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Physical evaluation, Powder analysis and Chromatographic Separation. of *Platycladus oriental* (L)

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ABSTRACT

Platycladus orientalis (L.) Franco, commonly known as Chinese arborvitae, has been the subject of extensive pharmacognostical, phytochemical, and chromatographic investigations due to its diverse medicinal properties. Pharmacognostical studies have detailed the plant's anatomical features, including its needle-like leaves with resin canals and distinctive cone structures, which aid in accurate identification and quality control. Microscopic examinations have revealed the presence of starch grains and essential oils within the mesophyll tissues, underscoring its therapeutic potential.A thorough pharmacognostical study of Platycladus orientalis leaves establishes diagnostic macroscopic and microscopic features—scale-like flattened shoots with resin-filled stomatal bands and characteristic transverse sections—ensuring correct plant identification and detection of adulterants alongside five other Cupressaceae species via microscopy and TLC profiling. Physicochemical parameters (loss on drying, total ash, acid-insoluble ash, extractive values) complement preliminary phytochemical screening, which reveals abundant flavonoids triterpenoids and phenolics in methanolic extracts Together, these integrated pharmacognostic, phytochemical and chromatographic approaches provide a robust framework for the quality evaluation and standardization of P. orientalis in both research and herbal medicine contexts.

KEYWORDS: medicinal properties, Physicochemical, standardization, quality

INTRODUCTION

Pharmacognosy, the applied science of the biological, biochemical, and economic aspects of natural drugs and their constituents, has been a significant part of human history since ancient times. Plants, fungi, and animals have been the oldest sources of natural drug products, with some having hallucinogenic properties. Some well-known drugs of plant origin include ephedrine, caffeine, morphine, and camphor. Fungi are second only to plants in their usefulness as medicinals, with some having psychoactive properties and being adopted for ritual or spiritual use. Animals also provide useful natural medicinal products, such as the poison from the skin of frogs.[1]

Medicinal plants still play an important role in emerging and developing countries of Asia, both in preventive and curative treatments, despite advances in modern western medicine. The ancient civilizations of India, China, Greece, and Arab developed their own systems of

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medicine independent of each other, but all were predominantly plant-based. Ayurveda, the oldest organized system of medicine, is considered the oldest (6000BC) among organized traditional medicine.[1,2]

The World Health Organization (WHO) estimates that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs. The subject has evolved significantly over time, with branches such as botany and plant chemistry being closely related. The history of pharmacognosy includes stages such as Ayurveda, Papyrus Ebers, Hippocrates, Aristotle, Theophrastus, Diascorides, Pliny, and Galen.

Plant profile:

Botanical Name	**:**	***Platycladus orientalis*(L) Franco**
Famil y	**:**	Cupressaceae
Synonym	**:**	Biota orientalis (L.) Endl.
		Thuja orientalis L.
Domain	**:**	*Eukaryota*
Kingdom	**:**	*Plantae*

: Geographical Distribution:

Native: China, Japan, Korea, Republic of, Russian Federation, Taiwan, Province of China, Ukraine

Exotic: France, Iran, New Zealand, Poland.

2.1.3: Cultivation[3]

Requirements

USDA Hardiness Zone	:	6 to 9
AHS Heat Zone	:	Not defined for this plant
Light Range	:	Sun to Full Sun
pH Range	:	5 to 7
Soil Range	:	Mostly Sand to Clay Loam
Water Range	:	Normal to Moist

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Propagation method:

Seed - best sown when ripe in the autumn in a cold frame. Stored seed germinates best if given a short cold stratification. It can then be sown in a cold frame in late winter. Plants make very little growth in their first year. When they are large enough to handle, prick the seedlings out into individual pots and grow them on in the greenhouse for at least their first winter. Plant them out into their permanent positions in late spring or early summer, after the last expected frosts. If there is sufficient seed it is worthwhile trying a sowing in an outdoor seed bed in April. Grow the plants on for at least two years before planting them out in the winter. Cuttings of half-ripe wood, 5 - 8cm with a heel, July/August in a shaded frame. Forms roots by the end of September but should be overwintered in a frame. Cuttings of almost ripe wood, 5 - 10cm with a heel, September in a cold frame. Forms roots in the following summer. Plant out in autumn or spring [4,5]

DESCRIPTION OF PLANT:

It is a small, slow-growing tree, to 15-20 m tall and 0.5 m trunk diameter (exceptionally to 30 m tall and 2 m diameter in very old trees). The foliage forms in flat sprays with scale-like leaves 2-4 mm long. The cones are 15-25 mm long, green ripening brown in about 8 months from pollination, and have 6-12 thick scales arranged in opposite pairs. The seeds are 4-6 mm long, with no wing.

Medicinal uses:

The leaves are used as antibacterial, antipyretic, antitussive, astringent, diuretic, emmenagogue, emollient, expectorant, febrifuge, haemostatic, refrigerant and stomachic. Their use is said to improve the growth of hair. They are used internally in the treatment of coughs, haemorrhages, excessive menstruation, bronchitis, asthma, skin infections, mumps, bacterial dysentery, arthritic pain and premature baldness. The leaves are harvested for use as required and can be used fresh or dried. This remedy should not be prescribed to pregnant women. The seed is aperient, lenitive and sedative. It is used internally in the treatment of palpitations, insomnia, nervous disorders and constipation in the elderly. The root bark is used in the treatment of burns and scalds. The stems are used in the treatment of coughs, colds, dysentery, rheumatism and parasitic skin diseases. [6,7]

Other uses :

Timber: Wood close-grained and knotty, heartwood dark brown, sapwood white or cream. The timber is used for gateposts and furniture.

Ornamental: Chinese arbovitae is planted in many gardens especially for its striking pale green foliage with odd-looking

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glaucous cones. Boundary or barrier or support: It tolerates pruning and is therefore used as a hedge plant.

2-MATERIAL AND METHODS

2.1 Collection of plant

Area of Collection:

The leaves were collected from local area of VBSPUniversity, Uttarpradesh

Month of Collection:

The leaves were collected in the month of August by hand picking method.

2.2 Authentication [4]

Herbaria Preparation:

Preparation of herbaria is carried out by certain methods. Other-wise the material collected will be spoiled within a short time. There are some steps:

(i) Selection

The plant specimen identified should be disease free with all parts intact without any injuries or deformities. The plant can be uprooted, root is to be cleaned and washed. Plant twigs having leaves and flowers must be collected in case of flowering plants.

(ii) Pressing

After collection of the plant, they should be pressed immediately in the field condition. Wilting the plant material should be avoided

(iii)Technique of Pressing

Collected plant specimen can be kept in newspaper sheets. News paper sheets are arranged alternately be blotting paper sheet. These paper sheets are pressed. A wooden press or an aluminium press can be used. Spreading of plant material inside the sheets and weight on press are to be done carefully. Standard size of the press measures 32 X 48cm.

(iv) Drying

Blotting paper sheets are changed 2 to 3 times for proper soaking of moisture from the plant materials. Paper changing in the press is done carefully for fifteen to twenty days by observing the condition of material.

(v) Mounting

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Good quality herbarium sheets are used for pasting or fixing materials. Heavy white sheet card boards are good for the mounting. Properly dried materials are fixed on the sheet by glue or gummed cell phone tape.

(vi) Labeling and Identification

Labeling and identification are done after fixing. A label is attached to the right hand corner of the herbarium sheet. The identification information carries locality, botanical name of the plant and vernacular name if any, family, soil, habit, uses, distribution and other entries as it deemed proper. The name of the collector is mentioned last.

(vii) Protection of Prepared Herbaria Sheet

Proper sanitation of storage condition is to be maintained. Mould, fungi, insects also create problem for herbaria sheet. Thoroughly dried and well ventilated warm conditions can save from fungal infection. Otherwise fungicides can be sprayed. Naphthalene balls and moth balls are kept in shelves to provide protection against insect invasion.

2.3 PHYSICAL EVALUATION:[1]

2.3.1 Loss on drying :

Materials :

(a) Powder drug 5 gm.

(b) Glass stoppered shallow weighing bottle

(c) Hot air oven

(d) Desiccators

Procedure :

A glass stoppered shallow weighing bottle was dried and weighed. 5gm of the powdered drug was transferred to the bottle. The bottle covered with stopper and weighed. The sample was then distributed as evenly as possible by gentle sidewise shaking to a depth not exceeding 10mm. The loaded bottle was kept in hot air oven; the stopper was removed and left in the oven. The powdered drug was dried for 30 minute at a temperature of 105ºC. After drying was completed the hot air oven was opened and the bottle was closed promptly, allowed to cool at room temperature in a desiccator before weighing. The bottle and the contents were then weighed. The procedure was continued until constant weight is obtained. [8]

2.3.2 Extractive value:

2.3.2.1 Methanol Soluble Extractive

Materials :

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(a) Powder drug 5 gm.

(b) Stoppered conical flask (250 ml.)

(c) 90% methanol (Bengal Chemical and Pharmaceutical ltd.)

(d) Flat bottom shallow dish.

Method :

5 gm. of the air dried drug was coarsely powdered, taken in a stoppered conical flask and maturated with 100 ml. of ethanol (90%) for 24 hours. It was shaken frequently during the first 6 hours and allowed to stand for 18 hours. There after it was filtered rapidly taking precaution against loss of ethanol and then 25 ml of the filtrate was evaporated in tared flat bottom shallow dish, dried at 105^0C and weighed. The percentage of ethanol soluble extractive was calculated with reference to the air dried drug. [5]

2.3.2.2 Water Soluble Extractive:

Materials :

(a) Powder drug

(b) Stoppered conical flask 250 ml.

(c) Distilled water

(d) Chloroform (Merck)

(e) Flat bottom shallow dish.

Method :

5 gm. of the air dried drug was coarsely powdered, taken in a Stoppard conical flask and macerated with 100 ml. of chloroform water for 24 hours, It was shaken frequently during first 6 hours and allowed to stand for 18 hours. Therefore it was filtered rapidly taking precaution against loss of chloroform water, then 25 ml. of the filtrate was evaporated in tarred flat bottom shallow dish, dried at 105^0C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug. [8]

2.3.2.3 Benzene Soluble Extractive

Materials :

(a) Powder drug

(b) Stoppered conical flask 250 ml.

(c) Benzene (RFCL Limited)

(d) Flat bottom shallow dish.

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Method :

5 gm of air dried drug was coarsely powdered, taken in a stoppered conical flask and macerated with 100 ml. of benzene for 24 hours. It was shaken frequently during first 6 hours and allowed to stand for 18 hour. Thereafter it was filtered rapidly taking precaution against loss of petroleum ether. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottom shallow dish, dried at 105^0C and weighed. The percentage of petroleum ether soluble extractive was calculated with reference to the air dried drug. (Table No.7)

2.3.3 ASH VALUE:[4]

2.7.3.1 Total Ash:

Material :

(a) Powder drug

(b) Silica crucible

(c) Muffle furnace

(d) Ash less filter paper.

Method :

2 gm of air dried drug was weighed accurately in a silica crucible and incinerated at a temperature not exceeding 450^0C, until free from carbon, cooled and weighed. As carbon free ash cannot obtain in this way then the charred mass was exhausted with hot water. The residue was collected on ash less filter paper and the residue was incinerated with filter paper until the ash is white. Then the filtrate was added, evaporated to dryness and incinerated at a temperature not exceeding 450^0C. The percentage of ash with reference to air dried drug was calculated. (Table No.7)

2.3.3.2 Water Soluble Ash

Material :

(a) Ash of 2 gm. Powder drug

(b) 25 ml. distilled water

(c) Silica Crucible

(d) Ash less filter paper

(e) Muffle furnace

(f) Desiccator

Method :

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2 gm air dried drug was weighed accurately in a silica crucible and incinerated at a temperature not exceeding 450^0C. The ash obtained was weighed and boiled for 5 minutes with 25 ml of distilled water and insoluble matter was collected on an ash less filter paper, washed with hot water and incinerated for 15 minutes at a temperature not exceeding 450^0C. The weight of the insoluble matter was subtracted from the weight of the ash; the difference tin weight represents the water soluble ash. The percentage of water soluble ash with reference to the air dried drug was then calculated. [3]

2.3.3.3 Acid Insoluble Ash

Materials :

(a) Ash of 2 gm. Powder drug

(b) 2 M hydrochloric acid (25 ml.)

(c) Ash less filter paper

(d) Silica Crucible

(e) Muffle furnace

(f) Desiccator

Method :

The ash was boiled for 5 minute with 25 ml. of 2 M. hydrochloric acid and the insoluble matter was collected on an ash less filter paper, washed with hot water, incinerated, cooled in a desiccator and weighed. The percentage of acid insoluble ash with reference to the air dried drug was then calculated

2.4 Macroscopy

The Macroscopy of leaves of *platycadus orientalis* shows

- **Colour**- Dark green,
- **Odour**- Characterstic
- **Taste**- Characterstic,
- **Size-** 2 to 3 mm,
- **Shape**- scale-like, deltoid

Other Characterstics-:

- Leaf arrangement: whorled
- Leaf type : simple

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- Leaf margin : entire

- Leaf venation : none, or difficult to see

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2.5 Microscopy

The transverse section of leaves of *platycadus orientalis* shows

- Upper & lower epidermis consist of thick brown coloured layers of which some outer cells were broken..
- Oil glands are present irregularly.
- Xylem fibers are present.
- Starch granules are seen neer the oil glands.

2.6 Powder analysis

2.6.1 Organoleptical Properties:-

Colour - Green

Odour - Characteristic

Taste - Characteristic

2.6.2 Powder Microscopy:-

The Leaf powder was cleared by boiling with 20% chloral hydrate solution. Different slides were prepared by taking –

(1) Powder alone

(2) Powder + Iodine Solution 2%

(3) Powder + Phluroglucinol 2% and Conc. Hydrochloric acid.

and observed under microscope.

Observation :

- Stone cells or sclereids were present
- Prismatic calcium oxalate crystals
- Phloem fibres
- Oil Gland

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- Reticulate and Scalariform vessels
- Secretory cells

2.7-ANALYTICAL STUDY

2.7.1 Chromatographic Study:

Chromatography term is comes from Greek word (from Greek chroma "color" and graphein "to write") and its principles were first discovered by Mikhail Tswett in 1906. Chromatography is an analytical technique employed for the purification and separation of organic and inorganic substances. Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for more advanced use. Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytes in a mixture In this technique, there are two phases, one is mobile phase and other is stationary phase. The separation is based on differential partitioning between the mobile and stationary phases. This technique is useful for the fractionation of complex mixure, separation of closely related compounds, such as isomers and in the isolation of unstable [10,11]

substances.

Thin layer chromatography (TLC)

TLC is an important technique for separation and identification of different class of natural product. This technique allows the separation of different components by the differential migration of solute between two phages, a stationary and mobile phage. The adsorbent (silica) acts as stationary phage and solvent systems were used as mobile phage. Polar compound have less affinity for solute, stick to it and move slowly up as mobile phage moves. The compounds will comparatively move quickly up the plates, so Rf values will be more, mixture of compounds will be separate according to polarities [12,13]

Principles of TLC

TLC is based on the two principle (i) adsorption (ii) partition. In adsorption, One or more compounds are spotted on a thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flows through because of capillary action. The compounds move according to their affinities towards the adsorbent. The component with more affinity towards the stationary phase travels slower. The component with lesser affinity towards the

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stationary phase travels faster. Thus the component are separate on a thin layer chromatographic plate on the affinity of the components towards the stationary phase. Once separation occurs individual components are visualized as spots at respective level of travel on the plate. Their nature is identified by means of suitable detection techniques. For the qualitative results, TLC can also provide a chromatographic measurement known as an Rf value. The Rf value is the "retardation factor" or the "ratio to-front" value expressed as a decimal fraction.

$$\text{The Rf value can be calculated as: } Rf = \frac{\text{Distance travels by solute}}{\text{Distance travels by solvent}}$$

3- RESULTS

3.1 Treatment of powder drug with different Chemical Reagents.

The powder drug on treatment with different chemical reagents show different colour when seen on naked eye (Table No 1).

Table No.1 POWDER ANALYSIS WITH CHEMICAL AGENTS

Reagents	Colour observed
Powder as such	Green
Powder + Concentrated HCL	Amber Green
Powder + Concentrated HNO_3	Yellow
Powder + Concentrated H_2SO_4	Brawnish Black
Powder + Glacial acetic acid	Brawnish Green
Powder + 5% NaOH solution	Yellowish Green

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Powder + 5% KOH solution	Yellowish Green
Powder + 5% Ferric chloride solution	Yellowish Brown
Powder + Picric acid	Yellowish Green
Powder + Ammonia	Light green

3.2 Fluorescence Analysis:-

The fluorescence characteristics of powder drug were studied under U.V. light after treating with different chemical reagents and reported. (Table No. 2)

Table No. 2 FLUORESCENCE ANALYSIS OF POWDERED DRUG

Reagents	Fluorescence Observed
Powder as such	Green
Powder + 1N NaOH in methanol	Light Green
Powder + 1N NaOH in water	Light Green
Powder + 50% Hydrochloric acid	Dark green
Powder + 50% Sulphuric acid	Dark Green
Powder + 50% Nitric acid	Flurance Green
Powder + Petroleum ether	Light green
Powder + Chloroform	Dark Green
Powder + Picric acid	Green
Powder + 5% Ferric chloride solution	Green
Powder + 5% Iodine solution	Light green
Powder + Methanol	Dark green

TABLE NO. 3.3 PHYSICAL EVALUATION PARAMETERS

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Sl. No.	Parameter	Values (%)(w/w)
1.	Loss on Drying	22%
2.	Ash Values	
	A. Total Ash	76.5%
	B. Acid insoluble ash	16.9%
	C. Water soluble ash	82.3%
3.	Extractive Values	
	A. Water soluble extractive	0.80%
	B. Methanol soluble extractive	1.16%
	C. Benzene soluble Extractive	0.40%
4.	Swelling Index	0.28

PHYTOCHEMICAL INVESTIGATION OF *Platycladus orienalis* (L) Franco LEAVES EXTRACTS:

3.4.1 Drying and Pulverization:

The collected plant material (Leaf) was shade dried at room temperature, then they are pulverized in mixer grinder to coarsely powdered drug and passed through mesh size 40 sieve.

3.4.2 Preparation of Extracts by Successive Solvent Extraction:

Materials:

(a) Soxhlet apparatus (JSGW)

(b) Petroleum ether LR. (RFCL Limited)

(c) Benzene LR (RFCL Limited)

(d) Methanol LR (Bengal Chemicals and Pharmaceuticals Limited.)

(e) Triple Distilled water

(f) Shade dried leaves of plant drug

Procedure:

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The leaves were dried in shade and powdered to get a coarse powder. About 500gm of dry coarse powder was extracted with petroleum ether (40-60^{0}C) by continuous hot percolation using soxhlet apparatus. The extraction was continued for 72hours. The petroleum ether extract was filtered and concentrated to a dry mass by using vacuum distillation. A greenish brown waxy residue (15.20gm) obtained. Subsequently the dried powder was extracted with benzene for 72hours. The benzene was then concentrated and a greenish black residue (25.185gm) was obtained. The mark left after benzene extraction was dried and extracted with methanol for 72hours. The methanolic extract then filtered and concentrated by vacuum distillation. A brown colour residue was obtained (42.250gm). The mark left after methanolic extraction was dried and extracted with distilled water by simple maceration. The macerated product was concentrated by vacuum distillation. A brown colour residue was obtained. (35.570gm).

TABLE NO. 3.4 PERCENTAGE OF EXTRACTS

Sl. No.	Extracts	% Yield
1.	Petroleum Ether	3.04
2.	Benzene	5.03
3.	Methanol	8.45
4.	Aqueous	7.11

3.5 CHROMATOGRAPHIC SEPERATION

Thin Layer Chromatographic Seperation (TLC)

Sample: Aqueous and Petroleum extract of Ocimumcanum&Platycladusorientalis

Plates: Precoated TLC Silica gel 60 plates (MERCK)

Activation of Plates: The plates are then activated in hot air oven about 100^o C for 30 minutes. Then they are kept for short periods in desiccators.

Method: (Ascending Development): The plates after spotting of the sample are placed in the chromatography chamber containing solvent at the bottom. The flow of solvent is from bottom to top.

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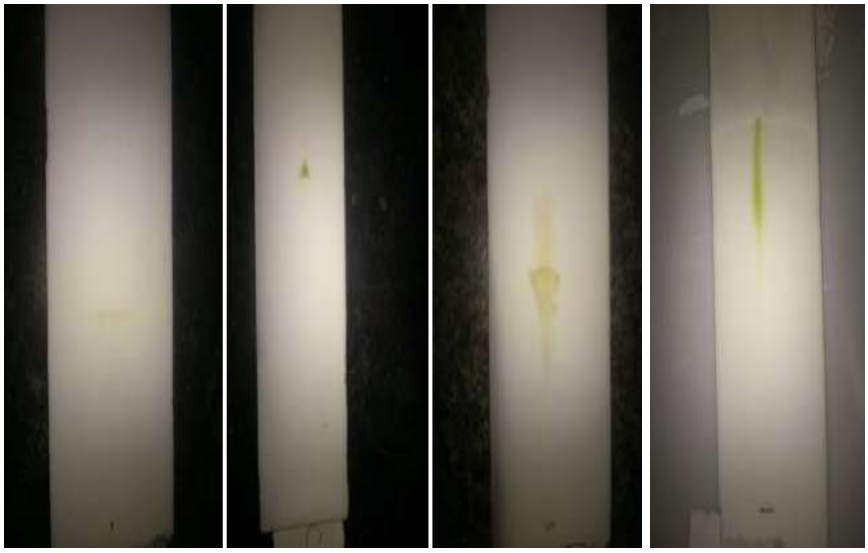
Solvent system: By trial and error method, the solvent system was selected basing on the type of extract to be separated on TLC plate, nature of phytoconstituents present in the extracts as per phytochemical test, different solvents in various proportions were tried keeping in view of their polarity index.



A **B** **C** **D**

TLC profile of different extracts of Platycladusorientalis

SlN.o	Image CODE	Solvent system	Ratio	Plant extract	Isolated compound	RF value
1	**A**	Acetic acid-water-n butanol	10:10:30	PO(AQ)	Phenolic Comp	0.2884
2	**B**	Acetic acid-methanol-chloroform	10:35:65	PO(PE)	Phenolic Comp	0.7764
3	**C**	Ethyl acetate-methanol-water	8:11:8	PO(PE)	Phytosterol	0.7559
4	**D**	Ethyl acetate-	8:11:8	PO(AQ)	Phytosterol	0.6862

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		methanol-water				

Where : PO=Platycladusorientalis , AQ=Aqueous,PE=Petroleum ether

SUMMARY AND CONCLUSION

The study focuses on the treatment of powder drug with different chemical reagents, resulting in different colors and fluorescence characteristics. The powder drug was tested under UV light and found to have various properties.

Physical evaluation parameters included loss on drying, ash values, extractive values, swelling index, and total ash. The collected plant material was shade dried at room temperature, pulverized, and powdered to obtain a coarse powder. Extracts were prepared by successive solvent extraction using a soxhlet apparatus, petroleum ether, benzene, methanol, triple distilled water, and shade dried leaves of the plant drug.

The extracted powder was then concentrated using vacuum distillation, resulting in a greenish brown waxy residue (15.20gm). The dried powder was then extracted with benzene for 72 hours, resulting in a greenish black residue (25.185gm). The mark left after benzene extraction was dried and extracted with methanol for 72 hours, resulting in a brown color residue (42.250gm). The macerated product was concentrated by vacuum distillation, resulting in a brown color residue (35.570gm).

Thin Layer Chromatographic Seperation (TLC) was used to separate the extracts of Ocimumcanum and Platycladusorientalis. The plates were activated in a hot air oven and kept in desiccators for short periods before being placed in the chromatography chamber containing solvent at the bottom. Different solvent systems were selected based on the type of extract to be separated on the TLC plate, the nature of phytoconstituents present in the extracts, and the polarity index.

The TLC profile of different extracts of Platycladusorientalis showed different compounds, such as acetic acid-water-n-butanol, phenolic compound, ethyl acetate-methanol-water, and phenolic compound.

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