Study of phenolic content and urease and alpha-amylase inhibitory activities of methanolic extract of *Rumex acetosella* roots and its sub-fractions in different solvents

Dildar Ahmed*, Qaria Mumtaz Mughal, Saba Younas and Muhammad Ikram
Department of Chemistry, Forman Christian College (A Chartered University), Ferozpur Road, Lahore, Pakistan

Abstract: The present study aimed to establish relationship between urease and alpha-amylase inhibitory activities on the one hand and on the other between anti-enzymatic activities and total phenolic contents of the methanolic extract of roots of *Rumex acetosella* and its fractions in various solvents. The methanolic extract and its fractions in chloroform, ethyl acetate, n-butanol and water showed remarkable inhibitory activities against both urease and alpha-amylase, there was a close correspondence between urease and alpha-amylase inhibitory activities of the plant samples. The n-butanol fraction which had the highest total phenolic content (252.19±2.32 µg of Gallic Acid Equivalents/mg of dry mass of the sample) showed prominent activity against both urease and alpha-amylase indicating a possible role of phenolics in inhibiting the activities of these enzymes. The samples displayed enzyme inhibitory activities in a dose dependent manner and their effectiveness was comparable with that of the standards, thiourea (for urease) and acarbose (for alpha-amylase). The samples were manifold more effective against urease than alpha-amylase; 2.8 mg/mL of MeOH extract produced about 81% inhibition in alpha-amylase activity, while only 10 µg/mL of the extract was required to create the same inhibition in urease activity. The IC_{50} values of methanolic, chloroform, ethyl acetate, n-butanolic, aqueous and standard solutions were 1.29, 1.31, 1.90, 1.38, 0.85 and 1.20 (mg/mL) respectively against alpha-amylase and 0.99, 3.89, 1.76, 0.91, 0.85 and 0.97 (µg/mL) respectively against urease. The total phenolic content in MeOH, hexane, chloroform, ethyl acetate, n-butanol and water fractions was 108.88±2.65, 43.70±1.90, 34.44±2.30, 230.71±1.78, 252.19±2.32 and 94.07±2.25 respectively.

Keywords: *Rumex acetosella* roots, phenolics, urease, alpha-amylase inhibitory activities.

INTRODUCTION

Urease is an important enzyme that converts urea into ammonia. Its activity has been shown to have link with a number of clinical conditions like development of gastrointestinal ulcers, kidney stones and pyelonephritis. In gastrointestinal track, urease is excreted by bacterium *helicobacter pylori* as a mechanism to survive in the acidic environment of the stomach. The enzyme converts urea into ammonia which neutralizes the acid around the microorganism. It has been estimated that more than 80% of humanity in the developing nations and overall more than 50% worldwide suffer from *H. pylori* infection (Logan and Walker, 2001). By inhibiting urease activity, the infection by *H. pylori* can, thus, be avoided. Urease inhibitors are therefore considered to be potential anti-ulcer drugs (Hassani *et al*., 2009; Arfan *et al*., 2010). Another possible application of urease inhibitors is in the field of agriculture where urea fertilizers are converted by microbial urease into ammonia. Abnormally enhanced release of ammonia into atmosphere has adverse environmental and economic consequences which can be minimized by the use of suitable urease inhibitors (Giocchini *et al*., 2002; Harrison and Webb, 2001; Ledgard, 2001).

**Corresponding author:* e-mail: dildarahmed@fccollege.edu.pk

Alpha-amylase inhibitors can play a role in control of diabetes mellitus by controlling blood glucose level, or hyperglycemia. Although a number of types of diabetes have been identified, 90 percent of the diabetic patients are victims of diabetes type 2. While type 1 diabetes is the result of the inability of pancreas to produce insulin, the type 2 is the outcome of abnormal secretion of insulin or inability of body to use the insulin it produces (Jarald *et al*. 2008; Funke and Melzig, 2006). The patients of type 1 diabetes have to depend on exogenous insulin for survival. Type 2 diabetes appears to be one of the major human health concerns in the 21st century both in the developed as well as developing countries (Odhav *et al* 2010). According to WHO (2011) there were more than 171 million patients of diabetes world-wide in 2006, and the number will increase to 366 millions in 2030 if the trend goes unchecked. The enzyme alpha-amylase converts starch polymers into smaller which subsequently change into glucose (Hara and Hond, 1990). Inhibition of alpha-amylase has been shown to reduce the bio-availability of glucose (Payan, 2004; Matsui *et al*., 2001; Braodhurst *et al*., 2000). Based on these studies, it is postulated blood glucose level can be regulated by the use of alpha-amylase inhibitors (Kim *et al*., 2000; Ali *et al*., 2006). A number of synthetic alpha-amylase inhibitors, like acarbose, are available but their prolonged use may cause severe side effects (Fred-Jaiyesimi *et al.,* 2009).

Natural alpha-amylase inhibitors from plants are a better option to control postprandial hyperglycemia with no or very little side effects (Sudha *et al* 2011; McCue and Shetty, 2004; Kasote *et al*., 2011; al-Azzawie and al-Hamdani, 2006; Funke and Melzig, 2006).

Rumex acetosella (family Polygonaceae) is a herb found in many parts of the world (Hatfield, 2004). The plants of Rumex, a large genus containing about 250 species, are known for different pharmacological activities including antimicrobial, anti-inflammatory, anti-analgesic and antioxidant (Yadav *et al.,* 2010; Tavares *et al.*, 2010; Gautam *et al*., 2010). In folkloric medicine, *R. acetosella* is used for the treatment of tumors, cancers, and diseases related to liver and urinary/kidney functions (Kemper, 1999). Phytochemical investigations on the plant by a number of workers has yielded flavonoids, phenolic compounds and terpenoids (Yuan *et al.,* 2001; Shuying *et al*., 2011; Abd el-Fattah *et al*., 1994; Cui *et al*., 2005). Our previous work showed the plant leaves possessed notable antioxidant and free radical scavenging properties. The present work aimed to investigate urease and alpha-amylase inhibitory activities of the methanolic extract of the roots of *R. acetosella* and its sub-fractions in chloroform, ethyl acetate, n-butanol and residual water. The total phenolic contents of the methanolic extract and its fractions in different solvents were also determined to explore possible relationship between phenolics and anti-enzyme activities of the plant samples.

MATERIALS AND METHODS

Collection and preparation of plant material

Rumex acetosella plants were collected from the northern hill station Abbottabad, Pakistan, in June 2010. Roots along with the adjoining woody part of the herb were separated and kept in shade at room temperature to dry for 15 days before they were crushed and ground to obtain a fine powder. The powder (200 g) was extracted in 100% methanol (900 mL) at room temperature for 15 days. The extract was filtered and the solvent was evaporated under reduced pressure, using rotary evaporator, at 30°C to obtain crude methanolic extract (10.398 g). For fractionation, a suspension of the methanolic extract (8.0 g) was prepared in water (80 mL) and extraction was carried out with hexane, ethyl acetate, chloroform and n-butanol respectively. The hexane, ethyl acetate, chloroform, n-butanolic and residual aqueous fractions so obtained were dried under reduced pressure and weighed to calculate percent yields on the basis of dried methanolic extract.

Chemicals

Folin-Ciocalteu reagent was purchased from Sigma Aldrich while Gallic acid from Scharlau-Switzerland. Urease (Jack Bean) was purchase from Avonchem and alpha-amylase (*Aspergillus oryzae*) from Unichem and acarbose from Baeyer, Pakistan. All other chemicals used in the study were of analytical grade. UV-Visible Spectrophotometer UVD-3200 Labomed, Inc. was used to record absorbance.

Total phenolic contents

The total phenolic content was determined using the method reported by Slinkard *et al.* (1977). Plant samples were prepared by dissolving 3 mg of the MeOH extract or any of its fractions in 10 mL of methanol. In a glass cuvette, 40 µL of a plant sample or the standard (Gallic acid), 3.16 mL of distilled water and 200 µL of Folin-Ciocalteu reagent were mixed, and after an interval of 8 min, 600 µL of sodium carbonate (7%) solution was added. The glass cuvette was then incubated at 40°C for 30 min. Absorbance was recorded against a blank at 765 nm and the total phenolic contents present in different samples were expressed as micrograms per milliliter of Gallic Acid Equivalents (µg/mL of GAE). The blank consisted of 40 µL of methanol instead of plat sample.

Urease inhibitory assay

Urease inhibitory activities of the methanolic extract of *Rumex acetosella* roots and its sub-fractions in different solvents were determined according to the protocol reported by Ghous *et al.* (2010) based on the method proposed by Weatherburn (1967). After dissolving the plant sample (methanolic extract or any of its fractions) in phosphate buffer (25 mL, pH 7.0), it was stored at 4°C till further use. Then, different dilutions (0.1-20 µg/mL) of this solution were made to prepare test solutions of the extract. To a test solution (1 mL) of sample or standard taken in a test tube, 15 µL of urea (0.08 g/mL), 485 µL of phosphate buffer and 2.5 mL of Reagent 1(consisting of 5 mg/mL urease, sodium salicylate 60 mmol/L, EDTA 1 mmol/L sodium nitroprusside 5 mmol/L, and phosphate buffer 120 mmol/L) was added, and after incubating the mixture at 37°C for 5 min, 2.5 mL of Reagent 2 (consisting of NaOH 400 mmol/L, and NaOCl (sodium hypochlorite) 10 mmol/L and phosphate buffer 120 mmol/L) was added. After incubating the reaction mixture at 25°C for 10 min, absorbance was measured on UVD-3200 Labomed, Inc. UV-visible spectrophotometer at 625 nm against the blank. The blank contained 500 µL of phosphate buffer, and 2.5 mL of each of the Reagent 1 and 2. The Reagent 2 was added without prior incubation of the mixture. The control contained all the reagents except the sample. The percent inhibition was determined using the formula:

$$\% \text{ Urease Inhibition} = [(A_c - A_s)/A_c] \times 100$$

Here A_s is the absorbance of the sample under study while A_c is the absorbance of the control. Each experiment was repeated thrice and average was calculated. Thiourea was used as a positive control. The % Inhibition was plotted versus the concentration of a sample and a logarithmic regression curve was established to calculate the IC_{50}

Table 1: Procedure to determine urease inhibitory activities of methanolic extract of *Rumex acetosella* roots and its fractions

	Extract (mL)	Urea (µL)	Buffer (µL)	Reagent 1 (mL)	Incubation at 37°C (min)	Reagent 2 (mL)	Incubation at 25°C (min)
Test	1	15	485	2.5	5	2.5	10
Blank	1	-	500	2.5	-	2.5	-
Control	-	15	485	2.5	5	2.5	10

Table 2: Procedure to determine alpha-amylase inhibitory activities of methanolic extract of *Rumex acetosella* roots and its fractions

	Extract (mL)	DMSO (mL)	Enzyme (mL)	Pre-Incubation at 25°C	DNS (mL)	Starch (mL)	Incubation at 25°C	DNS (mL)	Heating at 85°C	Dilution with water (mL)
Test	1	-	1	30 min	--	1	3 min	1	15 min	9
Blank	1	-	1	30 min	1	1	3 min	-	15 min	9
Control	-	1	1	30 min	-	1	3 min	1	15 min	9

value for each sample which is the concentration of the given sample required to inhibit the activity of urease by 50 percent.

Alpha-amylase inhibitory assay

The alpha-Amylase inhibitory activities of the plant samples were carried out according to the method reported by Nickavar *et al*. (2006). The starch solution (0.5% w/v) used as the substrate was prepared by boiling potato starch in distilled water for 15 min. The enzyme solution was prepared by dissolving 1 mg of alpha-amylase in 20 mM phosphate buffer (100 mL, pH 6.9).

The sample solutions were prepared in DMSO (dimethyl sulfoxide) in different concentrations (0.1 to 3.6 mg/mL). The DNS solution (20 mL 96 mM 3,5-dinitrosalicylic acid, 12 g sodium potassium tartrate in 8 mL of 2 M NaOH and 12 mL deionized water) was used as the coloring reagent of reaction. Three sets of experiments were conducted: test, blank and control. A mixture of 1 mL of each of the test and enzyme solutions, in a test tube, was incubated at 25°C for 30 min. Then, after taking out 1 mL from this mixture, 1 mL of the abovementioned starch solution was added and the mixture was incubated at 25°C for 3 min. Finally, 1 mL of the DNS solution was added. The tube was then covered and heated in water bath at 85°C for 15 min. After cooling the tube, the reaction mixture was diluted with distilled water (9 mL). It was mixed well and the absorbance was recorded at 540 nm. In case of blank, the DNS solution was added prior to the addition of the starch solution, while rest of the method was same as for the test. For control, all procedure was again the same except that plant extract was replaced by 1 mL of DMSO. Acarbose, a well-known anti-diabetic medicine, was used as a positive control. The percentage inhibition was calculated by the formula: % Inhibition = $[(A_c – A_s) /A_c] \times 100$

Here A_s is the absorbance of the given sample while A_c the absorbance of control. The IC_{50} values for alpha-amylase inhibition were calculated by the same method used for urease inhibition.

STATISTICAL ANALYSIS

Each test was conducted three times to ensure reproducibility and the results were expressed as mean of three readings. One way ANOVA was applied and the results were correlated.

RESULTS

Total phenolic contents

The phenolic contents of MeOH extract of the roots of *R. acetosella* and its sub-fractions in different solvents were measured in terms of Gallic acid equivalents and the results are shown in table 3. The n-butanolic fraction showed the highest total phenolic content (252.19 µg/mL of GAE), while the chloroform fraction showed the lowest (34.44 µg/mL of GAE). Second highest phenolic content was in ethyl acetate fraction (230.71 µg/mL of GAE) followed by residual aqueous (94.07 µg/mL of GAE).

Table 3: Total phenolic content of methanolic extract of roots of *Rumex acetosella* and its fraction in various solvents expressed as µg of Gallic Acid Equivalents/mg of dry mass of the sample.

S.No.	Extract/Fraction	Phenolic content
1.	Crude Methanolic Extract	108.88 ± 2.65
2.	n-Hexane	43.70 ± 1.90
3.	Chloroform	34.44 ± 2.30
4.	Ethyl acetate	230.71 ± 1.78
5.	n-Butanol	252.19 ± 2.32
6.	Aqueous	94.07 ± 2.25

Urease inhibitory assay

Urease is a nickel-containing enzyme found in plants, fungi and bacteria. Urease inhibitory activity of the plant samples was determined by indophenol method based on Berthelot reaction, and the results are exhibited in Table 4. In this method, urease is allowed to catalyze the conversion of urea into carbon dioxide and ammonia. The ammonia thus produced is reacted with salicylate and sodium hypochlorite in the presence of sodium nitroprusside forming an indophenol product (λ_{max} is 625 nm). The reaction can be shown as follows:

$$H_2N\text{-}CO\text{-}NH_2 + H_2O \xrightarrow[\text{Urea}]{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{Salicylate} + NaOCl \xrightarrow[OH^-]{\text{Nitroprusside}} \text{Dicarboxy indophenol}$$

The intensity of the colour is a measure of the urease activity. An inhibitor retards urease activity and thus formation of indophenol is suppressed resulting in the decrease of absorbance.

Alpha-amylase inhibitory assay

The alpha-Amylase inhibitory activity of plant samples of *Rumex acetosella* was determined using the chromogenic method designed by Bernfeld (1955) with some modifications and the results are displayed in the Table 5. In this method alpha-amylase is allowed to hydrolyze starch and maltose so produced reacts with 3,5-dinitrosalicylate and the reaction is monitored spectrophotometrically at 540 nm.

DISCUSSION

The high content of phenolic compounds in polar solvents was due to the fact that phenolics contain polar OH groups. It is known that the phenolic compounds possess great potential to scavenge free radicals and show antioxidant activities (Miliauskas *et al*., 2004).

The methanolic extract of the roots of *Rumex acetosella* and its sub-fractions in different solvents exhibited remarkable urease inhibitory activity. In general, the activity was comparable to that of thiourea which was used as standard. Both the samples and standard showed inhibitory activity in a concentration dependent manner. As the Table 4 shows, the IC_{50} values of residual aqueous and n-butanolic fractions, 0.85 and 0.91 respectively were lower than that of thiourea (0.97), which was almost equal to that of methanolic extract (0.99).

The polar fractions having higher total phenolic contents

Table 4: Dose dependent urease inhibitory activities and IC_{50} values of extract/fractions of *Rumex acetosella* roots in different solvent against thiourea standard

S. No.	Concen. (µg/mL)	%Inhibition					
		MeOH	$CHCl_3$	EtOAc	n-BuOH	H_2O	Thiourea
1	0.1	02.34±0.98	____	00.96±2.33	03.45±1.75	04.01±0.67	03.87±3.56
2	0.20	13.93±0.22	06.82±0.99	10.21±1.14	15.34±0.33	18.65±0.14	16.45±1.84
3	0.40	24.56±0.45	14.34±0.56	22.56±0.32	26.12±1.55	27.06±0.56	28.68±1.39
4	0.60	33.11±1.29	23.61±1.76	31.90±0.48	32.34±2.98	39.65±0.09	39.83±1.76
5	0.80	41.76±2.20	29.45±1.48	38.74±0.29	45.44±1.79	48.07±0.41	46.09±1.05
6	1.00	52.23±0.88	37.90±1.33	45.04±0.75	56.98±1.86	55.19±1.77	51.04±2.54
7	2.00	62.46±0.25	44.73±0.59	52.85±0.28	60.41±0.17	63.37±1.09	55.21±1.53
8	4.00	66.26±1.04	51.41±0.23	59.30±0.23	64.03±0.79	70.78±0.18	59.45±0.26
9	6.00	70.56±0.22	56.66±0.38	63.92±0.31	70.27±0.40	73.50±1.68	64.23±0.33
10	8.00	75.32±1.28	60.62±0.25	69.05±0.36	75.09±0.46	77.73±0.14	70.23±1.14
11	10.00	81.89±1.44	64.38±0.65	72.86±0.69	79.13±0.10	82.93±0.74	74.98±1.45
12	12.00	85.26±0.62	67.50±0.08	75.39±0.52	82.80±0.05	84.58±0.79	79.67±2.76
13	14.00	89.08±0.17	71.58±0.37	77.72±0.13	86.48±0.37	88.72±1.78	83.61±0.67
14	16.00	92.26±1.06	73.61±0.27	80.71±0.46	89.29±0.40	93.88±0.71	89.50±0.45
15	18.00	94.61±0.21	78.83±0.39	83.39±0.30	91.77±0.07	95.11±0.46	92.13±0.66
16	20.00	96.35±0.13	81.29±0.13	87.74±0.27	93.39±0.75	97.36±0.13	95.03±3.94
IC_{50} (µg/mL)		0.99	3.89	1.76	0.91	0.85	0.97

Table 5: Dose dependent alpha-amylase inhibitory activities and IC_{50} values of extract/ fractions of *Rumex acetosella* roots in different solvents against acarbose standard

S. No.	Conc. (mg/mL)	%Inhibition					
		MeOH	$CHCl_3$	EtOAc	n-BuOH	H_2O	Acarbose
1	0.1	02.01±0.21	___	___	01.79±2.13	02.77±1.93	03.53±1.65
2	0.20	10.06±1.89	06.88±2.56	07.14±0.22	09.13±0.67	11.23±0.66	14.11±0.48
3	0.40	19.76±2.30	14.20±1.76	11.45±0.78	15.43±0.19	23.47±0.99	20.63±0.74
4	0.60	27.31±0.11	25.15±0.89	21.32±0.81	22.78±0.77	35.56±3.34	29.72±1.23
5	0.80	39.98±1.50	33.46±0.45	28.79±0.36	32.64±0.27	44.71±3.66	37.44±2.22
6	1.18	44.51±1.17	43.65±0.92	32.05±0.50	43.20±0.34	57.89±0.82	45.51±0.49
7	1.47	56.51±0.49	55.07±0.48	39.66±0.83	51.27±0.48	69.31±1.82	54.89±0.28
8	1.84	64.85±0.36	62.55±1.19	47.07±1.19	67.00±0.41	76.17±0.71	66.77±0.45
9	2.30	72.87±0.86	67.24±0.97	62.58±0.97	72.95±0.92	83.20±0.21	78.00±0.36
10	2.80	81.33±0.10	70.47±0.28	75.24±0.28	79.11±0.66	90.59±0.36	86.95±0.92
11	3.60	91.79±1.03	74.61±1.47	83.37±1.93	85.59±0.98	94.85±0.51	93.88±0.86
IC_{50} (mg/mL)		1.29	1.31	1.90	1.38	0.85	1.20

showed better urease inhibitory activity that may indicate a possible involvement of phenolic compounds in urease inhibition. This is in accordance with the findings of many other workers (Randhir and Shetty, 2007).

The methanolic extract of the roots of *Rumex acetosella* and its fractions in various solvents showed excellent alpha-amylase inhibitory activity that increased with the increase in concentration. The activity was comparable to that of acarbose used as standard. The IC_{50} value of MeOH extract was almost equal to that of acarbose while residual aqueous fraction was more potent with IC_{50} being 0.85.

It is notable that the n-butanol and aqueous fractions were most powerful against both urease and alpha-amylase indicating that more polar and water soluble natural products have higher potential to inhibit these enzymes. These findings confirm the results found by other works (Rohn *et al*., 2002; Funke and Melzig, 2005; Pulok *et al*., 2006).

CONCLUSION

The assays exhibited that the roots of *Rumex acetosella* have remarkable ability to inhibit enzymatic activities of urease and alpha-amylase. The polar fractions that were most effective against urease, were also most potent against alpha-amylase. These fractions had the highest total phenolic content as well. The data, thus, indicated a possible relationship between the phenolic compounds and inhibition of these enzymes as well as between urease and alpha-amylase activities. Chemical investigation of the polar fractions to isolate the actual chemical substances responsible for anti-enzymatic activity may possibly yield effective drugs for alpha-amylase and urease inhibition.

REFERENCES

Abd el-Fattah H, Gohar A, el-Dahmy S, and Hubaishi A (1994). Phytochemical investigation of *Rumex luminiastrum*. *Acta Pharm. Hung.,* **64**(3): 83-85.

Al-Azzawie H and Al-Hamdani MSS (2006). Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. *Life Sci*., **78**: 1371-377.

Ali H, Houghton PJ and Soumyanath A (2006). α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. *J. Ethnopharmacol.,* **107**: 449-455.

Ali SI and Qaiser M (1986). A phytogeographical analysis of the phanerogames of Pakistan and Kashmir. *Proc. R. Soc. Edinburg.,* **89B**: 89-101.

Anderson WP (1999). Red Sorrel (*Rumex acetosella*). *In*: Anderson, Wood Powell, Perennial Weeds: Characteristics and identification of selected herbaceous species. Iowa State University Press, Iowa, pp.170-171.

Anonymous (2011). Flora of North America, http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=242414256 (March 12, 2011).

Arfan M, Ali M, Ahmad H, Anis I, Khan A, Choudhary MI and Shah MR (2010). Urease inhibitors from *Hypericum oblongifolium* wall. *J. Enzym. Inhib. Med. Ch.,* **25**(2): 296–299.

Bernfeld P (1955). Amylases α and β. *Meth Enzymol*, **1**: 149-58.

Braodhurst CL, Polansky MM and Anderson RA (2000). Insulin-like activity of culinary and medicinal plant aqueous extracts *in vitro. J. Agric. Food Chem.,* **48**: 849-852.

Cui HH, Wang ZY, Wang ZQ, Li RM and Kang YH (2005). Study on the optimal harvesting time of *Rumex gmelini* by analyzing the contents of principal

components. *Zhongguo Zhong Yao Za Zhi*, **30**(11): 808-811.

Kasote DM, Bhalerao BM, Jagtap SD, Khyade MS and Deshmukh KK (2011). Antioxidant and alpha-amylase inhibitory activity of methanol extract of *colocasia esculenta* corn, *Pharmacologyonline*, **2**: 715-721.

El-Zaawely AA, Xuan TD and Tawata S (2005). Antioxidant and antibacterial activities of *Rumex japonicus* Houtt. Aerial parts. *Biol. Pharm. Bull.*, **28**(12): 2225-2230.

Fan JP and Zhang ZL (2009). Studies on the chemical constituents of *Rumex crispus*. *Zhong Yao Cai.* **32**(12): 1836-1840.

Fred-Jaiyesimi A, Kio A and Richard W (2009). α-Amylase inhibitory effect of 3b-olean-12-en-3-yl (9Z)-hexadec-9-enoate isolated from *Spondias mombin* leaf. *Food Chem.*, **116**: 285-288.

Funke I and Melzig MF (2006). Traditionally used plants in diabetes therapy - phytotherapeutics as inhibitors of á-amylase activity. *Rev. Bras. Farmacogn.*, **16**(1): 1-5.

Funke I and Melzig MF (2005). Effect of different phenolic compounds on a-amylase activity: screening by microplate-reader based kinetic assay. *Pharmazie*, **60**: 796-797.

Gautam R, Karkhile KV, Bhutani KK and Jachak SM (2010). Anti-inflammatory Cyclooxygenase (COX)-2, COX-1 Inhibitory, and Free Radical Scavenging Effects of *Rumex nepalensis*. *Planta Med.*, **76**(14): 1564-1569.

Ghous T, Akhtar K and Choudhry AM (2010). Screening of selected medicinal plants for urease inhibitory activity. *Biology and Medicine*, **2**(4): 64-69.

Giocchini P, Nastri A, Marzadori C, Antisari LV and Gessa C (2002). Influence of urease and nitrification inhiitors on N losses from soils fertilized with urea. *Biol. Fert. of Soils*, **36**: 129-135.

Hagerman AE, Ried KM, Jones GA, Sovik KN, Ritchard NT and Hartzfeld PW (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. *J. Agric. Food Chem.*, **46**: 1887-1892.

Hara Y and Hond M (1990). The inhibition of a-amylase by tea polyphenols. *Agric. Biol. Chem.*, **54**: 1939-1949.

Harrison R and Webb J (2001). A review of the effect of N fertilizers type on gaseous emissions. *Adv. Agron*., **73**: 65-108.

Hassani ARS, Ordouzadeh N, Ghaemi A, Amirmozafari N, Hamdi K and Nazari R (2009). *In vitro* inhibition of helicobacter pylori urease with non- and semi-fermented *Camellia sinensis*. *Indian J. Med. Microbiol.*, **27**(1): 30-34.

Hatfield G (2004). *Encyclopedia of Folk Medicine Old World and New World Traditions* (1st Ed.) ABC-CLIO, Santa Barbara, California, pp.120-121.

Holm LR, Doll J, Holm E, Pancho J and Herberger J (1997). *Rumex acetosella* L. (Polygonaceae: Buckwheat Family). World Weeds: Natural Histories and Distribution. John Wiley Sons, New York, pp.683, 685, 688, 691.

Jarald E, Joshi SB and Jain DC (2008). Diabetes and herbal medicines. *Iranian J. Pharmacol. Ther.*, **7**: 97-106.

Kemper KJ (1999). Sorrel, Longwood Herbal Task Force: ttp://www.mcp.edu/herbal/default.htm (April 2, 2011).

Kim JS, Kwon CS and Son KH (2000). Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. *Biosci. Biotechnol. Biochem.*, **64**(11): 2458-2461.

Ledgard SF (2001). Nitrogen cycling in low input legume-based agriculture, with emphasis on legume/grass pastures. *Plant and Soil*, **228**: 43-59.

Logan RP and Walker MM (2001). Clinical Review: ABC of the upper gastrointestinal track. Epidemiology and diagnosis of *Helicobakter pylori* infection. *Bacteriol. Med. J.*, **323**: 920-922.

Matsui T, Ueda T, Oki T, Sugita K, Terahara N and Matsumoto K (2001). A-glucosidase inhibitory action of Natural acylated anthocyanins, 1. Survey of natural pigment with potent inhibitory activity, *J. Agric. Food Chem.*, **49**: 1948-1954.

McCue PP and Shetty K (2004). Inhibitory effects of Rosmarinic acid extracts on porcine pancreatic amylase *in vitro*. *Asia Pacific J. Clin. Nutr.*, **13**(1): 101-106.

Miliauskas G, Venskutonis PR and Van-Beck TA (2004). Screening of radical scavenging of some medicinal and aromatic plant extracts. *Food Chem.*, **85**: 231-237.

Nickavar B and Yousefian N (2009). Inhibitory effects on six *Allium* species on α-amylase enzyme activity. *Iranian J. Pharm. Res.*, **8**(1): 53-57.

Odhav B, Thangaraj K, Nonhlanhla K and Himansu B (2010). Screening of African traditional vegetables for their alpha-amylase inhibitory effect. *J. Med. Plants Res.*, **4**(14): 1502-1507.

Payan F (2004) Structural basis for the inhibition of mammalian and insect alpha-amylases by plant protein inhibitors. *Biochemica et Biophysica Acta - Proteins & Proteomics*, **1697**: 171-180.

Pulok KM, Kuntal M, Kakali M and Peter JH (2006). Leads from Indian medicinal plants with *Hypoglycemic poternrtials. J. Ethnopharmacol.*, **106**: 1-28.

Randhir R and Shetty K (2007). Improved α-amylase and Helicobacter pylori inhibition by fenugreek extracts derived via solid-state bioconversion using Rhizopus oligosporus. Asia Pac. J. Clin. Nutr., 6(3): 382-392

Rohn S, Rawel HM and Kroll J (2002). Inhibitory effects of plant phenols on the activity of selected enzymes. J. Afric. Food Chem. 50: 3566-3571.

Shuying G, Bo F, Ruonan Z, Jiankang M and Wei W (2011). Preparative isolation of three anthraquinones from rumex japonicus by high-speed counter-current chromatography. *Molecules*, **16**(2): 1201-1210.

Slinkard K and Singleton VL (1977). Total phenol analysis: Automation and comparison with manual Methods. AM J ENOL VITICULT, **28**: 49-55.

Sudha P, Zinjarde SS, Bhargava SY and Kumar AR (2011). Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. *BMC Complement. Altern. Med.,* **11**: 5.

Tavares L, Carrilho D, Tyagi M, Barata D, Serra AT, Duarte CM, Duarte RO, Feliciano RP, Bronze MR, Chicau P, Espírito-Santo MD, Ferreira RB, dos Santos CN (2010). Antioxidant capacity of Macaronesian traditional medicinal plants. *Molecules,* **15**(4): 2576-2592.

Weatherburn MW (1967). Phenol hypochlorite reaction for determination of ammonia. *Anal. Chem.*, **39**: 971-974.

Wijayal CH, Rahminiwati M, Wu MC and Diana L (2011). Inhibition of α-Glucosidase and α-Amylase activities of some Indonesian herbs: *In*: Vitor Study, The 12th Asean Food Conference, 16-18 June 2011, Bitec Banger Bangkok, Thailand.

World Health Organization (2011). http://www.who.int/mediacentre/factsheets/fs312/en/index.html (Accessed January 11, 2012)

Yadav, Kumar S, Jain S, Pundir P, Jadon RK, Sharma S, Khetwal A, Gupta KS, Molan AL and Faraj AM (2010). The effects of condensed tannins extracted from different plant species on egg hatching and larval development of *Teladorsagia circumcincta* (Nematoda: Trichostrongylidae), *Folia Parasitol (Praha),* **57**(1): 62-68.

Yuan Y, Chen WS, Zheng SQ, Yang GJ, Zhang WD and Zhang HM (2001). Studies on chemical constituents in root of *Rumex patientia* L. *Zhongguo Zhong Yao Za Zhi.*, **26**(4): 256-258.