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Short communication

Amylase inhibitory activity of some macrolichens in Mazandaran province, Iran

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Abstract

Introduction: α-amylase is a major form of amylase found in humans and other mammals. It is the special key enzyme involved in carbohydrates breakdown. Inhibition of this enzyme could be used in treatment of diabetes. In this study, the effect of ten Iranian macrolichens on alpha amylase were tested.

Methods: Different concentrations of the extracts (25, 50 and 75 mg/ml) were incubated with enzyme substrate solution and activities of enzyme were measured and acarbose was used as the positive control. Thin layer chromatography (TLC) *and* gradient-elution high performance liquid chromatography (HPLC) were *used* to *determine* the *phytochemical* compounds of the extracts.

Results: The extracts showed a dose dependent inhibitory effect on amylase as *Usnea articulata*> *Ramalina pollinaria*> *R. hyrcana* > *Cladonia rei*> *Flavoparmelia caperata*> *Parmotrema chinense*> *Punctelia subrudecta*> *P. borreri*> *Hyperphyscia adglutinata*> *Peltigera praetextata*. The highest inhibition of amylase was 60% at extract concentrationa 75 mg/ml in *U. articulata*. TLC and HPLC for this species proved the presence of the compounds as usnic acid, fumarprotocetraric acid and protocetraric acid.

Conclusion: This study showed that, macrolichens have inhibitory properties against α-amylase and determination of the type of enzyme inhibition by these macrolichen extracts could be provided by successful use of macrolichen chemicals as drug targets.

Keywords:
α -glucosidase;
Enzymatic reaction;
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Introduction

Diabetes mellitus is a serious endocrine disorder that affects the metabolism of carbohydrates and responsible for increased risk of diseases such as atherosclerosis, renal failure, blindness and diabetic cataract (Hemmati et al., 2010). Alpha amylase is a protein enzyme that involved in the breakdown of long chain carbohydrates. Alpha glucosidase breaks down starch and disaccharides to glucose. These two inhibitors (e.g. α-amylase and α-glucosidase enzymes) are the potential targets in the development of lead compounds for treatment of diabetes (Saxena and Vikram, 2004; Fujisawa et al., 2005; Thomson et al., 2007; Skamagas et al., 2008). For an unmemorable time, natural products from plants and lichens are used in the management of diabetes. More than 800 secondary compounds have been discovered from lichen-forming fungi and most

of these are unique. These secondary compounds have several possible biological roles including antibacterial, antifungal, anti-HIV, anticancer, anti-protozoan and etc. Literatures in amylase inhibitory activity of lichens, in particular, lichens of Iran, are scanty (Maulidiyah et al., 2015; Zhou et al., 2011; Kim et al., 2008; Thilagam et al., 2013).

The aim of this study therefore, is to provide insights regarding the amylase inhibitory property of ten macrolichens namely *Flavoparmelia caperata* (L.) Hale, *Parmotrema chinense* (Osbeck) Hale & Ahti, *Cladonia rei* Schaer., *Hyperphyscia adglutinata* (Flörke) H. Mayrhofer & Poelt, *Ramalina hyrcana* Sipman, *Ramalina pollinaria* (Westr.) Ach., *Punctelia borreri* (Sm.) Krog, *Punctelia subrudecta* (Nyl.) Krog, *Peltigera praetextata* (Flörke ex Sommerf.) *Zopf,* and *Usnea articulata* (L.) Hoffm. on α-amylase enzyme.

Materials and methods

Collection and identification of macrolichens

The macrolichens namely The macrolichens namely *Flavoparmelia caperata* (L.) Hale, *Parmotrema chinense* (Osbeck) Hale & Ahti, *Cladonia rei Schaer*., *Hyperphyscia adglutinata* (Flörke) H. Mayrhofer & Poelt, *Ramalina hyrcana* Sipman, *Ramalina pollinaria* (Westr.) Ach., *Punctelia borreri* (Sm.) Krog*, Punctelia subrudecta* (Nyl.) Krog, *Peltigera praetextata* (Flörke ex Sommerf.) Zopf, and *Usnea articulata* (L.) Hoffm. were collected from Zirab area (Mazandaran province), during February 2003. They were identified based on morphological, anatomical and color tests using standard keys. The vouchers were deposited in the herbarium of B (Botanic Garden and Botanical Museum Berlin-Dahlem).

Extraction of powdered macrolichen material

The dried macrolichens materials (50 g) were ground to fine powder and extracted by soxhlet apparatus using 80% methanol (250 ml) as solvent. The resulted extracts were filtered by using Whatman filter paper no.1 and concentrated at 40°C under reduced pressure. The condensed methanol extract was stored at 4°C until use. The extracts were dissolved in 5% dimethylsulfoxide (DMSO) for the experiments (Dias and Urban, 2009).

Detection of secondary metabolites by TLC and HPLC

Compounds were characterized by standardized thin layer chromatography (TLC) (Culberson 1972; Culberson and Johnson, 1982) and gradient-elution high performance liquid chromatography (HPLC) (Elix et al., 2003) methods. Thin layer chromatography in solvent A (180 ml toluene: 60 ml 1-4, dioxine: 8 ml acetic acid) was performed to detect secondary metabolites (Culberson and Johnson, 1982).

***In vitro* amylase inhibitory activity**

Amylase inhibitory activities of different concentrations (25, 50, 75 µl) were determined by method of Thilagam et al., 2013. The enzyme (0.5%) was prepared in phosphate buffer (pH 6.8). In short, 500 µl of different concentrations (2, 1, 0.5 mg/ml) of extracts and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing 0.5 mg/ml porcine pancreatic α-amylase solution were incubated at 36°C for 11 min. After preincubation, 200 µl of 1% starch solution in 0.1 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each test tube and the mixture was re-incubated for 1 h at 37 °C. The enzymatic reaction was stopped by adding 1 ml of DNS (3,5-dinitrosalicylic acid) color reagent. The same was performed for control (as acarbose) where extract was replaced by buffer. The test tubes were then incubated in a boiling water bath for 5 minutes and cooled down to the room temperature. To each tube, 10 ml of distilled water was added and the absorbance (A) of the mixture was taken at 540 nm. The percentage (%) inhibition was calculated using formula (Karunaratne et al., 2014; Giancarlo et al., 2006): %Inhibition = [A_{540} control - A_{540} extract /A_{540} control] x100

Statistical analysis

For the extracts and standard compound, three samples were prepared for each assay. The data was presented as mean ± standard deviation of three tests.

Results

The secondary metabolites detected in macrolichen materials are shown in Table 1. Usnic acid was detected in *U. articulata* and *R. hyrcana*. Evernic acid *was characterized in R. pollinaria* (Table 1).

α-amylase activity was determined at different initial

Table 1: Secondary metabolites detected by TLC and HPLC in studied macrolichens.

macrolichens	secondary metabolites			
Flavoparmelia caperata	protocetraric acid	caperatic acid	atranorin	
Parmotrema chinense	stictic acid	constictic acid	atranorin	
Cladonia rei	homosekikaic acid			
Hyperphyscia adglutinata	-	-	-	
Ramalina hyrcana	sekikaic acid	usnic acid		
Ramalina pollinaria	protocetraric acid	norstictic acid	salazinic acid	evernic acid
Punctelia borreri	lecanoric acid			
Punctelia subrudecta	lecanoric acid	atranorin	chloroatranorin	
Peltigera praetextata	-	-	-	
Usnea articulata	usnic acid	fumarprotocetraric acid	protocetraric acid	

Table 2: Inhibition of amylase by different concentrations of macrolichen extracts.

	amylase inhibitory activity (%)		
macrolichens	25*	50	75
Usnea articulata	35±0.12	50±0.01	60±0.02
Ramalina pollinaria	31±0.04	47±0.07	51±0.13
Ramalina hyrcana	30±0.01	38±0.02	42±0.07
Cladonia rei	29±0.02	32±0.12	35±0.02
Flavoparmelia caperata	21±0.08	29±0.03	32±0.05
Parmotrema chinense	25±0.04	28±0.13	31±0.02
Punctelia subrudecta	19±0.02	22±0.06	27±0.04
Punctelia borreri	14±0.07	18±0.06	23±0.01
Hyperphyscia adglutinata	13±0.01	15±0.06	17±0.04
Peltigera praetextata	10±0.09	13±0.08	14±0.07

*: different concentrations

substrate concentrations and the results are shown in the Table 2. The extract caused a dose dependent on inhibition of amylase activity. Among lichens, *U. articulata* caused higher inhibition of enzyme activity followed by *R. pollinaria*, *R. hyrcana*, *C. rei*, *F. caperata*, *P. chinense*, *P. subrudecta*, *P. borreri, H. adglutinata* and *P. praetextata* (Table 2).

Discussion

Hyperglycemia can be a serious problem in management of diabetes because in long term, acute and chronic complications can occur if blood glucose concentration is not kept in normal levels (Sudha et al., *2011*). *Type 2 diabetes* which formerly named

non-insulin-dependent on diabetes is the most prevalent form of diabetes accounting for 90% of cases throughout the world. One promising therapeutic approach to decrease the hyperglycemia, is to retard and reduce the digestion and absorption of ingested carbohydrates through the inhibition of carbohydrate hydrolyzing enzymes. One such drug is acarbose. It reduces postprandial hyperglycemia and is used to treat type 2 diabetes. It inhibits alpha-glucosidase enzymes in the brush border of the small intestines and pancreatic alpha-amylase. However, this and other related drugs are known to have gastrointestinal side effects such as abdominal pain, flatulence and diarrhea in the patients (Mohamed et al., 2012; Sudha et al., 2011; Verma et al., 2012). Therefore, it becomes necessary to identify the amylase inhibitors from natural sources having lesser side-effects. Natural α-amylase inhibitors of herbal origin especially lichen compounds are an attractive therapeutic approach to control post-prandial hyperglycemia via reducing the glucose release from starch and delaying carbohydrate absorption. These compounds are able to inhibit activity of the carbohydrate hydrolyzing enzymes in small intestine and potentially useful in control of diabetes. Most known lichen compounds with biological activities are phenolic metabolites (such as orcinol and β-orcinol), anthraquinones (such as endocrocin and parietin), dihydroxy dibenzofurans (such as usnic acid), depsides (such as anziaic, gyrophoric and diffractaic acids), depsidones (such as lobaric acid and salazinic acids), depsones (such as picrolichenic acid), γ-lactones (such as protolichesterinic, nephrosterinic and rocellaric acids) and pulvinic acid derivatives (such as calycin, epanorin and *pulvinic acid*). These substances exhibit a great diversity of biological effects including antimicrobial, anti-inflammatory, analgesic, antipyretic and antiproliferative and cytotoxic activities, and there has been a growing interest in the pharmaceutical properties of compounds derived from lichens (Rashmi et al., 2014; Basile et al., 2015; Goel et al., 2011; Řezanka et al., 2001).

Conclusion

This research supports the use of lichens in folklore medicine for the treatment of diabetes. The potent antidiabetic properties of the studied macrolichens are due to presence of compounds inhibiting α-amylase enzyme (like usnic acid, fumarprotocetraric acid and protocetraric acid in *Usnea articulata*). However, further investigations are needed to demonstrate the antidiabetic mechanism of usnic acid, fumarprotocetraric acid, protocetraric acid, norstictic acid, salazinic acid, evernic acid, sekikaic acid, homosekikaic acid, caperatic acid, atranorin, stictic acid, constictic acid, lecanoric acid and chloroatranorin.

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Conflict of interest

The authors declare that there are no conflicts of delineations.

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