Send Orders for Reprints to reprints@benthamscience.ae

58 *Current Enzyme Inhibition,* 2014, *10,* 58-67

The Inhibitory Effect of Some Algerian Plants Phenolics Extracts on the α - Glucosidase and α - Amylase Activities and Their Antioxidant Activities

Ihcen Khacheba[1], Amar Djeridane[1], Abdelkarim Kameli[2] and Mohamed Yousfi[*,1]

[1]*Laboratoire des Sciences Fondamentales, Université Amar Telidji. Laghouat- Algérie*

[2]*Ecole Normale Supérieure - Kouba - Algeria*

Abstract: The aim of this study consisted in extracting and quantifying phenolic and flavonoïds compounds of five selected Algerian plants. The second step was devoted to studying the effects of phenolic compounds on the kinetics catalyzed by two enzymes belonging to the class of hydrolase (the α - amylase and the α - glucosidase) responsible for the digestion of sugars. Finally, we assessed the potential antiradical of our extracts.

The results indicate that the phenolic extracts from these plants have inhibitory effects on both enzymes, with K_i values in µg/ml range (18.19-72.14 µg / ml) for the α - amylase and (52.26 - 203.90 µg / ml) for α – glucosidase. The antioxidant activity test shows that our phenolic extracts exhibit good antioxidant capacity comparatively to antioxidants taken as reference with IC_{50} values which vary from 0.044 µg / ml to 0.452 µg / ml. This work contributes to understanding the role of natural polyphenols in the regulation of oxidative stress and normalization of glycemic disorders.

Keywords: α - Amylase, antioxidant activity, DPPH, α - glucosidase, inhibition effect, phenolic extracts.

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolisc disorder caused by an absolute or relative lack of resistance to insulin. It is characterized by hyperglycemia and accompanied by various chronic vascular complications [1-3]. About 171 millions people worldwide have diabetes, which is likely to be more than double by 2030 and around 3.2 million deaths every year are attributable to complication of diabetes; six deaths every minute [4, 5].

The best way to control postprandial plasma glucose level is with a medication in combination with dietary restriction and an exercise programme [6]. One of the therapeutic approaches for the decreasing of postprantal hyperglycemia is to retard absorption of glucose by the inhibition of carbohydrate – hydrolysing enzymes, for example amylase and glucosidase, in the digestive organs [6-8].

For this reason, postprandial hyperglycemia may be treated by amylase glucosidae inhibitors, for example, acarbose, voglibose and miglitol which are widely used but also reported to cause various side-effects [1, 8]. Therefore, safer natural amylase and glucosidase inhibitors have been reported from plant sources [1, 9-12].

Polyphenols are known to inhibit the activity of digestive enzymes such as amylase, glucosidase, pepsin, trypsin and lipases. Synergy between phenolics may play a role in mediating amylase inhibition and therefore have the potential to contribute to the management of diabetes mellitus [13, 14].

Polyphenols, flavonoïds in particular, may act as inhibitors of amylase and glucosidase (similar to acarbose, miglitol and voglibose) leading to a decrease in post – prandial hyperglycemia [15, 16]. It was suggested that increased reactive oxygen species levels are an important trigger for insulin resistance in numerous settings, and antioxidant therapy might be a useful strategy in prevention of DM [17-20].

Our study is in keeping with the general pattern of bringing our contribution to the development of the vegetable kingdom in the region of Laghouat as a source of natural bioactive substances. In this context, we are interested in five local plants; the choice of these plants is based on the fact that these latter are not known among herbalists in the treatment of diabetes, due to their use at a very small scale, and because no study has been so far undertaken for the development of these plants.

Therefore, in search of new natural inhibitors of amylase and glucosidase, the present work aims at studying the effect of phenolics extracts of the five selected plants have on the reaction of kinetics catalyzed by enzymes belonging to the class of hydrolases such as α - amylase and α – glucosidase, using an *in vitro* spectrophometric model, and to evaluate the antioxidant activity of these phenolic extracts in order to discover a relationship between the antioxidant and inhibitors activities.

2. MATERIALS AND METHODS

2.1. Materials

Five plants were evaluated in this study, namely *Agatophora alopecuroïde* (Chenopodiaceae), *Genista corsica* (Fabaceae), *Hammada elegans* (Chenopodiaceae), *Helianthemum kahiricum* (Cistaceae), *Salsola baryosma* (Chenopodiaceae). The plants were collected from «Sidi – Makhlouf » town, situated 40 Km North of the town of Laghouat in the steppe region of Algeria, in October 2008, March

*Address correspondence to these authors at the Laboratoire des Sciences Fondamentales, Université Amar Telidji. Laghouat- Algéria; Tel: + 0021329920066; Fax: + 002132992006; E-mails: yousfim8@gmail.com; ihcbio@yahoo.fr

1875-6662/14 $58.00+.00

2009, and October 2009, and were kept in dark at room temperature. The plants were identified with the contribution of the members of the laboratory of Fundamental Sciences, University of Laghouat and a voucher specimen of each plant was kept in the laboratory. All chemicals were purchased from Sigma (USA), Aldrich (Milwaukee, USA) and Fluka Chemie (Buchs, Switzerland). All the solvents used were of analytical grade.

2.2. Extraction of Phenolic Compounds

The air-dried aerial parts of each plant were finely powdered. Five grams of each powder were extracted for 24 h with 100 mL of 80/20 (v/v) hydro - alcoholic solvent (methanol/water) at room temperature.

The extract was filtered, then the residue was extracted for the second time with 100 ml of the same hydrau-alcoholic solvent for 24 h at room temperature. After removal of methanol under reduced pressure in a rotary evaporator at 40 °C, the remaining aqueous solution of the extraction was defatted twice with petroleum ether to remove lipids. Then, the aqueous fraction was extracted firstly with dichlomethan and secondly with ethyl acetate. The organic fractions were dried with anhydrous sodium sulphate, and then evaporated to dryness using a rotary evaporator. The dried residue was dissolved in 10 mL of methanol and kept at 4°C.

2.3. Determination of Total Phenolics Compound

The amount of total phenolics in the samples was determined with the Folin-Ciocalteu reagent using the method of Singleton and Ross (1965) [21]. The procedure is as follows: 100 µL of each sample was added to 500 µL of the aqueous solution of Folin-Ciocalteu reagent at 10%. After 2 min incubation at room temperature, 2 mL of 2% (w/v) sodium carbonate in water were added. Blanks were prepared by replacing the reagent by water to correct for interfering compound. After 30 min of incubation in the dark at room temperature, the absorbance of all samples was measured at 760 nm using the Shimadzu 1601 visible spectrophotometer. The gallic acid was used as a standard and all the assays were carried out at least in triplicate.

2.4. Quantification of Flavonoids Content

The flavonoids content in the extracts was determined spectrophotometrically according to Lamaison and Carnat (1991) [22], using a method based on the formation of the complex flavonoids-aluminium, having an absorption maximum at 409 nm. Rutin was used for the calibration curve. 1mL of diluted sample was mixed with 1mL of 2% aluminum chloride methanolic solution. After incubation at room temperature for 20 min, the absorbance of the reaction mixture was measured at 409 nm with a Shimadzu 1601 visible spectrophotometer and the flavonoids content is expressed in mg per g rutin equivalent (RE) of dry weight material.

2.5. Evaluation of Antioxidant Activity (DPPH Assay)

The free radical scavenging capacity of the phenolics extracts of our plants was determined using $DPPH^{\cdot}$ test. Radical scavenging activity of plant extracts against stable $DPPH^{\cdot}$ (2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically according to the method of Brand Williams *et al*. [23], by the slightly modification as described below.

The solution of $DPPH^{\cdot}$ in methanol (250 µM) was prepared daily before measurements. Various concentrations of 100 µL of sample solution diluted in methanol were added to 1mL of the $DPPH^{\cdot}$ radical solution. The mixture was then shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The decrease in absorption was measured at 517 nm using the same spectrophotometer. Absorption of a blank sample containing the same amount of methanol and $DPPH^{\cdot}$ solution was prepared and measured daily.

% Scavenging of the $DPPH^{\cdot}$ free radical was measured using the following equation:

% DPPH radical-scavenging = $[(A_0 - A_S) / A_0] \times 100$, and the antioxidant activity of the extract was expressed as an IC_{50} value defined as the concentration (in mM) of the extract that inhibited the formation of DPPH radicals by 50%. The DPPH radical scavenging activity obtained for each plant extract was compared with that of Trolox, vitamin C and BHA.

A_0 : Absorbance of control

A_s : Absorbance of test Sample

2.6. Assay for Fungal α - Amylase Inhibitory Activity

The fungal α - amylase inhibitory activity was determined according to a literature method [24], on its substrate starch using neocuproïne as a reagent. The method is based on the reducing power of the maltose product that reacts with a basic solution of glycine - copper (A solution) with blue color, developing a yellow-orange color in the presence of néocuproïne (B solution).

In brief, 200 µL of sodium phosphat buffer containing 6 mM NaCl, (pH = 6,9) was mixed with 100 µL of soluble starch (0,05%) as a substrate and 100 µL of suitable aliquots of our phenolic extracts whereas 100 µL of the buffer was used in the place of the plant extract for the blank sample. After thoroughly mixing, both sample and blank test tubes were pre – incubated at 37°C for 10 min, then the reaction was started by the addition of 100 µL of α – amylase from *Aspergillus oryzae* (13 units; one unit of enzyme activity is defined as the amount of enzyme required to release one micromole of maltose from strach per minute under assay conditions). After incubation at 37°C for 5 min, the reaction was stopped by adding 1 ml of A solution and 1 mL of B solution. The reaction mixture was incubated at 100°C for 10 min; after that, the tubes were cooled with tap water.

Enzyme activity was quantified by measuring optical density proportionally to the quantity of the maltose equivalents released from starch at 450 nm, and the inhibitory activity was calculated using the following formula:

Inhibitory activity (%) = $[(A_0 - A_s) / A_0] \times 100$

A_0 : Absorbance of control without inhibitor

A_s : Absorbance of test Sample with inhibitor

2.7. Assay for Yeast α – glucosidase Inhibitory Activity

Yeast α – glucosidase inhibitory activity was determined by a method we developed us on its substrate sucrose, using iodine as an oxydant. The method is based on the oxidizability of the aldehyde group of glucose by iodine in the basic middle.

Each molecule of glucose released in the reaction mixture reacts with iodine excess causing a decrease in the concentration inducing a parallel decrease in the intensity of the brown color characteristic of iodine which is measured at 405 nm after acidification.

To study the inhibitory activity of our phenolic extracts on the α - glucosidase, 1 mL of the substrate sucrose prepared in phosphate buffer (pH = 7) is mixed with 100 µL of each phenolic extract diluted in the same phosphate buffer and then incubated for 10 min at 37°C. The reaction was started by adding 100 µL of the enzyme followed by an incubation period of 30 min at 37°C. The enzymatic reaction was stopped by adding 250 µL of NaOH, then 1 mL of the iodine solution was added. The reaction mixture was gently agitated and then incubated in the dark for 30 min; after that, 500 µL of HCl solution was added to the mixture. The absorbance was measured at 405 nm using the same spectrophotometer used previously against a white. In this method, a witness was required. The inhibitory activity was calculated using the following formula :

Inhibitory activity (%) = $[(A_0 – A_s) / A_0] \times 100$

A_0 : Absorbance of control without inhibitor

A_s : Absorbance of test Sample with inhibitor

3. RESULTS AND DISCUSSION

3.1. Total Phenolic Content

The plant polyphenols including flavonoids have proven beneficial to many diseases, so they are considered therapeutic as a preventive agent from several diseases including those associated with oxidative stress, such as cancer and cardio – vascular diseases [25], and have been extensively studied as natural powerful antioxidants that can replace synthetic antioxidants [13, 19, 26, 27]. In addition to their biological properties, they have the capacity to inhibit the activity of a number of digestive enzymes such as α-amylase, α - glucosidase, pepsin, trypsin and lipases, in particular flavonoids and their sweet derivatives. It is precisely this subject which was under extensive study [14-16].

The phenolic extracts of five plants were tested for their antioxidant status and inhibitory activity towards yeast α – glucosidase and fungal α – amylase. A number of spectrophotometric methods for quantification of phenolic compound in plant materials were developed. These tests were based on different principles and were used to determine the various structural groups present in phenolic compounds [28]. The phenolic content of each plant extract was estimated by the Folin–Ciocalteu procedure, and the amount of polyphenols in the plants was calculated from the calibration curve of gallic acid previously realized and expressed in milligrams equivalents gallic acid per gram of dry matter, while the quantification of flavonoids in our extracts was determined by complexation with trichloride aluminum from the calibration curve of rutin and expressed in milligrams of rutin equivalent per gram of dry matter (Table **1**).

The content of phenolic compounds (mg/g) in the methanolic extracts, varied between 0.27 and 3.02 mg/g. The highest amounts were found in the ethyl acetate fractions while the lowest amounts were found in dichloromethane fractions. The plant *Helianthemum kahiricum* showed the highest values in total phenols in the two dichloromethane and ethyl acetate fractions against the plant *Salsola baryosma* which showed the lowest value.

The amount of total phenolic compounds in all the tested plants is less than that in other studies on other plant species in the region of Laghouat [29], but near to another studies of Yousfi *et al*. [30]. This lowness among of our plants may be related to a poverty of our plant in polyphenols, and probably to favorable biotic conditions our plants grew in which did not stimulate the biosynthesis of these molecules. The content of flavonoïds (mg/g), in rutin equivalents varied

Table 1. Total amount of plant phenolics compound and flavonoids.

Name of Plant	Total Phenolics (mg GAE/g dw)[a]	Flavonoids Content (mg RE/g dw)[b]
Dichloromethane fraction		
Agatophora alopecuroïde	0.27 ± 0.03	N . D
Genista corsica	0.37 ± 0.06	N . D
Hammada elegans	0.96 ± 0.05	0.20 ± 0.07
Helianthemum kahiricum	1.00 ± 0.09	0.74 ± 0.03
Salsola baryosma	0.19 ± 0.02	N . D
Ethyl acetate fraction		
Agatophora alopecuroïde	1.71 ± 0.07	1.55 ± 0.52
Genista corsica	2.60 ± 0.31	2.56 ± 0.04
Hammada elegans	1.45 ± 0.14	1.08 ± 0.02
Helianthemum kahiricum	3.02 ± 0.15	1.75 ± 0.46
Salsola baryosma	1.08 ± 0.01	0.77 ± 0.02

[a] milligrams of gallic acid equivalent per gram of dry weight of plant.
[b] milligrams of rutin equivalent per gram of dry weight of plant.
N.D Non Detected.

from 0.20 to 2.56. The highest amounts of flavonoïds were found in ethyl acetate fractions of *Genista Corsica*, All the ethyl acetate fractions showed the presence of flavonoïd while two of the five plants studied showed the presence of flavonoïds in dichloromethane extracts which can be explained by the fact that the studied plants contained a polar class of flavonoïd; and for that reason the solvent dichloromethane, with a low polarity, limited their passage in the plants: *Agatophora alopecuroides, Genista Corsica* and *Salsola baryosma*.

3.2. Antioxidant Activity by the DPPH Method

Various studies on natural products have proven that there are particularly phenolic compounds which are responsible for their antioxidant activity [13, 19, 25]. Phenolic compounds are known as powerful chainbreaking antioxidants and are very important plant constituents because of their scavenging ability due to their hydroxyl groups. [30]. It was suggested that the increase levels of free radicals is an important trigger for insulin resistance [20]. Limit production of free radicals represents a therapeutic target for glycemic control [31].

In this context, we evaluated the antioxidant power of our phenolic extracts in the hope of finding the existence of a relationship between antioxidant activity and the inhibitory capacity against the α - amylase and α - glucosidase view that antioxidant therapy may be a useful strategy in the treatment of diabetes.

The evaluation of antioxidant activity of our phenolic extracts was conducted by an *in vitro* chemical test. In this test, we were interested in measuring the activity of the free radical scavenging by the antioxidant fractions of our phenolic extracts, using the stable radical 1,1-diphenyl-2- picrylhydrazyl (DPPH).

DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule and, because of its odd electron, the methanolic solution shows a strong absorption band at 517 nm, which decreases in the presence of free radical scavengers. This scavenging activity has been largely used as a quick and reliable parameter to assess the *in vitro* general antioxidant activity of plant extracts, especially that assigned to phenolic compounds i.e phenolic acids and flavonoids [26, 32].

In the current study, the scavenging activities of DPPH exerted by our phenolic extracts as well as that of Trolox, vitamin C and BHA (Butylated hydroxyanisole) used as synthetics antioxidants and taken as reference, were calculated from the linear % inhibition – concentration curves. The concentration of inhibitors was expressed in µg / mL.

The antioxidant efficacy of our extracts was determined by calculating the IC_{50} parameter which represents the inhibitor concentration (antioxidant) needed to achive 50% reduction of free radicals. The results are summarized in (Table **2**).

The IC_{50} values of the phenolic extracts of our plants generally vary from 0.044 µg/ml to 0.452 µg/ml. The highest value was recorded for the extract ethyl acetate of *Salsola baryosma* plant, where asthe lowest value was recorded for ethyl acetate extract of the plant *Agatophora alopecuroïde.*

All the plant extracts showed a beneficial effect against free-radical damage compared to the standards antioxidant, Trolox, Vitamin C and BHA.

The ethyl acetate extract of the plant *Agatophora alopecuroïde* is a powerful antioxidant where as the ethyl acetate extract of the plant *Salsola baryosma* is a weak antioxidant.

We note that the dichloromethane extracts were less active against the DPPH ˙compared to the ethyl acetate extracts. Two hypotheses can be put forward to explain their lesser effect: either that these extracts do not contain enough polyphenolic compounds due to the little polar character of

Table 2. Free radical (DPPH) scavenging activity of the 5 plant extracts.

Name of Plant	IC_{50} (µg/ml)
Dichloromethane fraction	
Agatophora alopecuroïde	0.15 ± 0.00
Genista corsica	0.11 ± 0.02
Hammada elegans	0.32 ± 0.04
Helianthemum kahiricum	0.20 ± 0.01
Salsola baryosma	0.27 ± 0.04
Ethyl acetate fraction	
Agatophora alopecuroïde	0.04 ± 0.00
Genista corsica	0.20 ± 0.01
Hammada elegans	0.10 ± 0.00
Helianthemum kahiricum	0.10 ± 0.00
Salsola baryosma	0.45 ± 0.01
Antioxidant standards	
BHA	9.00 ± 0.02
Trolox	10.93 ± 0.33
Vitamine C	9.73 ± 0.30

the dichloromethane, or that the polyphenols do not correspond to antioxydants with interesting properties.

The ethyl acetate fractions of *Agatophora alopecuroïde, Hammada elegans* and *Helianthemum kahiricum* were the best inhibitors and their inhibitory effects were almost 10-fold more potent than those of Trolox, Vitamin C and BHA which is classified as a potent standard antioxidant [33-38]. Dichloromethane fractions of *Agatophora alopecuroïde, Helianthemum kahiricum* and *Salsola baryosma* had significant potency and were 5-fold more active than the standard. Other extracts had a weaker activity 2 and 3 - fold than that of Trolox vitamin C and BHA.

We note that the increase in the content of phenolic compounds is proportional to the antioxidant activity in acetate and dichloromethane extracts except for two plants: *Agatophora alopecuroïde* and *Hammada elegans* for the ethyl acetate extracts and *Hammada elegans* and *Helianthemum kahiricum* for the dichloromethane extracts, while the increasing rates flavonoids is inversely proportional to the antioxidant activity extracted two plants *Agatophora alopecuroïde* and *Salsola baryosma*.

This result shows clearly that the plants containing high levels of polyphenols have the strongest antioxidant power, and those which have a high rate in flavonoids have the lowest antioxidant powers. This implies that there is no influence of the concentration of flavonoids on the antioxidant activity, but the type of molecules does act on free radicals. The activity of flavonoids to scavenge radicals depends mainly on their structure. It is known that only flavonoids of a certain structure and particularly the hydroxyl and double bond positions in the molecule determine antioxidant properties; in general, these properties depend on the ability to donate hydrogen, or an electron to a free radical [39].

We might also note that the ethyl acetate phenolic extract of plant *Helianthemum kahiricum* has a near activity to that of *Hammada elegans* plant although the percentage of flavonoids in the latter is higher than in *Helianthemum kahiricum.* On the other hand, ethyl acetate extracts of *Genista corsica, Agatophora alopecuroïde, Salsola baryosma and Hammada elegans* plants have the same percentage of flavonoids, but they have completely different IC_{50} values. From this, we can conclude that the antioxidant capacity of these plants could mainly be due to the percentage and / or the presence of certain molecules potentially active of flavonoids in the phenolic extracts.

A detailed examination of the phenolic composition of the plant extracts is required for a comprehensive assessment of the individual compound exhibiting antioxidant activity.

The results of this study show that these plants can be used as an easily accessible source of natural antioxidants, either as a possible food supplement, or in the pharmaceutical industry.

However, the components responsible for the antioxidant activity of these plants are currently not known, and further work should be performed on their isolation and identification.

3.3. Inhibition of Fungal α - amylase and Yeast α - glucosidase

In order to find a natural inhibitor against the fungal α - amylase and yeast α - glucosidase, we studied *in vitro* the effects of our phenolic extracts on the activities of the two enzymes, at varying concentrations of extracts and substrates, to identify plants with inhibitory abilities on the enzymes.

The enzymatic activities of α - amylase and α - glucosidase were titrated using starch as a substrate for α - amylase which releases maltose, and sucrose substrate for α - glucosidase which releases glucose with a spectrophotometer detection after reaction with complexing agents.

The two enzymes show Michaelis kinetics. The kinetic parameters of both enzyme (V_{Max}, k_{cat} and K_M) were calculated using a Lineweaver - Burk plot [$1/v = f(1/S)$] and the results are given in Table **3**.

To identify plants with inhibitory capacities, we subjected our phenolic extracts to inhibition assay at the same concentration of extracts. These tests showed that all phenolic extracts had a significant inhibition on both enzymes α - amylase and α - glucosidase.

To investigate the type of enzyme inhibition and to determine the inhibition constants (K_i) for each extract, the α - amylase and the α - glucosidase, activities were assayed in the presence of different concentrations of the substrates (0.4 - 1.11 g/l) for α - amylase , (5 - 30 mM) for α - glucosidase and different concentrations of polyphenol extracts (88 - 258 µg/ml). The K_i value (18.19 - 203.90 µg/ml), was obtained from a Lineweaver - burk plots (Fig. **1**, **2**, **3** and **4**) which showed that the inhibition by all the investigated plants was competitive, mixed and non- competitively mixed with low K_i values of the order of µg/ml. The results are summarized in (Table **4**).

In the Lineweaver – burk graphs, we studied the inhibitory effect of all phenolic fractions, except for the dichloromethane fraction of the plant *Agatophora alopecuroide* which presented a very low rate inhibition of 2.64% on α - glucosidase and which prompted us to eliminate the study of its inhibitory effect.

Table 3. Kinetic parameters of the α - amylase and the α - glucosidase.

Parameters	α - Amylase	α - Glucosidase
K_M	1.01 ± 0.2 g/l	10.93 ± 0.25 mM
V_{max} (µM/min)	24.17± 0.02	176.04 ± 0.01
k_{cat} (min^{-1})	0.12 ± 0.1	1.01 ± 0.15
k_{cat}/K_M	0.14 ± 0.5 l $min^{-1}g^{-1}$	0.092 ± 0.6 mM^{-1} min^{-1}
$1/k_{cat}$ (min)	8.53 ± 0.1	0.99 ± 0.15

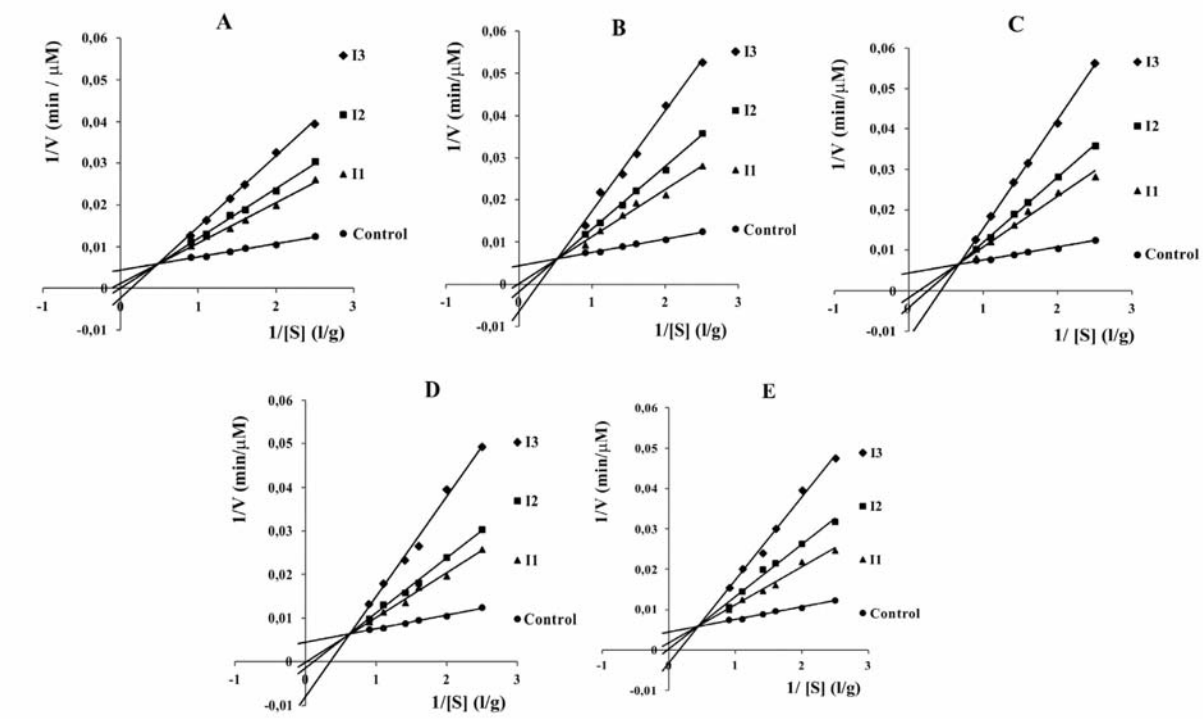

Fig. (1). Lineweaver - burk plots of inhibition of α - amylase according to the total phenol concentration of the ethyl acetat fraction plant of **A**: *Agatophora alopecuride*; **B**: *Gensita corsica*; **C**: *Hellanthemum kahiricum*; **D**: *Hammada elegans*; **E**: *Salsola baryosma*. Activity was determined by formation of maltose at several substrate concentrations ranging from 0.4 to 1.11 g/1. And three concentrations of inhibtor I1, 12 and 13.

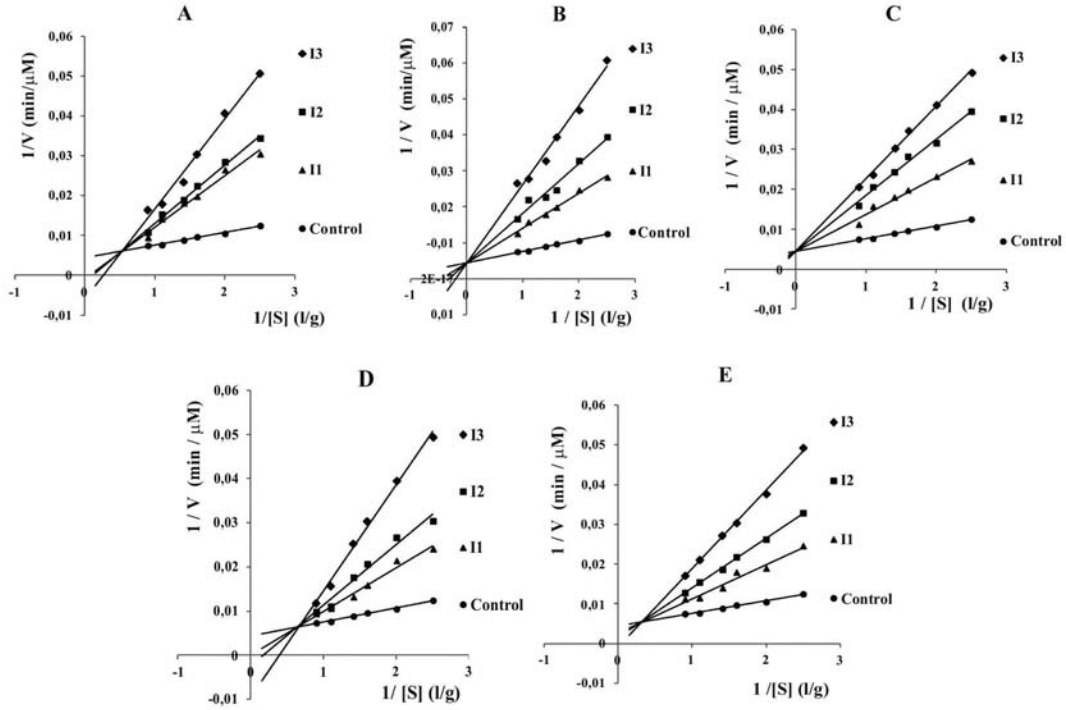

Fig. (2). Lineweaver - burk plots of inhibition of α - amylase according to the total phenol concentration of the dichloromethance fraction plant of **A**: *Agatophora alopecuride*; **B**: *Gensita corsica*; **C**: *Hellanthemum kahiricum*; **D**: *Hammada elegans*; **E**: *Salsola baryosma*. Activity was determined by formation of maltose at several substrate concentrations ranging from 0.4 to 1.11 g/1. And three concentrations of inhibtor I1, 12 and 13.

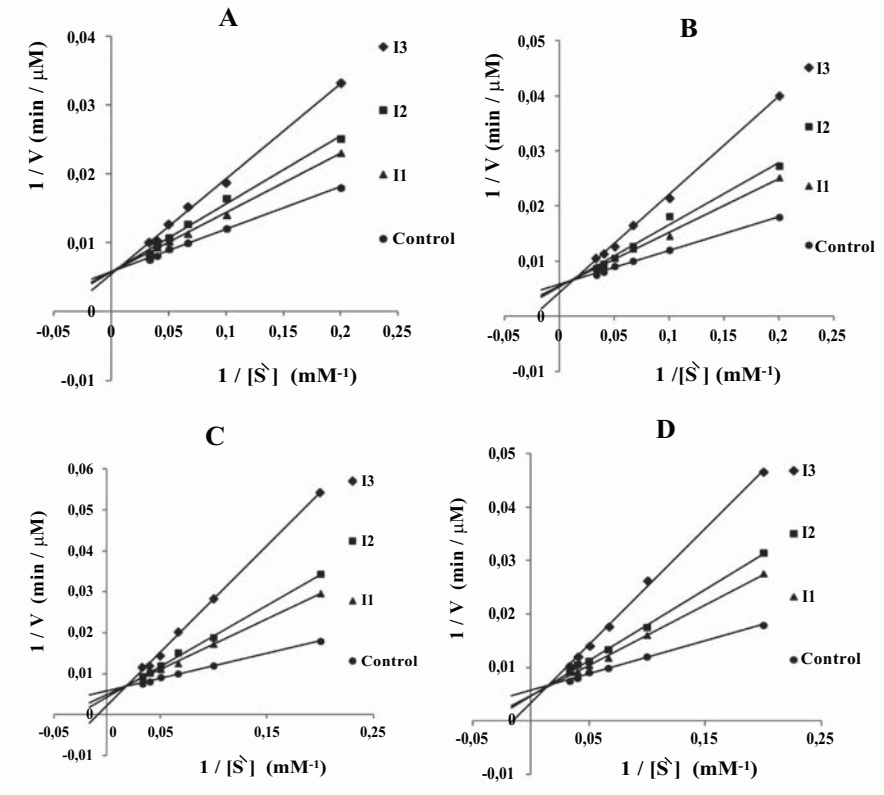

Fig. (3). Lineweaver - burk plots of inhibition of α - glucosidase according to the total phenol concentration of the dichloromethance fraction plant of **A**: *Agatophora alopecuride*; **B**: *Gensita corsica*; **C**: *Hellanthemum kahiricum*; **D**: *Hammada elegans*.

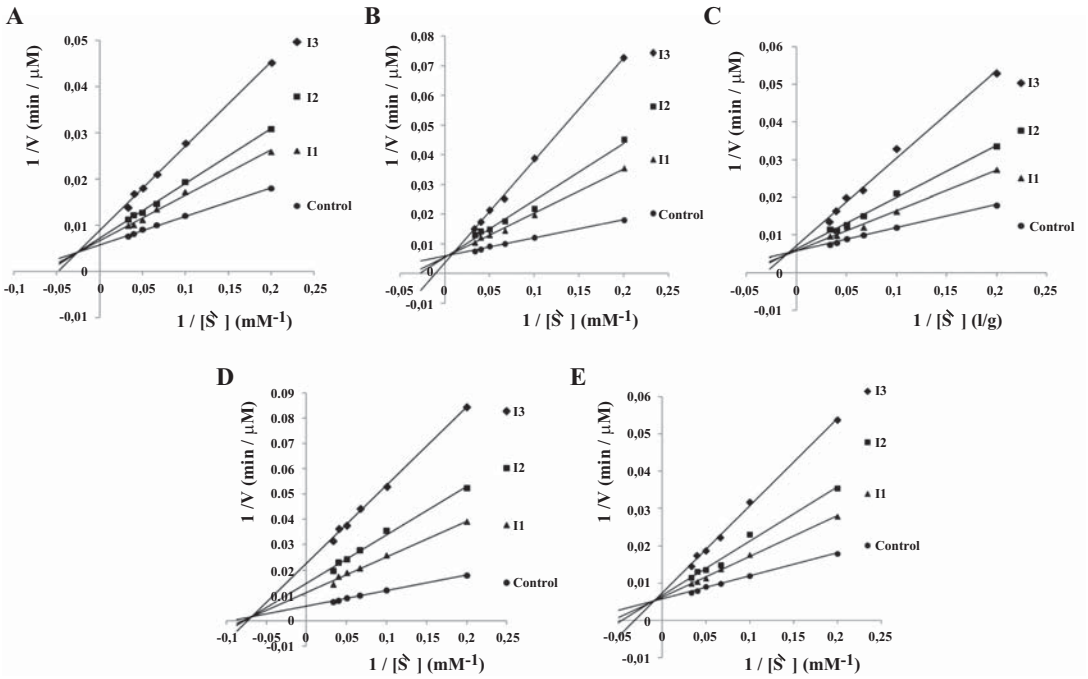

Fig. (4). Lineweaver - burk plots of inhibition of α - glucosidase according to the total phenol concentration of the ethyl acetate fraction plant of **A**: *Agatophora alopecuride*; **B**: *Gensita corsica*; **C**: *Hellanthemum kahiricum*; **D**: *Hammada elegans*; **E**: *Salsola baryosma*. Activity was determined by formation of glucose at several substrate concentrations ranging from 5 to 30 mM. And three concentrations of inhibtor I1, 12 and 13.

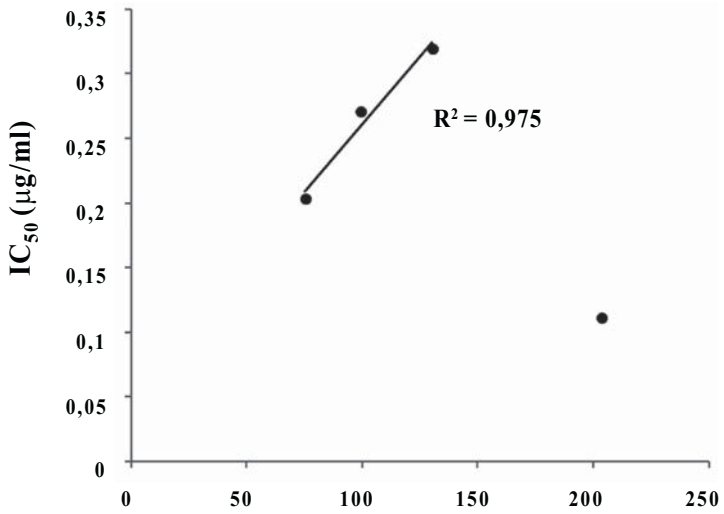

Fig. (5). Relationship between the inhibitor consantes K_i of α - glucosidase and the IC_{50} values of the 5 plants.

Table 4. K_i and Inhibitor Type for Total Polyphenol Extracts Obtained for α - amylase and α - glucosidase.

Name of Plant	K_i (µg/ml)	Type of inhibition
Dichloromethane fraction / α - amylase		
Agatophora alopecuroïde	72.14	Mixed
Genista corsica	46.67	Mixed
Hammada elegans	39.34	Mixed
Helianthemum kahiricum	29.70	Mixed
Salsola baryosma	48.23	Mixed
Ethyl acetate fraction / α - amylase		
Agatophora alopecuroïde	57.42	Mixed
Genista corsica	42.96	Competitive
Hammada elegans	43.44	Competitive
Helianthemum kahiricum	18.19	Mixed
Salsola baryosma	50.75	Mixed
Dichloromethane fraction / α - glucosidase		
Agatophora alopecuroïde	Not calculated	Not determined
Genista corsica	203.90	Competitive
Hammada elegans	130.08	Mixed
Helianthemum kahiricum	75.30	Mixted
Salsola baryosma	99.32	Mixted
Ethyl acetate fraction / α - glucosidase		
Agatophora alopecuroïde	125.52	Non compétitive mixed
Genista corsica	52.26	Mixed
Hammada elegans	66.00	Non -competitive mixed
Helianthemum kahiricum	82.72	Non - competitive mixed
Salsola baryosma	87.06	Non - competitive mixed

The best inhibitors were the phenolic extracts of the ethyl acetate fraction. The best value was recorded for the ethyl acetate fraction of *Helianthemum kahiricum* on α - amylase with a K_i value of 18. 19 µg/ml and the dichloromethane fraction of *Genista corsica* was least potent with a K_i value of 203.90 µg/ml in α - glucosidase.

All the plants showed different values of inhibition constants with the majority being close to each other and all above 30 µg/ml. This can be explained by the fact that the two fractions dichloromethane and ethyl acetate may have phenolic molecules with close chemical structure and were able to react differently against the two enzymes, and as the inhibition phenomenon is the result of a synergy between several molecules [20], these values could be lower if the molecules responsible for inhibition in the two solvents of extraction were together in the same reaction medium.

On the other hand, by comparing the values of inhibition constants obtained after the effects of two fractions on the two enzymes, it was found that those recorded for the α - amylase were less than those of α - glucosidase indicating that the compounds constituting our sample had more affinity for the α - amylase as α - glucosidase.

The inhibitory activity was proportional to the concentration of phenolic compounds.

Also, it may be thought that the inhibitory potency of the plant extracts is not limited to the phenolic content but may be due to the presence of some individual active phenolic compounds.

Thus the inhibitory potency of an extract cannot be explained just on the basis of its phenolic content but, also requires its proper characterization. There are several reasons to explain the ambiguous relationship between inhibitory potency and total phenolics: total phenolics content did not include all the possible inhibitors; the synergism among the inhibitors in the mixture accounted for the inhibition; however, it was not only dependent on the concentration of individual inhibitors, but on the structure and interaction among them as well.

In the hope to finding a positive correlation between the inhibitory activities of α - amylase, α - glucosidase and antioxidant activities, we plotted the variation of the inhibition constants K_i and the IC_{50} values in all the plants studied.

Positive correlations were found in almost all the extracts with a correlation coefficient of $R^2 > 0.90$. All fractions showed a variation of IC_{50} inversely proportional to that of K_i values reflecting a group of plants with the same trends in antioxidant, and inhibitory activity may be due to the same mode of action of phenolic compounds used in the tests, or to the presence of the same class of substances in each plant with different strengths. But the curve of the variation of IC_{50} and the K_i values in the dichloromethane extracts of α - glucosidase (Fig. **5**) showed that for a significant antioxidant activity, there is an increase of the inhibitory activity.

This result informs that the inhibitory activity of α - glucosidase in this case depends on two important factors: the content of phenolic compounds in each plant and the structure of individual phenols responsible for this activity.

These results reveal that the inhibitory activity against the two enzymes (α - amylase and α - glucosidase) depends on a significant factor which is the content of phenolic compounds and flavonoids in each plant.

In this study, it is difficult to be conclusive about the most active class of phenolic compounds, and the observed effect of *Helianthemum kahiricum* extract is probably due to the direct effect of some individual phenolic compounds.

CONCLUSION

Herbal medicine has been used for many years by different cultures around the world for the treatment of diabetes. Many natural resources have been investigated with respect to the suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine.

This study investigated the effect of phenolic extracts from five local plants gathered around the town of Laghouat in the steppe region of Algeria, focusing on the inhibitory effects on α - glucosidase and α - amylase.

The results obtained through this test show that the majority of these plants have significant inhibitory effects on both enzymes. The values of the constants (K_i), thus obtained, indicate that these plants can be investigated in Pharmacotherapeutic, including the ethyl acetate fraction of "*Helianthemum kahiricum"* which presented the lowest values of inhibition constant.

Our study is the first report on potential inhibition of these plants extracts of digestive enzymes, α - glucosidase and α - amylase. In conclusion, the results from this study give scientific support to the use of these plants in traditional medicine for the treatment of diabetes. This study would be helpful to explain the pharmacological mechanism and also to develop medicinal preparations, nutraceutical or functional foods for diabetes and related symptoms.

Further isolation of the bioactive compounds responsible for the inhibition of the two enzymes must be done to elucidate their molecular structure and to study their mechanism of action to confirm their antidiabetic activity. In addition, more experiments must be carried out *in vivo* to pave the way to the development of new agents for the treatment of diabetes and its complications.

This work provided new knowledge ethnopharmacological and phytochemicals about local plants in the region of Laghouat, and helped to highlight the role of natural compounds in the regulation of oxidative stress and normalization of blood sugar disorders.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict on interest.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Feng, J.; Yang, X.W.; Liu, T.H. Bio-assay guided isolation and identifictaion of α - glucosidase inhibitors from the leaves of *Aquilaria sineensis*. *Phytochemistry,* **2011**, *72*, 242-247.

[2] Gholamhoseinian, A.; Fallah, H.; Sharifi-far, F.; Mirtajaddini, M. The inhibitory effect some Iranian plants extracts on the Alpha - glucosidase. *Basic Med. Sci.,* **2008**, *11*, 1-9.

[3] Nickavar, B.; Mosazadeh, G. Influence of three Morus species extracts on α - amylase activity. *Iran. J. Pharmaceut. Res.,* **2009**, *8*(2), 115-119.

[4] Shruti, S.; Sandesh, S.; Sung-Yum, S. Chaenomeles Sinensis : A Potent α-and β - glucosidase Inhibitor. *Am. J. Pharmacol. Toxicol.,* **2009**, *4*(1), 8-11.

[5] Deutschländer, M.S.; van de Venter, M.; Roux, S.; Louw, J.; Lall, N. Hypoglycaemic activity of four plant extracts traditionally used in South Africa for diabetes. *Ethnopharmacology,* **2009**, *124*, 619-624.

[6] Kim, Y.-M.; Wang, M.-H.; Rhee, H.-I. A novel α - glucosidase inhibitor from pine bark. *Carbohydr. Res.,* **2004**, *339*, 715-717.

[7] Hasenah, A.; Houghton, P.J.; Sounyanath, A. α - amylase inhibitory activity of some Malaysian plants used to treat diabetes ; with

particular reference to *Phyllanthus amarus*. *Ethnopharmacology,* **2006**, *107*, 449-455.

[8] Shobana, S.; Sreerama, Y.N.; Malleshi, N.G. Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: Mode of inhibition of α - glucosidase and pancreatic amylase. *J. Food Chem.,* **2009**, *115*, 1268-1273.

[9] Le Berre–Anton, V.; Bompard-Gilles, C.; Payan, F.; Rougé, P. Characterization and functional properties of the α - amylase inhibitor _ α-AI/ from kidney bean _ Phaseolus Íulgaris/ seeds. *J. Biochim. et Biophys. Acta*, **1997**, *1343*, 31-40.

[10] Nagaraj, R. H.; Pattabiraman, T. N. Purification and properties of an α - amylase inhibitor specific for human pancreatic amylase from proso (*Panicium miliaceum*) seeds. *J. Biosci.,* **1985**, *7*, 257-268.

[11] Santimone, M.; Koukiekolo, R.; Moreau, Y.; Le Berre, V.; Rougé, P.; Marchis-Mouren, G.; Desseaux, V. Porcine pancreatic α - amylase inhibition by the kidney bean (Phaseolus vulgaris) inhibitor (α-AI1) and structural changes in the α - amylase inhibitor complex. *Biochim. et Biophys. Acta,* **2003**, *1696*, 181-190.

[12] Shinde, J.; Taldone, T.; Barletta, M.; Kunaparaju, N.; Hu, B.; Kumar, S.; Placido, J.; Zito, S. W. α - glucosidase inhibitory activity of *Syzygium cumini* (Linn.) Skeels seed kernel *in vitro* and in Goto-Kakizaki (GK) rats. *Carbohydr. Res.,* **2008**, *343*, 1278-1281.

[13] Apostolidis, E.; Kwon, Y. I.; Shetty, K. Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type II diabetes and hypertension. *Innovative Food Sci. Emerg. Technol.,* **2007**, *8*, 46-54.

[14] Chethan, S.; Sreerama, Y.N.; Malleshi, N.G. Mode of inhibition of finger millet malt amylases by the millet phenolics. *Food Chem*., **2008**, *111*, 187-191.

[15] Andrade – Cetto, A.; Becerra-Jiménez, J.; Càrdenas-Vàzquez, R. Alpha - glucosidase - inhibiting activity of some Mexican plants used in the treatment of type II diabetes. *Ethnopharmacology*, **2007**, *116*, 27-32.

[16] Kalidas, S.P.; McCue, P. Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase *in vitro*. *Asia Pacific J. Clin. Nut*r., **2004**, *13*(1), 101-106.

[17] Changer, S.M.; Kuttan, R. Antidiabetic activity of *Aegle marmelos* and its relationship with its antioxidant properties. *Indian J. Physiol. Pharmacol.,* **2004**, *48*(1), 81-88.

[18] Changer, S.M.; Kuttan, R. Antidiabetic and antioxydant activity of *Terminalia belerica. Indian J. Exp. Biol.,* **2009**, *47*, 270-275.

[19] Ma, C.M.; Sato, N.; Li, X.Y.; Nakamura, N.; Hattori, M. Flavan-3-ol contents, anti-oxidative and α - glucosidase inhibitory activities of *Cynomorium songaricum*. *Food Chem*., **2010**, *118*, 116-119.

[20] Ranga, R,R.; Tiwari, A.K.; Reddy, P.P.; Babu, S.K.; Madhusudana, A.K.; Madhusudana, R.J. New furanoflavanoids, intestinal α - glucosidase inhibitory and free-radical (DPPH) scavenging, activity from antihyperglycemic root extract of *Derris indica* (Lam.). *Bioorg. Med. Chem.,* **2009**, *17*, 5170-5175.

[21] Giner – Chavez, B.I. Condensed tannins in tropical forages. These Ph. D, Cornell University, Ithaca, USA., **1996**.

[22] Bahorun, T. Substances naturelles actives : La flore mauricienne, une source d'approvisionnement potentielle. *Food Agric. Res*., **1997**, 83-94.

[23] Brand-williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensmittelwissenschaft Technologie*., **1995**, *28*, 25-30.

[24] Dygert, S.; Li, L.H.; Florid, D.; Thoma, J.A. Determination of reducing sugar with improved precision. *Anal. Chem*., **1965**, *13*, 367-374.

[25] Nickavar, B.; Alinaghi, A.; Kamalinejad, M. Evaluation of the antioxidant properties of five Mentha species. *Iran. J. Pharmaceut. Res.,* **2008**, *7*(3), 203-209.

[26] Shahin, S.A.; Naresh, K.; Abhinav, L.; Angad, S.; Hallihosur, S.; Abhishek, S. Utpal Bora. Indian medicinal herbs as sources of antioxidants. *Food Res. Int*., **2008**, *41*, 1-15.

[27] Xiuzhen, H.; Tao, S.; Hongxiang, L. Dietary Polyphenols and Their Biological Significance. *Int. J. Mol. Sci.,* **2007**, *8*, 950-98.

[28] Naczk, M.; Shahidi, F. Extraction and analysis of phenolics in food. *J. Chromatogr. A.,* **2004**, *1054*, 95-111.

[29] Djeridane, A.; Yousfi, M.; Brunel, J.M.; Vidal, N.; Ajandouz, E. H.; Stocker, P. Inhibition of porcine liver carboxylesterase by a new flavone glucoside isolated from *Deverra scoparia*. *Chem.-Biol Interact.,* **2008**, *172*(1), 22-26.

[30] Djeridane, A.; Yousfi, M.; Nadjemi, B.; Maamri, S.; Djireb, F.; Stocker, P. Phenolic extracts from various Algerian plants as strong inhibitors of porcine liver carboxylesterase. *Enzyme Inhibit. Med. Chem.,* **2006**, *21*, 719-26.

[31] Pournaras, C. Pathologies vasculaires oculaires. *Elsevier Masson.,* **2008**, 183-181.

[32] Raquibul, S.M.H.; Mokarram, Md. H.; Raushanara, A.; Mariam, J.; Ehsanul, Md. H.M.; Rahman, S. DPPH free radical scavenging activity of some Bangladeshi medicinal plants. *Med*. *Plants Res*., **2009**, *3*(11), 875-879.

[33] Liang – Liang, Z.; Yi- Ming, L.; Hai-Chao, Z.; Shu-Dong, W.; Jia-Hong, C. Condensed tannins from Mangrove Species *Kandelia candel* and *Rhizophora mangle* and their antioxidant activity. *Molecules*, **2010**, *15*, 420-431.

[34] Limmy, T. P.; Narendhirakannanr, R. T. *In - vitro* antioxidant studies on ethanolic extracts of leaf, stem and root of *Sida rhombifolia* L. *Int. J. Pharm. Biol. Sci.,* **2010**, *1*(2), 1-10.

[35] Priyarani, M.; Venkatesan, J.; Binilraj, S.S.; Sasidharan, I.; Admakmriamma, K.P. Antioxidant and cytotoxic potential of acetone and methanolic extracts of frech and dray barks of *Cinnamomum zeylanicumverum*: *In-Vitro* study. *Cell Tissue Res.,* **2010**, *10*(1), 2131-2138.

[36] Randhavane, P.V.; Narwade, S.K.; George, S.; Karale, B.K. Synthesis and biological screening of some halogenated thiadiazoles and triazoles. *Indian J. Chem*., **2010**, *49*, 89-95.

[37] Reddy, K.Y.; Saravana, K.A.; Mohana, L.S.; Surendar, A. Antioxidant properties of methanolic extract of *Oxalis corniculata*. *Int. J. Phytopharmacol.,* **2010**, *1*, 43-46.

[38] Sasikumar, J.M.; Gincy, M.M.; Teepica, D.P.D. Comparative studies on antioxidant activity of methanol extract and flavonoid fraction of *Nyctanthes arbortristis* leaves. *Electronic J. Environ. Agric. Food Chem.,* **2010**, *9*(1), 227-233.

[39] Marfak, A. Radiolyse gamma des flavonoïdes : étude de leur réactivité avec les radicaux issus des alcools : formation de depsides. *Thèse de doctorat de l'université de Limoges.,* **2003**, 30-40.

[40] Adediwura, F.J.; Abo, K.; Wilkins, R. α - Amylase inhibitory effect of 3b-olean-12-en-3-yl (9Z)-hexadec-9-enoate isolated from *Spondias mombin* leaf. *Food Chem*., **2009**, *116*, 285-288.

Received: January 20, 2013 **Revised: May 06, 2013** **Accepted: May 07, 2013**