Phytochemical analysis, phytotoxic and alpha amylase inhibition activity of *Cyanodondactylon*

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Abstract

Herbal medicines that obtained from medicinal plants are safe and oldest natural products used for treatment of different diseases. The present study was undertaken to evaluate the phytochemical analysis, phytotoxic activity and alpha amylase enzyme inhibition activity of methanolic extract of *Cyanodondactylon*. For this whole plant was collected and shade dried and grinded to get the powder. The extraction was carried out by cold percolation in methanol. The methanolic extract was subjected to phytochemical analysis for the absence or presence of secondary metabolites using standard protocol. *In-vitro* phytotoxic activity was performed by adopting the standard protocol. The alpha amylase enzyme inhibition activity of plant extract was carried out by using starch as substrate, pancreatic alpha amylase as the enzyme, and acarbose as standard. Phytochemical analysis displayed the presence of different chemical constituents such as alkaloids, flavonoids, tannins, polyphenols, terpenoids etc. The result of *in-vitro* phytotoxic bioassay revealed that the plant extract showed moderate activity with percentage growth regulation 75% in 1000 µg/ml and 30% in10 µg/ml. The alpha amylase enzyme inhibition was 96.2% at 1000 µg/ml while 37.06% at 40 µg/ml concentration. The inhibition was found dose dependent.

Keywords: **Phytotoxic, phytochemical, α- amylase, diabetes, *Cyanodondactylon***

Introduction

Medicinal plants are the main sources of large number of potent and powerful drugs. Globally medicinal plants have been used as a source of medicine and 80-85% of populations rely on these medicinal plants using the extacts or their active components to meet their primary health care (Igacimuthu et al. 2006 &Elujoba et al. 2005). Chemical compounds isolated from natural sources are healthier and safer alternate to the synthetic drugs (Rai et al. 2007). Pharmacologically active constituents are isolated from the different parts of medicinal plant like root, stem, flower, fruit, seed etc. Pharmacological activities of plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, glycosides, tannins and terpenoids present in these plants. Such active phytoconstituents isolated from plant can use as lead compounds or pharmacologically active agent for drug discovery process. Diabetes mellitus, one of the major public health problems worldwide, is the metabolic disorder which leads to the deficiency in the production of insulin by pancreas and the body does not use insulin properly or insulin resistance. Diabetes is considered as one of the fifth leading cause of death in the world (Vats et al. 2004 & Kumar et al. 2006). Cardiovascular diseases, neuropathy, nephropathy and retinopathy are among the major risk that is associated with diabetes (Rubin et al. 2012). At least 171 million peoples have diabetes and this number is probable to more than double by 2030 (Chaudhary et al. 2012). In the Asian region the defect in insulin secretion in nonobese type 2 diabetes is more common than insulin resistance (AASD, 2013). Available antidiabetic drugs for the treatment of diabetes are with low efficacy and side effects. Different extracts from medicinal plants have also been used traditionally to manage diabetes globally, and these are considered as relatively inexpensive, less toxic and with little or no side effects (Gupta et al. 2008). Medicinal plants also contain some toxic phytoconstituents, however the side effects due to these constituents are less common as compare with synthetic drugs (Calixto et al. 2000).

Cyanodondactylon is a perennial weed, belongs to the family poaceae, widely distributed throughout the world. People have been using this plant since many years for the various medicinal properties like anthelmintic, anti-diuretic, anti-inflammatory and hepatoprotective activity, antidiabetic activity, jaundice and renal problems (Kanimozhi et al, 2012*).* *Cyanodondactylon*is the rich source of secondary metabolites like glycosides, saponins, tannins, proteins, flavonoids

and steroids (Kumar et al. 2011). The plant is the folk medicine for anasarca, calculus, carbuncles, cancer, cough, hypertension, snakebite, gout, fever, stones, skin diseases, and rheumatic infections (Kaup et al. 2011). The plant is traditionally used as an agent to control diabetes in India (Kritikar&Basu, 1980). The extract of *Cyanodondactylon* leaf has been reported to be antidiabetic (Singh et al. 2007 &Rai et al. 2010). The present study aims to explore the phytochemicals as secondary metabolites, alpha amylase enzyme inhibition activity and phytotoxic activity of methanolic extract of *Cyanodondactylon*.

Materials and methods

Chemicals and reagents
Chemicals used in this study were methanol (Merck, Germany), porcine pancreatic alpha amylase. All the needed chemicals used in this research work were of the commercially available analytical grade.

Plant collection and extract preparation
Cyanodondactylon was collected from Chitwan district of Nepal based on their ethnobotanical uses. The plant was identified by Rita ChhetriResearch Officer, National Herbarium and Plant Resources, Ministry of Forests and Soil Conservation, Godawari, Nepal. 200 g powder of plant was extracted by cold percolation method in methanol (400-600 ml) at room temperature for 48 hours with frequent agitation. The mixture was filtered through clean cotton and filtrate was concentrated at the temperature lower than the boiling point of methanol under reduced pressure by rotary evaporator to get the crude extract. Finally the concentrated extracts were fridge dried to yield a dry powder.

Phytochemical screening
Phytochemical screening is the method of finding the chief secondary metabolites present in the plant extracts. The phytochemicals as secondary metabolites were investigated by following the standard protocol.The analysis of the presence of main groups of natural constituents present in the plant extract was done by the color reaction using different specific reagents (Ciulei 1982).

Alpha amylase inhibition assay
Alpha amylase inhibition assay was performed using a standard protocol where the undigested starch due to enzyme inhibition was detected at 630 nm (blue, starch-iodine complex) described by Kala et al. (2016). The stock solution of the plant extract was made by dissolving 10 mg in 10 ml of dimethylsulphoxide (DMSO) (1000 µg/ml). Substrate was prepared by dissolving 200 mg starch in 25 ml of NaOH (0.4 M) by heating at 100°C for 5 minutes. After cooling, pH was adjusted to 7.0 and the final volume was made up to 100 ml using distilled water. Acarbosewas used as Positive control. 400 µl of substrate solution was pre-incubated at 37 °C for 5 minutes with 200 µl of acarbose or plant extract at varying concentrations (40, 80, 160, 320, 640 and 1000 µg/ml), followed by 200 µl of 50 µg/ml α-amylase (20mM phosphate buffer with 6.7 mMNaCl, pH 6.9), and incubated at 37°C for 15 min. Termination of the reaction was carried out by adding 800 µl of HCl (0.1M). Then, 1000 µl of iodine reagent (2.5 mM) was added, and absorbance was measured at 630 nm. The assay was carried out in triplicates using spectrophotometer. Percentage of inhibition was calculated using the formula;

$$\% \text{ Inhibition} = (1-[Abs2-Abs1/Asb4-Abs3]) \times 100$$

Where,
Abs1 is the absorbance of the incubated mixture containing plant sample,   alpha amylase and starch. Abs 2 is the absorbance of incubated mixture of sample and starch.Abs3 is the absorbance of the incubated mixture of starch and α-amylase.Abs4 is the absorbance of incubated solution containing starch.

Statistical analysis
Data were recorded as mean of (±) standard deviation of three determinations of absorbance for each concentration, from which linear correlation coefficient (R^2) value was calculated using MS Office Excel 2007. The linear regression for a straight line is, $y = mx + c$, using this regression equation, concentration of extract were calculated.

In-vitro phytotoxic bioassay
E-Medium was prepared by mixing various constituents in 1000 ml distilled water and pH was adjusted between 6.0 to7.0 adding potassium hydroxide pellets(stock solution).Working E-medium was prepared by mixing 100 ml of stock solution and 900 ml of distilled water.30 mg for crude extract is dissolved in 1.5 ml of methanol serving as stock solution.Three flasks were inoculated with 10, 100 and 1000 µl of solution pipetted from the stock solution for 10, 100 and 1000 µg/ml. Solvent was allowed to evaporate overnight. 20 ml of working E. medium was added and then plant of *Lemna minor*, each containing a rosette of two to three fronds, to each flask (total 20 fronds). Other flasks supplemented with E-medium and reference (standard drug) plant growth inhibitors and promoters serving as negative

and positive controls, respectively. The flaks were kept in growth cabinet for seven days.Plants were examined daily during incubation. Number of fronds were counted and recorded per flasks on day 7.Results were analyzed as growth regulation in percentage calculated with reference to the negative control (Atta-ur-Rehman, 1991&Hideji, 1982).

Calculation

$$\%Regulation = 100 - \frac{\text{No. of fronds in test}}{\text{No. of fronds in negative control}} * 100$$

Results and discussion

Methanolicextract of *Cyanodondactylon* showed the good source of secondary metabolites such as flavonoids, terpenoids, polyphenols, glycosides, reducing sugars, cardiac glycosides, anthraquinone, carotenoids and saponin. It showed that plant can be used as source of secondary metabolites to isolate the active compounds for the different biological activities.

Table 1: Phytochemical analysis of plant extract

Polyphenols	+	Reducing sugar	-
Steroids	-	Tannin	+
Alkaloids	-	Cardiac glycoside	+
Flavonoids	+	Anthraquinone	+
Terpenoids	-	Carotenoids	+
Glycosides	+	Saponin	+

Key: + = Present   - = Absent

Table 2: *In-vitro*Phytotoxic activity

Name of the plant	Conc. of Plant extract (µg/mL)	No of fronds		% growth regulation	Concentration of standard drug (µg/mL)
		Sample	Control		
Lemna minor	1000	05		75%	
	100	09	20	55%	0.015
	10	14		30%	
K Keys: Standard drug Paraquat				Incubation condition= (28±1ºC)	

The phytotoxicity of the methanolic extract of *Cyanodondactylon*investigated on *Lemna minor* was observed to have dose dependent activity because low activity was found in 10 µg/ml (30% inhibition) and high activity was found in 1000 µg/ml (75% inhibition) respectively. Moderate phytotoxic activity was found in the 100 µg/ml (55% inhibition). Significant phytotoxicity was shown in 100 and 1000 µg/ml with 55% and 75% growth inhibition (Table 2).
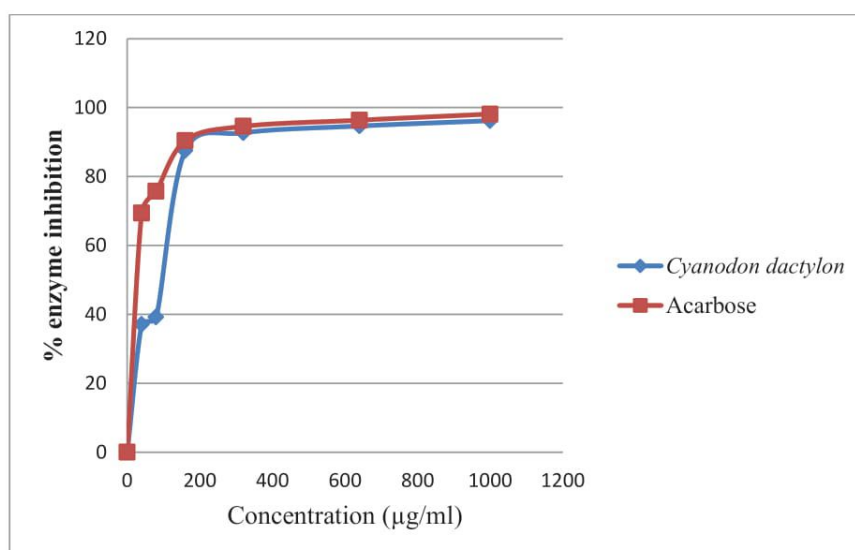

Figure1: Inhibition of alpha amylase activities by plant extract

Alpha amylase inhibitory activity of plant extract was determined using quantitative starch-iodine method. The result of α amylase inhibition activity is shown in figure 1. The result shows the plant extract have the similar enzyme inhibition activity as that of standard drug acarbose. Hence, the plant extract is the source of potent alpha amylase enzyme inhibitory agent.

The result showed a dose dependent increase in percentage inhibitory activity of α- amylase by the plant extract. Reddy et al. (2010) has analysed the oral administration of ethanolic extract of *A. precatorius*. The serum glucose value of 131.16 ± 1.939 mg/dl was found whereas standard glibenclamide showed the value of 96.5 ± 1.607 mg/dl. This result shows the similarities with the result reported by previous scholar. Hence, here the α-amylase inhibitory activity of the plant extract might be due to the presence of analogous phytoconstituents which were marked during phytochemical analysis.

Conclusions

In summary the present study firstly depicts the potential of the plant extract of *Cyanodondactylon* on phytotoxic and α-amylase inhibitory activity, which indicate that the plant might be considered as a potential source in the search of new drugs. This study provides some scientific support for the traditional use in diabetes management and other ailments. But, further experiment is needed to find out whether the plant extract possesses antidiabetic activity under *in-vivo* conditions.

Acknowledgements

References

Asian Association for the Study of Diabetes (AASD), (2013), Promoting research for better diabetes care in Asia: Kyoto declaration of diabetes. *Journal of Diabetes Investigations*, 4(2): 222.

Atta-ur-Rehman, (1991).Studies in Natural Product Chemistry, Netherlands, Elsevier Science Publishers, B. V. 9: 383-409.

Calixto, J., B. (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents) Braz, *Journal of Medical Biology Reserch*, 33:179–189.

Chaudhary, P., Goel, B. &Ghosh, A. K. (2012).Antidiabetic activity of *Adina cordifolia* (Roxb) leaves in alloxan induced diabetic rats, *Asian Pacific Journal Trop Biomed*, 2 1 (3): 1630-S1632.

Ciulei, I. (1982). Methodology for analysis of vegetable drugs.Practical manuals on industrial utilization of medicinal and aromatic plant, Bucharest.*Phytochemistry*982; 63: 97-104.

Elujoba, A. A., Odeleye, O. M. &Ogunyemi, C. M. (2005). Traditional medicine development for medical and dental primary health care delivery system in Africa, *African Journal of Traditional, Complementary and Alternative Medicines*, 2(1):46–61.

Gupta, R., Bajpai, G. K., Johri, S. &Saxena, A. M. (2008).An overview of Indian novel traditional medicinal plants with antidiabetic potentials. *African Journal of Traditional, Complementary and Alternative Medicine*. 5(1):1–17.

Hideji, Y., Oshida, A., Ikuta, H. & Adachi, T. (1982). Phenolic plant growth inhibitors from the flowers of *CucurbitaPepo*, *Phytochemistry*, 21(8): 1935-37.

Ignacimuthu, S., Ayyanar, M. &Sivaraman, S. K. (2006). Ethnobotanical investigations among tribes in Madurai district of Tamil Nadu (India), *Journal ofEthnobiologyEthnomedicine*, 2(25):1–7.

Kala, M. J., Sharma, T. S. &Upadhyaya, K. W. (2016).*Journal of Pharmacy and Pharmaceutical Sciences*, 5: 606-616.

Kanimozhi, D. &Bai, R. V. (2012).Evaluation of Phytochemical antioxidant antimicrobial activity determination of bioactive components of ethanolic extract of aerial and underground parts of *Cynodondactylon* L. *International Journal of Scientific Research and Reviews*, 1(2):33-48.

Kirtikar, K. K. &Basu, B. D. (1980). Indian Medicinal Plants, Second Ed. Lali Mohan Publication, India: 2650.

Kumar, A.,Sawarkar, H. A.,Deshmukh, V. S.,Mishra, K. K., Singh, M., Verma, T. &Kahyap, P. (2011). *Cynodondactylon* (L) Pers: Pharmacological actions and medicinal applications. *International Journal of Herbal Drug Research*, 1(1): 1-7.

Kumar,G. P. S., Arulselvan, P., Kumar, D. S. & Subramanian, S. P. (2006). Anti-diabetic activity of fruits of *Terminaliachebula* on streptozotocin induced diabetic rats, *Journal of Health Science*, 52(3): 283–291.

Rai. P. K., Rai, N. K., Rai, A. K. &Watal, G. (2007). Role of LIBS in elemental analysis *of P. guajaua*responsible for glycemic potential.*Institute of Science and Technology*, 35:507-522.

Rashmi, S., Nayanatara, K., Leigelin, A., Bernhardta, K., Gorantla, R., Sandeep, V., Shettyd, S., Ramesh Paib, S. &Arunkumare, B. (2011). Antihyperlipedemic activity of *Cynodondactylon* extract in high-cholesterol diet fed Wistar rats, *Genomic Medicine, biomarkers and Health Sciences*, 3(3-4): 98-102.

Reddy, M., Dharma, T. N., Bhagvan, R. & Reddy, P. K. (2010).*Pharmcology*Online: 701-705.

Rubin, R., Strayer, D. S., Rubin E. & Rubin's Pathology. (2012). *Clinicopathologic Foundations of Medicine*, 6th ed. Philadelphia: Lippincott Williams & Wilkins, 1081–1098.

Singh, S. K., Kesari, A. N., Gupta, R. K., Jaiswal, D. &Watal, G. (2007). Assessment of antidiabetic potential of *Cynodondactylon* extract in streptozotocin diabetic rats. *Journal of.Ethnopharmacology*, 114:174-179.

Vats, V., Yadav, S. P. & Grover, J. K. (2004).Ethanolic extract of *Ocimum sanctum* leaves partially attenuates streptozotocin-induced alterations in glycogen content and carbohydrate metabolism in rats, *Journal of Ethnopharmacology*, 90(1):155–160.