important mechanism of phenolic antioxidant action (Mohamed et al., 2009). Many reports have revealed that there is a direct correlation between antioxidant activities and reducing power of certain plant extracts (Benzie and Szeto, 1999; Koleva et al., 2002). The reducing power of the presently investigated herbs indicated that among studied herbs *A. altilis*, *P. vulgaris* and *S. barbata* are better sources of antioxidants with high reducing power (2.32, 2.165 and 1.397, respectively, which these values are higher than that of BHT (absorbance of 1.13). Meanwhile, this value is lower for *C. fruticosa*, *C. nutans* and *M. charantia* (0.944, 0.833 and 0.503, respectively). At the same concentrations, the reducing powers of ultrasonic extract and soxhlet extract from *Agrimonia pilosa* had a value of 1.373 and 1.292, respectively (Chunhuan et al., 2009). At 0.5mg/ml concentration this of the extract obtained using acetone, ethyl acetate, methanol or of 0.52, 1.737, 0.903, respectively (Jayaprakasha et al., 2000) and that derived from barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. trees had absorbances from 1.34 to 1.87 at 10 mg/ml (Bushra et al., 2007). Comparison with some herbs from former works more emphasized antioxidant activity of *A. altilis*, *P. vulgaris* and *S. barbata*. Moreover, the reducing power property indicates that these compounds are electron donors, and can reduce the oxidized intermediates

of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Yen and Chen, 1995). Therefore, the bioactive components of *A. altilis*, *P. vulgaris* and *S. barbata* extracts can also act as primary and secondary antioxidants, sacavenging free radicals, and can inhibit the lipit peroxidation.

3.3. $ABTS^+$ radical scavenging activity

It is known that $ABTS^+$ is an excellent substrate for peroxidases frequently used to study antioxidant properties of natural compounds. The $ABTS^+$ radical scavenging activities were measured at six concentrations, ranging from 5 -160 µg/mL. There were significant differences in inhibition (%) between herbs ($P<0.05$) in Fig.4. Of all the tested herbs, *P. vulgaris* showed an outstanding scavenging activity with a sacaveging value of 98.54% at 160 µg/mL concentration. Interestingly, three herbs still being *A. altilis*, *P. vulgaris* and S. *barbata* showed high scaveging value (92.22, 98.54 and 95.93 %, respectively) and other herbs showed scaveging capacity being at lower level (85.6, 80.9 and 76.15 % for *C. fruticosa*, *C. nutans* and *M. charantia*), respectively.

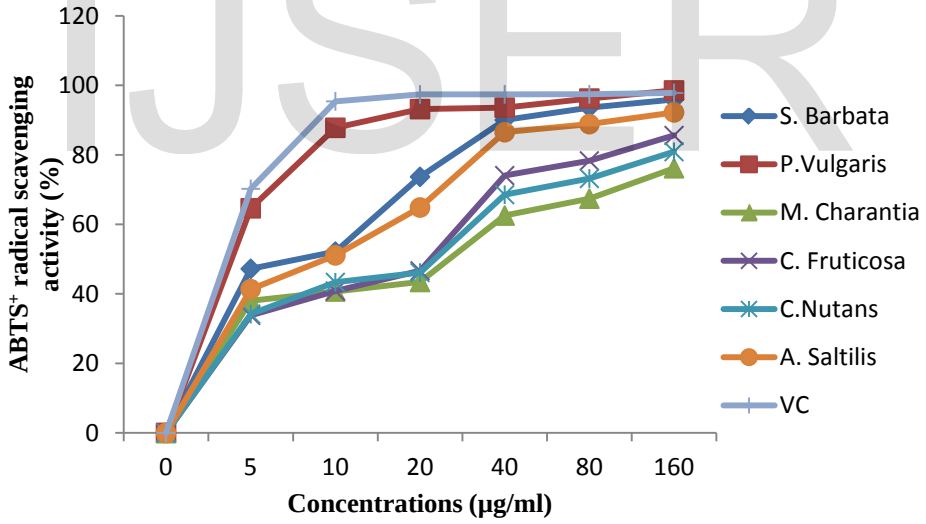


Figure 4. $ABTS^+$ radical scavenging activity of herbs and Vitamin C (VC)

The data demonstrated emphatically more significant relationships between reducing potential and $ABTS^+$ radical scavenging. The higher reducing power value, the higher the $ABTS^+$ radical scavenging activity. The comparative IC_{50} values of *P. vulgaris*, *A. altilis*, S. *barbata* being higher inhibitions and Vitamin C were <5, 6.656±0.3, 10.048±0.83 and <5 µg/ml, respectively. Thus, the $ABTS^+$ radical scavenging activity decreased in the following order: VC ≈ *P. vulgaris*> *A. altilis*> *S. barbata.*

Previously some plant extracts have been reported to $ABTS^+$ radical scavenging activity, such as IC_{50} values of soxhlet and ultrasonic extracts from *Agrimonia pilosa* were 7.31±0.3 and 5.88±0.25 µg/ml (Chunhuan et at, 2009), at 50 µg/ml concentration, the stalk extracts of *Jangwongeubje* exhibited the highest $ABTS^+$ radical scavenging activity (84.36%) (Lei et al., 2012). Whereas, that derived from *P. vulgaris, A. altilis, S. barbata* had higher values at the same concentration. This indicated that $ABTS^+$ radical scavenging activity of these herbs found in the present study was relatively high.

3.4. DPPH free radical-scavenging activities of herb (%)

DPPH is long-lived nitrogen radical. Many antioxidants that react quickly with transient radicals such as the peroxyl radical may react slowly or may be even inert to DPPH (Huang et al., 2005). To understand more clearly the antioxidant effects of studied herb medicines on the DPPH assay, the radical scavenging activity of extracts were measured, ranging from 5 to 320 µg/mL (Fig 5). Extracts of *P. vulgaris* had the highest DPPH radical scavenging activity with value of 90.83 % at 320 µg/ml, the lowest being for *M. charantia* with value of 41.73 % at the same concentration. The plant extracts from *P. vulgaris, S. barbata* and *A. altilis* ranked as the top three most active plant extracts among them, exhibited strong activity on scavenging DPPH radicals with the determined IC_{50} values 23.97±2.69, 35.49±2.3 and 37.37±2.65 µg/ml, respectively (Table 4). Another three plant extracts were shown IC_{50} over 78 µg/ml (78.91 - >200 µg/ml). Basically, a higher DPPH radical-scaveing activity is associated with a lower IC_{50} value. This data indicated that three herbs of *P. vulgaris, S. barbata* and *A. altilis* had better antioxidant potential among selected herbs but with IC_{50} being higher than that of BHT.

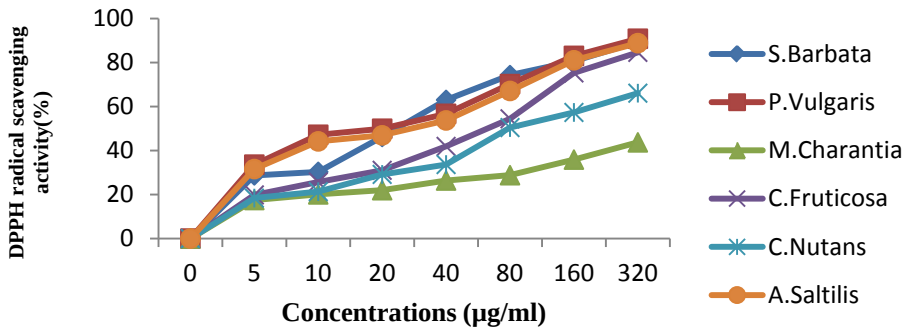


Figure 5. DPPH free radical scavenging activity of herbs (%).

The general evaluation of the IC_{50} showed in table 4 represent the concentration needed to reach 50% of the total antioxidant activities . The higher the IC_{50} values show the lower the antioxidant capacity. The lowest IC_{50} values of $ABTS^+$ radical scavenging activity and DPPH radical scavenging activity were *P. vulgaris* which were <5 and 23.97±2.69 µg/ml, respectively. The IC_{50} of *M. Charantia L.* was too high to be determined for $ABTS^+$ radical scavenging activity and DPPH radical scavenging activity (24.497±1.982 and >200 µg/ml, respectively).

Table 4. IC_{50} (µg/mL) of antioxidant capacities and α- amylase inhibition of herb medicine extractions

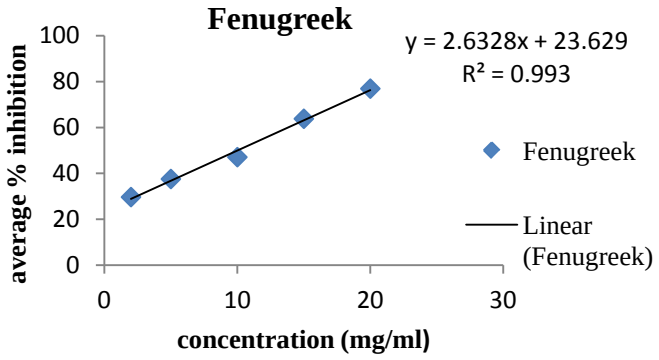
Herb	IC_{50} of $ABTS^+$ radical scavenging activity	IC_{50} of DPPH radical scavenging activity	IC_{50} of α- Amylase inhibition
S. barbata	6.656±0.3c	37.37±2.65c	15.15±0.59d
P. vulgaris. L.	<5	23.97±2.69d	29.02±1.09
M. charantia L.	24.497±1.982a	>200	22.53±0.36c
C. fruticosa	19.799±0.7b	78.91±0.75b	9.07±0.16e
C. nutans	20.87±0.212ab	117.25±0.39a	32.53±0.94a
A. altilis	10.048±0.83c	35.49±2.34c	10.72±0.304e

Some results in the present study were in agreement with earlier reports. Various researchers have reported the antioxidant capacity of these herbs. Such as, DPPH radical scavenging activity IC_{50} values of 1.33±0.001 mg/ml from methanolic leaves extract of *C. nutans* (Ruhaiyem et al., 2015); 64.6 and 73.5 µg/mL from extracts of 1-year-old buds and 6-month-old buds (Ali et al., 2014). IC_{50} value for DPPH radical scavenging capacity of *P. vulgaris* was 113µg/ml (Lie et al., 2005). While there are also results being not in agreement with earlier reports. For example, the DPPH radical scavenging of *M. Charantia* at 2000 µg/mL was 45.95 and 21.14 and 15.49% for ethanol extracts from ripe fruit, ripe seed and unripe fruit, respectively (Ozusaglam and Karakoca, 2013). These could be explained that antioxidant activities depend on the presence of compounds in the tested extracts. Generally, the antioxidant properties are associated with the presence of compounds which exert their action by breaking the free radical. Total phenolics content of a spice has been usually found to correlate highly

with $ABTS^{+}$, DPPH, and O^{2-} scavenging activities of that spice, and therefore, phenolics were considered as the main contributors responsible for free-radical scavenging of a spice (Katsube et al., 2004; Tsai et al., 2005). In accord with the observations of these workers, it was observed herein that a significant correlation existed between the total phenolics content of a spice and its DPPH-scavenging activity. Moreover, it was also correlated with the total phenols which could be responsible for the antioxidant activity (Bhalodia et al., 2011). In additon, also as a result of various methods in drying method, extract method, maturity at harvest, growing condtions, different organs but in the present study, results indicated that there is an obviously antioxidant capacity dominance of some selected herbs.

3.5. *In vitro* inhibitory alpha amylase assay

In the present study extracts of selected herb medicines with known anti-diabetic activity were investigated for their potential to inhibit α-amylase activity, ranging from 2 to 20 mg/ml (Fig. 6 and 7) as comparable with Fenugreek. Amongst the selected plants, extract of *C. fruticosa, A. altilis* (96.08 and 87.3 % respectively) at 20 mg/ml concentration show higher amylase inhibition than Fenugreek (76.94%). This was followed *S. barbata* > *M. charantia L* > *P. vulgaris. L.* > *C. nutans* (59.57; 42.69; 37.73 and 32.12 %, respectively). The EC_{50} values were calculated and the results showed in the Table 4. The higher the IC_{50} value, the lower the alpha amylase inhibition. The data also clearly indicate one more time that *C. fruticosa* was with highest inhibition capacity (lowest IC_{50} value of 9.07±0.16 mg/ml. Moreover, the highest IC_{50} value of α-amylase inhibition was *C.nutans* (32.5±0.94 mg/ml).



R^2 values represented mean data set of n=3

Figure 6. *In vitro* alpha amylase inhibitory assay for standard fenugreek

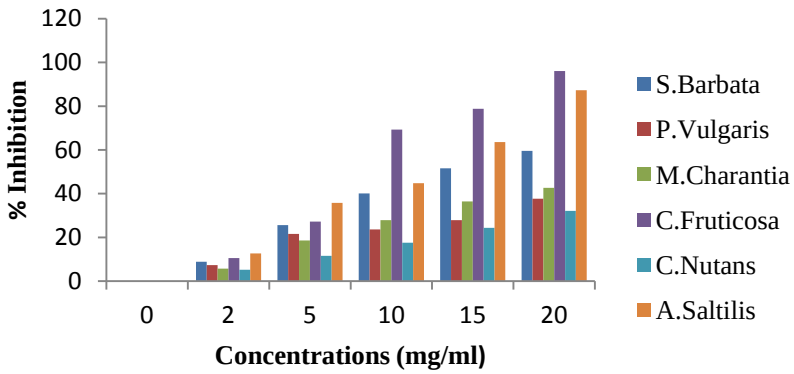


Figure 7. *In vitro* alpha amylase inhibitory assay for extracts of selected herb medicine

Diabetes mellitus is a metabolic disorder may be due to enhanced cellular oxidative stress and reduced antioxidant activity. Polyphenols and flavonoids are natural antidiabetic agents, which interferes the production of free radicals, reduce oxidative stress inhibitit digestive enzyme, thus lowering postprandial glucose. This is obvious with some herbs such as *A. altilis* and *S. barbata* treated in this work. Total phenolics and flavonoids are high, anpha amylase inhibition capacity and antioxidant are too. However, the results showed that *C. fruticosa* had hightest alpha amylase inhibition capacity whereas the total phenolics of this herb (20.62 mg GAE/g) is not as high as that of *A. altilis* and *S. barbata* (30.72 and 29.93 mg GAE/ g, respectively). Upon this study, it may be stated that total phenolics and flavonoids; antioxidant acitivity had coherent relationships but alpha amylase inhibition does not depend completely on total phenolics and flavonoids. The unclear relationship between the alpha amylase inhibition capacity and the total phenolics may be explained that it may be due to the diversity and complexity of the natural mixtures of phenolic compounds or the dominant effect of any extraordinary phenolic group in these plant extracts. Each herb contains proprietarily different phenolic compounds with corresponding effects of alpha amylase inhibition capacity. Is there any relationship between the phytochemical compound and alpha amylase inhibition capacity ?. Therefore, a more detailed investigation with this relationship is progress.

3.6. Conclusion

In conclusion, this study provides scientific proofs for six herb's traditional usege. Our results reveal that methanol and ethanol are effective solvents to extract the natural products extraction from plants because of their higher efficiency. The higher antioxidant activity is possessed by *P. vulgaris*, *A. altilis* and *S. barbata* being interesting sources of antioxidative plant. It has been also

corroborated in this study that *C. fruticosa, A. altilis* and *S. barbata* are strong α-amylase inhibitors especially *C. fruticosa* with excellent inhibition capacity (IC_{50} of 9.07± 0.16). The results support the traditional use of these plants as anti-diabetic drugs and adjuvant antioxidant and may contribute new treatment strategies for diabetic disease in the future. Diverse laboratory *in vitro* studies have shown the health effects of these herbs, but these and the human clinical evidence are still restrained, so a in-depth investigation of the attributes of these plants and to identify the compounds responsible for the alpha amylase inhibition capacity is in progress. Moreover, future research is necessary to actually define the different benefits particularly for the ailments like viral diseases and cancers where synthetic drugs are not effective because of lesser safety margin and higher cost. Health benefits of the different bioactive principles need to be evaluated more.

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