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Phytochemical Investigation and HPTLC Screening of Thuja Orientalis

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ABSTRACT
Thujaoccidentalis,commonlyknownasTreeVitaeorwhitecedar,ishome-grown in Europe as an ornamental tree .Thujaoccidentalis , commonly known asTreeVitaeor whitecedar ,ishome-grownto easternNorthAmericaandisgrownin Europe as an ornamental tree.Thujaorientalis(commonly-peacock,family-Cu-pressaceae)isagenusof coniferous trees.T.orientalis is a Evergreen , monoecious trees or shrubs thatgeow to 10-60 feet tall long . The shoots are flat , the leaves are like scales.leavesareGrowingwithresinglandsarrangedin aflattenedfanshape.Theplantwasfirstrecognized as aremedybynative Indiansin canadaparasiticworms. Theessential oil derived from the leaves is toxic.a -thujone is useful as an insecticideand an antihelminthic agent for the treatment of parasitic worms . present study the physicochemical, preliminary Phytochemical and HPTLCidentificationwerecarriedoutphysicochemicaltestsforthesamplesThujaorientalis leaf were performed viz. loss on drying gat 105°C, total ash content,acidinsolubleash,alcoholsolubleextractive, watersolubleextractive,benzeneandacetonesolubleextractivewerecarriedout. Phytochemical studies of Thujaorientalis has been shown the presenceofvariousversatileconstituentssuchasflavonoids,triterpenoids,vitamin c, stibene, derivatives and many others like resveratrol, piceatannol, pallidol,perthenocissin and phytosterols.Out of which ascorbic acid,triterpene,beeta-sitosterol,ketosteroid,twoassymmetrical tetracyclic, triterpenoids and calciumwereidentifiedasmajorconstituentsofthisplant.

KEYWORDS: Physicochemical, Phytochemical, HPTLC-Fingerprinting.

I. INTRODUCTION

With the emerging interest around the world in adopting and studyingtraditionalsystemsandharnessingtheirpotentialevaluationoftherichheritageofIndiantraditionalmedicineonthebasisof varioushealthcaresystemsisessential .Their leaves contain essential oils used to treat fungus infections , cancer , molesandWiththeemerginginterestaroundtheworldinadoptingandstudyingtraditionalsystemsandharnessingtheirpotentialevaluationoftherichheritageofIndian traditional medicine on the basis of various health care systems is essential[1]. However , athujoneisatoxicsubstancethatdisruptsneurologicalsignalsinthebrain.Ingestionof the essential oils of thuja leaves can cause death .Oil of thuja contains thujonewhich hasbeen studied for itsGABA (gamma- aminobutyric acid) receptorantagonistic,withpotentiallylethalproperties [2].
Ayellowdyeisobtainedfromtheyoungbranches[3].Thujaisalsooccasionallyusedfortreatingdiseasesofskin ,blood,Gastrointestinaltract,kidney,hrain,wartyexcrescences,spongytumors[4]. Platyclodus is a monotypicgenus of evergreen coniferous trees in the cypreesfamily cupressaceap , containing only one species , platycladusorientalis , alsoknownas Chinesethuja[5].
Thuja species are used as food plants by the larvae of some Lepidoptera speciesincludingautumnalmoth,theengrailedandjuniperpug.Thefoliageisalsoreadilyeatenbydeer,andwheredeerpopulationdensityishigh,canadverselyaffectthegrowth of young trees and the establishment of seedlings [6] Current researchsuggests that Thuja originated in the Americas and migrated to East Asia via theBering Landbridge in the Miocene.Fossil records show that Thuja was significantlymore widely distributed during the late Cretaceous and early Tertiary than we seetoday [7] .Thuja is a monophyletic genus that sits within the order Pinales in theCupressaceae.Thuja is in the Cupressoid clade and is sister to the genus Thujopsisis supported with 100 % bootstrap support and 1.0 posterior probability . Withinthe

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genus the taxonomy is in flux , but most recent research based on molecularanalysis of plastomes in the genus Thuja showed evidence for a new grouping , withtwosisterclades:T.standishiiandT.koraiensistog etherandT.occidentalisandT.sutchuenensistogether,w ithT.plicatasistertoT.occidentailsandT.sutchuenensis[8] .
Cedarwoodoilandcedarleafoil,whicharederivedfrom Thujaoccidentalis
, have different properties and uses [9] . The natives of Canada used the scaledleaves of Thujaoccidentalis(Eastern White Cedar) to make a tea that has beenshowntocontain50mgofvitaminCper100grams; thishelped preventand treatscurvy[10].Inthe19thcenturyThujawascommonl yusedasanexternallyappliedtinctureorointmentforthe treatmentofwarts,ringwormandthrush,[11].Anda local injection of the tincture was used for treating venereal warts [12] . A 2017trial showed that its extract effectively killed both gram - positive and gram -negativebacteria[13].

Collectionandprocessingofplant'smaterial

The Fresh leaves of thujaorientaliswere collected from the Department ofDEENDAYALRESEARCHINSTITUTECHITR AKOOT.**[Figureno.1]**Theleavesof thujaorientaliswherecollectedinMarch2022fromthe Department ofDEENDAYALRESEARCHINSTITUTECHITR AKOOT.ThecollectedPlantwasauthenticated with the biological department. Then the collected leaves werewashed three times And then cut into small pieces of leaves. **[Figure no.2]** Thenput the cut leaves to drying at sun light for few days .**[Figure no.3]** The driedplantsampleswasgroundinelectricgrindertogetf inepowderformforfurther use**.[Figure no.4]** These were stored in air tight glass containers untilrequiredfor analysis andthuja

Aim & Objectives

Duringthecourseofpresentinvestigationhavetakenth efallowingobjectivespertainingtothepharmacologno sticalanalysisofThujaOrientalis(leaf)
MainobjectivesoftheDissertationwork

1. PhysicochemicalofThujaOrientalis(leaf)
   - LossOn Drying
   - WaterSolubleash
   - AlcoholSolubleash
   - Totalash
2. PhytochemicalevaluationofThujaorientalis(leaf)
3. HPTLC-FingerprintingofThujaOrieantlis(leaf)


Fig.1–Collected leaf


Fig.2–Washedleaf

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Fig.3–Dryleaf

Fig.4–LeafPowder

II. MATERIALS &METHODS

Methanol(ARgrade).Folinandciocalteu'sPhenolreagent,MolischreagentConc.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-chemicalparameters.
- DeterminationofMoistureContent(Lossondryingat105°C).
- Determinationofalcoholsolubleextractive.
- Determinationofwatersolubleextractive.
- DeterminationofAshvalues.
- Determinationoftotalash.
- DeterminationofAcid-insolubleash.
- Phytochemicalqualitativeanalysis.
- Carbohydrate.
- Testforalkaloids.
- Testforflavonoids.
- Testforsaponins.
- TestforProteins.
- TestforGum.
- Testfor tannins.

MethodologyforHighPerformanceThin-LayerChromatography:High Performance Thin-Layer Chromatography of the test solutions of samplethujaorientalis was carried out on Silica Gel 60 F254 precoated plates (0.2 mmthickness; from Merck India Limited Mumbai). A TLC applicator from CamagLinomat-5 (Camag Switzerland 140443) was used for band application and photodocumentationunit(CamagReprostar-3:140604)was usedfordocumentationofchromatographicfingerprints.

III. RESULTS & DISCUSSION

Theresultsofphysicochemical analysis are given in Table 2 to 8 , Phytochemical analysis aregiveninTableno.9 andRfvalueofHPTLCfingerprintsprofileofThujaorientalisaregiveninTableno. 10.

The total ash value is an indicative of total amount of inorganicmaterial after complete incineration and the acid insoluble ashvalue is an indicative of silicate impurities, which might havearisen due to improper washing of the ingredients. Ash value isusefulindeterminingauthenticityandpurityofthedrugandalsothesevaluesareimportantquantitativestandards,theextractivevalues,alcoholsoluble,watersoluble,benzenesolubleandacetone soluble indicates the amount of active constituents ingiven amount of plant material when extracted with respectivesolvent. The loss on drying value fungal oryeast growth. In our study all the findings are within prescribedlimitsofayurvedicPharmacopoeia ofindia.

Table-2.LossOnDrying(LODValueOfThujaorientalisleaf)

S.N.	EMPTYPETRIDISH +2GMPOWERW.T.	AFTER HOURSDRYINGW.T	AFTER½HOURSDRYINGW.T	DIFFERENCE
1	16.1211	16.0586	16.055	0.0661

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2	16.9635	16.9016	16.8985	0.065
3	19.8785	19.8168	19.8133	0.0624
			Total	**0.1935**

Sampleweight–2gm
Averagewt.Difference–0.1935/3=0.0645LOD=0.0645×100/2
LOD=3.22%

Table– 3.WaterSolubleExtractiveValueOfThujaorientalis(leaf)

S.N.	**PETRIDISHPREW.T**	**PETRIDISHFINALW.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**

SAMPLEWEIGHT -2gm
AverageWeight Difference=0.0949×500=47.45%

Table- 4.EthanolSolubleExtractiveValueOfThujaorientalis(leaf)

S.N.	**PETRIDISHPREW.T**	**PETRIDISH FINALW.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**

SAMPLEWEIGHT –2gm
AverageWeightDifference=0.2096×500=104.81%

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Table–5.BenzeneSolubleExtractiveValueOfThujaorientalis(leaf)

S.N.	PETRIDISHPREW.T	PETRIDISH FINALW.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**

SAMPLEWEIGHT – 2gm
AverageWeightDifference =》 0.0178×500 = 8.9%

Table- 6.AcetoneSolubleExtractiveValueOfThujaorientalis(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.TotalAshvalueofThujaorientalis (leaf)

S.N.	Crucibleweight	Crucibleweight +2gmsample	$_1$stWeight	$_2$ndWeight	$_3$rdWeight	Difference
1	19.5948	21.5948	19.7299	19.7291	19.7292	0.1344

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2	17.3151	19.3151	17.4507	17.4501	17.4496	0.1345
					Totalweight-	0.2689

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

Table-8.AcidinsolublevalueofThujaorientalis(leaf)

S.N	**W.t.ofEmptycrucible W.t**	**1st dayweight**	**2nd dayweight**	**Difference**
1	17.3258	17.3254	17.3244	0.0014
2	19.6053	19.6047	19.6042	0.0011
			Total	0.0025

Sampleweight=2gm
Averageweightdifference=0.0025/2
Acidashvalue=0.0015×100/2
Totalashvalue=0.075%

Table–9.Preliminaryphyto-chemicalinvestigation

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

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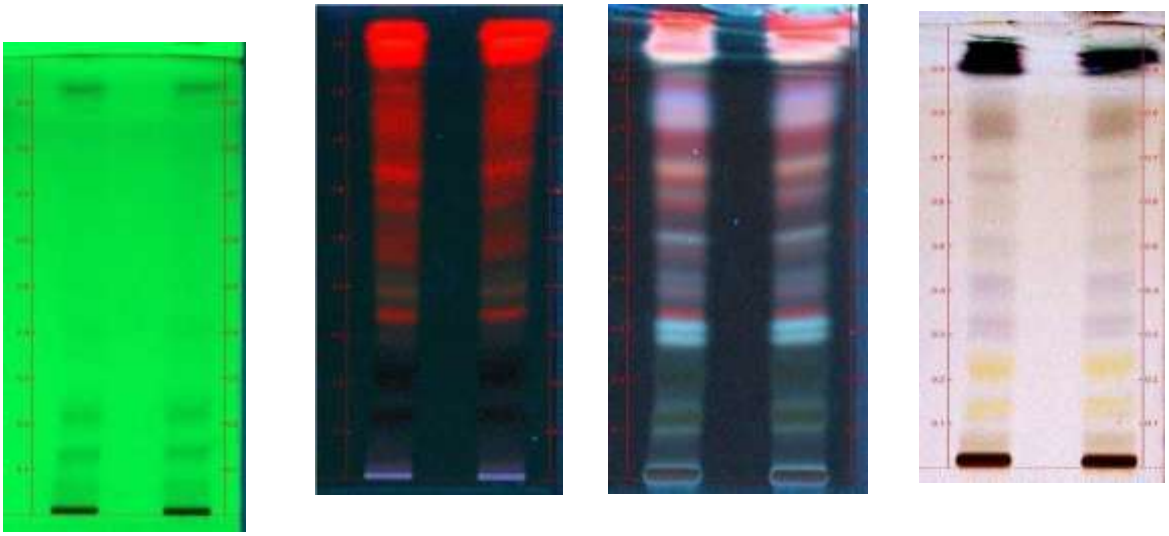
3.	**Flavonoids**	**Shinoda test**	+	*	*	+
		FluroscenceTest	*	*	*	+
4	**Saponins**	**Froththest**	*	*	*	+
5	**Protein**		+	+	+	+
6	**Gum**		*	*	*	-
7	**Gelatin**		*	*	*	-
8	**Steroids**		*	*	*	-

(*)Notdone
(+)Present
(-)Abesent

HPTLC fingerprint profile of the test solution is depicted in (Fig. 5, 6, 7 & 8) indicatesthepresenceofdifferenttypesofphytochemicals.Developmentoffingerprintprofilewouldserve as a reference standard of the authentic sample. The TLC plate was examined under254nm, 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 ofthe bands obtainedwere recorded and given inTable 10.

HPTLCfingerprintsprofileofThuja



A B A B A B A B

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Fig.5:254nm Fig.6:366nm Fig.7: 366nmAfter derivatization Fig.8: 254nmAfter derivatization

After derivatization :–
WhereTrackA:testsolutionof Thuja&TrackB:testsolutionof Thuja

Table-10:$R_f$valuesof HPTLCfingerprintsprofileofThuja

S. No.	R_f values	254nmbeforederivatization	366nmbeforederivatization	366nm afterderivatization	254nmAfterderivatization
1	Rf 1	0.08(black)	0.14(brownishred)	0.10(sky blue)	0.14(yellow)
2	Rf 2	0.14(black)	0.22 (brownishred)	0.30 (skyblue)	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)

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IV. DISCUSSION

Qualitative phyto-chemical analysis were performed in benzene, acetone, ethanolandwaterextracts,variousphytochemicalslike Alkaloids,carbohydrates,flavonoids, protein,resinandsoponin were present in studied sample of ThujaOrientalis. Which could make the drugusefulforpotentialandpreventivehealthcareneeds.The polyphenols were identified and quantified from drug powder, from methanolextracts The quantification of total polyphenols was performed by UV-Vis spectralmethod at 500 nm. The total polyphenols were expressed in gallic acid. The resultsshow thattheleavesarerichinpolyphenols.ThequalitativeTLCanalysiswasperformedusing:silicaplates(Merck) withfluorescence 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 CAMAG10x10 cm Twin trough chamber and visualized under UV at 254 nm and 366 nmafter derivatization using 5% methanolicsulphuric acid reagent. The Rf values andcolorsoftheresolvedbands inchromatogrammewerecalculated.LOD was found 3.22% in our studied sample which indicates the drug in safeandcapabletopreventmicrobialgrowth.physicochemicaltestcarriedoutandfoundwatersolubleextractive value where found 47.45% , Alcohol extractive value 104.81% ,Benzeneextractivevalue8.9%,Acetoneextractive value13.9%.total Ash and Acid insoluble ash was calculated and found 6.75% and0.075%.preliminaryphytochemicalscreeningwas donetoidentifythepossibility of active constituentsfor extracts of thedrugsin differentsolvents. Benzene, Acetone, Ethenol, and Water were screened for phytochemicalandvariousphytochemicalslikealkaloids,flavonoids,

saponin,protein,carbohydrate,werepresentinourstudysamples. Which indicatesthedrugstherapeuticpotentialofcure diseases.HPTLCScreeningwasdoneandplatewasobserved at254nm&366nmbefore&afterderivetisationwith5%methanolicH2SO4.At254nmmeasurespot. seenatRf0.08,0.014and0.094.At366nmmajorspotatRf0.14(brownishred),0.22(brownishred) ,0.34(red) ,0.50(red),0.62(red),0.80(red),0.90(red).Similarly366 nm afterderivetisation major of seen at Rf0.10(skyblue),0.30(skyblue)0.50(skyblue), 0.60(red),0.74(pink), 0.90(red).Blue,red,brown,fluorescence,colourmajor indicatesthepresentofessentialoilcompounds.

V. CONCLUSION

ThedrugThujaorientalishasbeenwidelyuse dintraditionalpracticesassingle drug and in different formulations . It is one of the main drug wellexplained in all Ayurvedic classics. For giving a validation to its therapeuticproperties and to standardize the drug the preliminary phytochemical analysisof the drug had been carried out. From the phytochemical evaluation of theThujaorientalis drug, the quantitative increase of its active phytoconstituentswas clearlyseen.Thiscertainlyincreasesthepotencyofthedrug.

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