International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 16-05-2021, Accepted: 31-05-2021, Published: 14-06-2021
Volume 6, Issue 3, 2021, Page No. 655-658

Studies on quality control and standardization parameters of *Cupressus* species

Sanjay Dwivedi[1*], Sumeet Dwivedi[2]
[1] Research Scholar, Faculty of Pharmacy, Oriental University, Indore, Madhya Pradesh, India
[2] Professor and Principal, University Institute of Pharmacy, Oriental University, Indore, Madhya Pradesh, India

Abstract
About 80% of the Indian population depends on traditional system of medicine for the treatment of several diseases. Increasing population relying on herbal plants and the product prepared from the herbs and because of the same plant need to be scientifically screened out for the quality standards. In the present investigation two Cupressus species *viz*., Cupressus torulosa D. Don ex Lamb, and Cupressus vietnamensis (Farjon & Hiep) Q.P. Xiang & J. Li were evaluated for quality parameters. In this study various standardization parameters were studied out and were reported.

Keywords: *Cupressus* species, standardization parameters, quality control

Introduction

Cupressus is one of genera of evergreen conifers within the family Cupressaceae having the common name cypress or cypress. It is considered a polyphyletic group. Based on genetic and morphological analysis, the genus *Cupressus* is found in the subfamily Cupressoideae [1-2]. They are evergreen trees or large shrubs, growing to 5–40 m tall. The leaves are scale-like, 2–6 mm long, arranged in opposite decussate pairs, and persist for three to five years. On young plants up to two years old, the leaves are needle-like and 5–15 mm long. The cones are 8–40 mm long, globose or ovoid with four to 14 scales arranged in opposite decussate pairs; they are mature in 18–24 months from pollination. The seeds are small, 4–7 mm long, with two narrow wings, one along each side of the seed. Many of the species are adapted to forest fires, holding their seeds for many years in closed cones until the parent trees are killed by a fire; the seeds are then released to colonise the bare, burnt ground. In other species, the cones open at maturity to release the seeds [3]. *Cupressus torulosa* D. Don ex Lamb. (Himalayan cypress or Bhutan cypress), is native to the mountainous northern regions of the Indian Subcontinent, primarily the Himalayas. It is a large tree, growing up to 45 m (150 ft) in height. [4-6] *Cupressus vietnamensis* (Farjon & Hiep) Q.P. Xiang & J. Li. (Vietnamese golden cypress), is native to the Vietnam, also found in Himalaya regions. The tree is 10-15 m tall in height [7-9]. So, far no any systematic study was carried out in evaluating the standardization parameters of leaves of selected plant, therefore, the present work was undertaken to revel and develop the quality control parameters for standardization of selected herb.

Fig 1: *Cupressus torulosa* D. Don ex Lamb.

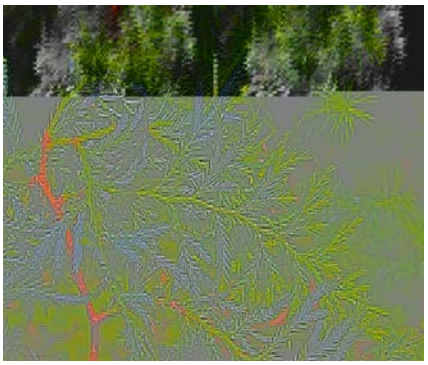


Fig 2: *Cupressus vietnamensis* (Farjon & Hiep) Q.P. Xiang & J. Li.

Material and Methods

Collection of herbs and their authentication

The leaves of *Cupressus torulosa* D. Don ex Lamb. (CTL) and *Cupressus vietnamensis* (Farjon & Hiep) Q.P. Xiang & J. Li. (CVL) were collected in the months of July-December 2020 from the Himalaya region and identified & authenticated by Dr. S. N. Dwivedi, Retd. Prof. and Head, Department of Botany, Janata PG College, A.P.S. University, Rewa, (M.P.) and was deposited in our Laboratory. Voucher specimen No. J/Bot/2020-CTL012 & CVL013 was allotted.

Development of Quality control/Standardization parameters of selected herbs [10-13]

Pharmacognostical Evaluation

Macroscopic studies

The macroscopy of different parts of the plant such as color, odor, size, shape, taste, surface characters and fractures were carried out.

Physicochemical Evaluation

The dried parts were subjected to standard procedure for the determination of various physicochemical parameters.

Determination of foreign organic matter (FOM)

Accurately weighed 100 g of the drug sample and spread it out in a thin layer. The foreign matter should be detected by

inspection with the unaided eye or by the use of a lens (6X). Separate and weigh it and the percentage present was calculate. The results are given in Table no. 15.

Determination of moisture content (LOD)
Place about 10 g of drug (without preliminary drying) after accurately weighing in a tared evaporating dish and kept in oven at 105^0 C for 5 hours and weigh. The percentage loss on drying with reference to the air dried drug was calculated.

Determination of ash value
The determination of ash values is meant for detecting low-grade products, exhausted drugs and sandy or earthy matter. It can also be utilized as a mean of detecting the chemical constituents by making use of water-soluble ash and acid insoluble ash.

Total ash
Accurately about 3 gms of air dried powder was weighed in a tared silica crucible and incinerated at a temperature not exceeding 450^0C until free from carbon, cooled and weighed and then the percentage of total ash with reference to the air dried powdered drug was calculated. The percentage of total ash with reference to the air-dried drug was calculated.

Acid insoluble ash
The ash obtained in the above method was boiled for 5 minutes with 25ml of dilute HCl. The residue was collected on ash less filter paper and washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Water soluble ash
The ash obtained in total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight at a low temperature. The weight of insoluble matter was subtracted from the weight of the ash. The difference in weights represents the water soluble ash. The percentage of water soluble ash with reference to the air dried drug was calculated.

Determination of swelling index
Swelling index is determined for the presence of mucilage in the seeds. Accurately weigh 1 g of the seed and placed in 150 ml measuring cylinder, add 50 ml of distilled water and kept aside for 24 hours with occasional shaking. The volume occupied by the seeds after 24 hours of wetting was measured.

Determination of extractive value
This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which as yet no suitable chemical or biological assay exists.

Cold maceration
Place about 4.0g of coarsely powdered air-dried material, accurately weighed, in a glass-stoppered conical flask. Macerate with 100ml of the solvent specified for the plant material concerned for 6 hours, shaking frequently, then allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent, transfer 25 ml of the filtrate to a tared flat-bottomed dish and evaporate to dryness on a water bath. Dry at 105°C for 6 hours, cool in a desiccator for 30 minutes and weigh without delay. Calculate the content of extractable matter in mg per g of air dried material. For ethanol-soluble extractable matter, use the concentration of solvent specified in the test procedure for the plant material concerned; for water-soluble extractable matter, use water as the solvent.

Successive Extraction of selected herbs
Sample were shattered and screened with 40 mesh. The shade dried coarsely powdered plant material (250gms) were loaded in Soxhlet apparatus and was extracted with petroleum ether (60-62°C), Chloroform, ethanol and water until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator and percentage yield were determined.

Preliminary phytochemical screening of extracts
The various extract obtained after extraction were subjected for phytochemical screening to determine the presence of various phytochemical present in the extracts. The standard procedures were adopted to perform the study.

Tests for carbohydrates
Molisch's test
To the Sample 2-3 drops of 1% alcoholic - napthol solution and 2 ml of conc. sulphuric acid was added along the sides of the test tube. Appearance of purple to violet ring at the junction of two liquids shows the presence of carbohydrates.

Fehling test
To the sample add fehling reagent, appearance of brick red precipitate shows presence of carbohydrates.

Test for glycosides
Legal's test
To the sample add 1 ml of pyridine and few drops of sodium nitropruside solutions and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

Borntrager's test
Sample was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink color, showing the presence of glycosides.

Baljet's test
To the sample add picric acid, orange color shows presence of glycosides.

Test for alkaloids
A small portion of the sample was stirred separately with few drops of dilute hydrochloric acid and was tested with various reagents for the presence of alkaloids. The reagents are

- Dragendroff's reagent - Reddish brown precipitates
- Wagner;s reagent - Reddish brown precipitates
- Mayer's reagent - Cream color precipitates
- Hager's reagent - Yellow color precipitate

Test for proteins and free amino acids

Small quantities of the sample was dissolved in few ml of water and treated with following reagents.

- Million's reagent: Appearance of red color shows the Presence of protein and free amino acid.
- Ninhydrin reagent: Appearance of purple color shows the Presence of Proteins and free amino acids.
- Biuret's test: Equal volumes of 5% sodium hydroxide solution & 1% copper sulphate solution was added. Appearance of pink or purple color shows the presence of proteins and amino acids.

Test for tannins and phenolic compounds

A small quantity of the sample was taken separately in water and test for the presence of phenol compounds and tannins was carried out with the following reagents.

- Dilute Ferric chloride solution (5%) - Blue color or green color
- 10% lead acetate solution - White precipitates

Test for flavonoids

Alkaline reagent test

To the test solution add few drops of magnesium hydroxide solution, intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicates presence of flavonoids.

Shinoda's test

Small quantities of the sample was dissolved in alcohol, to them piece of magnesium followed by conc. hydrochloric acid drop wise added and heated. Appearance of pink, crimson red, green to blue color shows the presence of flavonoids**.**

Tests for fixed oils and fats

Spot test

A small quantity of sample was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

Saponification test

Few drops of 0.5 N alcoholic potassium hydroxide were added to a small quantity of sample along with a drop of phenolpthlein, the mixture was heated on a water bath for 1-2 hours, formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Tests for steroids and triterpenoids

Libermann-burchard test

Treat the sample with few drops of acetic anhydride, boil and cool. Then add con. sulphuric acid from the side of test tube, brown ring is formed at the junction two layers and upper layer turns green which shows presence of steroids and formation of deep red colour indicates presence of triterpenoid.

Salkowski test

Treat the sample with few drop of conc. sulphuric acid, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

Test for mucilage and gums

- Small quantities of sample was added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitates was dried in oil and examined for its swelling property for the presence of gum and mucilage.
- To the sample add ruthenium red solution, pink color shows presence of mucilage.

Test for waxes

To the test solution add alcoholic alkali solution, waxes get saponified.

Results and Discussion

The leaves of *Cupressus torulosa* D. Don ex Lamb. (CTL) and *Cupressus vietnamensis* (Farjon & Hiep) Q.P. Xiang & J. Li. (CVL) collected from Himalaya region were identified morphologically and compared with standard available monograph. The dried plant part leaves of *Cupressus torulosa* D. Don ex Lamb. (CTL) and *Cupressus vietnamensis* (Farjon & Hiep) Q.P. Xiang & J. Li. (CVL) were subjected to standard procedure for the determination of various physicochemical parameters. The results were presented in table 1. The shade dried coarsely powdered plant material of *Cupressus torulosa* D. Don ex Lamb. (CTL) and *Cupressus vietnamensis* (Farjon & Hiep) Q.P. Xiang & J. Li. (CVL) was extracted with petroleum ether, Chloroform, ethanol and water. The extracts obtained were evaluated for pH, color and % yield. The results are presented in table 2. The various extract obtained after extraction were subjected for phytochemical screening to determine the presence of various phytochemical present in the extracts. The standard procedure was adopted to perform the study. The results were mentioned in table 3.

Table 1: Physicochemical Evaluation of *Cupressus* species

S/No.	Parameters	CTL	CVL
1.	FOM	1.23±0.02	1.03±0.03
2.	LOD	2.02±0.11	1.90±0.07
3.	TA	5.25±0.02	5.02±0.01
4.	AIS	1.12±0.11	0.98±0.02
5.	WSA	2.02±0.02	1.85±0.01
6.	SI	1.19±0.01	0.85±0.11
7.	WSEV	15.35±1.21	14.28±1.02
8	ESEV	8.36±1.08	7.03±1.05

Note: All values are expressed as Mean±SEM, n=3

Abbr.: *Cupressus torulosa* D. Don ex Lamb. (CTL) and *Cupressus vietnamensis* (Farjon & Hiep) Q.P. Xiang & J. Li. (CVL)

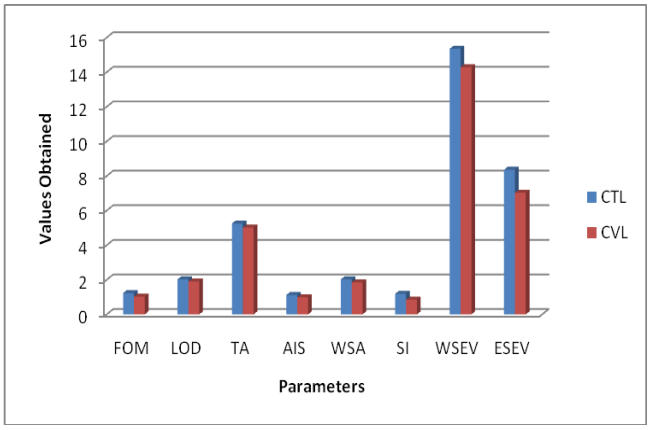
Table 2: Estimation of % yield of various extract of *Cupressus* species

S/No.	Extract	Parameters			
		Nature of Extract	Color	pH	% Yield (w/w)
1.	PEECTL	Semi Solid	Light greenish	7.0	0.85
2.	CECTL	Semi solid	Greenish Black	7.1	1.25
3.	EECTL	Solid Powder	Light Green	7.1	4.65
4.	AECTL	Solid Powder	Light Green	7.0	9.32
5.	PEECVL	Semi Solid	Light greenish	7.1	0.64
6.	CECVL	Semi solid	Greenish Black	7.2	1.12
7.	EECVL	Solid Powder	Light Green	7.1	4.50
8.	AECVL	Solid Powder	Light Green	7.0	8.98

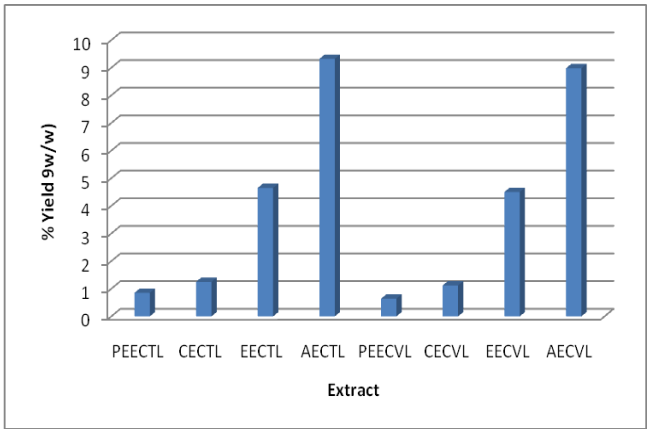
Table 3: Preliminary phytochemical screening extract of *Cupressus* species

S/No.	Constituents	CTL				CVL			
		PEE	CE	EE	AE	PEE	CE	EE	AE
1.	Carbohydrates	-	-	-	-	-	-	-	-
2.	Glycosides	-	-	-	-	-	-	-	-
3.	Alkaloids	-	-	-	-	-	-	-	-
4.	Protein & Amino acid	+	+	+	+	+	+	+	+
5.	Tannins & Phenolic compounds	-	-	+	+	-	-	+	+
6.	Flavonoids	+	+	+	+	+	+	+	+
7.	Fixed oil and Fats	-	-	-	-	-	-	-	-
8.	Steriods & Triterpenoids	+	+	+	+	+	+	+	+
9.	Waxes	-	-	-	-	-	-	-	-
10.	Mucilage & Gums	-	-	-	-	-	-	-	-

Abbr.: +=Present; -=Absent



Graph 1: Physicochemical Evaluation of *Cupressus* species



Graph 2: % yield of extract *Cupressus* species

Conclusion

Assessment of quality control and standardization parameters of the medicinal plants is of great interest and importance in order to reveal quality, safety and efficacy of medicinal plants. Ayurveda and traditional system of medicine healers treat diseases using the plants which have immense medicinal potentiality. But due to lack of standardization parameters correct identification of the plant is lacking, therefore development of QC parameters is of great interest. The present work was undertaken to revel the quality control and standardization parameters of leaves of *Cupressus torulosa* D. Don ex Lamb. and *Cupressus vietnamensis* (Farjon & Hiep) Q.P. Xiang & J. Li. In this study physicochemical, extraction and preliminary phytochemical screening of the selected plant material was done and reported.

References

1. Farjon A. Monograph of Cupressaceae and Sciadopitys. Royal Botanic Gardens, Kew, 2005.
2. Gadek PA, Alpers DL, Heslewood MM, Quinn CJ. Relationships within Cupressaceae sensu lato: a combined morphological and molecular approach. American Journal of Botany,2000:87:1044-1057.
3. Henry George Liddell, Robert Scott, A Greek-English Lexicon, on Perseus Digital Library
4. Zhang D, Christian T. "Cupressus torulosa". IUCN Red List of Threatened Species, 2013. e.T32131A2810351.
5. "Cupressus torulosa". World Checklist of Selected Plant Families (WCSP). Royal Botanic Gardens, Kew.
6. Earle, Christopher J. ed. "Cupressus torulosa". The Gymnosperm Database, 2018.
7. Thomas P. "Xanthocyparis vietnamensis". IUCN Red List of Threatened Species, 2013. e.T44028A2991576.
8. Thompson, Christian 2008-12-15. "First Contact in the Greater Mekong" (PDF). World Wildlife Fund. Retrieved, 2008-12-20.
9. Farjon A, Nguyen Tien Hiep, Harder DK, Phan Ke Loc, Averyanov L. "A new genus and species in the Cupressaceae (Coniferales) from northern Vietnam, Xanthocyparis vietnamensis". Novon,2002:12(2):179-189.
10. Kokate CK. "Practical Pharmacognosy; 4th ed. Vallabh Prakashan,2005:18:112-121.
11. Khandelwal KR. Practical Pharmacognosy, Thirteenth edition Nirali Prakashan, Pune, 2005, 149-156.
12. The Ayurvedic Pharmacopoeia of India. Part-I. 1st ed. New Delhi: published by Gov. of India ministry of health and family welfare department of Homoeopathy, 1999:3:225.
13. Quality control method for medicinal plant material, 1st edition, published by World Health Organisation Geneva, Delhi, 2002.