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Research Article

Phytochemical Profile, TLC Profiling and Antioxidant Potential of *Prunus Avium*

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Abstract

This work looks at the Chromatographic content of the hydro-alcoholic extract of *Prunus avium* leaf and its antioxidant activity. The presence of essential metabolites was confirmed by preliminary phytochemical screening for glycosides, flavonoids, alkaloids, steroids, volatile oils, and tannins, indicating the plant's ethnomedicinal value. To maximise the separation and visibility of constituent substances, TLC was done using three solvent systems of varied polarity. Under 254 nm and 365 nm UV light, distinct, well-resolved bands were observed, indicating a different range of polar and mid-polar phytochemicals. Further antioxidant activity was assessed using DPPH and ABTS radical scavenging assays. The extensive chromatographic and phytochemical profiles obtained in the study highlight the potential of P. avium as a valid phytotherapeutic candidate, setting the stage for future bioactive-guided reclusion and anticancer testing.

Keywords: *Prunus avium,* sweet cherry, Chromatographic content, TLC analysis, hydroalcoholic extract.

1. INTRODUCTION

Prunus belongs to the Rosaceae family, specifically the Amygdalaceae subfamily. It includes of over 430 species of evergreen and deciduous trees and shrubs, primarily located in the temperate zones of northern hemisphere. *Prunus avium* (Sweet cherry) is a temperate region fruit that is also most popular in regions rich in phytoconstituents, which are responsible for various medicinal properties. They have a lower glycemic index than other fruits and vegetables. Sweet cherry is also said to be a good source of vitamin C & other vitamins also giving them an advantage over other fruits and vegetables. High amounts of water, lower levels of fat, mainly saturated fat, which is also cholesterol-free, and a calorie deficit. The principal phytoconstituents responsible for pharmacological actions in the *Prunus* genus are saponins, sterols, alkaloids, terpenoids, flavonoids, tannins, and phenolic acids [1,2].

Sweet cherry also contain several phenolic chemicals, including phenolic acids and flavonoids (anthocyanins, flavan-3-ols, and flavanols) and (hydroxycinnamic derivatives) which have been linked to their antioxidant activity [3,4]. Polyphenols were quantified using HPLC in an ultrasound-assisted *P. avium* extract and Ferulic acid, quercetin, Coumaric, Gallic acid and syringic acids were found [5,6]. *Prunus Avium* includes diverse flavonoids such Aequinoctin, Chrysin, Galangin, Naringenin, Jaceidin, Tectochrysin, Dihydrotectochrysin and Kaempferol, as well as phenolic acids like Caffeic acid, p-Coumeric acid, o-Coumaric acid, and Chlorogenic acid [7]. *Prunus Avium* contains many chemical constituents with varying pharmacological activity, including antioxidant, antimicrobial, anti-fibrotic, anti-tumor, anti-estrogen, and anti-cancer, respectively [1].

2. MATERIALS AND METHODS

2.1 Preparation of plant material:

The Leaves of *Prunus avium* were collected from the natural habitat in Prayagraj, Uttar Pradesh, India and then shade dried to grind in a coarse powder.

2.2Process of Extraction for Phytochemical / Phytoconstituent of *Prunus avium*

Extraction of phytoconstituent was done by using the soxhlet apparatus extraction technique from *Prunus avium* leaves. The dried and powdered plant material (about 50 g) was accurately weighed and placed in a thimble made of Whatman filter paper. The thimble was inserted into the Soxhlet extractor attached to a round-bottom flask containing 250 mL of mixture of water and alcohol (50:50) solvent. The extraction was carried out at the solvent's boiling temperature for 6–8 hours until the solvent in the siphon tube of the extractor became colorless, indicating complete extraction of the phytochemicals. After extraction, the solvent was concentrated under reduced pressure using a rotary evaporator at to obtain a thick, viscous crude extract. The extract was then transferred into a pre-weighed glass dish and kept in a vacuum oven to remove any residual solvent and kept in a desiccator for further use (Figure 1) [8].

Figure 1: Extraction process of *Prunus avium* leaves extract. *https://BioRender.com*

2.3 Phytochemical Analysis

Different phytochemical tests were done on crude hydroalcohalic extract of the leaves of *prunus* to identify the phenols, alkaloids, glycosides, volatile oils, flavonoids, balsams, terpenes, tannins, and resins [9].

2.4Preparation of Thin layer Chromatography (TLC) plate & profiling of *Prunus avium* extract

To create a fingerprint of phytochemicals found in the hydroalcoholic extract, TLC was done. TLC plates from Merck the stationary phase consisted of pre-coated silica gel were used. A small volume of 1-1.5 µL of the hydroalcoholic extract was employed upon the plate by glass capillary, keeping in mind that spot was roughly ~1 cm above from the plate's lower side. To ensure reproducibility and constant migration, spot size and spacing were uniformly distributed among all sample [10,11].

Three different solvents were taken: 1- toluene: ethyl acetate (7:3, v/v), 2- methanol : ethyl acetate (2:8, v/v) 3- toluene : ethyl acetate (3:7, v/v) [12]. Spots were visualized using UV light of wavelengths of 254 and 365 nm [13,14].

2.5 Antioxidant activity

2.5.1 DPPH scavenging activity:

In a 96-well microplate, 5 µL of various test compound stock solutions (as detailed in the Excel sheet) were combined with 0.1mL of 0.1mM DPPH solution. The assay was conducted in triplicate, while duplicate blanks were prepared using 0.2mL of DMSO/ Methanol and 5 µL of test compounds at different concentration (as listed in the Excel sheet). The plate was then incubated in the dark for 30 minutes. Post incubation, absorbance was measured at 495nm using an iMark Microplate Reader (Bio-Rad) to assess DPPH decolorization. A reaction mixture containing 20 µL of deionized water served as the control. The radical scavenging activity was calculated as percent inhibition relative to the control, and the IC_{50} values were determined using GraphPad Prism 6 [15,16].

2.5.2 ABTS Radical Scavenging Ability:

ABTS (SRL-Chem-Cat no.-28042) radicles were prepared by mixing APS (2.45 mM) and ABTS (7mM) solution, which was diluted 100X to prepare ABTS free radical reagent. Add 10µl of different stock of the standard (Ascorbic Acid

-SD Fine- F13A/0413/1106/62, Concentration as per mentioned in excel sheet) and samples (As per mention in excel sheet) to the 200µl of ABTS free radical reagent in 96 well plate and incubated at RT for 10 min in dark. After incubation measure absorbance of the decolorization at 750nm using a microplate reader (iMark, BioRad). Results were presented with respect to negative control. IC-50 was calculated using Software Graph Pad Prism 6 [17,18].

3 RESULT

3.1 Phytochemical analysis

It showed the diverse classes of phytochemicals as glycosides, Alkaloid, steroids, flavonoids, starch and saponins in the different chemical tests.

3.2 TLC Profiling

Bright fluorescent spots were visible under 365 nm UV light, which suggested the presence of flavonoids and terpenoids. In the Toluene: Ethyl Acetate (7:3) three spots were seen, methanol: ethyl acetate (2:8) solvent system showed total 5 spots and Toluene: Ethyl Acetate (3:7) showed 9 visible fluorescent spots, range of Rf values from 0.41 to 0.81, indicating the diverse phytochemical profile, potentially encompassing phenolics compounds and sterols. (Figure 2)

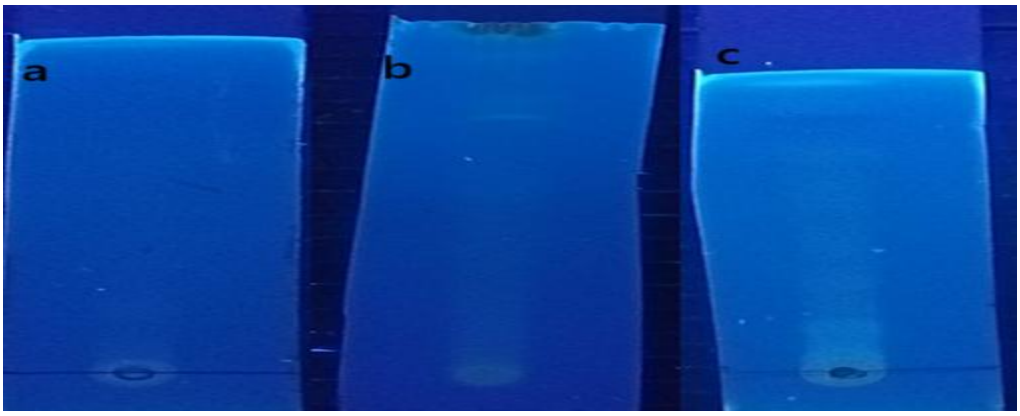

Figure 2: TLC profiling of *Prunus avium* bark employing multiple solvent systems and visualizing under 365nm UV light. (a) Toluene: Ethyl Acetate, 7:3 (b) Methanol: Ethyl Acetate, 2:8 (c) Toluene: Ethyl Acetate, 3:7.

3.3 Antioxidant activity

DPPH radicle scavenging activity was observed in *Prunus avium* at IC50 = 423.1 ± 0.041 µg/ml (Figure 3) while ABTS scavenging activity was observed at IC50 = 148.5 ± 0.92 µg/ml) (Figure 4).

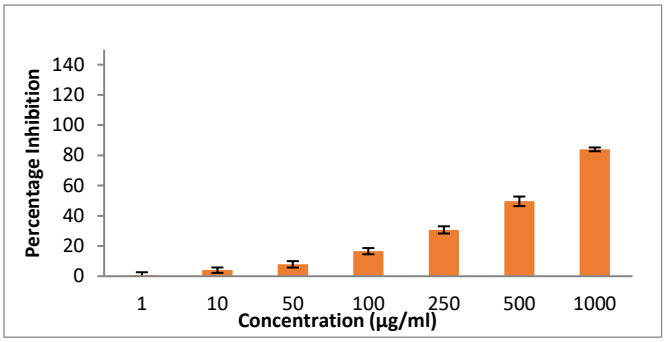

Figure 3: DPPH scavenging activity of *Prunus avium (Data are represented in Mean ± SEM)*

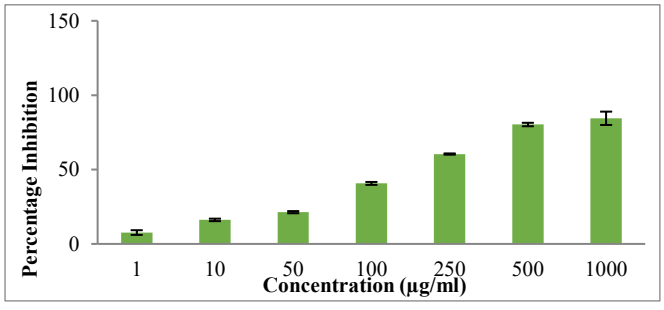

Figure 4: ABTS Radical Scavenging Ability of *Prunus avium (Data are represented in Mean ± SEM)*

4. DISCUSSION

The preliminary phytochemical screening of *Prunus avium* bark extract shown the presence of different classes of bioactive secondary plant metabolites, such as glycosides, alkaloids, steroids, flavonoids, starch, and saponins. These bioactive compounds are well known for its pharmacological properties, mostly antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. Flavonoids and saponins, for example, are known to show strong free radical scavenging activity, however steroids and alkaloids have been linked with anti-inflammatory and antimicrobial potential. TLC profile confirmed the chemical variety of *P. avium*. Light color fluorescent spots were seen in 365 nm UV light, suggesting the presence of flavonoids and terpenoids, both. The chromatographic separation using different solvent systems shows TLC profiles: Toluene: Ethyl Acetate (7:3) shows 3 different spots. Methanol: Ethyl Acetate (2:8) shows 5 different spots. Toluene: Ethyl Acetate (3:7) shows 9 different spots. The experimental Rf values 0.41 to 0.81, shows various phytochemical profile with different polarity. The higher number of spots in the Toluene: Ethyl Acetate (3:7) system signifies that this solvent system provided better resolution meant for separating various components. According to different literature, phenolic compounds, flavonoids, terpenoids and sterols are associated with antioxidant activity [19-22].

TLC thus proved to be an effective preliminary tool to visualize the complex chemical composition of the extract. The potential antioxidant activity of *P. avium* bark extract

was evaluated by using two standards in vitro assays: DPPH free radical scavenging and ABTS radical cation decolorization assays. The DPPH assay of the extract showed an IC_{50} value of 423.1 ± 0.041 µg/ml, and the ABTS assay exhibited an IC_{50} value of 148.5 ± 0.92 µg/ml. The lower IC_{50} value in ABTS compared to DPPH for *P. avium* shows that the extract may be more effective against the ABTS radical cation as compared to DPPH radicals. Flavonoids and phenolics serve as hydrogen or electron donors, neutralizing reactive oxygen species (ROS) and thus mitigating oxidative stress [23,24]. Nevertheless, the crude extract's IC_{50} values indicate that it contains a diverse array of compounds with variable antioxidant potential.

5. CONCLUSION

Qualitative screening alongside TLC analysis confirmed the presence of a wide spectrum of bioactive phytochemicals in the hydroalcohalic extract of *Prunus avium* leaves. The presence of several secondary metabolites, including flavonoids, steroids, and saponins with established pharmacological actions, demonstrates this medicinal plant's therapeutic potential. Effective TLC analysis using varied solvent systems produces a comprehensive profile of the phytoconstituents, indicating substantial chemical diversity and variations in compound mobility influenced by solvent polarity. Antioxidant activity results substantiate the observations of P. *avium's* traditional medicinal usage and warrant further exploration as a source of natural chemicals for the development of anticancer medicines.

Declaration of Competing Interest: The authors declare no competing interest.

Ethics approval and consent to participate: NA

Consent for publication: The publication of the material in print, online or other media formats as determined by the publisher.

Availability of data and materials: All data and materials related to this work are available in the manuscript.

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