**EFFECT OF ELECTROMAGNETIC RADIATION ON EUGENOL CONTENT DIFFERENT SPECIES OF *OCIMUM***

**SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT**

**FOR THE AWARD OF THE DEGREE OF**

**MASTER OF TECHNOLOGY**

**IN**

**BOTANY**

SUBMITTED BY:

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## Abstract

Eugenol is a secondary metabolites and essential components of various plants such as Ocimum, Clove, Cinnamon and Nutmeg. The eugenol has a medicinal properties help to cure toothache as well as it is also act as anticancer properties.

In the present study the effect of electromagnetic on eugenol concentration *Ocimum gratissimum* and *Ocimum sanctum*. It was observed that as the frequency of radiation increased up to a limited extend the concentration of eugenol also increased of about 7.50 GHz range, whereas the frequency increased a decline in the concentration of eugenol was observed.

The result were justified by using HPLC, TLC and UV spectrophotometer. Hence EMR radiation up to a limited extend expose to *Ocimum gratissimum* and *Ocimum sanctum* can increase the concentration of eugenol. So, that it can be used for wide range of application.

## Acknowledgement

I take this opportunity to present my votes of thanks to all those guidepost that really acted as lightening pillars to enlighten my way throughout this project that has led to successful and satisfactory completion of this study.

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I also acknowledge with a deep sense of reverence , my gratitude towards my parents and member of my family, who has always supported me morally as well as economically.

Finally, I wish to extend my warm thanks to everybody involve directly or indirectly in my work.

Thank you

Sharddha Suman

# DECLARATION

I, Sharddha Suman hereby declare that this project report entitled "**Effects of Electromagnetic Radiations on Eugenol content in *Ocimum sanctum*"** is carried out by me under the supervision of **Dr. Leena Parihar** for the partial fulfillment of degree of M.Sc Botany (Honours).

**Mentor signature**
Dr. Leena Parihar

**Student Name**
Sharddha Suman

## CERTIFICATE

This is to certify that Sharddha Suman has started research work entitled "**Effects of Electromagnetic Radiations on Eugenol content in *Ocimum sanctum*"** under my guidance and supervision. To the best of my knowledge, the present work is the result of investigation and study. No part of the project has been submitted for any other degree or diploma.

The project is fit for the partial fulfillment of the conditions for the degree of M.Sc Botany (Honours) from Lovely Professional University, Phagwara, Punjab.

**Signature of Supervisor**

Dr. Leena Parihar

Assistant Professor

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## Table of content

# CHAPTER-1

# INTRODUCTION

The spectrum is a scientific word which describes all the entire range of light of wavelength that exists. From radio waves to gamma rays, most of the light in the universe is, in fact invisible to us (Crockett, 2013). Electromagnetic radiations (EM) radiation is the radiation which make up the electromagnetic spectrum are X-rays, infrared light, microwaves, ultraviolet light, and gamma rays (Mattson, 2014). Sunlight is also a type of EM energy but visible light is come under a small portion of the EM spectrum, which contains a wide range of electromagnetic wavelengths (Lucas *et at*., 2015).The interaction of electromagnetic radiation on human particularly the brain concern with the increase use of mobile communication the effect on neuronal activity and various brain diseases like blood brain barrier permeability, neurotransmitter balance, genomic response, sleep, cognitive function, including brain tumor (Hossmann, *et al*., 2002).

Electromagnetic waves from mobile phone shows effects on human ejaculated semen. Mobile phones are used frequently in day today life. As the demand of using mobile phone increases, the expose of the harmful effect of electromagnetic radiation is also increased. Study was conducted to check the effect of electromagnetic radiation on the human semen resulted infertility in male. Some group was exposed to harmful electromagnetic radiation which showed a significant decrease in viability and sperm motility and increase in “reactive oxygen species (ROS) and DNA fragmentation index (DFI)” as compared to unexposed group. It concludes that mobile phone which leads to emits electromagnetic waves shows some oxidative stress in human semen and also causes mutation in DNA fragments. The study speculate that these radiation may negatively affect spermatozoa and impair male fertility (Veerachari *et al.*, 2012). Interest are fastly growing in today’s society for the potential causing harm or damage effects of radio frequency electromagnetic fields to the health of the common peoples of society. More specifically, here it is great importance for the effects of electromagnetic radiation (EMR) on the action of developing and function of the brain, especially with the being used wide spread use of telephones made up of cells that is cellular Rf (Retardation Factor) exposure is directly to the head. In 2006 WHO issued a Research Agenda for priority research into the effects of radio frequency fields. Among the research needs recognized were pre- and post-natal animal studies for radio frequency exposure and its capabilities of developing operations on cognitive performance. Therefore the rationale for the present study was that "behavioural studies with immature animals may provide a useful indicator of possible cognitive effects in children" (WHO 2006 Research Agenda for Radio Frequency Fields).

| Types of radiation | Frequency | Wavelength |
|---|---|---|
| Radio waves | $<3x10^{11}$ Hz | >1mm |
| Microwaves | $3x10^{11}$ - $10^{13}$ Hz | 1mm - 25um |
| Infra red | $1x10^{13}$ - $4x10^{14}$ Hz | 25um - 750nm |
| Optical waves | $4x10^{14}$ - $7.5x10^{14}$ Hz | 750nm - 400 nm |
| Ultra violet | $10^{15}$ - $10^{17}$ Hz | 400nm - 1nm |
| X-rays | $10^{17-}10^{-20}$ Hz | 1nm-1pm |
| Gamma rays | $10^{20-}$ $10^{24}$ Hz | $<10^{-12}$ m |

Snow *et al*., (1997)

**Table no. 1 Showing frequency and wavelength of electromagnetic radiations**

It has been proven that the rats which are exposed, to GSM 900 electromagnetic radiation, caused severe structural damage to the brain. In the pyramidal cell layer there is a darker, shrunken neurons in the hippocampus. Due to this damage there is a leakage of albumin from the blood brain barrier into the brain. Radio frequency field is the reason to increase the permeability of the blood brain barrier which provide a large plasma proteins like albumin to secret into the brain and resulted structural damages (Eberhardt *et al.*, 2008)."The hippocampus is an important as well as integral part of the brain's limbic system and its mineral corticoid and glucocorticoid receptors are involved in behaviour regulation as well as regulation of the hypothalamo-pituitary adrenal (HPA) axis", (Sapolsky *et al.*,1984*;* Gewirt *et al.*, 2000*;* Gold *et al.*, 2002*;* Ziegler and Herman, 2002*;* Kellner and Wiedemann, 2008). It is also a site of long-term potentiation (LTP)—the cellular mechanism believed to underlie learning and memory. In the hippocampus the damage of neurons lead to behavioural disturbances, impaired learning and memory, as well as negatively impact the functioning of the hypothalamo pituitary adrenal axis. Some other effects of electromagnetic radiation may also cause some harmful effects on brain. Many laboratory and clinical animal has been targeted to studies the effect of environmental conditions on the brain and behavioural development of them (Heim *et al.*, 1997*;* Daniels *et al.,* 2004). Some of the children for instance who have been disturbing experiences show an increase in depressive disorders, abnormal behaviour and anxiety disorders in adulthood (Nemeroff, 2004; Wals and Verhulst, 2005). Due to these effects the development of brain has retarded for life long. Studies was basically held by using rodent also shows early life trauma to observe affect behaviour in adulthood (Jezová *et al.,* 2002*;* Sullivan *et al.,* 2006). Rat pups to maternal are separated within 3 h between postnatal day 2 and 14 (Daniel *et al*., 2004).The rationale is the procedure of the significant to long term changes in behaviour as well as brain chemistry. These suggested that the brain is able to face the environmental factors at this age, and that harmful effect can cause long lasting damage to brain function. Exposure to electromagnetic radiation, as an environmental factor, would yield similar results. The exposed rat pups to EMR from post-natal day 2 to14 for 3 h per day. They hypothesized that exposure to electromagnetic radiation early in the life of rat pups will lead to abnormal brain development, impacting negatively on their behaviour during adulthood (Daniels *et al*., 2009). Our surrounding environment is subjected to expose to microwaves and (radio) electromagnetic radiation. As a result of spreading among in very large number use of wireless telecommunication. This yield produces a very large and heavily increases in electromagnetic pollution. The portable telephone that is mobile phones can be operated on wireless technology using a 900-1800 MHz (GSM) channel and 2200(UMTS/3G) signals (Verschaevel, 2009). There is an important of capability of being done adverse effects of mobile phone radiations. As a result of the very large increase in the using of these phones throughout the world. The potential situation that involve being exposed to danger of electromagnetic field (EMF) emitted by mobile phones on living systems has been studied in very short period with lots of efforts. Most of the detailed investigations were adapted amount of light on human health (Seitzh *et al.,* 2005). A literature review published between 2000-04 (Erogulo *et al.,* 2006). Many other studies were conducted on genetic (Ruediger *et al.,* 2009) and biological (Barterim *et al.,* 2004) effect of portable telephones on system that is relating to living things as biological system and including plants also.

Plants are well known for the production of organic compounds and oxygen. In the natural environment, they are also exposed to sequences without interruption of electromagnetic fields. Plant of tomato were exposed to a low level of electromagnetic fields (EMF) (900MHz 5V m-4) for 10 minutes to check change in the abundance of three wound-induced transcripts, plays a very important role in the early events of all plant responded to stress (Rouxd, 2006). There is suggestion that application of radio frequency field has a stimulus effects on tomato plants, resulting in accumulation of stress- related transcripts. Duckweed *(Lemna minor L.*) was exposed to EMF for almost two hours for the investigation of the physiological response of the plan (Tkale *et al.*, 2009). The influence of oxidative stress was induced, especially at 900 MHz, by exposure of the Duckweed to the exposure of non thermal radiofrequency field, most probably due to the effect on anti oxidative enzyme activities. Electromagnetic radiation have the inhibitory effects on the growth of root of Mung beans (*Vigna radiate*) has been investigated recently, Sharma, (2009). Important inhibition of the growth of root has been observed as a result of the application of a mobile phone electromagnetic field by the inducing reactive oxygen species generates the oxidative stress in the manner of time dependent *Allium cepa L.* Seed were exposed to frequency of radio EMFs (400 and 900 MHz, at different strength for two hours) for the investigation of the effects of this application on the growth of roots, mitotic activity and mitotic aberrations of *Allium cepa* L, meristem cell. It states that the root's development and germination rate were not renew depending on specified method, but there will be a "mitotic division and mitotic aberration such as lagging chromosomes, vagrant, chromosome sickness, and disturbed anaphase" were induced. The reason for the increase in the mitotic abnormalities may be explained with the imparing of the mitotic spindle due to electromagnetic radiation (Tkalec *et al*., 2008)

The genus *Ocimum*, (Lamiaceae), also called basil has long been recognized as a diverse and combination of essential oils. *Ocimum* contains average 50 to 150 species of herbs and shrubs from the tropical regions of Asia, Africa, Central and South America and semitropical (Bailey 1924, Hortus *III* 1976, Darrah 1980). Plants have square stems, fragrant opposite leaves, and whorled flowers on spiked inflorescences (Darrah, 1980). The medicinal plants are playing an important role on the traditional medical practises for curing various diseases. In prevention of treatment of cancer, tulsi play an important role. Health benefitting effects by reducing stress and improving both cellular and humoral immunity is also a major role of tulsi and thus may be a new approach in theraphy of cancer and prevention of ill effects of radiation (Singh; 2012). In Ayurveda and Siddha systems there are uses of different parts (leaves, stem, flower, root, seeds and even whole plant (Kumar *et al*.,2013). A small herb are spread throughout India, which has been recommended for the treatment of various diseases like bronchitis, cold, cough, bronchial asthma, malaria, diarrhoea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever, and as an antidote for snake bite, scorpion sting, insect bite, etc. (Prakash *et al*., 2004, Godhwani *et al*, 1988). Seed of *Ocimum sanctum* possess anticoagulase properties. Many species belong to this species possess many medicinal values and have high range of application on indigenous system of medicine. *Ocimum sanctum* has been been associated with alkaloids, glycosides, tannins and saponins. Rai et al ; 1996. The *Ocimum sanctum* has also been suggested to possess antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, antiemetic, antispasmodic, analgesic, adaptogenic and diaphoretic actions (Prakash *et al*., 2004). Using medicinal plant as a remedy does not cause any harmful effect to us more over it is less expensive, efficacy (Singh *et al*., 2011).

*Ocimum* gratissimum is mainly widespread over the tropical countries. It has been considered as a most chief used in folk medicine (Dubey *et al*., 2000). It is famous for food spice (Chiu *et al*., 2012). The whole plants and their essential oil have various applications in medicine (Asia and Africa). Their preparations are used to treat sunstroke influenza and headache. The seed are known to have laxative properties and can also act against gonorrhoea. Despite of that it is also used to treat fever inflammation of throat, ear, eyes, stomach pain and skin diseases (Orwa *et al*., 2009).

Eugenol is a phenyl propanoid which is chemically an allyl chain substituted guaiacol. It is weakly acidic, slightly soluble in water and is soluble in organic solvents. It is a clear to pale yellow liquid with characteristic and pleasant odour and spicy pungent taste. In

detergents and soaps large quantities of Eugenol are used for their spicy aroma but they cause discoloration due to their phenol structure. Eugenol has the ability to alleviate tooth pain and hence it is ideally used in dental clinics as a routine analgesic agent. The anticancer activity of several essential oil constituents including Eugenol have been investigated (Kamatou, 2012).Eugenol (4-allyl-2-methoxyphenol) is a fragrant compound that is commonly present in various sorts of plants, especially in spices and medicinal herbs. Eugenol is frequently used in dental and oral hygienic practice. Eugenol
has various significance such as dental analgesic anti-microbial, anticonvulsive and activities. Besides, anti-inflammatory and antioxidative activities of Eugenol Alzheimer's disease can be treated with the help of Eugenol. Eugenol can inhibit the excessive influx of calcium ion in neuron induced by β component it leads to the neural damage. Eugenol is also known to possess an antidepressant activity and hence increase expression of brain Neurotrophic derived factor. Thus Eugenol can be a good medicine for depression and Alzheimer's disease. For the treatment of Central Nervous System (CNS) i.e. Parkinson's disease Eugenol and analogue can also be used.

| Species | Alkaloids | Medicinal properties | References |
|---|---|---|---|
| *Ocimum gratissimum* | Eugenol, methyl eugenol, cis-ocimene, Thymol-p-cymene—y-terpinene, pinene, camphor, trans-ocimene germacrene- D, trans-carypohyllene, farnesene and l-bisabolene, | Treating gastrointestinal, good anti-diabetic and anti-cancerous properties disorders, | (Mondello et al., 2002), (Yayi et al.,2011) |
| *Ocimum sanctum* | Thymol or sesquiterpene alcohols eugenol methyl ether and carvacrol, ursolic acid flavonoids such as apigenin**,** polyphenols**,**anthocyanins and luteolin**,** eugenol**,** | Digestive problems, controlling ring worm and skin diseases,anti-inflammatory, antiarthritic, antistress, antipyretic**,**treating fever, cold, cough, bronchitis, | (Singh *et al.*,1996), (Prakash., *et al*., 2004), (Godhwani et al, 1988), (Yayi et al.,2011) |
| *Ocimum basilicum* | linalool and methyl chavicol, eugenol and 1,8-cineole | Lower the blood pressure, cleansing the body, lowering the stress, lowering cholesterol and as antispasmodic, blood cleanser, detoxifier | (Yayi et al, 2011) |

**Table no. 2** Different species of *Ocimum* with their Alkaloids and Medicinal properties

# CHAPTER-2

# TERMINOLOGY

**EMR:**

Electromagnetic radiation Radiation.

**HPLC:**

High Performance Liquid Chromatography.

**$CH_2Cl_2$** :

Dichloromethane.

**CNS**:

Central Nervous System.

**ACN:**

Acetonitril.

**OPA:**

Ortho Phosphoric Acid.

# CHAPTER-3

## REVIEW OF LITERATURE

EMR (electromagnetic radiation) are very short wavelength with high energy such as gamma rays and x-rays and may cause physical damage to matter that comes upon its ways or can also cause ionization. This can also effect the living organisms the EMR radiation posses high energy and hence can able to break the molecular bonds and cause damage to cell (Pietruszewski *et al*.,2007) in his study used EMR radiation that does not cause damage to the cell and hence called non ionizing radiation but these radiation sometimes cause certain biological effects. Microwaves also have less energy and hence incapable of disrupting the molecular bonding but the water present in tissue absorbed these radiation and these develops a strong current which cause heat generation in tissue, (Pietruszewski et al.,2007).The strength, density of any source of EMR is not only related to the strength level of the source, but increases rapidly as the distance from the source of EMR decreases. A familiar thing was observed that more nowadays people are very much used to mobile phones which may radiate harmful radiation near person's head. Cell phone, radiate very small range of EMR. So, even while they are very close, they are not considered as danger. EMR from cordless and hand-held radios have very less energy that is caused to ionization or damage to DNA present in human tissue. The widespread use of this technology, has risen concerned about the possible severe health issues that may occur, particularly brain cancer. Various studies have been conducted in United States and other countries which highlight these concerns, any association between EMR from these devices and cancer *(*Zamanian *et al.*, 2005). Electromagnetic waves which are released from mobile phone operating at 1800 give a shows the effect in mitotic division, germination, root growth of tips of *Lens culinaris Medik*. Electromagnetic waves which release from mobile phone will be one of the reasons of seed dormancy as compare seed germination emitted. The state of dormant seed the root growth decrease that is due to the oxidative stress (Akbal *et al.*, 2012). Harmful effect of electromagnetic radiation increase which create human health issue which include male infertility. Whereas Some sample were exposed to electromagnetic radiation and it was observed that there was motility as well as viability in the sperm sample under consideration, increase in reactive oxygen species and DNA fragment index compared to unexposed group. And hence the conclusion of this study was that the mobile phone emit electromagnetic waves which leads to oxidative stress in human semen and also cause mutation. It was speculate from study that these radiations may negatively affect spermatozoa and impair male fertility (Veerachari *et al.*, 2012*).* There is some essential oil found in different species of *Ocimum. Ocimum basilicum* contain (Z)-methylcinnamate, geraniol,(E)-methyl cinnamate, linalool, camphor, *Ocimum gratissimum* comprises eugenol,(Z)- bocimene, germacrene D, bcaryophyllene, *Ocimum sanctum* methyl eugenol, bcaryophyllene, germacrene D as main constituents (Jirovetz *et al.*, 2003). Electromagnetic radiation is emitted from some surrounding materials i.e. household appliances and mobile phones, (Salford *et al.*, 2003). When the presence of strongly stained areas in the brains of rats that were exposed to mobile phone electromagnetic radiations was studied and it was found that these darker neurons were particularly most prevalent in the hippocampal area of the brain. The aim of this study was to check effect of electromagnetic radiation on the brain cells. Since hippocampus which is responsible in a function of memory, learning, and emotional states. Here, the negative effects of EMR in subjects were studied on both male and female rats were exposed to EMR radiation to study electromagnetic effects on them. It was observed that some animal exposed to it shows less locomotors activity, increased grooming and a tendency of increased basal corticosterone levels. These observation suggested that harmful exposure of electromagnetic radiation may lead to abnormal brain functioning in animals (Daniele *et al*., 2009).

Tulsi in management of neurological (e.g convulsions & epilepsy), inflammatory and allergic disorders (Sen,1993). *Ocimum sanctum* help to fight against gastric ulcer because it have antiulcerogenic action of eugenol and essential oil extracted from Tulsi leaves, (Dey *et al.*, 1993); Lowering of uric acid level by extract of Tulsi leaves and eugenol claims the therapeutic potential of *Ocimum sanctum* in treatment of rheumatoid arthritis (Sarkar *et al.*,1990)

*Ocimum sanctum* (also Tulsi or Holy Basil) belongs to the family Lamaiceae aromatic plant present worldwide and an escaped weed. *Ocimum sanctum,* is known as Holy Basil in English and in Hindi they are aromatic herb having soft hairy branch. Widely present in India. It is a shrub reaching a height of 0.5 to 1.5 m. The leaves are 2-4 cm in length, (Dev *et al.,* 2011). The essential oil from *Ocimum sanctum* posse's antibacterial and anti fungal activity as well as it also has some anticoagulase factor. The *Ocimum sanctum* extracts are rich in alkaloids, saponins and tannin. The tulsi plants were found to be rich in fibre that may help to reduce the risk of colon cancer diabetes and some cardiovascular disease (Rai et al., 1996). In order to check the antifertility effect of O. sanctum (Kasinathan et al., 1972) the *O. sanctum* leaves were fed to the sterile male albino mice in the regular diet then the biochemical and histological changes in the reproductive organ of the mice was observed it was found that the adrenal,prostate glands, adrenal glands showed decreased in weight. There was no proper development of spermatid bundles,as well as few cells were found to be degenerative. It was also observed that the pH of seminal vessel was reduced and hence the spermatozoa motility was effected. hence the *Ocimum sanctum* act as a antifertility agent. *Ocimum sanctum* is enriched with therapeutic properties (Dharmani *et.al.*, (2004) conducted a study in which the antiulcerogenic in cold restraints, asperin, alcohol, pyloric ligation i.e. gastric ulcer models in sprauge and histamine induced duodenal (HST) cancer were tested and found *Ocimum sanctum* can healed the ulcer in 20 days. It posses uler healing activity, the *Ocimum sanctum* was very much effective to reduce the risk of hepatotoxic induced by paracetomol. A significant decrease in the level of serum enzyme was observed under the study conducted by (Chattopadhyay *et.al.*,1992). According to (Kath *et al.*, 2012). *O. sanctum* showed significant activity at the doses of about 100mg/kg can be very much helpful to prevent the induced by gastric ulcer induced by aspirin by inducing the contraction in rats uterus.

It carries numerous medicinal value and has solution to various diseases. It act as a antimicrobial,antiinflammatory,cardioprotective,adaptogenic,antidiabetic,hepatoprotectie, anticarcinogenic,radioprotective,immunomodulatory, neuroprotective, etc. It anticancer activity is proved by studies performed in biological model like fibrosacrcome cell culture papilomas in the skin of albino mice, mice having sarcoma-180 tumors etc, given a proof for its anticancer activity (Singh; *et al.*,2012). Different species of *Ocimum* contain different type of essential oils example eugenol, linalool, camphor, and methyl cinnamate. *Ocimum sanctum* contain several antibiotic compounds, these compound contributes a lot to human health and easily available throughout the year for cosumption. It also play a important role to maintain the nutritional value in our body. It can be said that it perform like a safe guard against various deficiencie (Singh.,2012,). Allergy is a common disease that has various manifestations .in certain cases allergy can also leads to death . various epidermological studies had revealed that it cause the upper and lower respiratory tract allergiesand one of the reason for these disease may be the increasing the environmental pollution. (Sridevi; et al.,2008) stated that the *Ocimum sanctum* was used in the pre historical times for the curement of various ailments such as antitode of snake, skin disease hepatic disorders, it has also being used for the treatment for the scorpion sting. New studies also revealed that the *Ocimum sanctum* leaves were also beneficiary in terms of having anticancer antianalgesic, antidiabetic.

*Ocimum gratissimum* is a small shrub having fragrant leaves, it is also known as tea bush. It has a brached stems, it is a shrub of about 1.9m height. The leaves are ovate too ovate-lanceolate are 10×5 cm. It coarsely crenate and has serrate margine. It is subacuminate to acuminate at the apex and cuneate and decurrent at the base. The leaves consist of pubscent and dotted on both the sides (Prabhu et al.,2009)Due to its strong aromatic odor the people of Nigeria used it as a flavouring agent in soups. Traditionally it is a best remedy for diarrhea (Okoli; et al.,2010). *Ocimum* species contain various essential oil that posses medicinal properties. These oils have chemopreventive, anticarcinogenic free radical scavenging and radio protective uses. When *Ocimum gratissimum* (OG) leaves were extracted in ethanol they have found to be have a significant by effect on chemical induced papilloma generation. This occurs by modulating the cytochrome P450 hydrocarbon hydroxylase etc. Recently this has been reported OG can reduce the oxidative toxicity activity just by consuming the extract of OG leaves orally. It also helps to enhance the particular activities of antioxidant enzyme in liver. The OG extract can be also be very much beneficiary in preventing H9c2 cell death inhibiting the apoptosis (

mitochondrial dependent apoptosis pathway), (Chiu; et al.,2012). Leismaniasis is caused by the genus of leishmania and it has severe consequences in human. This disease can be controlled with the help og oil from *Ocimum grastissimum* having Leishmanicidal activity. At the concentration of eugenol of about 100 to 1000µg/ml. The *Ocimum gratissimum* can efficiently inhibit the growth of Leishmania amazonesis and hence the oil of *Ocimum gratissimum* can be used as antileishmanicidal drug preparation, (Nakamura et al.,2005).

For the treatment of dermatophytosis the more effective and less toxic antifungal agent development is required. The medicines obtained from the *Ocimum gratissimum* can be used for the treatment of infectious disease. The extracts which are obtained from the leaves of *Ocimum gratissimum* were accessed for in vitro antifungal activity through agar dilution technique which are used against the dermatophytes. The antifungal activity is produced by the extract which are used against Microsporum canis, M. gypseum, Trichophyton rubrum and T. mentagrophytes. Trichophyton rubrum are one of the most common aetiological agent of dermatophytosis found in Goiania, state of Goias, Brazil was also at risk with dermatophyte. The eugenol and hexane fraction were the most effective. Hexan fraction stop the growth of dermatophytes at 125µg $ml^{-1}$ concentration and 80% of the dermatophytes growth is inhibited by the eugenol at the same concentration, the result obtained from the extraction of *Ocimum gratissimum* are mostly active against human pathogenic dermatophytes (Silva et al.,2005). It has been reported that the in vitro and in vivo growth of pathogen (i.e anthracnose disease of cowpea) or its germination process can be reduce or halted by using hot and cold extract of *Ocimum grastissimum* and *Cymbopogon citratus* on cowpea (Amadioha et al.,1999).

Eugenol and the essential oils also show the properties to be immune stimulant claiming the therapeutic potential *of Ocimum sanctum* in immunological disorders associated with immune suppression (Rajeshwari., *et al.*,1992). Eugenol is used as a flavouring agents in the food industry, has a different variety of biological activity, and can be performed as a biomarker. The leaves of *Ocimum*, contain maximum number of eugenol which are used as a herbal medicines, (Joshi., *et al.*,2011).Eugenol(l-hydroxy-2-methoxy-4-allylbenzene) play a important role, the active constituent present in *Ocimum sanctum*, it has a potential to cure the diseases. Although because of its great therapeutic potentials and wide occurrence in India the practitioners of traditional systems of medicine have been using, (Prakash*., et al*., 2005). *Ocimum* which are produce throughout the world is famous for its medicinal properties. Different parts of tulsi play a important role in medicinal value. Eugenol is a naturally occurring phenolic compound, it is count under one of the important compound of basil and exists to a lesser extent in oil of several other plants. It have a properties of analgesic, antiseptic, anti-inflammatory and antibacterial properties. Tulsi leaves possess a volatile oil which is very much effective against bacteria and insect. The essential components of these tulsi are eugenol, carvacsol, methyl eugenol, caryophylene, they also yield other substance such as apigenin and ursolic. The tulsi was pharmalogical investigated for antistress, antiinflamatory, antiasthmatic, antimicrobial, hypotensive, hypoglycaemia and analgesic activity. It also posses antioxidant activity, earlier the tulsi leave were very much used in demulcent, cough respiratory tract infection general stress syndrome, fungal infection and also as a deusetic (Thakur *et al*., 2009). Ayurvedic medicine is play a vital role from ancient period of time. *Ocimum* (Tulsi) is one of the important agent for extraction of some essential oils for experimental process as well as therapeutic use, (Hahn, *et al*.,2013). *Ocimum sanctum* have huge number of biological potential to cure several number of diseases, (Rahman, *et al*.,2011). *Leporinus macrocephalus*, a fish species was used as a test organism to evaluate the anaesthetic effect on *Leporinus macrocephalus*, was exhibited by eugenol. Fast and safe anaesthesia could be achieved by using dose of 37.5 mg/l of eugenol. Various toxic effects are caused by synthetic drugs and hence prevention and treatment of cancer can be achieved by natural agents that can inhibition and promortional events associated with carcinogenesis, the anticancer activity of several essential oil constituents including eugenol have been investigated. The cell viability of HeLa cell was reduced by eugenol. Anticancer activity of eugenol was tested alone and in combination with chemotherapeutic (gemcitabine). Dose dependent selective cytotoxicity towards HeLa cells was showed by eugenol in comparison to normal cell (Kamatou *et al*.,2012). *Aspergillus parasiticus* well known to produce mycotoxin which is very much toxic if consume. A study conducted by (Jayashree *et a*l.,2002) eugenol inhibited the aflatoxin production. A study conducted by Henglin et al., (2013) found that about 0.3% of eugenol provides about 80% better effect than that of the fungicides that are commercially available such as famoxadane and cymoxanil etc it was also observed that the eugenol was helpful in increasing the yield of the crops by 51.09%, hence from this study it can be said that eugenol can be used as a environmental friendly method to prevent the crops from fungal effect. Eugenol has a property to impart flavour, sensitizer, irritant it can also generate little anaesthesia. Sometime bacterial infection can also leads to increasing the risk of cancer. The eugenol can posses various antibacterial antiviral properties that can help the mankind to recover from dreadful diseases caused by pathogenic microbes (Pavitra et al.,2014). Eugenol is also have the hydrophobia property it facilitate the separation of the lipids from cell membrane and mitochondria of pathogenic organism. Organism cell wall is changed due to lipid losses and hence the permeability also increase. It has also been reported eugenol are capable of blocking proton motive force active transport, electron stream and hence cause coagulation of cell content. Eugenol has insecticidal effects and hence used in worldwide agricultural field. They have also some effects in immune regulation and reproductive regulation. It is used in therapy after combining with classic drugs eg; Aspirin, eugenol ester, Ibuprofen eugenol ester. Sometime eugenol were also reported to reduce the side effects of certain drugs( Kong et al.,2014).

# CHAPTER-4

## SCOPE OF STUDY

In our present lifestyle our fitness has become one of the most important part of life and allopathic medicines has become trend to boost our immunity and are mostly preferred by people from instant relief from various health issues, which may later on causes harmful effects in our body or fatal in some case, (Kumar *et al*.,2013). Natural drugs improvement application have been continued for thousands of years. As eugenol is a plant extract, it has a very wide range of application in history. In recent years, due to its low toxicity and less residual activity, eugenol has been now commonly used as a component of agriculture, forestry, animal husbandry, and veterinary medicine. Tulsi has various medicinal properties which is a boon against various dreadful diseases like "asthma, diabetes, as well as some common frequently occurring disease *viz* diarrhea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever" etc. Each part of a *Ocimum* is useful to mankind. Some important essential oils are used as antifungal, antibacterial as well as antiviral agents, (Singh *et al*.,2011). Electromagnetic radiation cause damages to plants that is it will inhibit the growth and development of the plants. Some of the damages such as crack, discoloration, and various forms of tissue necrosis.

# CHAPTER-5

# OBJECTIVES OF STUDY

The aim of the present work is to check the Effect of Electromagnetic Radiation on the Eugenol content in *Ocimum*”. Keeping in mind the importance of eugenol and the effect of electromagnetic radiation on living being the work is plan radiate various *Ocimum* sample with EMR and evaluating the effect of Eugenol content from different species of *Ocimum*.

## Chapter-6

## MATERIALS AND RESEARCH METHODOLOGY

**Equipments:**

Klystron microwave test bench this instrument availed by School of Biotechnology and Biosciences, Lovely professional University, Phagwara


**Figure no. 1** Klystron microwave test bench

**Materials Required:**

*Ocimum* species, Pots, Soil, FYM (Farm Yard Manure), sieve of size 2mm used in this project work were supplied by School of Biotechnology and Biosciences Lovely Professional University, Phagwara, Punjab.

The seedlings were collected from 2 different localities of Punjab (Ludhiana and Phagwara). Then these seedlings were exposed to EMR radiation with the help of "Klystron Microwave Test Bench". Different sets of seedling were made and are separated to study the effect of EMR at different range as well as different time duration.

In this research work the various frequencies were selected. 6.41 GHz, 7.50 GHz, 7.62 GHz for time duration 30min.

The pot was filled with soil, further the pretreated seedlings of *Ocimum* species are sown and then the pot are kept on 28 terrace for getting a sufficient amount of sunlight for the proper growth of plant and observe the "Effect of Eugenol Quantity after radiation".

**EXTRACTION OF EUGENOL:**

The leaves of the matured grown plants was collected and the co-distillation was carried out to extract eugenol from *Ocimum* species

**Co-distillation:**

1 g of crushed, leaves of *Ocimum* is added to 15 ml of distilled water in a round-bottom flask of 25-ml. Add spin bar and assemble the micro scale distillation apparatus. Leaves should be well wetted and soaked for 15 minutes. The crushed leaves suspension and heated by using a heating mantle. Temperature was maintained at 100°C, still bottom should be wrapped with that of the aluminium foil. The distillate was periodically transferred to screw cap centrifuge tube of 15 ml. The steam distillate was continued until 10-30 ml of distillate has been collected. To the water eugenol emulsion 2ml of $CH_2Cl_2$ was added this was mixed well. The layer are allowed to separate $CH_2Cl_2$ eugenol solution was added to 50 ml conical vial. Again 1ml of $CH_2Cl_2$ was added to the water eugenol emulsion. The mixture was thoroughly mix and again layer formation was allowed. This steps was repeated again, no water should be transfer during collection $CH_2Cl_2$ eugenol emulsion. In the eugenol $CH_2Cl_2$ solution 2-3 micro spatula of anhydrous sodium sulphate was added

**Evaporation:**

Transfer the dried eugenol $CH_2Cl_2$ solution to clean dry tared 50 ml conical vial. Then rinse the dried agent with $CH_2Cl_2$. Evaporate the $CH_2Cl_2$ using the hot water bath at approximately 40°-60° C. (Pavia *et al.*, 1999).

**Estimation of Eugenol contents from Thin Layer Chromatography:**

Thin layer chromatography was performed using silica gel. Silica gel was prepared by mixing the powdered silica with distilled water to make slurry of it. The prepared silica slurry were evenly spreaded on the TLC plates to form a thin firm layer plates. The slurry was allowed to solidified by air drying it for about an hour. Then the plates were activated by keeping it in hot air oven for 2 hours at 65°C. The sample i.e. extract from *Ocimum gratissimum* and *Ocimum sanctum* were loaded 1 inch above the base of the plates with the help of capillary tubes along with control and standard for better comparison.

The plates were air dried and kept in the chromatography chamber containing solvent i.e. a mixture of petroleum ether, toluene and ethyl acetate in ratio 7:2:1 respectively.

After the solvent reached the top of the plates, the plates were dried and exposed to iodine vapour chamber for 30 minutes for the development of colors and the distance travelled by solvent and solute is noted to calculate Rf value (Rana *et al*., 2011).

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

**Estimation of Eugenol content from spectrophotometer:**

After performing TLC, the sample having brown color indicating the presence of eugenol. This sample was swabbed from TLC plates and place in centrifuge tubes then 5ml of methanol was added to those tubes (Joshi et al.,2011).Then spin for 2000 rpm for 5 minutes was given to settle silica particles. The supernatants were used for spectrophotometric analysis by scanning it at 250-360 nm.

**Estimation of Eugenol content from HPLC** *(Ocimum gratissimum)*

1 gm of each radiated samples was dissolved in 15ml of methanol. Then the sample were subjected to sonication. The standard was prepared and 25 ml volume makeup was done. 10µl standard sample was loaded for HPLC. The retention peak time was observed and recorded. Similarly the samples were loaded and their retention time was observed and recorded. For HPLC analysis acetonitrile: water: Ortho phosphoric acid (ACN: Water: OPA) i.e. 80: 19.95: 0.05 was found to be suitable solvent for the separation of strong Eugenol dichloromethane extract from *Ocimum gratissimum*. (HHRC 2014)

The percentage of eugenol was calculated using a formula given below:

$$\frac{\text{Area of samples}}{\text{Area of standard}} \times \frac{\text{weight of standards}}{\text{weight of samples}} \times \text{purity of sample}$$

## CHAPTER-7

## RESULT AND DISCUSSION

The seedlings were irradiated on 6.41GHz, 7.50GHz as well as 7.62 GHz with timelines including 30minutes.

**Control 7.62 6.41 7.50**

**Figure no. 2** Showing the irradiated seedlings of *Ocimum sanctum*

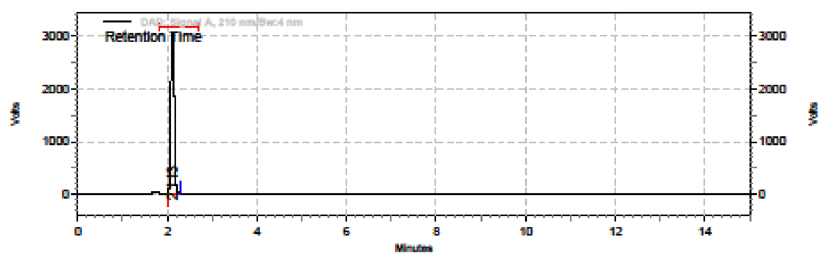

**Figure no. 3** Showing the irradiated seedlings of *Ocimum gratissimum*

**Result analysis from HPLC of species** *Ocimum gratissimum*:

Herbal Health Research Consortium Pvt.Ltd
Village Khayala Khurd, Amritsar(Punjab)

**Area % Report**

Data File: C:\EZChrom Elite\Enterprise\Projects\April 2015\Data\066 Eugenol Standard.dat
Method: C:\EZChrom Elite\Enterprise\Projects\April 2015\Method\Eugenol.met
Analysis Time 4/16/2015 10:12:28 AM
Vial Number Vial 41




**DAD: Signal A, 210 nm/Bw:4 nm**
**Results**

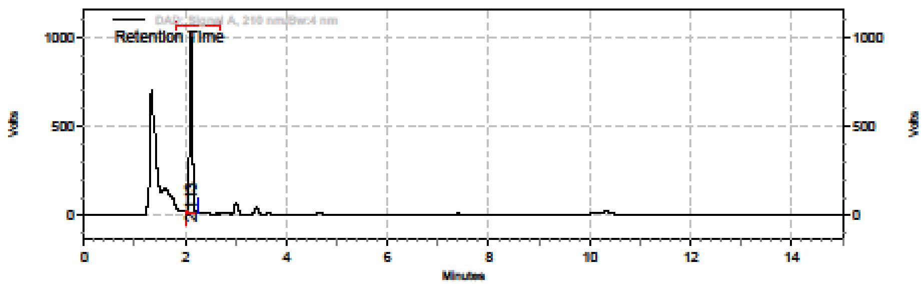
| Retention Time | Area | Name |
|---|---|---|
| 2.113 | 36373614 | Eugenol |
| Totals | 36373614 | |

**Graph no.1 Standard *Ocimum gratissimum***

Herbal Health Research Consortium Pvt.Ltd
Village Khayala Khurd, Amritsar(Punjab)

**Area % Report**

| | |
|---|---|
| Data File: | C:\EZChrom Elite\Enterprise\Projects\April 2015\Data\071 Tulsi A.dat |
| Method: | C:\EZChrom Elite\Enterprise\Projects\April 2015\Method\Eugenol.met |
| Analysis Time | 4/16/2015 11:46:37 AM |
| Vial Number | Vial 46 |




**DAD: Signal A, 210 nm/Bw:4 nm**
**Results**

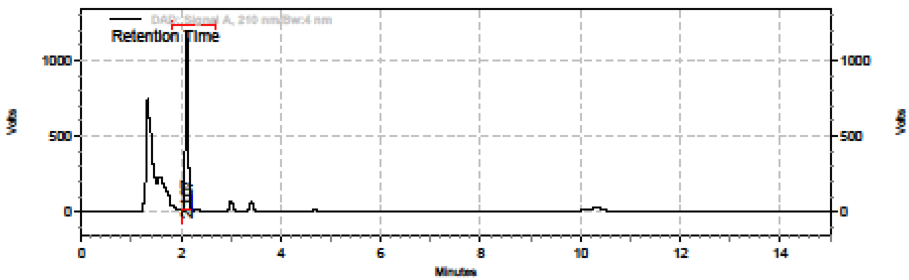
| Retention Time | Area | Name |
|---|---|---|
| 2.113 | 7880681 | **Eugenol** |
| Totals | 7880681 | |

**Graph no. 2 6.41 GHz *Ocimum gratissimum***

Herbal Health Research Consortium Pvt.Ltd
Village Khayala Khurd, Amritsar(Punjab)

**Area % Report**

| | |
|---|---|
| Data File: | C:\EZChrom Elite\Enterprise\Projects\April 2015\Data\072 Tulsi B.dat |
| Method: | C:\EZChrom Elite\Enterprise\Projects\April 2015\Method\Eugenol.met |
| Analysis Time | 4/16/2015 12:02:48 PM |
| Vial Number | Vial 47 |




**DAD: Signal A, 210 nm/Bw:4 nm**
**Results**

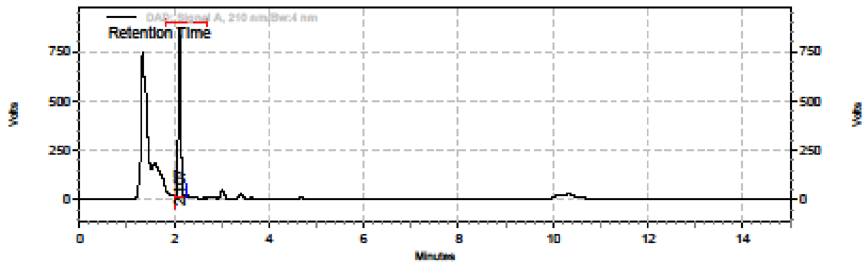
| Retention Time | Area | Name |
|---|---|---|
| 2.107 | 9182100 | **Eugenol** |
| Totals | 9182100 | |

**Graph no-3: 7.62 GHz *Ocimum gratissimum***

Herbal Health Research Consortium Pvt.Ltd
Village Khayala Khurd, Amritsar(Punjab)

**Area % Report**

| | |
|---|---|
| Data File: | C:\EZChrom Elite\Enterprise\Projects\April 2015\Data\073 Tulsi C.dat |
| Method: | C:\EZChrom Elite\Enterprise\Projects\April 2015\Method\Eugenol.met |
| Analysis Time | 4/16/2015 12:19:00 PM |
| Vial Number | Vial 48 |




**DAD: Signal A, 210 nm/Bw:4 nm**
**Results**

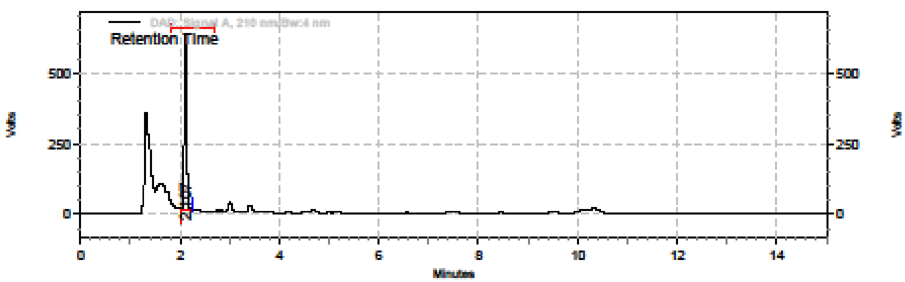
| Retention Time | Area | Name |
|---|---|---|
| 2.107 | 6640076 | Eugenol |
| Totals | 6640076 | |

**Graph no. 4: 7.50 GHz *Ocimum gratissimum***

Herbal Health Research Consortium Pvt.Ltd
Village Khayala Khurd, Amritsar(Punjab)

**Area % Report**

| | |
|---|---|
| Data File: | C:\EZChrom Elite\Enterprise\Projects\April 2015\Data\070 Tulsi Control.dat |
| Method: | C:\EZChrom Elite\Enterprise\Projects\April 2015\Method\Eugenol.met |
| Analysis Time | 4/16/2015 11:30:24 AM |
| Vial Number | Vial 45 |




**DAD: Signal A, 210 nm/Bw:4 nm**
**Results**

| Retention Time | Area | Name |
|---|---|---|
| 2.107 | 4814353 | Eugenol |
| Totals | 4814353 | |

**Graph no. 5: Control of *Ocimum gratissimum***

**Analysis of HPLC results:**

*Ocimum gratissimum* was species taken for quantification of eugenol with different range of Electromagnetic Radiation i.e., 7.50 GHz, 7.62 and 6.41GHz GHz with time duration of 30 minutes. The comparison was done with standard and control.

The high performance liquid chromatography analysis of the sample B was done and maximum eugenol content was found in the sample in which 7.50 GHz range of electromagnetic radiation was given. The eugenol content was 1.812% and cover the area about 9182100.

The minimum eugenol content was observed on the sample C which was subjected to radiation of about 7.62 GHz eugenol content was found to be about 1.310% and cover the area about 6640076. The third sample i.e sample A which was radiated at the range of 6.41 GHz having the eugenol content of 1.555% and cover the area about 7880681.

The control was that one which is not subjected to electromagnetic were found to have low eugenol concentration i.e 0.950% with the area of 4814353.The standard having the purity of 99% with area about 36373614.

The result has been observed that the *Ocimum gratissimum* seeds were subjected to electromagnetic radiation the eugenol content was significantly increase upto some extend. It was also observed that when they are subjected to high frequency of electromagnetic the eugenol content decrease. Hence the electromagnetic radiation upto some extend i.e upto 7.50 GHz for 30 minutes is beneficial to increase the eugenol content.

| Samples | Area | % of eugenol |
|---|---|---|
| Standard | 36373614 | 99% |
| Control | 4814353 | 0.950% |
| Sample A(6.41GHz) | 7880681 | 1.555% |
| Sample B(7.50GHz) | 9182100 | 1.812% |
| Sample C(7.62 GHz) | 6640076 | 1.310% |

**Table no. 3 Calculation of eugenol content via. HPLC**

**Analysis of TLC results:**

TLC plates are loaded with the sample of species *Ocimum gratissimum*.

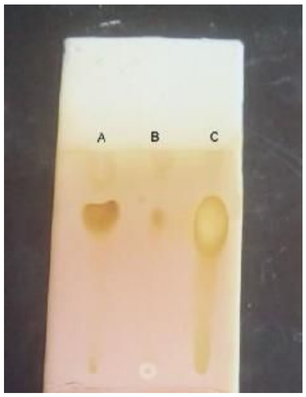


Figure no: 4

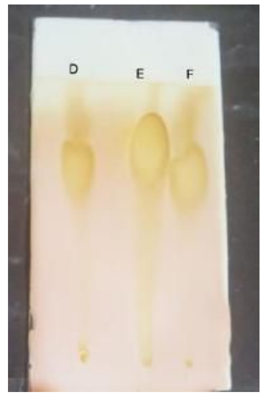


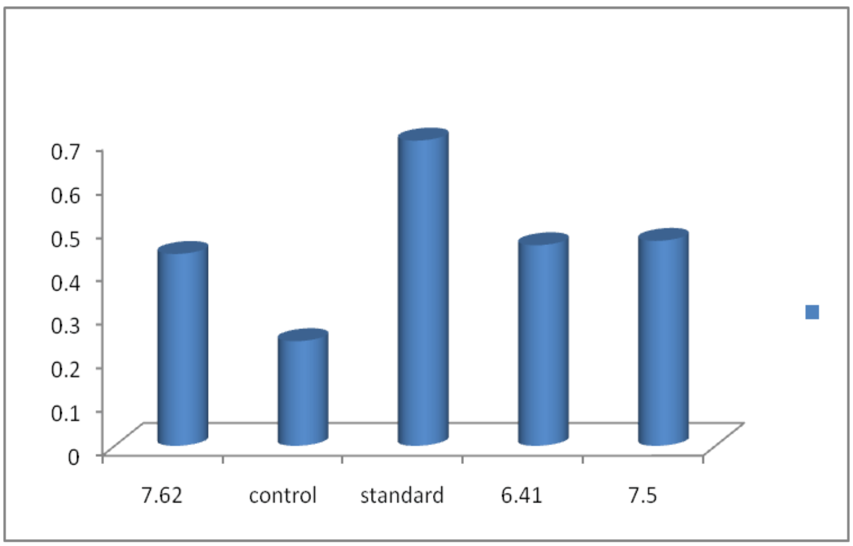
Figure no: 5

**Fig. 4 and 5 TLC plates showing color by eugenol of *Ocimum gratissimum***

A. Seedlings radiated at 7.62GHz
B. Control
C. Standard
D. Seedlings radiated at 6.41GHz
E. Seedlings radiated at 7.50GHz

| Samples | Rf value |
|---|---|
| 7.62 GHz | 0.44 |
| Control | 0.24 |
| Standard | 0.70 |
| 9.48 GHz | 0.46 |
| 7.50 GHz | 0.47 |

Table no,4 Rf values of *Ocimum gratissimum*




Graph 6, Showing the Rf values of *Ocimum gratissimum*

TLC plates are loaded with the sample of species *Ocimum sanctum:*


Figure no: 6


Figure no:7

**Fig. 6 and 7 TLC plates showing color by eugenol of *Ocimum sanctum***

G. Standard

H. Seedlings radiated at 6.41 GHz

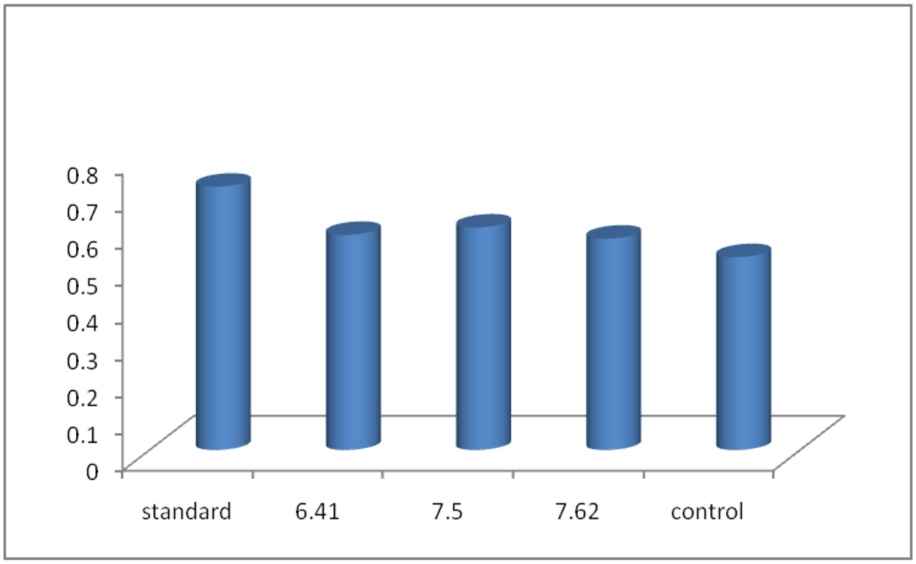
I . Seedlings radiated at 7.50 GHz

J. Seedlings radiated at 7.62 GHz

K. Control

| Sample | Rf value |
|---|---|
| Standard | 0.71 |
| 6.41 GHz | 0.58 |
| 7.50 GHz | 0.60 |
| 7.62 GHz | 0.57 |
| Control | 0.52 |

**Table no,5** Rf values of *Ocimum sanctum*




Graph no, 7 Showing the Rf values of *Ocimum sanctum*

**Result analysis by UV visible spectrophotometer of *Ocimum gratissimum:***

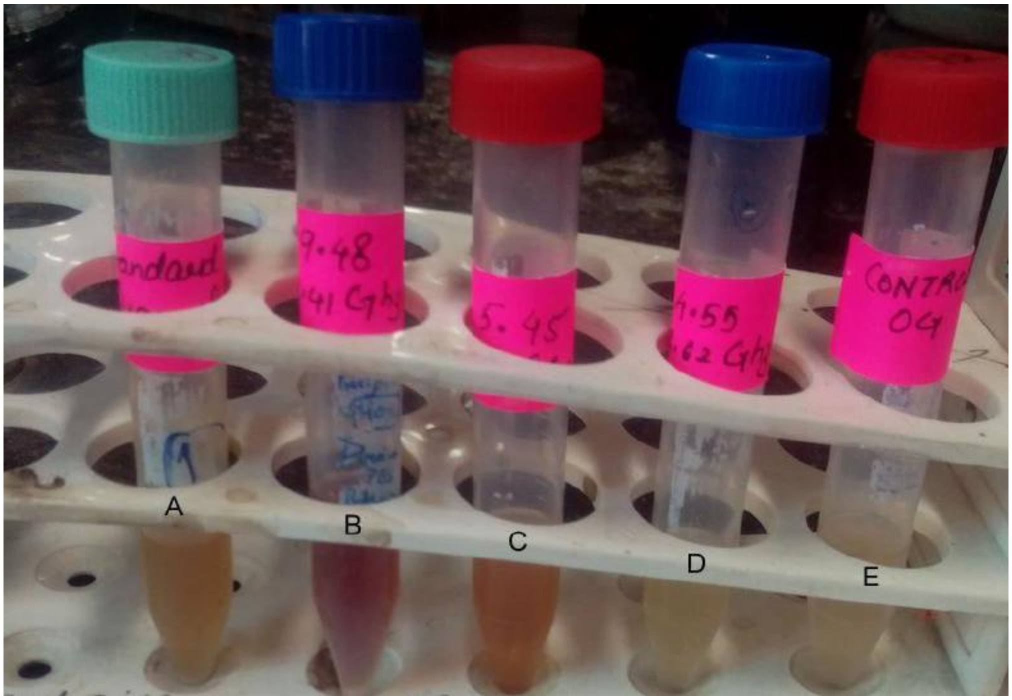


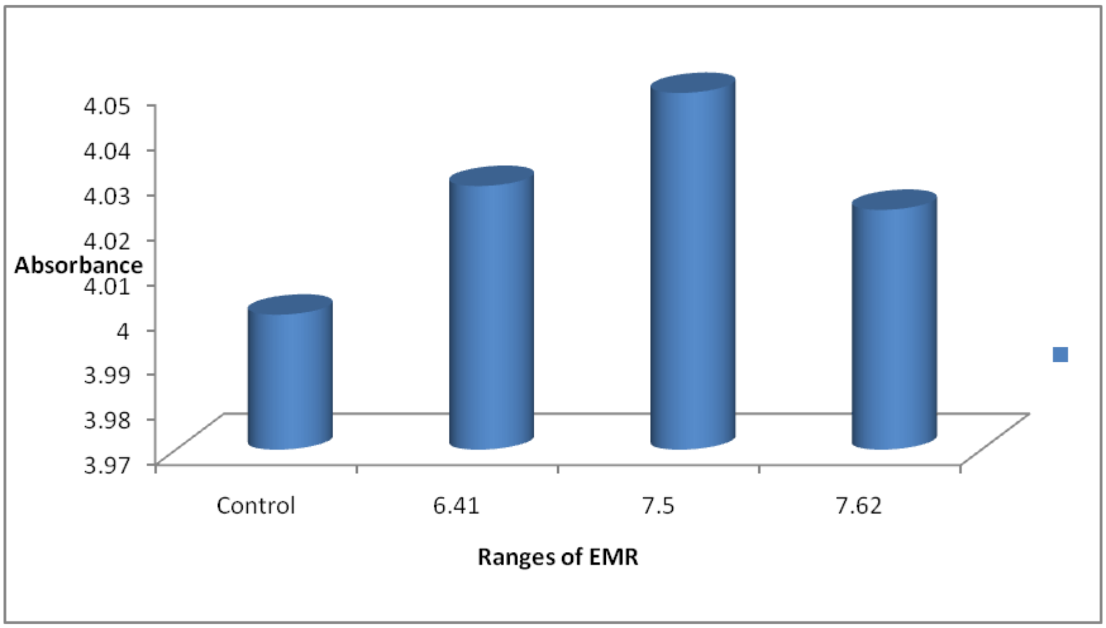

**Figure no: 8**

The above centrifuge tubes contain a mixture eugenol separated from TLC and methanol:

A. Standard
B. 6.41 GHz
C. 7.50 GHz
D. 7.62 GHz
E. Control

| Samples | Absorbance |
|---|---|
| Control | 4 |
| 6.41 | 4.0286 |
| 7.5 | 4.0493 |
| 7.62 | 4.0233 |

**Table no:6 Showing the absorbance of *Ocimum gratissimum* in different range of EMR**




Graph no. 8 Showing the absorbance of *Ocimum gratissimum*

After scanning the extract of methanol on 250-360 nm. It was observed that the compound absorbs maximum at 293nm. It has been observe as the range of electromagnetic radiation increase upto a limited extend the concentration of eugenol increase, the maximum content of eugenol was observed in 7.50 GHz i.e 4.05 absorbance and least eugenol was observed in the seedling radiated with 7.62 GHz and it has been also observed that 6.41 has moderately support to increase the eugenol concentration.

**Result analysis by UV visible spectrophotometer of *Ocimum sanctum*:**

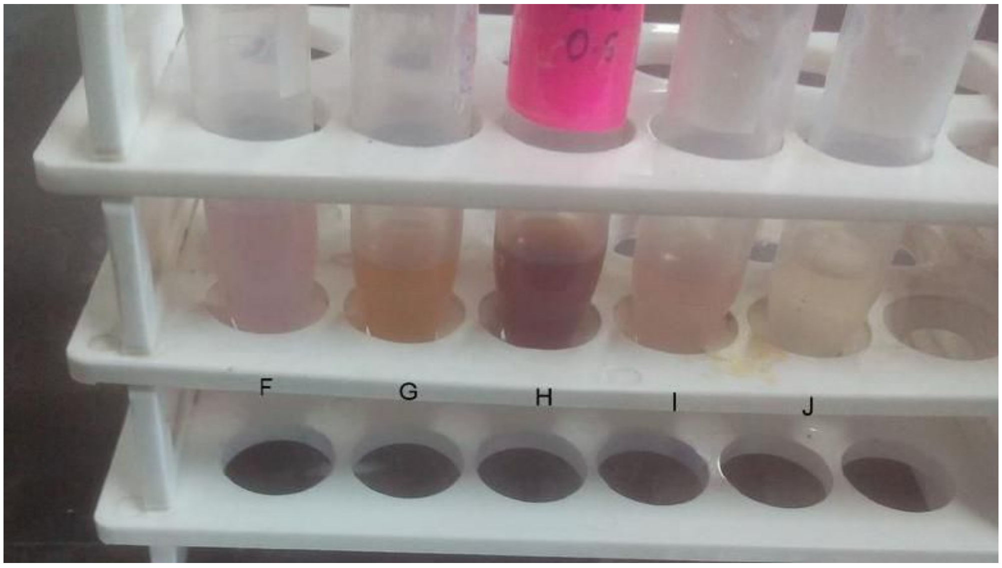



**Figure no: 9**

The above centrifuge tubes contain a mixture eugenol separated from TLC and methanol:

F.7.62 GHz

G.7.50 GHz

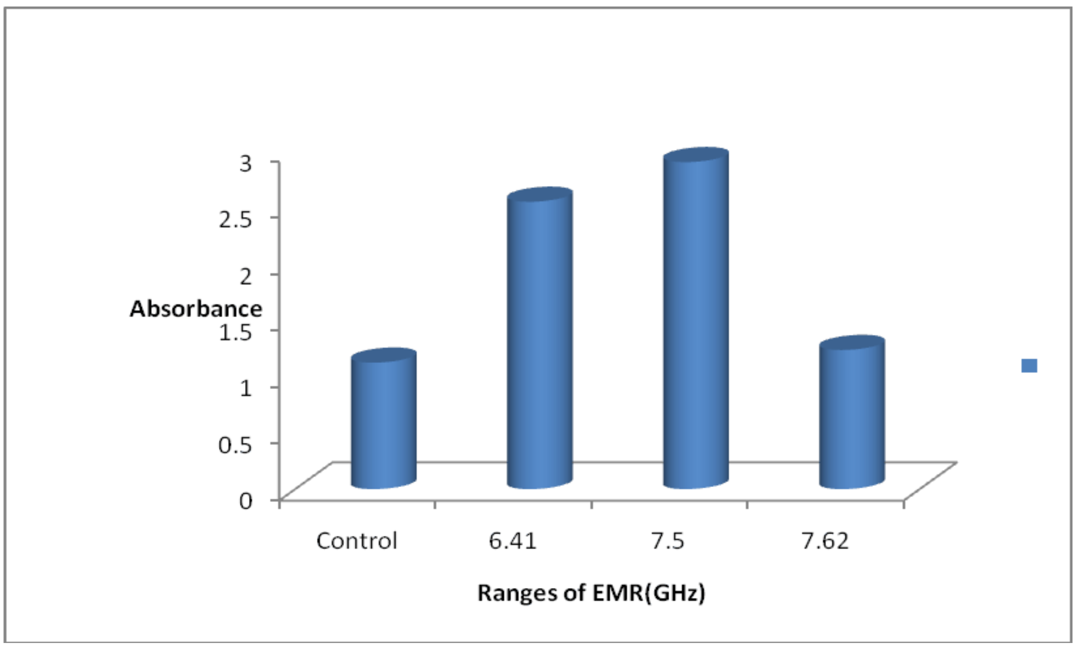
H.Standard

I.6.41 GHz

J.Control

| Sample | Absorbance |
|---|---|
| Control | 1.1215 |
| 6.41 | 2.5485 |
| 7.5 | 2.9003 |
| 7.62 | 1.2339 |

**Table no :7 Showing the absorbance of *Ocimum sanctum* in different range of EMR**




**Graph no:9** Showing the absorbance of *Ocimum sanctum*

After scanning the extract of methanol on 250-360 nm. It was observed that the compound absorbs maximum at 293nm. It has been observe as the range of electromagnetic radiation increase upto a limited extend the concentration of eugenol increase, the maximum conten of eugenol was observed in 7.50 GHz i.e 2.90 absorbance and least eugenol was observed in the seedling radiated with 7.62 GHz i.e 1.23 and it has been also observed that 6.41 i.e 2.54 has moderately support to increase the eugenol concentration.

As eugenol is a secondary metabolite so while exploring the seedling to EMR which is an stressful condition for plants the concentration of eugenol has increased and much increment in the frequency rate may hence effected the biosynthesis pathway of eugenol and hence the concentration of eugenol at higher concentration was decrease.

# CHAPTER-8

# CONCLUSION AND FUTURE SCOPE

Medicinal plants are big reservoir, it contain large number of essential oils with medicinal properties. "Tulsi is a queen of all the plants", Singh et al.,(2011) it play a important role in curing diseases like cold, cough, skin diseases, gastric ulcere, it also help to purifies the blood. It is also a good antibacterial, antiseptic, antifungal, antidepressant as well as antiviral agent. Medicinal plant did not cause any harmful effect in human body.

Eugenol is naturally occurring phenolic compound. Eugenol is used as flavouring agents in many food industries. It is also a remedy for skin diseases and body pain, it is also used as a dental remedy and oral hygiene.

Eugenol is one of the essential oil extract from *Ocimum* species. It play role in antifungal, antiseptic and antibacterial, anticarcinogenic, (Uma et al.,2001).

In the present study sample which is subjected with 7.50 GHz having highest eugenol content as compare to others and control. The result has been observed that the *Ocimum* seedlings were subjected to electromagnetic radiation the eugenol content was significantly increase. It was also observed that when they are subjected to high frequency of electromagnetic radiation the eugenol content decreases. Hence the electromagnetic radiation up to some extend i.e. up to 7.50 GHz for 30 minutes is useful to increase the eugenol content.

Now a day's eugenol is preferred to use in therapy after combining with some classics drugs. It is also used to reduce the side effects of certain drugs. Cancer is one of disease that can be reduce with the help of eugenol. It is having a property of antioxidant which help to prevent the formation of free radicals within the body before it can cause damage to any cells. This helps to reduce the rate of spontaneous mutation and hence reduce the risk of cancer (Pavithra et al., 2014).

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