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# Pharmacognostical standardization of leaves of *Thuja orientalis* (Linn.) Franco

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**ABSTRACT**

*Thuja orientalis (Linn.) Franco belongs to family Cupressaceae. It is commonly known as Tree of life and Orientale Arbor-Vitae in English. The leaves have been used as antibacterial, antipyretic, antitussive, astringent, emmenagogue, emollient, expectorant, febrifuge, haemostatic, refrigerant, stomachic, diuretic and used in alopecia. 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 Thuja orientalis (L.) Franco. The macroscopical and microscopical characters of leaves were studied. The transverse section of leaves indicated the arrangement of various cells in epidermis, hypodermis, palisade cells, spongy parenchyma and vascular bundles. Preliminary phytochemical screening of various extracts revealed the presence of glycosides, flavonoids, sterols, phenolic compounds, carbohydrates and amino acids. 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. These studies will be helpful in developing standards for quality, purity and sample identification of this plant.*

**Keywords**: *Thuja orientalis*, Cupressaceae, pharmacognostical, physico-chemical parameters.

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## INTRODUCTION

With the emerging worldwide interest in adopting and studying traditional system and exploiting their potential based on different health care system the evaluation of the rich heritage of Indian traditional medicine is essential [1]. The detailed pharmacognostical evaluation would give valuable information regarding the morphology, microscopical, physical characteristics and phytochemical evaluation of the crude drugs [2]. In order to quench the thirst for a new drug from the herbal origin, leaves of *Thuja orientalis* (Linn.) Franco has been chosen. Ethnomedicinal information suggests that *Thuja orientalis* (L.) Franco is being used as herbal medicine from ancient times and categorized as one of the fundamental herbs in Chinese materica medica where it is mainly used in treating conditions such as gout, rheumatism, diarrhoea, chronic tracheitis and hair loss. Thuja possess antitussive, expectorant, anti-inflammatory, antibacterial, antifungal, antioxidant activity [3]. The leaves yield an essential oil which is used as tonic, diuretic and antipyretic [4]. Thuja is used in homeopathy both internally and externally for tissue degeneration, tumours, warts and fungoid growths [5]. Standardization of the herbal raw drugs includes passport data of raw plant drugs, authentication, microscopic & molecular examination, identification of chemical composition of drugs [6]. Hence in this experimental work we make an attempt for the standardization of *Thuja orientalis* (Linn.) Franco leaves by carrying out its pharmacognostical, physicochemical evaluation and preliminary phytochemical screening.

## MATERIALS AND METHODS

### Plant material

The leaves of *Thuja orientalis* (L.) Franco were 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 reagents used for the study were of analytical grade and procedures were taken from official methods.

### 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 [7].

### 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 [8].

### 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 [9].

### Physicochemical parameters

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 [10, 11]. 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 plants [12, 13].

### Preliminary phytochemical screening

The preliminary phytochemical screening was carried out on the extracts obtained after successive 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 [14, 15].

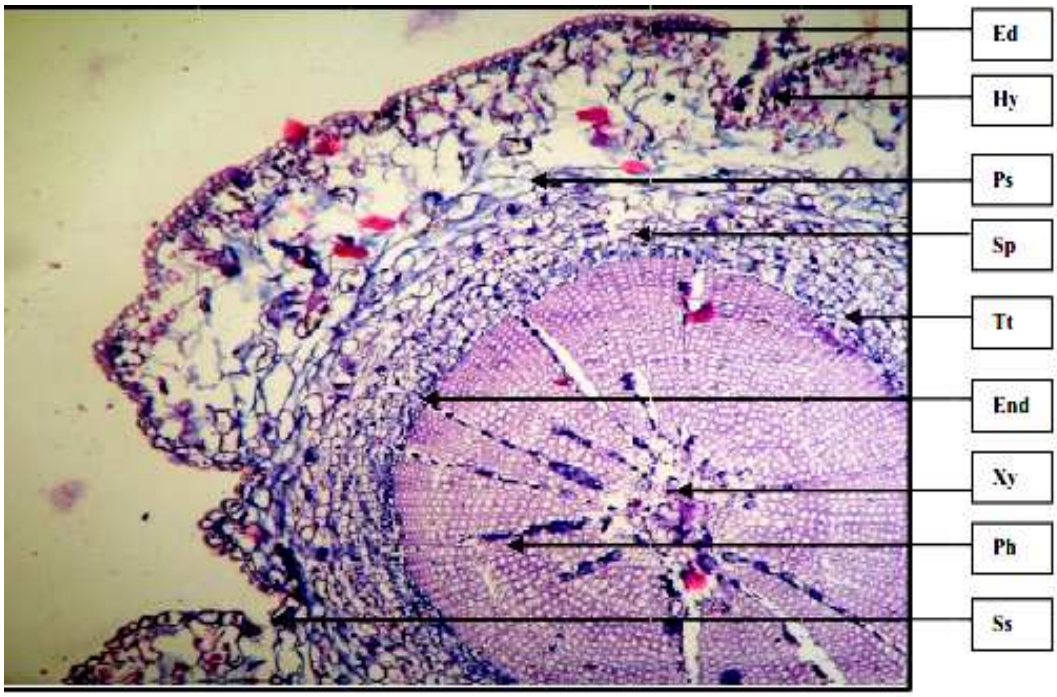
## RESULTS

### Macroscopical characters

The leaves of *Thuja orientalis* was greenish when fresh and brownish in colour when dried. Young leaves were small needle shaped with slightly bitter taste and camphoreous odour. The texture was stringy and outer surface was rough. Alignment of the leaves was persistent scale like arranged in opposite decussate pairs.

### Microscopical characters

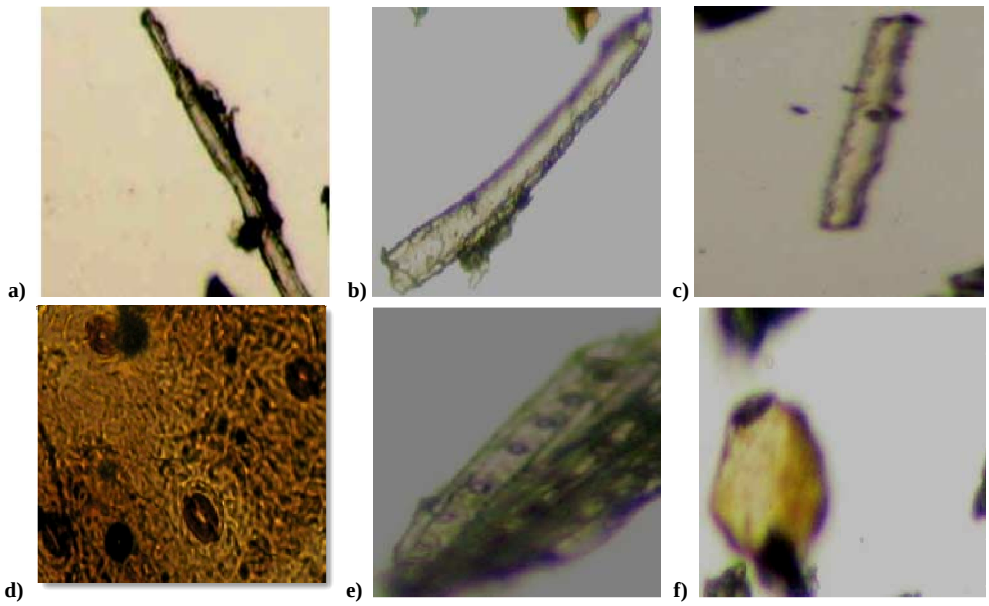
Transverse section of leaf showed single layered epidermis that was externally covered with a thick, striated cuticle. Beneath the epidermis single layered highly lignified, thick walled hypodermis was present. Due to xerophytic nature sunken stomata were seen in epidermal layer. Rows of elongated, closely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




*Ed:Epidermis, Hy:Hypodermis, Ps:Palisade cells, Sp:Spongy Parenchyma, Tt:Transfusion tissue, End:Endodermis, Xy:Xylem, Ph:Phloem, Ss:Sunken stomata*

**Powder Studies of *Thuja orientalis* (Linn.) Franco Leaves**

Powder studies shows the presence of lignified and non lignified fibres with long and narrow variably thickened wall and with few pits on it. Sclerides were found singly or in the group of two or three. Tracheids with pits were present singly and in groups. Parenchymatous cells were isodiametric and slightly elongated rectangular with moderately thickened wall with faint striations. Few parenchymatous cells showed numerous pits. Mucilage cells and starch granules were also seen.




**a) Fibres b) Unicellulr Trichomes c) Sclerides d) Sunken stomata e) Tracheids f) Oil cell**

**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 | 18.21 ± 0.54 % w/w |
| Water soluble ash value | 4.36 ± 0.66 % w/w |
| Acid insoluble ash value | 20.18 ± 0.76 % w/w |
| Sulphated ash value | 13.58 ± 0.88 % w/w |
| Loss on drying | 8.79 % w/w |
| Volatile oil | 1.00 % w/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) | Greenish Brown | Greenish Black | Brownish | 6.01 |
| Chloroform | Blackish | GreenishBlack | Brownish Black | 5.02 |
| Ethylacetate | Greenish Brown | Greenish | Brownish | 9.50 |
| Ethanol | Brownish | Greenish Black | Blackish Green | 12 |
| Aqueous | Brownish | Greenish Black | Dark Brownish | 7.7 |

**Fluorescence Analysis**

Fluorescence analysis is the easy method for the resolution study of crude drugs when physical and chemical methods do not produce adequate results. The 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.

**Table 3: Fluorescence analysis of *Thuja orientalis* (L.) Franco leaves powder**

| Treatment | Colour observed under | | |
|---|---|---|---|
| | Ordinary light | UV Light 254nm | UV Light 365nm |
| Powder + $HNO_3$ | Brownish | Greenish Brown | Blackish Brown |
| Powder + 1N HCL | Yellow | Greenish Yellow | Bluish Green |
| Powder + 1N KOH | Greenish Brown | Greenish Yellow | Greenish Black |
| Powder + 5% $FeCl_3$ | Brownish Blue | Greenish Brown | Blackish Blue |
| Powder + 5% Iodine | Reddish Yellow | Reddish Violet | Dark Brown |
| Powder + Picric acid | Yellowish Green | Yellowish | Dark Green |
| Powder + $HNO_3$+ $NH_3$ | Brown | Greenish Black | Black |
| Powder + Glacial Acetic acid | Brownish Yellow | Brownish Green | Brown |

**Quantitative Studies**

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

**Table 4: Quantitative studies of *Thuja orientalis* (L.) Franco leaves**

| Sr. No. | Estimation | Observation |
|---|---|---|
| 1. | Foaming Index | < 100 |
| 2. | Swelling Index | >1 |

**Table 5: Preliminary phytochemical screening of various extracts**

| Test | Petroleum ether | Chloroform | Ethanol | Aqueous |
|---|---|---|---|---|
| Alkaloids | – | – | – | – |
| Glycosides | – | – | + | + |
| Carbohydrates | + | + | + | + |
| Sterols | – | + | – | – |
| Saponins | – | + | + | + |
| Phenolics | – | + | + | + |
| Flavonoids | + | + | – | – |
| Protein & Amino acid | – | – | – | + |

*+ means present , - means absent*

**Preliminary Phytochemical Screening**

The successive extracts obtained were subjected to investigation for various phytoconstituents. It revealed the presence of different phytoconstituents like carbohydrates, glycosides, phenolics, flavonoids, saponin, protein, amino acid and sterols in different extracts as in Table 5.

## CONCLUSION

The scientists from past few decades are keen and sincere to evaluate traditionally used medicinally plants due to their specific hair growth properties, desirable action and reliable biological action. The leaves of *Thuja orientalis* (Linn.) Franco are still used in treatment of alopecia 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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