



## PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF *OCIMUM TENUIFLORUM* L. LEAVES

**Manonmani R**

Assistant Professor, PG and Research Department of Botany, Holy cross college (Autonomous), Tiruchirappalli, Tamil Nadu, India

**Kaviya K\***

PG and Research Department of Botany, Holy cross college (Autonomous), Tiruchirappalli, Tamil Nadu, India \*Corresponding Author

**ABSTRACT** *Ocimum tenuiflorum* L. is widely used as a medicine to cure various diseases. The present study was aimed to determine the various phytochemical compounds of tulsi leaves. The qualitative analysis revealed the presence of various secondary metabolites such as tannins, saponin, flavonoids, terpenoids, cardiac glycosides, proteins, leuco anthocyanin, anthocyanin, coumarins, phenol, alkaloids, xanthoprotein, phenol, emodin and carbohydrates. From the quantitative analysis it was found that phytochemical constitution with the highest quantity was flavonoid (0.013mg/g) followed by saponin (0.008mg/g), alkaloid (0.005mg/g), phenol (0.003mg/g), tannin (0.002mg/g) and terpenoid (0.001mg/g). From the GC-MS analysis of Methanolic leaves extract twenty compounds were identified. These compounds have various biological properties like antimicrobial, antioxidant, anti-inflammatory, antitumour etc. Hence this plant is beneficial to use as herbal medicine to cure various diseases.

**KEYWORDS :** *Ocimum tenuiflorum*, phytochemical, ethanolic extract, GC-MS, biological activities.

### INTRODUCTION

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs<sup>[1]</sup>. Traditional medical system has great value and also many medicinal plants have been identified from indigenous pharmacopoeias, because of which plants are still making imperative contribution to health care regardless of modern medicines which has many advances<sup>[2]</sup>. The medicinal values of plants lie in their Phytochemicals, which produce definite physiological actions on the human body. Phytochemicals are compounds present in plants that are used as food and medicine to protect against illness and to maintain human health<sup>[3]</sup>. The genus *Ocimum* comes under Lamiaceae family and is found in many parts of the World like tropical and sub-tropical regions of Asia, Africa and Central and South America<sup>[4]</sup>. It is a source of essential oils and aroma compounds, a culinary herb and an attractive, fragrant ornamental plant. Tulsi is considered as a sacred plant and is known as queen of medicinal plants from ancient period in India<sup>[5]</sup>. Aqueous extract of *O. tenuiflorum* L. leaves is used for common cold and fever. The whole plant (Leaves, stem, flower, root and seed) had been used in several traditional medicine systems like Ayurveda, Greek, Siddha, Roman, Unani for its range of therapeutic activities. The plant possesses anti-inflammatory, antioxidant, antitumoral, antifertility, antidiabetic, antifungal, antimicrobial, cardioprotective, analgesic, antispasmodic and adaptogenic properties and also effective in reducing the growth of a variety of cancer cell lines *in vitro*<sup>[6]</sup>.

### MATERIALS AND METHODS

#### Collection of the plant material

The leaves of *O. tenuiflorum* L. were collected from Allithurai, Tiruchirappalli District. The leaves were washed with sterile water and dried in shades. Then the leaves samples were powered in mechanical grinder.

#### Preparation of ethanolic extracts

The ethanolic extract of *O. tenuiflorum* L. was prepared by drenched 40gm of the dried leaves powder in 1litre of ethanol by using a hot percolation extractor for 24 hrs continuously. The leaves extract were filtered through Whatmann filter paper No.1 (125mm). The filtered sample extract was concentrated and dried by using a rotatory evaporator under reduced pressure.

#### Preliminary Phytochemical screening:

Chemical test were carried out in the ethanolic leaves extract using standard procedures to identify the constituents as described by many authors<sup>[7-15]</sup>.

#### Test for Tannins (Ferric Chloride Test)

To 2ml of extract, 2ml of distilled water was added in a test tube and then filtered. A few drops of 0.1% ferric chloride was added to the

filtrate. Green precipitate was regarded as positive for the presence of tannins.

#### Test for Phlobatannins (Precipitate Test)

To 2ml of extract, 2ml of 1% HCl was added and boiled. Red precipitate was regarded as positive for the presence of phlobatannins.

#### Test for Saponin (Foam Test)

To 5ml of extract, 5ml of H<sub>2</sub>O was added in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

#### Test for Flavonoids (Alkaline Reagent Test)

To 1ml of extract, a few drops of 10% lead acetate solution was added. A yellow colour indicates the presence of flavonoids.

#### Test for Steroids (Salkowski Test)

To 2ml of extract, 2ml of chloroform and two drops of conc. H<sub>2</sub>SO<sub>4</sub> were added.

Reddish brown ring was regarded as positive for the presence of steroids.

#### Test for Terpenoids (Salkowski Test)

To 2ml of extract, 2ml of chloroform and Conc. H<sub>2</sub>SO<sub>4</sub> were added. A reddish brown colouration of the interface indicates the presence of terpenoids.

#### Test for Cardiac Glycosides

To 1ml of extract, 2ml of glacial acetic acid, 1ml of ferric chloride, 1ml of distilled water and Conc. H<sub>2</sub>SO<sub>4</sub> were added. Formation of violet or brown ring was regarded as positive for the presence of cardiac glycosides.

#### Test for Anthocyanins<sup>[7]</sup>

To 2ml of extract, 2ml of HCl and 1ml of ammonia solution were added. Pinkish red to bluish colour was regarded as positive for the presence of anthocyanins.

#### Test for Leucoanthocyanins<sup>[8]</sup>

To 1ml of extract, 1ml of isoacetyl amyl alcohol was added. Observation of organic layer into red was regarded as positive for the presence of leucoanthocyanins.

#### Test for Emodin<sup>[8]</sup>

To 2ml of extract, 2ml of ammonium hydroxide and 3ml of benzene were added. Red colour was regarded as positive for the presence of emodin.

#### Test for anthroquinones<sup>[9]</sup>

To 2ml of extract, 1ml of benzene and 2ml of ammonia solution were

added. Pink, violet, or red colouration was regarded as positive for the presence of anthroquinones

#### Test for Xanthoproteins<sup>[9]</sup>

To 1ml of extract, 4 drops of ferric chloride was added. Blue black colouration was regarded as positive for the presence of xanthoproteins.

#### Test for Proteins (Xanthoprotein Test)

To 1ml of extract, 1ml of Conc.H<sub>2</sub>SO<sub>4</sub> was added and boiled. White precipitation was regarded as positive for the presence of proteins.

#### Test for Coumarins<sup>[10]</sup>

To 2ml of extract, 3ml of 10% sodium hydroxide was added. Yellow colour was regarded as positive for the presence of Coumarins.

#### Test for Phenols<sup>[10]</sup>

To 1ml of extract, few drops of ammonia solution were added. Reddish orange precipitate formation was regarded as positive for the presence of phenols.

#### Test for Glycosides (Liebermann's Test)

To 2ml of extract, 2ml of chloride and 2ml of acetic acid were added. Violet to blue to green colour was regarded as positive for the presence of glycosides.

#### Test for Alkaloids (Hager's Test)

To 2ml of extract, few drops of picric acid were added and shaken gently to extract the alkaloids base, yellow precipitate was regarded as positive for the presence of alkaloids.

#### Test for Carbohydrates (Molisch's Test)

To 2ml of extract, 2ml of distilled H<sub>2</sub>O, 2 drops of ethanolic alpha naphthol and 2ml of Conc.H<sub>2</sub>SO<sub>4</sub> were added. Formation of reddish violet ring was regarded as positive for the presence of carbohydrates.

#### Quantitative Phytochemical Analysis:

##### Determination of total phenols<sup>[11]</sup>

1gm of powder sample was boiled with 2ml of diethyl ether and taken 5ml of extract, added with 10ml of distilled water and then added 2ml of ammonium hydroxide and 5ml of concentrated amyl alcohol. Plant sample was undisturbed to react for 30 minutes for colour formation. It was measured at 505 nm.

##### Alkaloid determination<sup>[12]</sup>

5gm of plant sample was boiled with 10ml of 10% acetic acid in ethanol and covered, and allow to stand for 2 hours. Ammonium hydroxide was added to the plant extract. The solution was allowed to settle and precipitate was collected and washed with dilute ammonium hydroxide and then filtered, dried and weigh.

##### Tannin determination<sup>[12]</sup>

3gm of powdered sample was extracted and mixed with 10ml of distilled water. The solution was filtered through the filter paper. 5ml of extracted sample was taken into a testtube and added with 1ml of 0.1M FeCl in 0.1N HCl and 0.008M Potassium ferrocyanide. Observation was measured at 320 nm within 10mins.

##### Saponin determination<sup>[13]</sup>

1gm of powdered sample and 20ml of condensed ethanol extract were prepared. The concentrated sample was transferred into 250ml separate funnel and 1ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and ether layer was discarded. Add few pinch of n-butanol in 10ml of remaining solution and then sample was heated in water bath, after desertion sample dried in crucible to constant weigh.

##### Flavonoid determination<sup>[14]</sup>

3g of the powdered sample was added with 10ml of 80% aqueous methanol. Extraction was filtered through the filter paper. Filtrate was transfer into crucible and discarded sample dryness over water bath and weigh.

##### Terpenoid determination<sup>[15]</sup>

10 g of sample was soaked in alcohol (50ml) for 24hours. Filtered sample was extracted with petroleum ether (40ml) for 24hours. After vanishing to dryness and weigh.

#### GAS CHROMATOGRAPHY-MASS SPECTROSCOPY (GC-MS) ANALYSIS

Sampling of Plant material for GC-MS analysis 10gm of powdered leaves material was soaked in 20ml of absolute alcohol overnight and then filtered through Whatmann No.1 filter paper along with 2gm Sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with absolute alcohol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and was concentrated to 1ml. The extract contains both polar and non-polar phytochemicals. GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-5MS fused silica capillary column (30mm×0.25mm ID×1 μm df, composed of 5% Diphenyl/95% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; Helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 2 μl was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

#### Identification of Components<sup>[16]</sup>

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight and Structure of the components of the test materials were ascertained.

#### RESULTS AND DISCUSSION

##### Qualitative phytochemical analysis

Qualitative screening revealed that the presence of various constituents such as Include phlobatannin in the ethanolic leaves extract of *O. tenuiflorum* L. (Table- 1).

**Table 1: Qualitative phytochemical analysis of ethanolic leaves extract of *Ocimum tenuiflorum* L.**

S.NO	PHYTOCHEMICALS	SAMPLES
1	Tannin	+++
2	Phlobatannin	++
3	Saponin	++
4	Flavonoids	+++
5	Steroids	-
6	Terpenoids	++
7	Cardiac glycosides	+++
8	Leuco anthocyanin	+
9	Anthocyanine	+
10	Anthroquinone	++
11	Protein	+++
12	Coumarin	+++
13	Glycoside	-
14	Phenol	+++
15	Alkaloids	+++
16	Xanthoprotein	+
17	Emodine	++
18	Carbohydrate	+

Strongly present = +++, Moderately present = ++, Slightly present = +, absence = -

##### Quantitative phytochemical analysis

Quantitative test revealed that the presence of various phytochemical constituents in *O. tenuiflorum* L. leaves. The phytochemicals with different quantities were mentioned in Table- 2.

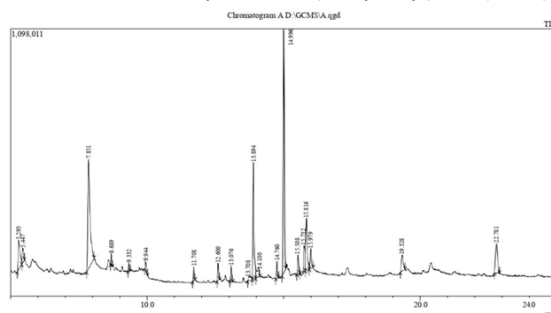
**Table :2 Quantitative phytochemical analysis of leaves extracts of *Ocimum tenuiflorum* L.**

S.NO	PHYTOCHEMICAL CONSTITUENTS	SAMPLES (mg/g)
1.	Flavonoid	0.013
2.	Tannin	0.002
3.	Saponin	0.008
4.	Alkaloid	0.005
5.	Phenol	0.003
6.	Terpenoid	0.001

**GC-MSANALYSIS**

The studies on the bioactive components in the Methanolic leaves extract of *O. tenuiflorum* L. by GC-MS analysis clearly showed the presence of twenty bioactive compounds. The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (peak area %) were presented in table-3. The GC-MS chromatogram of the twenty peaks of bioactive compounds detected were showed in Fig-1. The bioactive compounds identified in ethanolic leaves extracts were 4-Hepten-3-one, 4-methyl- (CAS) 4-Methyl-4-Hepten-3-One (5.55%), 1,2,3-Propanetriol, diacetate (CAS) Diacetin (1.83%), Cytidine (CAS) Cyd (23.55%), Zingiberene (0.87%), Hexadecanoic acid (CAS) Palmitic acid (0.69%), 1,2 – Benzoldicarbonsaeure,DI –HE (0.85%), Hexadecanoic acid (CAS) Palmitic acid(1.27%), Cyclopentane, heneicosyl- (CAS)

Heneicosane, 1-cyclopentyl (1.51), 2-Nonen- 1-ol, (E)- (CAS) trans-2-Nonenol ( 1.48%), 2-(4-hydroxy-2-butenyl)- 2-nitrocycloheptanone(1.21%), Hexadecanoic acid (CAS) Palmitic acid (12.50%), Phthalic acid, butyl ester, ester with butyl glycolate (CAS) (2.28%), Eicosa-5,8,11,14-Tetraynoic acid (1.49%), 5,8,11,14-Icosatetraynoic Acid(21.33%), dl-Citronellol(1.82%), 1,E-11,Z-13-Octadecatriene (3.22%),9,12,15 Octadecatrienoic acid, methyl ester (7.04%), 9- Octadecenoic acid (Z)- (CAS) Oleic acid (2.51%), Trans-2-phenyl-1,3-dioxolane-4-methyl octadec-9,12,15-trienoate (2.91%) and 1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (6.11%).



19	19.328	Trans-2-phenyl-1,3-dioxolane-4-m	<sup>28</sup> H <sup>40</sup> O <sup>4</sup>	440	2.91	Unknown
20	22.781	1,2-Benzenedicarboxylic acid, bis(2-ethylhexy	<sup>24</sup> H <sup>38</sup> O <sup>4</sup>	390	6.11	Antifouling and antimicrobial activity, Insecticides, Plasticizer <sup>[25]</sup>

## DISCUSSION

The phytochemicals are the chemical compounds produced by plants either the product of plant metabolism or synthesized for defense purposes. In the present investigation, the results of phytochemical analysis of *O. tenuiflorum* L. revealed the presence of various secondary metabolites such as tannins, phlobatannins, saponins, flavonoids, terpenoids, cardiac glycosides, leuco anthocyanins, anthocyanins, anthroquinones, phenols, proteins, coumarins, alkaloids, xanthoproteins, emodine and Carbohydrates. The quantitative analysis of *O. tenuiflorum* L. showed highest amount was flavonoid (0.013mg/g) followed by saponin (0.008mg/g), alkaloid (0.005mg/g), and phenol (0.003mg/g), tannin (0.002mg/g), and terpenoids (0.001mg/g).

The study on the phytochemical analysis by Sangeeta Sankhalkar, and Vrunda Vernekar<sup>[26]</sup> who investigated the quantitative and qualitative analysis of phenolic and flavonoid content in *Moringa oleifera* Lam. and *O. tenuiflorum* L. and the results showed the total flavonoid in *Ocimum* leaf was 4.47 mg/ml. Total phenolic content in a leaf of *O. tenuiflorum* L. was 2.28 mg/ml. Balasaheb Kale<sup>[27]</sup> who investigated the phytochemical analysis for various chemical constituents of *O. sanctum* and he found out various chemical constituents such as carbohydrates, flavanoids, alkaloids, saponins, glycosides, cardiac glycosides, anthraquinone, tannins and steroids. Praveen Garg and Rajesh Garg<sup>[28]</sup> investigated the phytochemical screening and quantitative estimation of total flavonoids of *O. sanctum* in different solvent extract and the results shows the occurrence of flavonoids, alkaloids, glycosides, saponins, tannins, phenolics, amino acid and diterpenes. The quantitative estimation showed total flavonoids content in methanolic leaves was 4.75 mg/100g.

GC-MS chromatogram of the methanolic leaves extract of *O. tenuiflorum* L. showed the presence of twenty phytoconstituents and possessed various biological activities. Cytidine (CAS) Cyd, 5,8,11,14-Icosatetraenoic acid and Hexadecanoic acid (CAS) Palmitic acid are the major compounds detected through GC-MS analysis. The identified known compounds exhibits various biological functions such as antioxidant activity, anti-inflammatory, antitumor, antimicrobial, immune stimulant, hepatoprotective, antiarthritic, antiandrogenic etc. GC-MS analysis of *Ocimum sanctum* L. by Senah Dohare *et al.*<sup>[29]</sup> determined twenty two phytoconstituents such as Eugenol, Bornyl acetate, Camphor, Selinene,  $\alpha$ -Pinene etc., Balasubramanian *et al.*<sup>[30]</sup> worked on GC-MS analysis of volatile compounds of the essential oil of Leaves of *O. sanctum* and they findout three compounds such as Benzene, 1, 2- dimethoxy4-(1-propenyl), Caryophyllene and Eugenol and they possessed various biological activities like antibacterial, anti-inflammatory, antioxidant, Cancer Preventive, Fungicide, antispasmodic, antiviral, insecticide etc.

## CONCLUSION

In the present study, the ethanolic leaves extracts of *O. tenuiflorum* L. revealed the presence of more than ten phto compounds and the GC-MS analysis showed the presence of twenty bioactive compounds. The presence of various bioactive compounds justifies that the leaves of *O. tenuiflorum* L. used for various ailments by traditional practitioners. The bioactive compounds found in this plant are being used for the pharmacological work especially the presence of antimicrobial properties can be used for the synthesis of new drug. However, the isolation of individual phytochemical constituent and subjecting it to biological activity will definitely give fruitful results. It could be concluded that, the presence of various bioactive compounds justifies the whole plant is used as a remedy for various ailments by traditional practitioners. So it is recommended as plant of pharmaceutical importance. It is more beneficial to use tulsi as an herbal medicine as compare to chemically synthesized drug.

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