



AkiNik

American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

A
J
E
O
N
P

American
Journal of
Essential
Oils and
Natural
Products

ISSN: 2321 9114

AJEONP 2013; 1 (1): 122-125

© 2013 AkiNik Publications

Received 13-8-2013

Accepted: 16-9-2013

In-vitro α -glucosidase inhibition and antioxidant activity of methanolic extract of *Centaurea calcitrapa* from Iraq

Raad A Kaskoos *

ABSTRACT

Centaurea calcitrapa (Family-Asteraceae), commonly known as 'Red star thistle', is a Turkish endemic species. The genus *Centaurea* comprises of about 500 species, many of which have been used as traditional medicines. The number of people in the world with diabetes has increased dramatically over the recent years. The treatment of type II diabetes is complicated by several risk factors inherent to the disease. Elevated postprandial hyperglycemia (PPHG) is one of the risk factors. One important approach for the treatment of type II diabetes mellitus is by decreasing the postprandial hyperglycemia. This is possible by inhibiting certain carbohydrate hydrolyzing enzyme like α -amylase and α -glucosidase. The objective of present study was to evaluate *in-vitro* antioxidant and α -glucosidase inhibitory activity of aerial parts of methanolic extract of *C. calcitrapa*. The extract showed strong α -glucosidase inhibition and antioxidant (DPPH scavenging) activity with IC_{50} 4.38 ± 0.31 mg/ml and 49.98 ± 3.78 μ g/ml, respectively. These results suggest the possible use of *C. calcitrapa* in the management of diabetes mellitus.

Keywords: *Centaurea calcitrapa*, Asteraceae, α -glucosidase, Antioxidant.

1. Introduction

Diabetes mellitus is a chronic disorder of metabolism caused by an absolute or relative lack of insulin. It is characterized by hyperglycemia (high blood sugar) in postprandial and/or fasting state, and its severe form is accompanied by ketosis and protein wasting [1]. It is also associated with a number of complications like retinopathy, neuropathy and peripheral vascular insufficiencies [2]. Glucose homeostasis is the key for the treating diabetes. The treatment of type II diabetes is complicated by several risk factors inherent to the disease. Elevated postprandial hyperglycemia (PPHG) is one of the risk factors [3]. PPHG is elevated by the action of α -glucosidase and α -amylase. Inhibition of these enzymes plays a major role in managing PPHG in diabetic patients. Inhibition of α -glucosidase enzyme activity leads to a reduction in disaccharide hydrolysis which has beneficial effects on glycemic index control in diabetic patients [4, 5].

Centaurea, a genus of about 500 species of herbaceous plants, is native to the Mediterranean region. It is widely used in Middle Eastern folk medicine [6, 7]. *C. calcitrapa*, *C. solstitialis* and *C. melitensis* reported to have hypoglycemic effects, and *C. calcitrapa*, *C. iberica* and *C. jacea* are reported to have antipyretic effects [8]. Aqueous extract of *C. aspera* shows hypoglycaemic effect in diabetic rats [9]. Antifungal activities were reported in constituents of *C. thessala* and *C. attica* [10], while chloroform extract from *C. musimomum* showed antiplasmodial effects [11]. Methanolic extract from *C. diffusa* demonstrated antibacterial activities [12] and similarly other species have been used for the treatment of various ailments. Keeping in view the fact that there are few reports of biochemical data available for *C. calcitrapa*, it was thought worthwhile to evaluate *in-vitro* antioxidant and α -glucosidase inhibitory activity of methanolic extract of its aerial parts.

Raad A Kaskoos

College of Pharmacy, Hawler Medical University, Erbil, Iraq

Correspondence:

Raad A Kaskoos

College of Pharmacy, Hawler Medical University, Erbil, Iraq

E-mail: raadkaskoos@gmail.com

2. Material and methods

2.1 Plant Material

The aerial parts of *Centaurea calcitrapa* were collected from the Shaqllawa, Iraq in the month of March-April, 2012. The sample was identified and a voucher number (PRL/2013/03) of the plant was kept for future reference.

2.2 Chemicals

Ascorbic acid and DPPH (2,2-diphenyl-1-picrylhydrazyl) were obtained from Sigma Chemicals Co., St. Louis, USA. α -Glucosidase and *p*-nitrophenyl- α -D-glucopyranoside (PNPG) was purchased from SRL, Bangalore, India. All other solvents and chemicals were of analytical grade.

2.3 Preparation of Methanolic Extract

The plant material was dried at temperature below 45 °C in shade and coarsely powdered in grinder. About 10 g of crude drug powder was weighed extracted in ultrasonic water bath at 50 °C for 30 min in methanol with a solid/solvent ratio of 1:10. Extract was concentrated in rotary evaporator (Hanshin, Korea) and dried in vacuum. The crude residue was reconstituted in methanol and filtered through 0.45 μ m membrane filter and stored at 4 °C in refrigerator till further study.

2.4 α -glucosidase Inhibitory Activity of Methanolic Extract of *Centaurea calcitrapa*

The α -glucosidase inhibitory activity was assessed by the standard method [13], with slight modifications. Briefly, a volume of 60 μ l of sample solution and 50 μ l of 0.1 M phosphate buffer (pH 6.8) containing α -glucosidase solution (0.2 U/ml) was incubated in 96 well plates at 37 °C for 20 min. After pre-incubation, 50 μ l of 5 mM *p*-nitrophenyl- α -D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at 37 °C for another 20 min. Then the reaction was stopped by adding 160 μ l of 0.2 M NaCO₃ into each well, and absorbance readings (A) were recorded at 405 nm by micro-plate reader and compared to a control which had 60 μ l of buffer solution in place of the extract. For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. The α -glucosidase inhibitory activity was expressed as inhibition % and was calculated as follows:

$$\% \text{ Inhibition} = \frac{A_{CO} - A_t}{A_{CO}} \times 100$$

Where,

A_{CO} is absorbance of the control and A_t is absorbance of the sample. The concentration of inhibitors required for inhibiting 50% of the α -glucosidase activity under the assay conditions was defined as the IC₅₀ value.

2.5 Antioxidant (DPPH scavenging) Activity of Methanolic Extract of *Centaurea calcitrapa*

The ability of a substance to scavenge DPPH free radicals was assessed by the standard method [14, 15], adopted with suitable modifications. The stock solution of methanolic extract was prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions 1000, 500, 250, 125, 62.5, 31.25, 15.62 and 7.81 μ g/ml

were prepared by serial dilution method. Diluted solutions (1 ml each) were mixed with 1 ml of methanolic solution of DPPH (1 mg/ml). After 30 min incubation in darkness at room temperature (25 °C), the absorbance was recorded at 517 nm. Control sample contained all the reagents except the plant extract. Percentage inhibition was calculated using equation given below:

$$\% \text{ Inhibition} = \frac{A_{CO} - A_t}{A_{CO}} \times 100$$

Where,

A_{CO} is absorbance of the control and A_t is absorbance of the samples.

IC₅₀ values were estimated from the % inhibition versus concentration plot using a non-linear regression algorithm.

3. Result and discussion

3.1 *In-vitro* α -glucosidase Inhibition Assay

The results of *in-vitro* α -glucosidase inhibitory study are summarized in Table 1. The methanolic extract of *C. calcitrapa* showed a concentration-dependent inhibition of enzyme. The highest concentration of 100 mg/ml tested showed a maximum inhibition of nearly 78.97 %. Figures 1, illustrate the inhibitory activity of methanolic extract of *C. calcitrapa* against α -glucosidase. Methanolic extract of *C. calcitrapa* seems to be less potent in α -glucosidase inhibitory potential compared to Acarbose. It may be that α -glucosidase is more sensitive towards Acarbose with the concentration required for 50% inhibition (IC₅₀) found to be 1.41 mg/ml. The IC₅₀ of methanolic extract of *C. calcitrapa* was found to be 4.38 mg/ml.

3.2 Antioxidant (DPPH scavenging) activity of *Centaurea calcitrapa*

The antioxidant activity of methanolic extract of *C. calcitrapa* was determined using a methanol solution of DPPH reagent. The antioxidant activity of methanolic extract of *C. calcitrapa* was expressed in terms of percentage of inhibition (%). Parallel to examination of the antioxidant activity of the extract, the values for standard ascorbic acid was obtained and compared with the antioxidant activity of methanolic extract of *C. calcitrapa*. The plot of % inhibition verses concentration given for ascorbic acid and extract is in Figure 2 was used to calculate IC₅₀ values. The examination of antioxidant activity of methanolic extract of *C. calcitrapa* showed concentration dependant response and varied from 6.34 \pm 0.98 to 85.58 \pm 1.11 % for 7.81 to 1000 μ g/ml, respectively. The IC₅₀ values of ascorbic acid and methanolic extract of *C. calcitrapa* were found to be 24.69 \pm 1.97 μ g/ml and 49.98 \pm 3.78 μ g/ml, respectively. The *C. calcitrapa* was also reported for their free radical scavenging activity (FRSA) in the DPPH screening assay for their *in-vitro* non-enzymatic inhibition of bovine brain lipid peroxidation and for their inhibition of xanthine oxidase (XO). In both antioxidant assays it shows strong antioxidant activity [16]. The *C. calcitrapa* can thus be regarded as promising candidate from natural plant sources of antioxidants with high value. The details of results were summarized in Table 2.

Table 1: α -Glucosidase inhibitory activity of methanolic extract of *Centaurea calcitrapa* compared to acarbose

| Acarbose Conc. (mg/ml) | % Inhibition | <i>C. calcitrapa</i> Conc. (mg/ml) | % Inhibition |
|--------------------------------------|-----------------------------------|------------------------------------|-----------------------------------|
| 10 | 87.05 \pm 0.27 | 100 | 78.97 \pm 2.11 |
| 5 | 81.82 \pm 0.48 | 50 | 73.46 \pm 1.24 |
| 2.5 | 63.82 \pm 0.97 | 25 | 64.74 \pm 1.98 |
| 1.25 | 51.24 \pm 0.48 | 12.5 | 54.73 \pm 0.97 |
| 0.625 | 35.44 \pm 0.32 | 6.25 | 46.83 \pm 1.38 |
| 0.3125 | 21.94 \pm 0.64 | 3.125 | 32.23 \pm 1.38 |
| 0.15625 | 16.81 \pm 0.68 | 1.56 | 13.68 \pm 1.15 |
| 0.07812 | 11.11 \pm 0.64 | 0.78 | 6.97 \pm 1.41 |
| IC₅₀ value (mg/ml) | 1.41 \pm 0.07 | - | 4.38 \pm 0.31 |

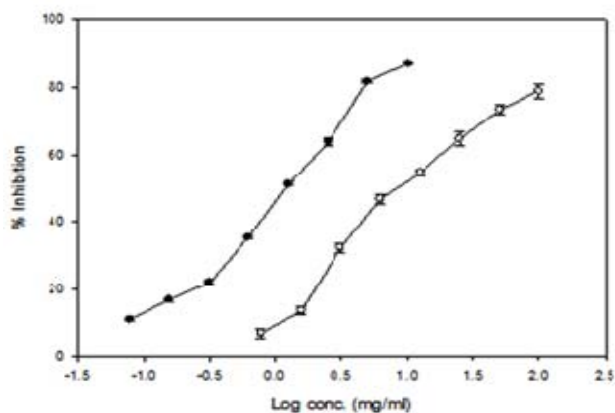


Fig 1: α -glucosidase inhibitory activity of methanolic extract of *Centaurea calcitrapa*

Table 2: Antioxidant (DPPH scavenging) activity of methanolic extract of *C. calcitrapa* compared to Vit C.

| Conc. (μ g/ml) | Vit. C | <i>C. calcitrapa</i> |
|---|------------------------------------|------------------------------------|
| 1000 | 93.45 \pm 0.46 | 85.58 \pm 1.11 |
| 500 | 92.74 \pm 0.77 | 83.33 \pm 0.98 |
| 250 | 88.44 \pm 0.47 | 77.09 \pm 3.27 |
| 125 | 84.15 \pm 1.68 | 64.33 \pm 0.77 |
| 62.5 | 70.55 \pm 0.92 | 45.60 \pm 1.24 |
| 31.25 | 53.68 \pm 1.53 | 32.92 \pm 1.08 |
| 15.62 | 43.14 \pm 1.24 | 21.67 \pm 1.23 |
| 7.81 | 26.68 \pm 0.92 | 6.34 \pm 0.98 |
| IC₅₀ value (μg/ml) | 24.69 \pm 1.97 | 49.98 \pm 3.78 |

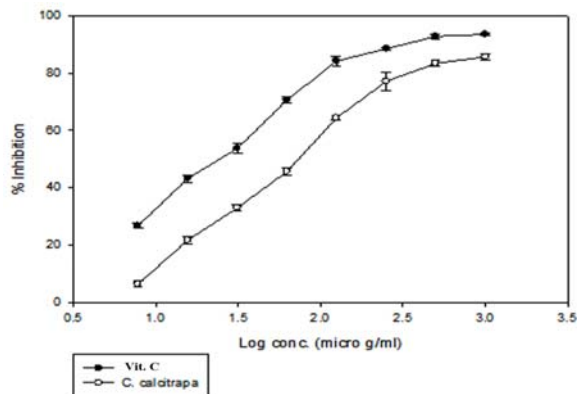


Fig 2: Antioxidant (DPPH scavenging) activity of methanolic extract of *C. calcitrapa*

4. Conclusion

The results of these *in-vitro* studies clearly indicated that aerial parts of methanolic extract of *C. calcitrapa* had strong α -glucosidase inhibitory (IC_{50} 4.38 mg/ml) and antioxidant activity (IC_{50} 49.98 μ g/ml). These attributes when combined in one plant are potentially useful to manage the glucose-induced hyperglycemia and related complications, and thus provide the biochemical rationale for further animal and clinical studies.

5. Reference:

- Bell GI. Molecular defects in Diabetes mellitus. *Diabetes* 1991; 40:413-417.
- Chehade JM, Mooradian AD. A rational approach to drug therapy of type 2 diabetes mellitus, disease management. *Drugs* 2000; 60(1):95-113.
- Gin H, Rigalleau V. Post-prandial hyperglycemia and Diabetes. *Diabet. Metabol.* 2000; 26:265-72.
- Notkins AL. Immunologic and genetic factors in type 1 diabetes. *J. Biol. Chem.* 2002; 277:4354-4358.
- Ahamad J, Naquvi KJ, Mir SR, Ali M, Shuaib M. Review on role of natural α -glucosidase inhibitors for management of diabetes mellitus. *Int. J. Biomed. Res.* 2011; 2(6):374-380.
- Salah S, Jager A. Screening of traditionally used Lebanese herbs for neurological activities. *J. Ethnopharmacol.* 2005; 97:145-149.
- Talhouk RS, Karam C, Fostok S, El-Jouni W, Barbour EK. Antiinflammatory bioactivities in plant extracts. *J. Med. Food.* 2007; 10:1-10.
- Kumarasamy Y, Middleton M, Reid R., Nahar L, Sarker S. Biological activity of serotonin conjugates from the seeds of *Centaurea nigra*. *Fitoterapia* 2003; 74:609-612.
- Masso JL, Adzet T. Hypoglycaemic activity of *Centaurea aspera* L. *Rev. Esp. Fisiol.* 1976; 32:313-316.
- Skaltsa H, Lazari D, Panagouleas C, Georgiadou E, Garcia B, Sokovic M. Sesquiterpene lactones from *Centaurea thessala* and *Centaurea attica* antifungal activity. *Phytochemistry* 2000; 55:903-908.
- Medjroubi K Sesquiterpene lactones from *Centaurea musimomum*, antiplasmodial and cytotoxic activities. *Fitoterapia* 2005; 76:744-746.
- Skliar MI, Toribio MS, Oriani DS. Antimicrobial activity of *Centaurea diffusa*. *Fitoterapia* 2005; 76:737-739.
- Dong HQ, Li M, Zhu F, Liu FL, Huang JB. Inhibitory potential of trilobatin from *Lithocarpus polystachyus* Rehd against α -glucosidase and α -amylase linked to type 2 diabetes. *Food Chemistry* 2012; 130:261-266.
- Hamad KJ, Ahmed Al-Shaheen SJ, Kaskoos RA, Ahamad J, Jameel M, Mir SR. Essential oil composition and antioxidant activity of *Lavandula angustifolia* from Iraq. *Int. Res. J. Pharm.* 2013; 4(4):117-120.
- Roby MHH, Sarhan MA, Selim KAH, Khalel KI. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. *Ind. Crops Prod.* 2013; 43:827-831.
- Pieroni A, Janiak V, Dürr CM, Lüdeke S, Trachsel E, Heinrich M. *In vitro* antioxidant activity of non-cultivated vegetables of ethnic Albanians in southern Italy. *Phytother Res.* 2002; 16(5):467-473.