Phytochemical Screening and Antimicrobial Activity of fruit extract of *Prunus cerasoides* Dehradun from Garhwal Himalaya

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Abstract-Phytochemical screening of the petroleum ether, chloroform, methanol, and aqueous extract of fruits of *Prunus cerasoides* and their in vitro antibacterial activity by agar disc diffusion method. All the extracts were found to contain alkaloids, flavonoids, glycosides, saponins except for the absence of tannins and steroids. The plant extracts were studied using *Staphylococcus aureus, Escherichia coli, Listeria Monocytogenes, Serrtia marccen-scens*, *Aspergillus flavus* for their antimicrobial activity.

Keywords: Phytochemical screening, methanolic extract, *Prunus cerasoides, Staphylococcus aureus, Serrtia marccen-scens.*

Introduction

Prunus cerasoides (syn. Prunus puddum) is native of Garhwal Himalayan and also wildly growing in sub-Himalayan tracts to montane zone 2400 metre high, Sikkim, Nepal, Bhutan, Myanmar, West China and North-East Verma[1]. It belong to the family Rosaceae, and locally known as Payu, Padam, Padmakha, and Himalayan cherry. Deciduous tree to 10 m high; bark reddish-brown exfoliating in thin circular strips. Leaves conduplicate in bud, elliptic or ovate-lanceolate, 3.5-8.5×2.5-3.5 cm, apex acuminate. In traditional medicine, the plant is used in Leprosy, Asthma and shown antipyretic activity[2]. According to the earlier investigation, much work has been carried out on stem bark, sapwood, seed of

the plant due to the presence of high concentration of flavonoids and flavonoid glycosides [3-7] but fruits are scantly studied. This prompted us to carry out the phytochemical screening and their antibacterial efficacy against various bacterial strains.

Figure-1 Ripe and dried fruits of *Prunus cerasoides*

Material and Methods

Plant materials

The fruits of the plants were collected from Dhanolti, located at North part of Garhwal Himalaya. Plant sample was authenticated by Department of Botany, HNB Garhwal University Campus Badshahi Thaul, Tehri, and voucher specimens were deposited in the Department. Shade dried leaves were coarsely powdered and subjected to successive solvent extraction by continuous hot extraction (Soxhlet). The extraction was done with different solvents in their increasing order of polarity such as petroleum ether (60-80^{0}C), chloroform, methanol and water. Each time, the mare was air dried and later extracted with other solvents. All the extracts were concentrated by distilling the solvent in a rotator flash evaporator. The dried extracts were dissolved in Dimethyl Sulphoxide (DMSO) and subjected to antimicrobial activity.

Phytochemical screening

Chemical tests were carried out on the methnolic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973)[8, 9, 10].

Test for tannins: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered.

10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Teat for flavonoids: Three methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harborne, 1973)[8, 10]. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Test for steroids: Two ml of acetic anhydride was added to 0.5 g Methanolic extract with 2 ml H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for cardiac glycosides (Keller-Killani test): Five ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Microorganism and Media

Gram positive bacteria: *Staphylococcus aureus* (ATCC29213), *Listeria monocytogens* (ATCC 19115), Gram negative bacteria: *Escherichia coli* (ATCC 25922), *Serrtia marcescens* (ATCC 21075); and fungus: *Aspergillus flavus* (ATCC 32612), were obtained from the Department of microbiology G.B. Pant Agriculture University Hill Campus, Ranichauri, Tehri Garhwal for the study. The bacterial and fungal stock cultures were maintained on Muller Hinton agar and Saboured- Dextrose agar slant, respectively, which were stored at 4^0 C.

Antimicrobial screening

The extracts were screened for their antimicrobial activity in vitro by disc diffusion method[11] using *S. aureus*, *L. monocytogens*, *E. coli*, *S. marcescens* and *A. flavus* at test organism. Agar cultures of the test microorganism were prepared.

Three to five similar colonies were selected and transferred to 5ml broth with a loop and the broth culture were incubated for 24 h at 37^0 C and suspension was checked to provide approximately 10^{10} colonies forming units per ml. 0.1 ml of organism's suspension were spread evenly on the agar plates. For screening, sterile 3mm dimeter disc (Whatman filter paper No 1) were impregnated with 0.2 ml of 1000 µg/ml of the various extracts of both the drugs, dried and then placed in inoculated plates of Muller Hinton agar and Saboured- Dextrose agar medium. DMSO solvent was used as negative control. The plates were inoculated at 37^0 C for 24 h and room temperature for 48 h for bacteria and fungi, respectively. After incubation for 24 and 48h, the results were recorded by measuring the zone of inhibition surrounding the disc. *Penicillin* (10 µg/disc) and *Gentamycin* (20 µg/disc) were used as reference slandered for bacteria and fungi respectively. Each experiment was done in triplicate.

Results and Discussion

Petroleum ether, methanol and aqueous extracts of *P. cerasoides* showed significance activity against E. coli and moderate activity against other organism except *L. monocytogens* and only the aqueous extract showed significant activity against *A. flavus* **(Table -1).**

Phytochemical screening of different extracts of *P. cerasoides* showed the presence of alkaloids, saponins, flavonoides and coumarins **(Table-2)**. Petroleum ether extracts of *Prunus cerasoides* were found to be more effective against *E. coli* and *S. marcescens* and *A. flavus*. When compared to other extracts of the plant. Phytochemical screening of the petroleum ether extract of *P. cerasoides* revealed the presence of alkaloids which suggest that these phytoconstituents may be responsible for their antimicrobial activity. Further studies are needed to isolate and characterize the bioactive principles to develop new natural drugs.

Table-1 Antimicrobial activity of *Prunus cerasoides* fruits extracts

Organism	ZONE OF INHIBITION OF EXTRACTS IN mm				
	PE	CE	ME	AE	STD
S. aureus	6	11	12	10	18
L. monocytogenes	9	NI	6	NI	18
E.coli	21	NI	15	17	20
S. marccenscens	8	7	9	8	18
A. flavus	NI	8	NI	19	*20

NI= No Inhibition, PE= Petroleum Ether ext, CE= Chroloform extract, ME= Methanol extract, AE=Aqueous extract; STD=Standared (*Penicillin, *Gentamycin*). Values are average of three determinations.

Table-2 Phytochemical screening of different extracts of *Prunus cerasoides* fruits

Phytoconstituents	*Prunus cerasoides*			
	PE	CE	ME	AE
Alkaloids	+	-	+	+
Glycosides	+	+	+	+
Saponins	+	-	-	-
Tannins	-	-	-	-
Flavonoids	+	+	+	+
Steroids	-	-	-	-

PE= Petroleum ether ext, CE= Chloroform extract, ME= Methanol extract, AE=Aqueous extract; + = Present, - = Absent.

Conclusion

The results obtained in the present study phytochemical screening assays confirmed the use of the plant and their species for further investigation potential natural bioactive compounds. It is the first report about antimicrobial effects of different extracts of *Prunus cerasoides* fruits against *S. marccenscens* and aqueous extract against *A. flavus*.

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Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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