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Analysis of Antioxidant, Antidiabetic Potential, and Corosolic Acid Content in *Prunus Padus* Leaves

Sudhir Kharyal* and Richa Puri

Department of Botany, Panjab University, Chandigarh - 160 014, India
**E-mail: Sudhir9805859219@gmail.com*

ABSTRACT

This research was conducted to detect corosolic acid, *in vitro* antioxidant and antidiabetic effect of *Prunus padus* leaf, also called Bird cherry and is a member of Rosaceae. It is a medium-sized tree with obovate, fine serrated leaves and white flowers. According to the HPLC Chromatogram of standard corosolic acid, leaf samples of *Prunus padus* displayed a peak with a retention time of 9.855 min. Quantitative analysis revealed a good concentration of corosolic acid i.e 8.032 µg/20 mg d w. *In vitro* studies showed potent antidiabetic and antioxidant activities DPPH radical scavenging test of *Prunus padus* showed 91.90 per cent inhibition and standard ascorbic acid showed 82.39 per cent inhibition at 250 µg/ml concentration. Acarbose and *Prunus padus* both have IC50 values of 193.62 ± 0.634 µg/ml and 114.72 ± 1.038 µg/ ml, accordingly, as determined by an alpha amylase inhibition experiment. The results demonstrate that *Prunus padus* possesses a good amount of corosolic acid and can be considered a natural source, which is very influential in neutralizing the reactive oxygen species and damage caused by diabetes and has strong antioxidant and antidiabetic effects.

KEYWORDS: *Prunus padus*; Corosolic acid; Antioxidant; Antidiabetic; HPLC

1. INTRODUCTION

Since the days of yore, humans have looked for cures in nature to recover from various diseases. Medicinal plants significantly contribute to the health and profit of the human race. Starting from the oldest medicine prescriptions to the current highly advanced synthetic or semi-synthetic medicines, medical science has made tremendous progress and is still burgeoning, but the human population is still consistently afflicted by various diseases. Oxidative stress, defined as disequilibrium between free radicals and antioxidants, is indeed the leading cause of insulin resistance, beta cell dysfunction, and finally type-2 diabetes and various other diseases.[1] Non-communicable diseases like diabetes and cardiovascular disease pose a great threat to human health in the 21st century.

Till now medicinal plants have attracted much attention as a great source of dietary antioxidants due to their capacity to biosynthesise antioxidants without involving enzymes and are capable of debilitating reactive oxygen species-induced oxidative damage.[2] Plants containing antioxidants protect beta cells and can prevent diabetes induced by reactive oxygen species.[3] Diabetes mellitus is characterised as a recurring disease marked by insufficiency in insulin production and action that leads to protracted hyperglycemia with the interruption in most metabolic processes of the body.[4]

Several therapeutic targets have been introduced to recover type-II diabetes, one of which is to inhibit α-Amylase and α-glucosidase, which in turn retards glucose uptake and reduces postprandial hyperglycemia**.** Current drugs in clinical use for inhibiting carbohydrate-digesting enzymes possess side effects like hypoglycemia, flatulence, diarrhea, etc. The call for therapeutic plants is surging in developing and developed countries because of the increased acceptance of plant-based medications, which are non-narcotic, without side effects, available at a bargain, and from time to time, they are the sole source of medical care for economically weaker section. According to reports, several plants have been used traditionally to treat diabetes. *Zingiber officinale* has been used traditionally to treat diabetes, asthma, joint pain, etc. *Syzygium cumini* bark is consumed by tribal people in Sikkim as a decoction to treat diabetes[7], and *Momordica charantia* juice has been shown to lower blood sugar levels in STZ-induced diabetic mice when administered orally.[8]

The bioactive profile of medicinal plants is solely responsible for their healing properties. One such crucial phytochemical is corosolic acid, a pentacyclic triterpenoid,

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chemically defined as 2 alpha-hydroxy ursolic acid.[9] Corosolic acid has been reported to be present in *Craraegus pinnatifida*[10], *Tiarella polyphylla*[11], *Lagerstroemia speciosa*[12] etc. Corosolic acid possesses plentiful medicinal properties such as anti-diabetic, antiproliferative, anti-inflammatory, and protein kinase (PKC) inhibitory activities.[14] The alpha-amylase enzyme is considered a perfect target by antidiabetic phytochemicals for treating type-2 diabetes mellitus.[15] Corosolic acid has been reported to be potent in the inhibition of carbohydrate digesting enzymes like alpha-amylase and alpha glucosidase.[16] Corosolic acid has been promulgated to decrease the level of blood sugar in KK-AY mice.[17]

Glucosol and Glucosupreme are commercially available products containing corosolic acid and are primarily marketed in the US and Japan as a supplement to control blood sugar and maintain a healthy weight. At present, many medicinal plants are still unexplored in Himachal Pradesh, India, for the presence of corosolic acid. Considering this side, this study was conducted for *in vitro* antidiabetic and antioxidant tests and quantitative detection of corosolic acid in *Prunus padus* leaves through the RP-HPLC method. *Prunus padus* L., also called Bird cherry, is widespread in Asia and northern Europe. It is a medium-sized tree with obovate, fine serrated leaves and white flowers, the bark is grayish brown and possesses an acrid odor (Fig. 1) *Prunus padus* inhibits alpha-glucosidase and demonstrates its antidiabetic potential.[18] *Prunus padus* possesses higher amounts of triterpene acids and has anti-inflammatory potential[19] and antioxidant properties in leaves and flowers.[20]

Figure 1. *Prunus padus* tree.

2. METHODOLOGY

2.1 Materials

Pure Corosolic acid standard (>98%) was purchased from Wuhan ChemFaces Biochemical Co., Ltd. in Wuhan, China Every other substance, including reagents, was of analytical grade.

2.2 Collection and Identification of Plant Material

A field excursion was carried out to University of Horticulture and Forestry, Dr. Y.S. Parmar, Nauni, Solan, H.P. for the collection of *Prunus padus* leaf samples. The collected plant samples were tentatively identified with the help of keys and descriptions given in various floras and monographs and later confirmed by matching with type specimens present in the herbarium at BSI, Dehradun.

2.3 Plant Extract Preparation

The leaf samples were completely cleaned with tap water, dried in the shade, ground into a powder, and then passed through 60 size mesh. A conical flask containing 10 g of powdered plant material and 20 ml of methanol was placed on an orbital shaker for 24 hours at room temperature. Filtered through Whatmann's filter paper No. 1 and left it to evaporate at room temperature to get a concentrated extract. The resulting extract was kept in the fridge at 8°C until use. The following equation was used to calculate the crude leaf extract's yield percentage.[21] Weight of crude extract/ weight of the powdered sample (g) x 100

2.4 Qualitative Phytochemical Screening

The phytochemical screening of methanolic *Prunus padus* leaf extract was conducted to analyse secondary metabolites like phenols, flavonoids, terpenoids, tannins, etc.

2.5 DPPH Radical Scavenging Assay (Antioxidant Activity)

Free radical molecules constitute DPPH, which turns discolored in the presence of antioxidants. The plant extract's capacity to scavenge free radicals was assessed using DPPH, and the absorbance was measured at 517 nm. Ascorbic acid was chosen as a standard. Inhibition per cent was estimated using the formula as follows:
Percent inhibition = $(A_{0\ (control)} - A_{1\ (extract)} \setminus A_{0\ (control)}) \times 100$

2.6 *In Vitro* α-amylase Inhibition Assay (Antidiabetic Activity)

The Alpha-amylase inhibition assay was used to determine the in vitro antidiabetic efficacy.[17] Plant extract concentrations ranging from 50 - 250 µ g were made in DMSO. The 20 mM sodium phosphate buffer was produced in 100 mL and 25 ml of 1 per cent starch solution in phosphate buffer was made ready at pH 6.9. 0.001 g of alpha-amylase enzyme solution was freshly blended in 100 ml of sodium phosphate buffer with pH 6.9 and 6.7 mM NaCl. The color mixture was made by mixing 20 ml, 96 mM DNSA, Potassium sodium tartrate in 8 mL, 2 M NaOH. The color reagent was then adulterated by adding 12 ml deionised water. Absorbance was studied at 540 nm and the positive control was chosen to be Acarbose. The following formula was used to calculate alpha-amylase inhibition percentage.

Percentage inhibition = Control (Absorbance) − Sample (Absorbance) / Control (Absorbance) x 100

2.7 Analysis of Corosolic Acid

RP-HPLC constitutes non-polar stationary phase and a polar, aqueous mobile phase. Although the polarity of the mobile and stationary phases have been inverted, reversed phase chromatography which uses a hydrophobic stationary phase and is the opposite of normal phase chromatography.[18] PDA detector (SPD-M20A, Shimadzu, Japan), UV detector, auto-sampler, Stationary phase- C_{18} (4.6 × 250 mm, 10 µm particle size), Nucleodur, and LC-solution software are all included with the RP-HPLC.[19]

2.8 Chromatographic Conditions

Acetonitrile served as the mobile phase while 0.1 per cent O-phosphoric acid served as the solvent B. Detector used was UV and PDA. Maximum absorption: 210 nm, Column Temperature: 30 ^{o}C. Flow rate: 1 ml/min, Injection volume: 20 µl and the diluents used was methanol.

2.9 Corosolic Acid Standard Stock Solution Preparation

Blank: Methanol was filtered through 0.22 µ millipore membrane filters and run into the HPLC system.

2.10 Standard Solution Preparation

To make a solution with a 1000 µg/ml stock, pure corosolic acid was mixed with 1 ml of methanol at a concentration of 10 mg. To make the appropriate dilutions, 0.01 ml of the stock was obtained, diluted up to 1 ml using diluents, filtered through 0.22 millipore membrane filters, and then added to the HPLC system.

2.11 Plant Sample Extraction

Prunus padus had a yield of 1.03 per cent (Leaf powder used 500.3g and 5.2 g was the obtained extract). To make a stock s olution, 20 mg of plant extract were placed in a vial and dissolved in 2 ml of methanol. To make the appropriate dilution, 0.1 ml of the stock solution was obtained, diluted up to 1 ml with diluents, filtered through 0.22 millipore membrane filters, and then injected for the HPLC testing.

3. RESULTS

3.1 Qualitative Analysis

Preliminary phytochemical analysis is an important step in the evaluation of active principal components present in medicinal plants. Phenols, flavonoids, tannins, and terpenoids were found in the Prunus padus leaf extract after qualitative analysis (Table 1).

3.2 *In Vitro* Antioxidant Activity

The DPPH carries an odd electron which gives deep purple color and absorbance at 517 nm. It ecolorizes on accepting an electron from the antioxidant which can be measured quantitatively from absorbance values. The DPPH radical scavenging increases with the rising concentration of plant extract 20 The methanolic leaf extract of *Prunus padus* showed 91.90 per cent inhibition and ascorbic acid showed 82.39 per cent inhibition at 250 µg/ ml concentration (Table 2).

Table 1. Qualitative phytochemical analysis of *Prunus padus* leaves

Components	Qualitative tests	Observation	Results
Phenol	Ferric chloride test	Bluish-black color	+ve
Flavonoids	Shinoda's test	Crimson red color	+ve
Terpenoids	Salkowski's test	Reddish-brown color	+ve
Tannins	Ferric chloride test	Greenish-black color	+ve

Table 2. % inhibition of DPPH by *Prunus padus* leaf extract and acorbic acid

Concentration (µg/ml)	% Inhibition Prunus padus	% Inhibition Ascorbic Acid
50	89.27 ± 0.036	42.63 ± 0.078
100	90.04 ± 0.035	54.09 ± 0.015
150	90.78 ± 0.031	67.29 ± 0.036
200	91.40 ± 0.054	73.80 ± 0.062
250	91.90 ± 0.027	82.39 ± 0.058

3.3 *In vitro* Antidiabetic Activity

Alpha amylase instigates the breakdown of starch into a simpler sugar. In the case of diabetes, the inhibition of alpha-amylase is an important target to reduce postprandial glucose level.[15] Alpha amylase inhibition (%) by *Prunus padus* leaf extract at varying concentrations from 50 to 250 µg/ mL (Table 3). As the concentration of the plant extract rose, the percentage of alpha-amylase inhibition also gets up. IC_{50} value for acarbose was 193.62 ± 0.634 µg/ mL whereas for *Prunus padus* extract, it was 114.72 ± 1.038 µg/ mL.

Table 3. % alpha amylase inhibition by plant extract and acarbose

Concentration (µg/ml)	% Inhibition Prunus padus	% Inhibition Ascorbic Acid
50	33.93 ± 0.446	13.69 ± 0.929
100	50.15 ± 0.258	13.69 ± 0.929
150	56.40 ± 0.258	40.92 ± 0.682
200	13.69 ± 0.929	55.51 ± 0.515
250	78.42 ± 0.258	62.95 ± 0.446

3.4 Analysis of Corosolic Acid

Analysis of corosolic acid was done with RP HPLC method equipped with PDA and UV detector.[24] HPLC chromatogram of standard corosolic acid showed a spike at 9.052 min. (Fig. 2) A leaf sample of *Prunus padus* showed a signal at 9.855 min., revealing the occurrence of corosolic acid (Fig. 3.). MS Excel software was employed to plot the calibration data and to calculate the concentration of corosolic acid in the plant sample. A calibration curve was created by linear regression based on the peak areas. Methanolic leaf extracts of *Prunus padus* showed the concentration of corosolic acid, i.e., 8.032 µg/20 mg dry / weight. Calibration equation- Y= 1498. 2x + 7838.8 and R^2= 0.9978

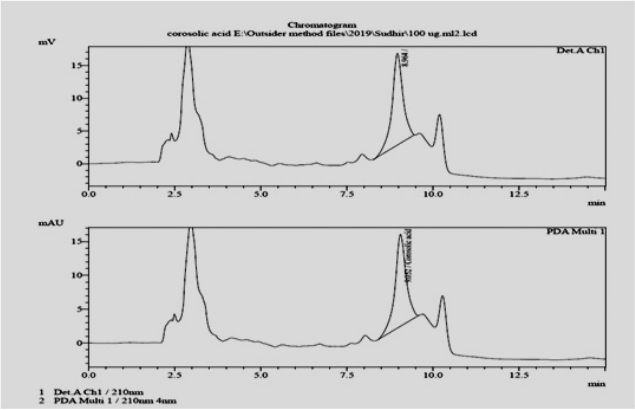

Figure 2. HPLC chromatogram of corosolic acid (standard).

S. No.	Compound Name	Retention time	Area	Tailing
1	Corosolic acid	9.05	306436	0.966

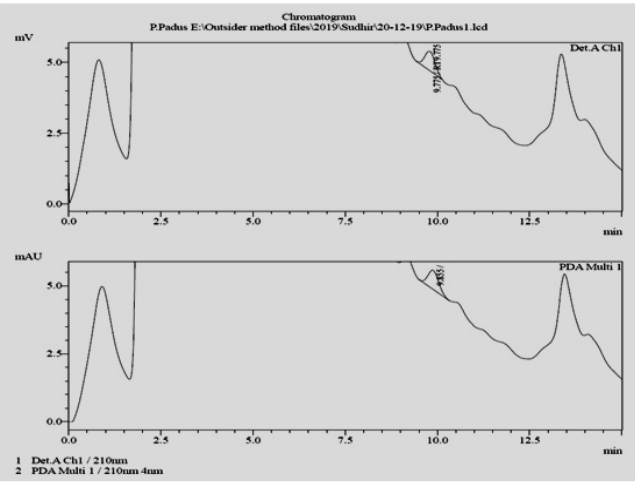

Figure 3. HPLC chromatogram of Prunus padus leaf extract.

Plant Sample	Retention time	Area	Tailing
Prunus padus	9.855	13854	1.185

4. DISCUSSION

The current study confirmed the presence of corosolic acid, a valuable phytochemical that is biosynthesised in the leaves of *Prunus padus*, as well as the plant was found to possess strong antioxidant and anti-diabetic properties. Corosolic acid has been proclaimed in earlier studies to possess numerous medicinal properties such as anti-diabetic, anti-inflammatory, and anti-obesity and protein kinase C inhibition activity.[13-14]

Oxidative stress i.e increased free radical formation accompanies diabetes and other diseases. It has been reported in many studies that increased free radical formation and less antioxidant activity promote the pathogenesis of diabetes. *Prunus padus* has been noted to contain polyphenols, which define a plant's ability to act as an antioxidant.[26] *Prunus padus* leaf extracts in methanol have reportedly been found to have potent hypoglycemic effects.[27] In addition to protecting beta-cell function and restoring insulin sensitivity, antioxidants shield the beta cell from oxidative damage. *Prunus padus* produces a variety of phytochemicals, including corosolic acid, which significantly boosts the antioxidant and anti-diabetic properties of the plant.

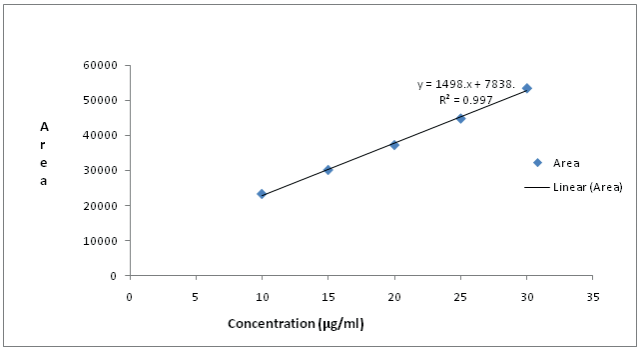

Figure 4. Standard calibration curve of corosolic acid.

Table 4. Calibration curve data of corosolic acid

S. No.	Conc.	Area
1	10	23317
2	15	30168
3	20	37263
4	25	44826
5	30	53444

5. CONCLUSION

The current investigation found that *Prunus padus* contains corosolic acid; 40 µg of corosolic acid was found in around 100 mg of leaf powder. *Prunus padus* is traditionally used as an antidiabetic plant without any harmful effects. Contrary to the current synthetic antidiabetic drugs which possess some side effects, corosolic acid has not been reported with any kind of adverse effect on human health.[28] It is worth noting that corosolic acid is a key player in significant antioxidant and anti-diabetic properties of *Prunus padus* which

could have a positive impact on the pharmaceutical sector. Further research can be carried out on other parts of *Prunus padus* or similar genera to explore more sources of corosolic acid which may have future possible scope as a drug ingredient with compelling pharmacological effects.

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CONTRIBUTORS

Mr Sudhir Kharyal has done MSc in Botany. He is currently pursuing PhD in Botany with specialisation in medicinal botany (Angiosperms) at Department of Botany, Panjab University, Chandigarh. His area of specialisation is angiosperms and medicinal botany.
In the current study, he worked on concept development, information gathering, experimental work, and text writing of this manuscript.

Prof Richa Puri holds the position of a Professor at Department of Botany, Panjab University, Chandigarh. She is expert in crop physiology, seed physiology, molecular markers to study taxonomy of bamboos, ethno botanical studies, research of medicinal and aromatic plants and their properties.
In the current study, she contributed in data analysis, article amendment for significant intellectual substance and publication version approval.