

# **EVALUATION OF ANTIOXIDANT PROPERTIES, CELLULAR EFFECT OF SELECTED PHYTOCOMPOUND OF *OCIMUM SANCTUM*.**

## **PROJECT WORK**

Submitted in partial fulfillment of the requirements for the award of Bachelor of  
Science in Biotechnology

by

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**SCHOOL OF BIO AND CHEMICAL ENGINEERING**

**DEPARTMENT OF BIOTECHNOLOGY**

# **SATHYABAMA**

**INSTITUTE OF SCIENCE AND TECHNOLOGY  
(DEEMED TO BE UNIVERSITY)**

**CATEGORY-1 UNIVERSITY BY UGC**

**Accredited with Grade "A++" by NAAC | 12B Status by UGC | Approved by AICTE  
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**APRIL - 2024**



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## DEPARTMENT OF BIOTECHNOLOGY

### BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Aloysius Vidhusha. M (411770003) who carried out the project entitled "Evaluation of antioxidant properties, cellular effect of selected phytochemical of *Ocimum sanctum*" under my supervision from January 2023 to April 2024.

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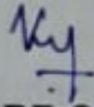
## DECLARATION

### ACKNOWLEDGEMENT

I Aloysius Vidhusha. M hereby declare that the Project Report entitled "Evaluation of antioxidant properties, cellular effect of selected phytochemical of *Ocimum sanctum*" done by me under the guidance of Dr. Saqib Hassan, Assistant Professor, Department of Biotechnology, Sathyabama Institute of Science and Technology (Internal) and Dr. Kunal Biswas, Assistant Professor (Research), Centre for Nanoscience & Nanotechnology, IRC (External) at Sathyabama Institute of Science and Technology is submitted in partial fulfillment of the requirements for the award of Bachelor of Science Degree in Biotechnology.

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## ABSTRACT

The phytochemicals present in plants are responsible for preventing diseases and promoting health have been studied extensively to establish their efficacy and to understand the underlying mechanism of their action. Such studies have included the identification and isolation of the chemical components and the establishment of their biological potency. Certain phytochemical in *Ocimum sanctum* were tested for their antioxidant properties using a DPPH assay. Eugenol is the selected phytochemical which is also tested for its antimicrobial property besides antioxidant properties to understand the effect of Eugenol on cellular level organisms such as *E. coli*. Antimicrobial activity assessment is performed using Disc diffusion method. This study can prove that naturally obtained antioxidants from plants can be a good alternative to artificially synthesized antioxidants which cause various side effects on human health.

**Keywords:** *Ocimum sanctum*, Eugenol, DPPH assay, Antioxidant, Antimicrobial, Disc diffusion

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## LIST OF ABBREVIATIONS

|                  |                                            |
|------------------|--------------------------------------------|
| DPPH             | 2,2-Diphenyl-1-picrylhydrazyl              |
| SOD              | Superoxide dismutase                       |
| GPx              | Glutathione peroxidase                     |
| GR               | Glutathione reductase                      |
| BHA              | Butyl hydroxyl anisol                      |
| BHT              | Butyl hydroxy toluene                      |
| GRAS             | Generally recognized as safe               |
| <i>O.sanctum</i> | Ocimum sanctum                             |
| <i>E. coli</i>   | <i>Escherichia coli</i>                    |
| ROS              | Reactive oxygen species                    |
| RNS              | Reactive nitrogen species                  |
| FtsZ             | Filamenting temperature-sensitive mutant Z |
| LB               | Lysogeny Broth                             |

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# **CHAPTER-1**

## **INTRODUCTION**

Our diets have changed as a result of our frequent use of processed foods and the substitution of fast food for healthier options. These foods' preservatives and dyes have the potential to create free radicals, which can lead to a several illnesses. Molecules having an unpaired electron are known as free radicals. This molecule becomes very reactive due to the presence of an unpaired electron. Aerobic organisms naturally produce free radicals as well [McCord, 2000]. Antioxidants are therefore necessary for the body to combat free radicals. Antioxidants can prevent the production of free radicals and the subsequent chain reaction that could cause damage to cells. To lessen its reactivity, an antioxidant will transfer hydrogen or electrons. Superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) are examples of antioxidant enzymes (primary antioxidants) that the body uses as a defense against free radicals [Zulaikhah, 2017]. However, the body is unable to combat an excessive amount of free radicals. To combat these free radicals, an antioxidant supply (secondary antioxidant) is therefore necessary. Although synthetic antioxidants like butyl hydroxyl anisol (BHA) and butyl hydroxy toluene (BHT) are highly effective and frequently employed in the processing industry, they can have negative health consequences on people, including promoting the development of cancer [Ayoub et al, 2017]. As a result, research into naturally occurring compounds' antioxidants and their potential to replace synthetic antioxidants has generated significant attention. Antioxidant components found in most plant-based meals include carotenoids, polyphenols, vitamin C, and ubiquinol. Basil leaf is one of the veggies that may be used as a natural antioxidant [Akbari et al,2022].

## 1.1 MORPHOLOGY AND PROPERTIES:

Common names for *Ocimum sanctum* include holy basil, tulsi, and tulsi. It is a strongly scented small annual herb that grows up to 18 inches tall and forms a low bush. Various parts of *O. sanctum*, such as leaves, stems, flowers, roots, seeds, and even the entire plant, have been used to treat a variety of conditions, including skin diseases, chronic fever, arthritis, malaria, diarrhea, dysentery, and insect bites [Prakash et al,2005]. Tulsi, a member of the Lamiaceae family of basil plants, is native to the Indian subcontinent, China, and Southeast Asia. It is widely grown as a crop in the tropical regions of Southeast Asia. Because of its therapeutic qualities, tulsi is referred to as "Mother Medicine of Nature" [Sah et al,2018]. In India, tulsi has been incorporated into lifestyle and medicinal practices that offer health benefits that contemporary science is only now beginning to uncover.



**Fig 1.1 *Ocimum sanctum***

Aromatic plants have volatile, pungent compounds that are present in one or more of their parts—the root, wood, bark, stem, leaf, flower, and fruit—and are known as essential oils [Meagher E et al,1993]. There are several intricate chemical compounds that contribute to the unique scent. Essential oils are often associated with fragrances and perfumes since these scents are greasy and represent the essence or active ingredients of the plant [King and Young,1999]. Because they evaporate when exposed to air at room temperature, these oils are also known as volatile or ethereal oils.

Essential oils are “limited volume, high value, highly concentrated goods” [Burt, 2004]. It manages psychological stress by promoting beneficial effects on memory through anxiolytic and antidepressant qualities, and it regulates metabolic stress by restoring normal blood pressure, cholesterol, and glucose levels. *O. sanctum* also has anti-hepatoprotective, antispasmodic, analgesic, anticancer, antidiabetic, antifungal, antibacterial, and cardioprotective qualities. The plant has an extensive amount of bioactive phytochemical components, including tannins, alkaloids, flavonoids, terpenoids, phenolics, and saponins, in addition to essential oils. This plant's polyphenols and flavonoids are primarily responsible for its numerous medicinal qualities, which include its potential as an antioxidant. [Kaurinovic et al, 2019]

## 1.2 PHYTOCHEMICAL:

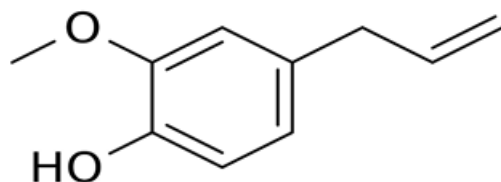
Phytochemicals are naturally occurring, physiologically active substances found in plants that provide health benefits for humans beyond those of macronutrients and micronutrients [Valko et al, 2003]. They enhance the color, flavor, and perfume of plants while shielding them from harm and disease. In general, phytochemicals are the compounds found in plants that shield their cells from environmental dangers such as pollution, stress, drought, UV radiation, and pathogenic invasion [Valko et al, 2007]. Because it contains certain compounds including estragol, linalool, eugenol, methyl cinnamate, limonene, and geraniol, basil oil has a high economic value. Although phytochemicals are categorized according to their biological functions, a single component may have many biological roles, such as acting as an antioxidant and an antibiotic.

Additionally, basil contains essential oils that include  $\alpha$ -terpineol, chavicol, linalool, and eugenol. It was reported that these substances were antioxidants. Essential oils are a group of secondary metabolites that are hydrophobic and may be extracted from plants [Aziz et al., 2018]. They are utilized in aromatherapy, alternative medicine, and perfumery. The majority of essential oils are actually hundreds of compounds that, when combined, give that specific plant's scent, even though a single ingredient may

have a distinct scent. Because these species are specifically recognized for having biological activity, such as antibacterial, antifungal, and antioxidant capabilities, research on the oils from aromatic and medicinal plants is expanding.

Many different plant sources, such as clove oil, nutmeg oil, cinnamon extract, and many more, contain eugenol, a phenolic component. The World Health Organization (WHO) has classified eugenol as GRAS and it is thought to be non-mutagenic [Nisar et al.,2021]. Eugenol is abundant in cloves, cinnamon, tulsi, and pepper. First, eugenol was isolated from the leaves and buds of the plant known as clove, *Eugenia caryophyllata*. Eugenol has attracted the interest of many researchers and opened the door to new studies into its use as a medicine to treat a variety of disorders because it is present in substantial amounts in the extracts of many medicinal herbs.

The two most successful sources of eugenol are thought to be clove and cinnamon, which contain 45–90% and 20–50% of eugenol, respectively. However, the main issues associated with these sources are greater cultivation expenses and commercial eugenol extraction. On the other hand, tulsi, ginger, bay, pepper, and other inexpensive and abundant begetters can be substituted for clove and cinnamon. Because these plant parts contain a significant amount of essential oils, eugenol is primarily found in the aerial portions of the plant, such as the leaves, bark, and flowers. Eugenol is also present in good amounts in tulsi leaves, typically between 40 to 71%. Nevertheless, the amount of eugenol in various plant components changes according to the season. [Özbek et al.,2022]



**Fig 1.2: Chemical structure of Eugenol**

Eugenol's medicinal potential has been the subject of numerous studies, which emphasize its significance as one of the main bioactive ingredients with a number of health-promoting properties. Eugenol has also been proposed as the cause of the medicinal properties of essential oils derived from different plants [Ulanowska et al., 2021]. Numerous investigations have been conducted to elucidate the mechanism underlying eugenol's medicinal action. Overall, the mechanistic explanation of eugenol's functional activity, which has been supported by numerous studies, includes its capacity to scavenge free radicals, inhibit the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), protect DNA and proteins, and increase the antioxidant capacity of cells [Anand et al., 2016].

### 1.3 ANTIOXIDANT:

In many physiological processes, including energy synthesis in mitochondria, phagocytosis, cell growth regulation, intracellular communication, and the detoxification of xenobiotics in the human body, reactive oxygen species are essentially created as the intermediate [Nagababu et al., 2010]. Tobacco smoke, pesticides, chemical pollutants, organic solvents, UV radiation, and exposure to other environmental conditions are some of the causes of excessive ROS generation. Stress brought on by the modern way of living increases the overproduction of free radicals, damages tissue, and worsens health issues.

Antioxidants are substances that can prevent or slow damage to cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures. They are sometimes called “free-radical scavengers.” Antioxidant is a substance that is present at low concentrations which significantly delays or prevents oxidation [Atasoy et al., 2021].

Plant-based antioxidants, particularly polyphenolic chemicals, significantly prevent oxidative stress-related illnesses and food rancidity caused by lipid oxidation. Plants produce polyphenols as secondary metabolites in response to environmental stressors. In addition to serving as reducing agents, metal chelators, and absorbents and

neutralizers of free radicals, polyphenols also effectively shield biological systems against deterioration under conditions of extreme oxidative stress [Cekic et al., 2022].

Cells require adequate levels of Antioxidants in order to prevent tissue damage caused by excessive production of reactive oxygen species. The roles of antioxidants are to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention [Bergamini et al., 2004].

### ***1.3.1 Antioxidant action:***

An antioxidant undergoes oxidative stress when it eliminates a free radical. As a result, the body has to continuously replenish its antioxidant reserves. Thus, an antioxidant may become ineffective against free radicals in some systems while being efficient against them in others. Furthermore, under some conditions, an antioxidant may even function as a pro-oxidant, producing harmful ROS and RN, for example. There are two ways that the antioxidant process can work: prevention or chain-breaking [Ranganathan et al,2012]. When a radical releases or steals an electron, a second radical is created for the chain breaking. The final one acts on another molecule in the same way until the free radical is either neutralized by an antioxidant that breaks the chain (carotenoids, vitamin C, E, etc.) or it just breaks down into a harmless product. Lipid peroxidation is a well-known illustration of this type of chain reaction. Superoxide dismutase, catalase, and glutathione peroxidase are examples of antioxidant enzymes that can prevent oxidation by slowing down the pace at which a chain is initiated. This can be achieved through either stabilizing transition metal radicals like copper and iron or scavenging free radicals that are just getting started [Johnsy et al,2012].

#### 1.4 ANTIMICROBIAL:

It has been demonstrated by science that eugenol exhibits pharmacological activity against a variety of bacteria, including both “Gram negative” and “Gram-positive” ones. Eugenol disrupts the cytoplasmic membrane and has the ability to dramatically increase the membrane's permeability profile. Furthermore, research on the effectiveness of eugenol indicates that it was successful in inhibiting the development of virulence factors such violacein, elastase, pyocyanin, and biofilm formation [Devi et al., 2010].

##### **1.4.1Antimicrobial action:**

Eugenol has demonstrated the ability to downregulate YidC, a highly conserved bacterial protein that is essential for membrane protein insertion, making it a potentially useful target in the field of antibacterial research. Furthermore, eugenol has shown efficacy in suppressing and eliminating biofilms generated by *Staphylococcus aureus* which is both methicillin-resistant and sensitive [Rubini et al., 2018]. Eugenol is also thought to reduce the virulence factors that *E. Coli* produces, namely VT1 and VT2. Eugenol's capacity to prevent the FtsZ assembly, which prevents bacterial cell division, is another intriguing discovery [Qiu et al., 2010].

## CHAPTER-2

### REVIEW OF LITERATURE

#### 2.1 PHYTOCHEMICAL EXTRACTION:

An experiment on “Essential oil eugenol: sources, extraction techniques and nutraceutical perspectives” was performed by [Khalil et al,2017] to elucidate the general properties, sources, extraction methods, therapeutic role and associated mechanisms of eugenol.

An experiment on the “Extraction, Purification and Equipment Designing for Eugenol” was performed by [ Sakhre et al, 2023]. The main purpose of this project is to find a suitable method to extract eugenol from sources widely available in the market and to make some products out of the eugenol extracted. An equipment design was also carried out as well as calculations to upscale the project if it were to be industrialized. Future scope which includes setting up of pilot plant in each household to reduce carbon footprint impact, recommending the usage of essential oils for a clean and better lifestyle, conducting plant designs and using the same methods extracting essential oils using other natural resources were also discussed.

#### 2.2 PHYTOCHEMICAL ANALYSIS:

“Analytical method development for determination of eugenol concentrations in selected species of *ocimum*” by [Joshi et al., 2015]. The UV and HPLC method proposed for the quantitative monitoring of eugenol in *Ocimum* leaf powder is rapid, simple, and precise. Hence from that UV and HPLC analysis it was concluded that the *Ocimum sanctum linn* contains higher concentration of Eugenol.

“Tulsi (*Ocimum sanctum*), an excellent source of phytochemicals” is a study conducted by [Borah et al.,2018]. The objective of the study was to analyze different phytochemical components of tulsi leaf. The study reveals that various secondary metabolites such as carbohydrates, tannins, flavonoids, saponins, glycosides, terpenoids, fatty acids and phenols 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 percent. Consequently, the amount of alkaloid and flavonoids ranged from 0.91 to 1.28 and 1.56 to 2.24 percentage respectively.

“Phytochemical analysis of medicinal herb (*ocimum sanctum*)” by [Panchal and Nayyar Par,2019]. Phytochemical studies Qualitative phytochemical investigation discovered presence of alkaloids compounds (Appearance of red color); flavonoids and tannins (The pink color shows the presence of flavonoids and blackish precipitate indicated the presence of tannins) and absence of flavonoids (Not observed pink coloration) in all mentioned extracts of plant. Salkowski’s test- (formation of brownish red color) showed positive result for aqueous extract. It showed negative result in case of protein, saponin, oil, steroids. The presence of these phytochemical components may be responsible for the observed antibacterial activity of the plant leaf extract.

“Studies on chemical, polyphenol content, flavonoid content, and antioxidant activity of sweet basil leaves (*Ocimum basilicum* L.)” by [Nguyen et al 2021]. The purpose of this study is to analyze the phytochemical profile, as well as to calculate the content of polyphenols and flavonoids and antioxidant activity of ethanolic and aqueous extracts from sweet basil leaves. The findings revealed that the leaves of sweet basil contained a wide range of pharmacologically active substances, such as alkaloids, coumarins, tannins, flavonoids, sugars, phenols, terpenoids and saponins. These findings have shown that this plant is a potential source of natural antioxidants.

### 2.3 ANTIOXIDANT ACTIVITY:

“Assessment of antioxidant activities of Eugenol by *in vitro* and *in vivo* Methods” by [Nagababu et al.,2011] describes the evaluation of the antioxidant activity of eugenol by testing the free radical scavenging activity and/or inhibition of radical production.

“Phytochemical screening and antioxidant activity of ethanolic extract and ethyl acetate fraction from basil leaf (*Ocimum basilicum* L.) by DPPH radical scavenging method” was performed by [Warsi and Sholichah,2017]. The antioxidant activity of the ethanolic extract and ethyl acetate fraction were tested qualitatively with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and phosphomolybdate. Its antioxidant activity was determined quantitatively using DPPH radical scavenging method. The research showed that antioxidant activity of the ethyl acetate fraction more potential than the ethanol extract of the basil leaf.

“Phytochemical screening and antioxidant activity of methanolic extract of *Ocimum sanctum* Linn. Leaves” by [Priyadarshini et al.,2019]. The phytochemicals present in plants are responsible for preventing disease and promoting health have been studied extensively to establish their efficacy and to understand the underlying mechanism of their action. Such studies have included identification and isolation of the chemical components, establishment of their biological potency both by *in vitro* and *in vivo* studies in experimental animals and through epidemiological and clinical-case control studies in man.

“Phytochemical and antioxidant profiling of *Ocimum sanctum*” by [Chaudhary et al.,2020] showed that the leaves of *Ocimum sanctum* were extracted in methanol (OsM) and sequentially fractionated with n-hexane (OsH), ethylacetate (OsE) and butanol (OsB) to find the best extraction solvent for antioxidants from the herb. Overall

data revealed that *O. sanctum* leaf extracts in butanol and ethylacetate with high polyphenolics and flavonoids, had strong antioxidant potential.

“Evaluation of antioxidant activity of *Ocimum Sanctum*-An *in vitro* study” performed by [Saravanan and Ramamurthy,2021] correlated the importance of antioxidant activity with Periodontitis.

“Phytochemical screening, total antioxidant capacity, and toxicity test of Basil leaf extract (*Ocimum x africanum Lour*)” by [Timotius et al.,2021]. The aim of this study is to determine the phytochemical profiling and antioxidant capacity of basil leaves extract. In this study it is found that basil leaves contain secondary metabolites and antioxidants capacity but not as much as vitamin C, even though basil leaves have antioxidant capacity not as much as vitamin C, basil leaves have another compound like Linalool that have antimicrobial and antioxidant activity, besides vitamin C also can induce acid reflux, so it can be concluded that basil leaves have secondary metabolites and the antioxidant capacity.

#### 2.4 ANTIMICROBIAL ACTIVITY:

“*In vitro* evaluation of the antimicrobial activity of Eugenol, Limonene, and Citrus extract against bacteria and yeasts, representative of the spoiling microflora of fruit juice” by [Bevilacqua et al 2009]. This article reports on the investigation on the bioactivity of eugenol, limonene, and citrus extract against three bacteria (*Lactobacillus plantarum*, *Lactobacillus brevis*, and *Bacillus coagulans*) and three yeasts (*Saccharomyces bayanus*, *Pichia membranifaciens*, and *Rhodotorula bacarum*), representing the spoilage microflora of fruit juices.

“Evaluating the anti-microbial effect of eugenol extracted from *ocimum sanctum*” by [Piyali et al., 2016]. The aim is to evaluate the antimicrobial property of eugenol extracted from both the powder and leaf samples of *Ocimum sanctum* (tulsi) and to have a comparative analysis of the synthetic eugenol and the naturally extracted eugenol from tulsi leaves. Further microbial assay is performed to evaluate the effect of eugenol. From the results, it can be concluded that the eugenol extracted from the powdered dry leaves and fresh leaves of tulsi has an anti-microbial effect and the membrane composed of eugenol has the capability to retain the eugenol.

“Progress on the antimicrobial activity research of clove oil and Eugenol in the food antiseptis field” by [Qiao Hu et al., 2018] researches find that both clove oil and eugenol express significant inhibitory effects on numerous kinds of food source microorganisms, and the mechanisms are associated with reducing the migratory and adhesion and inhibiting the synthesis of biofilm and various virulence factors of these microorganisms.

“A comprehensive review on Eugenol’s antimicrobial properties and industry applications: A transformation from ethnomedicine to industry” by [Mak et al., 2019]. This review covers eugenol’s antimicrobial activities, formulations to improve aqueous solubility, and applications in the food industry. Extensive scientific investigations validated the ethnomedicinal uses of eugenol as an antimicrobial agent. This is the first review on eugenol that details the antimicrobial potential of eugenol and its possible applications as a protectant in the food industry.

“Antibacterial activity of polyeugenol against *staphylococcus aureus* and *Escherichia coli*” by [Handayani., 2019]. The result showed that antibacterial activity not present at *Escherichia coli* while *Staphylococcus aureus* has the presence of antibacterial activity

## CHAPTER-3

### AIM AND OBJECTIVES

#### 3.1 AIM:

To evaluate the antioxidant property and cellular effect of particular phytochemical from *Ocimum sanctum*.

#### 3.2 OBJECTIVES:

- To extract the selected phytochemical using appropriate techniques.
- To analyze the extracted phytochemical using various techniques.
- To assess the antioxidant activity by performing DPPH assay.
- To evaluate the cellular effect of the phytochemical using Disc diffusion method.

## CHAPTER-4

### MATERIALS AND METHODOLOGY

#### 4.1 EXTRACTION OF EUGENOL: (HYDRODISTILLATION)

Fresh leaves of basil are washed and pat dried to prevent possible contamination. Arrange the Clevenger apparatus with continuous water supply to the condenser. Now fill the 1000 ml round bottom flask with 200 grams of basil leaves and 750 ml of distilled water.



**Fig 4.1: Hydro distillation of *Ocimum sanctum* leaves to obtain Eugenol**

Switch on the heating mantle and adjust the temperature to 90 degrees Celsius. Allow the process to take place for about 4 to 5 h. After 5 h switch off the heating mantle and cease the water supply. Now obtain the sample collected in the separating burette carefully in a sterile container.

#### **Storage:**

Store the sample at freezing temperature since the obtained phytochemical is a volatile oil which may evaporate easily at warm temperature.



**Fig 4.2: Clevenger apparatus**



**Fig 4.3: Eugenol extracted**

## 4.2 PHYTOCHEMICAL ANALYSIS:

### 4.2.1 Confirmatory test for phenol (FERRIC CHLORIDE TEST):

A ferric chloride test is performed to detect the presence of the phenol group in the phytochemical. To 1 ml of sample add 2 to 3 drops of ferric chloride solution and add 2 ml of methanol to it. Color change of sample from light green to dark brown indicates the presence of phenol group in the sample which is responsible for the antioxidant property of plant.



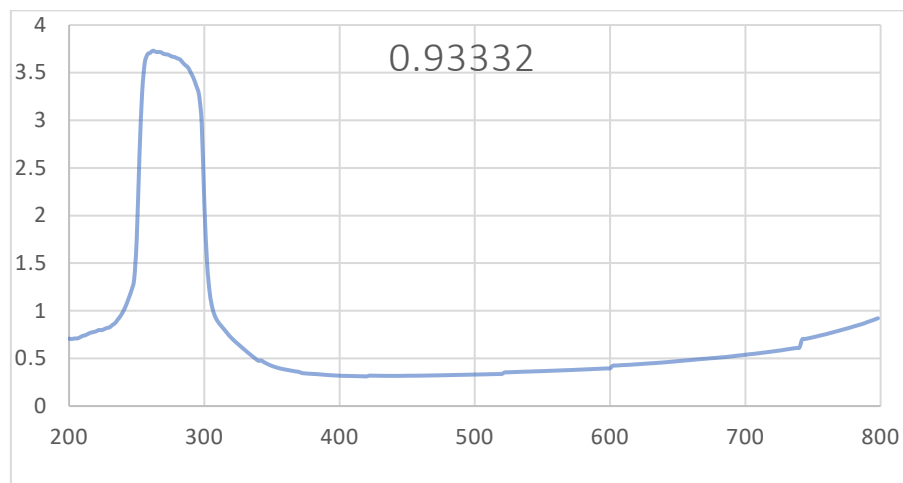
**Fig 4.4: Addition of ferric chloride solution and methanol to the sample result in color change to dark brown and indicate the presence of phenol group.**

## 4.2.2 Spectroscopic analysis:

### 4.2.2.1 UV spectroscopy

UV-visible spectroscopy is one of the most frequently employed technique for phytocompound analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Sample is placed between a light source and a photo detector, and the intensity of beam of light is measured before and after passing through the sample.

The absorbance of the standard solutions was determined at 282 nm. The UV spectroscopic analysis of eugenol extracted from *Ocimum sanctum* shows the absorbance between 250 to 280 nm.

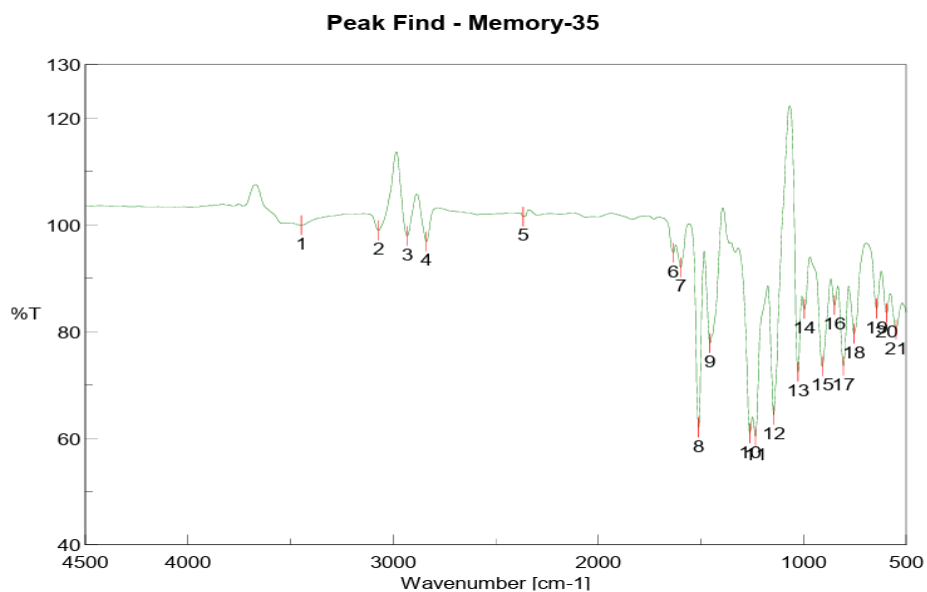


**Fig 4.5: UV- visible spectroscopic graph**

### 4.2.2.2 Fourier transform infrared spectroscopy

FTIR is the most powerful tool for identifying the types of chemical bond and functional groups present in compounds. The wavelength of light absorbed is characteristic of the chemical bond. By interpreting the infrared absorption spectrum, the chemical bond in a molecule can be determined.

The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peak values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on the ratio of its peak. The results of FTIR analysis confirmed the presence of alcohol, phenol, alkanes, aldehydes, aromatic compound, secondary alcohol, aromatic amines, and halogen compound.



| [ Result of Peak Picking ] |          |           |     |          |           |
|----------------------------|----------|-----------|-----|----------|-----------|
| No.                        | Position | Intensity | No. | Position | Intensity |
| 1                          | 3447.13  | 99.8952   | 2   | 3071.08  | 98.9372   |
| 3                          | 2930.31  | 97.9051   | 4   | 2837.74  | 96.8582   |
| 5                          | 2366.23  | 101.511   | 6   | 1634.38  | 94.7649   |
| 7                          | 1598.7   | 91.9948   | 8   | 1510.95  | 61.9526   |
| 9                          | 1455.99  | 77.8007   | 10  | 1261.22  | 60.8828   |
| 11                         | 1234.22  | 60.3513   | 12  | 1145.51  | 64.3709   |
| 13                         | 1026.91  | 72.4602   | 14  | 996.053  | 84.2145   |
| 15                         | 908.308  | 73.4538   | 16  | 849.49   | 84.9539   |
| 17                         | 805.135  | 73.5835   | 18  | 754.031  | 79.5631   |
| 19                         | 644.108  | 84.2523   | 20  | 594.932  | 83.4479   |
| 21                         | 548.649  | 80.3308   |     |          |           |

**Fig 4.6: FT-IR spectrum of compound isolated from *O. sanctum* essential oil**

The characteristic band at 3447 cm<sup>-1</sup> (for O-H group) indicate the presence of phenol group, 2930 cm<sup>-1</sup> (for C-H stretching), 2366 cm<sup>-1</sup> (for C-H stretching), 1598 cm<sup>-1</sup> (for C=O stretching), 1455 cm<sup>-1</sup> (for C-H bending), 1026 cm<sup>-1</sup> (for glycoside) were exhibited by the sample.

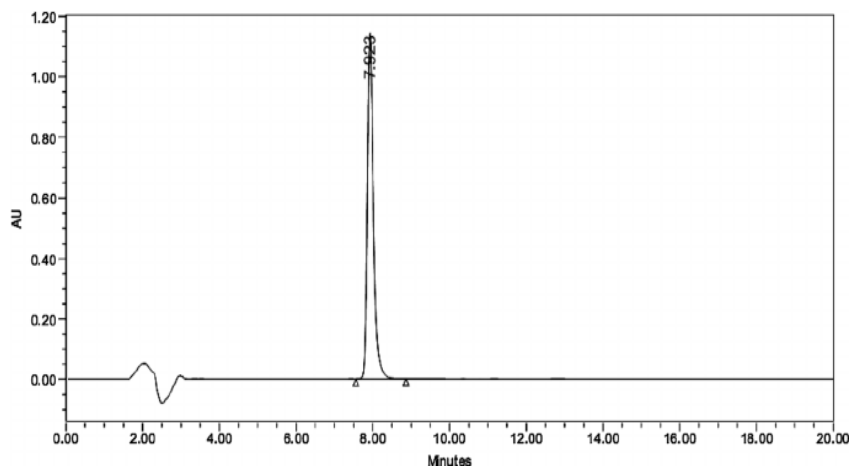
#### **4.2.3 Chromatographic analysis:**

##### **4.2.3.1 HPLC**

Separation using High Performance Liquid Chromatography is based on the affinity of the different compounds within the analyte to the mobile phase (eluent) and the stationary phase. The specific intermolecular interactions between the molecules of a component of the sample being taken up transitorily on to the stationary phase.

The greater the interaction with the stationary phase compared with the mobile phase, the longer the time spent interacting with the stationary phase, the longer the time spent on the column and the longer the retention time (R<sub>f</sub>) for that component. The power of the technique comes from the wide range of mobile and stationary phases that may be used to fine tune separations.

Retention time for Eugenol extracted from plants will exhibit between 7 to 14 minutes. Retention time for the sample is recorded at 7.923 minutes.



**Fig 4.7: HPLC graph of Eugenol extracted from *Ocimum sanctum***

## 4.2 ANTIOXIDANT ACTIVITY ASSESSMENT:

The 1,1-diphenyl-2-picrylhydrazyl radical is a stable free radical used to determine the antioxidant activity. The single electron of the nitrogen atom in DPPH is reduced to the corresponding hydrazine by taking a hydrogen atom from the antioxidants.

### **4.2.1 DPPH assay:**

#### *Sample stock solution preparation:*

Prepare sample solution by adding 10 microliters of eugenol sample to 25 ml of methanol.

#### *DPPH stock solution preparation:*

Now prepare the DPPH stock solution by adding 4 mg of DPPH to 100 ml methanol and covering it with aluminium foil. Keep the DPPH stock solution in the dark for 2 h due to its sensitivity to light.

#### *Dilution:*

Make a sample solution series of various concentrations by adding 1ml, 3ml and 5ml of sample solution to methanol and mark up the sample to 10 ml respectively. Mix the series solution by inversion. Now perform the DPPH assay by adding 1ml from each sample solution of different concentrations to 3 ml of DPPH stock solution and mark up to 10 ml with methanol.

#### *Storage:*

Place in the dark and allow the reaction to take place for about 30 minutes.

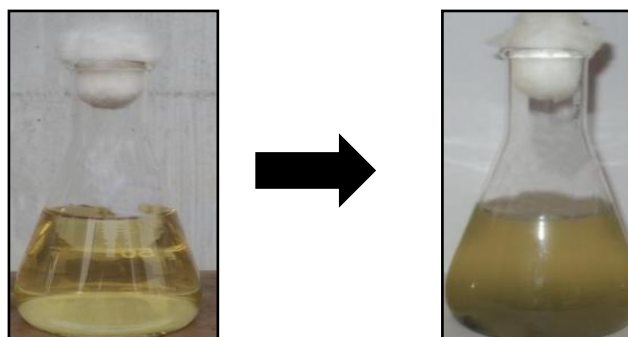


**Fig 4.8: DPPH assay workflow**

#### 4.4 ANTIMICROBIAL ACTIVITY ASSESSMENT:

##### 4.4.1 *E. coli* culture:

Prepare LB broth by adding 3.9 g of LB broth powder to 100 ml distilled water in a conical flask. Cover the conical flask with cotton plug and autoclave the mixture for 45 min at 121 degrees Celsius. Now inoculate 10 microliters of *E. coli* into the broth using micropipette and swirl to mix. Place the preparation in a shaking incubator and allow to grow for about 24 to 48 h.



**Fig 4.9: Preparation of *E. coli* culture**

##### Storage:

*E. coli* is maintained at freezing temperature of about -80 degrees Celsius to prevent the loss of the stain.

**Table 4.1 Composition of LB broth**

| MEDIUM          | CONCENTRATION |
|-----------------|---------------|
| LB broth powder | 3.9 g         |
| Distilled water | 100 ml        |

#### **4.4.2 Confirmatory test for *E. coli*:**

##### *Gram staining:*

Bacteria when stained with a primary stain and fixed by a mordant, some bacteria are able to retain the primary stain by resisting decolorization while other get decolorized by decolorizer. Those bacteria which retain the primary stain are gram positive while those bacteria which get decolorized and then get counterstained are gram negative.

##### *Procedure:*

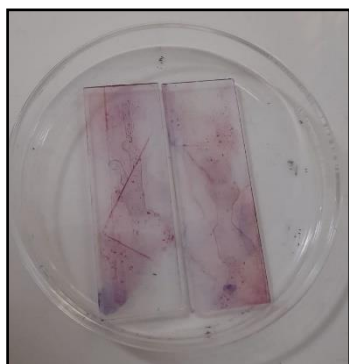
Add 2 to 3 drops of *E. coli* on the glass slide and spread it evenly. Heat fix the slide.

*Primary stain:* Add methylene blue stain on the slide and rinse it in running water after a minute.

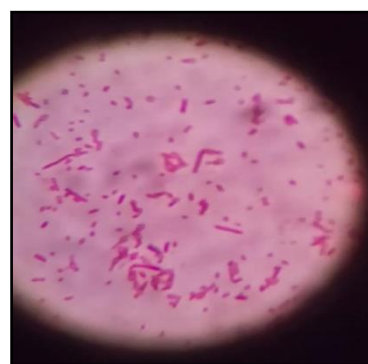
*Mordant:* Now add iodine solution and rinse it in running water after a minute.

*Decolorizer:* Add decolorizer and rinse it after 10 seconds.

*Counter stain:* Add safranin solution and rinse in running water after 30 seconds. Now visualize the slide under microscope.



**Fig 4.10: Slide view of gram staining**



**Fig 4.11: Microscopic view of gram staining**

#### **4.4.3 Disc diffusion:**

##### *Media preparation:*

Prepare growth medium for *E. coli* growth by adding 3.9 g of Muller Hilton agar in 100 ml of distilled water in a conical flask. Secure the conical flask with cotton plug and autoclave for 45 min. Pour the medium in a sterile petri plate and allow to solidify. Perform the procedure in laminar air flow cabinet to avoid contamination.

##### *Swabbing:*

After the medium got solidified sterile cotton swab is dipped into the *E. coli* suspension and spread the inoculum evenly over the plate to obtain uniform growth of the bacteria.

##### *Disc preparation:*

Prepare the antimicrobial disc by adding the sample to four sterile discs at various concentration ranging from 5,10,15,20 microliters. Allow the disc to dry and place the disc in the petri plate with appropriate distance between the disc.

*Storage:*

Secure the petri plate with parafilm and place it in the incubator for 24 h to allow *E. coli* growth.

***Table 4.2: Composition of Muller Hilton Agar***

| MEDIUM             | CONCENTRATION |
|--------------------|---------------|
| Beef infusion      | 300 ml        |
| Casein hydrolysate | 1.6 g         |
| Agar               | 1.5 g         |
| Starch             | 1.5 g         |
| Distilled water    | 1000 ml       |

## CHAPTER-5

### RESULTS AND DISCUSSION

#### 5.5 DPPH ASSAY:

Color changes from green to pale brown or pale violet will indicate the presence of antioxidant activity in the sample tested. Decolorization of deep violet color to pale brown indicated the antioxidant effect of the substance by mechanism of free radical scavenging activity.

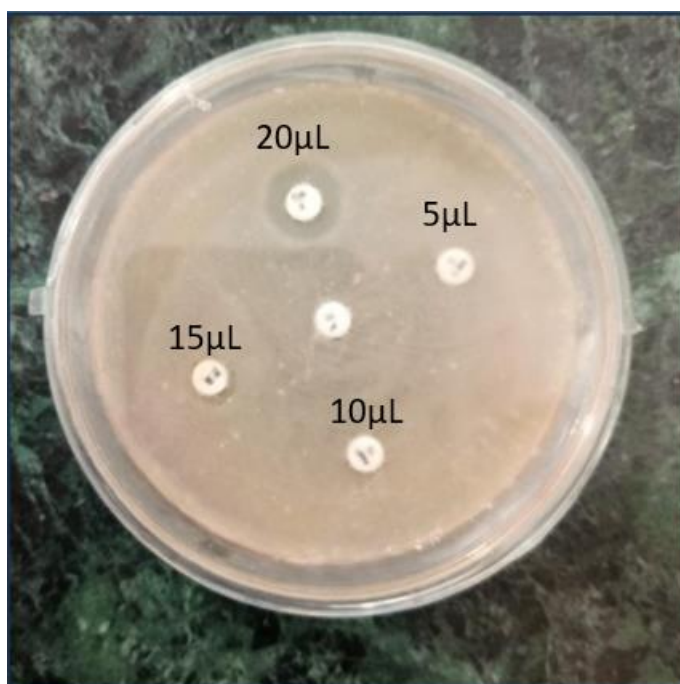
**Table 5.1 DPPH radical scavenging activity**

| <b>Sample</b>                 | <b>Percentage inhibition</b> |         |         |         |         |          |
|-------------------------------|------------------------------|---------|---------|---------|---------|----------|
|                               | 10µL/ml                      | 20µL/ml | 40µL/ml | 60µL/ml | 80µL/ml | 100µL/ml |
| <i>Ocimum sanctum</i> extract | 57.42%                       | 63.86%  | 67.32%  | 69.30%  | 75.74%  | 80.19%   |
| Marketed product              | 44.05%                       | 49.50%  | 57.92%  | 65.82%  | 69.86%  | 72.77%   |
| Ascorbic acid                 | 78.71%                       | 80.69%  | 82.17%  | 85.17%  | 89.10%  | 89.60%   |

From table 5.1 it is revealed that as concentrations of extract, marketed antioxidant and ascorbic acid increases, the DPPH scavenging activity also increases.

### 5.6 DISC DIFFUSION:

Disc with 20 microliters of plant extract eugenol shows slight antimicrobial activity. Against Gram-negative bacteria, eugenol is believed to act by damaging the cytoplasmic membrane. Being a hydrophobic molecule, it can easily penetrate the lipopolysaccharide cell membrane and enter the cytoplasm. Once present in the cell, it can cause alterations to the cell structure, resulting in the leakage of intracellular components.



***Fig 5.1: Disc diffusion***

## CHAPTER-6

### SUMMARY AND CONCLUSION

The experimental setup outlined in this paper offers a practical way to separate medicinal constituents from plant material, which is then subjected to a chemical analysis employing a growing number of analytical methods. Simple tests, like color testing, make the experiment relevant even in laboratories with little resources, while more complex methods, like UV and FTIR, are better suited for graduate and undergraduate students in college. We can determine *Ocimum sanctum*'s antioxidant activity within the limitations of the study. This chapter explains how to test eugenol's ability to scavenge free radicals and/or limit the generation of radicals in order to assess its antioxidant activity.

The test for Eugenol's antimicrobial activity was conducted. Gram-negative E. Coli bacteria, a subset of gram-positive bacteria, were used in antibacterial tests. Mueller Hinton Agar is an excellent medium for antibacterial testing and was utilized in this experiment. The FDA and WHO have endorsed MHA for antimicrobial testing due to its ability to support good pathogenic development and its combination of sulfonamides, trimethoprim, and mild tetracycline inhibitors. The instruments and agar medium that will be used for the tests must first be sterilized in an autoclave set at 121 degrees Celsius. In order to prevent interference with and contamination of antibacterial testing, sterilization is performed in order to eliminate live microorganisms and spores. Experiments involve creating multiple variants of concentrations. For active substances, these variations are 5 $\mu$ L, 10 $\mu$ L, 15 $\mu$ L, and 20 $\mu$ L, which are derived from the diameter of the greatest inhibitory zone measured when testing the active ingredients. Antibacterial activity tests were conducted over the course of a 24-h incubation period. The findings of these tests were defined by the existence of clear

zones surrounding the disc, which denoted the absence of bacterial colonies growing in that region. This clear zone is known as the inhibition area diameter.

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