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PHYTOCHEMICAL SCREENING, QUANTIFICATION AND IN VITRO ANTIDIABETIC ACTIVITY OF STRYCHNOS NUX-VOMICA LEAF EXTRACT

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ABSTRACT

The aim of this study was to indicate phytochemical screening, quantification and in vitro antidiabetic activity through potential inhibition α-amylase and α-glucosidase enzymes using the aqueous ethanolic extract of Strychnos Nux-vomica. The leaves of Strychnos Nux-vomica was dried under shade and powdered. The aqueous ethanol extract used for the in vitro study to monitor the antidiabetic activity. Various concentrations of the plant extract (100, 200, 300, 400 and 500 µg/ml) were prepared in aqueous ethanol solution and subjected to α amylase inhibitory and α Glucosidase inhibitory assay. Strychnos Nux-vomica aqueous ethanolic extract proved the α-amylase inhibitory activity with an IC50 values 310.86 ± 17.96µg/ml and α Glucosidase inhibitory activity with an IC50 values 550.34 ± 8.29 µg/ml. Thus the present study proves the hypoglycemic effect in the Strychnos Nux-vomica aqueous ethanol extract. The present study concludes that Strychnos Nux-vomica has antidiabetic activity.

Keywords: Strychnos Nux-vomica, in vitro antidiabetic α–amylase, α-glucosidase enzymes.

INTRODUCTION:

Diabetes mellitus is an endocrine disorder. It is described by hyperglycemia and conflicts the carbohydrate, protein and fat metabolisms [1,2].Diabetes mellitus is presently a rising global health concern and assessed number of 171 million diabetics worldwide in 2000 is estimated to increase to about 366 million by 2030[3]. α amylase and α glucosidase are the significant enzymes involved in breakdown the long chain carbohydrates and breakdown starch and disaccharides to glucose respectively.

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These digestive enzymes assist in intestinal absorption. Alpha amylase and glucosidase inhibitors are the prospective targets in the research development for the treatment of diabetes [4]. Plant extracts are used as a drug or supplement to treat cardiovascular diseases, diabetes and other metabolic syndromes that rise due to extreme production of oxidative stress [5]. In diabetes, alpha amylase plays a role in the increase of the postprandial blood glucose by directly breaking down the carbohydrate into their respective maltose and maltotriose [6].The objective of this study to examine the inhibition of α-amylase and α-glucosidase enzymes using the aqueous ethanolic extract of Strychnos Nux-vomica.

MATERIALS AND METHODS:

Collection of plant material and Sample extraction:

Leaves of Strychnos Nux-vomica was used for investigation obtained from Nellore district, Andhra Pradesh, India. The plant was authenticated by G.V.S Moorthy, Botanical survey of India, Coimbatore. Authentication number of plant is BSI/SRC/5/23/2013-14/Tech/682.

Sample extraction:

Leaves of Strychnos Nux-vomica is washed with distilled water, shade dried, podered for the solvent extraction process and extracted out with Petroleum Ether, Chloroform, Acetone, 50% Ethanol for qualitative and quantitative analysis were carried out .The crude extract was obtained by extracting 100 grams of dried plant powder in 500ml of Petroleum Ether, Chloroform, Acetone, 50% Ethanol in a water shaker for 72 hrs. Repeatedly solvent extraction was done with same solvent till colour less solvent obtained. Extract was further concentrated using a rotary vacuum evaporator at 45-50 °C and stored in 0-4°C in air tight container.

Qualitative analysis

Phytochemical screening was carried out following the methods of Horbone, 1984[7] and Kokate et al., 1995[8].

Test for alkaloids:

To 1 ml of the extract, a few drops of Wagner's reagent were added and the formation of a reddish brown precipitate indicates the presence of alkaloids.

Test for Flavonoids:

To 1 ml of the extract, magnesium turnings and 1-2 drops of concentrated hydrochloric acid were added. Formation of pink colour indicates the presence of Flavonoids.

Test for Phenols:

A small quantity of the extract was dissolved in 0.5ml of 20% sulphuric acid solution. Followed by addition of a few drops of aqueous sodium hydroxide solution, it turns blue presence of phenols.

Test for Tannins:

To 1 ml of the extract, few ml of 5% ferric chloride was added. The development of dark bluish black colour indicates the presence of tannins.

Test for steroids and sterols:

The extract was dissolved in 2ml of chloroform and equal volume of concentrated Sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterols compound in the extract.

Test for Saponins:

5 ml of the extract was taken in a test tube and a few drops of 5% sodium bicarbonate solution were added. The mixture was shaken vigorously and kept for 3 minutes. Formation of honey comb like froth shows the presence of Saponins.

Test for Glycosides:

The extract was dissolved in pyridine and freshly prepared sodium nitroprusside solution was added. The formation of pink to red colour indicates the presence of glycosides.

Test for Protein:

To 1 ml of extract, equal volume of 40% NaOH solution and two drops of 1% Coppersulphate solution were added. The appearance of violet colour indicates the presence of protein.

Test for amino acids:

To 1 ml of extract, 2 drops of freshly prepared 0.2% Ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or aminoacids.

Test for Carbohydrates:

Five ml of Benedict's solution was added to 2 ml of the extract boiled in a water bath. The appearance of red or yellow or green precipitate indicates the presence of reducing sugars.

Quantitative analysis

Determination of Carbohydrate:

Table 1 Qualitative analysis of Strychnos Nux-vomica leaves extracts

S.NO	PARTICULARS	PET ETHER	CHLOROFORM	ACETONE	50% ETHANOL
1	Alkaloids	+	+	+	+
2	Flavonoids	-	-	+	+
3	Phenolic& tannins	-	-	+	+
4	Steroids & sterols	-	-	-	-
5	Saponins	-	-	-	+
6	Glycosides	-	-	-	-
7	Protein	+	+	-	-
8	Carbohydrates	-	-	+	+

The total carbohydrate content was determined according to the method described by Krishnaveni [9]. 0.2 ml of sample (5 mg/ ml) was pipetted out and the volume was made upto 1 ml with distilled water. Then 1.0ml of 5% phenol reagent was added followed by 5.0 ml of 96% sulphuric acid. The tubes were kept at 20°C for 20 min. The absorbance was measured at 490nm.

Determination of Protein:

The total protein content was determined according to the method described by Lowry [10]. In brief, 0.25 ml of extract (5mg/ ml) was pipetted out and the volume was made upto 1.0ml with distilled water. 5.0 ml of alkaline copper reagent was added (50 ml of 2% sodiumcarbonate and 1 ml of 0.5% copper sulphate) to all the tubes and allowed it to stand for 10min.Then 0.5ml of Folin's Ciocalteau reagent was added and incubated in dark for 30min. The intensity of the colour developed was measured at 660nm.

Determination of total alkaloid content:

Total alkaloid content was determined according to the method described by Fazel [11]. One ml of the sample solution (5mg/ml) was transferred to a separating funnel and then 5ml of BCG solution (69.8 mg bromocresol green, 3 ml of 2N NaOH and 5 ml distilled water were mixed and raised to 1 L with distilled water) along with 5 ml of phosphate buffer (2 M sodium phosphate adjusted to pH 4.7 with 0.2 M citric acid) was added. The mixture was shaken and the complex formed was extracted thrice with chloroform by vigorous shaking. The extracts were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. The analysis was performed in triplicate and the results were expressed as atropine equivalent.

Estimation of Flavanoids:

The leaf extracts and the standard rutin were dissolved in methanol separately for the total flavonoid estimation. To each 1 ml of extracts the 4 ml of water followed by 0.3 ml of 5% sodium nitrate were added. After 5 minutes, 0.3 ml of 10% aluminum chloride solution was added and at the 6th minute, 2 ml of 1M sodium hydroxide was added. After proper mixing absorbance was measured using a spectrophotometer at 510 nm and a standard graph was plotted. The absorbance value obtained from extracts were interpreted from the standard graph to get the total flavanoids content expressed as milligram equivalents of rutin [12].

Table 2 Quantitative assay of the Phytoconstituent present in the Strychnos Nux-vomica Ethanolic leaf extract

S.No	Name of the Compound	Amount present in the sample	Method followed
1	Total carbohydrate(mg/g extract)	46.22 ± 7.51	Krishnaveni et al. (1984).
2	Protein (mg/g extract)	442.67 ± 22.83	Lowry etal. (1957).
3	Total alkaloid (mg AE/g extract)	18.00 ± 0.70	Fazel et al. (2008).
4	Flavonoid (mg RE/g extract)	2.22 ± 0.12	(Woisky and Selationo, 1998).
5	Total Saponins (mg DE/g extract)	2.45 ± 0.15	Hiai et al. (1976).

Values are means of three independent analyses of the extract ± standard deviation (n = 3).
AE – Atropine equivalent; TAE – Tannic acid equivalent; RE- Rutin equivalent; DE – Diosgenin equivalent

Table 3 Inhibition of α-amylase activity

Sample	Concentration (µg)	Percentage activity (%)	IC50 (µg/ml)
Strychnos Nux-vomica (50%V) Ethanolic extract	100	4.09 ± 0.54	310.86 ± 17.96
	200	10.38 ± 0.94	
	300	16.35 ± 2.37	
	400	22.64 ± 0.94	
	500	24.53 ± 0.94	

Values are means of three independent analyses of the extract ± standard deviation (n = 3).

Determination of total Saponin content:

Total Saponin content was determined by the vanillin-sulphuric acid method following the procedure of Hiai [13]. In brief, 0.5 ml of the sample (1mg/ml), 0.25 ml 8% (w/v) vanillin solution and 2.5 of 72% (w/v) sulphuric acid were added and thoroughly mixed in an ice water bath. The mixture was then warmed in a bath at 60°C for 10 minutes, then cooled in ice cold water. Absorbance of the mixture was determined at 535 nm versus reagent blank. The analysis was performed in triplicate and the results were expressed as diosgenin equivalent.

In vitro methods employed in antidiabetic studies

In vitro inhibition of α-amylase (Miller): [14]

3, 5-dinitrosalicylic acid assay (DNSA). The α- Amylase (0.5 mg/ml) was premixed with extract at various concentrations (100-500µg/ml) and starch as a substrate was added as a 0.5% starch solution to start the reaction. The reaction was carried out at 37°C for 5 min and terminated by the addition of 2 ml of DNS(3,5-dinitrosalicylic acid) reagent. The reaction mixture was heated for 15 min at 100°C and diluted with 10 ml of distilled water in an ice bath. α- amylase activity was determined by measuring spectrum at 540 nm. The % α-amylase inhibitory activity is calculated by the following formula.

% Inhibition = (Control OD-Sample OD / Control OD) × 100

The IC50 value was defined as the concentration of the sample extract to inhibit 50% of α-amylase activity under assay condition.

In vitro inhibition of α-glucosidase (Miller, 1959): [14]

The enzyme α-glucosidase inhibitory activity was determined by premixing α-glucosidase (0.07 Units) with 100-500 µg/ml of extract. Then 3mM p-nitrophenyl glucopyranoside was added as a substrate. This reaction mixture was incubated at 37°C for 30 min and the reaction was terminated by the addition of 2 ml of sodium carbonate. The α- glucosidase activity was determined by measuring the p-nitrophenyl release from p-nitrophenyl glucopyranoside at 400 nm. The % α-glucosidase inhibitory activity is calculated by the following formula

% Inhibition = (Control OD-Sample OD / Control OD) × 100

The IC50 value was defined as the concentration of the sample extract to inhibit 50% of α-glucosidase activity under assay condition.

RESULTS:

Qualitative analysis of different solvent extracts showed the presence of alkaloids, flavonoids, phenols, tannins, Steroids, sterols, Saponins, Glycosides, Protein and Carbohydrates were tabulated in table 1.

Quantitative analysis of the Ethanol extract:

The various active phytochemical compounds were quantified by various process with respect to the working concentration of

Table 4 Inhibition α- glucosidase activity

Sample	Concentration (µg)	Percentage activity (%)	IC50 (µg/ml)
Strychnos Nux-vomica (50%V) Ethanolic extract	100	5.80 ± 0.83	550.34 ± 8.29
	200	9.57 ± 0.59	
	300	12.24 ± 0.62	
	400	15.76 ± 0.24	
	500	17.96 ± 0.36	

Values are means of three independent analyses of the extract ± standard deviation (n = 3)

mg/gram of the extract with standard. The carbohydrate showed the presence of 46.22 ± 7.51 mg/gram and protein was442.67 ± 22.83 mg/gram of the ethanolic extract of Strychnos Nux-vomica leaves. The other quantification assay shows 18.00 ± 0.70 mg/gram, 18.00 ± 0.70 mg/gram and 2.45 ± 0.15 mg/grams of Total alkaloid, Flavonoid and Total Saponins respectively. The final data drafted were tabulated with reference methods followed in following table 2.

Inhibition assay α-amylase activity:

The maximum inhibition α-amylase activity was 24.53 at a concentration 500 µg/ml. The percentage inhibition at 100, 200, 300, 400 and 500 µg/ml concentrations of ethanolic extract of Strychnos Nux- vomica showed a concentration-dependent reduction in percentage inhibition. Thus the highest concentration 500 µg/ml tests showed a maximum inhibition of nearly 24.53 %. The percentage inhibition varied from 4.09 % - 24.53 % from the lowest concentration to the highest concentration. Table 3 represents the inhibitory activity of α-amylase.

In vitro α- glucosidase inhibition study

The maximum inhibition α-glucosidase activity was 17.96 at a concentration 500 µg/ml. The percentage inhibition at 100,200,300,400 and 500 µg/ml concentrations of ethanolic extract of Strychnos Nux- vomica showed a concentration-dependent reduction in percentage inhibition. Thus the highest concentration 500 µg/ml tests showed a maximum inhibition of nearly 17.96 %. The percentage inhibition varied from 5.80 % - 17.96 % from the lowest concentration to the highest concentration. Table 4 represents the inhibitory activity of α- glucosidase.

DISCUSSION:

Many herbal extracts used reported for their anti-diabetic activities and being used in Ayurveda for the treatment of diabetes[15].Diabetics are prone to many difficulties due to the nature of the disease. Long-standing diabetes can lead to heart, kidney and circulation problems, including stroke [16]. α-amylase and α-glucosidase are enzymes involved in carbohydrate breakdown and intestinal absorption respectively. Inhibition of the two enzymes would effect in lower blood glucose with rich carbohydrate diet [17].

In this study, in vitro α-amylase and α-glucosidase inhibitory studies demonstrated that (50%V) Ethanolic extract inhibitory activity. The percentage inhibition at 100,200,300, 400, 500 µg/ml concentrations of Strychnos Nux-vomica ethanolic extraction on α-glucosidase and α-amylase showed a concentration-dependent reduction in percentage inhibition. The highest concentration of Strychnos Nux-vomica ethanolic extraction tests showed a maximum inhibition of nearly 24.53 and 17.96 % of α-amylase and α- glucosidase respectively. Antidiabetic effect of Strychnos Nux-vomica ethanolic extraction showed the inhibitory effect against α-amylase and a-glucosidase.

CONCLUSION:

The result of the present study that indicates Aqueous Ethanolic (50%V) extract of Strychnos Nux-vomica proved that the alpha amylase and alpha glucosidase inhibitory activity. The plants may essentially contain herbal bioactive compounds inhibiting the enzyme activity. The present study is restricted to the preliminary screening of enzyme inhibitory activities of the plant extract. It might be used for therapeutic antidiabetic treatment in the diabetes mellitus. The obtained in vitro diabetes results that will be conformed further in vivo studies.

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REFERENCES:

1. J. P. Burke, K. Williams, K. M. Narayan and C. Leibson, S. M. Heffner and M. P.Stern, Diabetes Care., **26**, 1999 (2003).
2. 2. K. G. Alberti, P. Z. Zimmet, Diabetic Medicine., 15, 535 (1998).
3. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalenceof diabetes: estimates for the year 2000 and

projections for2030. Diabetes Care. 2004; 27(5): 1047–1053.
4. Subramanian R, Asmawi AZ, Sadikun A, J Pol Biochem Soc, 2008, vol. 55:391-398.
5. Chikaraddy A, Maniyar Y, Mannapur B, Hypoglycemic activity of ethanolic extract of Lawsonia inermis Linn. (henna) in alloxan induced diabetic albino rats, Int J Pharma Bio Sci,2(4): 287-292, (2012).
6. Dineshkumar B, Mitra A, Manjunatha. M, A comparative study of alpha amylase inhibitory activities of common anti-diabetic plants at Kharagpur 1 block, Int J Green Pharm, 4(2):115-121, (2010).
7. Horbone JB., (1984) 'Phytochemical Methods'. A guide to modern techniques of plant analysis 2nd edn. Chapman and Hall, New York.
8. Kokate C.K, Purohit A.P and Gokhale S.B., In: Pharmacognosy, 3rd edition. Niralin Prakashan, Pune. 1995.
9. Krishnaveni S, Balasubramanian T and Sadasivam S. Phenol Sulphuric acid Method. Food Chemistry, 1984; 15: 229.
10. Lowry OH, Roseobrough NJ, Farr AL and Randall RJ. Protein measurement with folin phenolsreagent. Journal of Biological Chemistry, 1957; 93: 265-275.
11. Fazel Shamsa, Hamidreza Monsef, Rouhollah Ghamooshi, Mohammadreza Verdian-rizi.Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J.Pharm. Sci, 2008;32: 17-20.
12. Woisky R. and Selationo R. A analysis of propels some parameter and procedure chemical quality control. J Agr Res, 1998, 37: 99-105.
13. Hiai, S., Oura, H., Nakajima, T., Color reaction of some sapogenins and saponins with vanillin and sulfuric acid. Planta Medica, 1976; 29: 116–122.
14. Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry., 31:426-428.
15. Matsui T, Tanaka T, Tamura S, Toshima A, Tamaya K, Miyata Y, Tanaka K, Matsumoto K. Alpha-Glucosidase inhibitory profile of catechins and theaflavins. J Agric Food Chem 2007; 55:99-105.
16. Pieroni A, Price LL. Eating and healing: Traditional food as medicine. Binghamton: Haworth Press; 2005.
17. Bischoff H (1994) Pharmacology of glucosidase inhibitor. Eur J Clin Invest 24:3–10.

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