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Research Article

PHYSICO-CHEMICAL AND PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF *THUJA OCCIDENTALIS* LINN. (CUPRESSACEAE) DRIED LEAVES

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ABSTRACT
Thuja occidentalis is a monoecious coniferous plant having a wide variety of medicinal value. It has hepatoprotective, anti-oxidant, anti-bacterial, anti-fungal, anti-cancer, anti-diabetic activity etc. In the present paper, *T. occidentalis* leaves has been standardized on the basis of organoleptic, physical and physico-chemical characteristics. Methanolic and hydro-alcoholic extracts have been prepared by microwave assisted extraction technique and subjected to preliminary phytochemical screening and thin layer chromatography (TLC). TLC fingerprinting profile has been obtained using different solvent systems.
KEYWORDS: Microwave extraction, physico-chemical, standardization, *Thuja occidentalis* Linn.

INTRODUCTION

Plant description

The 'Northern White Cedar' is a monoecious conifer with a height in the range 15 to 38 m, stunted or prostrate in harsh, frigid environment. Occasionally the trunk is divided into two to three secondary stems, often reproducing from fallen trunks. The bark is reddish or grayish brown, 6-9 mm thick, fibrous, and fissured. The leaves of the branchlets are 1.5 to 3-5 mm in length, acute, dull yellowish green on both surfaces. The pollen cones are 1-2 mm and reddish, the seed cones ellipsoid, 9-14 mm in length and brown in color.

The leaf arrangement of *Thuja occidentalis* is alternate with simple leaf and entire leaf margin. It has scaly leaves with less prominent leaf venation. The leaf is evergreen with persistence fragrance. The length of leaf blade is less than 2 inches. The color of leaf is green and when falls, no color change occurs.[1]

Medicinal potential of *T. occidentalis* (Cupressaceae) leaves

T. occidentalis ethanolic fraction has been reported to produce hepatoprotective potential against CCl_4 induced liver damage in rats. A dose of ethanolic fraction of *T. occidentalis* , 400 mg/kg p.o. exhibited significant protection from liver damage in acute and chronic CCl_4 induced liver damage model. Histopathological examination also supported the use of *T. occidentalis* for hepatoprotection. The fraction was found to possess good hepatoprotective activity.[2]

The antioxidant activity of aerial parts of *T. occidentalis* Linn. (Cupressaceae) ethanolic fraction was evaluated using different models viz. 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical, superoxide anion radical and hydroxyl radical scavenging. The antioxidant activity increased in a concentration dependent manner and about 100, 150, 200, 250 & 300µg/ml inhibited the $FeSO_4$ induced lipid peroxidation.[3]

T. occidentalis crude extract confirmed good antibacterial and antifungal activity against *Aspergilus niger*.[4] *T. occidentalis* is considered as antipsycotic homeopathic drug used mainly for wart like excrescenses upon mucus & cutaneous surface, vegetative condylomata and spongy tumours.[5] *T. occidentalis*, in small doses had been used against arthritis and gout. *T. occidentalis* tea helps to alleviate cold and headache and in syrups to alleviate cough.

T. occidentalis essential oil has been widely used in steam bath.[6] Thujone rich fraction separated from crude ethanolic extract of *T. occidentalis* had been reported to possess the anticancer potential in the malignant melanoma cell line A375 and confirmed the anti-proliferative and apoptosis-inducing property. *T. occidentalis* mother tincture had been used to treat various ailments, particularly moles and tumors.[7] *T. occidentalis* had confirmed the activity against breast cancer cell lines by inducing apoptosis (programmed cell death).[8]

T. occidentalis ethanolic fraction was also found to possess significant anti-ulcer activity in five different experimental models. It produced significant protection and showed increase in blood glutathione level due to the presence of rich amount of phenolic compounds.[9]

The immunopharmacological potential of *Thuja* has been investigated in various *in vitro* and *in vivo* test models.[10] *T. occidentalis* has been reported to produce anti-diabetic ativity.[11]

MATERIALS AND METHODS

Chemicals and reagents used

All the chemicals and reagents used were of analytical grade from CDH, LOBACHEMIE, HIMEDIA, RENKEM companies.

Procurement of plant material

Fresh aerial parts (leaves and twigs) of *Thuja occidentalis* were collected from the Maharshi Dayanand University Campus, Rohtak and authenticated by Dr. H. B. Singh, (Taxonomist) Head, Raw material Herbarium and Museum Division, National Institute of Science Communication And Information Resources (NISCAIR), New Delhi.

Physical evaluation

Determination of ash

Total ash

2 g of dried powdered leaves of *T. occidentalis* were placed in silica crucible in a uniform layer and ignited by gradually increasing temperature to 500-600° C. The ash so obtained was cooled, weighed and allowed to cool in desiccator for 30 min. The content of total ash was calculated in mg per g of air dried material.

Water - soluble ash

To the total ash containing crucible, added 25 ml of water and boiled for 5 min. Collected the insoluble matter in a sintered-glass crucible or on an ashless filter paper. Washed with hot water and ignited for 15 min. at a temperature not exceeding 450° C. Substracted the weight of the residue in mg per g of air dried material.

Determination of extractable matter

Hot extraction method

4 g of coarsely powdered air dried material was transferred to a glass-stoppered conical flask. Then 100 ml water was added, weighed, shaked well and allowed to stand for 1 h. Attached a reflux condenser to the flask and boiled gently for 1 h, cooled and weighed. Then it was readjusted to the original weight with water, shaked and filtered. Then 25 ml of the filterate was transferred to a tared flat-bottomed dish and evaporated to dryness on a water bath. It was then dried at 105° C for 6 h, cooled in a desiccator for 30 min, weighed immediately and calculated as mg per g of air dried material.

Cold maceration method

4 g coarsely powdered air dried leaves of *T.occidentalis* were transferred to a glass-stoppered conical flask and macerated with 100 ml of water for 6 h, shaked frequently and then allowed to stand for 18 h. It was then filtered and transferred to a tared flat-bottomed dish and evaporated to dryness on a water-bath. Finally, it was dried at 105˚C for 6 h and cooled in a desiccator for 30 min, weighed and calculated as mg per g of air dried material.

Ether soluble extractable material

4 g coarsely powdered air dried *T. occidentalis* was transferred to a glass-stoppered conical flask. Macerated with 100 ml of the required solvent for the given plant material for 6 h, shaking frequently, then allowed to stand for 18 h. Filtered rapidly taking care not to lose any solvent, transfer 25 ml of the filterate to a tared flat-bottomed dish and evaporated to dryness on a water-bath. Dried at 105℃ for 6 h, cool in a desiccators for 30 min and weighed and calculated as mg per g of air dried material.

Determination of water and volatile matter

Azeotropic method (toluene distillation)

Weighed 25 g *T. occidentalis* leaves, transferred to a distillation flask and added 150 ml toluene. Then the flask was heated gently with the distillation rate of 4 drops per second. As soon as the water has been completely distilled, rinsed the inside of the condenser tube with toluene. Further, the distillation was continued for 5 min. Stopped further heating , allowed the receiving tube to cool to room temperature and dislodge the droplets of water which adhere to the walls of the receiving tube. Allowed the water and toluene layers to separate and read off the volume of water. Calculated the content of water in % using the formula:

$100(n^1-n)/w$

Where , w = the wt. in g of the plant material

n = the no. of ml of water obtained in the first distillation

n^1 = the total no. of ml of water obtained in both distillations

Loss on drying (gravimetric determination)

Weighed accurately 5g of *T. occidentalis*, in a previously dried and tared flat weighing bottle and it was dried using two methods

a) in an oven at 100-105˚C for 5 h.

b) in a desiccator over phosphorus pentaoxide R under microscopic pressure or reduced pressure and at room temperature.

The sample was dried until two consecutive weighing do not differ by more than 5 mg. Calculated the loss of weight in mg per g of air dried material.

Determination of volatile oils

Volatile oil determination was done by steam distillation using dean starke apparatus. Distillate was collected in a graduated tube using xylene and the aqueous phase was

allowed to circulate into the distillation flask. For all determinations, rate of distillation was observed.

Determination of swelling index

Swelling index was determined as per official method and readings were taken in triplicate.

Determination of foaming index

The foaming index was determined as per WHO guidelines according to the given formula

Foaming index = 1000/a

Where a = volume in ml of the decoction used for preparing the dilution in the tube where foaming index is observed.

Determination of crude fibre content by Dutch method

Two grams of powdered drug sample was taken in a beaker and added 50 ml of 10% v/v nitric acid. It was boiled with constant stirring (till about 30 s after boiling started), strained through fine cotton cloth on a buchner funnel. Residue was given washing with boiling water and transferred to a beaker. 50ml of 2.5% v/v sodium hydroxide solution was added and heated till it boiled. Boiling point was maintained for 30 s, stirred constantly. It was again strained and washed with hot water. Residue was transferred in a cleaned and dried crucible, weighed and percent crude fibre was calculated for quantitative study. [12]

Preparation of methanolic and hydroalcoholic extracts of *T.occidentalis* by Microwave assisted extraction technique

Defatted powdered leaves of *T.occidentalis* were treated with methanol and hydroalcoholic solution to yield methanolic and hydroalcoholic extracts. Powdered defatted drug was treated with microwaves in domestic microwave oven. The drug contained in a conical flask was irradiated in a domestic microwave by 20 seconds on and 20 seconds off. The procedure was repeated for 7 minutes for methanolic extract and 10 minutes for hydroalcoholic extract. Then the extracts obtained were filtered and concentrated on water bath and dried in desiccator.

TLC profile of methanolic and hydroalcoholic extracts of *T.occidentalis*

Thin layer chromatography sampling of methanolic and hydroalcoholic extract were performed and different components were obtained using solvent systems toluene: ethyl acetate: formic acid: water, chloroform: methanol and toluene: ethyl acetate and observed under UV at 366 nm.

RESULTS AND DISCUSSION

T. occidentalis was evaluated for physical evaluation, including total ash, water soluble ash, acid insoluble ash, determination of extractable matter, determination of water and volatile matter, swelling index, foaming index and crude fibre determination by dutch method and the results are represented in table 1. The methanolic and hydroalcoholic extracts of *T. occidentalis* were prepared by microwave assisted extraction technique and the extractive values are given in table 1. The two extracts so prepared, were investigated for their phytochemistry and the results are mentioned in table 2. Fluoresence analysis of dried powder of *T. occidentalis* leaves, its methanolic and hydroalcoholic extracts, was prepared and the results are stated in table 3 and 4, also TLC profile of methanolic and hydroalcoholic extracts of *T. occidentalis* was studied using three different solvent system and the results are expressed in table 5. The solvent system, toluene: ethyl acetate: formic acid: water:: 100: 36: 36: 10 showed maximum constituents separated and their R_f values were found to be 0.78, 0.72, 0.35, 0.16.

CONCLUSION

Thuja occidentalis Linn. is used in traditional system of medicine against a variety of ailments and diseases. There is need to standardize a drug and the numerical standards reported in this study could be useful for the compilation of a suitable monograph for its proper identification. Also, to apply effective drug delivery system rather than conventional systems to any medicinally important herbal drug or formulation, it is necessary to carry out the standardization and to assess the quality and purity of a drug.

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Table 1: Standardisation parameters result of crude drug *T.occidentalis*

S. No.	Parameter	Value
1.	Ash value i) Total ash ii) Water soluble ash	 6.14% w/w 4.5% w/w
2.	Extractable matter i) Hot ii) Cold iii) Ether	 83.1 mg/g 39.67 mg/g 4.7 mg/g
3.	Foaming index	18.18
4.	Swelling index	2.4 ml/g
5.	Moisture and volatile matter	150 mg
6.	Crude fibre content	17.5% w/w

Table 2: Preliminary phytochemical investigation of dried leaves methanolic and hydroalcoholic extacts of *T.occidentalis* prepared by microwave extraction technique

S.no.	Treatment	Drug	Methanolic Extract	Hydroalcoholic Extract
1.	Test for carbohydrate Molisch's test Fehling's test Benedict's test	+ + + +	+ + + +	+ + + +
2.	Test for steroidal saponins Lieberman-Burchard rxn.	+ +	+ +	+ +
3.	Test for tannins and Phenolic compounds : 5% ferric chloride sol. Lead acetate sol.	+ -	+ -	+ -
4.	Test for alkaloids : Mayer's test Dragendorff's test Hager's test	- - -	- - -	- - -
5.	Test for non-reducing polysaccharides (starch) : Iodine test	+ +	+ +	+ +
6.	Test for flavonoids : 5% Sodium hydroxide solution.	+	+	+

Table 3: Fluorescence analysis of dried powder of *T.occidentalis* leaves

S.No.	Treatment	Normal light	UV at 254 nm	UV at 366 nm
1.	Dry powder	Green	black	Fluorescent green
2.	Powder + 5% NaOH	Yellowish green	Brown	Yellowish brown
3.	Powder + 5% KOH	Yellow	Blue black	Greenish blue
4.	Powder + 5% $FeCl_3$	Yellow	Dark brown	Greenish brown
5.	Powder + conc. H_2SO_4	Greenish yellow	Blue black	Fluorescent green
6.	Powder + conc HCl	Brownish yellow	Dark brown	Greenish brown
7.	Powder + conc. HNO_3	Orange brown	Blackish purple	Brownish black
8.	Powder + Iodine solution	Orange	Brown	Dark brown
9.	Powder + 5% HCl	Light yellow	Light blue	Greenish blue
10.	Powder + 5% H_2SO_4	Yellowish brown	Sky blue	Blue
11.	Powder + dil HNO_3	Yellow	Dark blue	Greenish blue
12.	Powder + Na_2CO_3	Green	Light yellow	Yellow
13.	Powder + alc. KOH	Light yellow	Light green	Pale yellow
14.	Powder + 1% $KMNO_4$	Purple	Brown	Dark brown
15.	Powder + $AgNO_3$	Pale yellow	Sky blue	Greenish blue

Table 4: Fluorescence analysis of methanolic and hydroalcoholic extracts of *T.occidentalis*

S.No.	Treatment	Normal Light		UV at 254 nm		UV at 366 nm	
		ME	HE	ME	HE	ME	HE
1	Dry powder	Light green	Brown	Blue voilet	Purple	Blue	Purple
2	P+5% NaOH	yellow green	Yellow	Bluish black	Bluish Black	Black	Black
3	P+5%KOH	Yellow	Black	Blue black	Blue Black	Greenish Blue	Brownish Blue
4	P+5%$FeCl_3$	Yellow black	Black	black	Black	Yellow Black	Black
5	P+5%H_2SO_4	Light green	Light Brown	black	Fluorescent Blue	Blackish Green	Blackish Brown
6	P+conc.H_2SO_4	Greenish yellow	Yellowish Green	Fluorescent green	Blue Black	Fluorescent Green	Fluorescent violet
7	P+conc. HCl	Yellow green	Yellow Brown	Dark blue	Dark Blue	Black Green	Black Brown
8	P+conc.HNO_3	Greenish brown	Dark Brown	black	Black	Green Black	Yellow Black
9	P+I_2 sol.	Orange red	Orange Red	black	Black	Light Brown	Dark Brown
10	P+5%HCl	Light green	Light Brown	Dark blue	Dark blue	Light Brown	Dark Brown
11	P+5%dil.HNO_3	Light green	Light Brown	black	Black	Grey Black	Black
12	P+5%Na_2CO_3	Yellow Black		Blue purple	Blue	Brown	Dark Brown
13	P+alc. KOH	Brown yellow	Yellow Brown	Blue black	Blue Black	Flouresent Green Yellow	Light Green Yellow
14	P+1%$KMnO_4$	brown	Dark Brown	brown	Dark Brown	Brown Black	Black

Where, P = Powdered extract, ME = Methanolic extract, HE = Hydroalcoholic extract

Table 5. TLC profile of methanolic and hydroalcoholic extracts of *T.occidentalis*

Methanolic extract	Hydroalcoholic extract	
Solvent system : Toluene: ethyl acetate: Formic acid: water :: 100: 36: 36: 10	Solvent system: Chloroform: methanol :: 9: 1	Solvent system: Toluene: ethyl acetate :: 9: 1
4 spots observed with R_f 1) 0.7741 2) 0.7258 3) 0.3548 4) 0.1612	2 spots observed with R_f 1) 0.5306 2) 0.2857	2 spots observed with R_f 1) 0.7142 2) 0.2857

Fig. 1. *Thuja occidentalis* leaves

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