



PHYTOCHEMICALS EXTRACT OF OCIMUM SANCTUM. (TULSI)

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Article History: Received: 12.12.2022

Revised: 29.01.2023

Accepted: 15.03.2023

Abstract

Ocimum sanctum also known as Tulsi or Holybasil is an aromatic plant and it belongs to the family Lamiaceae. It is widely used as medicine to cure various ailments. The objective of the study was to analyse different phytochemical components of tulsi leaf. The dried powder of Tulsi (50g) was placed in the thimble of Soxhlet apparatus and the experiment was done separately for methanol, ethanol and distilled water. The percentage yield was 8% w/w, 7% w/w, and 5% w/w respectively. The study reveals that various secondary metabolites such as carbohydrate, tannin, flavonoids, saponins, glycoside, terpenoid, fatty acids and phenol are present in tulsi leaf extract. From the quantitative analysis it was found that high amount of phenols are present in Tulsi leaf ranging from 1.6 to 7.6 percentages. Consequently the amount of alkaloid and flavonoids ranged from 0.91 to 1.28 and 1.56 to 2.24 percentages respectively. From the GC-MS analysis of methanolic extract three compounds were identified as major constituents viz., Eugenol, Benzene, 1, 2-dimethoxy-4-(2-propenyl), α -Farnesene and Cyclohexane, 1, 2, 4-triethenyl. These phyto-chemicals are known to possess antiseptic, analgesic, anti-inflammatory, antimicrobial, antistress, immunomodulatory, hypoglycemic, hypotensive and antioxidant properties. Hence it is more beneficial to use tulsi as a herbal medicine as compared to chemically synthesized drug.

Keywords: phytochemical, *Ocimum sanctum*, GC-MS, medicine.

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DOI: 10.31838/ecb/2023.12.s3.107

1. Introduction

The plant kingdom is an excellent source of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Medicinal plants are rich source of different types of medicines and produce various bioactive molecules. Herbal plant extracts are very useful and are the major sources of medicine which play vital role in controlling various types of pathogens (Doss, 2009) and as growth promoters. These are the cheaper source for therapeutics and viable solution for various pathogens. The medicinal plants extract have

now emerged as a good alternative as they are rich in a wide variety of secondary metabolites such as tannins, phenolics, alkaloids and flavonoids etc which enhances growth, innate immune response and disease resistance against pathogenic bacteria in human as well as in different organisms (Edoga et al., 2005). About 80% of individuals from developed countries use various medicinal plants as traditional medicines as anticancer drugs (Dewick, 1996), antimicrobial drugs (Phillipson, 1996), antifungal and in various proposes. The medicinal plants are rich sources of secondary metabolites which are chemically and taxonomically extremely diverse compounds with obscure function. A large number of phytochemicals are widely uses in human therapy, agriculture, veterinary, various scientific researches and in different areas (Vasu et al., 2009) along with inhibitory effects on all types of microorganisms in vitro (Cowan, 1999).

Ocimum sanctum L. commonly known as holy basil (Tulsi) is an herbaceous perennial, belongs to family Lamiaceae and is considered as one of the most important source of medicine and drugs with many secondary metabolites and essential oils recommended fortreatment of malaria, diarrhoea, bronchial asthma, dysentery, bronchitis, skin diseases, arthritis, painfuleye disease, chronic fever and eye diseases etc 5,6. In addition, *Ocimum sanctum* also shows anticancerous, antifungal, antimicrobial, antifertility, hepatoprotective, antispasmodic, cardio protective, antiemetic, antidiabetic, analgesic, adaptogenic, and diaphoretic properties 6-9. The pharmacological studies reported in the present research confirm the therapeutic value of *O. Sanctum*. Therefore, the present study looks into the extraction and preliminary phytochemical analysis of *O. Sanctum* leaves.

2. Materials and Methods

Collection of plant material: Leaves of *Ocimum sanctum* L. (tulsi) were collected from different sites of Dibrugarh District, Assam, washed with sterile water and dried in shades. Then the samples were powered in mechanical grinder.

Aqueous, methanol and ethanol extract: The dried tulsi

(50g) powder was placed in the thimble of Soxhlet apparatus and 500- 700 ml of distilled water, methanol and ethanol was used for extraction procedure and the experiment was done separately for all the two solvents and distilled water. The extraction was continued till clear solvent or water was seen in the thimble. The extract was concentrated using rotary evaporator. Then the extract was dried in a digital water bath till a dark green residue was obtained. The percentage yield of the extract was calculated using the following formula:

$$\text{Percentage yield} = \frac{\text{Final weight of the dried extract}}{\text{Initial weight of the powder}} \times 100$$

All the three extracts were kept in separate vials in the refrigerator till further use.

Qualitative phytochemical analysis: The extract was tested following standard biochemical methods as described below. **Test for proteins:**

Biuret's test: 2ml of Biuret reagent was added to 2ml of extract. The mixture was shaken well and warm for 5 min. Appearance of red or violet colour indicated presence of proteins.

Million's test: Crude extract was mixed with 2ml of Millon's reagent, if precipitate appeared which turned red on gentle heating confirmed the presence of protein. **Ninhydrin test:** Crude extract was mixed with 2 ml of 0.2% solution of Ninhydrin and boiled for some time, if violet colour appeared indicating the presence of amino acids and proteins.

Test for carbohydrates:

Fehling's test: Equal amount of Fehling A and Fehling B reagents were mixed and 2ml of it was added to the plant extract and then gently heated the sample. Appearance of brick red precipitate indicated the presence of reducing sugars.

Benedict's test: Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates. **Molisch's test:** 2ml of Molisch's reagent was added to 0.5 ml of crude extract and the mixture was shaken properly. After that, 2ml of concentrated H_2SO_4 was poured carefully along the side of the test tube. Appearance of a violet ring at the interface indicated the presence of carbohydrate.

Iodine test: 2ml of iodine solution was mixed with 0.5 to 1 ml of crude extract. A dark blue or purple coloration indicated the presence of the carbohydrate. **Test for phenol:** 2 ml of alcohol and 2-3 drops of ferric chloride solution was added to 1 ml of crude extract, bluegreen or black coloration indicated the presence of phenols

Test for tannin: 1 ml of distilled water and 2-3 drops of ferric chloride solution was added to 0.5 ml

of crude extract. A black coloration indicated the presence of tannin.

Test for flavonoids

Shinoda test: Crude extract was mixed with small amount of magnesium and concentrated HCl was added drop wise. Appearance of pink scarlet colour after few minutes indicated the presence of flavonoids.

Alkaline reagent test: 0.5 ml of crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for saponins: 1ml of crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for glycosides

Liebermann's test: Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H₂SO₄ was added. If colour change from violet to blue to green which indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Salkowski's test: 2ml of chloroform was mixed with crude extract. Then 2ml of concentrated H₂SO₄ was added carefully and shaken gently. A reddish brown colour indicated the presence of glycoside.

Keller-kilani test: 0.5 ml of crude extract was mixed with 2ml of glacial acetic acid containing 2-3 drops of 2% solution of FeCl₃. Then 2ml of concentrated H₂SO₄ was poured into the mixture. A brown ring at the interface indicated the presence of cardiac glycosides.

Test for steroid

(i) 2ml of chloroform was added to the crude extract of Tulsi. Then 2ml of each of concentrated H₂SO₄ and acetic acid were added into the mixture. The presence of steroids was indicated by appearance of a greenish coloration in the reaction mixture.

(ii) Crude extract was mixed with 2ml of chloroform and gently added concentrated H₂SO₄. A red colour was seen in the lower layer this indicated the presence of steroids. **Test for terpenoids:** Crude extract was mixed in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H₂SO₄ was added and heated for about 2 minutes. Presence of terpenoids was indicated by a greyish colour at the interface.

Test for alkaloids: 2ml of 1% HCl was mixed with crude extract and heated gently. After heating,

Mayer's And Wagner's reagents were added to the mixture. If precipitate was observed in the reaction mixture which indicated the presence of alkaloids.

Test for anthraquinone: 5ml of chloroform and 5 ml of ammonia solution was added to 0.2 gm of plant extract. Appearance of pink, red or violet colour indicated the presence of anthraquinone.

Oils & Fats: A small quantity of crude extract was pressed between two filter papers separately. An oily appearance on filter paper indicated the presence of fixed oil and fats.

Test for lactones

Baljet's test: Crude extract was treated with sodium picrate solution. Presence of lactone was observed by appearance of yellow to orange colour in the mixture.

Quantitative analysis of phytochemical in the plant extract:

Determination of total phenolic contents

(Singleton et al., 1999): The amount of total phenol for aqueous, methanol and ethanol extract were determined by Folin- Ciocalteu reagent method. 2.5 ml of 10% Folin- Ciocalteu reagent and 2 ml of 2% Na₂CO₃ were added to 0.5 ml of plant extract. The mixture was then incubated at room temperature for 30 minutes. Gallic acid was used as standard (1mg/ml). The absorbance of the sample was measured at 765nm. All the tests were done in triplicates and the results were determined from standard curve and were expressed as gallic acid equivalent (mg/g of extracted compound).

Determination of alkaloid (Harborne, 1973): 5 g of the sample was taken and 200 ml of 10% acetic acid in ethanol was added to the sample and allowed to stand for 4 hours. Then the solution was filtered and the extract was concentrated on water bath. Conc. NH₄(OH) was added drop wise and the whole solution was allowed to settle and the precipitate was then washed with dilute ammonium hydroxide and filtered. The residue was dried and weighed and this was the amount of alkaloid present in the plant material

Determination of flavonoids (Bohm & Kocipai-Abyazan, 1994): 10 g of plant sample was taken and extracted repeatedly with 100ml 80% methanol. Then the solution was filtered and the filtrate was transferred into an empty crucible and evaporated into dryness over water bath and weighed. The final weight dry weight was amount of flavonoids in the plant sample.

Preparation of stock solution

The extracts were reconstituted in methanol. Methanolic extracts (1 µl) were injected for GC-MS analysis.

Gas Chromatography-Mass Spectrometry analysis Methanolic extract of the leaves of *Ocimum sanctum* was subjected to GC-MS analysis on a GC-MS Clarus 500 Perkin Elmer system comprising a AOC- 20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Restek RtxR – 5, (30 meter X 0.25 mm)(5% diphenyl / 95% dimethyl polysiloxane), running in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 2.0 μ l was employed (split ratio of 10:1); injector temperature 280°C. The oven temperature was programmed from 50°C (for 1 min.), with an increase of 6 °C / min to 280 °C, then ending with isothermal for 15 min at 280°C. Mass spectra

were taken at 70 eV; a 0.5 seconds of scan interval and fragments from 40 to 550 Da. Total GC running time was 60 minutes.

Identification of Compounds

Interpretation on mass spectrum GC-MS was conducted using the database of department of Chemistry; Dibrugarh University. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the department of Chemistry library.

3. Results

The yield of residue after Soxhlet extraction and evaporation of 50 gm dried plant leaves in methanol, ethanol and water were as follows:

Table.1: Amount of plant extracts yield percentage in different solvents

Extract	Yield amount (%) W/W
Aqueous	5%
Methanol	8%
Ethanol	7%

The phytochemicals analysis in *Ocimum sanctum* (Tulsi) leaf extracts in the two solvents and aqueous conditions were summarized in Table 2. Various bioactive molecules were found in Tulsi leaf extract from the phytochemical screening. The amount of extraction is more in case of organic

solvent than that of water. From the quantitative analysis it was found that high amount of phenols are present in Tulsi leaf ranging from 1.6 to 7.6 percentages. Consequently the amount of alkaloid and flavonoids ranged from 0.91 to 1.28 and 1.56 to 2.24 percentages respectively.

Table.2: Qualitative phytochemical screening methanol extract of tulsi leaf

Phytochemicals	Aqueous extract	Methanol extract	Ethanol extract
Protein	-	-	-
Carbohydrate	-	+	+
Phenol	+	+	-
Tannin	-	+	+
Flavonoid	+	+	+
Saponin	-	+	+
Glycosides	+	+	+
Steroid	-	-	-
Terpenoid	-	+	+
Alkaloid	+	+	+
Anthraquinone	-	-	-
Fixed oils and fatty acid	-	+	-
Test for lactones	-	-	-

“+” present, “-” absent

Table.3: Percentage of total phenolic, alkaloid and flavonoid contents in plant extract

Extract	Phenolic	Alkaloid	Flavanoid
Aqueous	1.61±0.56	0.91±0.66	1.56±0.64
Methanol	7.61±0.55	1.28±0.03	2.24±1.02
Ethanol	4.61±0.56	0.94±0.58	1.91±0.56

Each value is the average of three analysis and ± standard deviation.

Table.4: Chemical constituents and the activity of some of the phytochemicals of *Ocimum sanctum*

Sl. No	Retention time (unit?)	Name of the compounds	Molecular weight	Molecular formula	Activity**
1.	7.20	Eugenol	164	C10H12O2	Anti-inflammatory, antioxidant, anticancer, Acaricide, Antibacterial, Antispasmodic, Antiviral, Insecticide
2.	7.70	α - Farnesene	93	C15H24	Acaricide, allergenic, analgesic, anaesthetic, antibacterial, antiinflammatory, antiedemic, antioxidant, antiviral, antitumor, antiulcer
3.	7.50	Benzene, 1, 2dimethoxy- 4-(1-propenyl)	178	C11H14O2	Insect-attractant, perfumery, flavour antibacterial, nematocidal
4.	13.36	Cyclohexane,1,2,4-triethenyl	162	C12H18	Antibacterial, anti-inflammatory, antiedemic, antispasmodic

**Source: Dr. Duke's phytochemical and ethnobotanical database (online database)

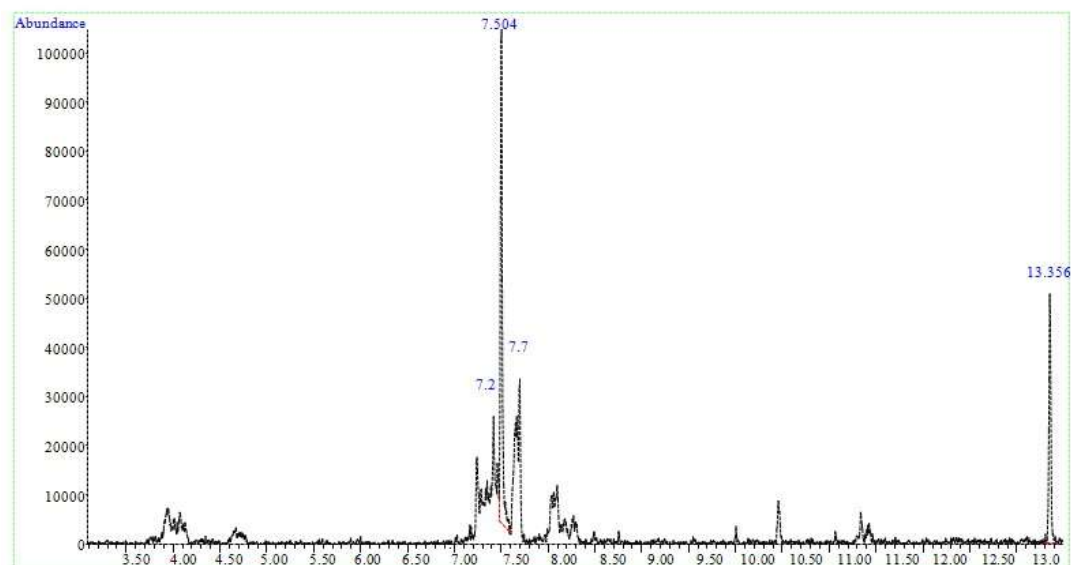


Fig.1: GC- MS chromatogram of the methanolic extract of the leaves of *Ocimum sanctum*

4. Discussion

Ocimum sanctum has various properties such as antistress, antiseptic, analgesic, anti-inflammatory, antimicrobial, immunomodulatory, hypoglycemic, hypotensive, cardioprotective and antioxidant (Williamson, 2002, Tanwar et al., 2015). Eugenol

(1-hydroxy-2-methoxy-4allylbenzene), the active constituents present in *O. sanctum* have been found to be largely responsible for the therapeutic potentials (Sailaja et al., 2010). This plant has various properties such as antistress, antiseptic, analgesic, anti-inflammatory, antimicrobial, immunomodulatory, hypoglycemic, hypotensive,

cardioprotective and antioxidant (Williamson, 2002, Tanwar et al., 2015). Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the active constituents present in *O. sanctum* have been found to be largely responsible for the therapeutic potentials (Sailaja et al., 2010).

The study reveals that various secondary metabolites such as carbohydrate, tannin, flavonoids, saponins, glycoside, terpenoid, fatty acids and phenol are present in tulsi leaf extract. Leaves of *Ocimum sanctum* contain water-soluble phenolic compounds and various other constituents, such as eugenol, methyl eugenol and caryophyllene that may act as an immunostimulant. Saponins act as antihyperlipidemic, hypotensive and cardiodepressive properties (Bairwa et al., 2012). The phytochemical constituents such as alkaloids, steroids, flavanoids, tannins, phenols and several other aromatic compounds of plants serve a defense mechanism against predation by many microorganisms, insects and other herbivore (Bonjar et al., 2004) Glycosides can act as cardiostimulants in cases of cardiac failure (Sood et al., 2005). Tannins have anti diarrheal and haemostasis properties (Asquith et al., 1986). Flavonoids are responsible for antioxidant and immunostimulatory properties. According to Cragg et al., 1999 and Khanna et al., 2003 alkaloids, glycosides, flavanoids and saponins are antibiotic principles of plants and these antibiotic principles are actually the defensive mechanisms of the plants against pathogens.

GC-MS chromatogram of the methanolic extract of *Ocimum sanctum* showed four major peaks (Figure.1) and has been identified after comparison of the mass spectra with the department of Chemistry library, DU, indicating the presence of four phytochemicals. It was observed that presence of Eugenol (Synonym: 2-Methoxy- 4-(2propenyl) phenol), Benzene, 1, 2-dimethoxy- 4-(2-propenyl) - (synonym: Methyl- Isoeugenol), α -Farnesene and Cyclohexane, 1, 2, 4- triethenyl were the major components in the extract. The phytochemicals that contribute to the medicinal property of the plant leaves is listed in Table No.1. Benzene, 1, 2-dimethoxy- 4-(1propenyl) (Methyl-Isoeugenol) has the property of Antifungal activity (Kurita et al., 1981), Nematicidal activity (Park et al., 2003) and Antifeedant activity (Katsumi, 1987). Eugenol is reported to possess Antimycotic (Azzouz et al., 1982) Antiviral (Bishop, 1995) Desinsection (Konstantopoulou et al., 1992) Antiparasitic (Pandey et al., 2000) Antioxidant (Ou et al., 2006) Anticancer (Hussain et al., 2011) and Ant insect activities (Pessoa et al., 2002).

Leaves extract of *O. sanctum* affected both specific and non-specific immune responses and disease resistance against fungal and bacterial infection (Santra et al., 2017). It stimulated both antibody response and neutrophil activity. The

experimental studies have shown that methanolic extract of *Ocimum sanctum* has anticancer effect by inhibition of nitric oxide synthesis (Kim et al., 1998). The use of medicinal plants acts as a source of antimicrobial agent also for aquaculture. Alcoholic extract increased step down latency and acetyl cholinesterase inhibition and so used in the treatment of cognitive disorders. *Ocimum sanctum* has been widely employed in traditional medicines. Hence phytochemicals from this plant can be used in variety of disorders afflicting mankind. The herbs are cheap, available in large quantity around us and they pose no danger to the living organisms, the environment and the consumers and hence greatly helpful for living organisms.

5. Conclusion

The presence of various bioactive compounds in the tulsi leaves justifies the uses for various ailments by living population. The results confirm the use of *Ocimum sanctum* plant as traditional medicinal properties and suggest that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by various pathogens. It is more beneficial to use tulsi as an herbal medicine as compared to chemically synthesized drug.

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