Journal of Medicinal Plants Studies

www.PlantsJournal.com

ISSN 2320-3862
JMPS 2016; 4(4): 215-218

Received: 30-05-2016
Accepted: 01-07-2016

Sarma Dhrubajyoti
State Drug Testing Laboratory (AYUSH), Jalukbari, Guwahati, Assam, India

Sarma Hiranmoy
State Drug Testing Laboratory (AYUSH), Jalukbari, Guwahati, Assam, India

Thakuria Arup Jyoti
State Drug Testing Laboratory (AYUSH), Jalukbari, Guwahati, Assam, India

Mazumder Zahidul
State Drug Testing Laboratory (AYUSH), Jalukbari, Guwahati, Assam, India

Sharma Neelakshi
Regional Drug Testing Laboratory, Sixmile, Guwahati, Assam, India

Sarma Nipan
State Drug Testing Laboratory (AYUSH), Jalukbari, Guwahati, Assam, India

Kakoty Dipu
State Drug Testing Laboratory (AYUSH), Jalukbari, Guwahati, Assam, India

Correspondence
Sarma Dhrubajyoti
State Drug Testing Laboratory (AYUSH), Jalukbari, Guwahati, Assam, India

A detail microscopic studies and pharmacognostical evaluation of *prunus cerasoides* D.Don (Rosaceae) leaf found in North East India (Meghalaya)

Sarma Dhrubajyoti, Sarma Hiranmoy, Thakuria Arup Jyoti, Mazumder Zahidul, Sharma Neelakshi, Sarma Nipan and Kakoty Dipu

Abstract
Prunus cerasoides (Family Rosaceae) the Himalayan Wild Cherry is a sacred plant in Hindu mythology. It is found in Sikkim, Nepal, Bhutan, Myanmar, West China and India. In the pharmacognostic study of *Prunus cerasoides* D.Don, some major studies like macroscopy, microscopy, phytochemical screening was carried out. Highest yield values were found with the methanolic extract. Multiple spots in the TLC indicates the presence of several active constituents in the leaf. Phytochemical screening were confirmed by the presence of Tannins, Alkaloids, Saponins, Steroids, Flavonoids, Glycosides, Carbohydrates.

Keywords: *Prunus cerasoides* D.Don, Microscopic, Phytochemical screening, Pharmacognostic, etc.

1. Introduction
The tree thrives in well-drained and moisture-retentive loamy soil. It will grow well with a bit of lime in the soil, but is likely to become chlorotic if too much lime is present [2]. It requires an open, sunny and sheltered location.
Prunus cerasoides (Family Rosaceae) the Himalayan Wild Cherry is a sacred plant in Hindu mythology. It is found in Sikkim, Nepal, Bhutan, Myanmar, West China and India [1].
In India the plant is restricted to sub-montane and montane Himalaya ranging from 1500-2400 m asl. In Garhwal Hills it is distributed abundantly in temperate zones of Pauri, Tehri, Chamoli and Uttarkashi districts. Locally it is known as 'Panyyan'. It is worshipped in all auspicious occasions by the inhabitants. People never cut the whole tree and use only its twigs in rituals as the wood are forbidden to be used as fuel. Thus it is common to observe quite old trees of *Prunus cerasoides* in the area. It blooms in October and lasts up to mid -December. Its pinkish- white flowers are the rich source of nectar and pollen for bees [3]. P. cerasoides is a medium sized tree which grows up to 30 metres in height. It flowers in autumn and winter, specifically in January and February. The fruit is astringent, and digestive. The juice of bark is applied externally to treat backaches. The stems are reported to be antipyretic, refrigerant and useful in vomiting, leprosy and leucoderma. Heartwood is moderately hard, strong, aromatic, astringent, bitter, acrid, refrigerant, antipyretic and tonic. It is useful in vitiated conditions of pitta, burning sensations, sprains, wounds, ulcers, leprosy and skin decolourations [4]. It has action similar to olive oil and is used in emollient preparations including nourishing creams, skin creams and cold creams. Berries are used to prevent abortion and used as tanning, and leaves are used as dyes, seeds used in treatment of stone and gravel in kidney.

2. Materials and methods
Collection of plant material
Leaves of P.cerasoides were collected from Shillong, Meghalaya with the help of some local khasi peoples [Figure-1].The Taxonomical identification was done by Botanical Survey of India, Shillong (Meghalaya). The collected leaves of P.cerasoides were then air dried, powdered after keeping some leaves in fixatives for microscopic studies and the dried leaf material were the stored in a air tight container for future use.

2.1 Pharmacognostic studies

Fresh leaves and stem were taken for morphological and histological studies. Coarse powder (60 #) was used to study microscopical characters, physicochemical parameters and phytochemical investigation. For the microscopical studies, transverse sections of leaves and stem were prepared and stained as per standard procedure [5]. The powder microscopy was performed according to the method of Khandelwal.

2.1.1 Macroscopic study

It refers to evaluation by means of organs of sense and includes the macroscopic appearance, color, odor, taste etc. of the drugs.

2.1.2 Microscopy study

A transverse section of fresh leaf of *P.cerasoides* was taken and cleaned. The sample was treated with chloral hydrate solution and different staining reagents and chemicals was used to detect the lignified cells in the cross sections and in the powder drugs [6]. The section was mounted on slides and studied under trinocular research microscope.

2.2 Quantitative estimation

Different physicochemical properties like moisture content, total ash, acid insoluble ash, extractive values of the leaf of *P.cerasoides* were determined using the methods described in the British Pharmacopoeia and Ayurvedic Pharmacopoeia.

2.3 Phytochemical Screening

The aqueous and methanolic extracts along with other solvent extracts of plant leaf materials were studied for various phytochemicals like alkaloids, carbohydrates, flavonoids, glycosides,gums and mucilages, phenols, tannins, reducing sugars, saponins, steroids, tannins and terpenoids by using precipitation and coloration reactions [7].

2.4 Extraction

500gm of powdered leaves of *P.cerasoides* were extracted successively with solvents like petroleum ether, benzene, chloroform, acetone and methanol respectively in a Soxhlet apparatus [7]. Each solvent extract was then concentrated by distilling off the solvent under reduced pressure.

TLC : Thin layer chromatography was carried out with the methanolic extract and maximum spots been separated on precoated silicagel G TLC plate with trial and error methods [8].

The physico-chemical parameters such as moisture content, pH (10% aqueous), total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive etc were carried out by the procedures as per pharmacopoeias [9].

Moisture content: 10g of powdered leaf material was taken in a dry and flat petri dish. The sample was dried in an oven at 105ºC for 5 hours. Dried until two consecutive weighing do not differ by more than 0.25%. Calculate the loss of weight in terms of percentage.

pH (10% aqueous): 1g of powdered sample was Dissolved in 100ml of distilled water, shaked frequently, then allowed it to stand for 18 hours. Filtered and check the pH using pH meter.

Total ash: Weigh accurately about 2-5g of dried plant material in a previously ignited and tarred crucible. Ignite it by gradually heating it to 500 – 600ºC until it is white. Cool in a desiccator and weigh. Calculate the content of total ash in terms of percentage.

Acid insoluble ash: To the crucible containing total ash, add 25ml of HCl (70g/l), cover with watch glass and boil gently for 5min. Rinse the watch glass with 5ml of hot water and add this liquid to the crucible. Collect the insoluble matter on an ashless filter paper and wash with hot water until the filtrate is neutral. Ignite the filter paper containing insoluble matter in crucible to constant weight. Cool in a desiccator and weigh. Calculate the content of acid insoluble ash in terms of percentage.

***Water-soluble extractive*:** Weigh accurately about 4g of air-dried material in a glass stoppered conical flask. Macerate with 100ml of distilled water for 6hours, shaking frequently, then allowed to stand for 18hours. Filter rapidly taking care not to lose any solvent, transfer 25ml of the filtrate to a tared flat bottomed petri dish and evaporate to dryness on a water bath. Dry at 105ºC for 6 hours, cool in a desiccator for 30 mins and weighed. Calculate the content of water- soluble matter in terms of percentage.

Alcohol soluble extractive: Weigh accurately about 4g of air-dried material in a glass stoppered conical flask. Macerate with 100ml of absolute alcohol for 6hours, shaking frequently, then allow to stand for 18hours. Filter rapidly taking care not to lose any solvent, transfer 25ml of the filtrate to a tared flat bottomed petridish and evaporate to dryness on a water bath. Dry at 105ºC for 6 hours, cool in a desiccator for 30 min and weighed. The content of alcohol-soluble matter in terms of percentage was calculated.

3. Result and discussion

The leaf of *Prunus Cerasoides* D.Don. (Rosaceae) had been investigated in a systematic way covering pharmacognostical and physicochemical aspects to rationalize its use as a drug of therapeutic importance.

3.1 Macroscopic characteristics

Macroscopically, the fresh leaf of *P. cerasoides* is 15 to 22 cm long, 7 to 12 cm wide and petiole 0.1 to 1.8 cm in length, simple, glabrous, broadly obovate in shape, acuminate apex with crenate, dentate margin and green in color

Fig 1: Leaf of the plant *Prunus cerasoides* D.Don

3.2. Microscopical characteristics

3.2.1. Leaf microscopy

TS of leaf passing through midrib region shows slight upper notch and large notch at lower surface. Upper and lower surface

of the leaf consists of rectangular thin walled epidermis, covered with thick cuticle followed by collenchymatous ground tissue; palisade cells reached up to the upper notched region. Palisade cell is single layered; midrib region show one median large size vascular bundle and two lateral vascular bundle. Vascular bundles are covered with fibrous bundle sheath which is very broad on lower side and 1 to 2 layers broad towards upper side. One group of sclerenchyma is present at upper notched side above the median vascular bundle. Xylem is arranged in cup shaped and surrounded by phloem facing toward the lower side (Figure 4). Xylem consists of vessels, tracheids, fibers and xylem parenchyma; inside the cup; cells are parenchymatous. Lateral vascular bundle also shows sclerenchymatous bundle sheath which encircles the vascular bundle. Sclerenchymatous bundle sheath is broad on both surfaces and only 1 or 2 layered on lateral side. TS passing through lamina region showed single layered palisade cells followed by several layers of spongy mesophyll embedded with lateral vascular bundles. *P.cerasoides* leaf surface shows the anomocytic stomata (Figure 2). Leaf surface also shows the presence of veins, vein islets, vein terminations (Figure 3) and palisade cells (Figure 3). Leaf constants such as stomatal number, stomatal index, palisade ratio, vein-islet number and veinlet terminations number were measured. The results are shown in Table 1.

Leaf surface of *P.cerasoides* leaf along with stomata, Vein islet and vein termination number.

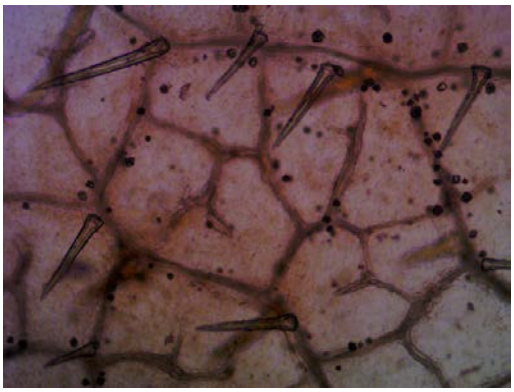

Fig 2.

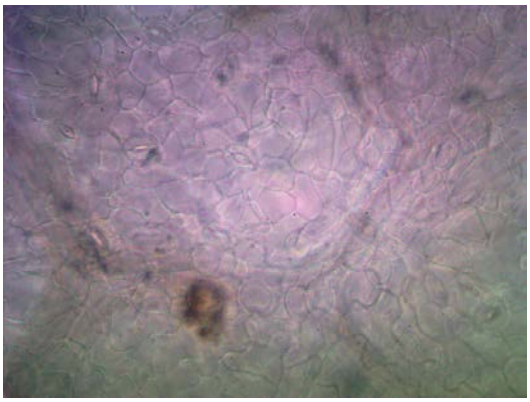

Fig 3.

Fig 2, 3: T.S of *P. cerasoides* leaf

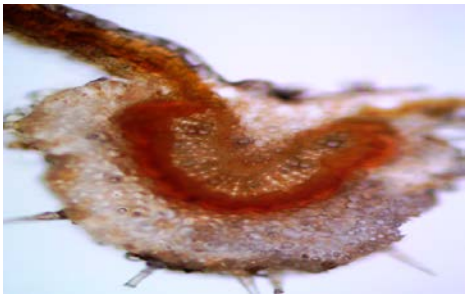

Fig 4.

Leaf constants (at 100X).

Table 1.

S. No.	Parameters	Value (in 1 mm^2 area)
1	Stomata number, upper surface	27.00
2	Stomata number, lower surface	177.00
3	Stomatal index, upper surface	7.55
4	Stomatal index, lower surface	30.88
5	Vein-islet number	6.00–8.00
6	Veinlet termination number	7.00–9.00
7	Palisade ratio	6.00–8.00 (per cell)

3.3. Determination of Quantitative standards

Table 2.

S. No.	Parameters	Values of 3 Replicates (%)	Mean (%)
1.	**Ash Value** **a)** Total ash	3.5% 3.1% 2.9%	3.16%
	b) Acid-insoluble ash	0	0
	c) water soluble ash	0	0
2.	**Extractive Value** **a)** Water soluble extract	22.80% 21.90% 21.10%	22.10%
	b) Alcohol soluble extract	27.20% 25.90% 29.20%	27.44%
3.	**Loss on drying**	2.60% 2.80% 2.70%	2.68%

Chromatographic Profile of Crude Extract of *Prunus cerasoides* D.Don

Fig 5: TLC under UV chamber

Fig 6: TLC on iodine vapour

The details of solvent system and the Rf values are mentioned in the Table-2.

Table 2.

Extract	Solvent System	No.Of Spots	Rf Values
Methanol	Carbon tetrachloride: Ethyl acetate (5:1)	4	0.40 0.65 0.70 0.84
Petroleum ether	Chloroform: Methanol (9:1)	1	0.70
Chloroform	n-Hexane : Ethyl acetate : Glacial acetic acid (5 : 1 : 0.5)	2	0.50 0.75

Discussion

Ethnomedically, the leaves of this plant were used by local people in the treatment of various disease conditions without standardization. The standardization of a crude drug is an integral part for establishing its correct identity. Before any crude drug can be included in an herbal pharmacopoeia, pharmacognostic parameters and standards must be established. Microscopic method is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials [10]. The pharmacognostic standards for leaves *P.cerasoides* found in this North Eastern region are carried out for the first time in this study. The macroscopical characters of the leaf can serve as diagnostic parameters. Ash values and extractive values can be used as reliable aid for detecting adulteration. These studies help in the identification of the plant materials. Ash values of drug give an idea of earthy matter or the inorganic composition and other impurities present along with drug. Extractive values are primarily useful for the determination of exhausted and adulterated drugs [11]. Extractive values are also useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in particular solvents. This analysis suggests that, leaves extract of *P.cerasoides* probably contain active agent(s) and this provides the basis for their folkloric use as a cure for some human ailments.

References

1. Dolui AK, Sarma HK. Traditional medicinal plants in Mizoram, Fitoterapia 2001; 72:141-161.
2. Bahyuguna R P, Jangwan J S, Kaiya T, Saaibara J. Puddumin-A a new flavanone glycoside from *Prunus cerasoides*, J. Nat Prod., 1987; 50(2):232.
3. Sharma 1970, Crane *et al.*, 1984, Gaur and Nawani 1989, Pratap 1997 and Gaur and Tiwari 2001.
4. Tripathi. Bio-efficacy of leaf extract of P.cerasoides D.Don (F. Rosaceae) on prostate and urinary disorders, 2005.
5. Mukharjee PK. Quality Control of Herbal Drugs, (Pub. by Business Horizons New Delhi), 2002.
6. Modern practical Botany, by Dr. B.P. Pandey (Pub. by S. Chand & company Pvt. Ltd.), I.
7. Practical pharmacognosy, Dr. CK Kokate. Pub. By Vallabh prakashan.
8. Trease, Evans. Textbook of pharmacognosy (Pub by: Harcourt Bruce and Co, Asia PTE Ltd), 1996.
9. Anonymous. The Ayurvedic Pharmacopoeia of India, (Pub by Ministry of Health and Family Wellfare, New-Delhi), 2011,
10. Mukharjee P K. Quality Control of Herbal Drugs, (Pub by Business Horizons New Delhi), 2002.
11. Trease and Evans. Textbook of pharmacognosy (Pub by : Harcourt Bruce and Co, Asia PTE Ltd), 1996,