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Original Article

Comparative Analysis of Secondary Metabolites and Anxiolytic Effects in Hydroponic and Soil-Grown Basil (*Ocimum basilicum*)

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ABSTRACT

Background: This study compares two different agricultural systems in order to identify the most effective basil crop that meets present and future demand at lower expenses and resource use. The major types of basil plants that are grown are basil grown in soil and basil grown in hydroponic system. **Materials and Methods:** In the Soxhlet extraction of basil leaves, basil leaves organic solvent (ethanol) was used. Phytochemical content, total phenolic and flavonoid content and thin layer chromatography of ethanolic extract were tested. High Performance Thin Layer Chromatography (HPTLC) analysis of the two basil extracts was done. The anxiolytic effects of *Ocimum basilicum* Leaf Extract (OBLE) were examined in Wistar rats on Elevated Plus Maze (EPM) test. The reference medication diazepam was compared with the results. **Results:** Basil grown hydroponically gave 26.46% w/w output compared to 8.20% w/w output from basil grown in soil. Findings indicate the presence of several phytochemicals, including carbohydrates, flavonoids, glycosides and phenols, in both basil leaf extracts. The quantitative analysis revealed a total phenolic content of 1.198 mg/g in soil basil, 0.652 mg/g in hydroponic basil and 299.9 mg/g in total flavonoid content in soil basil, and 54.9 mg/g in hydroponic basil. The soil-grown TPC and TFC basil were higher in quantity than hydroponic basil. The HPTLC analysis of the ethanolic extract identified eugenol as a major secondary metabolite. Both extracts showed similar effects in terms of anxiolytic activity. **Conclusion:** The study concludes that the hydroponic growth offers a viable approach for generating high-quality basil that possesses comparable anti-anxiety qualities to soil-grown basil, while potentially alleviating issues related to contamination from soil-borne pesticides and toxins.

Keywords: Anxiolytic Activity, Eugenol, Hydroponics, *Ocimum basilicum*, Secondary Metabolites.

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INTRODUCTION

Hydroponics is the cultivation of plants in nutrient-rich liquid mediums without soil, with roots submerged. It was first referred to as "hydroponics" in the early 1930s by Professor William Gericke.[1] This method is less labor-intensive, saves water, enables yearly crop growth, facilitates intensive farming practices, and supports high-yield food production in limited spaces.[2,3] Circulating hydroponic systems like nutrient film technology and deep-water culture are the most technically and environmentally sound designs compared to conventional crop production methods.[4]

Lettuce, the most often grown hydroponically worldwide, is an example of waste reduction in action.[5,6] Hydroponic leaves are edible and can sell for up to 40% more than conventional lettuce crops, offering healthier food and higher nutritional quality.[7] Hydroponics helps avoid drawbacks of traditional farming, such as excessive water consumption, acreage requirements, high fertilizer and pesticide concentrations, and soil erosion and degradation.[5] *Ocimum basilicum* commonly referred to as the "king of herbs," is a member of the Lamiaceae family. This annual plant typically produces flowers that are white to purple in color.[8] Known for its distinctive flavors, it is widely used as a culinary herb and consumed in large quantities.[9] The plant is widespread, particularly in tropical regions across Asia, Africa, and Central and South America.[10] It contains numerous phytochemical compounds that offer various health advantages.[11,12] The roles of metabolites are multifaceted and include defence, communication, structure, fuel, and stimulatory and inhibitory effects on enzymes in addition to their own catalytic activity typically as an enzyme's cofactor.[13-15] Recent years have witnessed an increase in interest in the potential to modify the synthesis of bioactive plant metabolites through the use of tissue culture

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technologies, mostly due to the growing commercial significance of secondary metabolites.[13,14] It is possible to regularly develop plant cell and tissue culture technologies in aseptic conditions, using plant parts such as foliage, stalks, underground structures, and growth points, for both the method of secondary metabolite extraction and multiplication.[16,17] Secondary metabolites are necessary for the survival of an organism; in contrast to primary metabolites, their absence causes long-term impairment of the organism's fertility, aesthetics, or survivability, or sometimes no change at all. Biosynthetically produced from primary metabolites, secondary metabolites comprise a diverse array of active chemicals. Inside the kingdom of plants, their range is more restricted.[13] Additionally, the pharmacological qualities of plant secondary metabolites are beneficial to human health.[14]

MATERIALS AND METHODS

Collection of plant materials

Ocimum basilicum leaves were purchased from Natura Life Naatu Marunda Kadai, Alagapuram Salem, Tamil Nadu, India. Hydroponic basil leaves of *Ocimum basilicum* were purchased from Mint Super Fresh Gold Gym building, high street Ram Ganga Vihar, Moradabad, Uttar Pradesh, India, washed with distilled water, and shade dried.

Extraction of plant part

The extract of powdered dried basil (41 g) and hydroponic basil (10.48 g) was defatted with petroleum ether, extracted with absolute ethanol, and concentrated using a rotary evaporator, yielding a dark green residue. The percentage yield was calculated using the formula:

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

Qualitative phytochemical analysis

Fehling, Shinoda, Dragendorff's, Mayer's, and Wagner's tests were conducted to analyze plant extracts for carbohydrates, flavonoids, alkaloids, and phenolic compounds. These tests were conducted using various solvents and reagents using standard protocol.[18]

Quantitative analysis of phytochemicals

Determination of total phenolic contents

Gallic acid solution (taken as a standard curve) was plotted by combining 25 mg gallic acid in 25 mL of distilled water resulting in a concentration of 1 mg/mL. A 25 mg test extract was dissolved in 25 mL of distilled water. Several doses of gallic acid (100 mg/mL, 200 mg/mL, 300 mg/mL, 400 mg/mL, and 500 mg/mL) were selected to ascertain the dry extract. After transferring 1 mL of each gallic acid concentration into standard 25 mL flasks, distilled water (9 mL) was then incorporated. Identical procedures were applied to both the samples and the control. To each flask, 1 mL of Folin-Ciocalteu solution (FC) and 10 mL of a 7% sodium carbonate solution, subsequently adding distilled water to achieve a final volume of 25 mL. The mixtures were left to incubate for 90 min, after which absorbance measurements were taken at 750 nm using a UV spectrophotometer. A standard graph was plotted for gallic acid, and the dry extract was quantified as an equivalent of gallic acid.[19]

Determination of total flavonoid contents

A standard curve of quercetin was plotted by combining quercetin (25 mg) in methanol (25 mL), to obtain 1 mg/mL solution. This solution (1 mL) was moved to a 10 mL volumetric flask, and 100 mg of methanol was included. Subsequently, 10 mL of methanol was employed to dissolve 10 g of an accurately measured extract. The resulting solution was further diluted to attain a concentration of 100 mg/mL. The extracts for this solution were mixed with 2.8 mL of water, 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, and 0.1 mL of potassium acetate (1 M). With volumes of 25, 50, 75 and 100 mL, a standard curve was drawn for the flavonoid quercetin. The absorbance was recorded at 415 nm. A calibration curve of absorbance vs concentration was constructed for quercetin. The overall flavonoid concentration was determined as the quercetin equivalent.[20]

Thin layer chromatography

Silica gel thin-layer chromatography plates are used to fingerprint essential oils. Basil plant extract is placed on the plates, which are pre-saturated with a solvent system ratio (chloroform to methanol; 9:1). The plates are then visualized at 365 nm using visible or ultraviolet light. The Retention Factor (R_f) values for isolated chemicals are determined using the formula. Thin-Layer Chromatography (TLC) is employed for this purpose. The Retention factor (R_f) values for the isolated chemicals are determined using the formula.[12]

$$\frac{Distance\ traveled\ by\ the\ compound}{Distance\ traveled\ by\ the\ solvent\ front}$$

High-Performance Thin-Layer Chromatography (HPTLC)

HPTLC procedures involved the use of a Lino mat with five semi-automatic samplers, where a solution of standards and sample extracts (10 µL) were applied as 6 mm bands, aided by a nitrogen stream at 0.5 MPa. The spraying dosage rate is 100 µL/s, with a personal volume of 0.2 µL. The band array was positioned 10 mm from the bottom of the plate and a minimum of 15 mm from each side. Following the evaporation of the sample solvent in the application band, chromatography was conducted in the automatic developing chamber utilizing methanol: ethyl acetate (1:9) as the mobile phase. The process involved specific steps: 3 min of humidity regulation with saturated $MgCl_2$, 10 min for chamber saturation, 10 min of plate pre-conditioning, and a 60 mm migration distance. Subsequently, the mobile phase residue was completely removed by heating at 80°C for 5 min. The

separation results on the HPTLC plate were then documented through imaging.

Experimental animals

Wistar albino male rats (total 24), between 120 and 150 g body weight, were allocated into four groups, comprising of 6 rats per group. Rats were sourced from the Animal House of Teerthanker Mahaveer Medical College and Research Centre. They were maintained under controlled conditions, with a temperature of 23±1°C, humidity of 55±10%, and a 12:12 light-dark cycle. Rats were maintained in plastic enclosures equipped with steel net tops. They were allotted one week to adjust to the laboratory environment. All *in vivo* studies received approval from the institutional animal ethics committee using protocol number CCSEA/1205/2024/6.

Grouping of animals

The control, standard and test drug group were prepared for experiment. The control group (Group I) received oral treatment with normal saline (1 mL/kg), standard group (Group II) was treated orally with diazepam (5 mg/kg), and the test group (Group III) was treated orally, with soil *Ocimum basilicum* extract (200 mg/kg). Similarly, for hydroponic extract, group I and II remain same and group III received test group orally, with *Ocimum basilicum* extract (200 mg/kg).

Behavioral evaluation (*In vivo* anxiolytic activity)

Elevated plus maze test

Two open arms measuring 50 cm×10 cm×40 cm and two closed arms measuring 50 cm×10 cm×40 cm were used in this maze test. The arms were positioned such that they faced each other, and an open ceiling extended from a common center platform measuring 10 cm×10 cm. The maze was positioned in a poorly illuminated room. The apparatus was positioned 50 cm above ground level. Each rat was individually placed at the maze's center, facing one of the enclosed arms. Subsequently, many entries and durations on both the arms were recorded over the 5 min observation period. An arm entry was established when all four paws of the rats were within the arm. A dispassionate "blind" observer conducted an observation.[21]

Statistical analysis

All values are shown as Mean±SD (Standard Deviation). The data was analyzed using GraphPad Prism 6.0. Statistical analysis was conducted using one-way ANOVA followed by Tukey's test. $p<0.05$ was accepted as a significant value.

RESULTS

Percentage yield of extract

The percentage yield of soil basil extract was determined to be 8.20%, while that of hydroponic basil was discovered to be 26.46%.

Phytochemical screening

The qualitative phytochemical analysis resulted in the existence of flavonoids carbohydrates, phenolic substances and essential oils.

Total phenolic content estimation

For both soil and hydroponic basil extract

The FC method quantifies the amount of material necessary to impede the oxidation of the standard reagent. The plant extract exhibited 0.145 absorbance. A calibration curve was constructed depicting absorbance against varying doses of gallic acid (Figure 1). The total phenolic content of the extracts was determined from the standard curve utilizing the regression equation (Y=0.0017x-0.0568; R^2=0.9931) and reported as mg of gallic acid equivalents based on dry weight. The calibration curve of the standard indicated that the total phenolic content of the basil

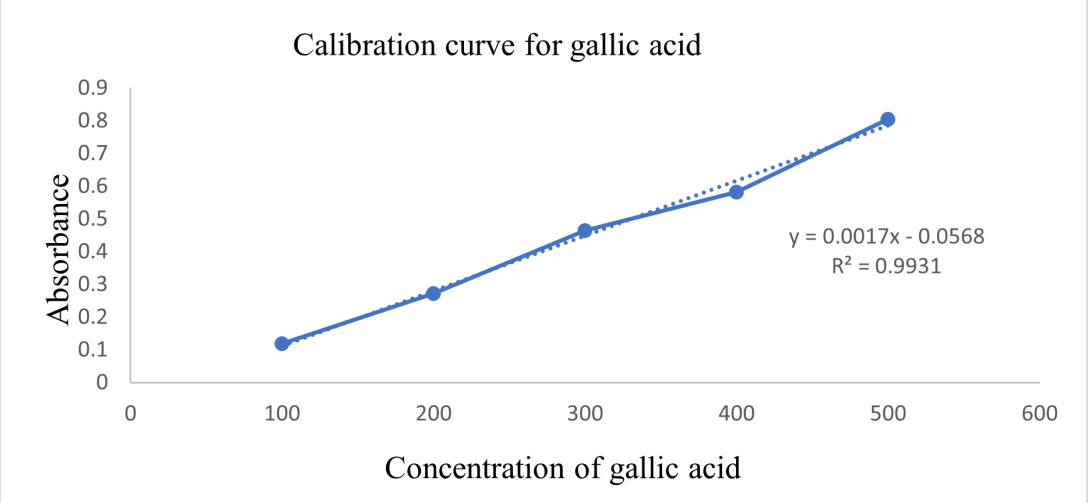

Figure 1: Calibration curve for gallic acid (standard).

ethanolic extract was 1.198 mg/g, whereas the hydroponic extract was 0.6520 mg/g.

Total flavonoid content estimation

For both soil and hydroponic basil extract

The total flavonoid content in the soil basil ethanolic extract was calculated as 299.9 mg/g and 54.9 mg/g from hydroponic. This was derived using the regression equation of the standard curve (Figure 2) for quercetin ($Y=0.2084x$; $R^2=0.9915$).

TLC for the *O. basilicum* leaves extract

The thin-layer chromatography of basil extract demonstrated the existence of three compounds with R_f values of 0.45, 0.63, and 0.81, utilizing a solvent system composed of chloroform and methanol in a 9:1 ratio. In a similar manner, the hydroponic extract exhibited three bands with R_f values of 0.54, 0.63, and 0.81.

HPTLC quantification

The HPTLC study on basil extracts from both soil and hydroponic systems utilized eugenol as the standard. The Retention factor (R_f) of standard eugenol was recorded at 0.75 for soil (Figure 3) and 0.77 for hydroponic (Figure 4). The R_f values of the basil extracts from both soil-cultivated and hydroponic systems corresponded with the R_f value of the standard eugenol, confirming the existence of eugenol in both extracts. The findings indicate that basil extracts from both cultivation techniques possess eugenol as shown in Figure 3.

In vivo anxiolytic activity

As depicted in Table 1, the control group for soil extract displayed typical anxiety behaviour with less time in the open arms and fewer open-arm entries. Diazepam (the standard anxiolytic drug) did reduce anxiety but without statistically significant results compared to the control. The OBLE-treated group exhibited the most profound anxiolytic effects, with increased time in the open arms and a greater number of open-arm entries, showing that OBLE may have potential anti-anxiety properties similar to or even greater than diazepam, though the lack of statistical significance needs further investigation.

In case of hydroponic extract in Table 2, the control group exhibited standard anxiety behavior, characterized by increased time in the closed arms and reduced entries into the open arms. The standard group exhibited a minor trend towards decreased anxiety; still, the behavioral modifications lacked statistical

Table 1: Effect of soil extract of *Ocimum basilicum* on an elevated plus maze test.

Groups	Drug and dose	Time spends in open arm Mean±Std	Time spends in close arm Mean±Std	No. of open arm entry Mean±Std	No. of close arm entry Mean±Std
I. Control	Distilled water (1 mL/kg).	174.27±0.96	197.43±1.46	2±0.63	3.33±0.74
II. Standard	Diazepam (5 mg/kg).	124.64±0.99^{ns}	167.70±1.66^{ns}	3.16±0.68^{ns}	2.83±0.89^{ns}
III Test Group	OBLE (200 mg/kg).	185.29±0.86^{ns}	113.10±0.94^{ns}	6.16±0.68^{ns}	5.16±0.68^{ns}

All values are expressed as the mean±SD (Standard deviation) (n=6 for each group); One-Way ANOVA followed by Tukey's multiple comparison test. ($p<0.05$; ^{ns} Not significant) v/s Control.

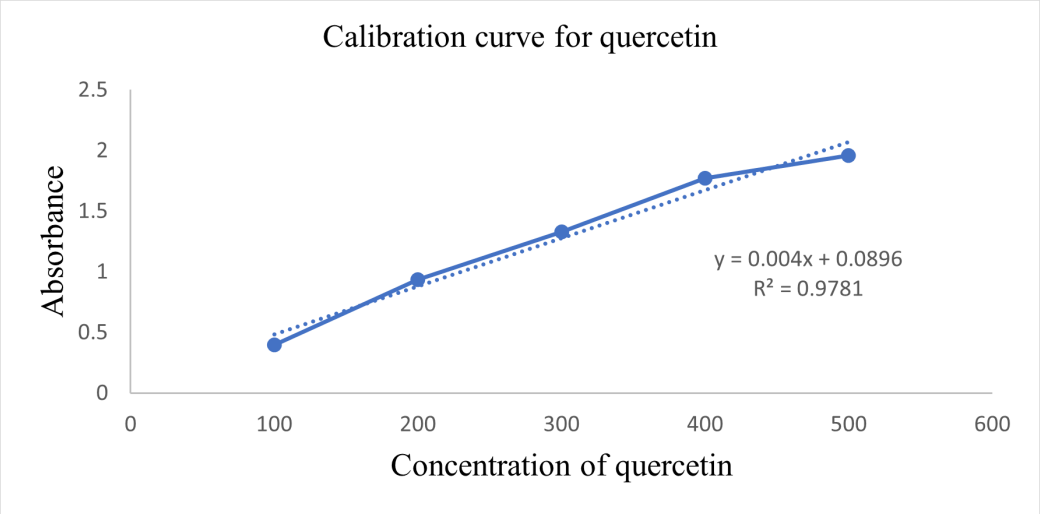

Figure 2: Calibration curve for quercetin (standard).

Table 2: Effect of hydroponic extract of *Ocimum basilicum* on elevated plus maze test.

Groups	Drug and dose	Time spends in open arm Mean±Std	Time spends in close arm Mean±Std	No. of open arm entry Mean±Std	No. of close arm entry Mean±Std
I. Control	Distilled water (1 mL/kg).	174.27±0.96	197.43±1.46	2±0.63	3.33±0.74
II. Standard	Diazepam (5 mg/kg).	124.64±0.99^{ns}	167.70±1.66^{ns}	3.16±0.68^{ns}	2.83±0.89^{ns}
III. Test Group	OBLE (200 mg/kg).	185.45±0.76^{ns}	113.26±0.76^{ns}	4.83±0.68^{ns}	4.5±0.95^{ns}

All values are expressed as the mean±SD (Standard deviation) (n=6 for each group); One-Way ANOVA followed by Tukey's multiple comparison test. (p<0.05; ^{ns} Not significant) v/s Control.

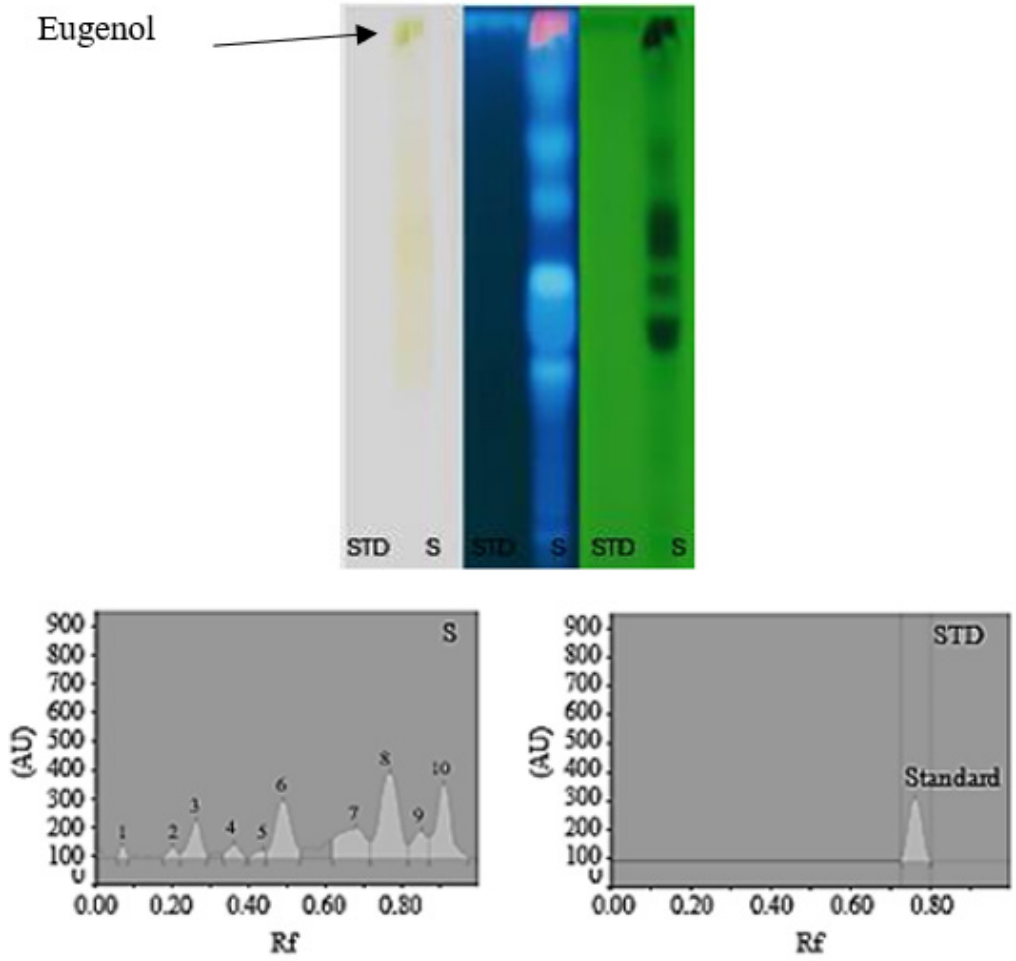

Figure 3: HPTLC chromatogram of soil basil extract contained bioactive compound eugenol in a solvent system of Toluene-ethyl acetate (9:3) mixture (STD- Standard; S-soil extract sample).

relevance when compared to the control group. In contrast, the test group (OBLE) demonstrated the most pronounced anxiolytic-like behavior, characterized by increased time spent in the open arms and a greater number of open-arm entries relative to both the control and diazepam groups. Nonetheless, the absence of statistical significance necessitates caution in the interpretation of these findings.

DISCUSSION

Anxiolytic, hypoglycemic, hypotensive, antioxidant, antiseptic, anti-inflammatory, anti-bacterial, immunomodulatory, and anti-stress are just a few of the many benefits of *Ocimum basilicum*. The higher percentage yield of *Ocimum basilicum*

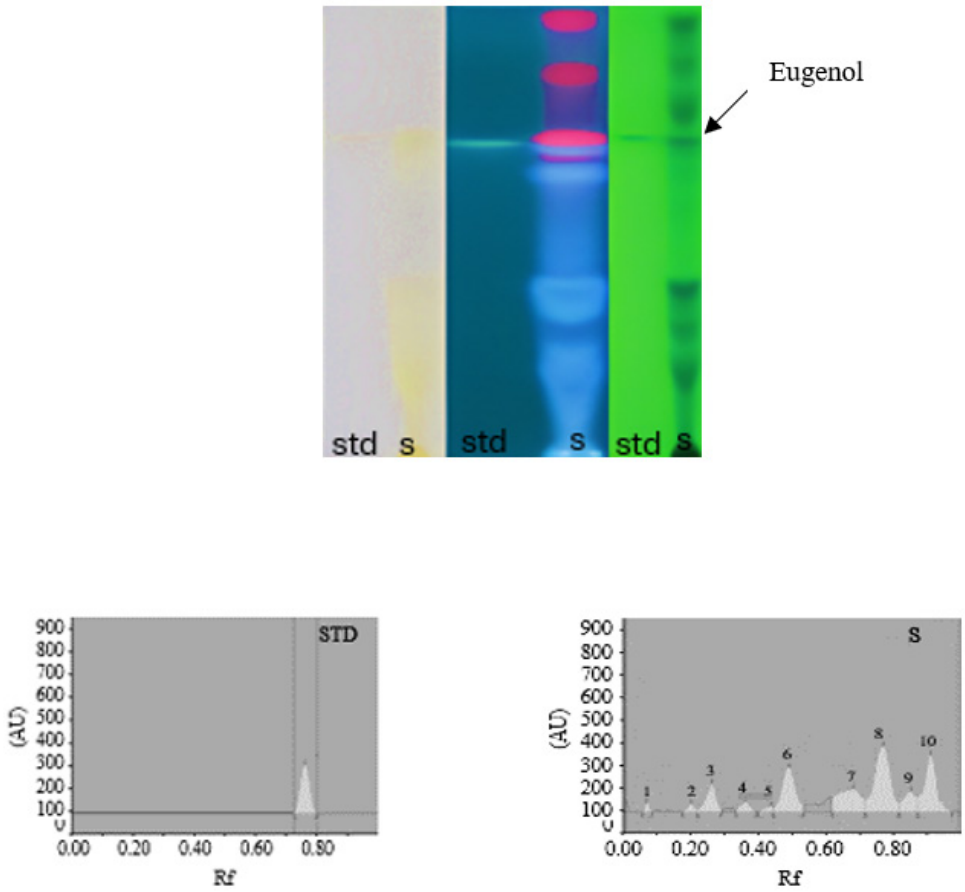

Figure 4: HPTLC chromatogram of hydroponic basil extract contained bioactive compound eugenol in a solvent system of Toluene-ethyl acetate (9:3) mixture (STD-Standard; S-hydroponic extract sample).

extract suggests a more efficient extraction process, possibly due to differences in plant metabolism under hydroponic conditions.

Phytochemical tests revealed the presence of bioactive compounds in hydroponic extracts, including carbohydrates, phenols, flavonoids, and alkaloids. The assessment of basil cultivated through a wide variety of ways such as hydroponic and soil-based systems is mandatory to carry out phytochemical screening to ensure quality and potential health benefits. These compounds, found in basil plants, contribute to their antioxidant properties.[22]

Spectrophotometric techniques were applied to measure the Total Phenolic Content (TPC), as well as the Total Flavonoid Content (TFC). The hydroponic extract had a TPC of 0.652 mg/g and the soil plant extract measured 1.198 mg/g. That means that the soil grown basil (basil cultivated in soil) contained more phenolic chemicals than basil grown hydroponically. Flavonoid content found in the TFC analysis consisted of 299.9 mg/g in the soil extract and 54.9 mg/g in the hydroponic extract. Concentration of flavonoids was found to be markedly elevated from the soil extract. Possibly the disparities are linked to different stress conditions or soil nutrient availability in hydroponic systems, where plants are grown, relative to soil, such that condition may influence the synthesis of secondary metabolites in plants. Quantification allows seeds of basil creaked in various conditions (in soil and hydroponics) to be subjected to analyze of the phytochemical profiles of plants cultivated in these conditions. Such results will help understand how cultivation tends to affect accumulation of advantageous chemicals, to then influence future agricultural, breeding efforts and ideal conditions determination. Understanding the influence of different elements, including light and nutrients and water, on the concentrations of the phenolic and flavonoid components will help establish optimal cultivation situations to promote these advantageous substances in basil.[23]

Isolation and identification of constituents from the extracts were carried out by Thin Layer Chromatography (TLC). In both results there were unique patches with R_f values that helped indicate the existence of several chemicals in the extracts. The extract of the soil based on R_f values show three areas around 0.45, 0.63 and

0.81. Three spots with R_f values of 0.54, 0.63 and 0.81 are shown by the hydroponic extract. The extracts are analogous because the compound categories show the same resemblance of R_f values; their amounts, however, may vary. Many phytochemicals in basil including phenolics, flavonoids and essential oils can be segregated and identified using Thin Layer Chromatography (TLC).[24]

Eugenol, a main phenolic constituent in basil, was identified by HPTLC in soil and hydroponic extracts. R_f values are close and eugenol presence is possible due to different growing conditions. This paper exists to emphasize that HPTLC, because it's crucial for profiling phytochemicals, supporting product quality, guiding agricultural practices and advancing health benefits research, is a laboratory tool that is essential today.[25]

Using the Elevated Plus Maze (EPM) test, the *in vivo* study investigated the effects of the extracts on anxiety related behaviour in subjects. Marked anxiolytic effect (p<0.03, time did not spend in the closed arms and consumption of time in the open arm) was observed at soil and hydroponic extract dosage of 200 mg/kg organoleptic. Both soil and hydroponic extracts showed comparable analytic effects.[26]

CONCLUSION

The study reveals that extracts from basil grown in hydroponics or soil have an anxiolytic effect and increase time spent in open arms, indicating that extraction, whether soil-based or hydroponic, does not affect the anxiolytic efficacy of basil extracts. While secondary metabolites may be influenced by agriculture treatments, such as pesticides, toxins and other pollutants of soil cultivated plants, and the hydroponic method offers a controlled, contaminant free environment, possibly producing purer extracts.

As with the previous concerns, the anxiolytic extracts were produced by both growth methods. In fact, hydroponic farming may serve as an alternative to soil-based growth, offering a much cleaner and safer source of medicinal plant material without sacrificing efficacy. Research is needed to compare secondary metabolite makeup of basil grown under both ways.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

EPM: Elevated Plus Maze; **HPTLC:** High Performance Thin Layer Chromatography; **SD:** Standard Deviation; **TLC:** Thin Layer Chromatography; **TPC:** Total Phenolic Content; **TFC:** Total Flavonoid Content.

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