

**ANTIMICROBIAL, ANTIOXIDANT AND EVALUATION OF
PHYTOCHEMICAL PROPERTIES OF CALLUS FROM *OCIMUM
AFRICANUM* CULTIVATED IN HUMIC ACID MEDIUM**

Submitted In Partial Fulfillment of The Requirements of The Award of
Bachelor of Science in Microbiology

Ms. B. PREETHI (40300048)

Ms. A. MANIMEGALAI (40300035)



SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

SATHYABAMA

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

Accredited with Grade "A" by NAAC | 12B Status by UGC |

Approved by AICTE

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

MAY - 2023

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DEPARTMENT OF BIOTECHNOLOGY

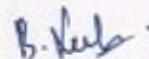
BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Ms. B. PREETHI (40300048) who carried out the project entitled "*Antimicrobial, Antioxidant And Evaluation Of Phytochemical Properties Of Callus From Ocimum africanum Cultivated In Humic Acid Medium*" under our supervision from september 2022 to April 2023 .



Internal Guide

Dr. PRAKASH. P



External Guide

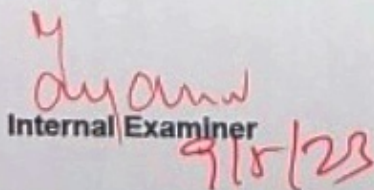
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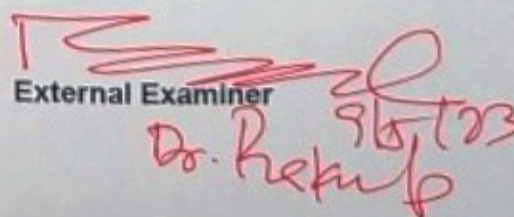
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DECLARATION

I am **Ms. B. PREETHI (40300048)** hereby declare that the Project Report entitled "Antimicrobial, Antioxidant and Evaluation of Phytochemical Properties of Callus From *Ocimum africanum* Cultivated In Humic Acid Medium" done by me under the guidance of **Dr. PRAKASH. P** and **Dr. LEEBA BALAN** at **BIONYME LABORATORIES PVT.LTD** is submitted in partial fulfillment of the requirements for the award of Bachelor of science degree in MICROBIOLOGY.

Date : 29/04/2023

Place : CHENNAI



Signature Of The Candidate

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I am pleased to acknowledge my sincere thanks to Board of Management of **SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

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I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of BIOTECHNOLOGY who were helpful in many ways for the completion

ABSTRACT

The potential of humic acid as a growth supplement in micropropagation of *Ocimum africanum* is reported in the present investigation. *Ocimum africanum* explants were grown in the MS media with 2-4D hormones growth factor and has been observed that it was effective for invitro regeneration of the explants. On the other hand, when humic acid was supplemented with the growth factor, there is a decrease in the contamination rate, increase in callus proliferation within reduced time. The optimum concentration of humic acid required was found to be 0.75 ml/200ml and 1ml/200ml for the effective growth of explants. These callus were taken and antimicrobial activity test was performed. This study revealed that HA callus methanol extract had a better growth reduction for Staphylococcus aureus , Escherichia coli and Candida albicans. GCMS results revealed that plant extract had 9 chemical components , sample callus had 4 chemical components and HA callus also had 4 chemical components. The antioxidant assay yielded an IC₅₀ of 83.47 ug/ml for control callus and IC₅₀ of 64.97 ug/ml for HA callus and this comparative study revealed that HA callus is more effective than control callus for its ability to scavenge free radicals .This study therefore supports the use of humic acid as a suitable growth supplement for the callus in micropropagation studies in plant tissue culture.

KEY WORDS : . *Ocimum africanum*, MS media, callus, explants, humic acid

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LISTS OF ABBREVIATIONS

1. **GCMS** – Gas Chromatography Mass Spectrometry
2. **HA** – Humic Acid
3. **MS** – Murashige and Skoog Medium
4. **%** – Percentage
5. **TLC** – Thin Layer Chromatography
6. **UV** – Ultra Violet
7. **OD** – Optical Density
8. **mg** – Milligram
9. **μg** – Microgram
10. **cm** – Centimeter
11. **R_f** – Retention Factor
12. **RT** – Retention Time
13. **ml** – Milliliter
14. **&** – And
15. **gms** – Grams

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CHAPTER 1

INTRODUCTION

1.1 PLANT BASIL

1.1.1 Medicinal Plants

Medicinal plants are plants that have been traditionally used for medicinal purposes to treat various ailments and diseases. These plants contain compounds that have therapeutic properties and can be used to treat or alleviate the symptoms of certain illnesses. These plants contain active compounds that have pharmacological effects and can be used to treat or prevent various diseases or health conditions.



Fig: 1.1: *Ocimum africanum*

They have been used for thousands of years by different cultures around the world. Many modern medicines are also derived from plants or their active compounds. Some examples of medicinal plants include aloe vera, garlic, ginger, chamomile, echinacea, ginkgo biloba, turmeric, and many others.

Here are some common features of medicinal plants:

Active compounds: Medicinal plants contain active compounds that have therapeutic effects on the body. These compounds may include alkaloids, flavonoids, terpenoids, phenolic compounds, and others.

Traditional use: Many medicinal plants have been used for centuries in traditional medicine systems around the world. They have been used to treat a variety of ailments, ranging from common colds to more serious conditions like cancer.

Biological activity: Medicinal plants have been shown to have biological activity in various studies. This means that they can affect the body in positive ways, such as reducing inflammation or improving immune function.

Safety: Medicinal plants are generally considered safe when used appropriately, although some plants may have toxic effects if used in excess. It is important to use medicinal plants under the guidance of a trained healthcare professional.

Availability: Many medicinal plants are readily available in nature or can be cultivated easily, making them accessible to people in many parts of the world.

Multiple uses: Many medicinal plants have multiple uses, such as treating different health conditions or even as a source of food or fiber.

Overall, medicinal plants offer a natural and often cost-effective approach to healthcare, and their use has been validated by centuries of traditional use and scientific research.

1.1.2 Herbal Plants

Herbal plants contain various chemical compounds that can have antimicrobial properties. The specific mechanism by which these compounds kill microorganisms can vary depending on the type of plant and the compound involved, but some common mechanisms include:

- 1) *Disruption of cell membranes:* Many herbal compounds can disrupt the cell membranes of microorganisms, leading to leakage of cellular contents and ultimately cell death. Examples of compounds that can disrupt cell membranes include saponins and essential oils.
- 2) *Inhibition of cell wall synthesis:* Some herbal compounds can inhibit the synthesis of the cell walls of bacteria and fungi, leading to their death. For example, the compound berberine found in goldenseal can inhibit the synthesis of the cell walls of some bacteria.

- 3) *Interference with microbial metabolism*: Certain herbal compounds can interfere with the metabolic processes of microorganisms, leading to their death. For example, some flavonoids found in plants like chamomile and Echinacea can interfere with the energy metabolism of bacteria and fungi.
- 4) *Generation of reactive oxygen species (ROS)*: Some herbal compounds can generate ROS such as hydrogen peroxide or superoxide, which can damage the DNA and proteins of microorganisms, ultimately leading to their death. Examples of compounds that can generate ROS include phenolic acids and alkaloids.

Overall, the antimicrobial properties of herbal plants are complex and can involve multiple mechanisms of action. Different compounds can have different targets and modes of action, which can make them effective against a wide range of microorganisms.

1.1.3 Humic acid

Humic acid is a natural organic substance that is formed from the decay of plant and animal matter over thousands of years. It is a complex mixture of various organic molecules, such as fulvic acid, humin, and other humic substances.

Humic acid is commonly found in soil, peat, coal, and other organic materials. It is known to have a number of beneficial properties for plants and soil, such as improving soil fertility, water retention, nutrient uptake, and root growth. It can also help to increase the effectiveness of fertilizers and reduce soil erosion.

Humic acid is often used in agriculture and horticulture as a soil conditioner and fertilizer additive. It can also be used in a variety of other applications, such as in animal feed, wastewater treatment, and as a component in some health supplements.

1.1.4 Plant Tissue Culture

Plant tissue culture is the process of growing and maintaining plant cells or tissues in a sterile and controlled environment outside of the plant. The technique involves the aseptic culture of plant cells, tissues, and organs in nutrient media under controlled environmental conditions of light, temperature, and humidity.

Plant tissue culture techniques can be used to propagate plants, produce disease-free plants, and regenerate whole plants from single cells or tissues. Plant tissue culture has many applications, including plant breeding, plant genetic engineering, and the production of pharmaceuticals and other useful compounds.

The basic steps of plant tissue culture include the preparation of explants (small pieces of plant material), sterilization of the explants, culture of the explants on a nutrient medium containing growth regulators, and maintenance of the cultures in appropriate environmental conditions. The explants may be obtained from various parts of the plant such as roots, stems, leaves, flowers, and seeds. Supplements are commonly used in plant tissue culture to provide additional nutrients, growth regulators, and other factors to promote cell division, differentiation, and regeneration.

1.1.5 *Ocimum africanum* Plant

Ocimum africanum is a type of basil plant that has a citrusy flavor and aroma, similar to lemon zest. It is also known as hoary basil, Thai *Ocimum africanum*, or *Maenglak* in Thai. The leaves of *Ocimum africanum* are typically light green, sometimes with a slightly grayish tint, and have a smooth, glossy texture.

Ocimum africanum is commonly used in Southeast Asian cuisine, particularly in Thai and Indonesian dishes. It pairs well with fish, poultry, and vegetables, and can also be used to flavor soups, curries, and stir-fries. In addition to its culinary uses, *Ocimum africanum* is also used in traditional medicine in some cultures, where it is believed to have properties that can aid in digestion, reduce inflammation, and promote relaxation. In addition to its culinary uses, *Ocimum africanum* is also used in traditional medicine to treat a variety of ailments, including coughs, colds, fever,

and digestive issues. It is believed to have antibacterial and anti-inflammatory properties. *Ocimum africanum* is a type of basil that has a citrusy flavor and aroma, with notes of lemon and mint. Its scientific name is *Ocimum basilicum citriodorum* and it is also sometimes referred to as "Thai *Ocimum africanum*" or "hoary basil". *Ocimum africanum* is commonly used in Thai, Indonesian, and other Southeast Asian cuisines, where it is added to soups, curries, stir-fries, and salads. It can also be used to flavor seafood, chicken, and vegetables, or to make tea or infused water.

Apart from its culinary uses, *Ocimum africanum* is also known for its medicinal properties. It is believed to have *anti-inflammatory, antiseptic, and antioxidant effects*, and has been used in traditional medicine to treat digestive issues, headaches, and other ailments. Some of the health benefits associated with *Ocimum africanum* include its ability to act as a natural *antibacterial and anti-inflammatory agent*, as well as its potential to help lower cholesterol levels and improve digestion. It's also a good source of *antioxidants and vitamins A and C*.

1.1.6 Micropropagation

The practise of artificially propagating plants vegetatively using cell or tissue culture techniques is known as micropropagation. Plants are generated invitro in this artificial propagation technique by vegetative or asexual reproduction. Plants can reproduce asexually, or by multiplying their vegetative components, or sexually, or by producing seeds. By creating numerous genetically identical copies of plants, a process known as clonal propagation, plants can be filled from a single person through asexual modes of reproduction. Since these plants don't produce functioning seeds like figs, grapes, bananas, etc., asexual reproduction through the multiplication of vegetative parts is the sole option for in vivo plant propagation. Application of cloning successfully

A developing mass of disorganised plant *parenchyma cells* is known as a plant callus (plural calluses or calli). Callus cells are the cells that cover a plant wound in living plants. Callus development is induced from plant tissue samples (explants) in biological research and biotechnology after surface sterilisation and plating onto tissue culture medium in vitro (in a closed culture vessel such as a Petri dish).[1] To

start callus formation or somatic embryogenesis, plant growth regulators including auxin, cytokinin, and gibberellin are added to the culture media. All significant groups of land plants have been subject to callus initiation descriptions.

The synthesis of a plant hormone is not localized to a specific tissue, but occurs in many different tissues, particularly in the meristematic regions. Furthermore, plant hormones are transported from the site of synthesis to act in distant tissues. Plant hormones influence a wide range of processes. Auxins, for example, have been found to influence a myriad of processes within a cell, including cell elongation, cell division, induction of primary vascular tissue, adventitious root formation, senescence, fruit growth, outgrowth of axillary buds and flower development. In some cases, the levels of endogenous hormones have been determined. All of Plant Media hormones and growth regulators are tested for use in plant tissue culture.

CHAPTER 2

REVIEW OF LITERATURE

2.1 SCIENTIFIC CLASSIFICATION

Kingdom – Plantae

Clade – Tracheophytes

Clade – Eudicots

Clade – Asteroids

Order – Lamiales

Family – Lamiaceae

Genus – *Ocimum*

Species – *Ocimum africanum*

Ocimum africanum is a hybrid basil and American basil *Ocimum basilium* and *Ocimum americanum*. The herb is grown in southern Asia and northern Africa. It has a lemon scent. It is used in cooking and for medicine. It can grow to 20-40 (8-20). It has white flowers in late summer. Leaves are narrow with slightly serrated edges. Leaves are similar to basil leaves. After flowering the seeds are formed. Seeds dry on the plant. It is more disease resistant than other basil. Moist well draining rich loamy soil is best for *Ocimum africanum*. Ph range between 6 and 7.5 is best for plant growth. Grows great in full sun or partial shades.

It has anti-inflammatory properties. It also has antibacterial properties. It has moderate levels of beta carotene which builds strong immune system and good for eye health. It also contains high levels of vitamin to which is essential for the production of clotting factors in the blood and plays a vital role in the bone strengthening and mineralization. *Ocimum africanum* contains minerals like potassium, manganese, copper, iron, magnesium. Potassium is an essential compound to control heart rate and blood pressure. Manganese is used as a co-factor for the antioxidant enzyme superoxide dismutase. The body converts beta carotene to vitamin A.

Two therapeutic herbs, hoary basil (*Ocimum americanum* L. syn. *O. canum* Sims) and holy basil (*Ocimum sanctum* L.), can be propagated in vitro using axillary shoot buds. On Murashige and Skoog medium (MS) enriched with benzyladenine (BA), shoot bud explants of both species were used to stimulate the development of multiple shoots. For *O. americanum* and *O. sanctum*, the ideal BA concentrations for shoot proliferation were 0.25 mg/l and 1.0 mg/l, respectively. The frequency of axillary branching and the development of numerous shoots increased noticeably when gibberellic acid (GA3), 0.5 mg/l, was added to the culture medium together with BA. *O. americanum* shoots were rooted on MS supplemented with half-strength The plantlets were successfully hardened off and planted in natural soil, where they developed normally. [**S Pattnaik et al. 1996**]

For basil (*Ocimum basilicum* L.), a successful plant regeneration protocol was created. The maximum frequency of 85% regeneration was seen in explants from seedlings that were 1 month old, with an average of 5.1 shoots per explant. Three different types of basil (Sweet Dani, methylcinnamate, and Green Purple Ruffles) were subjected to the regeneration technique. On Murashige and Skoog basal medium supplemented with thidiazuron (16.8 M) for roughly 30 days, callus and shoot induction was started. Rooted plantlets were moved and given 20 days to acclimatise in a greenhouse, where they underwent typical morphological development. This is the first account of a basil primary callus regeneration system that was successful in vitro. [**James E. Simon et al. 2000**]

Ethanol, methanol, and hexane extracts from *Ocimum basilicum* Labiatae (sweet basil) were investigated for their invitro antimicrobial properties. A total of 146 microbial organisms belonging to 55 bacteria, and four fungi, and a yeast species were studied using a disk-diffusion and minimal inhibition concentration (MIC) method. The result showed that none of the three extracts tested have antifungal activities and antibacterial effects. Both the hexane and methanol extracts, but not the ethanol extracts, inhibited three isolates out of 23 strains of *Candida albicans* studied. All three extract of *O. basilicum* were different in terms of their antibacterial activities. [**Ahmet adiguel et al. 2005**]

Essential oils were examined for antimicrobial activity against reference and clinical strains of *Salmonella Enteritidis*. Based on the size of the inhibition zone and the minimal inhibitory concentration, basil oil had the strongest antimicrobial activity against all the tested bacteria, and *S. Enteritidis* SE3 was the most sensitive strain to all the tested oils. Gas chromatography/mass spectrometry analysis revealed that the major constituents of the oil were linalool (64.35%), 1,8-cineole (12.28%), eugenol (3.21%), germacrene D (2.07%), terpineol (1.64%), and cymene (1.03%). The results from this study confirm the potential use of basil oil as an antimicrobial agent to control *S. Enteritidis* in food. [**Parichat PHUMKHACHORN et al.2010**]

The influences of cultivar on the phenolic composition and antioxidant properties of 15 different basil varieties was determined. Cultivar had a statistically significant effect on total phenolic levels ($p < 0.001$) and anthocyanin concentrations ($p < 0.001$). Rosmarinic ($p < 0.001$), chicoric ($p = 0.002$) and caffeic ($p = 0.001$) acid concentrations were affected by cultivar, although caftaric acid levels ($p = 0.083$) were not. Nine of the cultivars in this study contained chicoric acid in higher concentrations than rosmarinic acid. These are the first basil cultivars that have been identified in which rosmarinic acid is not the dominant phenolic acid. In addition, six of the cultivars in this study had caftaric and caffeic acid concentrations that were similar or higher than rosmarinic acid levels. For the basil cultivars in this study, the individual phenolic acid composition was found to be an important factor influencing the measured antioxidant capacity. [(**M.Kwee et al.2011**)]

Ocimum basilicum L., popularly known as sweet basil, is a Lamiaceae species whose essential oil is mainly composed of monoterpenes, sesquiterpenes and phenylpropanoids. The contents of these compounds can be affected by abiotic and biotic factors such as infections caused by viruses. The main goal of this research was an investigation of the effects of viral infection on the essential oil profile of common basil. Seeds of *O. basilicum* L. cv. Genovese were sowed and kept in a greenhouse. Essential oils of healthy and infected plants were extracted by hydrodistillation and analyzed by GCMS. However, methyleugenol contents were significantly decreased in infected plants. [**Alice Nagai et.al 2011**]

The extraction of humic acid was optimized using Response Surface Methodology (RSM) –Central Composite Design (CCD) by altering the concentration of the key constituents such as Lignite, Potassium hydroxide and Sodium hydroxide. Among 20 trials, the maximum humic acid was extracted in an amalgamation of lignite 5g, KOH 4g and NaOH 1g. The humic acid obtained after this process was subjected to evaluate the minerals and characterized by using FESEM-EDAX method. The growth parameters of *Vigna mungo* were studied after applying optimized humic acid [**Prakash P et al.2014**]

In vitro anticholinesterase activity of the volatile oils of both *O. basilicum* and *O. africanum* was investigated and both samples showed significant activity. The major constituents of the two oils were isolated using several column chromatographies. Linalool, 1,8-cineol and eugenol were isolated from the volatile oil of *O. basilicum* and camphor was isolated from the volatile oil of *O. africanum*. Results showed that scopolamine-induced cognitive dysfunction was significantly attenuated by administration of the volatile oils and their terpenoids, eugenol and camphor, in the passive avoidance task and inhibited brain acetylcholinesterase activity. [**M M Salama et.al 2014**]

Plants have been an important source of medicine with qualities of thousands of years. *Ocimum americanum* L. belonging to family Lamiaceae is a medicinal and aromatic herb with white or pale lilac flowers. 13 compounds were identified and they are reported as dibutyl phthalate, 1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester, 1,2-benzenedicarboxylic acid, bis (2-methyl propyl ester, 1,2-benzene dicarboxylic acid butyl 2-ethyl hexyl ester; 1,2-benzenedicarboxylic acid butyl 2-methyl propyl ester; phthalic acid; butyl 2-pentyl ester; phthalic acid, butyl hept-4-yl ester, phthalic acid, butyl hex-3-yl ester phthalic acid; Di(2-propyl pentyl) ester diisooctyl phthalate, Bis(2-ethyl hexyl) phthalate; Squalene; Trans-geranylgeraniol. GCMS analysis and the biological activity of each compound were discussed in present attempt. It can prove as an effective and cheaper drug for various human diseases. [**Shubhangi Nagorao 2016**]

A phyto-sociological and ecological study of naturally growing *Ocimum* species and their conservational strategies is carried out in Dakshin Dinajpur district, West

Bengal, India. The main objective of this work is to find out their (*O. americanum* and *O. africanum*) natural distribution, density, abundance and the dominance along with associated species. A total of 50 (1 × 1 m²) quadrats were made at two study sites, each site contained 25 quadrats. Coexistence of almost 57 species of families with both study species of *Ocimum* in two study sites. **[Subhas Chandra Roy et.al 2016]**

Mechanisms by which plants acclimatize to water deficit conditions can affect development and, especially in aromatic plants, the essential oil synthesis. To evaluate the effects of water deficit on *Ocimum africanum*, seedlings were exposed to water levels corresponding to 100, 80, 70 and 60% of the substrate field capacity. The different watering regimes corresponded to predawn water potentials of -0.5, -0.9, -1.6 and -1.9 MPa, respectively. Decreased stomatal conductance and increased glandular and tector trichome density were observed. **[Fabio Pinta Gomes et al.2016]**

Ocimum africanum Lour.(hairy basil, hoary basil or *ocimum africanum*) is an aromatic herb in Asian and Thai cuisine that has been used as a carminative, a digestion enhancement and remedy for gastrointestinal disturbance in Thai traditional medicine. This study was performed to evaluate the gastroprotective effect of *O. africanum* leaf extract in acid/alcohol-induced gastric lesion in rats. Pretreatment of the plant extract can prevent the gastric damage in a dose-dependent manner. The protective effect of the plant extract against acid/alcohol-induced gastric damage relates to its high antioxidative property and the ability to enhance the activities of three antioxidative enzymes, superoxide dismutase, catalase and glutathione peroxidase. **[Jaruwat Bunwijit et al.2017]**

The extracted from fresh leaves of four *Ocimum* species *Ocimum basilicum* L. (sweet basil), *O. africanum* Lour., *O. americanum* L. and *O. minimum* L. by different extraction methods hydrodistillation (HD), steam distillation (SD) and microwave assisted extraction (MAE) techniques. It has been concluded that MAE technique is considered the best method of extraction, not only in saving energy but also it requires least amount of plant materials (200 gm). GC-MS analyses revealed that β -linalool increases in its percentile in *O. basilicum* (77%) upon using MAE and *O.*

africanum (13%) by HD. Estragole (methyl chavicol) considered as a carcinogenic agent naturally present in family Lamiaceae with very low percentile. It increases dramatically in both volatile oils obtained by HD of *O. basilicum* (37%) and *O. minimum* (34%) as well as SD of *O. basilicum* (33%) and *O. africanum* (20%). **[M Dina Manar Eissa 2018]**

Essential oils are valuable natural products extracted from flowers, leaves, fruits, seeds, stems, roots, or resins of various plants. They are used as raw materials in many fields, including perfumes, cosmetics, spices, foods, nutrition and treatment for several health disorders. Essential oils are complex mixtures comprising many single compounds. Various This variation could be understood by studying the factors which influence the chemical composition of the essential oils, in particular under photochemical conditions. This review discusses the major components present in the essential oils, the methods of extraction from plants, stability, photochemical reactions of essential oil and their important components under different conditions and biological activities of essential oils. **[L Selva Roselin et al.2018]**

This present study evaluates the potential antifungal activity of selected essential oils viz.; *Tagetes minuta*, *Ocimum africanum*, *Eucalyptus citriodora* and *Mentha arvensis* against *Malassezia furfur*. The studies were carried out using agar well-diffusion method at 50 µl. The minimum inhibitory concentration (MIC) was calculated using microdilution method and the volatile oil were analyzed by using GC-MS. Result confirmed the potential efficacy of essential oil of *T. minuta*, followed by *E. Citriodora* showing the maximum zone of inhibition (mm) 31.07 ± 2.01 and 24.75 ± 0.05 , respectively, against *M. furfur*. The lowest MIC values of *T. Minuta* < 5 µl/ml was found to be effective against *M. furfur*. Essential oil of *O. africanum* was found less effective as it was unable to inhibit the growth of tested fungus. Results depict that the essential oil *T. minuta* may be used as an alternative to the other chemically made hair products. **[V Singh et al.2019]**

Comparative studies of the adaptation of green-and purple-leaved *Ocimum basilicum* varieties, which differ in carotenoid and anthocyanin contents, to high-intensity light have been carried out. The plants were grown for 21 d under white

lightemitting diode providing different light intensities [300, 550, and 1,350 μmol (photon) $\text{m}^{-2} \text{s}^{-1}$]. During the experiment, fresh mass and relative water content, photosynthetic pigment and anthocyanin content, amount of total free phenolic compounds, and the contents of thiobarbituric acid reactive substances and H_2O_2 were analysed. The obtained results are consistent with the concept of the important role of not only anthocyanin but also carotenoids in plant adaptation to high-intensity light. **[LA Stetsenko et al. 2020]**

The chemical composition and bioactive properties of two species of the *Ocimum* genus, namely *O. basilicum* and *O. citriodorum*. For this purpose, samples of these plants grown in Portugal were evaluated for their composition in phenolic and volatile compounds, and the infusion and hydroethanolic extracts were assessed for their in vitro antioxidant, antimicrobial, cytotoxic, and anti-inflammatory activities. In both samples rosmarinic acid was the major phenolic compound, predominant volatile compound. Despite *O. citriodorum* extracts showing slightly better activity against some strains, both types of extracts evidenced similar antimicrobial activity. The extracts also revealed interesting cytotoxicity, particularly the *O. citriodorum* hydroethanolic extract which was also the only one exhibiting anti-inflammatory activity. **[Chaimae majdi et al. 2020]**

Date palm variety Quntar, the effects of humic acid (HA; 0, 0.5, 2.5, and 5.0 mg L^{-1}) and zinc oxide nanoparticles (ZnONPs ; 0, 50, 75, and 150 mg L^{-1}) were investigated at several stages of micropropagation. The maximum callus induction (274 mg/jar), highest percentage of callus producing buds (83.34%), and average bud formation (14.2) per callus were seen in media supplemented with 2.5 mg L^{-1} HA and 50 mg L^{-1} ZnO-NPs . In terms of root regeneration and the number of roots per shoot, adding HA and ZnO-NPs to the medium was most successful. The greatest results were obtained using 2.5 mg L^{-1} humic acid and 75 mg L^{-1} ZnO-NPs . **[Al-Mayahi, A.M.W. 2021]**

The African *ocimum* Lour can serve as a good replacement for perennial lemon grass because it is a short-duration crop even among sweet basil. The quick-

growing and short-lived crops are ideal for the production of natural citral and essential oils. To evaluate the morphological variance for growth, seed characteristics, essential oil content, and chemical makeup, an experiment was run. Maximum growth metrics were seen to be under control, although the essential oil extracted from fresh DLB-7 herbage showed maximum seed swelling index, oil content, and citral A and citral B content. In essential oils, DLB-6 was shown to have the highest percentage of methyl chavicol. **[Parmeshwar Lal Saran et al. 2022]**

Lemon balm, mint, and basil were used to determine whether light modification (plants grown under pearl shade nets with 40 % shaded index or in un-shaded open field) could improve the quantity and quality of essential oils (EOs) and antioxidant activity. The yield of EOs of lemon balm, mint and sweet basil obtained after 120 min of hydrodistillation was 0.18, 2.0 and 1.12 mL/100 g, respectively. At the same time under shading conditions plants obtained more levels of EOs (0.22, 2.23, and 1.32 mL/100 g). Medicinal plants are a good source of natural antioxidants with potential application in the food and pharmaceutical industries. Medicinal plants can be recommended to grow in shading conditions for achieving optimal quality parameters. **[Zoran S Ilic et al. 2022]**

An aromatic herb in Asian and Thai cuisine that has been used as a carminative, a digestion enhancement and remedy for gastrointestinal disturbance in Thai traditional medicine. This study was performed to evaluate the gastroprotective effect of *O. africanum* leaf extract in acid/alcohol-induced gastric lesion in rats. Pretreatment of the plant extract can prevent the gastric damage in a dose-dependent manner. At 125 mg/kg of body weight, the plant extract attenuated the inflammation with% ulcer index of 5.08 ± 1.41 , whereas at doses of 250 and 500 mg/kg of body weight, the gastric lesion was completely prevented in the manner similar to what was observed in omeprazole pretreated animals. Preliminary phytochemical investigation demonstrated that certain phenolic compounds such as rosmarinic acid, caffeic acid and vanillic acid might take part in the protective effects. **[Betty omenbelle George et al. 2022]**

Humic substances (HS) are increasingly applied as biostimulants in agriculture, though their mechanism of action is not yet completely understood due to their

complex and heterogeneous composition. we isolated thirteen different humic and fulvic acids from different sources, such as soils, composts, peat, leonardite and lignite, and characterized the distribution of their carbon components by ^{13}C -CPMAS–NMR spectroscopy. While a multivariate statistical analysis of NMR results well-separated the HS characteristics based on their origin, humic materials generally increased callus growth, as expected, with largest effects being exerted by the mostly polar humic acids from composts. This work confirms that HS of various origin can act as sustainable biostimulants of plant growth, though the comprehension of their effects on plants biochemical activities requires further research and additional understanding of their molecular composition and conformational behaviour. **[A Nuzzo et al.2022]**

An optimization procedure for multistep temperature-programmed capillary gas chromatography was developed for the analysis of basil essential oil. The optimized conditions were obtained by using a split ratio of, a gas flow rate of 1.3 mL/min, and a heating rate of 2°C/min. A statistical study was performed based on hierarchical ascending classification and principal component analysis to elucidate the relationships between the basil varieties, their region of growth, and their essential oil composition. In addition to identifying several chemotypes, such as linalool, linalool/eugenol, linalool acetate, methyl eugenol, methyl chavicol, eugenol, methyl cinnamate E, and geraniol, a new compound, namely, octadecenamide (Z) (oleamide), was detected in the essential oil of *Ocimum basilicum* L. from Algiers (A1). **[A. L. de Oliveira 2022]**

Two high essential oil yielding hybrids, namely one interspecific hybrid between of *O. basilicum* and *O. kilimandscharicum* Gürke (HYBL-1) and another intraspecific hybrid of *O. basilicum* × *O. basilicum* (OBL-1) of basil were analyzed using GC, enantiomeric GC, NMR, enantio-GC–MS and GC–MS methods. Inter specific hybrid HYBL-1 contained high essential oil-rich in linalool (68.5%), camphor (8%), and 1,8-cineole (4.6%) as characteristic constituents among monoterpenoids. **[Ashish Kumar et al.2022]**

Intercropping and sole cropping of lemongrass and lemon-scented basil crops were evaluated to know the utility of lemongrass intercropping with lemon-scented basil

oil yield and chemical profiles by utilizing the space available during the initial lag phase to increase the essential oil yield and income of the farmer. The results showed that Intercropping of lemongrass variety Krishna with variety CIM Jyoti of lemon-scented basil recorded significantly higher essential oil yield than different cultivation/cropping of lemongrass and lemon-scented basil. **[kumar ashish et al.2022]**

The adoption of suitable irrigation levels (IRL), humic acid doses (HAD) and soil mulching (SM) are important tools for improving the morpho-physiological and biochemical traits of medicinal and aromatic plants. *Ocimum basilicum* L. cultivated under four IRL: IRL 100 = 100% FC–IRL 75 = 75% FC–IRL 50 = 50% FC–IRL 25 = 25% FC and four HAD: HA 0 = 0.0 L ha⁻¹–HA 10 = 10.0 L ha⁻¹–HA 20 = 20.0 L ha⁻¹–HA 40 = 40.0 L ha⁻¹ were applied in order to evaluate morpho-physiological and biochemical traits under the ecological condition. The plant height (PH), fresh herb yield (FHY), dry herb yield (DHY), dry leaf yield (DLY), protein ratio (PR), and main essential oil compounds (MEOC) of *Ocimum basilicum* L. In addition, PH, FHY, DLY, and EOY were highest at HA 40 and HA 20. HA particularly improved FHY, DLY, and the main essential oil compounds that can be considered plant bio stimulants , which were defined by several studies and regulations.**[Zehra aytac et al.2023]**

The effects of light on plants depend on the energy dose and spectral characteristics. We investigated how UV-A radiation with two different wavelengths, 365 and 385 nm, combined at different intensities as supplemental light to red-blue baseline illumination affects growth and selected physiological and biochemical traits of green leaf basil (*Ocimum basilicum* L.). We did not detect any negative effects of UV-A on plant growth and photosynthetic functions, even at high doses of short-wave UV-A. Furthermore, thylakoid processes were enhanced under UV-A, as evidenced by higher electron transport rates and increased photochemical efficiency. The increase in anthocyanin index (ARI) indicates the UV-screening response. UV-A increased the pool of xanthophyll cycle pigments and their ratio to chlorophylls. All this prevents photoinhibition and contributes to a high plasticity/efficiency of the basil's response to light environment. **[Mariana Cecilia Groharc et al. 2023]**

CHAPTER 3

AIM AND OBJECTIVE

3.1 AIM

To evaluate the antimicrobial activity and effectiveness of humic acid induced callus.

3.2 OBJECTIVE

- Cultivation of callus by using plant tissue culture methods.
- To identify and compare the substances present in control callus and humic acid callus by gas chromatography mass spectrometry.
- To determine the antimicrobial properties of callus and humic acid callus.
- To determine the concentration of humic acid at which the callus grows best.
- To identify the antioxidant effects of humic acid callus and to do a comparative study.
- To perform thin layer chromatography to separate the compounds from the humic acid callus methanol extract.
- To determine the phytoconstituents present in humic acid callus by using phytochemical test.

CHAPTER 4

MATERIALS AND METHODS

4.1 SAMPLE COLLECTION

Few explants were collected from the plant *Ocimum africanum*. Take the plant, remove the leaves and cut the stem into small pieces. Put these explants into a beaker containing few drops of disinfectant. Close the beaker with the help of a cloth and tie it with the thread. Keep the beaker on running tap water for 30 minutes. After 30 minutes drain the water from the beaker. Add hypochlorite solution to the beaker and swirl it for 20 minutes. After 20 minutes pour the hypochlorite solution from the beaker. Add sterile distilled water to the beaker and swirl it for 2 minutes and pour the water and repeat for five times.

4.2.1 STOCK PREPARATION

4.2.1 SODIUM HYPOCHORITE SOLUTION:

Take 100ml of distilled water and add 1ml of NaClO to it and stir it completely. Then mark the bottle with the date and NaClO and keep for sterilization in a cooker. After sterilization it can be used.

4.2.2 MERCURIC CHORIDE SOLUTION:

Take 100ml of distilled water and add 0.1g of HgCl₂ and dissolve it completely. Then mark the bottle with the date and HgCl₂ and keep for sterilization in a cooker. After sterilization it can be used.

4.3 MEDIA PREPARATION

For media preparation take 1 liter of distilled water. Add 30g/l of sucrose and 4.4g/l of MS medium to 1 liter of distilled water. Stir and completely dissolve the MS media and sucrose. Check the pH of the mixture. It should be between 5.6 – 5.8. If the pH

is less than 5.6 add few drops of 1N NaOH and bring the needed Ph. if the ph is above 5.8 then add few drops of 1N HCl and bring the needed ph. Ph meter rod should be cleaned before and after using it for correct enumeration of the ph. Split the 1-liter mixture into four equal parts each containing 250ml. Take a beaker and add 250ml of the mixture and add 2g of agar. Boil the mixture until the agar dissolves completely. After boiling, take 0.5mg/l of 2-4 D hormone and add it to the boiled mixture and stir it completely. Now pour this prepared media into six glass bottles equally each containing 30ml and label it has S-1. Now take the next 250ml of the mixture and add 2g of agar and boil it until the agar melts completely. After boiling add 1mg/l of 2-4 D hormone and stir it completely. Now pour this media into another six bottles each containing 30ml and mark them as S-2. Again, take the third 250ml and add 2g of agar and boil it until it dissolves completely and add 1.5 mg/l of 2-4 D hormone and stir it completely. Then pour the media into six glass bottles each containing 30ml of media and mark it as S-3. Take the last 250ml of the mixture and add 2g of agar to it and boil it. After boiling add 2mg/l of 2-4 D hormone. stir it completely and transfer it six glass bottles each containing 30ml and mark it as S-4. After the media completely cools, close the bottles and label the batches as s-1, s-2, s-3 and s-4 respectively in their caps. Keep these 24 bottles in a cooker. After five whistle keep the flame at low for 15 minutes. Keep the cooker aside for some time until the pressure releases completely. Then take the sterilized glass bottles containing medium and place them in plant tissue culture room. **[Ali Talati 2017]**

4.4 CULTURE PROCEDURE

Wipe the laminar air flow with ethanol and switch on the UV for 15 minutes. After 15 minutes switch off the UV and switch on the light and blower. Bring the beaker containing the explants to the laminar chamber. Remove the cloth from the beaker and add mercuric chloride solution and swirl it for 3 minutes. Then pour the HgCl₂ and add distilled water to the beaker and wash for five times each 2 minute. Take the forceps and surgical knife and sterilize it before using. Using the forceps take the plant explants and drain the excess water from it using a tissue paper. Take each leaf and cut the edges of the leaf and bring like a square or rectangle shape using the surgical knife. If the explants are stems then cut the edges. Take the

prepared S-1 ,S-2 ,S-3 and S-4 medium bottles and place the explants in these bottles. Place two to three explants in each medium bottle. Remove water if incase present in the bottle before placing the explants into it. After placing the explants close the lid and label it and seal it tightly with a tape. Then keep these culture bottles in plant tissue culture lab. [**Chimdessa E 2020**]

4.5 HUMIC ACID PREPARATION

Take 100ml of distilled water. Add 1g of humic acid to it and stir it well. Store it in a bottle.

4.5.1 HUMIC ACID INDUCED MEDIA PREPARATION

For media preparation take 1 liter of distilled water. Add 30g/l of sucrose and 4.4g/l of MS medium to 1 liter of distilled water. Stir and completely dissolve the MS media and sucrose. Check the pH of the mixture. It should be between 5.6 – 5.8. if the pH is less than 5.6 add few drops of 1N NaOH and bring the needed pH. If the pH is above 5.8 then add few drops of 1N HCl and



Fig: 4.5: Humic Acid

bring the needed ph. Ph meter rod should be cleaned before and after using it for correct enumeration of the ph. Split the 1-liter mixture into 5 equal parts each containing 200ml. take the first 200ml of the mixture, and add 100ul of humic acid and 1.6g of agar and boil it until the agar melts completely. After boiling add 0.5ml of 2-4 D hormone and stir it well. Pour this to 5 glass bottles equally and label it as HA-1. take the second 200ml of the mixture, and add 250ul of humic acid and 1.6g of agar and boil it until the agar melts completely. After boiling add 0.5ml of 2-4 D hormone and stir it well. Pour this to 5 glass bottles equally and label it as HA-2. take the third 200ml of the mixture, and add 500ul of humic acid and 1.6g of agar and boil it until the agar melts completely. After boiling add 0.5ml of 2-4 D hormone and stir it well. Pour this to 5 glass bottles equally and label it as HA-3. take the fourth 200ml of the mixture, and add 750ul of humic acid and 1.6g of agar and boil it until the agar melts completely. After boiling add 0.5ml of 2-4 D hormone and stir

it well. Pour this to 5 glass bottles equally and label it as HA-4. take the last 200ml of the mixture, and add 1000ul of humic acid and 1.6g of agar and boil it until the agar melts completely. After boiling add 0.5ml of 2-4 D hormone and stir it well. Pour this to 5 glass bottles equally and label it as HA-5. After the media completely cools, close the bottles and label the batches as HA 1, HA 2, HA 3, HA 4 and HA 5 respectively in their caps. Keep these 25 bottles in a cooker. After five whistle keep the flame at low for 15 minutes. Keep the cooker aside for some time until the pressure releases completely. Then take the sterilized glass bottles containing humic acid induced medium and place them in plant tissue culture room. [**Prakash P et al.2014**]

4.6 COMPOSITION OF MURASHIGE AND SKOOG MEDIUM

Table 4.6.1: Major Nutrients

MAJOR NUTRIENTS	STOCK (g)	DISSOLVED IN WATER (ml)	FOR 1 LITRE (ml)
$(\text{NH}_4)_2\text{NO}_3$	16.50 g	500 ml	50 ml
KNO_3	19.00 g		
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	4.40 g		
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	3.70 g		
KH_2PO_4	1.70 g		

Table 4.6.2: Micro Nutrients

MICRONUTRIENTS	STOCK (g)	DISSOLVED IN WATER (ml)	FOR 1 LITRE (ml)
MnSO ₄ .4H ₂ O	2.23 g	250 ml	2.5 ml
ZnSO ₄ .7H ₂ O	0.86 g		
H ₃ BO ₃	0.62 g		

Table 4.6.3: Minor Nutrients

MINOR NUTRIENTS	STOCK (g)	DISSOLVED IN WATER (ml)	FOR 1 LITRE (ml)
Na ₂ MoO ₄ .4H ₂ O	0.12 g	500 ml	1.0 ml
CuSO ₄ .5H ₂ O	0.01 g		
CoCl ₂ .6H ₂ O	0.01 g		

Table 4.6.4: Potassium

POTASSIUM	STOCK (g)	DISSOLVED IN WATER (ml)	FOR 1 LITRE (ml)
KI	0.08 g	250 ml	2.5 ml

Table 4.6.5: Iron

IRON	STOCK (g)	DISSOLVED IN WATER (ml)	FOR 1 LITRE (ml)
Na ₂ EDTA	1.86	250	5
FeSO ₄	1.39		

Table 4.6.6: Vitamins

VITAMINS	STOCK (g)	DISSOLVED IN WATER (ml)	FOR 1 LITRE (ml)
Thiamine HCl	0.01 g	100 ml	1 ml
Pyridoxine HCl	0.05 g		
Nicotinic acid	0.05 g		
Glycine	0.20 g		

4.6.1 MEDIA PREPARATION COMPOSITION:

Sucrose - 30g/L

Inositol - 100mg/L

Agar - 8g/L

4.7 EXTRACTION

4.7.1 PLANT EXTRACTION

- Whole plant of *Ocimum africanum* was washed and shade dried
- After complete drying , the samples were kept in the beaker containing 30ml methanol.



Fig: 4.7.1: Plant Extraction

4.7.2 CALLUS EXTRACTION

- Take the bottles labelled "S "from the tissue culture room.
- Collect the callus and weigh them
- Note down the wet weight readings
- Take a Petri dish and place a tissue paper over it and place the callus and allow it for drying
- After 1 week, take the callus and note down the dry weight value.
- Now add these dried "s "callus to another bottle containing 30ml of methanol.



Fig: 4.7.2: Callus Extraction

4.7.3 HUMIC ACID EXTRACTION

- Take the bottles labelled “HA “from the tissue culture room.
- Collect the callus and weigh them
- Note down the wet weight readings
- Take a Petri dish and place a tissue paper over it and place the callus and allow it for drying
- After 1 week, take the callus and note down the dry weight value.
- Now add these dried “HA “callus to another bottle containing 30ml of methanol.

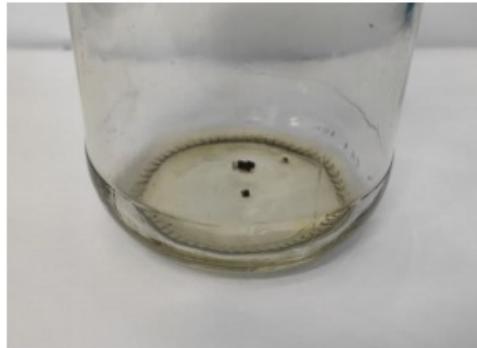


Fig: 4.7.3: Humic acid Extraction

CHAPTER 5

RESULT AND DISCUSSION

5.1 GROWTH OF CALLUS

5.1.1 MS Media Induced Callus

Table 5.1.1: MS media Induced callus

DATE	NUMBER OF BOTTLE	CODE OF MEDIUM	NUMBER OF EXPLANT	PRESENCE OF CONTAMINATED EXPLANT
10/9/22	10	S-1	1	Fungal
			2	Fungal
			1	Fungal
		S-2	3	fungal
			1	-
			2	Bacterial
		S-3	1	Fungal
			2	-
		S-4	3	Fungal
			3	Bacterial

5.1.2 Humic Acid Induced Callus

Table 5.1.2: Humic Acid Induced Callus

DATE	NUMBER OF BOTTLE	CODE OF MEDIUM	NUMBER OF EXPLANT	PRESENCE OF CONTAMINATED EXPLANT
13/9/22	13	HA-1	1	-
			1	-
			2	Fungal
		HA-2	2	Fungal
			2	Fungal
			1	-
		HA-3	2	Fungal
			1	-
			3	Fungal
		HA-4	1	Fungal
			2	Fungal
			2	Fungal
			2	-

5.2 PHYTOCHEMICAL TEST

- We carried biochemical test for plant extract, plant Callus and humic acid induced callus.
- The different qualitative chemical tests were performed for establishing the profile of the extract for its chemical composition.
- The following tests were performed on the extract to detect various phytoconstituents present in them.

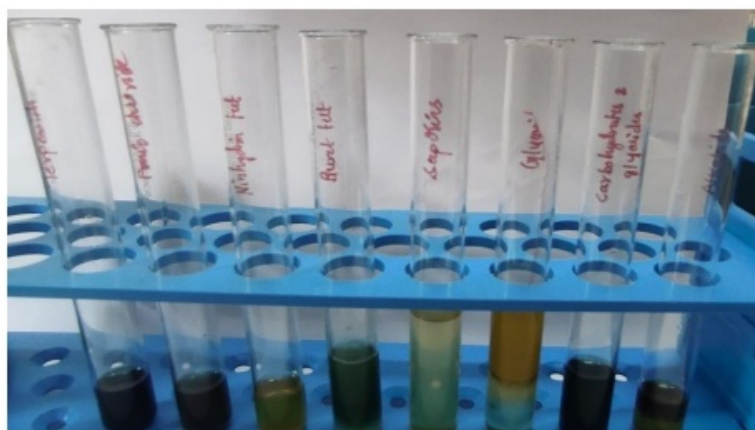


Fig: 5.2. phytochemical test

Table 5.2: Phytochemical analysis of different extracts of *Ocimum africanum*

BIOCHEMICAL TESTS	PLANT SAMPLE	HUMIC ACID INDUCEED CALLUS HA-4	HUMIC ACID INDUCEED CALLUS HA-5
Detection Of Alkaloids. Dragendrafts Test	Present of yellow precipitate	Present	Present
Detection Of Carbohydrates and Glycosides. Fehling's Test	Absent of red precipitate	Absent	Absent
Detection Of Glycosides' Borntragers Test	Absent of pink color	Absent	Absent
Detection Of Saponins Foam Test	Absent of foam	Absent	Absent
Detection Of Protein and Amino Acis. A) Buret Test B) Ninhydrin Test	A) Present of pink Layer B) Absent of purple color	A) Present B) Absent	A) Present B) Absent
Detection Of Phenolic Components Ferric Chloride Test	Absent of green color	Absent	Absent
Test For Teraponids	Present of yellow Precipitate to red precipitate	Present	Present

5.3 THIN LAYER CHROMATOGRAPHY

- Take TLC silica plate and cut them into 8cm x 4.5cm and label them
- Take the extracts of the plant, S and HA callus and place small drops of the extract to the respective markings.
- Take a beaker and add toluene and ethyl acetate.
- Take the marked plate and place it evenly into the beaker and close the lid.
- We can observe the rising up of extract on the plate.
- Let it rise until the top line.
- Take the plate out and let it dry for 2 minutes.
- Now place the TLC plate under UV cabinet and observe the readings.

THIN LAYER CHROMATOGRAPHY SYSTEMS

TLC SYSTEM – 1

- Add 3ml of toluene and 1ml of ethyl acetate to the beaker.

TLC SYSTEM – 2

- Add 2ml of toluene and 2ml of ethyl acetate to the beaker.

TLC SYSTEM - 3

- Add 1ml of toluene and 3ml of ethyl acetate to the beaker.

TLC SYSTEM – 4

- Add 2ml of methanol to the beaker.

CALCULATION OF RETENTION FACTOR (R_F) VALUE

$$R_f \text{ VALUE} = \frac{\text{SOLUTE FRONT}}{\text{SOLVENT FRONT}}$$

5.3.1 TLC SYSTEM 1

Table 5.3.1: TLC System 1

SPOTS	R _f value of different extracts		
	PLANT EXTRACT	S-CALLUS EXTRACT	HA- CALLUS EXTRACT
Spot 1	0.04	0.08	0.08
Spot 2	0.36	0.11	0.14
Spot 3	0.45	0.98	0.76
Spot 4	0.54	-	-
Spot 5	0.60	-	-
Spot 6	0.75	-	-
Spot 7	0.97	-	-

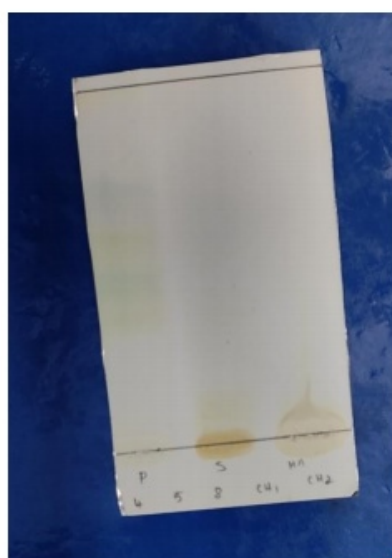


Fig: 5.3.1.1: TLC System 1

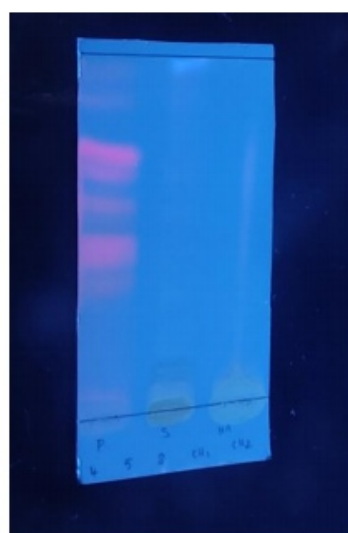


Fig: 5.3.1.2: TLC System 1

5.3.2 TLC SYSTEM 2 :

5.3.3 Table 5.3.2: TLC System 2

SPOT	R _f value of different extracts		
	PLANT EXTRACT	S-CALLUS EXTRACT	HA – CALLUS EXTRACT
Spot 1	0.04	0.10	0.17
Spot 2	0.17	0.26	0.51
Spot 3	0.82	0.33	0.60
Spot 4	0.91	0.51	0.72
Spot 5	1	0.69	-



Fig: 5.3.2.1: TLC System 2

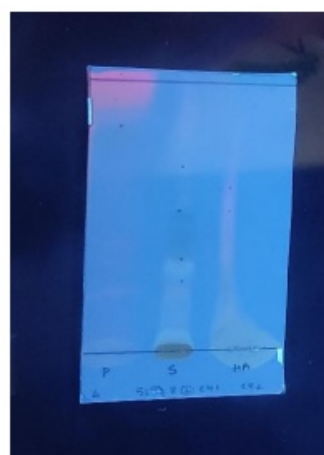


Fig: 5.3.2.2: TLC System 2

5.3.4 TLC SYSTEM 3 :

5.3.5 Table 5.3.3: TLC System 3

SPOT	R _f value of different extracts		
	PLANT EXTRACT	S-CALLUS EXTRACT	HA CALLUS EXTRACT
Spot 1	0.05	0.04	0.08
Spot 2	0.25	0.35	0.16
Spot 3	0.48	0.42	0.38
Spot 4	0.64	0.63	0.54
spot 5	0.77	0.67	0.73
Spot 6	0.97	0.86	-



Fig: 5.3.3.1: TLC System 3

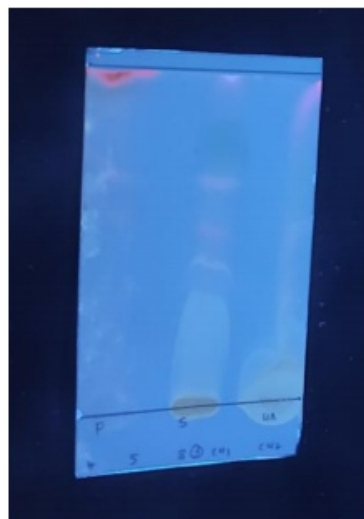


Fig: 5.3.3.2: TLC System 3

5.3.4 TLC SYSTEMS 4 :

Table 5.3.4: TLC System 4

SPOT	R _f value of different extracts		
	PLANT EXTRACT	S-CALLUS EXTRACT	HA CALLUS EXTRACT
Spot 1	0.54	2.72	0.07
Spot 2	0.64	0.55	0.5
Spot 3	0.73	0.76	0.88
Spot 4	0.80	0.88	-

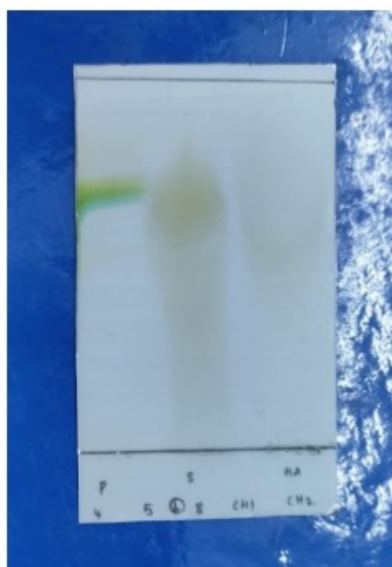


Fig: 5.3.4.1: TLC System 4



Fig: 5.3.4.2: TLC System 4

Thin layer chromatography was performed with different ratios of solvents. Toluene and ethylacetate was used as solvents from TLC system- 1 to 3 and methanol was used as solvent in TLC system - 4. The R_f values were calculated and plotted as table above.

Thin-Layer chromatographic separation and validated HPTLC method for quantification of Ursolic acid in Various *Ocimum* species was done by Kedar Kumar Rout, Rajesh Kumar Singh, Durga Prasad Barik, Sagar Kumar Mishra. Quantification and densitometric determination were performed after derivatization of the plate with methanol-sulphuric acid reagent in reflection/absorption mode at 540 nm. The limit of detection and limit of quantification were found to be 20 and 35 ng/spot, respectively. The response of ursolic acid was linear over the range of 40 to 440 ng/spot with a correlation coefficient of $r^2 = 0.9995$. (**Kedar Kumar et al. 2012**)

Phytochemical and pharmacological evaluation of the residue by-product developed from the *Ocimum americanum* (Lamiaceae) postdistillation waste was studied by Izabela Jasicka-Misiak, Mariia Shanaida, Natalia Hudz, Piotr Pawel Wieczorek. The study's revealed that the dry extract made from the herb American basil (*Ocimum americanum* L) was used to identify the extract's rosmarinic acid, caffeic acid, and rutin content by high-performance thin-layer chromatography. The extract included many polyphenols. The primary constituents were rutin, luteolin-7-O-glucoside, and rosmarinic acid. The extract's total phenolic content was 106.31 mg GAE/g, and its IC_{50} value for its ability to scavenge free radicals against 2, 2-diphenyl-1-picrylhydrazyl was 0.298 mg/mL. (**Izabela Jasicka-Misiak et al. 2021**)

5.4 GAS CHROMATOGRAPHY MASS SPECTROMETRY

5.4.1 CONTROL CALLUS:

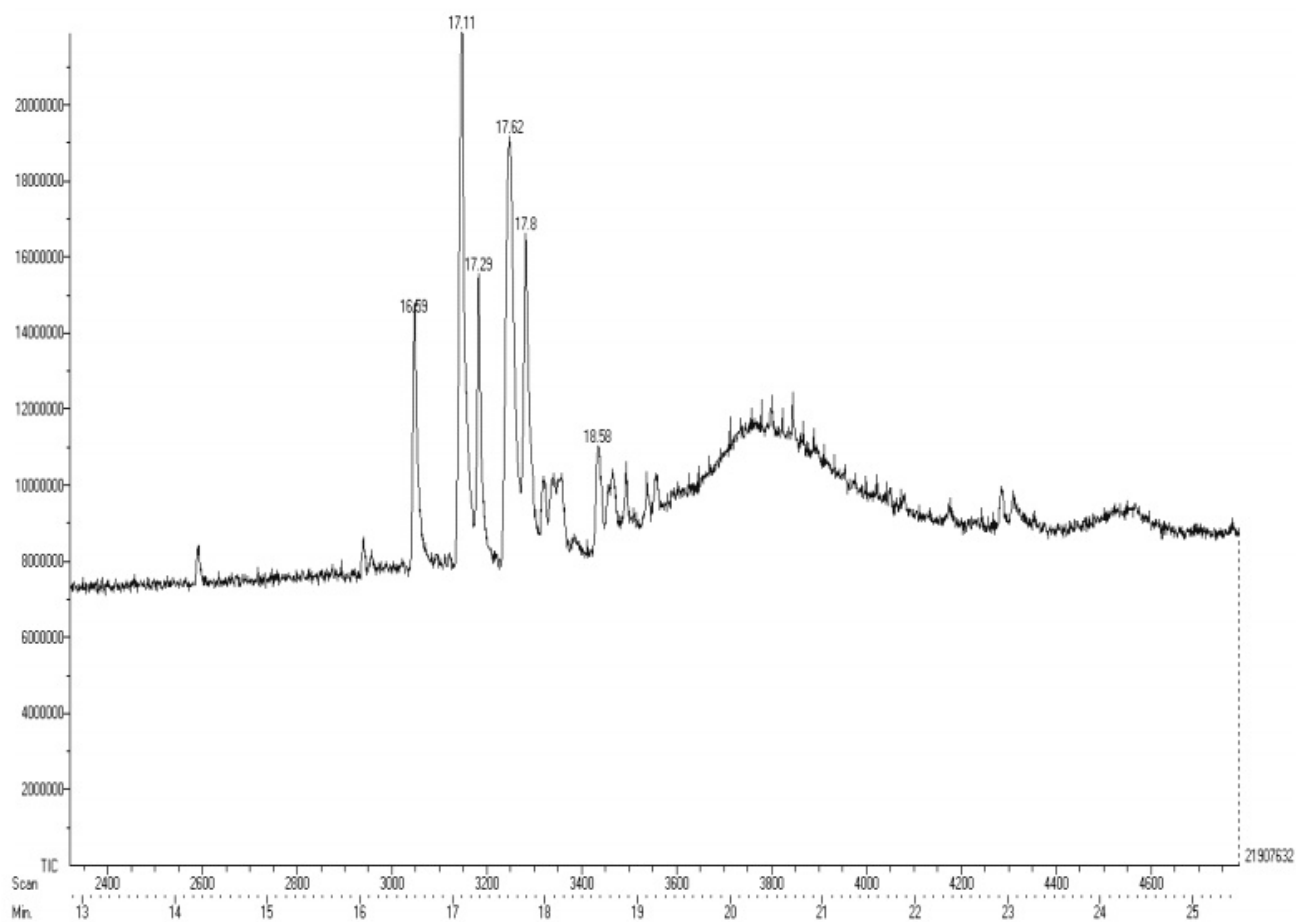
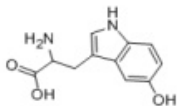
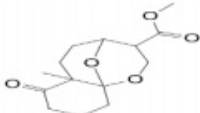
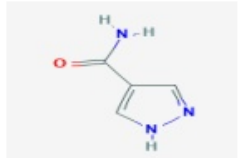
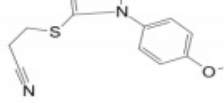


Fig: 5.4.1: Control Callus

Table 5.4.1: Control Callus

S.NO	RT VALUE	COMPOUND NAME	COMPOUND STRUCTURE	USES
1	16.59	DI-5-Hydroxytryptophan		Effective in treating a wide variety of conditions, including fibromyalgia, insomnia, binge eating associated with obesity, cerebellar ataxia, and chronic headaches.
2	17.11	5-Hydroxy-6-methyl-12,13-dioxatricyclo [7.3.1.0(1,6)]tridecane-10-carboxylic acid, methyl ester		-
3	17.8	Pyrazole-1-carboxamide,4-bromo-N-chloroacetyl		-
4	18.58	3-[1-(4-Methoxyphenyl)-1H-tetrazol-5-ylsulfanyl]-propionitrile		-

5.4.2 HUMIC ACID CALLUS EXTRACT:

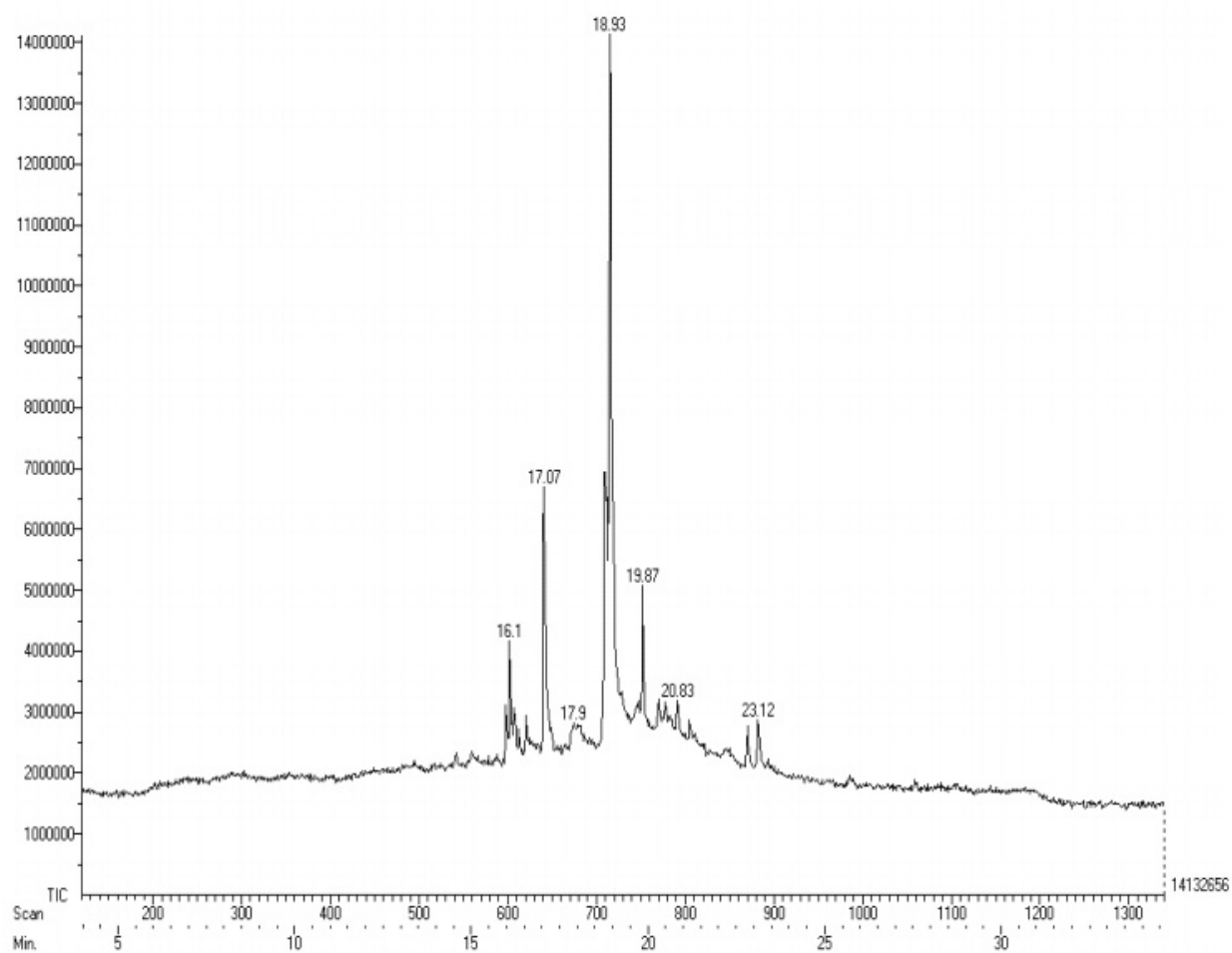
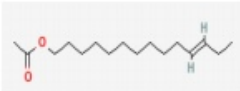
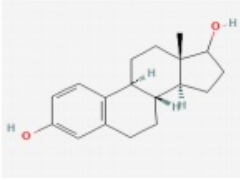
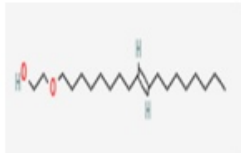
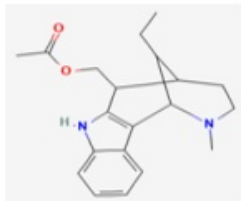


Fig: 5.4.2: Humic acid Callus Extraction

Table 5.4.2: Humic acid Callus Extraction

S.NO	RT VALUE	COMPOUND NAME	COMPOUND STRUCTURE	USES
1	16.1	11-tetradecyn- 1-ol acetate		Agrochemicals Pesticides
2	17.07	Hexadecenoic acid, methyl ester		Antioxidant, antimicrobial, and anti-inflammatory activities.
3	17,9	Estra- 1,3,5(10)-trien- 17a-ol		An estrogen and a human metabolite
4	18.93	phytol		Potential anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, antinociceptive, anti- inflammatory, immune- modulating, and antimicrobial effects

S.NO	RT VALUE	COMPOUND NAME	COMPOUND STRUCTURE	USES
5	19.87	Ethanol,2-(9-octadecenylloxy)-[2]		-
6	20.83	Dasycaroidan-1-methanol,acetate (ester)		Medicinal plants are important source of antibacterial compounds
7	23.12	Rutin		vitamin P acts as an antioxidant and protects against chronic diseases

5.4.3 PLANT EXTRACT

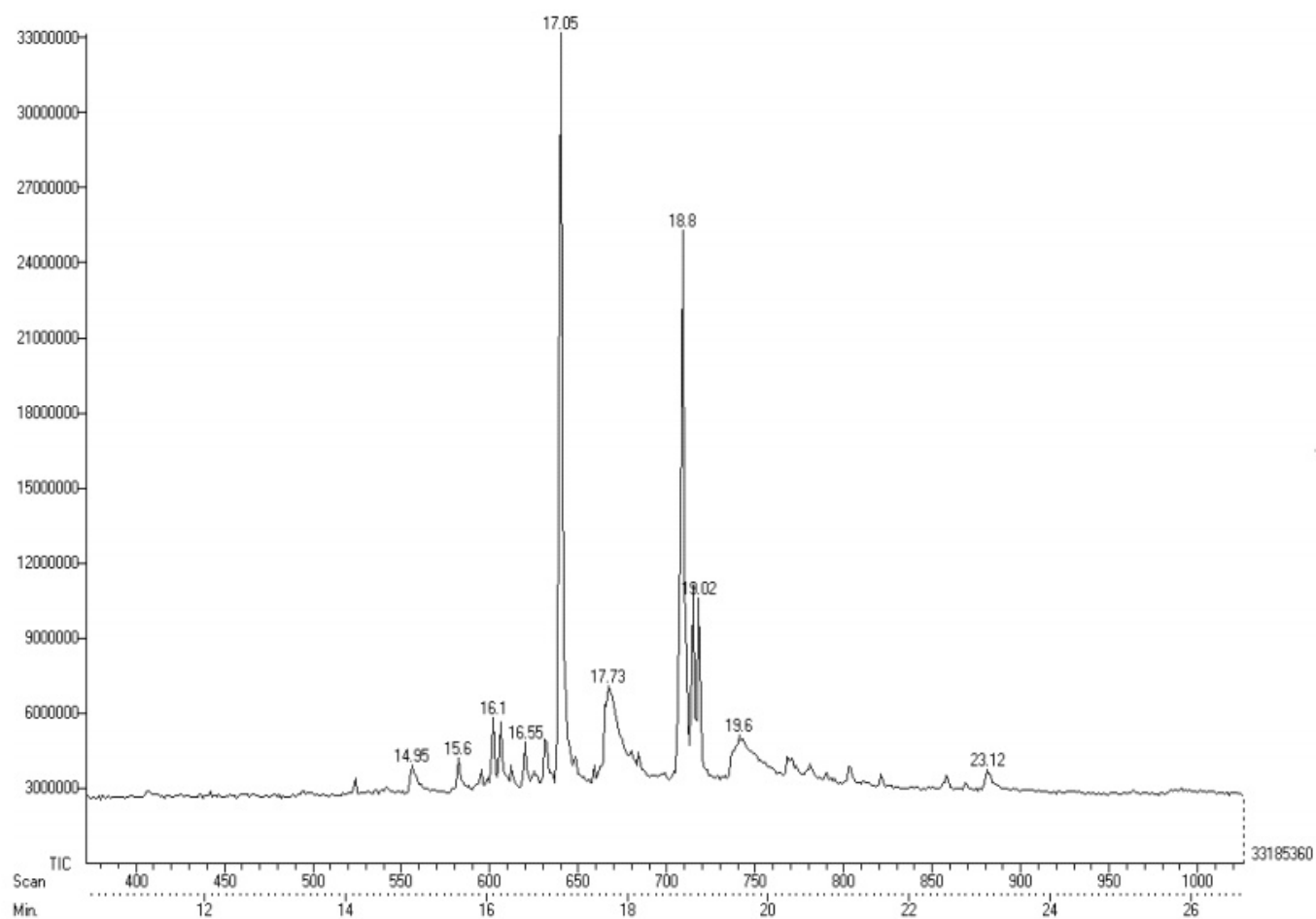
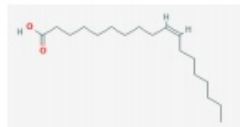
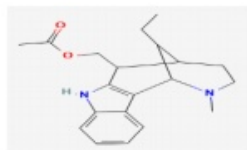


Fig: 5.4.3: Plant Extract

Table 5.4.3: Plant Extract

S.NO	RT VALUE	COMPOUND NAME	COMPOUND STRUCTURE	USES
1	14.95	Tridecanoic acid,12-methyl, methyl ester		It has a role as a plant metabolite.
2	15.6	1-Hexadecanol,2- methyl3		medicine to control diabetes mellitus as well as various other diseases.
3	16.1	E-11-methyl-12- tetradecen-1-ol- acetate		-
4	16.55	Z-10-methyl-11- tetradecen-1-ol- propionate		-
5	17.05	Hexadecenoic acid, methyl ester		Antioxidant, Antimicrobial, & anti- inflammatory activities
6	17.73	N-hexadecenoic acid		Anti-inflammatory agents

7	18.8	10-Octadecenoic acid, methyl ester		Antibacterial, antifungal, antioxidant, decrease blood cholesterol
8	19.02	Heptadecanoic acid ,16-methyl-, methyl ester		Treatment of skin cancer, antibacterial and antioxidant
9	19.6	Dasycarpidan-1-methanol, acetate		Medicinal plants are important source of antibacterial compounds

GCMS was performed for plant extract , sample callus and HA callus of *Ocimum africanum* . The study revealed that plant extract had 9 chemical components , sample callus had 4 chemical components and HA callus also had 4 chemical components. Hexadecenoic acid, methyl ester and Dasycarpidan-1-methanol, acetate components were found both in plant extract as well as in HA callus.

Diversity of the genus *Ocimum* (Lamiaceae) through morpho-molecular (RAPD) and chemical (GC–MS) analysis was studied by Tanmay Chowdhury, Amitava Mandal, Subhas Chandra Roy, Dilip De Sarker. The evaluation of morphological characteristics and chemical components included 18 qualitative and 17 quantitative measurements. The study reveals that the *O. africanum* and *O. basilicum* species, significant variability in physical features were found. *O. africanum* and *O. basilicum*, did not exhibit substantial difference in chemical composition. **(Tanmay Chowdhury et al. 2017)**

5.5 ANTIOXDIANT ASSAY

5.5.1 PLANT EXTRACT:

Table 5.5.1: Plant Extract

Conc. ug/ml	% OF INHIBITION
20	14.44
40	34.79
60	49.89
80	56.67
100	70.89
IC₅₀	60.13

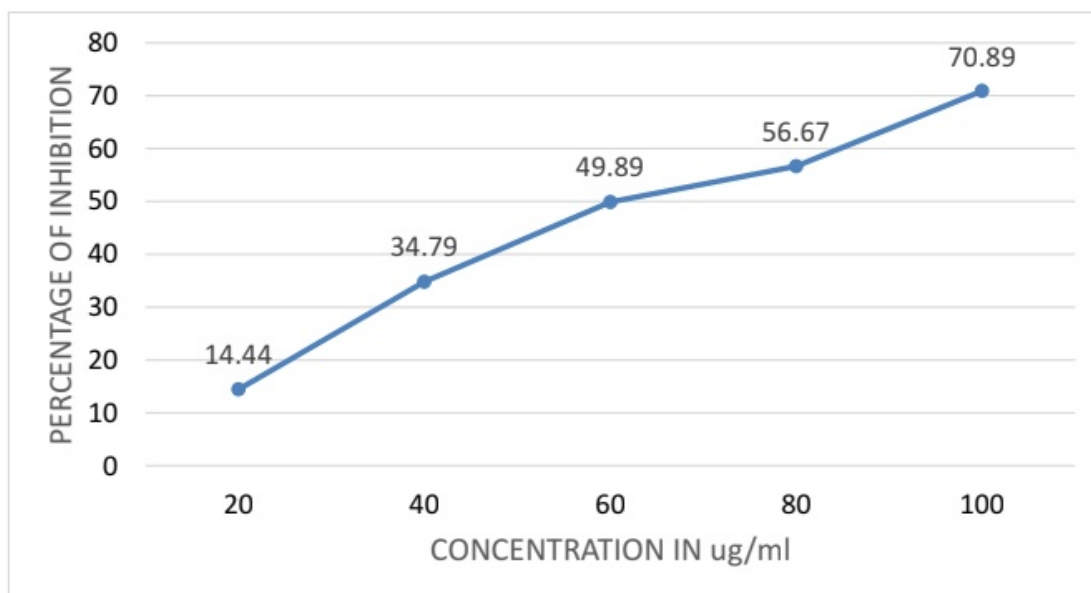


Fig: 5.5.1: Plant Extract

5.5.2 CONTROL

Table 5.5.2: Control

Conc. ug/ml	% OF INHIBITION
20	11.82
40	23.19
60	35.44
80	47.92
100	56.45
IC₅₀	83.47

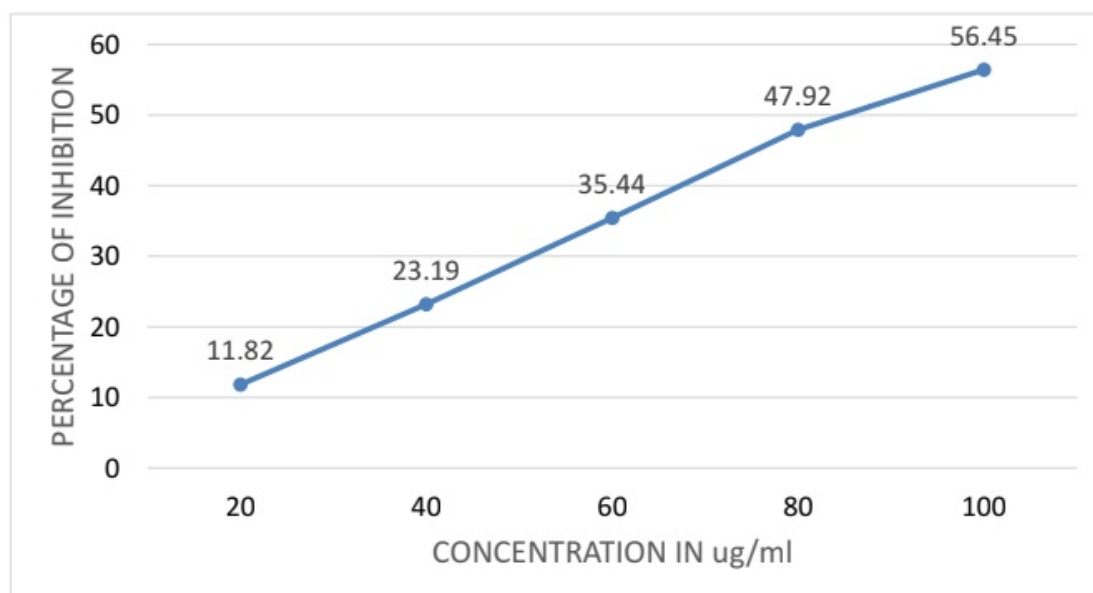


Fig: 5.5.2: Control

5.5.3 HUMIC ACID CALLUS EXTRACT:

Table 5.5.3: Humic Acid Callus Extract

Conc. ug/ml	% OF INHIBITION
20	16.19
40	30.85
60	46.17
80	59.95
100	73.96
IC₅₀	64.97

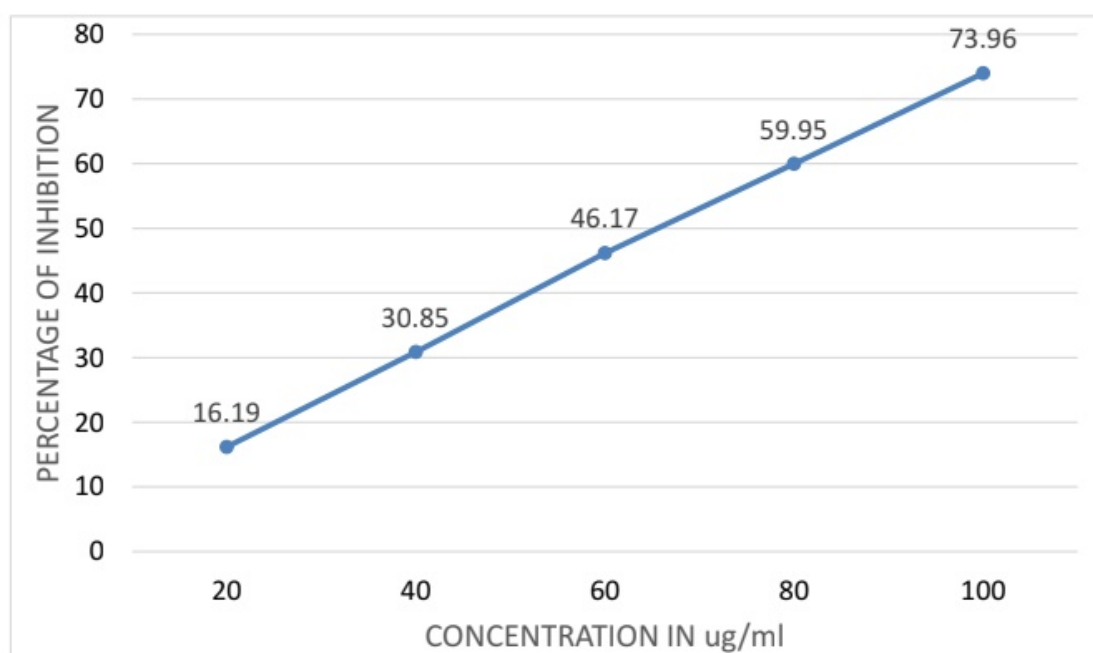


Fig: 5.5.3: Humic acid Callus

5.5.4 ASCORBIC ACID:

Table 5.5.4: Ascorbic Acid

Conc. ug/ml	% OF INHIBITION
20	33.46
40	47.09
60	55.60
80	71.62
100	79.27
IC ₅₀	51.58

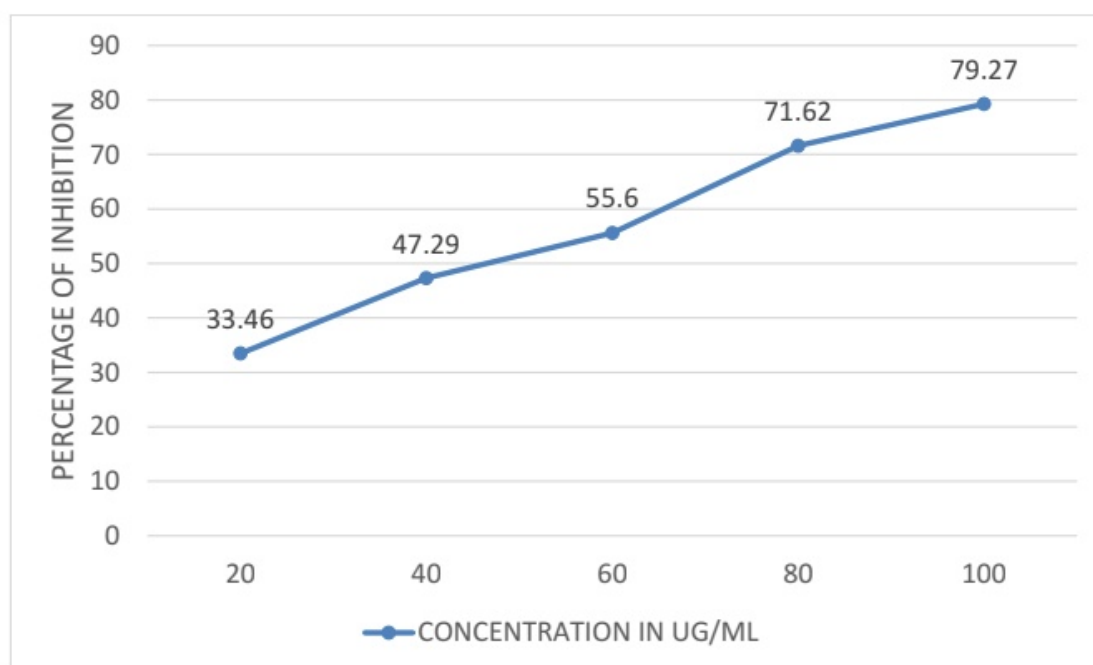


Fig: 5.3.4: Ascorbic acid

Antioxidant assay of methanol extract of *Ocimum africanum* cultivated in invitro cultivation was done. This study revealed that the lemon basil callus have antioxidant capacity. The antioxidant assay yielded an IC₅₀ of 83.47 ug/ml. A comparative study between HA callus and control callus revealed that HA callus is more effective than control callus with a difference of IC₅₀ value of 18.5. A comparative study between HA callus and ascorbic acid revealed that ascorbic acid is more effective than HA callus with a difference of IC₅₀ value of 13.39.

Phytochemical screening, total antioxidant capacity, and toxicity test of basil leaf methanol extract (*Ocimum africanum* lour) cultivated by invitro cultivation was done by David Limanan, Eny Yulianti, F Ferdinal on 1st Tarumanagara International conference on medicine and health (TICMIH 2021), 52-56, 2021. This study reveals that basil leaves have secondary metabolites and antioxidant capacity, but not as much as vitamin C. The antioxidant assay yielded an IC₅₀ of 174.04 g/mL. (**David Limanan et al. 2021**)

Phytochemical and pharmacological evaluation of the residue by-product developed from the *Ocimum americanum* (Lamiaceae) postdistillation waste was studied by Izabela Jasicka-Misiak, Mariia Shanaida, Nataliia Hudz, Piotr Paweł Wieczorek. The study's revealed that the dry extract made from the herb American basil (*Ocimum americanum* L was used to identify the extract's rosmarinic acid, caffeic acid, and rutin content by high-performance thin-layer chromatography. The extract included many polyphenols. The primary constituents were rutin, luteolin-7-O-glucoside, and rosmarinic acid. The extract's total phenolic content was 106.31 mg GAE/g, and its IC₅₀ value for its ability to scavenge free radicals against 2, 2-diphenyl-1-picrylhydrazyl was 0.298 mg/mL. (**Izabela Jasicka-Misiak et al. 2021**)

5.6 ANTIMICROBIAL ACTIVITY

5.6.1 ANTIMICROBIAL ACTIVITY OF CALLUS:

Table 5.6.1: Antimicrobial Activity of Callus

ORGANISM	Zone of inhibition (in mm)			
	50 ug/ml	75 ug/ml	100 ug/ml	ANTIBIOTIC Streptomycin (20 ug/ml)
<i>Staphylococcus aureus</i>	3	5	5	12
<i>Candida albicans</i>	-	3	5	10
<i>Escherichia coli</i>	5	10	8	3



Fig: 5.6.1.1: *Staphylococcus aureus*



Fig: 5.6.1.2: *Candida albicans*



Fig: 5.6.1.3: *E. coli*

5.6.2 HUMIC ACID INDUCEED CALLUS EXTRACT:

Table 5.6.2: Humic Acid Induced Callus Extract

ORGANISM	Zone of inhibition (in mm)			
	50 ug/ml	75 ug/ml	100 ug/ml	ANTIBIOTIC Streptomycin (20 ug/ml)
<i>Staphylococcus aureus</i>	3	5	10	20
<i>Candida albicans</i>	-	2	3	18
<i>Escherichia coli</i>	-	-	5	18



Fig: 5.6.2.1 *Staphylococcus aureus*

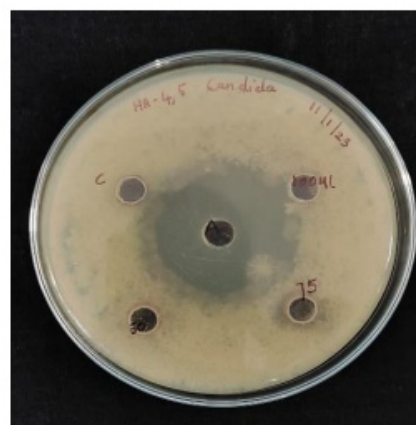


Fig: 5.6.2.2 *Candida albicans*

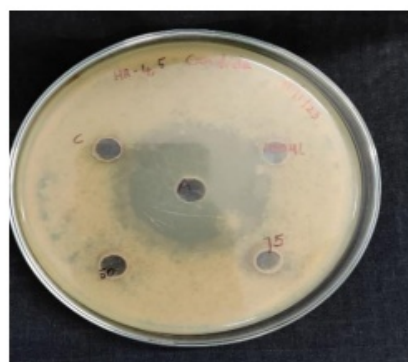


Fig: 5.6.2.3 *E. coli*

Antimicrobial activity of *Ocimum africanum* was done. This study reveals that the methanol extract of *Ocimum africanum* callus had a better growth reduction for *Staphylococcus aureus* , *E.coli* and *Candida albicans*.

Comparison of methods of extraction and antimicrobial activity of six *Ocimum* species against human pathogens was done by Manjula Bomma, Florence Okafor, SR Mentreddy, Leopold Nyochembeng, William Setzer, Bernhard Vogler. This study reveals that Essential oils of *Ocimum basilicum* (PI 358472) had a growth reduction of 57% for *S. pyogenes*, 72% for *E. coli*, methanolic extracts of different accessions of *Ocimum tenuiflorum* (MSR1) and *Ocimum basilicum* (PI 172996) showed a growth reduction of 74%, respectively, for *S. pyogenes* and 91% for *E. coli*. **(Manjula Bomma et al,2018)**

CHAPTER 6

SUMMARY AND CONCLUSION

The conclusion of Humic acid medium callus having potential activity of antimicrobial, antioxidant significant phytochemical compounds present and compare with control callus. In this study, the effect of humic acid on the growth of callus of *ocimum africanum* plant was investigated. A comparison study was also carried out between callus grown in MS medium and callus grown in HA induced MS medium. The effects of HA and soil applications of humic substances on the callus growth and grown at various HA concentrations were examined. The application doses of humic acid were 100ul, 250ul, 500ul, 750ul and 1000ul. Callus application of humic acid decreased the contamination rate of callus and also increased its growth rate. Callus growth was best at the concentration of 750ul and 1000ul. Supplements like 2-4 D hormone was also induced to the MS media at a concentration of 0.5ml/L. GCMS results of plant extract, callus and humic acid induced callus showed that compounds present in plant extract and HA induced callus were similar. Antioxidant assay also concluded that HA induced callus has an IC₅₀ value of 64.97 and callus with a value of 83.47 and thus shows HA callus has better antioxidant properties than callus. All together, these results provide evidence that HA can be used in plant tissue culture for better callus production and to decrease the contamination rate.

CHAPTER 7

REFERENCES

1. A Muscolo, M Sidari, V Cozzolino, A Nuzzo, S Nardi, Molecular characteristics of humic substances from different origins and their effects on growth and metabolism of *Pinus laricio* callus A Piccolo Chemical and Biological Technologies in Agriculture 9 (1), 72, 2022
2. Ali Talati Media Preparation for Plant Tissue Culture January 2017
3. Alice Nagai, Lígia ML Duarte, Déborah YAC Santos. Influence of Viral Infection on Essential Oil Composition of *Ocimum basilicum* (Lamiaceae) Natural product communications 6 (8), 2011.
4. Al-Mayahi, A.M. W. The effect of humic acid (HA) and zinc oxide nanoparticles (ZnO-NPS) on in vitro regeneration of date palm (*Phoenix dactylifera* L.) cv. Quntar Plant Cell Tiss Organ Cult 145, 445–456 (2021)
5. Ashish Kumar, RK Lal, CS Chanotiya, Akansha Dwivedi. The pre-eminence of agro-parameters and chemical constituents in the influence of harvest interval by traits× environment interaction over the years in lemon-scented basil Industrial Crops and Products 172, 113989, 2021
6. Chaimae Majdi et al. Phytochemical Characterization and Bioactive Properties of Cinnamon Basil (*Ocimum basilicum* cv. 'Cinnamon') and *Ocimum africanum* (*Ocimum × citriodorum*) Antioxidants (Basel). 2020.
7. Chimdessa E. Composition and Preparation of Plant Tissue Culture Medium. Tissue Bio Bioeng 3: 120. (2020)
8. Cymbopogon flexuous (Nees ...Kumar Ashish, AC Jnanesha, RK Lal, BK Dubey Acta. Intercropping and mixed herb distillation for high-quality oil yield using lemon-scented basil (*Ocimum africanum* Lour.) cv. CIM-Jyoti and lemongrass Ecologica Sinica 42 (4), 269-273, 2022
9. David Limanan, Eny Yulianti, F Ferdinal. Phytochemical Screening, Total Antioxidant Capacity, and Toxicity Test of Basil Leaf Extract (*Ocimum x africanum* Lour). 1st Tarumanagara International Conference on Medicine and Health (TICMIH 2021), 52-56, 2021
10. Dominik Vodnik, Žiga Vogrin, Helena Šircelj, Mariana Cecilia Grohar, Aljaž Medič, Klaudija Carović-Stanko, Toni Safner, Boris Lazarevič Phenotyping of basil (*Ocimum basilicum* L.) illuminated with UV-A light of different wavelengths and intensities Scientia Horticulturae 309, 111638, 2023

11. Godson Oseahumen Omoike, Betty Omenebelle George. In the antioxidant properties of *Ocimum africanum* leaves aqueous extract from the southeastern region of Nigeria after in vitro gastrointestinal digestion. *International Journal of Food Properties* 25 (1), 2000-2008, 2022
12. Izabela Jasicka-Misiak, Mariia Shanaida, Nataliia Hudz, Piotr Paweł Wieczorek. Phytochemical and pharmacological evaluation of the residue by-product developed from the *Ocimum americanum* (Lamiaceae) postdistillation waste. *Foods* 10 (12), 3063, 2021
13. Jaruwat Bunwijit, Bungorn Sripanidkulchai, Wanassanun Pannangrong, Jintana Junlatat, Kittisak Sripanidkulchai. Gastroprotective effect of hydroalcoholic extract of *Ocimum africanum* leaves. *Journal of Science & Technology* 39 (4), 2017
14. Kedar Kumar Rout, Rajesh Kumar Singh, Durga Prasad Barik, Sagar Kumar Mishra. Thin-Layer chromatographic separation and validated HPTLC method for quantification of Ursolic acid in Various *Ocimum* species. *Journal of Food and Drug Analysis* 20 (4), 22, 2012
15. L.A. Stetsenko¹, P.P. Pashkovsky¹, R.A. Voloshin¹, V.D. Kreslavski², V.I. Kuznetsov¹, S.I. Allakhverdiev¹. Role of anthocyanin and carotenoids in the adaptation of the photosynthetic apparatus of purple- and green-leaved cultivars of sweet basil (*Ocimum basilicum*) to high-intensity light. *Photosynthetica* 2020, 58(4):890-901
16. M Dina. Different Composition of Two *Ocimum* Species "Lamiaceae" Prepared by Different Methods and Analysed by GC-MS Spectroscopy, Manar Eissa in *Products and Services 2018 (i-CHIPS 2018)*
17. M G Tadros, M M Salama, M A Farag. In vitro and in vivo Anticholinesterase Activity of the Volatile Oil of the Aerial Parts of *Ocimum basilicum* L. and *O. africanum* Lour. Growing in Egypt. Shahira M Ezzat. *International Journal of Medical, Health, Biomedical, Bioengineering and Pharmaceutical Engineering*, 2014
18. Manjula Bomma, Florence Okafor, SR Mentreddy, Leopold Nyochembeng, William Setzer, Bernhard Vogler. Comparison of methods of extraction and antimicrobial activity of six *Ocimum* species against human pathogens. *Journal of Agriculture & Life Sciences* 5 (2), 2018
19. Marcone A. L. de. Optimization of GC-MS Method for Analysis of Basil Essential Oils from Algeria: Design of Experiments and Exploratory Pattern Recognition. Oliveira. Published 13 Oct 2022

20. Martielli Santana dos Santos¹, Lucinéia dos Santos Souza², Carlos Augusto Souza Costa², Fabio Pinto Gomes². Effects of water deficit on morphophysiology, productivity and chemical composition of *Ocimum africanum* Lour (Lamiaceae) African Larissa Journal of Agricultural Research 11 (21), 1924-1934, 2016
21. Melike Sayarer ¹, Zehra Aytaç ¹, and Mine Kürkçüoğlu. The Effect of Irrigation and Humic Acid on the Plant Yield and Quality of Sweet Basil (*Ocimum basilicum* L.) with Mulching Application under Semi-Arid Ecological Conditions Plants 2023, 12(7), 1522
22. Eileen M. Kwee, Emily D. Niemeyer variations in phenolic composition and antioxidant properties among 15 basil (*Ocimum basilicum* L.) cultivars Food Chemistry 128 (4), 1044-1050, 2011
23. Parmeshwar Lal Saran, Hiteksha I Damor, Mohan Lal, Rohan Sarkar, Kuldeep A Kalariya. Selection of Suitable Accession of *Ocimum africanum* Lour for Herbage and Citral Production Journal of Essential Oil-Bearing Plants 25 (3), 707-715, 2022
24. Pongsak RATTANACHAIKUNSOPON & Parichat PHUMKHACHORN Antimicrobial Activity of Basil (*Ocimum basilicum*) Oil against Salmonella Enteritidis in Vitro (2010) 74:6, 1200-1204
25. PRAGYA Pandey, R Upadhyay, V Singh. Screening of Antifungal Activity of Essential Oils and Its Chemical Composition Against Malassezia Furfur International Journal of Bio-Technology and Research 9 (2), 31-36, 2019
26. Ranjana Maurya, Pankhuri Gupta, CS Chanotiya, SS Dhawan, Shubham Srivastava, Anju Yadav, Ashish Kumar, Yvvs Swamy, RK Lal Investigation of monoterpenoids rich essential oils of two *Ocimum basilicum* L. varieties at different agro-climatic conditions in India Acta Ecologica Sinica 42 (2), 1-10, 2022
27. S Ilić, Lidija Milenković, Nadica Tmušić, Ljiljana Stanojević, Jelena Stanojević, Dragan Cvetković Lwt. Essential oils content, composition and antioxidant activity of lemon balm, mint and sweet basil from Serbia Zoran 153, 112210, 2022
28. S Pattnaik et al. In vitro propagation of the medicinal herbs *Ocimum americanum* L. syn. *O. canum* Sims. (Hoary basil) and *Ocimum sanctum* L. (holy basil) Plant Cell Rep. 1996 Aug.

29. Shubhangi Nagorao Ingole. Phytochemical analysis of leaf extract of *Ocimum americanum* L. (Lamiaceae) by GCMS method World Scientific News, 76-87, 2016
30. Suzan A Khayyat, L Selva Roselin Recent progress in photochemical reaction on main components of some essential oils Journal of Saudi Chemical Society 22 (7), 855-875, 2018
31. Tanmay Chowdhury, Amitava Mandal, Amit Kumar Jana, Subhas Chandra Roy, Dilip De Sarker. Study of phyto-sociology and ecology of naturally growing *Ocimum* species with their conservational strategies in Dakshin Dinajpur district of West Bengal Acta Ecologica Sinica 36 (6), 483-491, 2016
32. Tanmay Chowdhury, Amitava Mandal, Subhas Chandra Roy, Dilip De Sarker. Diversity of the genus *Ocimum* (Lamiaceae) through morpho-molecular (RAPD) and chemical (GC–MS) analysis. Journal of Genetic Engineering and Biotechnology 15 (1), 275-286, 2017
33. Winthrop B. Phippen, and James E. Simon. "Shoot Regeneration of Young Leaf Explants from Basil (*Ocimum Basilicum* L.)." In Vitro Cellular & Developmental Biology. Plant 36, no. 4 (2000): 250–54.