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Research Article

Amylase Inhibitory Activity of Some Macrolichens of Western Ghats, Karnataka, India

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ABSTRACT

Diabetes mellitus is an endocrinal chronic disease caused by altered carbohydrate metabolism and characterized by elevated blood glucose levels. The present study was conducted to determine amylase inhibitory activity of methanol extract of six macrolichens namely *Everniastrum cirrhatum* (Fr.) Hale, *Usnea sinensis*, *Ramalina conduplicans* Vain, *Ramalina hossei*, *Parmotrema pseudotinctorum* (des. Abb.) Hale and *Parmotrema tinctorum* collected from Western Ghats, Karnataka. The lichens were collected, powdered and extracted using methanol. The inhibitory activity of extracts of the lichens was determined against fungal amylase. The extracts caused a dose dependent inhibition of amylase activity. Among lichens, *R. conduplicans* caused higher inhibition of enzyme activity followed by *R. hossei*, *P. tinctorum*, *U. sinensis*, *E. cirrhatum* and *P. pseudotinctorum*. The inhibitory efficacy of lichens could be related to the presence of secondary metabolites present. The lichens of Karnataka could be exploited in the development of agents active in regulating postprandial glucose level and hence the lichens could possibly used to manage diabetes.

INTRODUCTION

Lichen represents a stable and self supporting symbiotic association between a fungus (mycobiont) and a photoautotrophic algal partner (photobiont). The thalli of these lichens are known to contain a variety of secondary metabolites (called lichen substances) which possess a wide range of bioactivities such as antioxidant, antibiotic, antimycobacterial, antiviral, anti-inflammatory, antioxidative, analgesic, antipyretic, antiproliferative and cytotoxic effects [1]. Bhadra wildlife sanctuary is located in the Western Ghats of the south interior Karnataka with cool climate throughout the year. A few studies have been carried on the biological activities of macrolichens from Bhadra wildlife sanctuary. The macrolichens of Bhadra wildlife sanctuary are shown to exhibit antimicrobial, larvicidal, anthelmintic, antioxidant, pancreatic lipase inhibitory, cytotoxic activity [2-14]. Literatures on amylase inhibitory activity of lichens, in particular, lichens of Karnataka, are scanty.

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The present work is carried out to determine amylase inhibitory activity of extracts of six macrolichens namely *Everniastrum cirrhatum* (Fr.) Hale, *Usnea sinensis*, *Ramalina conduplicans*, *Ramalina hossei*, *Parmotrema pseudotinctorum* and *Parmotrema tinctorum* collected from Bhadra wildlife sanctuary, Karnataka, India.

MATERIALS AND METHODS

Collection and identification of Lichen material

The lichens namely *Everniastrum cirrhatum* (Fr.) Hale, *Usnea sinensis*, *Ramalina conduplicans*, *Ramalina hossei*, *Parmotrema pseudotinctorum* and *Parmotrema tinctorum* were collected from the forest area of Western ghats, Karnataka, during February 2011. The lichens were identified based on morphological, anatomical and color tests [15]. The shade dried powdered lichen material was extracted with methanol, spotted on the silica plate and developed with solvent A (180ml toluene: 60 ml 1-4, dioxine: 8ml acetic acid) to detect secondary metabolites using standard protocols [16, 17].

Extraction of powdered lichen material

The dried lichen materials were ground to fine powder and extracted by soxhlet apparatus using methanol as solvent. The extracts were filtered using Whatman filter paper no.1 and concentrated at 40°C under reduced pressure [14].

Screening amylase inhibitory activity of lichen extract

The inhibitory activity of different concentrations of extract of the lichens was determined against amylase (Diastase (Fungal) 3240, Lobachemie Laboratory reagents and fine chemicals, Mumbai) by following the method Karthik *et al.*[11]. The enzyme (0.5%) was prepared in phosphate buffer (pH 6.8). Briefly, 500µl of different concentrations of lichen extracts and 500µl of 0.1M phosphate buffer (pH 6.8) containing amylase were incubated at 25°C for 10 min. After preincubation, 500µl of a 1% starch solution in 0.1M phosphate buffer (pH 6.8) was added to each tube and further incubated at 25°C for 10 min. The reaction was stopped by addition of 1ml of dinitrosalicylic acid reagent. The same was performed for control where extract was replaced with buffer. The test tubes were placed in a boiling water bath for 10 min and cooled. To each tube, 10ml of distilled water was added and the absorbance (A) was measured at 540nm. The percentage (%) inhibition was calculated using formula:

% Inhibition = [A_{540}Control - A_{540} Extract / A_{540}Control] x100

RESULTS AND DISCUSSION

The secondary metabolites detected in the powdered lichen materials are shown in Table 1. Usnic acid was present in *R. hossei*, *R. conduplicans* and *U. sinensis*. Salazinic acid was present in *R. conduplicans* and *E. cirrhatum*. Sekikaic acid was detected in *R. conduplicans* and *E. cirrhatum*. Lecanoric acid and Protolichesterinic acid were detected in *P. pseudotinctorum* and *P. tinctorum* respectively.

The amylase inhibitory activity of the lichen extracts was determined against Diastase (amylase). The extract caused a dose dependent inhibition of amylase activity. Among lichens, *R. conduplicans* caused higher inhibition of enzyme activity followed by *R. hossei*, *P. tinctorum*, *U. sinensis*, *E. cirrhatum* and *P. pseudotinctorum* (Fig. 1).

Diabetes mellitus (DM), an endocrinal chronic disease is caused by altered carbohydrate metabolism and characterized by elevated blood glucose levels. There are two main types of diabetes, type I and type II. The most prevalent form is non-insulin dependent DM (type II) accounting for 90% of cases throughout world. The control of hyperglycemia is critical in the management of diabetes. Pancreatic α-amylase is an enzyme that hydrolyzes the starch to oligosaccharides and maltose in small intestine. Membrane bound α-glucosidase hydrolyzes di- and oligosaccharides to glucose. Inhibition of these tow enzymes reduces the rate of starch digestion and result in decrease in post-prandial blood glucose levels especially in diabetic patients. One approach to decrease the hyperglycemia, especially after a meal, is to retard and reduce the digestion and absorption of ingested carbohydrates through the inhibition of carbohydrate hydrolyzing enzymes. Acarbose is one such drug which inhibits alpha-glucosidase enzymes in the brush border of the small intestines and pancreatic alpha-amylase. Other drugs that belong to this class are miglitol and voglibose. Acarbose reduces post-prandial hyperglycemia and is used to treat type-2 diabetes. However, these drugs are known to have gastrointestinal side effects such as abdominal pain, flatulence and diarrhea in the patients [11, 18-20]. Therefore, it becomes necessary to identify the amylase inhibitors from natural sources having lesser side-effects. Literatures on amylase inhibitory activity of lichens, in particular, lichens of Karnataka, are scanty. In an earlier study, Karthik *et al.* [11] showed insecticidal and amylase inhibitory activity of methanol extract of a macrolichen *Heterodermia leucomela* collected at Bhadra wildlife sanctuary, Karnataka, India. The extract of *H. leucomela* showed about 38% of inhibition of amylase enzyme activity at 25mg/ml concentration of extract.

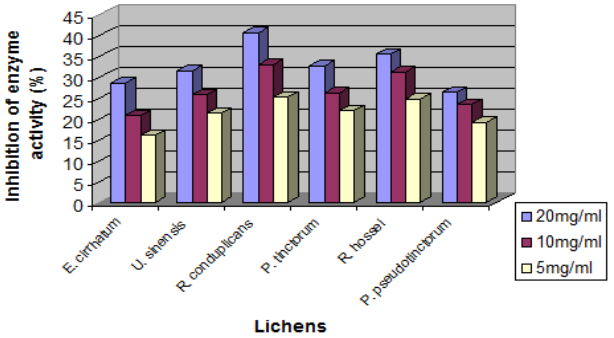

Figure 1: Inhibition of amylase by different concentrations of lichen extracts

Table 1: Secondary metabolites detected in lichens

Lichen species	Secondary metabolites detected				
	Usnic acid	Sekikaic acid	Salazinic acid	Lecanoric acid	Protolichesterinic acid
R. hossei	+	+	-	-	-
R. conduplicans	+	+	+	-	-
E. cirrhatum	-	-	+	-	-
P.pseudotinctorum	-	-	-	+	-
P.tinctorum	-	-	-	-	+
U. sinensis	+	-	-	-	-

CONCLUSION

A marked dose dependent inhibition of amylase by the extracts of lichens was observed in this study and the activity could be attributed to the presence of secondary metabolites. The lichens could be exploited in the development of agents active against pancreatic amylase which is involved in the rise of postprandial glucose level and hence the lichens could possibly used against diabetes.

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