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Pharmacognostical standardization of leaves of *Cupressus macrocarpa* Hartweg. ex Gordon

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ABSTRACT

Cupressus macrocarpa Hartweg. ex Gordon belongs to family Cupressaceae. It is commonly known as Monterey Cypress. Traditionally the decoction of leaves is used in rheumatism. The species of genus Cupressus are used to improve bladder tone and coadjuvant in therapy of urinary incontinence and enuresis. The present study was carried out to establish the pharmacognostical study along with preliminary phytochemical screening of petroleum ether, chloroform, ethanol and aqueous extracts of *Cupressus macrocarpa*. The macroscopical and microscopical characters of leaves were studied. The transverse section of leaves indicated the arrangement of various cells in epidermis, sunken stomata, hypodermis, spongy parenchyma and vascular bundles. The physico-chemical parameters such as total ash, acid insoluble ash, water soluble ash and sulphated ash value, loss on drying, extractive values, fluorescence analysis of extracts and powder treated with different chemical reagents were studied under ordinary light, short and long UV light. The foaming and swelling index of leaves were also studied. Preliminary phytochemical screening of various extracts revealed the presence of glycosides, flavonoids, sterols, phenolic compounds, carbohydrates and amino acids. These studies will be helpful in developing standards for quality, purity and sample identification of this plant.

INTRODUCTION

Cupressus macrocarpa Hartweg. ex Gordon commonly known as Monterey Cypress (Eckenwalder,1993) belongs to family Cupressaceae. Traditionally the genus *Cupressus* is used in rheumatism, whooping cough and styptic problem (Kuiate *et al*., 2006). It eliminates fluid retention and is used to promote venous circulation to the kidney and bladder area, to improve bladder tone and coadjuvant in therapy of urinary incontinence and enuresis (Price, 1993; Chidell, 1992).

Strobiles of *Cupressus macrocarpa* is used topically as acaricide in animals in the region of Pallars Sobira and Pallars Jussa situated in North West Catalonia (Agelet and Vallès, 2001). Standardization of herbal raw drugs includes passport data of raw plant drugs, authentication, microscopic & molecular examination, identification of chemical composition of drugs (Sekhon and Choudhary, 2011). Hence in this experimental work we make an attempt for the standardization of *Cupressus macrocarpa* leaves by carrying out its pharmacognostical, physicochemical evaluation and preliminary phytochemical screening.

MATERIALS AND METHODS

Plant material

The leaves of *Cupressus macrocarpa* was collected manually from Guru Jambheshwar University of Science & Technology, Hisar, Haryana in the month of October 2010 and authenticated by Dr. H.B. Singh, Head Raw Material Herbarium & Museum, New Delhi vide Ref. NISCAIR / RHMD / Consult /-2010-11/1485/83. A voucher specimen has been retained in the Department of Pharmaceutical Science, Guru Jambheshwar University of Science & Technology, Hisar. The plant material (1kg) was air dried at room temperature (30-40 ^{o}C) and then powdered to pass through a sieve of 1mm and further subjected to various studies.

Chemicals and Reagents

All the chemicals and solvents used for the study were of analytical grade and procedures were taken from official methods.

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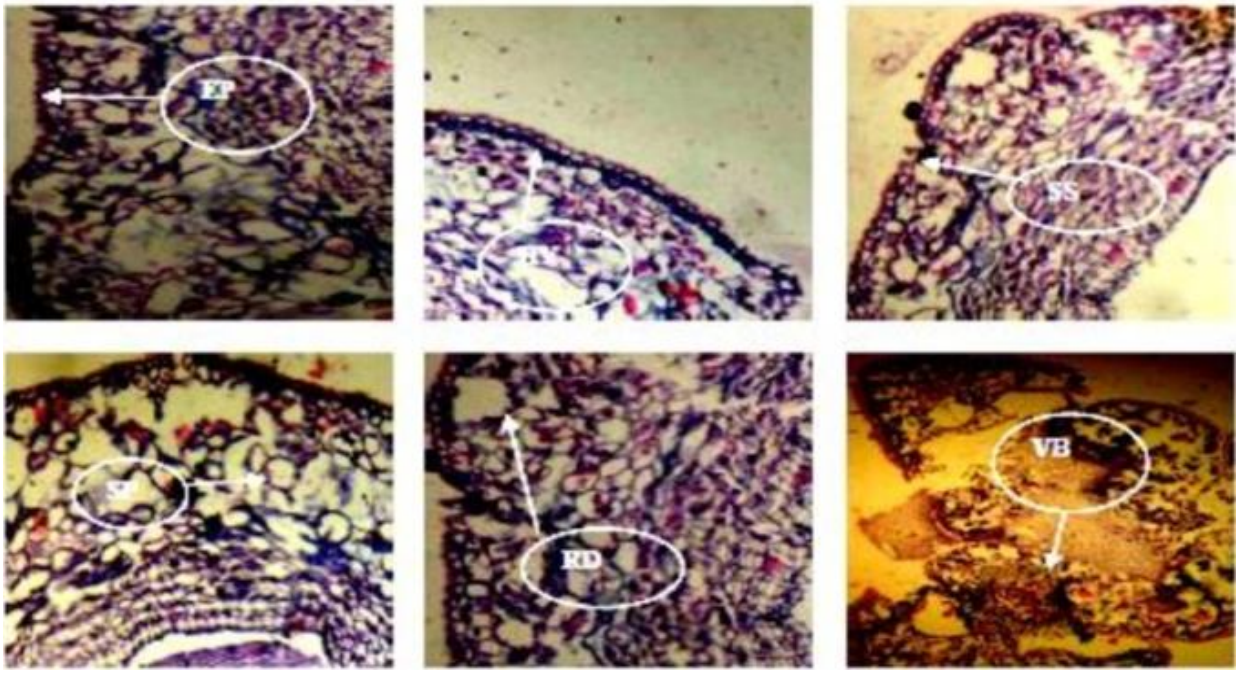

Fig. 1: Transverse Sections of the leaves of *Cupressus macrocarpa* Hartweg. ex Gordon. EP : Epidermis HY: Hypodermis, SS: Sunken Stomata, SP: Spongy Parenchyama, RD: Resin Duct, VB: Vascular Bundles.

Macroscopical characters

Untreated sample was examined and studied for their macroscopical characters such as colour, odour, taste, shape, size and texture. The alignment of leaves was also observed (Sharma and Singh, 2013).

Microscopical characters

Thin transverse sections of the leaves were cut using microtome (WES WOX Model, MT-1090 A) stained with 0.25% toluidine blue adjusted to pH 4.7 and observed under compound microscope. Transverse sections of 10 to 12 µm thickness were prepared. Photography was done by using zeiss primo star trinocular microscope attached with canon photomicrograph unit (Johansen and Brien, 1940).

Powder studies

For microscopical examination the powder was stained with phloroglucinol, concentrated hydrochloric acid and glycerine to study various anatomical features viz. sclerides, tracheids, unicellular trichomes, collenchyma, parenchyma and palisade cells (Trease and Evans, 1996).

Physicochemical parameter

The dried plant material was subjected for determination of physicochemical parameters. The ash values such as total ash, acid insoluble ash, water soluble ash and sulphated ash were determined according to standard procedures (Anonymous, 2002; Sharma and Kumar, 2012). The physicochemical parameters such as loss on drying, volatile oil content, extractive values, fluorescence analysis, foaming index, swelling index were determined according to official methods for quality control of medicinal plant (Singh *et al*, 2013).

Preliminary phytochemical screening

The preliminary phytochemical screening was carried out on the extracts obtained after successively extraction with petroleum ether, chloroform, ethanol and aqueous solvents. The dried extracts were treated with different chemical reagents for the detection of presence and absence of phytoconstituents (Kokate, 1994; Harborne, 1998).

RESULTS AND DISCUSSION

Macroscopical characters

The leaves of *Cupressus macrocarpa* were bright green when fresh and brownish yellow in colour when dried. Young leaves were small needle shaped with characteristic slightly bitter taste and camphoreous odour. The texture was stringy and outer surface was rough.

Microscopical characters

Transverse section of leaf showed single layered isodiametric epidermis that was externally covered with a thick, striated cuticle. Beneath the epidermis single layer highly lignified, thick walled hypodermis was present. Due to xerophytic nature sunken stomata were seen in epidermal layer. Rows of elongated, closely arranged, palisade parenchyma followed by radially elongated spongy parenchyma cells with small intercellular spaces were present. The mesophyll is chlorenchymatous with varying number of plate like infolding of the wall projecting into cell cavity. The endodermis is a single continuous layer of barrel shaped cells. The transfusion tissue is composed of parenchymatous cells, resin cells and tracheids cell with bordered pits on their tangential and transverse walls. Cells were irregular in shape and single bicollateral vascular bundles were present in the

central region. Transverse section of leaf is shown in Fig 1 and Fig 3.

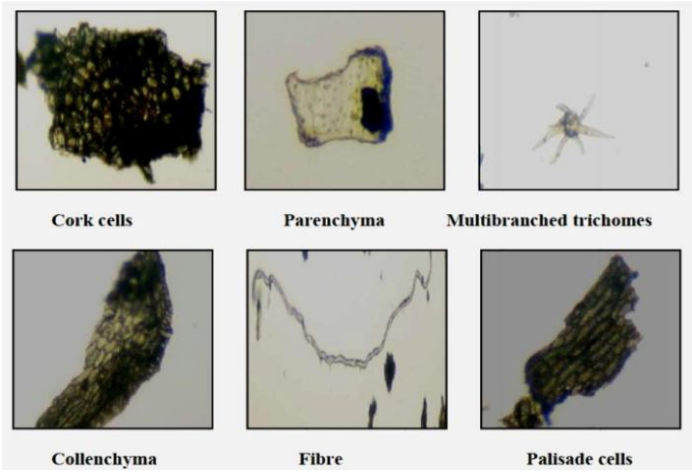

Fig. 2: Powder microscopy of *Cupressus macrocarpa* leaves.

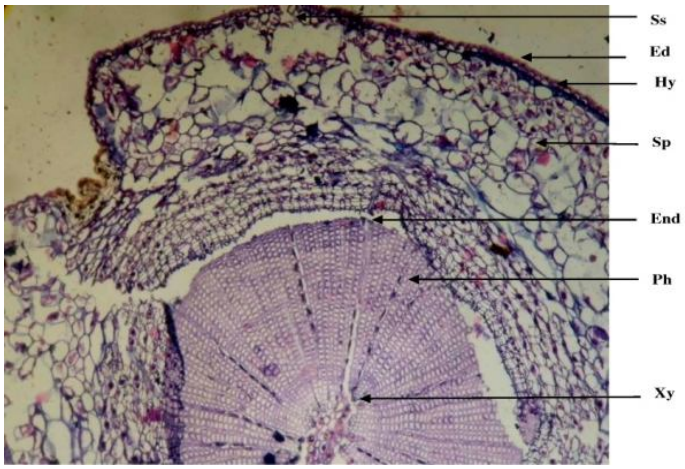

Fig. 3: Transverse sections of *Cupressus macrocarpa* hartweg. ex gordon leaf. SS: Sunken Stomata, ED: Epidermis, HY: Hypodermis, SP: Spongy Parenchyma, END: Endodermis, PH: Phloem, XY: Xylem.

Powder Studies of *Cupressus macrocarpa* Hartweg. ex Gordon Leaves

Powder studies shows the presence of lignified with long and narrow variably thickened walls. Parenchymatous cells were isodiametric or slightly elongated rectangular with moderately thickened wall and faint striations were present. Few cork cell, stone cells, starch grains palisade cells, multicellular trichomes were seen. Fibres were found scattered. Powder characteristics of *Cupressus macrocarpa* Hartweg.ex Gordon are shown in Fig 2.

Physico-chemical parameters

The physicochemical parameters such as total ash, water soluble ash, acid-insoluble ash and sulphated ash, loss on drying, volatile oil content were established and shown in Table 1. The extractive values by successive extraction method and fluorescence characteristics of extracts in visible and UV light are summarized in Table 2.

Table. 1: Ash values, Loss on drying, Volatile oil content.

Evaluation Parameter	Value
Total ash value	19.68 ± 0.32 % w/w
Water soluble ash value	5.69 ± 0.34 % w/w
Acid insoluble ash value	21.52 ± 0.88 % w/w
Sulphated ash value	14.42 ± 0.94 % w/w
Loss on drying	9.965 % w/w
Volatile oil	1.25 % v/w

Table. 2: Extractive values and colour of extract under different lights.

Extract	Colour of Extract			Extractive value (% w/w)
	Ordinary Light	UV Light 254nm	UV Light 365nm	
Petroleum ether (60-80°C)	Brownish Black	Greenish Black	Brown	7.56
Chloroform	Blackish	Greenish Black	Brown	6.58
Ethanol	Brownish	Greenish Black	Blackish	15.42
Aqueous	Blackish Brown	Greenish Black	Brownish	7.51

Table 3: Fluorescence analysis of *Cupressus macrocarpa* leaves powder.

Treatment	Colour observed under		
	Ordinary light	UV Light 254nm	UV Light 365nm
Powder + 1N HCL	Greenish Black	Greenish Brown	Black
Powder + 1N KOH	Brownish Green	Greenish Yellow	Black
Powder + 5% $FeCl_3$	Brownish	Greenish Brown	Black
Powder + 5% Iodine	Light Brown	Greenish Brown	Bluish Green
Powder + Picric acid	Yellowish Brown	Green	Black
Powder + HNO_3	Brownish Black	Greenish	Black
Powder + Glacial Acetic acid	Yellowish Brown	Greenish Yellow	Light Brown

Fluorescence Analysis

When physical and chemical methods produce inadequate results plant material may be identified from their adulterants on the basis of fluorescence nature. The powder of leaves was treated with different chemical reagents and observations are reported in Table 3.

Quantitative Studies

Quantitative studies for foaming index and swelling index were performed. The results are tabulated in Table 4.

Table. 4: Quantitative studies of *Cupressus macrocarpa* leaves.

Sr. No.	Estimation	Observation
1.	Foaming Index	< 100
2.	Swelling Index	< 1.1

Preliminary Phytochemical Investigation

The successive extracts obtained were subjected to investigation for various phytoconstituents. It revealed the presence of different phytoconstituents like carbohydrates, anthraquinone glycosides, cardiac glycosides, phenolics,

flavonoids, saponin, protein, amino acid and sterols in different extracts as in Table 5.

Table. 5: Preliminary phytochemical screening of various extracts.

S N.	Plant Constituents / Test Reagents	Petroleum ether	Chloroform	Ethanol	Aqueous
	ALKALOIDS				
1.	Mayer's reagent	–	–	–	–
	Dragendroff's reagent	–	–	–	–
	Hager's reagents	–	–	–	–
	Wagner's reagents	–	–	–	–
	GLYCOSIDES				
2.	Fehling solution	–	–	–	–
	Killer–Killani test	–	–	–	+
	Baljet test	–	–	+	+
	Borntrager test	–	–	–	–
	Legal test	–	+	+	+
	CARBOHYDRATES				
3.	Molish's reagent test	+	+	+	+
	Fehling solution test	–	–	–	–
	Benedict test	–	–	–	+
	STEROLS				
4.	Liebermann- Burchard test	–	+	–	–
	Salkowski test	–	–	–	–
	Triterpenoids	–	+	+	+
	SAPONINS				
5.	Foam test	+	+	+	+
	Sodium bicarbonate test	–	–	+	+
	RBC Haemolysis test	–	–	–	–
	PHENOLICS				
6.	**COMPUNDS & TANNINS**				
	Ferric chloride test	+	+	+	+
	Lead test	+	+	+	+
	FLAVONOIDS				
7.	Shinoda / Pew test	+	+	–	–
	Ammonia test	–	–	–	–
	Alkaline reagent test	–	–	+	+

+ means present , - means absent.

CONCLUSION

The scientists from past few decades are keen and sincere to evaluate traditionally used medicinal plants due to their desirable action and reliable biological action. The leaves of *Cupressus macrocarpa* Hartweg. ex Gordon are still used in treatment of various disorders in traditional system of medicine. The pharmacognostical standardization of this plant gives idea about identification, physical evaluation and monograph of this plant.

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