

AN APPROACH TO ANTI-DIABETIC POTENTIAL OF *PUTRANJIVA ROXBURGHII WALL* .LEAVES BY *IN VITRO* MODELS

ABSTRACT

Aim: An Approach to Anti-Diabetic potential of *Putranjiva roxburghii wall* .Leaves by *In vitro* models.

Study design: The current research program is an attempt to screen *invitro* anti-diabetic activity of ethanolic extract of *Putranjiva roxburghii wall* leaves by using – α amylase inhibitory activity and starch iodine assay.

Place and duration of study: Department of pharmacology ,SJM College of Pharmacy Chitradurga, between July to October.

Methodology: In this study, the crude extract was obtained by using soxhlet extraction method by using 70% ethanol, The extract was used to analyse the antidiabetic activity using *invitro* models namely α -amylase inhibition activity and starch iodine assay, all the experiments were performed in triplicates. The effect of leaf extract was compared with the standard drug metformin.

Results: The results of α -amylase inhibition activity showed that the extract inhibits the α amylase enzyme in a concentration (0.3,0.6,0.9,1.2 and 1.5mg/ml) with percentage inhibition range (6.02%-55.55%) for extract which is not significantly different as compared to metformin (16.12% - 69.89%). Similarly for starch iodine assay the extract inhibits the α -amylase enzyme in a concentration (same as above) of with percentage inhibition range (16.66%-75%) for extract which is not significantly different as compared to metformin (22.57% - 85.71%).

Conclusion: From the above finding of the study, *Putranjiva roxburghii wall* leaf extract has ability to inhibit α -amylase enzyme and exhibits antidiabetic activity.

Key words: *Diabetes Mellitus, Anti-Diabetic, α -amylase, Putranjiva roxburghii wall, Starch Iodine.*

INTRODUCTION

Diabetes Mellitus (DM) is a chronic and endocrine illness characterized by elevated levels of blood glucose, accompanied by disturbed metabolism of fats and proteins. Blood glucose rises because it cannot be metabolized in the cells, due to lack of insulin production by the pancreas or the inability of the cells to effectively use the insulin that is being produced (Roglic G, 2016). Major symptoms-polyuria, polydipsia, polyphagia, delayed healing of wounds and loss of strength. The most common forms of diabetes are type 1 diabetes (5%), which is an autoimmune disorder, and type 2 diabetes (95%), which is associated with obesity. Gestational diabetes is a form of diabetes that occurs in pregnancy, and other forms of diabetes are very rare and are caused

by a single gene mutation. There's no cure for diabetes. But with treatment and lifestyle changes, you can live a long, healthy life (Dwivedi M, 2020).

The first World Health Organization (WHO) Global Report on Diabetes was launched on World Health Day, April 7, 2016. Diabetes, historically recognized as a serious illness, has become more prevalent in recent decades, significantly affecting global health. Along with cardiovascular disease (CVD), cancer, and chronic respiratory disease, diabetes was highlighted in the 2011 Political Declaration on Noncommunicable Diseases (NCDs). In 2013, WHO member states adopted a global monitoring framework for NCDs, setting 9 targets to be achieved by 2025(Chan M, 2016).

Current treatments for DM primarily include insulin for Type 1 diabetes and lifestyle modifications and oral hypoglycaemic agents for Type 2. These hypoglycaemic agents have their limitations and are known to produce serious side effects. Therefore, the search for more safer, specific and effective hypoglycaemics agents has continued to be an important area of investigation with natural extracts from readily available traditional medicinal plants offering great potential for discovery of the new anti-diabetic drugs (C Jain, 2014).

Medicinal plants and their bioactive constituents can be used for treatment of DM throughout the world especially in countries where access to the conventional Anti-diabetic agents are inadequate. Hence, Medicinal plants and herbs are often used to cure or prevent diseases. According to WHO medicinal herbs account for 80% of raw materials used in herbal medicine. About 80% of the global population relies on plant-based medicine for their primary health care (Punitha R, 2006).

Putranjiva roxburghii wall. a moderate-sized evergreen tree from the Euphorbiaceae family, grows in moist forests and possesses numerous medicinal properties. It exhibits anti-inflammatory, antioxidant, antimicrobial, hypoglycaemic, and analgesic activities due to compounds like terpenoids, flavonoids, tannins, and alkaloids. High flavonoid content, highlighted in literature, prompted research on its potential antidiabetic effects (Paras Dar, 2018). Thus, the present study was carried out with an aim to investigate the antidiabetic effect of ethanolic extract of *Putranjiva roxburghii wall.* leaves on various biochemical alteration by *invitro* methods.

MATERIALS AND METHODS

The selected leaf material was identified and authenticated by a botanist, Chitradurga Karnataka. Fresh leaves were collected, the leaf part was dried separately at room temperature and pulverized. The powder obtained is subjected to Soxhlet extraction with the 70% ethanol solvent (Rajahamsa AL, 2013). The extract was taken for phytochemical studies. This showed the presence of flavonoids, alkaloids, glycosides, saponins, triterpenoids, tannins, and phenolic compounds.

***In-vitro* evaluation of anti-diabetic activity**

α- amylase inhibition activity (Dewangan H, 2017)

Using the 3,5-dinitrosalicylic acid (DNSA) technique, the α -amylase inhibition test was carried out. Amylase (10 mL), phosphate buffer (50 mL, 100 mM, pH=6.9), and plant extract (20 mL, 20-100 μ g/mL) at varied quantities are combined. The substrate was then added, and the mixture was incubated at 37°C for 30 minutes with 20 μ L of 1% soluble starch (in phosphate buffer, 100 mM, and pH 6.8). Following that, 100 μ L of di-nitro salicylic acid (DNSA) reagent was added to the mixture, and it was then given 10 minutes to boil. Absorbance at 540 nm was measured using a UV-visible spectrophotometer.

The α -amylase inhibition activity was calculated using the formula:

$$\% \text{inhibition} = \frac{\text{absorbance 1} - \text{absorbance 2}}{\text{absorbance 1}} \times 100$$

Where, Absorbance 1 – control, Absorbance 2 – standard.

Starch Iodine Assay (Abdullah N, 2017)

Approximately 1 mL of plant extract of different concentration (0.1–10 mg/mL) was taken in test tubes. A volume of 20 μ L of α -amylase was added to each test tube and incubated for 10 min at 37 °C. After the incubation, 200 μ L of 1% starch solution was added to each test tube and the mixture was further incubated for 1 h at 37 °C. Then, 8 mL of distilled water was added. Absorbance of the mixture was taken at 565 nm. Each experiment was done in triplicate. The α -amylase inhibitory activities were calculated as percentage inhibition. Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC₅₀) were determined graphically.

The Starch Iodine Assay was calculated using the formula:

$$\% \text{inhibition} = \frac{\text{absorbance 1} - \text{absorbance 2}}{\text{absorbance 1}} \times 100$$

Where, Absorbance 1 – control, Absorbance 2 – standard

Results

α -amylase inhibition activity

α -amylase plays a crucial role in carbohydrate digestion by breaking down starch into simpler sugar. It shows by slowing the digestion and absorption of carbohydrate. It aids in maintain better glycaemic control and reducing the risk of complications associated with fluctuating blood glucose level.

In the present study we evaluated *in-vitro* α amylase activity of crude ethanolic extract of *Putranjiva roxburghii wall.* leaves. There was a dose dependent increase in percentage inhibitory activity against α -amylase enzyme. At a concentration of 1.5 mg/ml of standard drug metformin showed percentage inhibition 69.89% and for the 1.5 mg/ml of leaves extract showed inhibition of 55.55%. The results are shown in table 1.

Table 1: Results of α -amylase inhibition activity.

Group	Absorbance 1	Absorbance 2	Absorbance 3	Average	% inhibition
Control	0.090	0.096	0.094	0.093	00
Metformin					
(mg/ml)					
0.3	0.079	0.069	0.086	0.078	16.12%
0.6	0.062	0.068	0.079	0.069	25.80%
0.9	0.066	0.071	0.063	0.066	29.03%
1.2	0.049	0.054	0.059	0.054	41.93%
1.5	0.032	0.029	0.024	0.028	69.89%
Test extract					

(mg/ml)					
0.3	0.072	0.082	0.091	0.083	6.02%
0.6	0.092	0.084	0.078	0.084	17.0%
0.9	0.088	0.093	0.090	0.090	26.66%
1.2	0.095	0.087	0.083	0.088	50.90%
1.5	0.071	0.063	0.057	0.063	55.55%

Starch iodine assay

Starch iodine, often used as a reagent to test for the presence of starch, plays a secondary role in antidiabetic activity. In studies related to diabetes, starch iodine can help assess the effectiveness of various treatments by evaluating carbohydrate digestion and absorption. For instance, it can be used to determine the effects of certain dietary fibres or phytochemicals on starch breakdown and glucose release.

In this present study we evaluated *in-vitro* starch iodine assay of crude ethanolic extract of *Putranjiva roxburghii wall.* leaves. There was a dose dependent increase in percentage inhibitory activity against α -amylase enzyme. At a concentration of 1.5 mg/ml of standard drug metformin showed percentage inhibition 85.71% and for the 1.5 mg/ml of leaves extract showed inhibition of 75%. The results are shown in table 2.

Table 2: Results of starch iodine assay.

DISCUSSION

Diabetes mellitus is a metabolic disorder that is characterized by hyperglycaemia and glucose intolerance, which is associated with impaired insulin secretion, peripheral sensitivity and eventual β -cell dysfunction (Ohiagu FO, 2021). Diabetes mellitus is a major public health issue with rising rates and long-term consequences are a leading cause of illness and mortality around the world, and it is linked to ongoing organ damage, dysfunction and failure (Rachakonda R, 2016). In the present study, the effect of Ethanolic Extract of *Putranjiva roxburghii* leaves exhibited significant antidiabetic activity and it was carried out by using *in vitro* models, like α -amylase inhibitory activity and starch iodine assay by using UV spectrophotometer.

α -amylase plays a crucial role in starch digestion, and its inhibition is a promising approach for managing

Group	Absorbance 1	Absorbance 2	Absorbance 3	Average	% inhibition
Control	0.008	0.007	0.007	0.007	00
Metformin					
(mg/ml)					
0.3	0.005	0.006	0.006	0.005	28.57%
0.6	0.003	0.002	0.004	0.003	57.14%
0.9	0.001	0.002	0.003	0.002	71.42%
1.2	0.0013	0.0014	0.0016	0.0015	78.57%
1.5	0.001	0.002	0.001	0.001	85.71%
Test extract					
(mg/ml)					
0.3	0.006	0.007	0.005	0.006	16.66%
0.6	0.003	0.006	0.004	0.004	25%
0.9	0.003	0.005	0.001	0.003	33.33%
1.2	0.005	0.004	0.006	0.005	70%
1.5	0.006	0.003	0.005	0.004	75%

postprandial hyperglycaemia. This study investigated the α -amylase inhibitory activity of several plant extracts, demonstrating their potential as anti-diabetic agents. The results indicated that certain extracts exhibited significant α -amylase inhibition, suggesting their efficacy in delaying carbohydrate digestion and subsequent glucose absorption. This aligns with previous research, which has shown that natural compounds can modulate digestive enzymes, leading to improved glycaemic control.

The starch-iodine assay is a biochemical test commonly used to evaluate the inhibitory effect of a compound (such as a plant extract) on starch breakdown, which is relevant in antidiabetic activity assessment. This assay specifically examines the inhibition of α -amylase, an enzyme that breaks down starch into simpler sugars, a key process in carbohydrate digestion and absorption.

In the current study, ethanolic extract of *Putranjiva roxburghii wall.* leaves exhibited the anti-diabetic activity by inhibiting the α -amylase enzyme and starch breakdown at different concentrations while it is compared with standard drug metformin.

CONCLUSION

The ethanolic extract of *Putranjiva roxburghii* leaves demonstrated significant antidiabetic activity by inhibiting α -amylase enzyme and reducing starch breakdown in *invitro* models, similar to the standard drug metformin. Phytochemical analysis revealed the presence of bioactive compounds like tannins, flavonoids, and phenols, which may contribute to this effect. These findings suggest that ethanolic extract of *Putranjiva roxburghii wall.* leaves could be a promising candidate for diabetes management, although further studies are needed to isolate the active compounds and clarify the mechanisms of action.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (Chat GPT, COPILOT etc) and text – to - image generators have been used during writing or editing of this manuscript.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This section is not applicable to our study, hence we followed in-vitro methods.

REFERENCES

1. Roglic G. (2016). WHO Global report on diabetes: A summary. International Journal of Non-Communicable Disease,1(1),3-8.
2. Dwivedi M, Pandey AR. (2020). Diabetes mellitus and its treatment: an overview. Journal of Advancement in Pharmacology,1(1),48-58.
3. Chan M. (2016). Global report on diabetes. World Health Organization,58(12),1-88.
4. C Jain, A Sign, P Kumar, K Gautam. (2014). Anti-Diabetic potential of flavonoids and other crude extract of stem bark of *Mangifera indica* Linn: A comparative study. Journal of scientific and Innovative Research,3(1),21-27.
5. Punitha R, Vasudevan K, Manoharan S. (2006). Effect of *Pongamia pinnata* flowers on blood glucose and oxidative stress in alloxan induced diabetic rats. Indian Journal of Pharmacology,38(1),62-63.
6. Paras Dar, Muhammad Faisal, Amara Dar, Usama Waqas. (2018). Journey of describing Current Traditional Medicine biological activities and chemical constituents in the leaves, stem, bark and seed of *Putranjiva roxburghii*. Current traditional Medicine,4(4),263-272.
7. Rajahamsa AL, Deepak KS, Rao TK, Kumar AP, Reddy GS, Potbhare MS. (2013). Multi-model confirmatory evaluation of anti-inflammatory, analgesic and antioxidant activities of *Putranjiva roxburghii Wall.* International Journal of Biomedical and Advance Research,4(12),921-932.

8. Dewangan H, Tiwari RK, Sharma V, Shukla SS, Satapathy T, Pandey R. (2017). Past and Future of *invitro* and *invivo* Animal Models for Diabetes A Review. Indian Journal of Pharmaceutical Education and Research,51(4),522-530.
9. Abdullah N, Kasim K. (2017). *In-vitro* antidiabetic activity of *Clinacanthus nutans* extracts. International journal of pharmacognosy and phytochemistry research,9(6),846-852.
10. Ohiagu FO, Chikezie PC, Chikezie CM. (2021). Pathophysiology of diabetes mellitus complications: Metabolic events and control. Biomedical Research and Therapy,8(3),4243-4257.
11. Rachakonda R, Nagasree DV. (2016). Prevalence and clinical profile of diabetes among pulmonary tuberculosis patients,2(2),6-10.

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