WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 3, 1564-1571. Research Article ISSN 2277– 7105

ALPHA-AMYLASE INHIBITION ASSAY AND QUALITATIVE ANALYSIS OF AQUEOUS FOLIAR EXTRACT OF PIPER BETLE

Varsha Chauhan, Dhananjay Kashyap and Neha Chauhan*

Department of Life Sciences, ITM University, Gwalior (M.P), India.

Article Received on
31 Dec. 2020,

Revised on 21 Jan. 2021,
Accepted on 11 Feb. 2021

DOI: https://doi.org/10.17605/OSF.IO/Z3CMK

***Corresponding Author**
Neha Chauhan
Department of Life Sciences, ITM University, Gwalior (M.P), India.

ABSTRACT

Piper betle is a member of Piperaceae family. It is a perennial dioecious, semi woody climber Stems strongly swollen at the nodes, papillose when young, soon entirely glabrous. Leaves alternate, simple and yellowish green to bright green in colour. Piper betle is a Vedic plant and its Vedic name is Saptasira6 and in Sanskrit it known as Tambool, Nagvelleri, Nagani, etc. and were used as remedy against various diseases. Piper betle is cultivated in India, China, Srilanka, Malaysia, Indonesia, Vietnam, Nepal and East Africa. Betel leaves have a strong pungent flavour and is widely used as a masticatory. It has shown various biological activities such as Antimicrobial, Anticancer, Antihistaminic, Anti-inflammatory, Antioxidant, Antihaemolytic, Antiulcer, Antidiabetic, Antiallergic, Antimalerial, Antituberculosis, Cytotoxic activity, Oral Hygiene, Wound Healing, etc. Traditionally it is used to treat Headache, Scanty, Weakness of nerves, Respiratory disorder, Constipation, Breast milk secretion, Inflammation and Boils. Many Research studies suggest herbal remedy of Piper betle to treat diabetes or overcome blood glucose level. Leave extract of Piper betle contains flavonoids and phenols which have antidiabetic effect. Result of this study revealed that leaves extract showed α-amylase inhibition in a dose dependent manner. The extracts showed maximum inhibition at a concentration of 5 mg/ml and which is decreased with decreasing concentrations i.e. 2.5 and 1 mg/ml. In conclusion, more research is required for developing a potential and valuable antidiabetic therapy by using α- amylase inhibition. Study also reveals that plant leaves also has phytochemicals in good quantity.

KEYWORDS: Piper betle, antidiabetic, phytochemicals.

INTRODUCTION

Diabetes is characterized by higher levels of blood glucose, it is chronic in nature and a metabolic disease. It will lead to serious damage to various vital organs such as eyes, heart, kidneys, nerves and blood vessels over a period of time.[1] About 422 million individuals worldwide have diabetes, particularly in low and middle-income countries, and 1.6 million deaths are directly attributed to diabetes annually.[1] The number of individuals with diabetes has risen from 108 million (1980) to 422 million (2014). Globally around 4% population was affecting and could be predictable to increase by 5.4% in 2025.[2,3] The most common is type 2 diabetes, it usually occurs in adults when the body becomes resistant to insulin or does not make enough insulin.[1] Type 1 diabetes is insulin-dependent diabetes, it is a chronic condition within which the pancreas produces little or no insulin by itself.[1]

The available therapy for diabetes includes various oral anti-diabetic agents such as sulfonylureas, thiazolidinedione's and α-glucosidase inhibitors etc. Henceforth antidiabetic drug discovery has shifted to focus on natural product and plant sources that having minimal side effects. Plants have played an important role in the new therapeutic agents for the antidiabetic drug.[3] Biological activities of the plant products used as alternative medicines to treat diabetes are in relevance to their chemical composition.[4,5] Herbal/Plant products are rich in flavonoids, phenolic compounds, coumarins, terpenoids and other constituents which help to reduce blood glucose levels.[4] Polyphenolic compounds which are present in plants can be used to regulate oxidation and stress-related chronic diseases, such as diabetes and cardiovascular diseases.[6] The antidiabetic activity of Piper betle hot water extract was the first scientific report in the world performed in rats.[2] The aqueous extract of Piper betel leaves possess marked hypoglycemic activity when tested in fasted normoglycaemic rat.[7] The normoglycaemic and streptozotocin induced diabetic rats using oral administration of hot water extract and cold ethanolic extract.[8] The ability of lowering blood glucose/sugar level of Streptozocin induced diabetic rat gives a suggestion that the extract have the insulin mimetic activity.[7] Amylase is an enzyme which hydrolyzes starch and glycogen as reserve carbohydrate in plants and animals respectively into reducing fermentable sugars, mainly maltose, and reducing limit dextrin's.[9] Amylase is mainly classified in three forms such as α-amylases, β-amylase and ɣ- amylase. The α-amylases are found in all types of organs and tissues.[9] The highest concentrations of amylase are found within the saliva and pancreas in animals. It is a major digestive enzyme having optimum pH is 6.7-7.0.[9] The pancreatic amylase hydrolyzes dietary starch into disaccharides and trisaccharides.[9]

Piper betle is a member of Piperaceae family. It is a perennial dioecious, semi woody climber. [8] Stems strongly swollen at the nodes, papillose when young, soon entirely glabrous. Leaves alternate, simple and yellowish-green to bright green in colour.[8] Piper betle is a Vedic plant and its Vedic name is Saptasira and in Sanskrit it known as Tambool, Nagvelleri, Nagani, etc. and were used as remedy against various diseases.[7] Piper betle is cultivated in India, China, Srilanka, Malaysia, Indonesia, Vietnam, Nepal and East Africa.[2,3,5,7,8,10] Betel leaves have a strong pungent flavour and is widely used as a masticatory.[2,10] It has shown various biological activities such as Antimicrobial, Anticancer, Antihistaminic, Anti-inflammatory, Antioxidant, Antihaemolytic, Antiulcer, Antidiabetic, Antiallergic, Antimalerial, Antituberculosis, Cytotoxic activity, Oral Hygiene, Wound Healing, etc.[11,12] Traditionally it is used to treat Headache, Scanty, Weakness of nerves, Respiratory disorder, Constipation, Breast milk secretion, Inflammation and Boils.[12] Many Research studies suggest herbal remedy of Piper betle to treat diabetes or overcome blood glucose level. Leave extract of Piper betle contains flavonoids and phenols which have antidiabetic effect.

MATERIALS AND METHODS

Solvent extraction of piper betle leaves

Fresh and disease free leaves of Piper Betle were collected from the botanical garden and used for solvent extraction. Collected leaves were washed with distilled water and shed dried at room temperature. The shed dried leaves were grinded and stored in air-tight container for the further use of extraction with solvents. Three different solvents such as petroleum ether, ethanol and distilled water were used for the extraction using Soxhlet method.

Phytochemical analysis

Qualitative analysis[13,14,15,16]

Qualitative analysis was done by the following methodology.

Test for alkaloids

To the extract added 1% HCl and 6 drops of Mayer's reagent and Dragendroff reagent. An organic precipitate indicated the presence of alkaloids in the sample.

Test for flavonoids

5 ml of dilute ammonia solution were added to a portion of aqueous filtrate of each plant extract followed by addition of conc. H2SO4. A yellow coloration is observed which confirms the presence of flavonoids and it disappears on standing.

Test of glycosides

Dissolve small amount of an alcoholic extract of the fresh or dried material in one ml of water. Add a few drops of aqueous NaOH solution. Yellow color indicates the presence of glycoside.

Test for steroids

2 ml of acetic anhydride was added to 0.5gm of ethanolic extract of each sample with 2 ml of H2SO4. The color change from violet to blue or green indicated the presence of steroids.

Test for tannins

5 ml of extract was added to few drops of 1% lead acetate. A yellow precipitate indicated the presence of tannins.

Test for terpenoids

5 ml of each extract was added to 2 ml of chloroform and 3 ml of conc. H2SO4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the terpenoids.

α- Amylase assay by DNSA method Alpha

α- Amylase assay was performed by following methodology of Juvekar et al. 2014 and Gayathri et al. 2013.[15,16] One twenty micro-liter of plant extract was mixed with 480 µl of distilled water and 1.2 ml of starch solution (1 g starch in 0.02 M sodium phosphate buffer containing 0.0067 M of sodium chloride in 100 ml) was added. The reaction was initiated by adding 600 µl of enzyme solution (1 mg of α- amylase in 10ml of 0.02 M of sodium phosphate at PH 6.9) were added into the mixture and kept at room temperature for 3 minutes. After 3 minutes 600 µl of the mixture was transferred into separate test tube which contains 300 µl of DNSA colour reagent (1 g 3,5-dinitrosalicyclic acid, 30 g sodium potassium tartrate and 20 µl of sodium hydroxide to final volume of 100 ml in distilled water), test tube were kept into the water bath for 15 minutes at 85-90˚C. After water bath sample was allowed to cool down at room temperature and 2.7 ml of distilled water was added into each test tube. The absorbance was recorded at 540 nm by using UV-spectroscopy (PerkinElmer). The control was prepared by using 120 µl of solvent in place of plant extract. The inhibition % was calculated by using formula.

$$\text{Inhibition \%} = \frac{(\text{Control 540} - \text{Sample 540}) \times 100}{\text{Control 540}}$$

RESULT AND DISCUSSION

Qualitative analysis

The qualitative estimation of Piper betle extract confirms the presence of some important phytochemical components which play a vital role in our life.

- Alkaloids are natural products that contain heterocyclic nitrogen atoms and are significant in protection and survival of plant, it was present in the extract.
- Flavonoids protect plants from different stress factors such as biotic and abiotic and are important antioxidants, it was present in the extract.
- Glycosides are stored in plants in inactive chemical form which reduces the sugars to utilize it, glycosides was present.
- Steroids are important component of plant call membrane which alter membrane fluidity and act as signaling molecules. They also control metabolism, immune function, salt and water balance, development of sexual characteristics, inflammation and injury, it was present in the extract.
- Tannin are commonly found in plant parts such as wood, bark of trees, buds, leaves, stem, fruits, seeds, roots and plant gall and they protect the plant, it was present in the extract.
- Terpenoids are secondary metabolites and occurs naturally in chemical compound, it is used in traditional herbal remedies and it was also present in the extract.

Table 1: Phytochemical properties of the ethanol extract of piper betle.

Sr no.	Phytoconstituents	Extract
1	Alkaloids	++
2	Flavonoids	++
3	Glycosides	++
4	Steroids	++
5	Tannins	++
6	Terpenoids	++

['+' = Present, '-' = Absent]

α- Amylase inhibition assay

α- amylase is a carbohydrate hydrolyzing enzyme which cleaves carbohydrate and produce monohydrates. In the present study, aqueous foliar extract of Piper betle has been used to find out the inhibition activity of α- amylase by using standard method of Naz et al. 2013.[17] Result of this study exhibited the leaves extract of Piper betle significantly inhibits the α-amylase in a dose dependent manner. Three different concentration i.e. 1 mg/ml, 2.5 mg/ml

and 5 mg/ml of aqueous foliar extract were used for the present study. The extract showed maximum inhibition at a concentration of 5 mg/ml and it is decreased with decreasing concentration i.e. 2.5 mg/ml and 1 mg/ml. At a dose of 1 mg/ml, 2.5 mg/ml and 5 mg/ml the aqueous foliar extract of this plant showed inhibition of 54.6%, 61.4% and 71.9% respectively.

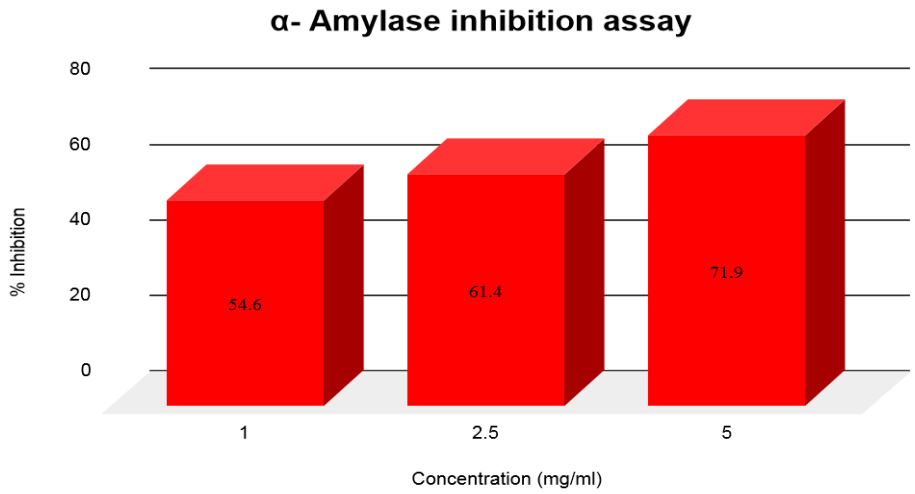

Table 2: α-Amylase inhibition assay in ethanolic foliar extract of piper betle.

Sample (concentration)	Volume (µl)	Distilled water (µl)	Starch (ml)	Enzyme solution (µl)	DNSA (µl)	Distilled water(ml)	Absorbance at 540nm ±SD
Control (solvent)	120	480	1.2	600	300	2.7	0.438 ±SD 0.270
Test 1 (1 mg/ml)	120	480	1.2	600	300	2.7	0.199 ±SD 0.013
Test 2 (2.5 mg/ml)	120	480	1.2	600	300	2.7	0.169 ±SD 0.009
Test 3 (5 mg/ml)	120	480	1.2	600	300	2.7	0.123 ±SD 0.011

[µl= microlitre; ml= millilitre; SD= standard deviation]

REFERENCES

1. T.S.Frode and Y.S.Medeiros; "Animal models to test drugs with potential antidiabetic activity", 2008; 115(2): 173-183. https://www.sciencedirect.com/science/article/pii/S0378874107005867.
2. Parthasarathi Perumal, Kavitha Saravanabhavan; "Antidiabetic and Antioxidant Activities of Ethanolic extract of Piper betle L. leaves in catfish, clarias gariepinus"; Volume Issue March, 2018; 11: 3. https://doi.org/10.22159/ajpcr.2018.v11i3.22393.

3. Horadugoda Gamage Sujatha Pushpakanthi Hewageegana, Liyanage Dona Ashanthi Menuka Arawwawala, Lakshmi Sriyani Rajapaksha Arambewela and Hettiarachchige Sami Ariyawansa; "Piper betle Linn: As a Remedy for Diabetes Mellitus"; IJRAP, 2011; 2(5): 1601-1603. https://www.ijrap.net/admin/php/uploads/671_pdf.pdf.
4. Sindhu.S.Nair, Vaibhavi Kavrekar, Anshu Mishra; "Evaluation of In Vitro Anti diabetic Activity of Selected Plant Extracts", 2013; 2(4): 12-19. http://ijpsi.org/Papers/Vol2(4)/version-1/B241219.pdf
5. A Malik, L Marpaung, P Simanjuntak and P Nasution; "Antidiabetic and cytotoxic activities of ethyl acetate extract of Piper betle Leaves"; doi:10.1088/1742-6596/1116/4/042017 https://iopscience.iop.org/article/10.1088/1742-6596/1116/4/042017/pdf
6. Leila Nouri, Abdorreza Mohammadi Nafchi and A.A.Karim; "Phytochemical, antioxidant, antibacterial, and α-amylase inhibitory properties of different extracts from betel leaves", 2014; 62: 47-52. https://www.researchgate.net/publication/265170835_Phytochemical_antioxidant_antibacterial_and_a-amylase_inhibitory_properties_of_different_extracts_from_betel_leaves.
7. D. Pradhan1, Dr. K. A. Suri, Dr. D. K. Pradhan and P. Biswasroy; "Golden Heart of the Nature: Piper betle L.", 1: 6. http://www.phytojournal.com/vol1Issue6/19.html
8. L.S.R.Arambewela, L.D.A.M.Arawwawala and W.D.Ratnasooriya; "Antidiabetic activities of aqueous and ethanolic extracts of Piper betle leaves in rats", 2005; 102(2): 239-245. https://www.sciencedirect.com/science/article/abs/pii/S0378874105004046?via%3Dihub
9. Kirti Rani, Rachita Rana and Sanchi Datt; "Review on characteristics and application of Amylases". 2015; 5(1): 1-5. https://www.researchgate.net/publication/272338614_REVIEW_ON_CHARACTERISTICS_AND_APPLICATION_OF_AMYLASES#:~:text=Amylases%20are%20enzymes%20which%20hydrolyzes,maltose%2C%20and%20reducing%20limit%20dextrins.
10. S.M.Subhani, Mohammad Mansoor and D. Srinivasa Rao; "Phytochemical and antidiabetic activity of Piper betle against alloxan induced diabetic rats", 2014; 3(3): 110-114. http://www.worldinventiapublishers.com/uploads/issues/issues_149251789458f60406b2cec.pdf
11. Sourav Mohanto, Shreyasi Datta and Surabhi Mandal; "PIPER BETEL LINN: A BRIEF STUDY"; International Journal of Current Medical And Pharmaceutical Research; 28

February, 2017; 3(02): 1290-1296. https://journalcmpr.com/sites/default/files/issue-files/IJCMPR-A--0444_1.pdf.

12. Rupa Sengupta and Jayanta K. Banik; "A REVIEW ON BETEL LEAF (PAN)"; IJPSR; 1 December, 2013; 1: 4519-4524. https://ijpsr.com/bft-article/a-review-on-betel-leaf-pan/
13. A. Kumar, G. Bartariya, B. Kumar 2017. Preliminary phytochemical screening of foliar extract of Saraca asoca (Roxb.). International Journal of Sciences & Applied Research, 2017; 4(9): 15-19. https://pdfs.semanticscholar.org/d20a/6043779f88bbcbc065c45a8ff52be428767e.pdf
14. A. Kumar, G. Bartariya, B. Kumar 2017. Preliminary phytochemical screening of foliar extract of Anthocephalus cadamba (Roxb.). International Journal of Sciences & Applied Research, 2017; 4(9): 20-24. https://pdfs.semanticscholar.org/6b47/0cd599046b92b41e37983f0fd32537001f4b.pdf
15. G. Bartariya, B. Kumar, A. Kumar Qualitative and quantitative analysis of phenols and flavonoids in foliar extract of Anthocephalus cadamba (Roxb.). Indo American Journal of Pharmaceutical Sciences, 2017; 4(10): 3664-3669. https://issuu.com/iajps.editor/docs/100.iajps100102017
16. G. Bartariya, A. Kumar, B. Kumar Qualitative and quantitative estimation of total phenolics and total flavonoids in leaves extract of Saraca asoca (Roxb.). Indo American Journal of Pharmaceutical Sciences, 2017; 4(11): 3863-3868. https://www.scribd.com/document/363542799/QUALITATIVE-AND-QUANTITATIVE-ESTIMATION-OF-TOTAL-PHENOLICS-AND-TOTAL-FLAVONOIDS-IN-LEAVES-EXTRACT-OF-SARACA-ASOCA-Roxb
17. R. Naz and A. Bano Phytochemical screening, antioxidants and antimicrobial potential of Lantana camara (Linn) in different solvents. Asian Pacific Journal of Tropic Disease, 2013; 3(6): 480-486. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4027341/.