AN APPROACH TO ANTI-DIABETIC POTENTIAL OF *Putranjiva roxburghii WALL*LEAVES BY *IN VITRO* MODELS

ABSTRACT

Aim: To evaluate the antidiabetic potential of Putranjiva roxburghii leaves using in vitro models, focusing on their ability to inhibit key enzymes involved in carbohydrate metabolism

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. 2024

Methodology: In this study, the crude extract was obtained by using the soxhlet extraction method using 70% ethanol, The extract was used to analyse the antidiabetic activity using *in-vitro* 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 a 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) with a 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 the ability to inhibit α-amylase enzyme and exhibits antidiabetic activity.

Keywords: 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 a lack of insulin production by the pancreas or the inability of the cells to effectively use the insulin that is being produced (Roglic , 2016). Major symptoms arepolyuria, 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 &Pandey, 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. In the 2011 Political Declaration on Noncommunicable Diseases (NCDs), diabetes was highlighted alongside cardiovascular disease (CVD), cancer, and chronic respiratory 2013, WHO member states adopted a global monitoring framework for NCDs, setting 9 targets to be achieved by 2025(Chan , 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 safer, specific, and effective hypoglycaemic agents has continued to be an important area of investigation with natural extracts from readily available traditional medicinal plants offering great potential for the discovery of new anti-diabetic drugs (Jain et al., 2014).

Medicinal plants are considered an important therapeutic aid in this regard toward reducing this severe ailment to a considerable extent. Strong medicinal systems like Ayurveda, Siddha, Unani, and Chinese are still showing potential and have been practiced for over 1500 years only because of their natural and motherly care through these magical plants. The majority of the people (>60%–80%) from developed as well as developing countries depend largely on these medicinal systems and rely on these special herbals. (Upadhyay et al.,,2024)

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Putranjiva roxburghii wall. a moderate-sized evergreen tree from the *putranjivaceae 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 et al., 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 fresh leaves were collected in the month of February, the leaf part was dried separately at room temperature (shade dry) and pulverized. The powder obtained is subjected to Soxhlet extraction with the 70% ethanol solvent.

The phytochemical screening is directly taken from the mentioned reference (Sarath & Sudha,2019)

List 1: chemicals and equipment used during experiments

Sl no	Chemicals and equipment
1	Metformin (standard antidiabetic drug)
2	Phosphate buffer (6.9)
3	Starch

4	DNSA (3,5 dinitro salicylic acid)
5	∝-Amylase kit
6	70% Ethanol
7	Soxhlet apparatus
8	Electronic balance
9	UV spectrophotometer
10	Incubator
11	pH meter
12	Micropipette
13	Beakers
14	Test tubes

In-vitro evaluation of anti-diabetic activity

Alpha - amylase inhibition activity (Dewangan et al., 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 µg/mL) at varied (0.3,0.6,0.9,1.2 and 1.5mg/ml) of concentration are combined. The substrate (here substrate refers to 1% soluble starch) was then added, and the mixture was incubated at 37°C for 30 minutes with 20 µL of 1% soluble starch (in phosphate buffer, 100 mM, and pH 6.8). Following that, 100 µL of di-nitro salicylic acid (DNSA) reagent was added to the mixture, and it was then given 10 minutes to boil by using a water bath Absorbance at 540 nm was measured using a UV-visible spectrophotometer. Yes, and all the experiment were performed in triplicate. The α- amylase inhibition activity was calculated using the formula:

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

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

Starch Iodine Assay (Abdullah & Kasim, 2017)

Approximately 1 mL of plant extract at varying concentrations (0.1–10 mg/mL) was placed in test tubes. To each tube, 20 µL of α-amylase enzyme solution was added, and the mixture was incubated at 37 °C for 10 minutes. Following incubation, 200 µL of 1% starch solution was added to each tube, and the reaction mixture was further incubated at 37 °C for 1 hour. After the second incubation, 8 mL of distilled water was added to stop the reaction. The absorbance of the resulting mixture was measured at 565 nm. All experiments were performed in triplicate. The α-amylase inhibitory activity was expressed as percentage inhibition, and the concentrations of extracts required to achieve 50% enzyme inhibition (IC_{50}) were determined.

$$\%\text{inhibition} = \frac{absorbance1 - absorbance2}{absorbance\ 1} \times 100$$

Where, Absorbance1 – control, Absorbance 2 – standard

Results

Alpha - amylase inhibition activity

Alpha -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**	**Mean** ±SD	**% inhibition**
Control	0.090	0.096	0.094	0.093	**0.093**±0.002	**00**
Metformin (mg/ml)						
0.3	0.079	0.069	0.086	0.078	0.078±0.01	**16.12%**
0.6	0.062	0.068	0.079	0.069	0.069±0.009	**25.80%**
0.9	0.066	0.071	0.063	0.066	0.066±0.004	**29.03%**
1.2	0.049	0.054	0.059	0.054	0.054±0.005	**41.93%**
1.5	0.032	0.029	0.024	0.028	0.028±0.007	**69.89%**
Test extract (mg/ml)						
0.3	0.072	0.082	0.091	0.083	0.083±0.01	**6.02%**

0.6	0.092	0.084	0.078	0.084	0.084±0.008	**17.0%**
0.9	0.088	0.093	0.090	0.090	0.090±0.004	**26.66%**
1.2	0.095	0.087	0.083	0.088	0.088±0.006	**50.90%**
1.5	0.071	0.063	0.057	0.063	0.063±0.009	**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.

Group	**Absorbance1**	**Absorbance 2**	**Absorbance 3**	**Average**	**Mean** ±SD	**% inhibition**
Control	0.008	0.007	0.007	0.007	0.007±0.001	**00**
Metformin (mg/ml)						
0.3	0.005	0.006	0.006	0.005	0.005±0.001	**28.57%**
0.6	0.003	0.002	0.004	0.003	0.003±0.002	**57.14%**
0.9	0.001	0.002	0.003	0.002	0.002±0.001	**71.42%**
1.2	0.0013	0.0014	0.0016	0.0015	0.0015±0.0001	**78.57%**
1.5	0.001	0.002	0.001	0.001	0.001±0.001	**85.71%**
Test extract (mg/ml)						
0.3	0.006	0.007	0.005	0.006	0.006±0.002	**16.66%**
0.6	0.003	0.006	0.004	0.004	0.004±0.002	**25%**

0.9	0.003	0.005	0.001	0.003	0.003±0.003	**33.33%**
1.2	0.005	0.004	0.006	0.005	0.005±0.001	**70%**
1.5	0.006	0.003	0.005	0.004	0.004±0.001	**75%**

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 et al., 2021). Diabetes mellites is a major public health issues 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 & Nagasree, 2016). In the present study, the effect of Ethanolic Extract of *Putranjiva roxburghii wall*. leaves exhibited significant antidiabetic activity and it was carried out by using *invitro* models, like α-amylase inhibitory activity and starch iodine assay by using UV spectrophotometer.

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

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