

IN-VITRO STUDIES ON α -AMYLASE & α -GLUCOSIDASE INHIBITORY ACTIVITY OF AERIAL PARTS OF *NEPTUNIA TRIQUETRA* [VAHL.] BENTH METHANOLIC EXTRACT & ITS FRACTIONS

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Abstract:

Neptunia species are group of plants belonging to fabaceae family. Some of the *Neptunia* species of genus are reported with anti-inflammatory, anti-oxidant, anti-diabetic & hepatoprotective activities. But antidiabetic activity of *Neptunia triquetra* was not investigated. Therefore in-vitro α -amylase & α -glucosidase activity is carried out to determine antidiabetic activity of *Neptunia triquetra*. The aerial parts of *Neptunia triquetra* were extracted by maceration using methanol. Later fractionation of methanolic extract is carried out by using toluene, ethyl acetate, and distilled water. The extract & its fractions underwent phytochemical analysis which indicated the presence of secondary metabolites such as alkaloids, flavonoids, steroids/triterpenoids, glycosides, tannins, phenolic compounds. The inhibitory activity was measured using established colorimetric assays such as the DNS method for α -amylase and pNPG method for α -glucosidase. The aqueous fraction showed highest in-vitro activity against α -amylase & α -glucosidase with IC₅₀ value of 5.17mg/ml & 5.73mg/ml, that can be comparable with the standard drug acarbose 5.14mg/ml & 5.01mg/ml respectively. While methanol extract and fractions showed moderate to low activity. In conclusion aqueous fraction of aerial parts of *Neptunia triquetra* methanolic extract can be used to control diabetes.

KEY WORDS: Diabetes, Acarbose, *Neptunia Triquetra*.

Abbreviations

PNPG	P-Nitro phenyl α D-glucopyranoside
DNS	Dinitrosalicylic acid
IC₅₀	Half-maximal inhibitory concentration
NTME	<i>Neptunia triquetra</i> Methanol Extract
T-NTME	Toluene <i>Neptunia triquetra</i> Methanol Extract
EA-NTME	Ethylacetate- <i>Neptunia triquetra</i> Methanol Extract
AQ-NTME	Aqueous- <i>Neptunia triquetra</i> Methanol Extract
DMSO	Dimethyl Sulfoxide
DM	Diabetes Mellitus

Introduction

Diabetes mellitus is a disorder that persists for longer time period, attributed with interruption in the metabolism of macronutrients. Increased glucose levels in blood is due to absence of insulin production [type-1 DM] or production of insulin which is not utilized by cells properly [type 2 DM] or both [Zheng et al., 2018].

Inhibition of α -amylase & alpha glucosidase enzyme systems cause decrease in digestion of carbohydrates which leads to decrease in glucose absorption due to decrease in conversion of polysaccharides into monosaccharides [Diaz-de-Cerio E et al., 2017].

Even though acarbose is used to manage diabetes, the patients using it suffer from abdominal distention, diarrhoea, flatulence, intestinal bloating that occur due to unrestricted blocking of α -amylase which cause atypical bacterial breakdown of sugars that are not digested in colon [Bischoff, 1994].

Neptunia triquetra [Vahl] Benth, belongs to fabaceae family which is also recognized as the yellow sensitive

plant found in India among grasses of cultivated lands. It is a small perennial, low prostrate herb with 1-2 pairs of pinnae, 2cm long, 8-13 pairs of leaflets, oblong rounded at apex with slender ascending compressed stems [Maheshwari, 2000]. It is used in Indian medicine for various ailments, it is used to cure eye infection using flowers, and fresh leaves juice was used as refrigerant, young stems were used as stimulant, astringent & earache with the help of its juice [Maheshwari, 2000]. The whole plant is a good tonic & used to treat jaundice, intestinal disease like diarrhoea and dysentery [Singh, 2007].

Preliminary studies on *Neptunia triquetra* have highlighted its rich phytochemical profile, particularly its high concentration of phenolic compounds & flavonoids, which are known to exert potent anti-oxidant & enzyme inhibitory activities. [Ahmed khaldoon yasir et al., 2019]

Although the plant is recognised for its medicinal potential, scientific validation of its anti-diabetic efficacy by inhibition of carbohydrate-metabolizing enzymes was not investigated previously. This reveals the necessity to further explore this medicinal plant scientifically. Therefore, the main purpose of the current investigation is to assess the in-vitro α -amylase & α -glucosidase inhibitory activity of aerial parts of *Neptunia triquetra*.

MATERIALS AND METHODS

Drugs and chemicals

α -Amylase obtained from Pancreases of porcine, Sodium potassium tartrate, Sodium dihydrogen phosphate, Disodium hydrogen phosphate, Starch, 3,5-Dinitrosalicylic acid, Yeast α -Glucosidase, Potassium dihydrogen phosphate, Dipotassium hydrogen phosphate, Para-Nitrophenyl- α -D-Glucopyranoside, Acarbose were procured from Taranath Scientific's, Hanumakonda, Telangana, India. All chemicals and solvents used meet stringent specifications

Plant material Collection

The aerial Parts of *Neptunia triquetra* [300gm] were collected from Maripeda Bangla, Khammam, and Telangana, India. Authentication of plant material was performed by Dr. MD. Mustafa [Taxonomist], Botany Department, Kakatiya University. The plant specimen [KU/UCPSC/56] was deposited in Pharmacognosy department, UCPS, Kakatiya University Hanumakonda, India. The running water is used to remove debris from plant by washing, then pulverized into small pieces, & dried under the shade.

Preparation of plant extracts

Aerial parts of *Neptunia triquetra* [150g of coarse powder] were macerated in a Florence flask using methanol for the time period of 1 week. The mixture was filtered after seven days & the maceration process was repeated with residual plant material. Then obtained

filtrate was combined and evaporated at ambient temperature to yield solid dark green extract. The resulting extract of methanol was then subjected to fractionation using toluene, ethyl acetate, & distilled water. The percentage yield of aerial parts of *Neptunia triquetra* methanol extract was 7.6%. The yields of the individual fractions were 1.873% [toluene], 0.406% [ethyl acetate], and 2.07% [aqueous]. All fractions were placed in a desiccator to remove moisture for further analysis.

Phytochemical tests

The Phytochemical tests were performed as per the standard protocol on the aerial parts of *Neptunia triquetra* methanolic extract and its fractions, so secondary metabolites like alkaloids, glycosides, terpenoids/steroids, flavonoids, saponins, tannins, phenolic compounds, can be identified

EVALUATION OF IN-VITRO α -AMYLASE & α -GLUCOSIDASE ENZYME INHIBITION ASSAY

Enzyme Inhibitory Assay of α - Amylase:

This procedure from McCue and Shetty [McCue and Shetty, 2004] was slightly modified to perform the following assay. The extracts were dissolved in DMSO at various concentrations [i.e., 1.25-10 milligram/ml] the 250 μ L of each extract from different concentrations were taken in different tubes and labelled them, to this 250 μ L of 0.02M sodium phosphate buffer [6.9-pH] possessing containing α -amylase solution [0.5 milligram/ml] was added to the each labelled tubes then at 37°C the solution was incubated for 15minutes, Later 250 μ L of 1% solution of starch in 0.02M sodium phosphate buffer [6.9-pH] was added with time interval & then incubated for 15minutes at 37°C [Momina, S. S et al., (2020)]. The 500 μ L of DNS reagent was added to retard the reaction. The tubes were incubated by placing them in boiling water for 5minutes & cooled at ambient temperature, using 5ml of distilled water the obtained reaction mixture was diluted, at 540nm absorbance was measure using UV-Visible spectrophotometer [Momina, S. S et al., (2020)].

The above procedure is carried out for preparation of control by substituting extract with distilled water. [Momina, S. S et al., (2020)].

To compare the sample activity, acarbose is used as positive control & the α -amylase inhibition was calculated using the formula given below.

$$\% \text{Inhibition} = \frac{\text{Abs}[\text{control}] - \text{Abs}[\text{extract}]}{\text{Absorbance}[\text{control}]} \times 100$$

α - Glucosidase Enzyme Inhibitory assay:

This procedure from Apostolidis et al. [2007] was slightly modified to perform the following pNPG assay. The extracts were dissolved in DMSO at various concentrations [i.e., 1.25-10 milligram/ml] the 50 μ L of each extract from different concentrations were taken in

different tubes and labelled them, to this 100µL of 0.02M phosphate buffer [6.9-pH] possessing containing α-glucosidase [yeast] solution [0.5 milligram/ml] was added & incubated for 15minutes at 37°C [Anuradha et al., 2021]. To the above reaction, 50µL of 0.1M phosphate buffer (pH6.9) containing 5Mmol/L P-Nitro phenyl-α-D-glucopyranoside solution was added & the reaction mixture was incubated in boiling water at 37°C for 5min, after cooling the solution 3mL of 100Mm Na₂CO₃ solution was added & liberated absorbance of P-Nitro phenol was measured at 405nm using (ELICO SL159 UV-Visible Spectrophotometer [Anuradha et al., 2021].

To compare the sample activity, acarbose is used as positive control & the α-glucosidase inhibition was calculated using the formula given below.

$$\% \text{Inhibition} = \frac{\text{Abs}[\text{control}] - \text{Abs}[\text{extract}]}{\text{Absorbance}[\text{control}]} \times 100$$

RESULTS:

Phytochemical analysis

Table 1: Preliminary Phytochemical analysis of *Neptunia triquetra* Methanolic Extract & its fractions are given in table below.

Class of chemical constituents & tests	NTME	T-NTME	EA-NTME	AQ-NTME
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Steroids/Triterpenoids	+	+	+	+
Glycosides/Carbohydrates	+	+	+	+
Saponins	-	-	-	-
Tannins	+	+	+	+
Phenolic compounds	+	+	+	+
‘+’: Present ‘-’: Absent				

Preliminary phytochemical screening of aerial parts of *Neptunia triquetra* methanolic extract & its fractions revealed the presence of alkaloids, flavonoids, glycosides, steroids/terpenoids, tannins & phenolic compounds.

In-vitro alpha amylase and alpha glucosidase enzyme inhibitory assay

The aerial parts of *Neptunia triquetra* methanolic extract & its fractions were tested for α-amylase and α-glucosidase enzyme inhibitory properties along with standard drug acarbose by colorimetric method.

In this study, at 10mg/ml concentration Methanol extract of aerial parts of *Neptunia triquetra* and its fractions exhibited [Toluene, Ethyl acetate, Aqueous] 88.06%, 74.2%, 76.06%, and 87.07%, α-amylase inhibitory activity [fig. 1] & 82.97% 55.47% 76.11% 84.13% α-glucosidase inhibitory activity [fig. 2] while standard drug Acarbose showed 92.5% α-amylase inhibitory activity & 95.5% α-glucosidase inhibitory activity at same concentration with IC₅₀ value of 5.14mg/ml for α-amylase & IC₅₀ value 5.01mg/ml α-glucosidase. Among the entire test sample the aqueous fraction exhibited most potent enzyme inhibitory activity with IC₅₀ value of 5.17mg/ml α-amylase & 5.73mg/ml α-glucosidase inhibitory activity. The aqueous fraction is highly comparable with standard drug acarbose, when compared with the other fractions and methanolic extract even though it is less potent than the acarbose.

Table 2: Effect of NTME, T-NTME, EA-NTME, AQ-NTME, & Acarbose on α- Amylase & α- glucosidase.

Test sample	IC ₅₀ of α-amylase	IC ₅₀ of α-glucosidase
Methanol	5.44±0.047	5.77±0.096
Toluene	6.24±0.019	8.36±0.041
Distilled water	5.17±0.014	5.73±0.076
Ethyl acetate	6.04±0.004	6.34±0.021
Acarbose	5.14±0.010	5.01±0.002

Figure1: Porcine pancreatic α-amylase inhibition by aerial parts of *Neptunia triquetra* methanolic extract, aqueous, ethyl acetate, toluene fractions and reference drug acarbose. Data is expressed in mean ±SD, [n=3].

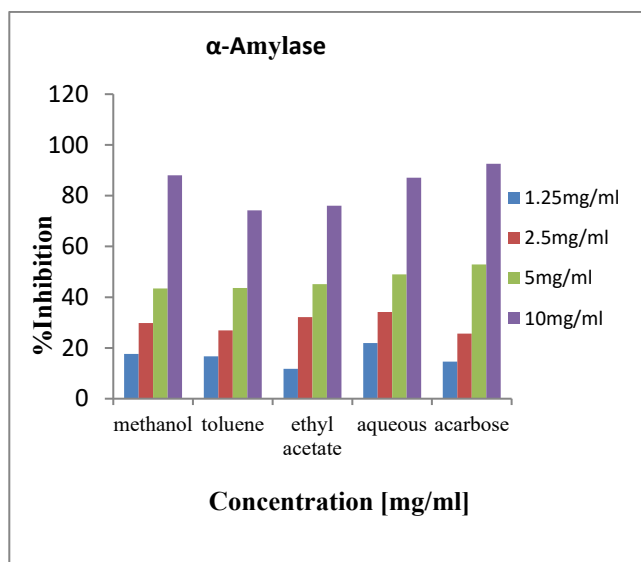
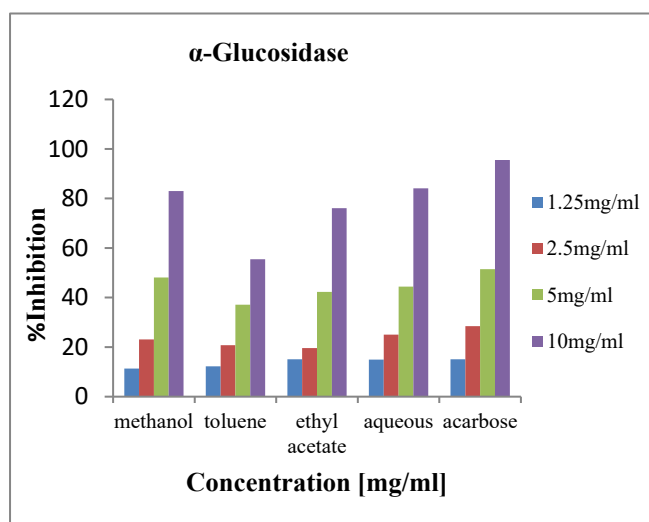


Figure 2: Inhibition of yeast α -glucosidase by aerial parts of *Neptunia triquetra* methanolic extract, aqueous, ethyl acetate, toluene fractions acarbose. Data is expressed in mean $\pm$ SD, [n=3].



Discussion:

Pancreatic α -amylase is a prominent enzyme found in the pancreatic juice hydrolyses complex polysaccharides to produce oligosaccharides, disaccharides while α -glucosidase located in the brush border cells of the small intestines, hydrolyses oligosaccharides and disaccharides to monosaccharides that are absorbed through the small intestine into hepatic portal vein and increase postprandial glucose levels [Asanuma-Date et al., 2012].

α -amylase and α -glucosidase Inhibition is important in decreasing post prandial blood glucose level by minimizing conversion of polysaccharides into oligosaccharide, disaccharide which gets reduced into monosaccharides [Zhu, Z. Y., Liu, W., Lu, J. J., &

Zhang, Y, 2021]

The phytochemical analysis of the aerial parts of *Neptunia triquetra* methanolic extract and its fractions [toluene, aqueous, ethyl acetate] shown the presence of secondary metabolites such as alkaloids flavonoids steroids/triterpenoids glycosides, tannins, phenolic compounds, that exhibit anti-hyperglycemic activity by inhibiting the α -amylase and α -glucosidase.

The flavonoids, phenolic compounds are responsible for major medicinal properties, including antioxidant property, which is associated with many disease, one of the disease include diabetes therefore, phytochemical screening is performed to know the presence of the flavonoids and phenolic compounds which helps in managing diabetes.

The studies performed showed potent dose-dependent inhibition against α -amylase and α -glucosidase for Aqueous fraction of *Neptunia triquetra* when compared with acarbose, while other extracts [NTME > EA-NTME > T-NTME] showed moderate to less activity.

Conclusion:

From the performed phytochemical screening of aerial parts of *Neptunia triquetra* we can conclude that various secondary metabolites are present in this plant. The enzyme inhibition studies of aerial parts of *Neptunia triquetra* methanolic extract, & toluene, ethyl acetate, aqueous fractions of methanolic extract showed significant α -amylase and α -glucosidase enzyme inhibitory activity when compared with standard acarbose, however aqueous fraction showed more activity when compared with methanolic extract, toluene & ethyl acetate fractions. However, further study is needed to conform the phytochemical responsible for the activity.

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Declarations

Conflict of interest: No conflict of interest

Ethical approval: Not applicable

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