



## **Phytochemical Analysis of Different Parts obtained from *Ocimum sanctum* Linn.**

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### **ABSTRACT**

*Ocimum sanctum* L. which is also known as holy basil and one of the traditionally important plant in India. The present study investigates the qualitative and quantitative analysis of the major bioactive constituents of the plant in some solvents (chloroform, ethanol and methanol). Plant parts like leaves, stem and roots were collected from local region of Uttarakhand. Qualitative phytochemicals such as alkaloids, flavanoids, glycosides, carbohydrates, terpenoids, proteins and tannins were detected. Qualitative phytochemical i.e., alkaloids, glycosides saponins, flavonoids and tannin were found higher concentration in the extract. The obtained data from *Ocimum sanctum* L. confirmed its wide application for therapeutic purpose in alternative therapy.

**Key words:** *Ocimum sanctum* L., Qualitative phytochemicals, Secondary metabolites, Flavonoids.

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### **INTRODUCTION**

Plants have been used by the human being since start of civilization for variety of purposes like feeding, clothing, sheltering, hunting etc [37]. Apart from this according to WHO, 80% of world population lives in rural areas rely on folk medicines which are the part of their traditional system and knowledge related to them is transferred from generation to generations or by written literature [23]. Medicinal use of different herbal preparations is already mentioned in various ancient Hindu literatures like Ayurveda, which is totally based on harnessing therapeutic potential of different herbs [29] yet none of these has a status comparable to "Tulsi" or holy basil because it became an important part of various Hindu religious traditions.

Tulsi (*Ocimum sanctum*), possesses potential therapeutic characters and use to treat many diseases like bronchial asthma, chronic fever, colds cough, malaria, dysentery, convulsions, diarrhea, arthritis, emetic syndrome, skin diseases, insect bites etc. and also used in the treatment of gastric, hepatic, cardiovascular and immunological disorders etc. It also heals various clinical conditions like eye disorders (glaucoma, cataract and chronic conjunctivitis), catalepsy, snake and scorpion bites etc [35].

*O. sanctum* is rich in many nutrients and biologically active compounds which may fortify its nutritional and pharmacological properties present in its natural form. *O. sanctum* is a source of Monoterpenes and Sesquiterpenes like neral, camphene, cholesterol and stigma steroid. The whole plant contains variety of biological compounds including flavonoids, triterpenoids, tannins, saponins etc. [33]. The whole plant of *O. sanctum* contains compounds like cirsilineol, cirsimaritin, isothymusin, rosmarinic acid, apigenin and eugenol [18]. Parts of whole plant contains different constituents in different amounts like leaves contain high content of essential oil includes octane benzene, toluene, camphene, limocene, ledol etc. Tulsi do not contain caffeine or other stimulants which increase physical endurance, the odour of *O. sanctum* is aromatic due to presence of volatile oil in leaf. The oil contains phenols, aldehydes, terpenes etc. [24].

Tulsi shows different activity against bacterial, viral and fungal components as the aqueous fraction and methanolic fraction of *O. sanctum* shows antifungal activity against several dermatophytic fungus [10]. Leaves of Tulsi help to prevent and reduce physical and mental stress because of having powerful adaptogenic property [4]. Tulsi have some antibacterial properties as it is a powerful herb used by humans from old times having properties of aqueous alcoholic, chloroform extract and oil obtained from leaves [22]. Benzene extract of *O. sanctum* leaves treated with albino rats decreased the total sperm count and sperm motility results androgen deprivation due to anti androgenic property of *O. sanctum*. Sperm testosterone level increased whereas level of FSH and LH sperm count were reduced in rabbits [1].

Many antibiotics are developed for the treatment of bacterial infections. Scientists search for antimicrobial substances from various sources such as medicinal plants, one of them is "Tulsi" having diverse uses and one of the holiest and commonly used medicinal plant many of them used in fungal infections and fresh juice used treatment of several diseases like bronchitis and skin diseases [28].

Hence there is need for discovery of new safer and effective drug. Medicinal herbs could be alternate source for treatment of these pathogens. Some essential oil of *O. sanctum* demonstrated highly active antifungal agents against dermatophyte. The major active substances in this oil are Eugenol and its derivatives [2]. Investigation was undertaken to screen whole part extract of aromatic *O. sanctum* for their antibacterial activity against bacteria and whole part of Tulsi.

## **MATERIAL AND METHODS**

### **Reagents and chemicals**

Petroleum ether, chloroform, ethyl acetate, ethanol, Solvents and chemicals used were purchased from Merck and Sigma-Aldrich., anhydrous sodium carbonate, aluminiumtrichloride (AlCl<sub>3</sub>), sodium nitrite, sodium chloride potassium acetate, ferric chloride, butylated hydroxytoluene (BHT), diethyl ether, ammonia solution, acetone, ethanol, hydrochloric acid, sodium hydroxide, phosphate buffer, potassium ferricyanide, ammonium molybdate, sodium phosphate, trichloroacetic acid, glacial acetic acid and sodium nitroprusside. All the chemicals used in this study were of analytical grade.

### **Procurement of plant material**

The whole plant of *O. sanctum* (Tulsi) was used for the experimental purpose in present work. The collected plant was identified according to various literatures [17].

### **Preparation of plant material**

Leaves, seeds and roots will be washed with distilled water to remove any dirt particles. The washed parts will be shade - dried for 10-12 days and will be converted into coarse powder by grinder and then stored at room temperature for further processing [17].

### **Extraction of metabolite from *O. sanctum***

The extraction will be done using Soxhlet method. In this method powdered plant material will be subjected to successive extraction with Chloroform, Ethanol, Methanol [16]. The obtained extracts will be filtered out, distilled off on water bath and stored under refrigeration for future processing.

### **Identification tests for active compounds**

Standard protocols will be followed to check the presence of principal components like alkaloids, glycosides, terpenoids and steroids, flavonoids, reducing sugar and tannin.

#### **Test of Alkaloids**

5 mg extract of *Ocimum Sanctum* (Tulsi) will transferred in the test tube and then 1% hydrochloric acid will be added following gentle heated. Red colour indicate the presence of alkaloids [9].

#### **Test for Flavonoids**

Plant extract will be treated with 1.5 ml of 50% methanol solution and warmed with metal magnesium followed by adding 5 - 6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and Orange color for flavones [32].

#### **Test for Terpenoids**

Plant extract will be treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid [32].

#### **Glycosides**

50mg of the extract was dissolves in concentrated HCl and hydrolysed for 2 hours on a water bath. 2ml of the filtrate was mixed with the 3ml of the chloroform in a test tube and shaken well. When the chloroform layer was separates out, 10% ammonia solution was added to it. Glycosides were indicated by the formation of the pink colour.

#### **Phenolic Compounds**

50mg of the dried extract was dissolved in the 5ml of the water. To this add 5% neutral ferric chloride solution slowly. A dark green colour indicates the presence of the phenolics.

#### **Amino Acid**

100mg of the extract was dissolved in 10ml of distilled water and filtered through Whatmann filter paper number 44. 2ml of the aqueous filtrate was treated with two drops of the ninhydrin solution. Generation of the purple colour means that the test is positive.

#### **Proteins**

A solution of the extract was prepared by dissolving 100mg of the extract in 10ml of the water. Filter this solution by using Whatmann filter Paper No. 1. In a test tube containing 2ml of the filtrate, add 1drop of the 2% copper sulphate solution. Now add 1ml of 95% ethanol followed by the addition of the excess KOH pellets. Pink or violet colour in the ethanolic layer formed.

### Carbohydrates

100 mg of the extract was treated with 5 ml of the double distilled water and filtered through Whatmann filter paper number 1. Two drops of the alcoholic solution of the  $\alpha$ -naphthol was added to 2 ml of the filtrate and shaken well. After this 1 ml of the concentrated sulphuric acid was added from the side of the test tube. Generation of the purple to violet ring at the junction implies the test to be positive.

### Saponin

50mg of the extract was mixed with the 20ml of the distilled water in a graduated cylinder. Shake this cylinder for 15 min. appearance of the 2cm layer foam indicates the presence of the saponins.

## RESULTS AND DISCUSSION

Medicinal plants constitute an effective source of both traditional and modern medicines, now 80% of rural population depends on medicinal plants as primary healthcare [20-26]. The term phytochemical is used for the plant chemicals with the varied structure and function collectively. Phytochemical may serve for the different kinds of functions for the protection and reproduction such as colour and odour for the protection and insect attraction for the pollination, hormonal function for growth and signaling, antifeedants and toxins for insects protection, allelochemicals for defence against herbivory and phytoalexins for pathogen defense [14, 31-35]. Use of the medicinal plant include fresh or dried part, whole, chopped, powdered or an advance form of the plant typically made through extraction using the different solvents in most of the traditional system of the medicine. The term phytomedicines usually refer to the medicines derived from the different parts of the plants including seeds, berries, leaves, bark, roots and flowers. Although there are no apparent morphological characteristics in medicinal plants growing with them, yet they possess some brilliant qualities or virtue that makes them medicinally important [11, 2 25, 3].

### Percentage yield of different extracts of *Ocimum sanctum*

After the extraction process, the crude plant extracts from leave, stem and seeds was obtained for the study of phytochemicals. To obtain an actual yield of extraction, different plant extracts with solvent were kept on a vacuum evaporator for complete vaporization of solvent and the extract was totally concentrated. Different types of a solvent such as chloroform, ethanol and methanol were used for the process of the yield of extraction and to evaluate the standard extraction efficiency for a particular plant or different parts of plants. The percentage yield is a very important phenomenon in the extraction of phytochemicals analysis. Results of the percentage yield of different extracts of *Ocimum sanctum* is given in table 1.

**Table 1: Extractive yield in % of different extract of *O.sanctum***

Extracts	Yield % (w/w)		
	Leaves	Stem	Seeds
Chloroform	2.2	2.9	4.4
Ethanol	4.1	4.5	5.2
Methanol	3.6	3.8	4.9

In the case of *Ocimum sanctum* (Table 1), the percentage yield of chloroform leaves, stem and seed extracts is 2.2%, 2.9% and 4.4% respectively, percentage yield of methanol leaves, stem and seed extracts is 3.6%, 3.8% and 4.9%, respectively and percentage yield of ethanol leaves, stem and seed extracts is 4.1%, 4.5% and 5.2%, respectively. According to the result of percentage yield, it is clear that ethanol was an excellent solvent for the extraction of *Ocimum sanctum*. The yielding of *Ocimum sanctum* using ethanol and methanol solvents was higher than chloroform solvent. *Ocimum sanctum* exhibited higher yield in ethanolic seed extract (5.2%), followed by ethanolic stem extract (4.5%) and ethanolic leaves extract (4.1%) than methanolic seed extract (4.9%) followed by methanolic stem extract (3.8%) followed by methanolic leaves extract (3.6%). While chloroform extracts exhibited minimum yield than methanol and ethanol extracts. Hence, we can use ethanol and methanol solvent for further studies.

### Qualitative Phytochemical Analysis of extracts of *O. sanctum*

A small amount of crude solvent extracts of *Ocimum sanctum* was taken to test for alkaloids, glycosides, saponins, flavonoids, phenol, proteins and carbohydrates separately for each extract of all samples. These phytochemical tests were performed by using Kokate [20] methods. In this method, the little amount of each extracts of both plants is suspended into sterile distilled water and to make the concentration of 1mg/ml. The results of phytochemicals are mentioned separately in the table 2.

Preliminary phytochemical screening of the leaves, stem and seed extracts of *Ocimum sanctum* has shown the presence of the various phytochemicals *viz.*, alkaloids, flavonoids, diterpenes, saponins, proteins, amino acids and sugars in different solvent extracts. The most common and largest classes of plant metabolites are phenolic compounds that have various chemical properties *viz.*, anti-inflammation, antiaging, anticarcinogenic, improvement of endothelial function, cell proliferation activities, cardiovascular protection, inhibition of angiogenesis [13]. *Ocimum sanctum* is potential medicinal plants due to availability and variation

of phytochemicals [38]. From the previous result, it can be seen that successfully phytochemical extraction depends on the solvent type used during the extraction process [26].

In present work, three different types of solvents (chloroform, ethanol and methanol) were utilized to obtain leaves, stem and seed extracts and used for qualitative phytochemical analysis using standard chemical tests. The methanolic and ethanolic extracts have shown the occurrence of mostly phytochemical components compared to other solvents. It is observed from the table that flavonoid component was positively proved by methanolic extracts of leaves, stem and seed of *Ocimum sanctum* and ethanolic extracts of stem and seed of *Ocimum sanctum*. The methanolic and ethanolic leaves, stem and seed extracts of *Ocimum sanctum* revealed positive results for saponins. Diterpenes exhibited positive results only in the stems of *Ocimum sanctum* in all extracts (chloroform, ethanol, methanol). Proteins and amino acids exhibited positive results on the methanolic leaves and stem extracts of *Ocimum sanctum* and ethanolic extracts of only leaves of *Ocimum sanctum*. Only seed extracts of *Ocimum sanctum* proved the occurrence of Glycosides. All the phytochemical tested in this study is exhibited negative results on chloroform leaf and stem extracts of *Ocimum sanctum* except only diterpenes. From the above results, it is clear that the *Ocimum sanctum* revealed the presence of diterpenes, saponins, flavonoids, amino acid, proteins and carbohydrates. These phytochemicals were present in all the plant parts however, the chloroform extract not proved the presence of more phytochemicals in this research.

**Table 2: Qualitative phytochemical screening of *O. sanctum***

Phytoconstituents	Chloroform			Methanolic			Ethanolic		
	Leaves	Stem	Seed	Leaves	Stem	Seed	Leaves	Stem	Seed
Alkaloids	-	-	+	-	-	+	-	+	+
Glycosides	-	-	+	-	-	+	-	-	+
Flavonoids	-	-	+	+	+	+	-	+	+
Phenolics	-	-	+	-	-	+	-	-	+
Amino acids	-	-	+	+	+	-	+	-	-
Carbohydrates	-	-	-	+	+	+	+	-	+
Proteins	-	-	-	+	+	-	+	-	-
Saponins	-	-	+	+	+	+	+	+	+
Diterpenes	-	+	-	-	+	-	-	+	-
Terpenoids	-	+	-	-	+	-	-	+	-

Phytochemicals have physiological activity and medicinal properties and it give rise to discovery of drug and their development [36]. There are some important bioactive phytochemicals present in medicinal plants such as Alkaloids, flavonoids, phenols, diterpenes, carbohydrates, proteins, glycosides and essential oils [8]. Flavonoids, glycosides, alkaloids, proteins, tannins, and phenols are major phytochemicals presented in *Ocimum sanctum* as secondary metabolites [27, 28].

Like the previous report, we also concluded that *Ocimum sanctum* has antioxidant nature due to the increased flavanoids component. Hence, further study of these phytochemicals can be done by quantitation of these phytochemicals. Thus, antioxidant-rich leaf, stem and seed extracts of *Ocimum sanctum* serve as a reservoir of nutraceuticals that increase the oxidative stress and helps in inhibition and deduction of the degenerative disease with subsequent health benefits [19].

Hence, variety of solvents (chloroform, methanol, ethanol) extracts could be seen as a virtuous source of high phytochemical compounds. Thus, there is beneficial to use these three solvents for qualitative analysis of plants [7, 8].

#### **Qualitative Phytochemical Analysis of *O. sanctum***

Phytochemicals present in all medicinal plants can be categorized as primary and secondary metabolites. Primary metabolites consist of sugar, proteins, lipids, amino acids etc., while secondary phytochemicals as alkaloids, glycosides, flavonoids, glycosides etc. They are mostly used in agriculture, human therapy, scientific research and several other areas [12]. It is desirable to the knowledge of the chemical constituents of plants because this evidence will be helpful for synthesizing complex chemical substances [39]. During the ongoing study, the phytochemicals were evaluated in collected plant species and only flavonoids, saponins and diterpenes present in some extract as secondary metabolites. Flavonoids are the only component, which is present in approximately all parts extracts of *Ocimum sanctum*. Hence, further estimation of flavonoids done by Total flavonoids content tests (TFC).

#### **Determination of total flavonoid content (TFC) test**

For the valuation of TFC firstly prepared the calibration curve of Quercetin (flavonoid). Quercetin was taken as standard in different concentrations ( $\mu\text{g/ml}$ ) and measured the absorbance of quercetin at 420nm.

According to this calibration curve, the flavonoid content of the leaves and stem were determined by using the following equation:  $Y = 0.040 X + 0.009$ ,  $R^2 = 0.999$ , where X is the absorbance and Y is the Quercetin equivalent (QE) (Table 3).

**Table 3: Preparation of calibration curve of Quercetin**

S. No.	Concentration ( $\mu\text{g/ml}$ )	Absorbance (mean) $\lambda_{\text{max}} = 420\text{nm}$
0	0	0
1	5	0.230
2	10	0.435
3	15	0.615
4	20	0.805
5	25	1.022

Methanolic and ethanolic extracts of studied plants are used for total flavonoids content test because of the presence of flavonoids in these extracts as secondary metabolites. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve:  $Y = 0.040 X + 0.009$ ,  $R^2 = 0.999$ , where X is the absorbance and Y is the Quercetin equivalent (QE) (Table 4)

**Table 4: Total flavonoid content of *Ocimum sanctum* extract**

Estimation	Methanolic extract			Ethanolic extract		
	Leaves	Stem	Seed	Leaves	Stem	Seed
Absorbance	1.901	0.730	1.987	-	1.117	2.10
Total Flavonoids (mg/100mg)	4.69	1.82	4.85	-	2.99	5.11

The results revealed for *Ocimum sanctum*, that higher TFC was obtained in ethanolic seed extract (5.11 mg/100mg) followed by methanol seed extract (4.85mg/100mg). However, ethanol leaf extract has no flavonoid content according to this study.

Flavonoid component has antioxidant activity due to the presence of their phenolic hydroxyl groups and it is capable of scavenging the reactive oxygen species [34] and it is effective on human health and their nutrition. Flavonoids component showed their activity by scavenging or chelating mechanism [30]. Flavonoids components are easily oxidized and donating electrons to scavenge free radicals and form a chelate complex with metal ions. The hydroxyl group of flavonoids is substituted with aromatic [6]. The higher flavonoid component in *Ocimum sanctum* is correlated with increased antioxidant activity [21]. It has been recorded in earlier study that a virtuous amounts of total flavonoid contents present in methanolic extracts of *Ocimum sanctum* [15]. It has been also reported that the flavonoid component and antioxidant activity possess a linear correlation [5].

## CONCLUSION

There are various reports available that indicate tulsi is a good source of flavonoids *Ocimum* species are extensively usage in the traditional medicine system owing to presence of the high amount of flavonoids and phenolic components. It has also proven that the effectiveness of flavonoids extraction depend on the plants and nature of solvent used. Researchers determined that ethanol was a good solvent for the flavonoids extraction in various medicinal plants. Hence present work can be seen as a potential source of useful drugs. Further studies are in progress of this plant for the purpose of isolation, identification, characterization and explanation of the structure of the phytochemical compounds.

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