Chemical Characterization and Analytical Study of *Ocimum canum* and *Platycladus orientalis* Seeds Extract

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ABSTRACT:
Exhaustive ethno practice studies showed that *Ocimum canum* and *Platycladus orientalis* seeds extract have potent medicinal value. Aqueous and petroleum ether extracts and seeds powder were selected for this research. In this article, physicochemical behaviour, fluorescence analysis and TLC has been studied to elucidate the characterization of the selected plants.
Keywords: Chemical, Characterization, *Ocimum canum*, *Platycladus orientalis*

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INTRODUCTION:
Human and plants have been unavoidably linked from the beginning of time. Living in isolated forest regions, prehistoric man observed and constantly employed the plant material nearby for a variety of purposes in general and the relief of diseases in particular. [1] Man's interest in plants stems from their ability to meet his basic requirements for food, shelter, and remedies. [2] The majority of medications utilized in the past came from plants, which for a long time served as man's sole source of chemistry. [3-4] Due to their potency, lack of contemporary alternatives, and cultural preference, medicinal herbs are used by many people around the world. [5-6] The plant kingdom is an abundant source of organic chemicals that have been used for therapeutic and other related purposes. *Ocimum canum* and *Platycladus orientalis* seeds extract (aqueous and petroleum ether) were characterized through physicochemical behaviour, fluorescence analysis and thin layer chromatography.

MATERIALS AND METHODS:

For research, both medicinal plants' powder was employed. According to Jackson and Snowdown, the premise for this test was the colour variation and flavor.

Measurements were made of the physicochemical parameter's total ash, water soluble ash, and acid-insoluble ash. Under ordinary light, UV light, and other lights, the extractive values and colour of extracts were assessed. (254 nm and 365 nm).

When physical and chemical approaches do not yield satisfactory results, fluorescence analysis is the quick and simple tool for the resolution research of crude pharmaceuticals. On the basis of fluorescence characteristics, the plant material can be distinguished from their adulterants. Different chemical agents were used to treat the leaf powder, and observations under regular and UV light were made. (254 nm and 365 nm).

Total ash:
Place 2-4g of the ground, precisely weighed material in a crucible that has already been lit and tared. (usually of platinum or silica). Spread the material out evenly, then gradually raise the heat to between 500 and 600 °C until

it ignites and turns white, demonstrating the absence of carbon. Weigh after cooling in a desiccator. If this method is unsuccessful in producing carbon-free ash, cool the crucible and wet the leftover material with approximately 2 ml of water or a saturated ammonium nitrate R solution. Dry in a water bath, then put on a hot plate and light an even flame. After 30 minutes of cooling in a suitable desiccator, immediately weigh the residue. Find the total ash content in mg per g of air-dried material.

Acid-insoluble ash:

Add 25 ml of hydrochloric acid (70g/l) TS to the crucible containing the whole ash, cover with a watch glass, and slowly boil for 5 minutes. Add 5 cc of hot water to the watch-glass to rinse it, then pour this liquid into the crucible. Gather any insoluble material using an ashless filter paper, and then wash the paper in hot water until the filtrate is neutral. Transfer the filter paper holding the insoluble material to the original crucible, let it dry on a hotplate, and then light it up until the weight is constant. After 30 minutes of cooling in a suitable desiccator, immediately weigh the residue. Determine how much acid-insoluble ash there is in each gramme of air-dried material.

Water-soluble ash:

Add 25 ml of water to the crucible containing the complete ash, and then boil it for 5 minutes. Collect the insoluble material on an ashless filter paper or in a crucible made of sintered glass. After a thorough hot water wash, ignite for 15 minutes in a crucible at a temperature no higher than 450°C. Subtract the weight of the entire ash from the weight of this residue in mg. Determine the amount of water-soluble ash in mg per g of the material that has been air-dried.

Thin Layer Chromatography (TLC) Techniques:

The TLC techniques were in the following:

Adsorbent materials:

A thin layer of an appropriate adsorbent material was bonded to a suitable plate made of glass, aluminium foil or plastic. Adherence of the adsorbent to the plate is usually assured by mixing a binder/ binding agent such as calcium sulfate with the stationary phase. While selecting an adsorbent, one has to consider the characteristics of the compounds to be separated.

Preparation of TLC plates:

The methods were pouring, dipping, spraying and spreading. The most commonly used procedure for preparing layers was by spread method. The usual thickness of TLC plates for identification was 0.25 mm. Usually a slurry of the adsorbent is prepared in a suitable solvent. For preparing the slurry silica gel G was shaken vigorously with water. After the slurry was applied to the plate, it was necessary to leave the plates undisturbed on the level or until the surfaces become dry. This steps usually takes 10-20 minutes.

Activation of the adsorbent materials:

After making thin layer on the plates, the next work was to remove the liquid associated with the thin layer as completely as possible. This can be done by drying the thin layer plate for 30 mins in air and then in an oven at 110^0C for another 30 mins. The drying process makes the adsorbent layer active.

Sample preparation:

Drugs were frequently applied to thin layer in the form of solution. The solid samples of test materials in which the substances to be analyzed were present in the predominant quantity were dissolved in a suitable solvent.

Sample application:

The solution to be analyzed were applied to the layer by means of different types of micro capillary pipettes (5 to 10 microliters). Sample volumes that can be applied spot wise were 0.5 to 5 microliters on thin layers.

Mobile phase selection:

The choice of mobile phase either as single solvent or a mixture was depended on the compounds to be separated and stationary phase to be used. The solvents were not reacted with the substances to separated. The commonly used organic solvents examples methanol, ethanol, acetone etc.

Development:

Due to non-flexibility of glass plates, ascending development techniques were commonly used in TLC. The plate was placed in the chromatographic chamber at an angle of 45^0. It was important in TLC that the development

chamber was perfectly saturated with the vapors of mobile phase. At the end of the development time, the plate was removed from the tank. Then, solvent front was marked and the plate was dried.

Location procedure:

For the location of the separated components, it was employed physical and chemical detection methods. UV light was main physical method used for the visualization. If the separated fractions don't respond to UV light, suitable reagents known as visualizing agents/spraying reagents were applied.

Calculation of R_f values:

After the location of the separated compounds, the next step was identification. This was done by calculating R_f values of different compounds.

The values of R_f can vary from zero to one. It was qualitative tool and used for identification.

$$R_f = \frac{\text{Distance travelled by the solute from the base line}}{\text{Distance travelled by the solvent front from the base line}}$$

RESULTS:

Table 1. Organoleptic Study:

Parameters	***Ocimum canum (O.C.)***	***Platycladus orientalis (P.O.)***
Color	Yellow to light brown	Brown
Odor	Aromatic	Camphoreous
Taste	Astringent	Slightly Bitter

Physicochemical Behaviour:

Table 2. Evaluation Parameters:

O.C.- Total ash value	16.58 ± 0.46 % w/w
O.C.- Water soluble ash value	9.34 ± 0.38 % w/w
O.C.- Acid insoluble ash value	22.26 ± 0.74 % w/w
P.O.- Total ash value	14.62 ± 0.22 % w/w
P.O.- Water soluble ash value	18.50 ± 0.66 % w/w
P.O.- Acid insoluble ash value	24.76 ± 0.88 % w/w

Table 3. Extractive values and colour of extract under different lights:

Extract	**Color of Extract**		
	Ordinary light	**UV Light 254nm**	**UV Light 365nm**
O.C. Aqueous Extract	Light Brown	Brownish	Blackish Blue
O.C. Petroleum Ether Extract	Yellowish Brown	Greenish Brown	Yellowish
P.O. Aqueous Extract	Brown	Dark Brownish	Brownish Green
P.O. Petroleum Ether Extract	Dark brown	Greenish Black	Dark Green

Table 4. Fluorescence Analysis of Seed Powders:

Taken about 0.5gms of plant powder into clean and dried test tubes. To each tube 5ml of different solvents were added as mentioned below:

Powder (O.C.)	**Color observed under**		
	Ordinary light	**UV Light 254nm**	**UV Light 365nm**
Powder as such	Yellow to light brown color	Greenish Brown	Light green
Powder + 1N Sodium hydroxide in methanol	Brown	Blackish	Whitish grey
Powder + (Nitric acid + Ammonia)	Light yellow	Greenish Brown	Greenish Black
Powder + 1N Sodium hydroxide in water	Brown	Brownish	Greenish Black
Powder + 50% Hydrochloric acid	Light yellow	Brownish	Greenish
Powder + 50% Sulphuric acid	Reddish yellow	Brownish	Greenish Black
Powder + 50% Nitric acid	Light green	Brownish Black	Greenish Black
Powder + Petroleum ether	Whitish grey	Brownish	Reddish yellow
Powder + Chloroform	Green	Blackish Green	Light green
Powder + Picric acid	Pale green	Dark Brownish	Whitish grey
Powder + 5% Ferric chloride solution	Light yellow	Blackish Brown	Brown
Powder + 5% Iodine solution	Fluorescent green	Bluish Green	Light brown
Powder + Methanol	Green	Greenish Black	Black
Powder + Ammonia	Green	Blackish Blue	Orange
Concentrated Hydrochloric acid	Light Green	Dark Brown	No change
Concentrated Sulphuric acid	Green	Dark Green	Light yellow
Concentrated Nitric acid	Orange	Black	Light brown
Glacial acetic acid	No change	Brown	Yellow
5% Sodium hydroxide	Light yellow	Reddish Violet	Yellow

solution			
5% Potassium hydroxide solution	Light brown	Yellowish	Brown
5% Ferric chloride solution	Yellow	Greenish Black	Yellowish brown
Powder (P.O.)	**Color observed under**		
	Ordinary light	**UV Light 254nm**	**UV Light 365nm**
Powder as such	Brown	Brownish	Greenish Black
Powder + 1N Sodium hydroxide in methanol	Greenish Brown	Yellow	Greenish Black
Powder + (Nitric acid + Ammonia)	Greenish Yellow	Greenish Brown	Greenish
Powder + 1N Sodium hydroxide in water	Greenish Yellow	Brownish Blue	Greenish Black
Powder + 50% Hydrochloric acid	Greenish Brown	Reddish Yellow	Greenish Black
Powder + 50% Sulphuric acid	Reddish Violet	Yellowish Green	Greenish Brown
Powder + 50% Nitric acid	Yellowish	Brown	Reddish Violet
Powder + Petroleum ether	Greenish Black	Brownish Yellow	Yellowish
Powder + Chloroform	Brownish Green	Light brown	Orange
Powder + Picric acid	Brown	Yellow	Black
Powder + 5% Ferric chloride solution	Light brown	Blackish Blue	Brownish
Powder + 5% Iodine solution	Black	Dark Brown	Greenish Brown
Powder + Methanol	Orange	Dark Green	Yellowish Green
Powder + Ammonia	No change	No change	Blackish Green
Concentrated Hydrochloric acid	Light yellow	Light yellow	Light Green
Concentrated Sulphuric acid	Light brown	Light brown	Green
Concentrated Nitric acid	Light brown	Yellow	Orange
Glacial acetic acid	Yellow	Yellow	No change
5% Sodium hydroxide solution	Brown	Brown	Light yellow

5% Potassium hydroxide solution	Yellowish brown	Reddish Violet	Light brown
5% Ferric chloride solution	Yellowish brown	Greenish Yellow	Yellow

Chromatographic Seperation:

Thin Layer Chromatographic Seperation (TLC)

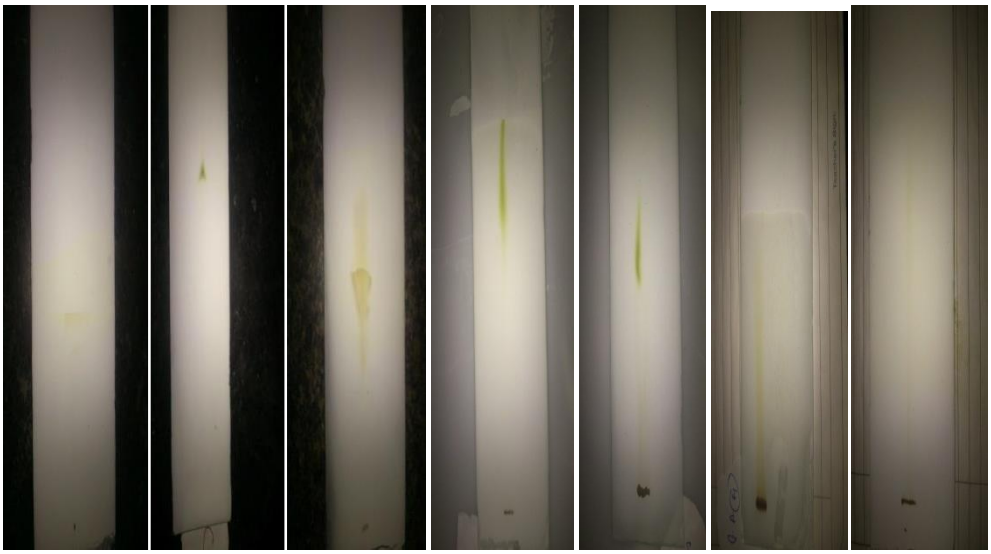
Sample: Aqueous and Petroleum extract of *Ocimum canum* & *Platycladus orientalis*

Plates: Precoated TLC Silica gel 60 plates (MERCK)

Activation of Plates: The plates are then activated in hot air oven about 100° 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.

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) (E) (F) (G)

Figure 1. TLC

Table 5. TLC profile of different extracts of *Ocimum canum* & *Platycladus orientalis*

Sl. No.	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**	Tolune-ethylacetate-formic acid	2.5:1:1	OC(AQ)	Flavonoid	0.5486
3	**C**	Acetic acid-methanol-chloroform	10:35:65	PO(PE)	Phenolic Comp	0.7764
4	**D**	Ethyl acetate-methanol-water	8:11:8	PO(PE)	Phytosterol	0.7559
5	**E**	Ethyl acetate-methanol-water	8:11:8	PO(AQ)	Phytosterol	0.6862
6	**F**	Chloroform-methanol	1:1	OC(PE)	Tri terpenoids	0.7692
7	**G**	Ethyl acetate-methanol-water	8:11:8	OC(PE)	Phytosterol	0.7872

Where: OC= Ocimum canum, PO=Platycladus orientalis, AQ=Aqueous, PE=Petroleum ether

CONCLUSION:

Organoleptic Studies:

Organoleptic testing was done for the assessment of flavor, odor, appearance and mouthfeel of the particular plant sample/s. The organoleptic testing is essential in ensuring sample to comply with the standard references and requirements. These study also helpful for further identification of the drug sample. Organoleptic analysis was also identified the sample's shelf life.

Physicochemical Studies:

The initial phytochemical analyses were conducted to investigate the significant and useful in identifying chemical elements in plant material that may result in their quantitative estimation as well as in identifying the source of pharmacologically active chemical compounds. An essential tool for spotting medicine adulteration or inappropriate management was the physicochemical study of plant pharmaceuticals. Particular attention was paid to the total ash when assessing the quality and purity of medications.

Fluorescence Analysis:

When exposed to UV light, numerous chemical components found in plant material displayed the characteristic of fluorescence. The characteristic of fluorescence was displayed by several chemical components found in the plant material. Some exhibit fluorescence in the visible spectrum during the day. Many natural compounds fluoresce under ultraviolet light but not in a way that can be seen during the day. Despite not being fluorescent, some chemicals were frequently transformed into fluorescent derivatives by utilizing various chemical reagents. As a result, we can frequently evaluate the quality of some crude medications using fluorescence, which is the most crucial factor in pharmacognostical evaluation. For a more thorough identification and characterization of the chosen medicines, the fluorescence analysis was undertaken.

Phytochemical Screening:

According to preliminary phytochemical analysis, *Ocimum canum* petroleum ether extract contains fixed oils, lipids, phytosterols, and triterpenoids whereas *Ocimum canum* aqueous extract contains carbohydrates and flavonoids. Similar to this, the aqueous extract of *Platycladus orientalis* contains carbohydrates, phytosterols, phenolic compounds, etc. The petroleum ether of *Platycladus orientalis* also contains phytosterols, tannins, phenolic compounds, saponins, etc.

Both research institutes and laboratories had a financial interest in the phytochemical study of the medicinal plant for the production of new medications for the treatment of various diseases.

Thin Layer Chromatography:

It was unknown what made plants and plant extracts active. Plant samples were subjected to thin layer chromatography in order to separate the constituent parts. The metabolic status of a plant was also distinguished using thin layer chromatography depending on its location and environmental factors. TLC is also used to verify a sample's purity when a chemical analysis may be done in conjunction with an actual reference. Additional spots on the plate indicate the presence of an impurity.

- *Platycladus orientalis*: Aqueous extract (Phenolic compound and phytosterol were isolated)
- *Platycladus orientalis:* Petroleum Ether extract (Phenolic compound and phytosterol were isolated)
- *Ocimum canum:* Aqueous extract (Flavonoids were isolated)
- *Ocimum canum:* Petroleum Ether extract (Tri terpenoids and Phytosterol were isolated)

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