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Phytochemical Investigation and HPTLC Screening of Thuja Orientalis

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ABSTRACT
Thuja occidentalis , commonly known as Tree Vitae or white cedar , is home - grown in Europe as an ornamental tree .Thuja occidentalis , commonly known as Tree Vitae or white cedar , is home - grown to eastern North America and is grown in Europe as an ornamental tree. Thuja orientalis(commonly - peacock , family - Cu - pressaceae) is a genus of coniferous trees.T.orientalis is a Evergreen , monoecious trees or shrubs that geow to 10-60 feet tall long . The shoots are flat , the leaves are like scales.leaves are Growing with resin glands arranged in a flattened fan shape . The plant was first recognized as a remedy by native Indians in canada parasitic worms. The essential oil derived from the leaves is toxic. A - thujone is useful as an insecticide and an antihelminthic agent for the treatment of parasitic worms . present study the physicochemical, preliminary Phytochemical and HPTLC identification were carried out physicochemical tests for the samples Thuja orientalis leaf were performed viz. loss on drying gat 105°C, total ash content, acid insoluble ash, alcohol soluble extractive, water soluble extractive, benzene and acetone soluble extractive were carriedout. Phytochemical studies of Thuja orientalis has been shown the presence of various versatile constituents such as flavonoids, triterpenoids, vitamin c, stibene, derivatives and many others like resveratrol, piceatannol, pallidol, perthenocissin and phytosterols.Out of which ascorbic acid,triterpene,beeta-sitosterol,ketosteroid,two assymmetrical tetracyclic, triterpenoids and calcium were identified as major constituents of this plant.

KEYWORDS: Physicochemical, Phytochemical, HPTLC-Fingerprinting, Total Ash value, Loss on Drying, water soluble extractive value.

I. INTRODUCTION

With the emerging interest around the world in adopting and studying traditional systems and harnessing their potential evaluation of the rich heritage of Indian traditional medicine on the basis of various health care systems is essential .Their leaves contain essential oils used to treat fungus infections , cancer , moles and With the emerging interest around the world in adopting and studying traditional systems and harnessing their potential evaluation of the rich heritage of Indian traditional medicine on the basis of various health care systems is essential [1] . However , a thujone is a toxic substance that disrupts neurological signals in the brain . Ingestion of the essential oils of thuja leaves can cause death .Oil of thuja contains thujone which has been studied for its GABA (gamma - aminobutyric acid) receptor antagonistic , with potentially lethal properties [2] . A yellow dye is obtained from the young branches [3] .Thuja is also occasionally used for treating diseases of skin , blood , Gastrointestinal tract , kidney , hrain , warty excrescences , spongy tumors [4]. Platyclodus is a monotypicgenus of evergreen coniferous trees in the cyprees family cupressaceap , containing only one species , platycladus orientalis , also known as Chinese thuja [5] .

Thuja species are used as food plants by the larvae of some Lepidoptera species including autumnal moth , the engrailed and juniper pug . The foliage is also readily eaten by deer , and where deer population density is high , can adversely affect the growth of young trees and the establishment of seedlings [6] Current research suggests that Thuja originated in the Americas and migrated to East Asia via the Bering Landbridge in the Miocene.Fossil records show that Thuja was significantly more widely distributed during the late Cretaceous and early Tertiary than we see today [7] .Thuja is a monophyletic genus that sits within the order Pinales in the Cupressaceae.Thuja is in the

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Cupressoid clade and is sister to the genus Thujopsis is supported with 100 % bootstrap support and 1.0 posterior probability . Within the genus the taxonomy is in flux , but most recent research based on molecular analysis of plastomes in the genus Thuja showed evidence for a new grouping , with two sister clades : T. standishii and T. koraiensis together and T. occidentalis and T. sutchuenensis together , with T.plicata sister to T. occidentails and T. sutchuenensis [8] .

Cedarwood oil and cedar leaf oil , which are derived from Thuja occidentalis , have different properties and uses [9] . The natives of Canada used the scaled leaves of Thuja occidentalis (Eastern White Cedar) to make a tea that has been shown to contain 50 mg of vitamin C per 100 grams ; this helped prevent and treat scurvy [10] . In the 19th century Thuja was commonly used as an externally applied tincture or ointment for the treatment of warts , ringworm and thrush , [11] . And a local injection of the tincture was used for treating venereal warts [12] . A 2017 trial showed that its extract effectively killed both gram - positive and gram - negative bacteria [13] .

Collection and processing of plant's material

The Fresh leaves of thuja orientalis were collected from the Department of Deendayal Research Institute Chitrakoot. **[Figure no.1]** The leaves of thuja orientalis where collected in March 2022. The collected Plant was authenticated with the biological department. Then the collected leaves were washed three times And then cut into small pieces of leaves. **[Figure no.2]** Then put the cut leaves to drying at sun light for few days .**[Figure no.3]** The dried plant samples was ground in electric grinder to get fine powder form for further use**.[Figure no.4]** These were stored in air tight glass containers until required for analysis and thuja.

Aim & Objectives

During the Research of present investigation have taken the fallowing objectives pertaining to the pharmacolognostical analysis of Thuja Orientalis(leaf)

Main objectives of the Research work

1. Physicochemical study of Thuja Orientalis (leaf)
   - Loss On Drying
   - Water Soluble ash
   - Alcohol Soluble ash
   - Total ash
2. Phytochemical evaluation of Thuja orientalis (leaf)
3. HPTLC - Fingerprinting of Thuja Orieantlis (leaf)

Fig. 1 – Collected leaf

Fig. 2 – Washed leaf

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Fig.3 – Dry leaf

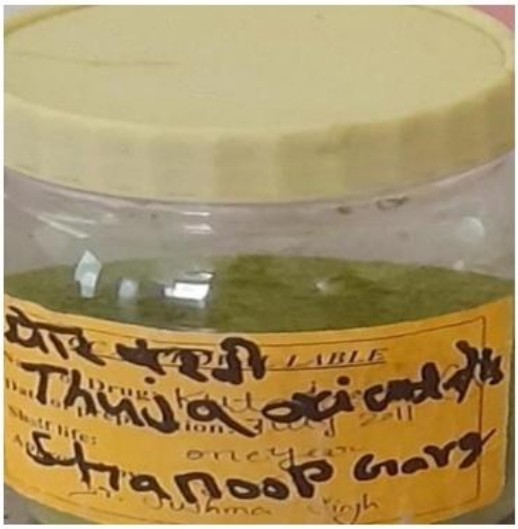

Fig.4 – Leaf Powder

II. MATERIALS & METHODS

Methanol (AR grade). Folin and ciocalteu's Phenol reagent, Molisch reagent
Conc. HCL , H_2SO_4 Dragondrof's reagents, Ethanol, Na_2CO_3, NaOH,CuSO4.5H2O ,
Potassium sodium tartrate, Phosphate buffer, Sodium sulphide (0.1N), Thiourea (0.3N),.

- Physico-chemical parameters.
- Determination of Moisture Content (Loss on drying at 105°C).
- Determination of alcohol soluble extractive.
- Determination of water soluble extractive.
- Determination of Ash values.
- Determination of total ash.
- Determination of Acid-insoluble ash.
- Phytochemical qualitative analysis.
- Carbohydrate.
- Test for alkaloids.
- Test for flavonoids.
- Test for saponins.
- Test for Proteins.
- Test for Gum.
- Test for tannins.

Methodology for High Performance Thin - Layer Chromatography: High Performance Thin-Layer Chromatography of the test solutions of sample thuja orientalis was carried out on Silica Gel 60 F254 precoated plates (0.2 mm thickness; from Merck India Limited Mumbai). A TLC applicator from Camag Linomat-5 (Camag Switzerland 140443) was used for band application and photo documentation unit (Camag Reprostar-3: 140604) was used for documentation of chromatographic fingerprints.

III. RESULTS & DISCUSSION

The results of physicochemical analysis are given in Table 2 to 8 , Phytochemical analysis are given in Table no. 9 and Rf value of HPTLC fingerprints profile of Thuja orientalis are given in Table no. 10 .

The total ash value is an indicative of total amount of inorganic material after complete incineration and the acid insoluble ash value is an indicative of silicate impurities, which might have arisen due to improper washing of the ingredients. Ash value is useful in determining authenticity and purity of the drug and also these values are important quantitative standards, the extractive values, alcohol soluble, water soluble, benzene soluble and acetone soluble indicates the amount of active constituents in given amount of plant material when extracted with respective solvent. The loss on drying value fungal or yeast growth. In our study all the findings are within prescribed limits of ayurvedic Pharmacopoeia of india.

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Table- 2.Loss On Drying (LOD Value Of *Thuja orientalis leaf*)

S.N.	EMPTY PETRIDISH +2GMPOWER W.T.	AFTER HOURS DRYING W.T	AFTER ½ HOURS DRYING W.T	DIFFERENCE
1	16.1211	16.0586	16.055	0.0661
2	16.9635	16.9016	16.8985	0.065
3	19.8785	19.8168	19.8133	0.0624
			Total	**0.1935**

Sample weight – 2gm
Average wt. Difference – 0.1935/3 = 0.0645 LOD=0.0645 × 100 / 2 LOD = 3.22%

Table – 3. Water Soluble Extractive Value Of *Thuja orientalis* (leaf)

S.N.	PETRIDISH PRE W.T	PETRIDISH FINAL W.T	DIFFERENCE
1	35.2399	35.3304	0.0905
2	32.0425	32.1315	0.0890
3	33.2018	33.307	0.1052
		Total	**02847/3**

SAMPLE WEIGHT - 2gm
Average Weight Difference = 0.0949 ×500 = 47.45 %

Table - 4. Ethanol Soluble Extractive Value Of *Thuja orientalis* (leaf)

S.N.	PETRIDISH PRE W.T	PETRIDISH FINAL W.T	DIFFERENE
1	35.5178	35.5723	0.0545
2	36.6149	36.6674	0.0525
3	31.9668	32.0162	0.0494
		Total	**0.6289/3**

SAMPLE WEIGHT – 2gm
Average Weight Difference = 0.2096× 500 =104.81%

Table – 5. Benzene Soluble Extractive Value Of *Thuja orientalis*(leaf)

S.N.	PETRIDISH PRE W.T	PETRIDISH FINAL W.T	DIFFERENCE
1	43.7366	43.7551	0.0185
2	45.1256	45.1430	0.0174
3	43.7536	43.7711	0.0175
		Total	**0.0534/3**

SAMPLE WEIGHT – 2gm
Average Weight Difference =》 0.0178×500 = 8.9%

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Table - 6. Acetone Soluble Extractive Value Of *Thuja orientalis* (leaf)

S.N.	PETRIDISH PREW.T	PETRIDISH FINALW.T	DIFFERENCE
1	43.2802	43.3085	0.0283
2	44.0280	44.0561	0.0281
3	43.3187	43.3457	0.027
		Total	**0.0834/3**

SAMPLE WEIGHT – 2gm
Average Weight Difference =》 0.0278 × 500 =13.9%

Table -7. Total Ash value of *Thuja orientalis* (leaf)

S.N.	Crucible weight	Crucible weight + 2gm sample	1st Weigh t	2nd Weight	3rd Weight	Difference
1	19.5948	21.5948	19.7299	19.7291	19.7292	0.1344
2	17.3151	19.3151	17.4507	17.4501	17.4496	0.1345
					Total weight-	0.2689

Sample Waight- 2gm
Average W.t difference- 0.2689/2
Total Ash- 0.135×100/2
Ash-6.75%

Table- 8. Acid in soluble value of *Thuja orientalis* (leaf)

S.N	W.t.of Empty crucible W.t	1st day weight	2nd day weight	Difference
1	17.3258	17.3254	17.3244	0.0014
2	19.6053	19.6047	19.6042	0.0011
			Total	0.0025

Sample weight = 2gm
Average weight difference =0.0025/2
Acid ash value =0.0015×100/2
Total ash value = 0.075%

Table – 9. Preliminary phyto- chemical investigation

S.N	Phytochemical	Test	Benzene	Acetone	Ethanol	D.Water
1.	**Carbohydrate**	**Fehling test**	*	*	*	+
		Benedict test	*	*	+	*
2.	**Alkaloid**	**Wagner's test**	+	+	*	*
		Mayer's test	+	-	*	+

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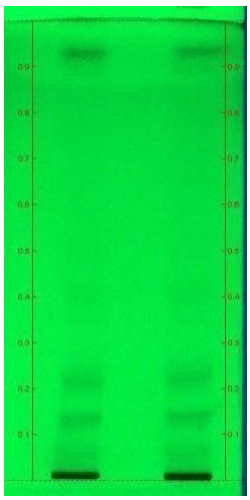
		Dragendorff's test	+	-	*	+
		Hager's test	-	-	*	+
3.	**Flavonoids**	**Shinoda test**	+	*	*	+
		Fluroscence Test	*	*	*	+
4	**Saponins**	**Froth test**	*	*	*	+
5	**Protein**		+	+	+	+
6	**Gum**		*	*	*	-
7	**Gelatin**		*	*	*	-
8	**Steroids**		*	*	*	-

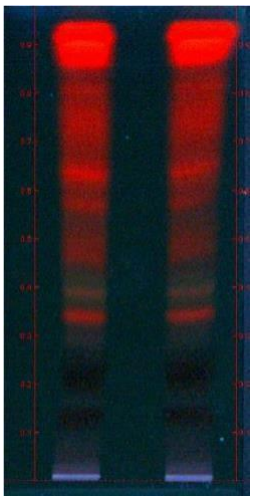
(*) Not done (+) Present (-) Abesent

HPTLC fingerprint profile of the test solution is depicted in (Fig. 5, 6, 7 & 8) indicates the presence of different types of phytochemicals. Development of fingerprint profile would serve as a reference standard of the authentic sample. The TLC plate was examined under 254nm, 366nm before derivatization and after derivatization 366nm & 254nm. The R_f values and colours of the bands obtained were recorded. It shows major spots and the R_f values and colours of the bands obtained were recorded and given in Table 10.

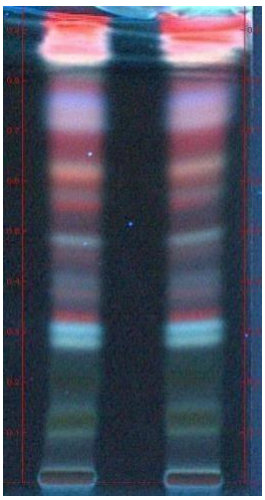
HPTLC fingerprints profile of Thuja



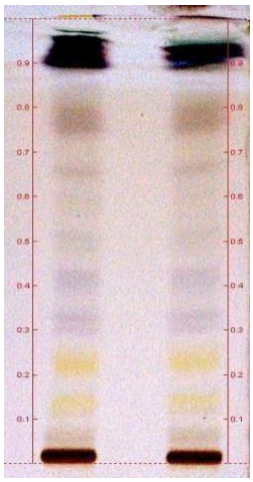
A B
Fig.5:254nm



A B
Fig.6: 366nm derivatization



A B
Fig.7: 366nm After derivatization



A B
Fig.8: 254nmAfter

After derivatization :–
Where Track A: test solution of Thuja & Track B: test solution of Thuja

Table-10: Rf values of HPTLC fingerprints profile of Thuja

S. No.	R_f values	254nm derivatization b	366nm before derivatizatio n	366nm afte r	254nm After derivatizatio n

				derivatizatio n	
1	Rf 1	0.08 (black)	0.14(brownish red)	0.10(sky blue)	0.14(yellow)
2	Rf 2	0.14(black)	0.22 (brownish red)	0.30 (sky blue)	0.24(yellow)
3	Rf 3	0.94(black)	0.34(red)	0.50(sky blue)	0.30(brownish blue)
4	Rf 4	-	0.50(red)	0.60(red)	0.40(brownish blue)
5	Rf 5	-	0.62 (red)	0.74(pink)	0.68(brown)
6	Rf 6	-	0.80(red)	0.90(red)	0.78(brown)
7	Rf 7	-	0.90(red)	-	0.90(black)

IV. DISCUSSION:-

Qualitative phyto-chemical analysis were performed in benzene, acetone, ethanol and water extracts, various phytochemicals like Alkaloids, carbohydrates, flavonoids, protein, resin and soponin were present in studied sample of Thuja Orientalis. Which could make the drug useful for potential and preventive healthcare needs. The polyphenols were identified and quantified from drug powder, from methanol extracts The quantification of total polyphenols was performed by UV-Vis spectral method at 500 nm. The total polyphenols were expressed in gallic acid. The results show that the leaves are rich in polyphenols. The qualitative TLC analysis was performed using: silica plates (Merck) with fluorescence indicator to 254 nm, a mixture of toluene, ethyl acetate: formic acid (7-3:5 v/v) as mobile phase. The development of the plate is done in the CAMAG 10x10 cm Twin trough chamber and visualized under UV at 254 nm and 366 nm after derivatization using 5% methanolic sulphuric acid reagent. The Rf values and colors of the resolved bands inchromatogramme were calculated. LOD was found 3.22% in our studied sample which indicates the drug in safe and capable to prevent microbial growth. physicochemical test carried out and found water soluble extractive value where found 47.45% , Alcohol extractive value 104.81% ,Benzene extractive value 8.9%, Acetone extractive value 13.9%. total Ash and Acid insoluble ash was calculated and found 6.75% and 0.075%. preliminary phytochemical screening was done to identify the possibility of active constituents for extracts of the drugs in different solvents. Benzene, Acetone, Ethenol, and Water were screened for phytochemical and various phytochemicals like alkaloids, flavonoids, saponin, protein, carbohydrate, were present in our study samples. Which indicates the drugs therapeutic potential of cure diseases.

HPTLC Screening was done and plate was observed at 254 nm & 366 nm before & after derivetisation with 5% methanolic H2SO4 .At 254 nm measure spot. seen at Rf 0.08, 0.014 and 0.094. At 366 nm major spot at Rf 0.14 (brownish red), 0.22(brownish red) , 0.34 (red) ,0.50(red), 0.62 (red), 0.80(red), 0.90(red). Similarly 366 nm after derivetisation major of seen at Rf 0.10(sky blue), 0.30(sky blue) 0.50(sky blue) , 0.60(red), 0.74(pink), 0.90 (red). Blue , red, brown, fluorescence , colour major indicates the present of essential oil compounds.

V. CONCLUSION

The drug Thuja orientalis has been widely used in traditional practices as single drug and in different formulations . It is one of the main drug well explained in all Ayurvedic classics. For giving a validation to its therapeutic properties and to standardize the drug the preliminary phytochemical analysis of the drug had been carried out. From the phytochemical evaluation of the Thuja orientalis drug, the quantitative increase of its active phytoconstituents was clearly seen. This certainly increases the potency of the drug.

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