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# *IN-VITRO* ANTI-DIABETIC AND HYPOLIPIDEMIC ACTIVITY OF ETHANOLIC EXTRACT OF *PRUNUS AVIUM.*

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**ABSTRACT**

Sweet cherry (*Prunus avium*) which belongs to *Rosaceae* family widely cultivated in Europe. The present study was designed to screen the pharmacological activities such as antidiabetic and hypolipidemic activity of ethanol extract of *Prunus avium* fruit *In-vitro.* The *Prunus avium* fruits were collected and dried in hot air oven at 37 $^{0}$C, extracted using ethanol by Maceration extraction technique. The antidiabetic activity was assessed by alpha-amylase inhibition method. The hypolipidemic activity was assessed by pancreatic lipase inhibition assay *In-vitro.* The preliminary phytochemical screening showed the presence of carbohydrates, alkaloids, flavonoids, glycosides, terpenoides, proteins, saponins, phenols, oils and fats. The *Prunus avium* fruit extract was found to be an effective anti-diabetic activity in *In-vitro* against alpha-amylase enzyme at $IC_{50}$ value of 6.02µg. The ethanol extract of *Prunus avium* fruit shows significant inhibition of pancreatic lipase enzyme at $IC_{50}$ value of 5.909 µg.

## 1. INTRODUCTION:

Wild cherry (*Prunus avium L.*) is one of the main hardwood species investigated in Europe since the beginning of the 1980s. Wild cherry wood is highly sought after for aesthetic applications such as paneling and cabinet-making, compared to tropical woods[1]. Sweet cherry, a fleshy non-climacteric stone fruit belongs to the genus Prunus and is mainly grown in countries falling in temperate climates. It is one of the most widely appreciated fruit for its taste, sweetness, colour and myriads of nutrients[2]. It is one of the first trees to flower in the spring and produces masses of white blossom. It matures at around 60-80 years, when trees are typically 20-25 m in height with trunk of 50-70 cm in diameter [3]. Prunus avium or sweet cherry is cultivated most widely in temperate region across the world, particularly in Turkey, United State and Iran. The phytochemicals extracted from sweet cherry act as a secondary metabolite. It consists of anthocyanins, perillyl and phenolic compounds including cyanide-3-rutinoside and flavonol p-coumoroquinic acid having importance in anti-cancer and anti-oxidation activities[4]. Additionally, other non-phenolic phytochemicals reported in the sweet cherry were volatile compounds, carotenoids, serotonin and melatonin. Their contents are more concentrated in exocarp than in the flesh of cherries[5]

**ANTI-DIABETIC:** Diabetes mellitus, characterized by hyperglycemia, is a genetically and clinically heterogeneous group of disorders with common feature of glucose intolerance. Based on WHO

recommendation, diabetes mellitus is classified into four major subtypes: type I (insulin dependent diabetes mellitus, IDDM), type II (noninsulin dependent diabetes mellitus, NIDDM) other specific types and gestational diabetes mellitus[6]. Cherry fruit and its by-products contain several phytochemicals mainly anthocyanins, phenolic acids, and flavan-3-ols, which have demonstrated biological activities such as antioxidant and antidiabetic properties. The literature reports compelling evidence of the benefits ascribed to the long-term consumption of phenolics in the prevention of several oxidative stress-induced diseases such as diabetes mellitus(DM)[7]. Diabetic condition can be treated by using following drugs [8].

**Sulphonylureas:** *First generation*: Acetohexamide, Chlorpropamide, Tolazamide, Tolbutamide

*Second generation*: Glibenclamide,Glipizide, Glimipride, Gliclazide**.**

**Biguanides:** Metformin, Phenformin.

**HYPOLIPIDEMIC:**

Hyperlipidemia is a common disease around the world affected both developed and developing countries, in this disease blood level of lipids is elevated more than normal range. Elevated lipids levels (cholesterol, fats, and triglyceride) predispose the patient to a various serious and sometimes lethal complications such as cardiovascular disease, cerebral strokes, hepatic and renal dysfunction[9]. So, to reduce hyperlipidemia condition several hypolipidemic drugs are used[10,11].

**Statins:** lovastatin, simvastatin, atorvastatin, pravastatin

**Bile Acid Sequestrants:** cholestyramine, colestipol.

## 2. METHODOLOGY:

**Collection and authentication of prunus aviun:** *Prunus avium* were collected from the Sri Rajarajeshwari fruits and vegetables shop, RR Nagar, Bengaluru. The plant has authenticated by the Dr. Thejash Kumar M.P. Coordinator and Head of the Department of Botany, Bharathi College, Bharathinagara, Maddur Tq, Mandya Dist. and Karnataka.

**Grinding and drying of prunus avium:**

The collected fruits (500 gm) were first carefully washed and sorted to remove any damaged or unripe specimens. Once selected, they were ground. This sample was then subjected to a drying process in a hot air oven, maintained at room temperature, for a period of 10 Days. This extended drying time ensured that all moisture was effectively removed, preventing any potential spoilage or degradation of the sample. After the drying process was complete, the powdered fruit was promptly transferred into an airtight container, which served to protect it from humidity and contaminants. This meticulous preparation allowed for the preservation of the sample's qualities, ensuring that it remained in optimal condition until the analysis could be conducted, thereby providing reliable and accurate results.

**PREPARATION OF PRUNUS AVIUM:**

The dried sample (90 g) was processed using a maceration extraction technique with 70% ethanol solvent system. The dried material was combined with 500 ml of ethanol and the flask was sealed tightly with aluminum foil, kept at room temperature for 48 hours. Following this initial period, the flask remainedon a magnetic stirrer for an additional two days to ensure thorough extraction. The resulting filtrate was then transferred to a ceramic plate to facilitate the evaporation of the solvent, a process that took one week to yield the extract of *Prunus avium.* Once the extraction was complete, the weight of the final product was recorded (11.355 gm), and it was subsequently tested for solubility. To further investigate its potential benefits, a preliminary phytochemical screening was conducted to identify the presence of various phytoconstituents in the extract and to evaluate its antidiabetic and hypolipidemic activity.

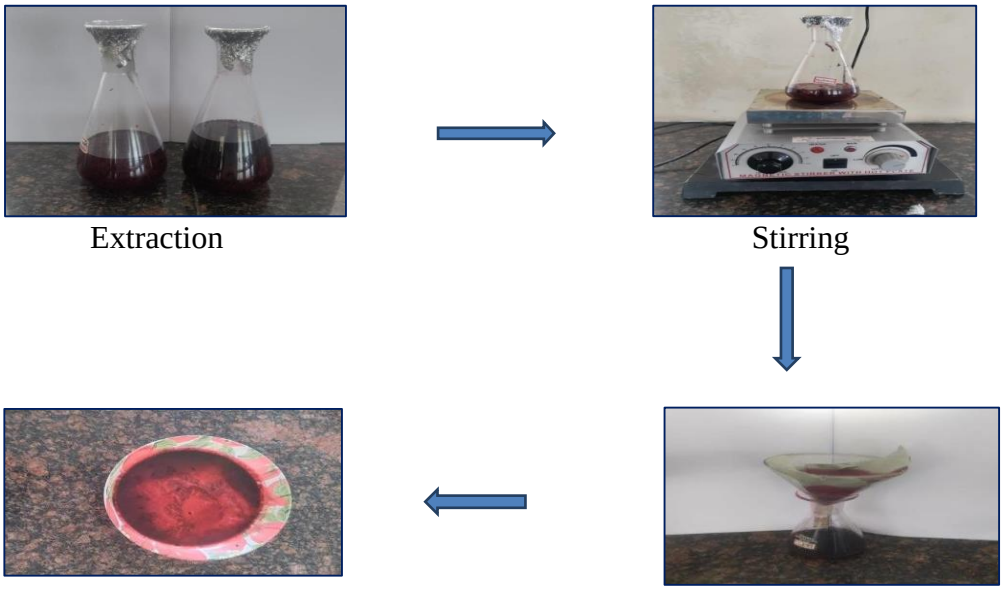


**Figure 1: Process of preparation of ethanolic extract of *Prunus avium***

**3. PROCEDURE: Anti-diabetic activity: By alpha amylase inhibition assay**

- Extract : 1µg/ml
- Alpha-amylase: 1µg/ml
- 0.1Mof phosphate buffer
- Starch : 0.1percent

**Control**

100µl of buffer
+
100µl of enzyme
+
100µl of buffer

**Test**

100µl of extract
+
100µl of enzyme
+
100µl of buffer

Incubated at room temperature for 10 min

Add 100µl of starch

Incubated at room temperature for 30 min

Add 1µl of DNSA reagent

5 min boiling on water bath

Cooled at room temperature and diluted with 10 ml of distilled water

Absorbance at 540nm.

**Table1: Alpha amylase inhibition assay**

| SL NO. | Concentration (µg) | % inhibition |
|---|---|---|
| 1. | 2 | 21.07 |
| 2. | 4 | 32.35 |
| 3. | 6 | 54.70 |
| 4. | 8 | 65.1 |
| 5. | 10 | 76 |

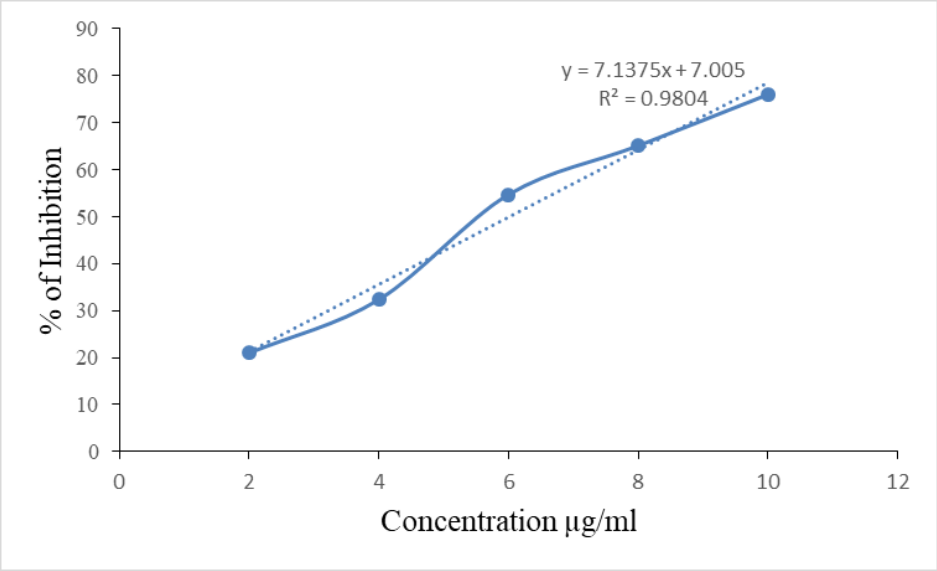


**Figure 2: Alpha-amylase inhibition activity**

**PANCREATIC LIPASE INHIBITION ASSAY:** In the present study to establish hypolipidemic potential of fruit extract, we carried out *In- vitro* lipase inhibition assay. The fruit extract inhibited pancreatic lipase enzyme with IC50 value of 5.909µg.

| Sl. No. | Concentration (µg) | % inhibition of Pancreatic lipase enzyme |
|---|---|---|
| 1. | 2 | 22 |
| 2. | 4 | 39 |
| 3. | 6 | 53 |
| 4. | 8 | 62 |
| 5. | 10 | 77 |

**Table 2: Pancreatic lipase inhibition assay**

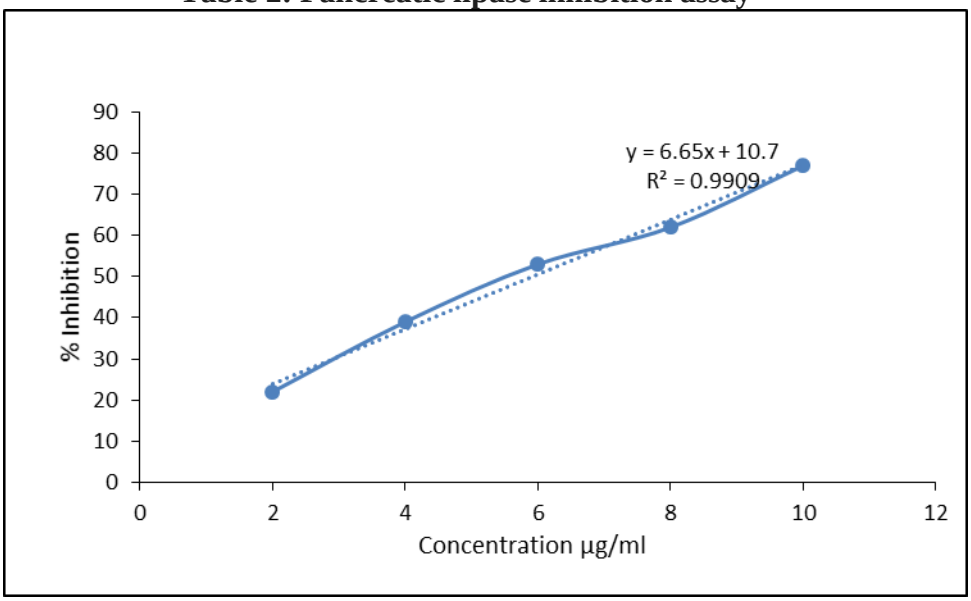


**Figure 3: Pancreatic lipase inhibition activity**

**Hypolidemic activity**: **By pancreatic lipase inhibition assay:**

**Principle:** The principle of a pancreatic lipase enzyme inhibition assay is to measure the activity of pancreatic lipase and assess how specific inhibitors affect this activity. Pancreatic lipase is an enzyme that catalyzes the hydrolysis of triglycerides into free fatty acides and glycerol in the digestive system. Inhibitors can interfere with the enzymes activity either by competing with the substrate (competitive inhibition) or by binding to the

enzyme or enzyme-substrate complex (non-competitive inhibition).

**Chemicals:** P-nitrophenyl butyrate (Pnpb), 0.1MTris HCL buffer (ph 8), pancreatic lipase.

**Procedure:**

Pancreatic lipase activity was determined by measuring the hydrolysis of p-nitrophenyl butyrate (p-NPB) to p-nitrophenyl using a method reported previously. The 0.1 mg/ml of enzyme solution was prepared by reconstituting porcine pancreatic lipase using 0.1 M Tris-HCl buffer (pH 8). Then, 5 µl of test sample was mixed with 90 µl of enzyme buffer, and incubated for 15 min at 37°C. After incubation, 5 µl of 10 mM p- nitrophenyl butyrate (p-NPB) was added to enzyme mixture and the reaction was allowed to proceed for further 15 min at 37°C. After incubation, the absorbance of p- nitrophenyl released was measured at 405 nm using a UV spectrophotometer Furthermore, a positive control, Orlistat was used to ensure the reliability of results. Relative pancreatic lipase activity (%) was calculated as (the activity of the compound with the substrate-the activity of the compound without the substrate)/ (Absorbance control) x 100.

**4. RESULT:**

**ANTIDIABETIC ACTIVITY: Alpha-amylase inhibition assay:** In the present study to establish antidiabetic activity of fruit extract, we carried out in-vivo alpha amylase inhibition assay. The fruit extract inhibited alpha amylase enzyme with the IC50 value of 6.02µg.

**PANCREATIC LIPASE INHIBITION ASSAY**: In the present study to establish hypolipidemic potential of fruit extract, we carried out *In- vitro* lipase inhibition assay. The fruit extract inhibited pancreatic lipase enzyme with IC50 value of 5.909µg.

**DISCUSSION:**

In the present study we subjected *Prunus avium* fruit extract to evaluate its antidiabetic and hypolipidemic activity *In-vitro*. Also extract is subjected to preliminary screening for its secondary metabolites. The results obtained indicate that *Prunus avium* possess antidiabetic and hypolipidemic activity. The antidiabetic effect of *Prunus avium* was evaluated by alpha amylase inhibition assay. The hypolipidemic effect of *Prunus avium* was assessed by pancreatic lipase inhibition assay by using *In-vitro* assay method. Alpha amylase activity was measured by *In-* vitro method. The fruit extracts prunus avium possess the alpha amylase inhibitory activity, thus the intensity of color decreases and the absorbance 540nm. The fruit extract of Prunus avium exhibited $IC_{50}$ value of 6.02 µg. Pancreatic lipase activity was measured *In- vitro* by hydrolysis of p- nitrophenyl butyrate (p-NPB) in the presence of pancreatic lipase enzyme. Further the process was quantified lipase inhibitory activity, thus the intensity of color decreases and the absorbance is measured at 405 nm. The fruit extract of *Prunus avium* exhibited $IC_{50}$ value of 5.909 µg.

**CONCLUSION:** In the present study, results indicate that the ethanol extracts of *Prunus avium* fruits possess hypolipidemic activity and antidiabetic activity. These activities may be due to the strong occurrence of chemcial moities present in fruits of *Prunus avium.* The current investigation leads to conclusion that *Prunus avium* fruit have antidiabetic activity and hypolipidemic activity.

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**Conflict of interest:** The authors declare no conflict of interest.

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