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# Chemical Characterization of Ocimum Sanctum in Bhilai – Durg Region of Chhattisgarh

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ABSTRACT

**Objective**- Medicinal plants have been widely used throughout the human history. Large number of chemical compounds are found in the plants which is used for certain biological functions and these chemical compounds play a vital role in defending against the pathogenic attack from insects, fungi, viruses etc.
**Method**- Some method which are used in characterization process are Proximate analysis of leaves of Ocimum sanctum (Collection of samples Proximate analysis), Pyrolysis extract, Fourier Transform Infrared Spectroscopy analysis, Determination of Zinc concentration. Thermo gravimetric analysis of Ocimum santum leaves.
**Result & discussion-** The leaves of Ocimum sanctum were subjected to proximate analysis. The alcoholic extractives of Ocimum sanctum leaves results reveal how far they differ in their qualities and it gives a finger print out of the sample purity. Ashes gives us the idea of mineral matter contained in the plant which is responsible for pharmacological effect Higher total ash value shows that it had higher mineral content [9] and higher value of acid insoluble ash shows that it has higher digestibility property when the plant is consumed.



## INTRODUCTION

Medicinal plants act as a great source of economic value all over the people. People are more aware about the adverse effects of chemical drugs, questioning and different assumptions worked out for allopathic medicines and their high costs. People show more interest and faith on the use of medicinal plants as it is affordable, safe and efficient with no side effects. So the recent years have witnessed the tremendous increase in the interest of multinational pharmaceutical companies and the domestic manufacturer of herbal medicines. Though ample information and literature is available on the various uses of the herbal medicines but scientific documentation on proximate composition and the chemical constituents is very scarce. Of course every part of the medicinal plant has constituents which are found to be very effective and so it is responsible for the pharmacological activity and also help in the development of medicines and industrial by products. Medicinal plants are rich in the active constituents which is active in pharmacological activity. Chhattisgarh state is significantly rich in diverse types of plants having medicinal importance and so it is declared as Herbal state in July 2001.[10,11,17]

Ocimum sanctum is also known as queen of herbs shown in figure-1. It belongs to family Lamiaceae. It is a shrub which is erect

and branched, 30-60 cm tall having hairy stems with green or purple leaves which have petioles and an ovate up to 5cm long; flowers are purple in colour in close whorls[1]. It is cultivated for medicinal and religious purpose. In Hindu mythology, this shrub is closely related with Goddess figure and is worshiped by Hindus. It is mentioned by Charaka in the Charaka Samhita, Ocimum sanctum is helpful for adapting to stress.

All parts of the plant Ocimum sanctum is very useful in the treatment of diseases like bronchitis, dysentery, arthritis, skin diseases, eye disease, insect bites, malaria, diarrhea etc. Ocimum sanctum have significant anti stress properties, antipyretic, carminative, diaphoretic, expectorant, vermifugal etc. Oil extracted from the leaves is used as pest repellent, antibacterial and insecticide[2]. It also possesses the properties of anti fertility, analgesic, antispasmodic, antifungal and antdiabetic. Decoction of leaves is used for gastritis and hepatic disorders. It lowers the level of uric acid levels and used as anti-inflammatory agent. Chewing of leaves of Ocimum sanctum before meals improves the appetite. It also reduces the chance of ulcers. It also removes the excess cough from lungs and nasal passage.

## METHODS & METHODOLOGY

### Proximate analysis of leaves of Ocimum sanctum[3-8]

### Collection of samples

The studies were undertaken on medicinal plants Ocimum sanctum of family Lamiaceae. The choice of plant parts were leaves of Ocimum sanctum which was collected from Chhattisgarh and was taxonomically authenticated. A care was taken to select healthy plants and the plant parts for the study were collected fresh and dried for a week to be involved in the proximate analysis.

### Proximate analysis[3-8]

### Extractive values

About 5g of the dried and finely coursed powder is mixed with 100 ml of 90 % ethanol in a closed flask. The flask was frequently shaken during the first 6 hours and allowed to stand for 18 hrs. Then the mixture was rapidly filtered to minimize the loss of ethanol and 25 ml of the filterate was evaporated to dryness in a tarred flat bottomed shallow dish. The residue was dried at $105^0$C for minutes and then weighed. The procedure was performed twice more from the filtrate.

### Ash values[7]

### Total ash and sulphated ash values

A silica crucible was heated for about 30 min to red hot and cooled in a desiccator to note down its weight. About 3 g of the powdered sample were weighed and then dried at 100-105$^o$C for 1 hr and ignited to constant weight in a muffle furnace at 600-$625^0$C until a carbon free ash is formed. The crucible was allowed to cool in a desiccator after each ignition and care was taken to avoid catching fire. The weight of the carbon free ash was determined. The procedure was repeated to obtain a standard deviation to ensure consistency and then tabulated.

The same procedure was carried out adding dilute sulphuric acid to determine the yield of sulphated ash.

### Acid Insoluble ash

About 1g of the total ash (from total ash) was boiled with 25 ml of 2M hydrochloric acid for 5 min. The acid insoluble was separated by filtration on an ash less filter paper in Gooch crucible .The content on the ash less filter paper was washed with hot water and ignited and then weighed to obtain the percentage of ash with reference to the air dried samples.

### Water soluble ash

About 1g of the total ash was boiled with 25 ml of water for 5 min and then filtrated to retain the insoluble matter on ash less filter paper. The content was ignited for 15 min at a temperature not exceeding $450^0$C then weighed. The difference between the amount of ash subjected and weight of insoluble ash was accounted as the water soluble ash value.

### Loss on drying

About 10 g of each specimen under study were accurately weighed and transferred to a charred china dish which was known for its

weight and kept in a hot oven at 100-105$^0$C for an hour. Then the sample was weighed along with china dish to deduct the actual weight of tarred china dish. The weight of the powder was noted to calculate the percentage loss on drying with reference to air dried specimen.

### Pyrolysis extract of Ocimum sanctum

#### Experimental set up and procedure for pyrolysis

The experimental set up consist of a heating chamber in which the dried leaves of Ocimum sanctum are placed separately turn by turn and that is then closed very tightly so as to avoid any leakage of gas as the result of pyrolysis.

A pressure cooker of 3 liter capacity is used as a heating chamber as Aluminium used here is one of the better conductors of heat. The heating is done by 1000 W Ni- chrome heating coils which are attached at the periphery of pressure cooker and heating is done to 600$^0$C. The entire arrangement is packed inside an Aluminum vessel with a lining of asbestos fibers which help in insulation and do not permit the loss of heat. A screw fitting is given at top of cooker to make it air tight and for the exit of fuming gases there is one suitable passage at the top of the cooker. Also a temperature sensor is provided which continuously monitors the temperature inside the vessel. The outlet of the reactor is connected to a condenser which condenses the gases coming of the outlet. Just at the other end of the condenser a measuring cylinder is placed where the gases being condensed is collected.

The dried leaves are fed in to the heating chamber and it is closed very tightly using screw and bolt. Once the heating is started and after reaching a suitable temperature the reaction begins and the vapours that are released comes out of the reactor outlet which is connected to the condenser where the vapours are condensed and collected in the measuring cylinder Most of the non condensable vapours are simply released. The product mainly consists of pyrolytic oil and water which then is separated based on density difference.

#### Fourier Transform Infrared Spectroscopy analysis of Ocimum sanctum

FTIR spectrum helps to identify the functional group present in Ocimum sanctum. FTIR spectra are recorded in KBr by sophisticated computer controlled FTIR Perkin Elmer spectrometer with He- Ne Laser as reference. The pyrolysis extract of sample Ocimum sanctum were scanned at room temperature and a spectral range of 4000-400 cm$^{-1}$.

#### Determination of Zinc concentration in Ocimum sanctum leaves

Analysis of Zinc concentration in the pyrolysed extract of Ocimum sanctum leaves were determined by Atomic Absorption Spectrophotometer Perkin Elmer 2380 model using suitable hollow cathode lamps.

#### Thermogravimetric analysis of Ocimum santum leaves

TGA were performed with TGA 4000, Pyris 6 TGA .Weight of Ocimum sanctum leaves taken were 6.910mg. These were loaded separately on quartz pan and mounted in instrument. Initial conditions of temperature were 30$^0$C and switch the gas to $N_2$ at 20 ml/ min. Temperature programming were heating rate from 30$^0$C to 400$^0$C at 10$^0$C in nitrogen and hold for 1 min at 30$^0$C.

## RESULT & DISCUSSION

The leaves of Ocimum sanctum were subjected to proximate analysis and results were as mentioned in **Table-1**. The alcoholic extractives of Ocimum sanctum leaves were 4.04% w/w and colour of the residue were light brown. The results reveal how far they differ in their qualities and it gives a finger print out of the sample purity. Ashes gives us the idea of mineral matter contained in the plant which is responsible for pharmacological effect Higher total ash value shows that it had higher mineral content [9] and higher value of acid insoluble ash shows that it

has higher digestibility property when the plant is consumed.

FTIR spectrum confirmed the presence of functional group in the leaves of Ocimum sanctum. The more intense band occurring at 3398.30 $cm^{-1}$, 2926.66 $cm^{-1}$, 2357.38 $cm^{-1}$, 1646.82 $cm^{-1}$, 1033.76 $cm^{-1}$ and 778.31 $cm^{-1}$ corresponds to O-H/ N-H/ C-H/C=O stretching, bending, vibrations respectively indicates the presence of alcohol, amines, amides, amino acids, meta substituted compounds in the leaves of Ocimum sanctum[10] as mentioned in F**igure 3.**

The very strong absorption band observed around 3373-3422 $cm^{-1}$ may be due to the presence of bonded N-H/C-H/O-H stretching of amines and amides[11]. The very strong absorption band observed in 1600-1660 $cm^{-1}$ region indicates the presence of amino acids. The strong absorption band observed between 3200-3400 $cm^{-1}$ indicates the presence of polymeric hydroxyl derivatives. Vibration of N-H shows the presence of primary amine[12]. The band observed at near 2848 cm-1 represent C-H symmetric stretching of methylene group in aliphatic compounds[13]. C=C stretching region falls with the range 1511-1561 $cm^{-1}$. Similarly the Chelated C=O stretching vibrations lie towards the lower wave number side that is within the range 1621-1635 $cm^{-1}$ [14].

This helps to elucidate the chemical structure and effort was taken to understand the significance of functional groups as bio active constituents for the treatment of various diseases. The active constituent present in Ocimum sanctum is Eugenol. The chemical name is 1-hydroxy-2-methoxy-4-allyl benzene. % of Eugenol contained in Ocimum sanctum is 0.27%.

Carboxylic acid present in the plants act as a main pharmaceutical agent in treatment of diseases like ulcers, jaundice, headache, stomatitis, fever, edema and rheumatic joint pains. Amine, amides and amino acids are the main group of protein synthesis.

Zinc concentration in Ocimum sanctum leaves were determined by Atomic Absorption Spectrophotometer and it is recorded and mentioned in table-2 .The study reveals that there was high concentration of Zinc in the leaves of Ocimum sanctum. Zinc plays an important role in enzyme catalysis and maintaining the structure[15]. It activates 300 enzymes and it is an important trace mineral for DNA synthesis, cell division and protein synthesis[16]. Zinc being a strong and effective antioxidant, it also improves the immune system and helps in brain development[17]. It also has anti ulcer activity, wound healing power, anti inflammatory effect etc.

TGA curve is helpful for chemical characterization of the medicinal sample taken and is helpful for pharmaceutical applications. TGA curve of leaves of Ocimum sanctum is depicted in figure-4. The study reveals that the initial degradation at $100^0$C is due to the loss of moisture and further increase in temperature releases carbon dioxide gas from the sample. Maximum degradation took place between 200-$400^0$C[18]. There are two types of weight loss. Weight loss in the first phase is due to loss of moisture and then loss of weight in the second phase is due to the decomposition of polysaccharides[19]. Highest initial degradation temperature is observed in the leaves of Ocimum sanctum so this reveals that this sample has less activation energy and they degrade fast to yield the residue at very high temperature. It has minimum initial temperature of sample decomposition which proves its thermal stability and this supports its role and activity in therapeutics and preparation of medicines[20].

## CONCLUSION

The review paper on chemical characterization of Ocimum sanctum leaves reveals the proximate analysis, structure of the sample, thermal stability, zinc concentration and its therapeutic action, functional groups, chemical constituents, elucidate the chemical structure, to understand the significance of functional group as it act as bio active constituents. The techniques employed can be applied in future as a valid method for the authentication of herbal medicines.

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**Table 1.** Proximate analysis on leaves of Ocimum sanctum

| S.No. | Experimental studies | Tulsi leaves %w/w |
|---|---|---|
| 1. | Total ash value | 15.21 |
| 2. | Water soluble ash | 46.72 |
| 3. | Acid insoluble ash | 17.6 |
| 4. | Sulphated ash | 15.6 |
| 5. | Loss on drying | 16.26 |

**Table 2.** Concentration of zinc in sample of Ocimum sanctum

| S.No. | Sample used | Concentration of Zinc(mg/kg) |
|---|---|---|
| 1. | Ocimum sanctum leaves | *20.0* |

**Fig. 1.** Ocimum Sanctum

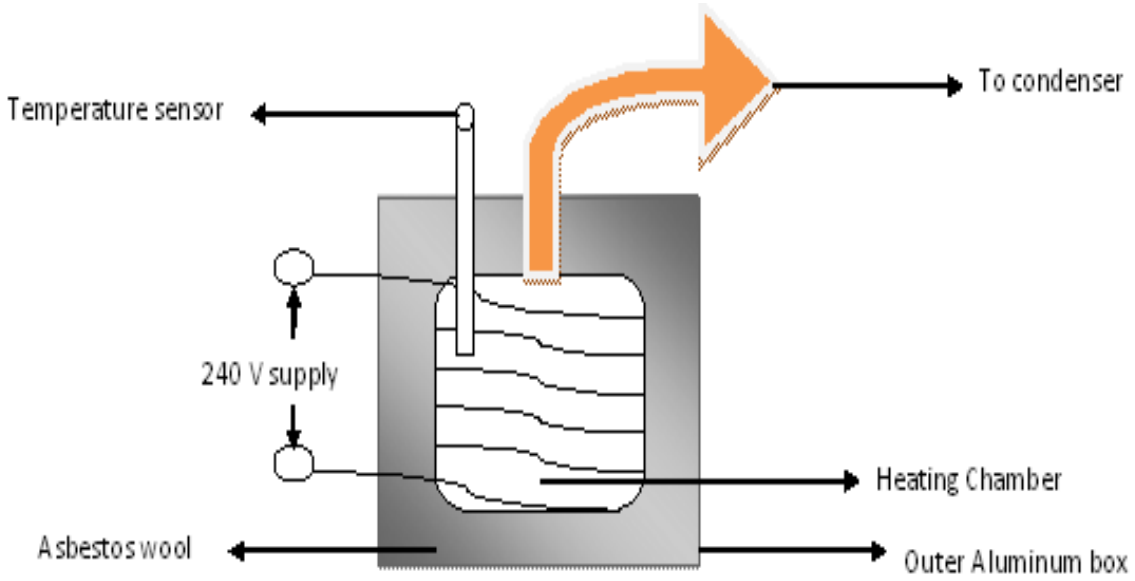


**Fig. 2.** Pyrolysis reactor

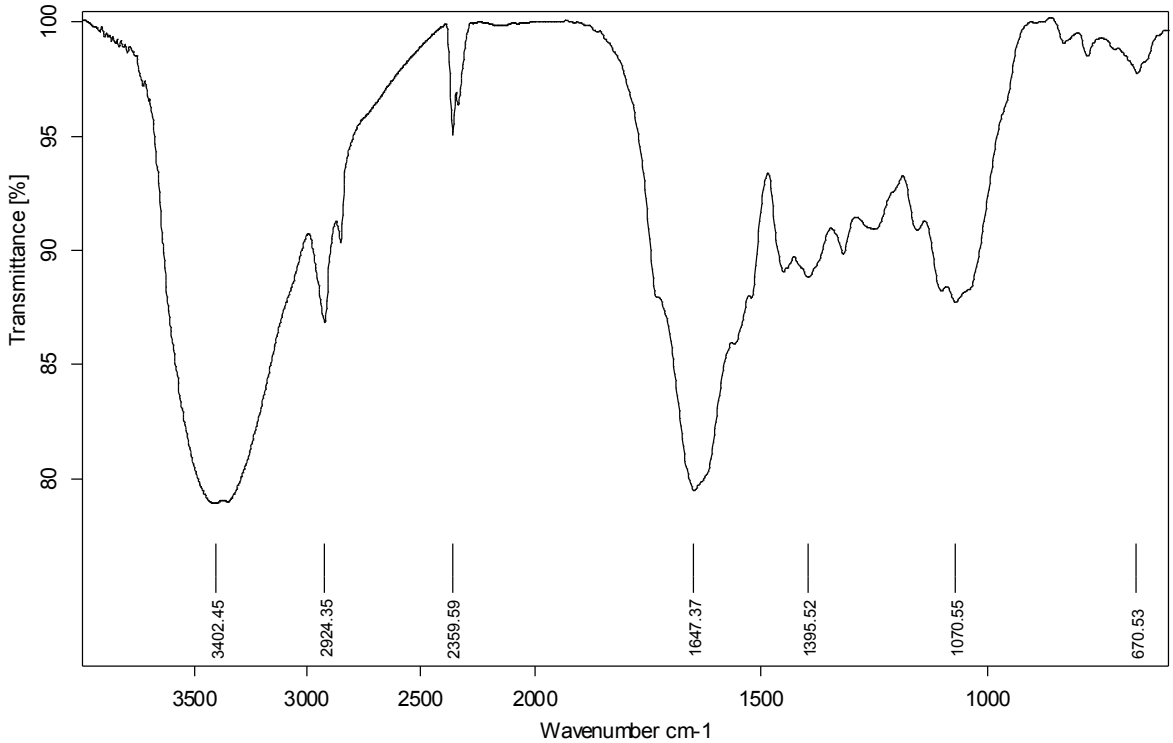


**Fig. 3.** FTIR Spectra of leaves of Ocimum santum

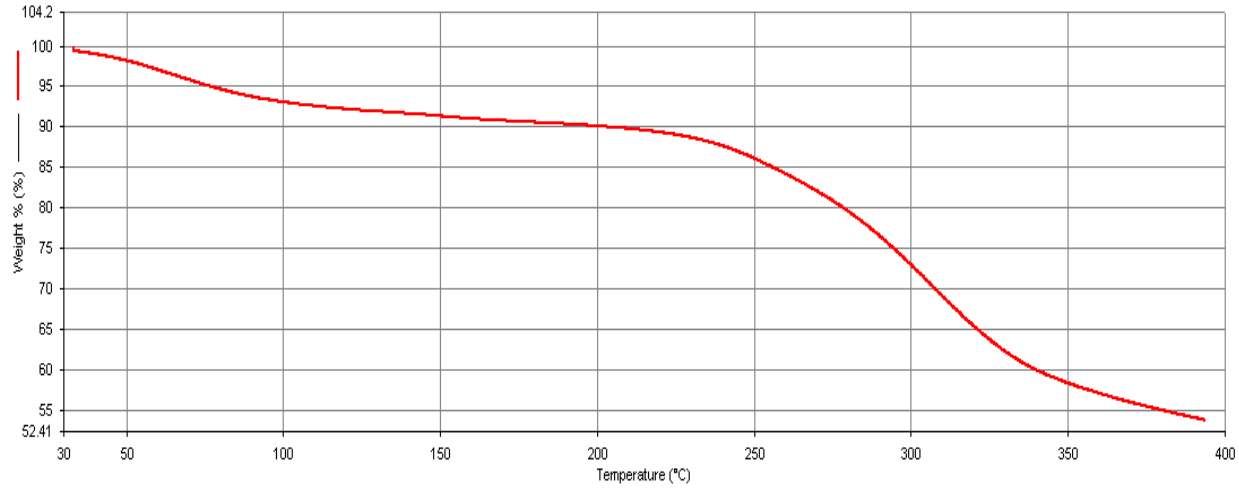


**Fig. 4.** Thermo gravimetric analyses of Ocimum sanctum leaves