Advances in Bioresearch
Adv. Biores., Vol 14 (5) September 2023: 301-306
©2023 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.14.5.301306

Advances in Bioresearch

ORIGINAL ARTICLE

In-vitro antioxidant and antidiabetic activity evaluation of *Vitex peduncularis* leaves Extracts

Pradeep Kumar Tiwari[1], Mohan Lal Kori[1], Neha Jain[1*]
[1]Vedica College of B. Pharmacy
Ram Krishna Dharmarth Foundation University, Bhopal (M.P.)
*Address for correspondence
Dr Neha Jain
Email id: nehasniper@gmail.com

ABSTRACT
Vitex Peduncularis is a moderate sized tree found in Bangladesh, India and many other countries. The plants of this genus have an overabundance of ethanopharmacological usage for treating a range of human ailments. This study evaluated the antioxidant and antidiabetic potential of Vitex Peduncularis using an in-vitro model. The antioxidant activity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl and superoxide anion radical scavenging abilities of the Ethyl acetate and Ethanol and aqueous extracts of Vitex Peduncularis. In vitro antidiabetic potential was assessed by evaluating the inhibitory effects of the extracts on the activities of α-amylase, α-glucosidase, maltase and sucrase. Ethanol extract displayed significantly higher ($p < 0.05$) DPPH and superoxide radical scavenging and hydroxyl radical scavenging activities. The ethanol extract displayed significantly higher ($p < 0.05$) inhibition of α-glucosidase, and strongest inhibition of α amylase. It can be concluded that Vitex Peduncularis extracts possessed antioxidant and antidiabetic activities, through the inhibition of diabetes-related enzymes.
Keywords: Vitex Peduncularis, α-glucosidase, DPPH

Received 22.05.2023 Revised 11.06.2023 Accepted 26.08.2023

How to cite this article:
Pradeep Kumar T, Mohan L K, Neha J. *In-vitro* antioxidant and antidiabetic activity evaluation of *Vitex peduncularis* leaves Extracts. Adv. Biores., Vol 12 (5) September 2023: 301-306.

INTRODUCTION

Diabetes mellitus (DM) is a major public health issue affecting more than 400 million people worldwide [1]. This metabolic disorder progressively leads to chronic microvascular, macrovascular and neuropathic life-threatening complications. DM is caused either by deficiency of insulin secretion, damage of pancreatic β cell or insulin resistance related to non-use of insulin. Inclination to sedentary lifestyle may be the major reason for the continual rise in the number of diabetic patients globally which is expected to strike 366 million in 2030 in the elderly population (>65 years) [2].The various complications associated with DM includes nephropathy, neuropathy, cardiovascular and renal complications, retinopathy, food related disorders and so on. Type 1 DM and type 2 DM are the 2 types of DM. Type 1 DM is an autoimmune disorder that affects pancreatic cells which reduces or impairs the production of insulin while type 2 DM is a result of impairment of pancreatic beta cells that hinder the individual's ability to use insulin [3]. It is well established that diabetes mellitus is a condition with a range of abnormalities like micro and macro vascular complications. The high degree of hyperglycemia in patients with DM associates with macrovascular complications as coronary artery disease, peripheral arterial disease, stroke, and with microvascular complications as diabetic nephropathy, neuropathy, and retinopathy. The formation of several proinflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor (TNF) in diabetic patients emerge the chronic inflammation in the progression of DM and its complications. Oxidative stress, inflammation, and dyslipidemia contribute to the development of diabetes associated complications. Therefore, it is important to develop antidiabetic agents targeting diabetes and its associated complications. The major conventional classes of drugs for the treatment of hyperglycemia includes sulfonylureas (enhance release of insulin from pancreatic islets); biguanides (reduces hepatic glucose

production); peroxisome proliferator-activated receptor-γ (PPARγ) agonists (boosts the action of insulin); α-glucosidase inhibitors (interferes with absorption of glucose in the gut). These classes of drugs are either administered as monotherapy or given in combination with other hypoglycaemics. Severe hypoglycemia, weight gain, lower therapeutic efficacy owing to improper or ineffective dosage regimen, low potency and altered side effects due to drug metabolism and lack of target specificity, solubility and permeability problems are the major drawbacks associated with the use of the above-mentioned conventional drugs [4]. Medicinal plants that have been traditionally used to produce a variety of compounds with known therapeutic properties. The plants of this genus have an overabundance of ethanopharmacological usage for treating a range of human ailments. The antidiabetic potentials of this have not been explored yet. So, the aim of present study is to evaluate the antioxidant and antidiabetic potentials of different extracts of Vitex Peduncularis using an in-vitro model.

MATERIAL AND METHODS

Plant material and extract preparation

The leaves of Vitex Peduncularis leaves were collected from the Bhopal (M. P.). The plant was identified and authenticated. Fresh leaves were used for Pharmacognostical studies. The leaves were powdered to 60# separately and stored in airtight containers and used for further studies. Vitex Peduncularis leaves powders were extracted sequentially with petroleum ether, ethyl acetate and ethanol using continuous hot extraction method i.e. soxhlet extraction. The aqueous extracts were get by maceration method.

Chemicals and reagents: Porcine pancreatic a-amylase, rat intestinal a-glucosidase, 1,1- diphenyl-2-picrylhydrazyl, gallic acid, acarbose and *para*- nitrophenyl–glucopyranoside were products of Sigma–Adrich Co., St. Louis, USA while starch soluble (extra pure) 1,1-diphenyl-2-picryl hydrazyl (DPPH), DMSO, Ascorbic acid Sodium nitro prusside, Griess reagent (1%w/v sulphanilamide, 2% w/v H3PO4 and N-(1-naphthyl) ethylene diamine di hydrochloride), Hydrogen peroxide (H2O2) Thiobarbituric acid Sodium phosphate buffer, 1mM ferric chloride Nitro blue tetrazolium Phenazene methosulphate, Nicotinamide adenine phosphate di nucleotide was obtained from J. T. Baker Inc.,. Other chemicals and reagents were of analytical grade and distilled water was used.

Preparation of sample: Extracts were dissolved in dimethysulphoxide (DMSO) to give stock solutions of 1.0 mg/mL and different concentrations (25, 50, 75, 100 and 200 µg/mL) of the extracts were prepared using a serial dilution method with distilled water. All extracts were stored at 4 ◦C prior to analysis.

DPPH free radical scavenging ability: The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical was evaluated by a modified method of Saha et al. [9]. Different concentrations (25, 50, 75, 100 and 200 µg/mL) of the extracts (100 mL) were mixed with 100 mL of 0.4 mmol/L methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm. The DPPH free radical scavenging ability of each extract was subsequently calculated with respect to the reference (which contains all the reagents without the test sample). Ascorbic was used as a positive control.

Hydroxyl radical scavenging ability: The ability of the plant extracts to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose was carried out using the modified method described by Oboh and Rocha [10]. Briefly, 50 mL freshly prepared different concentrations (25, 50, 75, 100 and 200 µg/mL) of the extracts was added to a reaction mixture containing 25 mL 20 mM deoxyribose, 100 mL 0.1 M phosphate buffer, 10 mL 20 mM hydrogen peroxide and 10 mL 500 mM $FeSO_4$, and the volume was made up to 200 mL with distilled water. The reaction mixture was incubated at 37 ◦C for 30 min, and the reaction was stopped by the addition of 50 mL of 2.8% TCA (trichloroacetic acid), this was followed by the addition of 50 mL of 0.6% TBA solution. The mixtures were subsequently incubated for 20 min and the absorbance was measured at 532 nm by UV-Visible spectrophotometer (Shimadzu UV-Vis1800). Ascorbic was used as a positive control.

Nitric Oxide (NO) radical scavenging activity: Nitric oxide (NO) was generated from sodium nitro prusside (SNP) and was measured by the Griess reagent (1% w/v sulfanilamide, 2%w/v H3PO3 and 0.1% w/v N-(1-Naphthyl) ethylene diamine dihydrochloride). SNP in aqueous solution at physiological PH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by the use of griess reagent. Scavengers of NO compete with oxygen leading to reduced production of NO. SNP (1ml of mM) was mixed with 1ml of selected plants extracts in different concentrations in DMSO, incubated at 25 ºC for 180 minutes. To 1ml of the incubated solution, 1ml of griess reagent was added. The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with N-(1-naphtyl) ethylene diamine dihydrochloride was read at 546nm by UV-Visible spectrophotometer (Shimadzu UV-Vis1800). All the test analysis were run in triplicate and averaged.

 © 2023 Author

Lower absorbance of reaction mixture indicates higher free radical scavenging activity. Ascorbic was used as a positive control.

Superoxide radical scavenging ability: Superoxide radical scavenging activity of the extracts was based on the method described by Liu et al. (1997) [11]. Superoxide radicals were generated in 25 mL of Tris-HCl buffer (16 mM, pH 8.0) containing 25 mL of NBT (50 mM) solution, 25 mL NADH (78 mM) solution and different concentrations (25, 50, 75, 100 and 200 µg/mL) of extracts (50 mL). The reaction started by adding 1 mL of phenazine methosulphate (PMS) solution (10 mM) to the mixture then incubated at 25 °C for 5 min The absorbance measured at 560 nm. Ascorbic acid was used as a positive control.

***In-vitro* antidiabetic activity**

α-Amylase inhibitory assay: α-Amylase inhibitory assay was carried out using a modified method of McCue and Shetty (2004) [12]. *Vitex Peduncularis* extract (25, 50, 75, 100 and 200 µg/mL) (100 ml) was took in a conical flask and added 100 mL of 0.02 M sodium phosphate buffer (pH 6.9) containing a-amylase solution. This solution was pre-incubated at 25 °C for 10 min, after which 100 mL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals and then kept at 25 °C for 10 min. The reaction was terminated by adding 200 mL of dinitrosalicylic acid (DNS) reagent. The flask was then kept in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 mL distilled water and the absorbance was measured at 540 nm using a uv spectrophotometer. The control was prepared using the same procedure replacing the extract with distilled water while activity of the standard was tested by replacing the extract with acarbose. The α-amylase inhibitory activity was calculated as percentage inhibition, and 50% inhibition of enzyme activity (IC50) were determined.

$$\% \text{ Inhibition} = \frac{\text{A control} - \text{A extract}}{\text{A control}} \times 100$$

α-Glucosidase inhibitory assay:

α-Glucosidase inhibitory assay was carried out using a modified method by Adisakwattana *et al*. [13]. Rat intestinal acetone powder (100 mg) was homogenized in 3 mL of 0.9% NaCl solution. After centrifugation (30 min), the crude enzyme (100 mL) was incubated with 5 mM *p*-nitrophenyl glucopyranoside (pNPG), 25 mM maltose or 50 mM sucrose in 0.1 M phosphate buffer, pH 6.9 and 50 mL of the different concentrations (25, 50, 75, 100 and 200 µg/mL) of extracts at 37 °C for 30 min. The reaction mixture was stopped by adding 50 mL of 0.1 M Na_2CO_3. The enzyme activities were determined by measuring the absorbance at 405 nm (α-glucosidase). The control was prepared using the same procedure replacing the extract with distilled water while activity of the standard was tested by replacing the extract with acarbose. Percentage inhibition 50% inhibition of enzyme activity (IC50) was determined.

$$\% \text{ Inhibition} = \frac{\text{A control} - \text{A extract}}{\text{A control}} \times 100$$

Statistical analysis: Statistical analysis was performed using GraphPad Software. The data were analyzed by one way analysis of variance (ANOVA). All the results were expressed as mean SEM for triplicate determinations.

RESULTS

Analysis of the free radical scavenging activities of the selected *Vitex Peduncularis* leaves extracts revealed a concentration dependent free radical scavenging activity resulting from reduction of DPPH, NO, Hydroxyl radical and superoxide radical radical to non-radical form. The scavenging activity of Ascorbic acid, a known antioxidant used as positive control, was however higher. The order of reduction potential was found more by ethanol extract.

The ethanol extract of *Vitex Peduncularis* leaves at varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity compared to other extract. Results revealed that all the tested extracts showed the percentage of inhibition in a dose dependent manner. Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins. All the results showed hydroxyl radical scavenging activity in a dose dependent manner.

The ethanol extract of *Vitex Peduncularis* leaves at varied concentrations showed remarkable inhibitory effect of Hydroxyl radical scavenging activity compared to ethyl acetate and aqueous extract. Superoxide is a reactive oxygen species, which can cause damage to the cells and DNA leading to various diseases. The ethanol extract of *Vitex Peduncularis* leaves at varied concentrations showed remarkable inhibitory effect of superoxide radical activity scavenging compared to ethyl acetate and aqueous extract. The ethanol extract of *Vitex Peduncularis* leaves extract at varied concentrations showed remarkable inhibitory effect of superoxide radical scavenging activity compared to ethyl acetate and aqueous extract.

 © 2023 Author

The IC50 value for the free radical scavenging abilities of the Vitex Peduncularis leaves extracts is presented in Table 1. Ethanol extract had the lowest IC50 for DPPH (25.26 mg/mL), which is significantly lower ($p < 0.05$) than other extracts and comparable to standards (ascorbic acid). Ethanol extract possessed the lowest IC50 (35.69 mg/mL) for hydroxyl radical scavenging ability. Aqueous extract also displayed the significant IC50 value for the inhibition of superoxide anion radicals, which is significantly different from other extracts and standard (ascorbic acid). The in vitro alpha amylase inhibitory studies demonstrate that the ethanol extract having good alpha amylase inhibitory activity. The percentage of inhibition at various concentrations of Vitex Peduncularis leaves showed a concentration dependent reduction in the activity. The highest concentration (200µg/ml) showed maximum inhibition of nearly 95%.
Figure 5-6 shows the inhibitory effects of different extracts of *Vitex Peduncularis leaves extract* against a-amylase and α-glucosidase. In the a-amylase assay, there were significant differences ($p < 0.05$) in the percentage inhibition of the enzyme amongst all the extracts tested except at 25 and 50 µg/mL. At all concentrations tested, there were significant differences ($p < 0.05$) amongst all the extracts in the α-glucosidase assay and α-amylase. However, at higher concentrations, ethanol extract exhibited highest inhibition which is significantly different than ethyl acetate and aqueous extracts. The IC50 values for the inhibition of a-amylase and a-glucosidase by *Vitex Peduncularis* leaves extract. Ethanol extract exhibited the lowest (52.71 µg/ml) IC50 for the inhibition of a-amylase and α-glucosidase (58.65 µg/ml) this is significantly comparable with ($p < 0.05$) the standard (acarbose).

DISCUSSION

The antioxidant activities of plants may act by preventing the production of free radicals or by neutralizing/scavenging free radicals produced in the body or reducing the transition metal composition. The results showed that ethanol extract had the lowest IC50 for the DPPH, superoxide anion scavenging, hydroxyl radical and nitric oxide inhibition ability. The DPPH radical scavenging ability of a plant extract is based on its ability to decolourize the deep purple colour measured from changes in absorbance. This implies that aqueous extract decolourize DPPH most and so possessed the best ability to scavenge the DPPH radicals. Superoxide radical anion (O2—) originates from the one-electron reduction of free molecular oxygen by nicotinamide adenine dinucleotide phosphate oxidase, which is the membrane-bound enzyme. This radical was also effectively scavenged by ethanol extract of Vitex Peduncularis leaves compared to the other extracts and had similar results to the standard (ascorbic acid). The control of postprandial plasma glucose levels is important in the treatment of diabetes mellitus and its associated complications. Inhibition of enzymes involved in the metabolism of carbohydrates such as a-amylase and α-glucosidase, is an important therapeutic approach for reducing postprandial hyperglycemia. α- Amylase found in the saliva and pancreatic juice, catalyses the hydrolysis of polysaccharides (such as starch) to disaccharides (like maltose and sucrose).
Though ethanol extract of Vitex Peduncularis leaves displayed the more inhibition of α-amylase (IC_{50} 64.71 µg/ml) and comparable to standard (acarbose) (IC_{50} 62.91 µg/ml). α-Glucosidases are located in the mucosal brush border of the small intestine and catalyse the conversion of disaccharides to monosaccharide like glucose. In this study, ethanol extract of Vitex Peduncularis exhibited the strongest inhibition of α-glucosidase. This is depicted by the lowest IC50 value of ethanol extract (76.65 µg/ml). The antioxidant and antidiabetic activities of different extracts of *Vitex Peduncularis* may be due to the presence of phytochemicals such as alkaloids, tannins, saponins and cardiac glycosides.

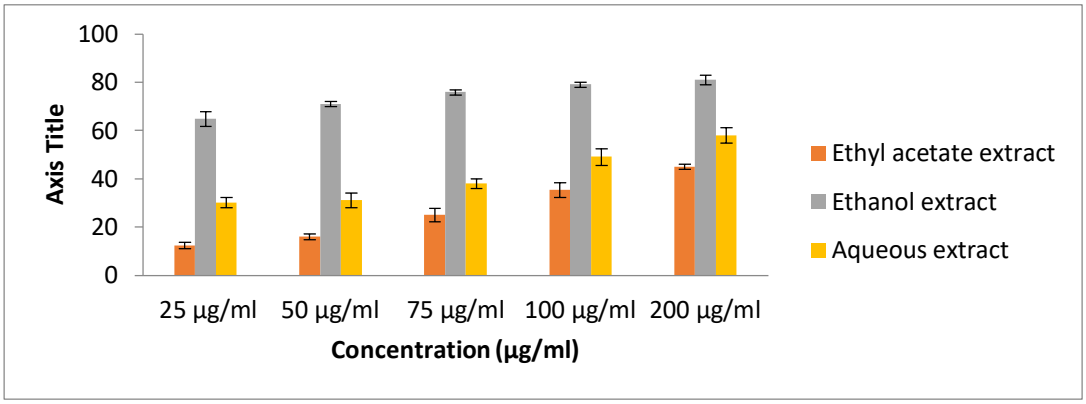

Fig. 1: Results of *Vitex Peduncularis* leaves extracts on DPPH radical scavenging model

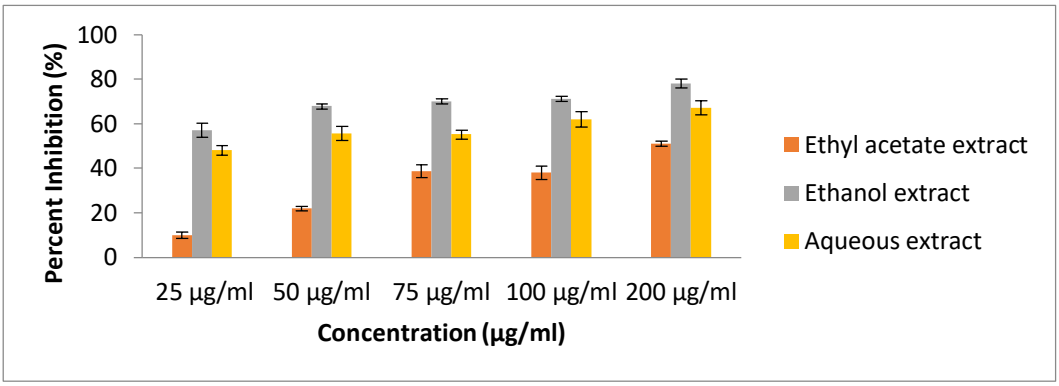

Fig. 2: Results of *Vitex Peduncularis* leaves extracts on Nitric oxide radical scavenging model

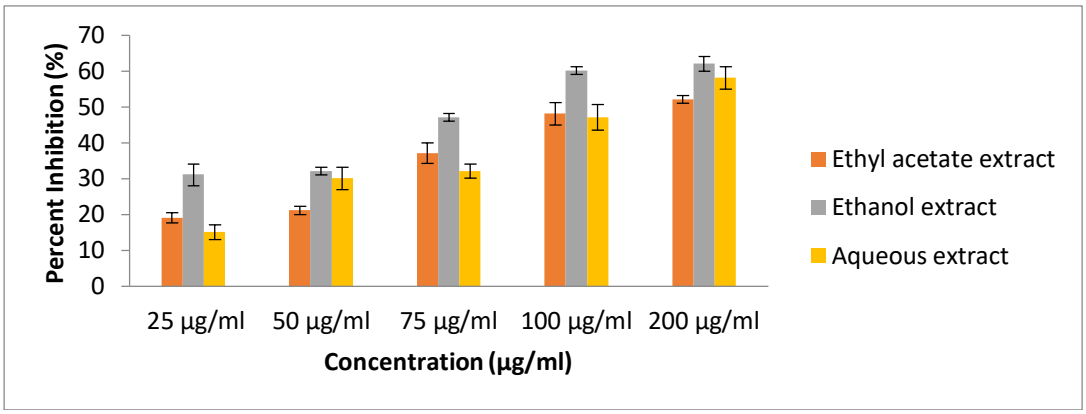

Fig. 3: Results of *Vitex Peduncularis* leaves extracts on Hydroxyl radical scavenging model

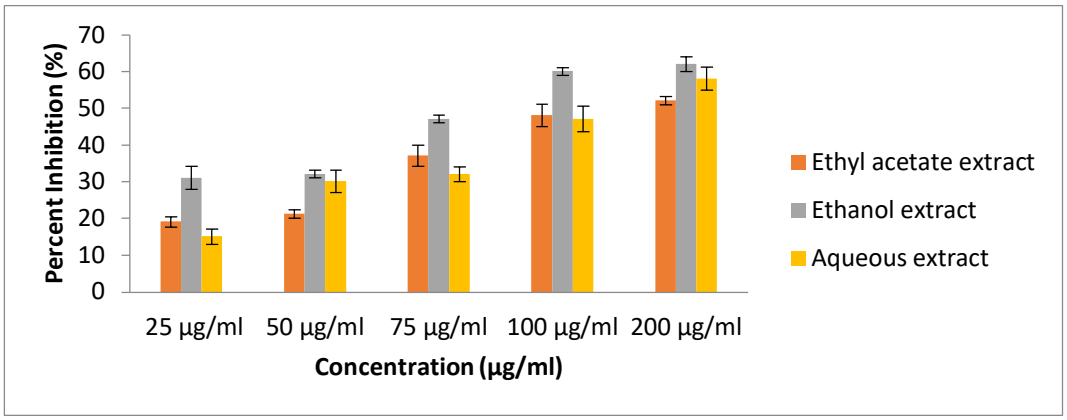

Fig. 4: *Vitex Peduncularis* leaves extracts on Super oxide radical scavenging activity

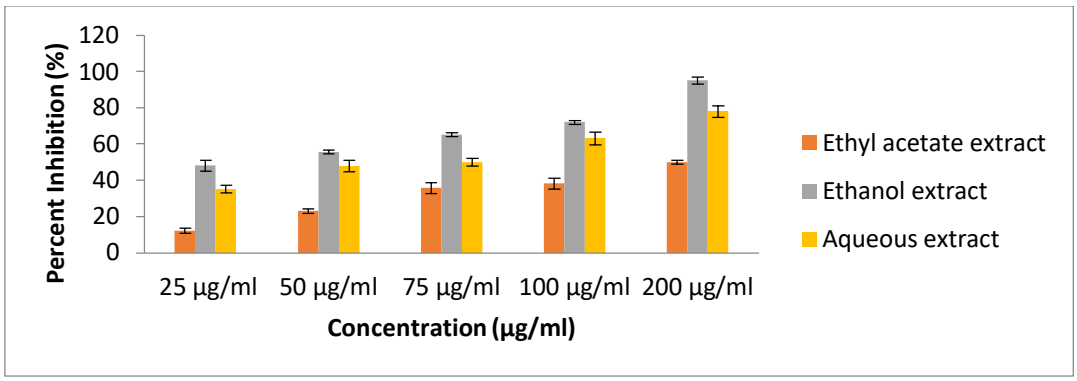

Fig. 5: In vitro alpha amylase inhibitory activity of *Vitex Peduncularis* leaves extracts

 © 2023 Author

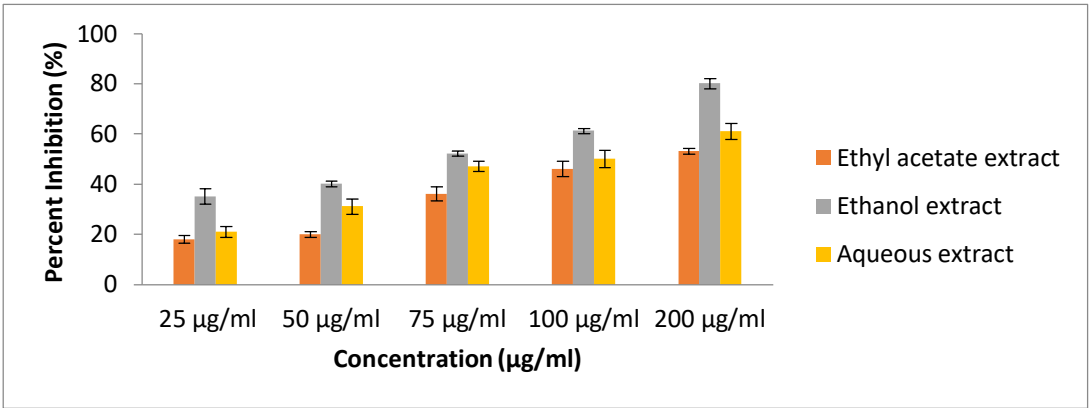

Fig. 6: *In vitro* Alpha Glucosidase inhibitory activity of *Vitex Peduncularis* leaves extracts

CONCLUSIONS

This study showed that *Vitex Peduncularis* leaves extracts possessed antioxidant and antidiabetic activities. One of the possible mechanisms of antidiabetic action of this plant is through the inhibition of diabetes-related enzymes. Therefore, this study supports the usage of Vitex Peduncularis leaves in the management of diabetes.

REFERENCES

1. R. Khursheed, S. Singh, S. Wadhwa, B. Kapoor, M. Gulati, R. Kumar, et al., (2019). Treatment strategies against diabetes: Success so far and challenges ahead, Eur. J. Pharmacol. 862; 172625, https://doi.org/10.1016/ j.ejphar.2019.172625.
2. S. Wild, G. Roglic, A. Green, R. Sicree, H. King, (2004). Global Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030, Diabetes. Care 27; 1047–1053.
3. C. Wong, H. Al-Salami, C. Dass, (2017). Potential of insulin nanoparticle formulations for oral delivery and diabetes treatment, J. Control. Release 264; 247–275.
4. A. Chaudhury, C. Duvoor, V.S.R. Dendi, S. Kraleti, A. Chada, R. Ravilla, et al.,(2017). Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management, Front. Endocrinol. 8; 1–12, https:// doi.org/10.3389/ fendo.2017.00006.
5. M. Okur, I. Karantas, P. Siafaka, (2017). Diabetes Mellitus: A Review on Pathophysiology, Current Status of Oral Pathophysiology, Current Status of Oral Medications and Future Perspectives, ACTA Pharm Sci. 55; 61, https://doi.org/10.23893/ 1307-2080.aps.0555.
6. A. Mayorov, (2011). Insulin resistance in pathogenesis of type 2 diabetes mellitus, Diabetes. Mellitus. 14; 35–45.
7. A. Ojha, U. Ojha, R. Mohammed, A. Chandrasekhar, H. Ojha, (2019). Current perspective on the role of insulin and glucagon in the pathogenesis and treatment of type 2 diabetes mellitus, Clin Pharmacol. 11. 57–65, https://doi.org/10.2147/ CPAA.S202614.
8. K. Kaku, (2010). Pathophysiology of Type 2 Diabetes and Its Treatment Policy, JMAJ 53. 41–46.
9. Saha, M.R., Hasan, S.M.R., Akter, R., Hossin, M.M., Alam, M.S., Alam, M.A., et al., (2008). In vitro free radical scavenging activity of methanol extract of the leaves of Mimusops elengi Linn. Bangladesh J. Vet. Med. 6, 197–202.
10. Oboh, G., Puntel, R.L., Rocha, J.B.T., (2007). Hot pepper (Capsicum annuum, Tepin and Capsicum chinese, Habanero) prevents Fe2+-induced lipid peroxidation in brain: in vitro. Food Chem. 102, 178–185.Liu, F., Ooi, V.E.C., Chang, S.T., 1997. Free radical scavenging activity of mushroom polysaccharide extracts. Life Sci. 60, 763–771.
11. Liu, F., Ooi, V.E.C., Chang, S.T., (1997). Free radical scavenging activity of mushroom polysaccharide extracts. Life Sci. 60, 763–771.
12. McCue, P.P., Shetty, K., (2004). Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase in vitro. Asia Pac. J. Clin. Nutr. 13, 101–106.
13. Adisakwattana, S., Charoenlertkul, P., Yibchok-Anun, S., (2009). Alpha-glucosidase inhibitory activity of cyanidin-3-galactoside and synergistic effect with acarbose. J. Enzyme Inhib. Med. Chem. 24, 65–69.

Copyright: © 2023 Author. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.