Research Article

Inhibitory Effect of *Eruca sativa* Mill. on Carbohydrate Metabolizing Enzymes *in vitro*

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ABSTRACT

The aim of this research is to investigate *in vitro* the biological effect of two extracts (water and ethanol) of *Eruca sativa* Mill**.** leaves (edible part) on carbohydrate hydrolyzing enzyme activities (α-amylase, α–glucosidase and β-galactosidase). Results showed that both extracts exhibited inhibitory effect on the enzymes in a linear relationship with the concentration of inhibitors (dose-dependent), accompanied with insignificant change, to some extent, in a low dose. Ethanol extract showed more potent reducing power than water as inhibited α-amylase by 32.00±1.21, 36.35±2.69, 55.22±4.92, 69.38±8.22 and 78.40±7.29% at concentrations of inhibitor 10, 50, 100, 500 and 1000µg/ml respectively, and α–glucosidase by 28.05±2.63, 37.80 ± 2.55, 44.21±2.87, 49.19±1.59 and 62.50±2.24% as well as β-galactosidase by 51.35±5.24, 56.62±6.53, 64.49±11.82, 67.82 ±3.94 and 66.86 ±3.79% as compared to the standard antidiabetic Acorbase.

Keywords: *Eruca sativa* Mill., α–amylase, α– glucosidase, β-galactosidase, anti-diabetic

INTRODUCTION

Diabetes mellitus is a debilitating and often life-threatening disease with increasing incidence in rural populations throughout the world. A scientific investigation of traditional herbal remedies for diabetes may provide valuable leads for the development of alternative drugs and therapeutic strategies. Alternatives are clearly needed because of the inability of current therapies to control all of the pathological aspects of diabetes, and the high cost and poor availability of current therapies for many rural populations, particularly in developing countries. This review provides information on more than 1200 species of plants reported to have been used to treat diabetes[1].

Eruca sativa Mill. (Synonym: Rocket, Rucola, Taramira, Water cress), belonging to Cruciferae or Brassicaceae family, has gained greater importance in recent years, as a vegetable and spice, especially among Europeans. Investigation of the aqueous extract of its leaves revealed the presence of flavonoidal compounds[2]. The structurally unique glucosinolates were identified in the leaves [3-6]. *E. sativa* Mill. leaves (edible part of the plant) are considered as important potential cancer preventive agents[2,7], it also proved to be an excellent source as antioxidant [8,9]. Sulforaphane, which is one of the most potent indirect antioxidant, is isolated until the date from *Eruca* and it is considered as potent antigenotoxic factors in rocket[10].

The seed oil of Rocket ameliorated hyperglycemia and oxidative stress. The effect of leaves (edible part of the plant) on diabetes was not studied, which encourage the authors to test the inhibitory effect against carbohydrate metabolic enzymes *in vitro* to assess the potential development of nutraceuticals from *E. sativa* Mill. for the treatment of diabetes

MATERIALS AND METHODS

Plant material

Leaves of *Eruca sativa* Mill. (Fig.1) were collected from Beni-Suef Gogernorate, September, 2012. The plant was kindly authenticated by a specialist from Botany Department in Faculty of Sciences, Beni-Suef University, Egypt. A voucher specimen no. BUPD 33 is desposited in Pharmacognosy Department, Faculty of Pharmacy, Beni-Suef University, Egypt.

Figure 1: *Eurca sativa* Mill.

(http://mob120.photobucket.com/albums/o193/rusticat/FOLIA/arugula123.jpg)

Preparation of extracts

The fresh leaves (250 g) were extracted by cold maceration in a glass container with ethanol till exhaustion. Combined extracts were concentrated by

distillation of the solvent under reduced pressure. The residue left was stored in a dark container, in a refrigerator for the biological study.

Another amount of fresh leaves (250g) were extracted with distilled water (1 L), on cold, using a sonicator. The water was filtered and dried using a lyophillizer. The residue left was stored in a refrigerator, in a dark container till the biological study.

Chemicals

All the chemicals used were of analytical grade (Merck, Sigma and Aldrich), the kits from (Sigma Chemical Company (USA), Biosystems (Spain) and Biodiagnostic (Egypt), the carbohydrate metabolizing enzymes (purified enzymes); α–amylase, α-glucosidase and β-galactosidase (EC3.2.1.1, EC3.2.1.20 and EC3.2.1.23 respectively) were obtained from Sigma Chemical Company (NY), USA.

Determination of carbohydrate hydrolyzing enzymes (α–amylase, α-glucosidase and β-galactosidase)

The methods followed are described in our previous communication[11].

RESULTS

The inhibition percent of both water and ethanol extracts of *Eruca sativa* Mill. (Table 1), showed appreciable activities. The ethanol extract was more prominent in carbohydrate inhibitory activity at different concentrations when compared to the standard Acarbose. A dose–response relationship is found in the carbohydrate inhibitory activities; the activity increased as the concentration of extracts increased for each individual one. However, water and ethanol extracts were able to inhibit α- amylase to give significant inhibitory percent of 18.26±3.97, 24.55±4.03, 32.56± 2.07, 37.14±0.89 and 44.59±1.55%, for water extract at concentrations of inhibitor 10, 50, 100, 500 and 1000µg/ml respectively, while ethanol extract showed reducing power of 32.00 ± 1.21, 36.35 ± 2.69, 55.22 ± 4.92, 69.38±8.22 and 78.40±7.29%, respectively at the same concentrations of inhibitor as compared to Acarbose which is recorded significant reducing effect reached to 33.11±1.23, 39.13±3.22, 48.36±4.10, 56.56±1.69 and 72.34±2.69%, respectively at the same concentrations of inhibitors.

Table 1: Carbohydrates metabolizing enzymes inhibition percentage of *Eruca sativa* Mill. leaves

Groups	α-amylase			α-glucosidase			β- galactosidase		
Concentrations	Acarbose	Water extract	Ethanol extract	Acarbose	Water extract	Ethanol extract	Acarbose	Water extract	Ethanol extract
10 µg/mL	33.11±1.23^{a}	18.26±3.97^{a}	32.00±1.21^{a}	45.10±3.12^{a}	18.47±4.56^{a}	28.05±1.63^{a}	65.20±10.12^{a}	17.24±6.39^{a}	51.35±5.24^{a}
50 µg/mL	39.13± 3.22^{b}	24.55±4.03^{b}	36.35±2.69^{b}	78.30±8.10^{b}	25.49±1.76^{b}	37.80±2.55^{b}	76.90±12.11^{b}	32.35±8.72^{b}	56.62±6.53^{b}
100 µg/mL	48.36 ±4.10^{c}	32.56±2.07^{c}	55.22±4.92^{c}	85.30±10.80^{c}	33.55±3.08^{c}	44.21±2.87^{c}	80.33±13.80^{b}	33.53±11.38^{b}	64.49±11.82^{c}
500 µg/mL	56.56 ±1.69^{d}	37.14±0.89^{d}	69.38±8.22^{d}	90.20±5.98^{d}	39.01±1.78^{d}	49.19±1.59^{d}	88.23±5.98^{c}	42.13±2.44^{c}	67.82±3.94^{c}
1000 µg/mL	72.34 ±2.67^{e}	44.59±1.55^{e}	78.40±7.29 ^{e}	98.54±11.55^{c}	55.11±2.70^{e}	62.5±2.24^{e}	90.44±10.90^{c}	40.08±5.11^{c}	66.86±3.79^{c}
LSD 5%	5.88	5.18	5.80	5.90	5.93	5.80	6.1	5.93	5.80

Enzymes are expressed as %. Data are mean ±SD of 3 replicates. Statistical analysis is carried out using one way analysis of variance (ANOVA), combined with post hoc and Co-Stat computer program, where unshared superscript letters between groups are significant differences at *P* values <0.05.

The most reducing capacity is also considered for ethanol extract which is capable of significantly inhibition activity of α–glucosidase to 28.05±2.63, 37.80 ± 2.55, 44.21±2.87, 49.19±1.59 and 62.50±2.24%, respectively with various concentrations of inhibitor (10–1000µg/ml), and more or less did so in a linear concentration–dependent manner. The reducing power of *Eruca sativa* Mill. water extract which has been used as one of α–glucosidase capability indicators is shown to be came later ethanol extract and exhibits significant reducing activity of 18.47±4.56, 25.49±1.76, 33.55±3.08, 39.01±1.78 and 55.11±2.70%, respectively, as compared to Acarbose standard (45.10±3.12, 78.30±8.10, 85.30±10.80, 90.20±5.98 and 98.54± 11.55%, respectively).

The inhibition of β-galactosidase by water and ethanol extract of *Eruca sativa* Mill. appear to be also a dose dependent and provided additional support for the previous finding by having that ethanol exhibited the strongest reducing activity at various concentrations of inhibitor and they were found to have the highest significant reducing activities of 42.13±2.44 and 67.82±3.94%, respectively at concentration of inhibitor 500µg/ml. The extent of β-galactosidase inhibition activity appears with 1000µg/ml inhibitors to demonstrate significant reducing activity of 40.08± 5.11 and 66.86± 3.79%, respectively for water and ethanol extract as compared to Acorbase standard (65.20± 10.12, 76.90± 12.11, 80.33± 5.98, 88.23±5.98 and 90.44± 10.90%, respectively).

From the manipulated results, we can deduce that, significant increase in reducing activity with increase extract concentrations (linear-relationship).

DISCUSSION

Several natural sources have been investigated for the suppression of glucose by different mechanisms. One of the therapeutic approaches for treating diabetes is to decrease the post–prandial hyperglycemia. This is done by retarding the absorption of glucose through the inhibition of carbohydrate hydrolyzing enzymes α-

amylase, α-glucosidase and β–galactosidase in the digestive tract. Inhibition of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the post prandial plasma glucose rise[12]. The fact that α–glucosidase and α-amylase showed different inhibition kinetics seems to be due to structural differences related to the origin of the enzymes[13].

The expression of the carbohydrate inhibitory activities is thought to be concomitant with the development of reductions as *Eruca sativa* Mill. species is known to be free radical chain terminators and antioxidant[10]. Therefore, it was considered important to determine the reductive capacity of the plant extracts as these may indicate their potential as ant-diabetic effect. Results showed interesting and comparatively potent β-galactosidase inhibitory activity as may be potentially useful in control of obesity and diabetes. The biological activity of the *Eruca sativa* Mill., water and ethanol by inhibiting the carbohydrate metabolizing enzymes *in vitro*, are used as preliminary marker for the potency of the plant under study.

Eruca sativa Mill. extracts reduce the release of glucose from carbohydrates, resulting in a dose-related delay in, or reduction of, the post-prandial increase in blood glucose and triglycerides, diminshed prevalence of diabetic nephropathy, as well as increased insulin binding in muscle[14]. This may be explained on the basis that *Eruca sativa* Mill. contains a number of bioactive molecules as phenolic compounds, carotenoids, glucosinolates.

The anti-amylase inhibitory activity may be due to the ability of phenolic compounds to interact with and/or inhibit proteins enzymes[15]. The same authors[15] added that, phenolic substances that are able to form quinines (such as caffeic acid, chlorogenic acid, gallic acid, etc) are more reactive than those phenolics that cannot form quinines and suggested that semi-quinones formed may react with amino acid side chains and free thiol groups on the enzyme.

Several authors attributed the hypoglycaemic action of many anti- diabetic marine and plant organisms to the presence of antioxidants such as phenolic and flavonoids as well as through enzyme inhibitors 3-O-galloylepicatechin and 3-O-galloylcatechin as they inhibited the digestive enzyme α-glucosidase and may prevent the progressive impairment of pancreatic beta-cell function due to oxidative stress, thus reduce the occurrence of type 2 diabetes[11,16,17]. It was found that, some constituents in marine and antidiabetic plants are concerned with stimulation of glucose uptake by peripheral tissues and inhibition of endogenous glucose production which may be involved in hypoglycemic and hypolipidemic mechanisms such as phenolic acids, flavonoids (quercetin, campherol, rutin), tannins and polysaccharides[18]. So, the hypoglycemic action of many antidiabetic drugs may be due to insulin–mediated by mechanisms in common with antidiabetic gliclazide drug[17].

CONCLUSION

It could be concluded that, the extracts of *Eruca sativa* Mill. exhibited inhibitory effect on carbohydrate hydrolyzing enzymes (α-amylase, α-glucosidase and β-galactosidase) in linear relationships to some extent with concentrations of inhibitor (dose dependant) and ethanol extracts showed more inhibition activity than water.

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