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Research Article

Screening of In vitro α-amylase Inhibitory Activity of Wild Orchids of Nepal

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Abstract

Orchids are the source of compounds like phenols, alkaloids, phenanthrenes widely used as a therapeutic agent. Inhibition of pancreatic α-amylase could be a better therapeutic approach in decreasing levels of post-prandial hyperglycemia. This study aimed to evaluate α-amylase inhibition of some orchid species to assess their inhibitory potential on PPA (porcine pancreatic α-amylase). Methanol extracts of the whole plant of *Gastrochilus distichus* (GDW), the pseudobulbs of *Otochilus albus* (OAP), the whole plant of *Papilionanthe uniflora* (PUW), pseudobulbs of *Eria graminifolia* (EGP), the leaves and pseudobulbs of *Pholidota articulata* (PAL and PAP) and stems of *Vanda cristata* (VCS) were screened for their phytoconstituents and role in α-amylase inhibition by modified 3,5-Dinitrosalicylic acid method. *V. cristata*, *E. graminifolia* and *G. distichus* extract showed moderate inhibition of α-amylase with IC_{50} of 582.73 µg/ml, 710.89 µg/ml, 798.78 µg/ml respectively when compared to acarbose (26.85 µg/ml). Phytochemical analysis revealed the presence of alkaloids, tannins, glycosides, flavonoids, saponins and steroids with the major phytoconstituents. This study concluded that *V. cristata*, *E. graminifolia* and *G. distichus* exhibited moderate α-amylase inhibitory activity and they could be a potent source for antidiabetic phytochemicals.

Keywords: Antidiabetic, α-amylase, Extract, Orchids, Phytoconstituents

Introduction

Diabetes mellitus (Type II) (T2DM) is a chronic metabolic disorder associated with an abnormally high level of blood glucose as a result of inadequate production of insulin or inconsiderateness of cells to the action of insulin (WHO, 2016). According to the International Diabetes Federation (IDF), the global prevalence of T2DM in adults was 536.6 million (10.5%) in 2021, with 783.2 million (12.2%) living with diabetes by 2045 (Sun et al., 2022). According to WHO, 436,000 Nepalese people have diabetes, and this figure is expected to rise to 1,328,000 by 2030 (Shrestha et al., 2020). T2DM is one of the primary threats to human health accounting for 90% of the cases worldwide (WHO, 1999; Olokoba et al., 2012). Therefore, a decrease in post-prandial hyperglycemia could be better therapeutic approach for the management of diabetes (Ceriello, 2005). Stimulation of endogenous insulin secretion, enhancement of the action of insulin at the target tissues, oral hypoglycemic agents, such as acarbose, biguanides, sulfonylureas and including inhibition of the carbohydrate hydrolyzing enzymes such as pancreatic α-amylase and α-glucosidase in the digestive tract are the major used ways for the

treatment of T2DM (Olokoba et al., 2012). However, there is a burden of unwanted side effects like abdominal pain, flatulence and diarrhoea in the patients and high secondary failure rates (Eichler et al., 1984; Dey & Attele, 2003; Chaudhary et al., 2017). Such limitations of currently available antidiabetic drugs have endorsed researchers all over the world to investigate alternative antidiabetic remedies. Particularly, the herbal source is considered with the hope of discovering new bioactive compounds that can be used or developed into safe, inexpensive antidiabetic remedies (Sudha et al., 2009; Grover et al., 2002; Ali et al., 2006). These natural products can be used in reduction of glucose by inhibiting the activity of carbohydrate hydrolyzing enzymes, such as PPA (porcine pancreatic α-amylase). Inhibition of this enzyme interrupts overall carbohydrate digestion, causing a marked reduction in the rate of glucose absorption; thereby reducing the post-prandial plasma glucose rise.

In Nepal, several attempts have been made to document the useful antidiabetic medicinal plants with existing traditional practices which are being used by local people (Joshi, 2011). Although the use of orchids for the treatment of diabetes with traditional remedies and practices has still not been documented. Orchids have been used as remedies for human diseases for centuries (Gutierrez, 2010; Singh & Duggal, 2009; Pant & Raskoti 2013; Pant et al., 2022). Linking indigenous knowledge and ethnopharmacological studies to modern research activities can provide a reliable approach, and chances of drug discovery much more effectively from orchids (Farnsworth, 1988; Bulpitt, 2005; Gurib-Fakim, 2006; Paudel et al., 2019). Being a potential source of novel compounds and the possibility of having medicinal properties, orchids can be used for the ever-demanding lifesaving drugs (Gurib-Fakim, 2006; Pant, 2013; Paudel et al., 2017; Yang et al., 2006). Therefore, the present study is focused on exploring the α-amylase inhibitory activity of wild orchids; *Gastrochilus distichus*, *Otochilus albus*, *Papilionanthe uniflora*, *Eria graminifolia*, *Pholidota. articulata*, and each was screened to identify its phytoconstituents qualitatively. *Vanda cristata*, *Pholidota articulata* have already reported medicinal benefits and are already being used in traditional medicine to treat various disorders (Pant, 2013) but before this study, no one had determined that any of these species inhibited the activity of pancreatic α-amylase.

Materials and Methods

Collection of plant materials

The plant materials of *P. uniflora* (pseudobulb), *G. distichus* (whole plant), *E. graminifolia* (pseudobulb) were collected from Daman of Makwanpur district, *O. albus* (pseudobulb) and *V. cristata* (stem) were collected from Chitlang of Makwanpur district and *P. articulata* (pseudobulb and leaves) was collected from Kathmandu district of central Nepal. The collected orchid species were identified using literature and confirmed by taxonomist Dr. Keshav Raj Rajbhandari. The voucher specimen of the selected plants was deposited in Tribhuvan University Central Herbarium (TUCH) (voucher number – P001 for *G. distichus*, voucher number – P002 for *E. graminifolia*, voucher number – P003 for *V. cristata*, voucher number – P004 for *P. uniflora*, voucher number – P005 for *O. albus*, voucher number – P006 for *P. articulata*).

Drying and extraction

The collected plant materials of orchids were shade-dried and grinded into a fine powder using an electric grinder. The fine powder was introduced to extraction in the ratio of 1:10 (w/v) with methanol in the sonicator. Rotavapor was used for drying solvent at a low temperature and reduced pressure. The extract was put in a glass vial at 4 °C.

Chemicals

Chemicals such as soluble starch, PPA (porcine pancreatic α-amylase), methanol, DMSO (dimethyl sulfoxide), DNSA (3,5-dinitro salicylic acid), sodium potassium tartrate tetrahydrate, ferric chloride, mercuric chloride, potassium iodide, chloroform, magnesium ribbon, hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium phosphate buffer, acarbose, α-amylase, and methanol were obtained from Hi-Media Laboratory, India.

Qualitative phytochemical analysis

Methanol extract from plant materials was tested for the presence of alkaloids, tannins, saponins,

steroids, terpenoids and flavonoids following a standard protocol (Trease & Evans 1983; Harborne, 1998).

Test for tannins: The plant extract (50 mg) was dissolved in distilled water (5 ml) in a clean test tube. The resulting aqueous plant extract solution (2 ml) was taken in a separate test tube. Then, the ferric chloride solution ($FeCl_3$; 0.1%; 2 ml) was added. The observation for the appearance of blue-black or greenish brown colouration.

Test for saponins: The aqueous solution of plant extract was prepared in a clean test tube. It was shaken vigorously for 5 minutes. The observation for the persistent froth for 5 minutes was done indicating the presence of saponins.

Test for steroids and terpenoids: The plant extract (50 mg) was dissolved in methanol (2 ml) in a clean test tube. The resulting methanolic solution was completely dried with the help of a boiling hot water bath. Then, chloroform (2 ml) was poured into the dried test tube. Again, concentrated sulfuric acid (2 ml) was added to it from the side wall of a test tube to make two layers. Then, the observation for the yellowish ring between two layers and converting yellow colour to red or dark red was made.

Test for flavonoids: The plant extract (50 mg) was dissolved into methanol (5 ml). The resulting methanolic solution of plant extract (2 ml) was taken in a separate test tube and concentrated hydrochloric acid (HCl; 2-3 drops) was added to it. Then, the magnesium ribbon pieces (3 mm×5 mm) were added to the resulting solution. The resulting mixture was allowed to stand for 10 minutes. The observation for the appearance of pinkish colouration on a mixture was done. A portion of methanolic solution (2 ml) in another test tube was taken as a positive control to distinguish the pinkish colouration appearance on a test solution**.**

Test for alkaloids: The plant extract (50 mg) was dissolved in methanol (5 ml) in a test tube. The mixture was filtered through the Whatman No. 1 filter paper. Thus, obtained filtrate was mixed with HCl (1%, 2 ml) in a test tube. Then, the mixture was heated in a steam bath. After that, the mixture was treated with Mayer's reagent (6 drops in each replica). Finally, the observation of the appearance of turbidity on a mixture was done.

In vitro α-amylase inhibition assay

The α-amylase inhibition assay of the extracts was performed by quantification of the reducing sugar liberated. Based on a decrease of maltose liberated inhibition activity of extracts was determined. The modified 3,5-dinitro salicylic acid (DNSA) method was adopted to estimate the maltose equivalent (Miller, 1959). The plant extract of different plant parts was diluted in DMSO to give a final concentration of 640 µg/ml, 320 µg/ml and 160 µg/ml, 80 µg/ml and 40 µg/ml. About 0.5ml of selected plant extracts were pre-incubated with 0.5 ml of α-amylase for 30 minutes then 0.5 ml (1% w/v) of the starch solution was added and the mixture was further incubated at 37°C for 10 minutes. About 0.5 ml DNSA reagent (30 g of sodium potassium tartrate tetra-hydrate in 20 ml of 2 M NaOH and 1 g of 3,5-dinitro salicylic acid) was added to terminate the reaction, and the content was heated in a water bath (80-90ºC) for 5 minutes. A blank solution was prepared without plant extract and acarbose was used as a positive control. The absorbance was measured at 540 nm using a spectrophotometer. The reducing sugar released from starch was estimated as maltose equivalent from a standard graph. The percentage of inhibition of α-amylase was calculated using the following equation.

$$\textbf{\% inhibition} = \frac{A1-A2}{A1} \textbf{x 100\%}$$

Where, A1 is the absorbance of the enzyme and starch, and A2 is the absorbance of the enzyme, starch and plant extracts.

Statistical analysis

The experiment for all test sample was carried out in triplicate for α-amylase inhibition. The percentage α-amylase inhibition was presented in the form of mean ± SD and the IC_{50} value of the extracts was calculated using a polynomial regression equation.

Results and Discussion

A decrease in post-prandial hyperglycemia is beneficial for the treatment of diabetes (Eichler, 1984; Tundis et al., 2010; Layer et al., 1986; Mangold, 1977). Digestion of dietary starch by α-glucosidase and α-amylase plays a significant role in raised blood glucose thus inhibition of the α-amylase enzyme is a very useful tool in the

management of hyperglycemia (Mangold, 1977; Tundis et al., 2010). Plant bioactive compounds are extraordinary therapeutic alternatives for the treatment of diabetes around the world. For diabetes management, plants with hypoglycemic properties are being used for a longtime (Bailey, 1989; Grover, 2002; Bnouham, 2006). In the present study, six wild orchids were screened for their PPA inhibitory potential. Crude extracts of plant materials of orchids showed α-amylase inhibition against the porcine pancreatic α-amylase (PPA) (Table 1). The inhibitory assay demonstrated that most of the crude methanol extracts inhibited the porcine pancreatic α-amylase. The crude extracts of *V. cristata* (VCS), *E. graminifolia* (EGP) and *G. distichus* (GDW) showed the moderate inhibitory activity against PPA with 49.36%, 41.13% and 38.54% respectively. *P. uniflora* (PUW), *O. albus* (OAP) and *P. articulata* (PAL and PAP) extracts demonstrated the lowest inhibitory activity (6.03%, 12.65%, 19.32% and 23.26%) against porcine pancreatic α-amylase at highest concentrations i.e., 640 µg/ml.

Table 1: Percentage α-amylase inhibition of methanol extract of wild orchid species and acarbose, and their IC50 values

Plant sample	**Concentration (µg/ml)**	**α-amylase inhibition (%) Mean ± SD**	**IC_{50} (µg/ml)**
Gastrochilus distichus whole plant (GDW)	40	-	798.78
	80	-	
	160	6.19±0.07	
	320	21.65±0.16	
	640	38.54±0.16	
Eria graminifolia pseudobulb (EGP)	40	-	710.89
	80	6.17±0.09	
	160	23.66±0.13	
	320	30.87±0.16	
	640	41.13±0.10	
Vanda cristata stem (VCS)	40	-	582.33
	80	11.72±0.11	
	160	23.24±0.16	
	320	38.83±0.13	
	640	49.36±0.16	
Papilionanthe uniflora whole plant (PUW)	40	-	5691.66
	80	-	
	160	-	
	320	-	
	640	6.03±0.04	
Otochilus albus pseudobulb (OAP)	40	-	2357.73
	80	-	
	160	-	
	320	6.12±0.07	
	640	12.650.09	
Pholidota articulata leaves (PAL)	40	-	1268.80
	80	-	
	160	-	
	320	12.44±0.06	
	640	23.26±0.13	
Pholidota articulata pseudobulb (PAP)	40	-	1539.61
	80	-	
	160	5.33±0.13	
	320	13.30±0.10	
	640	19.32±0.06	
Acarbose	40	63.24±0.10	26.85
	80	83.96±0.16	
	160	90.45±0.19	
	320	95.37±0.07	
	640	97.20±0.10	

The concentration of extract (inhibitor) required for 50% inhibition of alpha-amylase (IC_{50}) was determined from corresponding dose-response curves of percentage inhibition versus concentration of inhibitor. The IC_{50} value of extracts was compared with those of acarbose, a known α-amylase inhibitor. Methanol extract of *V. cristata* appeared to be a moderate inhibitor of α-amylase (IC_{50}: 582.73 µg/ml) followed by *E. graminifolia* (710.89 µg/ml) and *G. distichus* (798.78 µg/ml) as compared to acarbose. However, there is a significant difference in the inhibition capacity of the methanol extract of other selected orchids (Table 1). Phytochemical analysis of these methanol extracts revealed the presence of alkaloid, flavonoid and tannin in all the selected orchid species, whereas saponin was detected in *E. graminifolia*, *G. distichus*, *V. cristata* whereas steroids and terpenoids were detected in all plants except *O. albus* and *E. graminifolia* (Table 2).

There was a dose-dependent increase in percentage inhibitory activity against α-amylase by all 7 extracts. Glycosides, saponins, alkaloids, flavonoids, phenols, triterpenes and steroids present in crude extract act as α-amylase inhibitors (Dastjerdi, 2015; Kim, 2010). Besides the compounds Palmitic acid (23.51%), Methyl 9-octadecenoate (53.43%), Alpha-Bisabolol,2-Methyl-Z,Z-3, 13-octadecadienol, Hexadecanoic acid, Docosenoic acid, , 15-methyl-hexadecanoic acid, 10-Octadecenoic acid methyl ester were previously reported in *Vanda cristata* play a significant inhibitor of alpha-amylase in the present study (Joshi et al., 2020). The presence of a such compound in methanol extract of selected orchids may act against diabetes mellitus either through their capacity to avoid glucose absorption or to improve glucose tolerance by competitive inhibition of sodium-dependent glucose transporter. Substrate molecule when bound to the active site of α-amylase, forms an enzyme-substrate complex. After that, the enzyme hydrolyses the α-bond of a starch molecule to form an oligosaccharide. Inhibitor forms hydrogen bonds with an amino acid residue of either the active site or another enzyme. Thus, the substrate molecule does not fit on the active site of an enzyme, resulting in the inhibition of glucose hydrolyzing enzymes (Perez, 1998).

Retardation of starch hydrolysis is one of the mechanisms of selected orchid species in exhibiting their hypoglycemic effect, eventually lowering PPHG. All the groups of phytochemicals have earlier been reported from these plants (Chand et al., 2016) but no study has been carried out to prove porcine pancreatic alpha-amylase inhibitory activity. Since selected species were among the least studied orchid species, this study encourages the researcher that orchids can be used against PPHG. Besides this, the nature of the phytochemical responsible for inhibitory activity has not been determined and needs to be characterized by further studies.

Table 2: Phytoconstituents in methanol extract of selected orchid species.

Plant sample	Alkaloid	Flavonoids	Saponin	Steroids & Terpenoids	Tannin
*Eria graminifolia p*seudobulb *(*EGP)	+	+	+	_	+
*Gastrochilus distichus w*hole plant (GDW)	_	+	+	+	+
Otochilus albus pseudobulb (OAP)	+	+	_	_	+
Papilionanthe uniflora whole plant (PUW*)*	+	+	_	+	+
Pholidota articulata leaves (PAL)	+	+	_	+	+
Pholidota articulata pseudobulb (PAP)	+	+	_	+	+
Vanda cristata stem (VCS)	++	++	+	+	+

Conclusion

The results of the study indicate that methanol extracts of selected orchids showed moderate α-amylase inhibitory activity. Among the orchids tested, the stem of *V. cristata* was the most effective inhibitor of alpha amylase.

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