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RESEARCH ARTICLE

Biochemical and Molecular Characterization of Five Basil Cultivars Extract for Enhancing the Antioxidant, Antiviral, Anticancer, Antibacterial, and Antifungal Activities

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ABSTRACT

Genetic diversity is crucial for understanding the various characteristics of plants, such as their appearance, function, chemical structure, and genetic composition. This study investigated five different basil cultivars, lemon, sweet, Italian, white, and red French, for genetic diversity by ISSR and SCOT analysis and its effect on essential oils' yield and antioxidant, antiviral, anticancer, and antimicrobial activities. The genetic diversity of five basil cultivars was determined using ISSR and SCOT-PCR molecular markers. A total of 60 loci were produced by the ISSR-PCR reactions, 26 of which were polymorphic, while 16 were unique. The level of polymorphism varied from 55.65 to 88.89 %. The molecular profile generated by SCOT-PCR revealed 54 loci, 26 of which were polymorphic, while eight were unique. The level of polymorphism ranged from 50 to 72.72%. The most unique bands were found in the BLe cultivar, 11 by the ISSR markers and 7 by the SCOT markers. The molecular results influence the biological activities of basil cultivars. The GC/MS detected twenty-four compounds; Linalool and methyl cinnamate were the main VOC compounds in the basil profile. The lemon basil essential oil (Ble) had the highest phenolic content (368 mg/g) compared to the other cultivars' EOs; therefore, it exhibited the highest scavenging activity, reducing 89 % of DPPH radicals followed by local basil (Bl). The Ble showed considerable antiviral activity against the bovine viral diarrhea virus (BVDV) as a model for the hepatitis C virus (HCV). Ble's cytotoxic concentration 50% (CC50) was 1400 µg/ml, demonstrating the best antiviral efficacy. Additionally, Ble and Bl exhibited significant antimicrobial activity against pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and pathogenic fungi, i.e., *Fusarium oxysporium*, *Aspergillus niger*, *Penicillium chrysogenum*, *Helminthosporium solani*, *Alternaria alternata*, *Pythium aphanidermatum*, *Botrytis cinerea*, and *Rhizoctonia solani*). The anticancer activity of basil EOs was examined against MCF7 cancer cell lines; BLe cultivar reduced 85 % of the viability of MCF7 cancer cells. The examined basil EOs exhibit strong phyto-medical potential based on their chemical structure and antioxidant, antiviral, antimicrobial, and anticancer properties. Based on the molecular and biochemical analysis results, the BLe and Bl cultivars was the best for medicinal use future breeding strategies.

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INTRODUCTION

Basil (*Ocimum* sp.) is a medicinal herb belonging to the mint family (Lamiaceae). It has a long history of use in traditional medicine and is highly valued in cooking. While originally found in tropical areas of Southeast Asia and Central Africa, basil has only recently become widely cultivated and used worldwide (Naidu *et al*., 2016; Zhakipbekov *et al*., 2024). Basil comprises diverse bioactive compounds, such as phenolic acids, flavonoids, and essential oils (Nadeem *et al*., 2022). These active compounds give basil a distinctive aroma, flavor, and medicinal attributes. Furthermore, they exhibit a variety of advantageous properties, such as antioxidant (Naidu *et al*., 2016)., anticancer (Perna *et al*., 2022), anti-inflammatory (Takeuchi *et al*., 2020), antimicrobial impacts (Dube *et al*., 1989). Historically, many diseases have been managed with the herb's extract, including skin issues, respiratory infections, and digestive disorders (Kunnumakkara *et al*., 2018). Basil may also have potential applications in both the avoidance and cure of chronic diseases, including cardiovascular disease (Mahmoud and ELDarder, 2016), cancer (Shimizu *et al*., 2013), and diabetes (Widjaja and Savira, 2019). Recent research has also produced encouraging results in this regard.

Basil is an ancient plant used for millennia (Mahmoud and ELDarder, 2016). Traditional Egyptian cuisine uses herbs for food and therapeutic purposes. According to archaeological findings, sacred basil (*Ocimum sanctum*) was utilized as a culinary seasoning and for medicinal intentions (Mahmoud and ELDarder, 2016). A wide range of basil cultivars are regarded as abundant in essential oil (0.1–0.7%). Up to this point, the plant essential oil has been associated with over 200 chemical compounds, such as methyl chavicol (estragole), Linalool, and 1,8-cineole. The fragrance industry uses aromatic compounds as flavorings and disinfectants (Kliszcz *et al*., 2021). Furthermore, these compounds possess antibacterial and antioxidant properties (da Silva *et al*., 2022). Essential oils (EOs) have demonstrated antiviral properties against various harmful viruses. They can disrupt viral infections and damage the protective outer layers of viruses, rendering them inactive (Da Silva *et al*., 2020; Pellegrini *et al*., 2023). Ocimum plants are a rich source of antiviral compounds. Wild Amazonian basil (*O. forsskaolii*) contains ursolic acid, which fights hepatitis C. Sweet basil (*O. basilicum*) and O. *carnosum* possess various compounds like 1,8-cineole, camphor, and trans-anethole that show activity against a range of viruses, including HIV, herpes, and several others (Tshilanda *et al*., 2020). Also, *Ocimum basilicum* has been tested against seven different species of rice pathogenic fungi, including *Alternaria brassicicola*, *Bipolaris oryzae*, *Aspergillus flavus*, *Fusarium moniliforme*, *Fusarium proliferatum* and *Rhizoctonia solani*, and it has been determined that basil essential oil inhibits spore germination and mycelial growth (Tangpao *et al*., 2022).

Basil is successfully cultivated in many locations. Although French basil is among the most widely grown varieties, its average yearly yield of 5.891 tons is only 1603 Faddan across a cultivated area of 1603 Faddan. Furthermore, additional basil species, including lemon, Thai, and African basil, are grown in Egypt (Sabry *et al*., 2019). Due to their unique aroma, flavor, and medicinal properties, each species of basil is indispensable in Egyptian cuisine. Basil diversity research is a complex and diverse endeavor requiring knowledge of genetics, genomics, and chemical composition. Particularly for basil plants, identifying distinctive biochemical characteristics and genetic markers is an essential component of plant breeding (Akbari *et al*., 2019).

Biological activities and biochemical characteristics are frequently used to indicate whether these plants can be utilized for sustenance, medicine, or treating prevalent diseases; however, the genetic markers offer a more comprehensive and accurate approach to monitoring genetic diversity among a population of basil plants (Ríos-Rodríguez *et al*., 2021). In recent years, there has been an increase in the use of genetic markers in breeding programs; this trend has been facilitated by advances in molecular biology that have enabled the rapid screening of large quantities of individuals for specific genetic markers using high-throughput genotyping platforms. By employing these markers, tracing the genetic transmission of particular attributes, such as disease resistance, and developing novel varieties with advantageous traits (Gossa *et al*., 2024) or yield improvements (Khater *et al*., 2021). Breeders can create new varieties with enhanced productivity, quality, marketability, and adaptability to changing environmental conditions by identifying and utilizing distinctive genetic resources.

Inter-simple sequence repeat (ISSR) markers are a class of PCR-based markers renowned for their cost-effectiveness, high polymorphism, and reproducibility. These attributes have extensively utilized ISSR in genetic diversity investigations about plant species (Nair, 2023). Prior research has used ISSR markers to assess the genetic diversity of Ocimum species and accessions originating from India and Iran (Aghaei *et al*., 2012; Gupta *et al*., 2021).

A category of molecular markers known as SCoT (Start Codon Targeted) markers binds to the start codon region of genes encoding proteins. The SCoT markers identify genetic variation between and within plant species by amplifying DNA fragments containing variable and conserved regions. Using SCoT markers to differentiate closely related basil plant species and cultivars of the same species has succeeded (Alves *et al*., 2019). The exceptional resolution exhibited by SCoT markers renders them highly advantageous in investigating genetic associations among diverse basil populations and varieties. By comparing SCoT profiles of various basil plants, it is possible to discern genetic clusters and ascertain the extent of relatedness among distinct *Ocimum* varieties (Gupta *et al*., 2021). The data above inform conservation initiatives, breeding programs, and the creation of novel varieties possessing desired characteristics. A review of the relevant literature identified the need for more comprehensive research on the genetic structure and phytochemical diversity of five local and international basil cultivars using ISSR and SCoT markers. Consequently, the objective of the current investigation was to examine the correlation between five *O. basilicum* cultivars grown under identical environmental conditions concerning the antioxidant, antiviral, antimicrobial, and anticancer activities of basil extracts and their essential oil characteristics. The acquired

data are critical in establishing the superior ecotype for subsequent utilization in breeding, cultivation initiatives, and medicinal applications.

MATERIALS AND METHODS

Plant materials: The basil seeds used in these studies were classified based on their origin (Table 1). The seeds were germinated, planted, and randomly collected at each cultivar's beginning of the flowering stage. The plants were dried in the shade at room temperature for 14 days.

Table 1: The *Ocimum* accessions in this study.

NO	code	Latin name
1	BL	*Ocimum basilicum* L. sweet basil
2	BR	*Ocimum basilicum* L. 'Grant Vert Red'
3	BW	*Ocimum basilicum* L. 'Grant Vert white'
4	BI	*Ocimum basilicum* L. 'Italian Large Leaf'
5	BLe	*Ocimum basilicum citriodora* 'Mrs. Burns' Lemon'

BL, local basil; BR, red Basil; BW, white Basil; BI, Italian Basil; BLe, lemon basil.

Isolation of the essential oils: Essential oils (EOs) were extracted using hydro-distillation in Clevenger-type equipment (VWR, Radnor, PA, USA). Precisely 100 grams of the solid portion of the above-ground component was submerged in water and subjected to boiling for 3 hours. The essential oil that was gathered was dehydrated using anhydrous sodium sulfate. The oil was retrieved and kept at 4°C (Torre *et al*., 2021).

Essential oil analysis

Sample preparation for GC-MS analysis: A quantity of 10 µl from the essential oils was mixed with 1 ml of GC-grade n-hexane. The new mixture was agitated for 1 min, and 1 µl was injected into the GC–MS using the autosampler injector (de Sousa *et al*., 2023).

Gas Chromatography-Mass Spectrometry System (GC/MS): The volatile compounds, VOCs, were analyzed using GC/MS. The GC/MS had a Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS (Thermo Scientific, USA). The capillary column contains fused silica as a stationary phase, with dimensions of 30 m length, 0.251 mm inner diameter, and 0.1 mm film thickness. For GC/MS detection, an electron ionization system with an ionization energy of 70 eV was used; Helium gas was used as the carrier gas at a constant flow rate of 1mL/min. The injector and MS transfer line temperature were set at 280 °C. The oven was set to increase its temperature from an initial value of 40°C and gradually raised to 280°C. A percentage relative peak area was utilized to investigate the identified VOCs. The volatile compounds were identified by comparing their respective RT and MS with those of different Library data of the GC/MS system (Adams, 2012; de Sousa *et al*., 2023; Żukowska and Durczyńska, 2024).

Total phenolic content: The total phenolic content (TPC) in extracts of basil cultivars was measured at a wavelength of 760 nm using a spectrophotometer (JENWAY, UK) using the modified Folin-Ciocalteu technique (Saad *et al*., 2021). 1 ml of extract, 0.5 ml of sodium carbonate 7.5 %, and 0.5 ml of Folin reagent were mixed and incubated for 30 min. The gallic acid linear equation calculated the absorbance, y = 0.005x + 0.1455.

Biological activity of Basil essential oil

Antioxidant activity (DPPH assay): The scavenging power of basil essential oil was measured according to (Saad *et al*., 2021). The EOs were mixed with DPPH (Sigma, USA) solution and incubated for 30 min at room temperature in the dark. The developed color was measured at 517 nm. % scavenging activity was calculated using the following equation:

$$\%\ \text{Scavenging activity} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100 \quad (1)$$

Anticancer activity

Cell culture: The MCF-7 breast adenocarcinoma cell line was acquired from (ATCC, USA). The cells were cultured in DMEM media with streptomycin at 100 mg/mL, penicillin at 100 U/mL, and heat-inactivated fetal bovine serum (FBS) at a concentration of 10%. The culture was maintained in a humidified environment with a 5% (v/v) CO_2 atmosphere at 37 °C.

Cytotoxicity assay: The viability of breast cancer cell lines (MCF-7) was assessed by measuring their ability to convert the yellow dye 2,5-diphenyl-2H-tetrazolium bromide (MTT) to a purple formazan, a process dependent on healthy mitochondria. Around 100,000 breast cancer cells were added to a 96-well plate containing a culture medium (2 ml). The microtiter plate was incubated for a day at 37°C and CO_2. The medium was replaced with a fresh one supplemented with FBS and basil cultivars EOs, then incubated for two days (Mosmann, 1983). The cells were collected using a trypsin-EDTA buffer and treated with trypan blue to distinguish viable cells. The live cell count was determined, and the findings were presented as the percentage of inhibition of MCF-7 cell lines (Thabrew *et al*., 1997; Chunarkar-Patil *et al*., 2024). The Bio-Rad microplate reader (USA) was used to measure the absorbance at 595 nm to evaluate the effects of basil essential oil on cell viability.

$$\%viability = \frac{Reading\ of extract}{Reading\ of\ negative\ control} \times 100 \quad (2)$$

Antimicrobial Activity

Tested Microorganisms: The antibacterial efficacy of basil cultivar EOs was assessed against bacterial strains, Gram-positive and Gram-negative pathogens: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, one clinical yeast (*Candida albicans* ATCC 10231), and pathogenic fungi (*Fusarium oxysporium, Aspergillus niger, Penicillium chrysogenum, Helminthosporium solani, Alternaria alternata, Pythium aphanidermatum, Botrytis cinerea*, and *Rhizoctonia solani*).

Disc assay: The antimicrobial activity of the five basil cultivars (Local, Lemon, Italian, red, and white basils) was assessed using the disc diffusion assay, following the methodology of Singh *et al.* (2016) with some modifications for the five basil cultivars. The McFarland standard solution (0.05) was made by mixing (0.5 mL) $BaCl_2$ and (99.5 mL) H_2SO_4 to obtain a solution with equivalent turbidity to 1.5×10^8 (CFU/ mL) cell density for bacteria and *Candia* and 1×10^6 spore/ml for fungi. 200 µL

of each bacterial and fungal culture was spread on nutrient agar or potato dextrose agar plates. 6 mm discs impregnated with varying basil cultivars' concentrations were added to plates. The antimicrobial activity was quantified by measuring the width of the inhibitory area during a 24 h incubation at 37 °C for bacteria and Candida and five days at 28 °C for fungi.

MIC and MFC determination: The minimum inhibitory concentration (MIC) refers to the least concentration of basil EO capable of inhibiting the development of bacteria. The Minimum Inhibitory Concentration (MIC) for bacteria and yeasts was determined using the previously established technique (Alowaiesh *et al*., 2023) with some adjustments. The Mueller-Hinton broth (MHB, Oxiod, UK) was utilized for bacterial cultures, while the potato dextrose broth from the same supplier was used for yeast cultures. The bacteria were incubated at a temperature of 37 ◦C for 24 h, while the yeasts were incubated at a temperature of 25 ◦C for 48 h and fungi at 28 °C for 5 days. The minimum fungicidal concentration (MFC) measurement for fungi was conducted using the gradient plate technique, following the previously reported procedure (Alsubhi *et al*., 2022; Saad *et al*., 2021).

Antiviral activity

Cultures and viruses: A cell culture experiment was conducted using Madin-Darby bovine kidney cells (MDBK) infected with a cytopathic bovine viral diarrhea virus genotype 1 (BVDV1a) strain. The cytotoxicity and antiviral activity of basil cultivars' essential oils were evaluated in 96-well and 6-well plates. The MTT colorimetric assay was used to measure the cytotoxicity of these oils (Cueto *et al*. 2011).

Cytotoxic assay: Cytotoxicity and antiviral activity were assessed using CC50 (50% cytotoxic concentration) and IC_{50} (50% inhibitory concentration). The antiviral activity assays were conducted using basil essential oils' maximum non-toxic concentration (MNTC), which did not cause cell damage.

$$\% \, \boldsymbol{cell\ viability} = \frac{\boldsymbol{Abs\ control - Abs\ EOs\ treated\ cells}}{\boldsymbol{Abs\ control}} \quad \textbf{(3)}$$

Plaque reduction assay: The plaque reduction test was used to evaluate the antiviral effectiveness of basil essential oils. A virucidal experiment was performed by mixing a fixed amount of BVDV virus suspension with varying doses of essential oil. These mixtures were incubated at room temperature, and aliquots were added to cells for viral adsorption. After removing excess inoculum, the infected cells were incubated with a nutrient-rich medium containing agarose and horse serum. The plates were later fixed and stained to count the viral plaques. The antiviral effectiveness of each EO concentration was measured by comparing the number of viral plaques in treated cells to untreated control cells. The selectivity index (SI) was calculated by dividing the CC50 (50% cytotoxic concentration) by the IC50 (50% inhibitory concentration) (Lanave *et al*., 2024).

Molecular analysis of *Ocimum basilicum* L. cultivars

DNA extraction: Five basil cultivar seeds were seeded in a Petri plate. Genomic DNA was isolated from 0.1 g of each sample's recently harvested and juvenile leaves using the protocol described by (Dellaporta *et al*., 1983). The obtained DNA was quantified using a microtiter plate reader at a wavelength of 280 nm. Its purity was then corrected based on the method described by Dairawan and Shetty (2020). The DNA samples were maintained at a temperature of -20°C until they were examined using PCR.

ISSR and SCoT analysis: The molecular-level genetic characterization of the examined basil cultivars was conducted using ISSR and SCoT-DNA markers. These approaches rely on amplifying small sections of the target genomic DNA utilizing ISSR and Scot primers (Williams *et al*., 1990). Table 2, provided in this study, displays the names and sequences of ISSR and SCoT primers.

The ISSR-PCR procedure used a final volume of 15 µl, while the Scot-PCR procedure used a final volume of 10 µl (Table 3). The PCR amplification protocol commenced with an initial denaturation step at 95°C for 4 minutes, followed by 35 cycles consisting of denaturation at 95°C for 90 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 90 seconds. The program concluded with a final extension step at 72°C for 10 min.

Agarose gel electrophoresis of PCR products: The agarose gel electrophoresis for visualizing the PCR products was made using the method described by Armstrong and Schulz (2008) with several modifications. Heat 1.5 mg of agarose in a water bath in 100 ml Tris-Borate EDTA (TBE) solution. After the temperature dropped to 55°C, 5 µl of ethidium bromide was introduced into the melted gel. The liquefied gel was transferred into a small gel device, followed by the instant insertion of the comb. The comb was then withdrawn after the gel solidified and coated with TBE buffer. Each well was filled with eight µL of PCR products and operated at a voltage of 80 V. The PCR products were seen under UV light using a UV spectrophotometer (Jenway, 6305) at a wavelength of 280 nm. The bands of ISSR and Scot-PCR products were enumerated, evaluated, and ordered based on their ranking. The acquired bands were compared to the bands of the DNA ladder (Non-Liner Dynamic Lth, USA).

All pieces that were bright and visible were assigned a score of (1) if they were present or (0) if they were absent (see Supplementary Tables 1,2). The bands were analyzed by considering the relative movement of their various sizes.

Statistical analysis: CoStat software (version 6.4, Monterey, CA, USA) (Snedecor and Cochran, 1989) was used to analyze the variance (ANOVA) test for five basil cultivars' biochemical characteristics. The data were derived as mean ± SD, and the differences were considered statistically significant at $p < 0.05$.

The bands generated by ISSR and SCOT-based PCR reactions were categorized as either absent (0) or present (1). Each locus was considered an independent event, regardless of its presence or absence. The bands and patterns of the five cultivars were compared to identify genetic diversity. The percentage of polymorphism (%) was measured by dividing the total number of loci scored by the number of polymorphic loci. The Dice coefficient (Dice, 1945) was employed to determine the genetic

Table 2: Sequence and operon codes of the ISSR and SCoT primers used.

No.	Codes	ISSR Primers	Codes	SCoT primers
		Sequence 5' to 3'		Sequence 5' to 3'
1	ISSR825	ACACACACACACACT	SCoT 2	CAACAATGGCTACCACCC
2	UBC835	AGAGAGAGAGAGAGAGYC	SCoT 3	CAACAATGGCTACCACCG
3	UBC826	ACACACACACACACACC	SCoT 6	CAACAATGGCTACCACGC
4	UBC827	ACACACACACACACACG	SCoT 14	ACGACATGGCGACCACGC
5	UBC808	AGAGAGAGAGAGAGAGC	SCoT 19	ACCATGGCTACCACCGGC
6	UBC868	GAAGAAGAAGAAGAAGAA	SCoT 33	CCATGGCTACCACCGCAG
7	UBC901	CACACACACACACACARY		

Table 3: Buffer constituents of ISSR and SCOT analysis.

Solutions constituents	ISSR volume (µl)	SCOT volume (µl)
Genomic DNA (50 ng/µl)	1.5	1.5
Primer (20 ppm)	1	1
dNTPs (2.5 ppm)	2.5	1.3
Tris HCl buffer (10 mM, pH 8.3)	2	1.5
Taq DNA polymerase	0.3	0.2
$MgCl_2$ (2 ppm)	0.5	0.3
Sterile distilled water	7.2	4.2

similarities between the varieties. The IBM SPSS statistics software (MJ, 1993) was employed to conduct the calculation. The clustering analysis method was processed using the STATISTICA 8 software to construct a phylogenetic dendrogram (Berk, 2005; CH, 2007).

RESULTS

Chemical characterization of basil cultivars

Essential oils content: Essential oil yields were 1.0%, 0.5%, 0.3%, 0.2% and 0.15% for *O. basilicum* L, citriodora Mrs. Burns' Lemon (BLe), sweet basil (BL), Grant Vert Red (BR), Grant Vert white (BW) and Italian Large Leaf (BI); respectively. The results of the GC-MS analysis of the oils are shown for five *Ocimum basilicum* L. cultivars in Table 4, where the eluents are provided in the order of the HP5MS column elution. The primary components identified in the EO of *Ocimum basilicum* L. cultivars included were 1.8-Cineole, Linalool, Methyl cinnamate, Eugenol, β-Pinene, β-Thujone, α-Bergamotene and α-Cadinol.

The main components found in the essential oil of BLe were Methyl cinnamate (42.02 %), Linalool (33.5 %), and 1.8-cineole (6.01 %). In comparison, the EO of (BL) Methyl cinnamate (33.5 %), Linalool (26.3 %), and 1.8-cineole (5.3 %) were the major components. Similarly, Methyl cinnamate was dominant in BR (31.2) and was followed by linalool (24.3) and 1.8-cineole (5). Conjointly with the previous three cultivars, Methylcinnamate was dominant in BW (29.5) and BI (29.3). Linalool was followed by reaching a ratio of 22.5% in BW and a higher ratio in BI (25.3%). On the contrary, 1.8-Cineole in BW was higher than BI at 4.5% and 4.1%, respectively.

Eugenol was found in all EOs, and BL exhibited the highest content (3.1%), followed by BLe (3.08), whereas BW had the lowest (2.1%). β-Pinene was found in high ratios in both BLe and BL, while it was a trace in BR and BW and disappeared in BI. α-Bergamotene was present in high ratios in BLe (3.22%) and BL (2.9%), while it was a trace in BR, BW, and BI, lowered 2.1%, 1.2%, and 0.9%, respectively. The levels of α-Cadinol and β-Thujone in BLe were exceptionally high and not as high in other cultivars. According to the ratios of Methylcinnamate, 1.8-cineole, Eugen ol, Linalool, α-Bergamotene, and other compounds, the two cultivars BW and BI displayed the most similar chemotype pattern overall.

Table 4: Detected essential oils profile of *Ocimum basilicum L.* by GC/MS.

NO	Components	RT (min)	BLe	BL	BR	BW	BI	P value
1	α-Pinene	11.88	0.45^{a}	0.3^{ab}	0.1^{b}	0.1^{b}	0.03^{b}	0.026
2	Camphene	13.75	0.05	0.04	0.01	0	0	0.095
3	β-Pinene	15.81	1.42^{a}	1.3^{b}	1^{bc}	0.8^{c}	ND	0.011
4	β-Myrcene	19.31	0.51^{ab}	0.55^{a}	0.4^{b}	0.35^{c}	0.23^{d}	0.001
5	Bornylene	20.28	0.31^{a}	0.23^{b}	0.12^{c}	0.9^{d}	0.02^{e}	0.0001
6	Eugenol	20.62	3.08^{ab}	3.1^{a}	2.9^{b}	2.1^{b}	2.2^{b}	0.021
7	1.8-Cineole	21.13	6.01^{a}	5.3^{b}	5^{bc}	4.5^{c}	4.1^{c}	0.016
8	2-Hexenal	21.16	0.03	0.01	0.02	0	0	0.9
9	Sulcatone	26.85	0.01^{a}	0	0	0	0	0.98
10	Fenchone	29.75	0.01	0.03	0.02	0.01	0	0.091
11	3-Methyl-hepta-1,6-dien-3-ol	30.23	0.01^{b}	0.07^{a}	0.02^{b}	0.01^{b}	0.01^{b}	0.026
12	β-Thujone	30.85	3.12^{a}	2.9^{ab}	2^{b}	1.8^{b}	1.1^{c}	0.019
13	Caprylyl-acetate	33.36	0.06	0.06	0.01	0.02	0.03	0.91
14	2,4-Heptadienal	33.80	0.04	0.03	0.02	0.03	0.01	0.92
15	Linalool	36.75	33.5^{a}	26.3^{b}	24.3^{b}	22.5^{c}	25.3^{b}	0.019
16	Germacrene D	37.86	0.15^{a}	0.12^{ab}	0.02^{b}	0.11^{ab}	0.09^{b}	0.036
17	α-Bergamotene	38.35	3.22^{a}	2.9^{b}	2.1^{b}	1.2^{c}	0.9^{c}	0.020
18	Terpinene-4-ol	38.49	0.03	0.01	0.01	0.01	0.01	0.989
19	Benzene acetaldehyde	39.90	0.04	0.03	0.04	0.02	0.03	0.955
20	Borneol L	42.43	0.44^{ab}	0.5^{a}	0.4^{ab}	0.2^{b}	0.09^{c}	0.010
21	delta-Guaiene	43.13	0.56^{a}	0.41^{b}	0.32^{c}	0.28^{d}	0.16^{e}	0.0001
22	Geraniol	47.66	0.05	0.02	0.01	0.02	0.02	0.0925
23	Methyl cinnamate	56.53	42.02^{a}	33.5^{b}	31.2^{b}	29.5^{c}	29.3^{c}	0.023
24	α-Cadinol	58.83	4.1^{a}	3.2^{b}	3^{b}	2.8^{c}	2.1^{c}	0.019

a-e in the same row indicates significant differences between basil verities in VOC content.

Total phenolic content of basil extracts: Table 5 lists basil extracts' total phenolic contents (TPC) from various sources. The different agroclimatic (seasonal, climatic, and geographical) conditions of the places may cause this variation in TPC content. The findings showed that other essential oils and extracts had variable phenolic content. Among the basil extracts, the highest TPC (368 mg GAE/g) was obtained from BLe basil, followed by 301, 230, 150, and 99 mg PE/g for BL, BR, BW, and BI basil extracts, respectively.

Biological activities of basil extract

Antioxidant activity: Using the stable DPPH as a reagent, the antioxidant activity of the basil extracts was evaluated in terms of their capacity to donate hydrogen or to scavenge DPPH radicals. The findings in Figure 1 demonstrate the variation in the antioxidant capability of the tested essential oils. The five tested *Ocimum basilicum* L. cultivars demonstrate considerable antioxidant activity either in the first cutting or the second one. The results indicated that the second cutting had higher antioxidant activity than the first

cutting in basil cultivars, as the first cutting ranged from 32 - 65 %, while the second cutting ranged from 50 % to 89 %. The antioxidant activities of five tested *Ocimum basilicum* L. cultivars are shown in Figure 1. BLe exhibited the highest free radical scavenging activity (89 %), whereas BI had the lowest (50 %) in the second cutting.

Antibacterial and anti-Candida activity: Table 6 and Figure 2 demonstrate that the development of pathogenic bacteria and Candida was considerably suppressed by the ethanolic extract of *Ocimum basilicum* L. cultivars (BLe, BL, BR, BW, and BI); the sizes of the inhibition zones (mm) increased with concentrations. EC was the most resistant microorganism in tested microbes with IDZ of 25-29 mm in all treatments, followed by CA. BC was the most vulnerable bacteria to essential oils of *Ocimum basilicum* L. cultivars concentrations.

Table 5: Total phenolic content of basil extracts (mg GAE/g of dry extract).

Cultivar	Phenolic content mg/g GAE
BLe	368±2.9^{a}
BL	301±3.5^{b}
BR	230±5.3^{c}
BW	150±2.1^{d}
BI	99±0.9^{e}
P value	0.00001697

Table 6: Determination of the antimicrobial effect of basil essential oils of five cultivars.

Cultivar	conc (µg/mL)	Inhibition Zone Diameter (mm)		
		Bacteria		Candida
		Bacillus cereus	*Escherichia coli*	*Candida albicans*
BLe	200	15±0.2^{f}	11±0.2^{f}	14±0.5^{de}
	400	27±0.5^{bc}	21±0.1^{c}	25±0.1^{b}
	800	33±0.6^{a}	29±0.2^{a}	28±0.2^{a}
BL	200	13±0.1^{g}	9±0.3^{g}	11±0.6^{g}
	400	25±0.2^{c}	23±0.5^{bc}	22±0.1^{c}
	800	29±0.4^{b}	25±0.1^{b}	26±0.2^{b}
BR	200	12±0.8^{g}	8±0.2^{g}	12±0.1^{ef}
	400	18±0.9^{e}	14±0.4^{e}	15±0.2^{d}
	800	22±0.2^{d}	18±0.7^{d}	16±0.1^{d}
BW	200	10±0.1^{h}	ND	10±0.3^{f}
	400	13±0.2^{g}	10±0.2^{f}	11±0.3^{ef}
	800	19±0.1^{e}	15±0.1^{e}	13±0.2^{e}
BI	200	9±0.5^{h}	ND	ND
	400	12±0.1^{g}	9±0.6^{g}	ND
	800	17±0.5^{ef}	11±0.1^{f}	10±0.1^{f}
P value		<0.00001	<0.00001	<0.00001

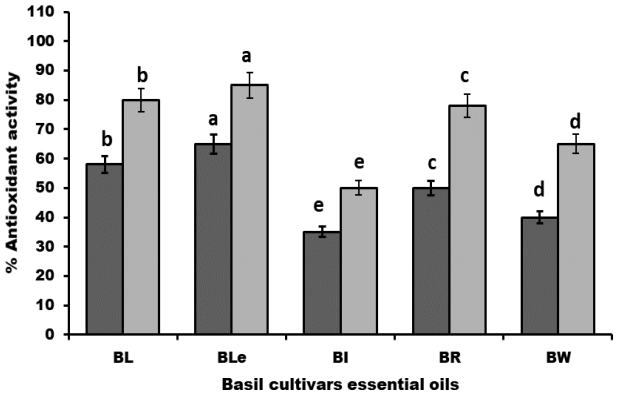

Fig. 1: Antioxidant activity of various basil cultivars (lemon, local, white, red, and Italian basil) against DPPH radicals.

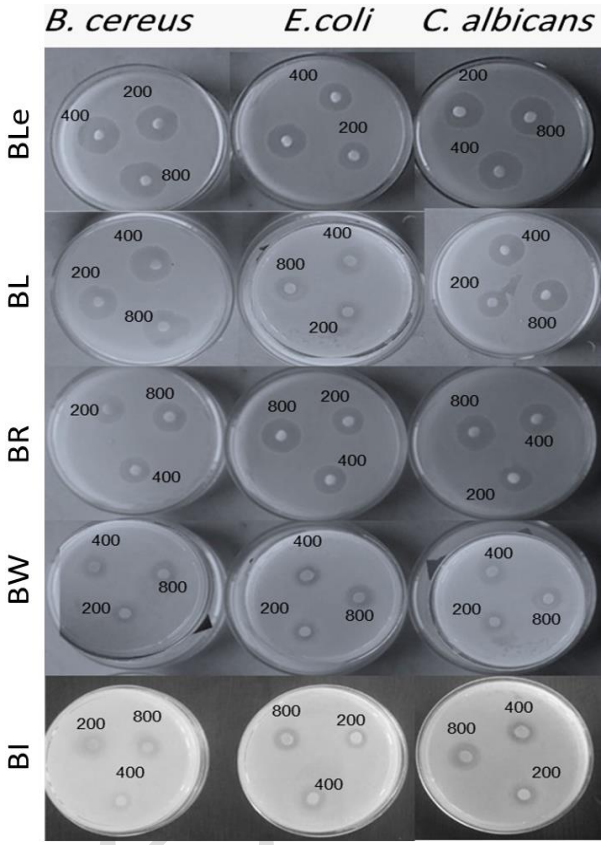

Fig. 2: Antimicrobial activity of *Ocimum basilicum* L. cultivars extracts at different concentrations (200,400 and 800 µg/mL).

Antifungal activity of basil essential oil: This study tested the antifungal activity of five basil cultivars' essential oils against eight phytopathogenic fungi (*Fusarium oxysporuim*, *Aspergulls niger*, *Penicillium chrysogenum*, *Helminthosporium solani*, *Rhizoctonia solani*, *Alternaria alternata*, *Pythium aphanidermatum*, and *Botrytis cinerea*). The inhibition zone diameters (IZDs) increased in a concentration-dependent manner (Table 7). Lemon basil essential oil (Ble) had the highest antifungal activity against the tested fungi; meanwhile, Italian basil essential oil (BI) had the lowest activity. *F. oxysporuim*, *P. chrysogenum*, *A. alternata*, and *B. cinerea* were the resistant fungi against all basil EOs concentrations with the lowest IZDs. The IZDs of the highest concentration of Ble ranged between 24-34 mm, followed by 21-31 mm for LE; meanwhile, the lowest IZDs (18-28 mm) were of BI. No IZDs were observed in BI at 50 µg/mL against *F. oxysporuim*, *P. chrysogenum*, *A. alternata*, and *B. cinerea*.

The basil essential oils of all tested cultivars inhabited the growth of pathogenic fungi, where Ble was the powerful antifungal where inhabited the growth studied fungi in the range of (20-40 µg/mL), followed by LE, where MIC was 25-40 µg/mL; meanwhile, the lowest EOs was BI with highest MIC of 40-85 µg/mL. The BR and BW had medium MIC values (Figure 3A). On the other hand, the Ble killed the tested fungi at the concentration range of 30-75 µg/mL; meanwhile, the highest MFC was in BI (75-150 µg/mL) (Figure 3B).

Antiviral activity of basil essential oil against HCV: The Ble showed considerable antiviral activity against the bovine viral diarrhea virus (BVDV) as a model for the

Table 7: Antifungal activity of five basil essential oils against the pathogenic fungi.

Pathogenic fungi	Concentration (µg/mL)/ inhibition zone diameters (mm)				
	Local basil essential oil (BL)				
	50	100	200	400	800
F. oxysporuim	9c	12d	16d	19e	21f
A. niger	15a	19a	23a	26a	30ab
P. chrysogenum	11bc	15c	18cd	20d	25d
H. solani	12b	17b	19c	22c	26c
Rhizoctonia solani	14ab	18ab	21b	24b	28b
Alternaria alternata	11bc	14c	20b	23b	25d
P. aphanidermatum	12b	18ab	22ab	26a	31a
Botrytis cinerea	10c	13d	17d	20d	23e
	Lemon basil essential oil (Ble)				
F. oxysporuim	12c	15d	19d	22e	24e
A. niger	18a	22a	26a	29a	33ab
P. chrysogenum	14bc	18c	21cd	23d	28cd
H. solani	15b	20b	22c	25c	29c
Rhizoctonia solani	17ab	21b	24b	27b	31b
Alternaria alternata	14bc	17c	23b	26b	28cd
P. aphanidermatum	15b	21ab	25ab	29a	34a
Botrytis cinerea	13c	16d	20d	23d	26d
	Italian basil essential oil (BI)				
F. oxysporuim	–	9d	13d	16e	18e
A. niger	12a	16a	20a	23a	27a
P. chrysogenum	–	12c	15c	17d	22cd
H. solani	9b	14b	16c	19c	23c
Rhizoctonia solani	11ab	15ab	18b	21b	25b
Alternaria alternata	–	11cd	17b	20b	22cd
P. aphanidermatum	9b	15ab	19ab	23a	28a
Botrytis cinerea	–	10d	14d	17d	20d
	French Red basil essential oil (BR)				
F. oxysporuim	9d	11e	15d	18e	20e
A. niger	14a	18a	22a	25a	29a
P. chrysogenum	10c	14c	17	19d	24cd
H. solani	11b	16b	18bc	21c	25c
Rhizoctonia solani	13ab	17ab	20b	23b	27b
Alternaria alternata	10c	13c	19b	22c	24cd
P. aphanidermatum	11b	17ab	21ab	25a	30a
Botrytis cinerea	9d	12d	16c	19e	22d
	French White basil essential oil (BW)				
F. oxysporuim	9c	9d	13e	16e	18e
A. niger	13a	16a	20a	23a	27a
P. chrysogenum	10bc	12c	15cd	17d	22cd
H. solani	11b	14b	16c	19c	23c
Rhizoctonia solani	12ab	15ab	18b	21b	25b
Alternaria alternata	10bc	11cd	17bc	20b	22cd
P. aphanidermatum	10bc	15ab	19ab	23a	28a
Botrytis cinerea	9c	10d	14d	17d	20d

The column's lowercase letters indicate significant differences ($p<0.05$).

human hepatitis C virus (HCV). The antiviral properties were evaluated by the MTT test and plaque reduction assay (J 8). The results were measured using CC50 (50% cytotoxic concentration), IC50 (inhibitory concentration for 50% of plaques), and SI (selectivity index). The CC50 of BLe was 1.4 mg/ml, demonstrating the best antiviral efficacy (SI = 5.60) in the virucidal assay.

The basil EOs of the five cultivars had considerable antiviral activity against the HCV, where Ble reduced 90% of virus plaque, followed by an 85% reduction in BL, while the lowest antiviral activity recorded in BI, where reduced the plaque by 55 % (Figure 4).

Anticancer activity: Figures 5A, B, C, and 6 show that each essential oil of five basil cultivars has an anticancer effect on human breast cancer cells. BLe reduces the viability of MCF7 cancer cells by 85%. In comparison, (BL) inhibited 81.28 % of cancer cell viability, BR) reduced the viability of breast cancerous cells by 53.78%, plus (BW) breast malignant cells' viability was decreased by 47.92 %, exceeding (BI) lowered breast malignant cells' survivability by 3.03 %; these results are correlated with

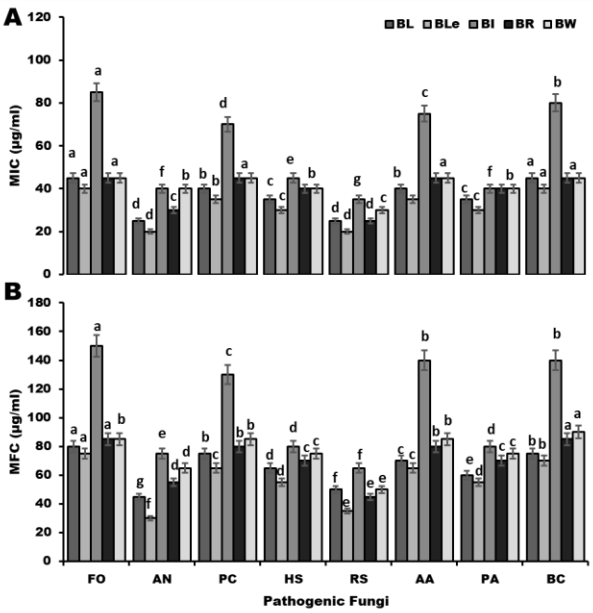

Fig. 3: Antifungal activity of *Ocimum basilicum* L. cultivars extracts expressed as (A) MIC and (B) MFC µg/mL against the pathogenic fungi.

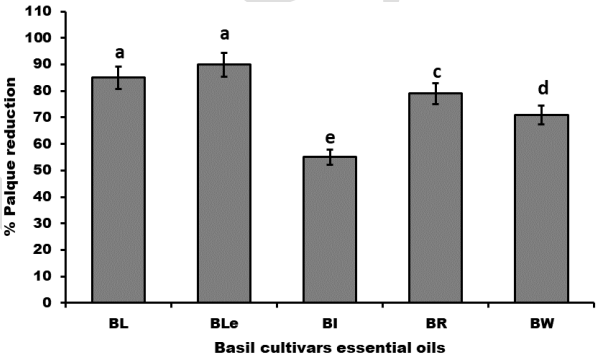

Fig. 4: Antiviral activity of *Ocimum basilicum* L. cultivars extracts against HCV expressed as the percentage of plaque reduction.

Table 8: Cytotoxicity of five basil essential oils on Human hepatitis C Virus.

Basil cultivars	CC50	IC50	SI
BL	1550d	300d	5.17b
BLe	1400e	250e	5.60a
BI	3350a	800a	4.19c
BR	1900c	450c	4.22c
BW	2150b	600b	3.58d

The column's lowercase letters indicate significant differences ($p<0.05$). Cytotoxic concentration (CC50), inhibitory concentration (IC50), selectivity index (SI). Local basil essential oil (BL), Lemon basil essential oil (Ble), Italian basil essential oil (BI), Frensh Red basil essential oil (BR), and Frensh White basil essential oil (BW).

microscopic images. On the other hand, Figure 3B shows the IC50 of treatments inversely correlated with % inhibition, where the IC50 of BLe was lowest after DOX (14 µg/mL), followed by BL (17 µg/mL) compared to BI with 85 µg/mL.

Molecular characterization of basil cultivars

Genetic structure by ISSR-PCR: The sequence and operon codes of the ISSR and SCoT primers used are listed in Table 9. Figure 7 shows the electrophoretic banding patterns of ISSR primers produced from the studied *Ocimum basilicum* L. cultivars, and their survey is presented in Table S1. The primer SR825 produced nine

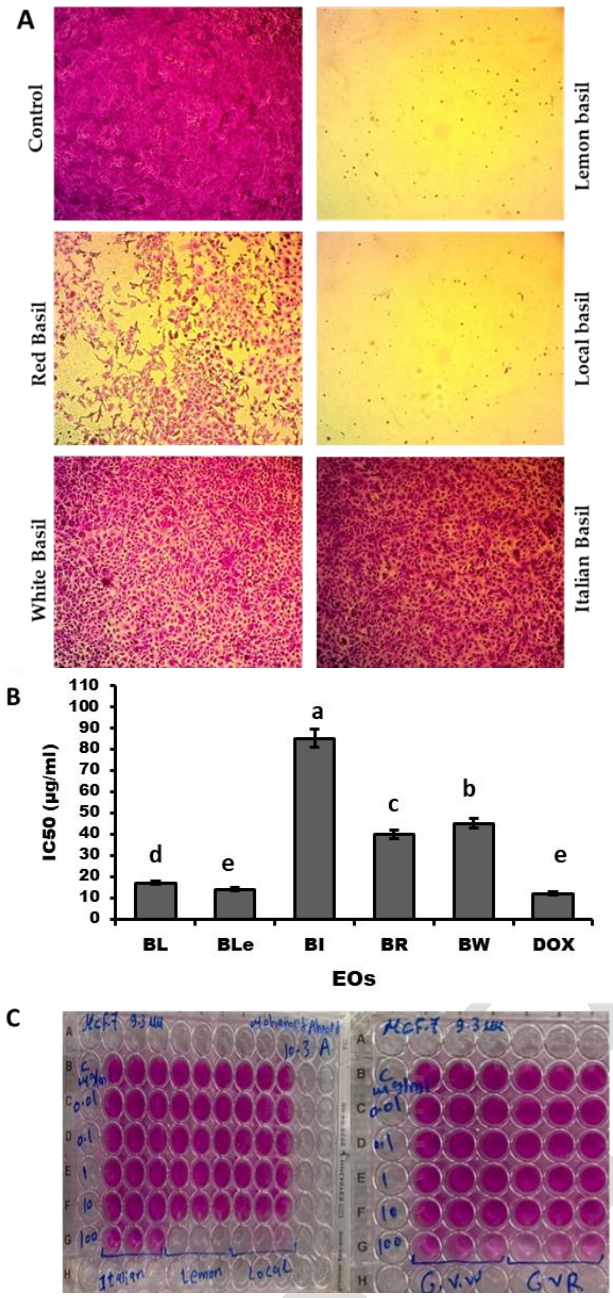

Fig. 5: Cytotoxicity of basil essential oil on the vitality of MCF7 cancer cells,

bands ranging from 11390 to 340 bp. The primer UBC 835 yielded 13 bands ranging from 1350 to 230 bp. The primer UBC826 produced five bands ranging from 1150 to 510bp. The primer UBC827 gave nine bands ranging from 1570 to 300bp. The primer UBC808 produced seven bands ranging from 1040 to 400 bp. The primer UBC868 produced nine bands ranging from 1300 to 405bp. The primer UBC901 yielded eight bands ranging from 1350 to 360 bp. These bands were distributed differently among the studied *Ocimum basilicum* L. cultivars. Seven ISSR primers amplified 60 bands, and 42 of these bands (70%) were polymorphic in the *Ocimum basilicum* L. cultivars. (Table 10).

The ISSR primer ISSR825 amplified two unique bands in the BLe cultivar at positions 770 and 390, which might serve as a molecular marker for characterizing this cultivar. Also , this primer manifested four common bands in all

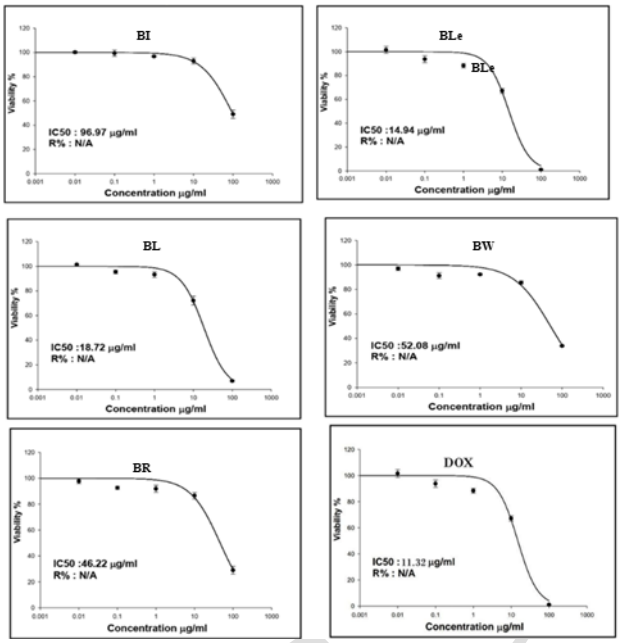

Fig. 6: Cytotoxicity of basil essential oil on the vitality of MCF7 cancer cells (IC50).

cultivars at positions1390,1000,650 and 480. The ISSR primer UBC835 amplified two unique bands in BW and BLe cultivars at positions 620 and 420, which might serve as molecular markers characterizing both cultivars. The same primer manifested five common bands in all cultivars at the positions 650,470,330,300 and 240. However, the ISSR primer UBC826 amplified only two common bands at the positions 570 and 510. The ISSR primer UBC827 amplified four common bands at the positions 950,650,530 and 370., The ISSR primer UBC808 amplified four unique bands for the cultivars: three in BLe at positions 880,750and 530 and one in BW at position 400, which might serve as a molecular marker for characterizing this cultivar. The same primer manifested one common band in all cultivars at position 670.

The ISSR primer UBC868 amplified three unique bands for the BLe cultivar at positions 1120,965 and 860, respectively, serving as molecular markers for this cultivar. The same primer manifested one typical band in all cultivars at position 640. However, the ISSR primer UBC901 amplified five unique bands, three in the BI cultivar at positions 1350,945 and 805 and two in the BLe cultivar at positions 590 and 505. These bands might be used as molecular markers to distinguish and characterize these cultivars. The same primer manifested one common band in all cultivars at position 360.

Interestingly, the seven ISSR primers that were used exhibited wide variations in banding patterns among the studied *Ocimum basilicum* L. cultivars. It is worth noting that such variations were reflected in a case of genetic, molecular polymorphism in *Ocimum basilicum*. Table (9) data illustrates polymorphisms detected by ISSR primers over all the studied *Ocimum basilicum* L. cultivars. The ISSR primers varied in the production of polymorphic bands. Polymorphism % ranged from 55.65 to 88.89 %. The primers UBC825 and UBC827 manifested the lowest polymorphism. But, the primers UBC868 gave the most significant polymorphism.

Genetic diversity and relationships among the studied *Ocimum basilicum* L. cultivars based on ISSR-PCR products: The similarity coefficients among *Ocimum bacilicum* L. cultivars based on the total number of the seven ISSR-PCR products illustrated that the most significant similarity coefficient was observed between the BR and BW cultivars (0.80), followed by BR and BL. In comparison, the lowest similarity coefficient was detected between the cultivars BW and BLe (0.48), followed by between BR and BLe.

The genetic Euclidean distances among the *Ocimum basilicum* L. cultivars based on the total numbers of the seven ISSR-PCR products revealed that the most comprehensive distance was observed between cultivars BW and BLe (5.57), followed by BR and BLe. However, in this regard, the nearest genetic distance was detected between BR and BW (3.46), followed by between BR and BL.

The constructed dendrogram of the studied *Ocimum bacilicum* L. cultivars based on the total numbers of the seven ISSR-PCR products is given in Figure (9A). The cut-off point is 10 Euclidean distances, and the *Ocimum bacilicum* L. cultivars are grouped into two clusters. The first cluster(I), the largest one, cluster II, comprises BLe. I divided the largest cluster into two sub-clusters. Subcluster I is the largest sub-cluster. Sub-cluster II comprised (BI) forming a single sub-cluster by itself. Sub-cluster I comprised BW and BR, while group two comprised BL.

SCoT-PCR: The electrophoretic banding patterns of SCoT primers produced from the studied *Ocimum basilicum* L cultivars are illustrated in Figure (8), and their survey is listed in Table (S4). The primer SCoT2 produced ten bands, with sizes ranging from 1460 to 320 bp. The primer SCoT19 yielded eight bands with banding sizes ranging from 1580 to 290 bp. The primer SCoT6 produced seven bands with sizes ranging from 2000 to 500 bp. But, the primer SCoT3 gave nine bands with sizes ranging from 1890 to 490bp. Also, the primer SCoT14 produced nine bands with sizes ranging from 1600 to 390 bp. But, the primer SCoT33 produced 11 bands with banding sizes ranging from 2900 to 480bp.

These bands of all SCoT primers were distributed widely and differently among the studied *Ocimum basilicum* L cultivars. The results show that 6 SCOT primers amplify 54 bands, and 34 polymorphism bands are present in these bands (63%) in the (*Ocimum basilicum* L.) cultivars. (Table 10)

The SCoT primer SCoT2 amplified three unique bands in BLe at positions 1460,1220, and 680, which might serve as a molecular marker for characterizing this cultivar. Also, this primer manifested five typical bands in all cultivars at positions 1080, 960, 875,760, and 440. The SCoT primer SCoT19 amplified only one unique band in BI at position 290, which might serve as a molecular marker for this cultivar. The same primer manifested three common bands in all cultivars at the positions1580,1470 and 820. However, the SCoT primer SCoT6 amplified one unique band in the BLe cultivar at position 1160, which might serve as a molecular marker for characterizing this cultivar. The same primer manifested three standard bands in all cultivars at positions 1020,720 and 500. The SCoT primer SCoT3 amplified one unique band in the BLe cultivar at position 1890, which might serve as a molecular marker for characterizing this cultivar. The same primer manifested three typical bands in all cultivars at positions 1400, 1120, and 770. The SCoT primer SCoT14 amplified only three common bands at positions 850,770 and 470. However, the SCoT primer SCoT33 amplified two unique bands in the BLe cultivar at positions 680 and 480. These bands might be used as molecular markers to distinguish and characterize these cultivars. The same primer manifested three standard bands in all cultivars at positions 1100,850 and 710.

Interestingly, the six SCoT primers exhibited wide variations in banding patterns among the studied *Ocimum basilicum* L. cultivars. It is worth noting that such variations were reflected in a case of genetic, molecular polymorphism in *Ocimum basilicum*. Data in Table (10) illustrate polymorphisms detected by SCoT primers over all the studied *Ocimum basilicum* L. cultivars.

The SCoT primers varied in the production of polymorphic bands. Polymorphism % ranged from 50 to 72.72%. The primer SCoT2 manifested the lowest polymorphism. But, the primers SCoT33 gave the greatest polymorphism.

Genetic diversity among the studied *Ocimum basiicum* L. cultivars by SCoT-PCR products: The similarity coefficients among *Ocimum bacilicum* L. cultivars based on the total number of the six SCoT-PCR products stated that the most significant similarity coefficient was observed between the cultivars BR, BI, and BR and BW (0.84), followed by between BW and BI. In comparison, the lowest similarity coefficient was detected between the cultivars BI and BLe (0.50), followed by BLe and BL.

The genetic Euclidean distances among the *Ocimum basilicum* L. cultivars based on the total numbers of the six SCoT-PCR products revealed that the most comprehensive distance was observed between BI and BLe (5.29), followed by the distance between BR and BLe. However, the nearest genetic distance was detected between BR and BW, and BR and BI (3.00) followed by BW and BI.

The constructed dendrogram of the studied *Ocimum bacilicum* L. cultivars based on the total numbers of the six SCoT-PCR products is given in Figure (9B). The cut-off point is 10 Euclidean distances, and the *Ocimum bacilicum* L. cultivars are grouped into two clusters. The first cluster(I), the largest one, cluster II, comprised the BLe cultivar. I divided the largest cluster into two sub-clusters. Subcluster I is the largest sub-cluster. Sub-cluster II comprised (BL) forming a single sub-cluster by itself. Sub-cluster I comprised two groups. Group one comprised BR and BI, and group two comprised BW.

Genetic diversity among the studied *Ocimum basiicum* L. cultivars by ISSR and SCoT-PCR products: The similarity coefficients among *Ocimum bacilicum* L. cultivars based on the total number of the seven ISSR and six SCoT-PCR products are given in Table (8). The most significant similarity coefficient was observed between the cultivars BR and BW (0.82), followed by between BW, BI, BR, and BI (0.79). In comparison, the lowest similarity coefficient was detected between the cultivars BI and BLe (0.52), followed by BR and BLe (0.53).

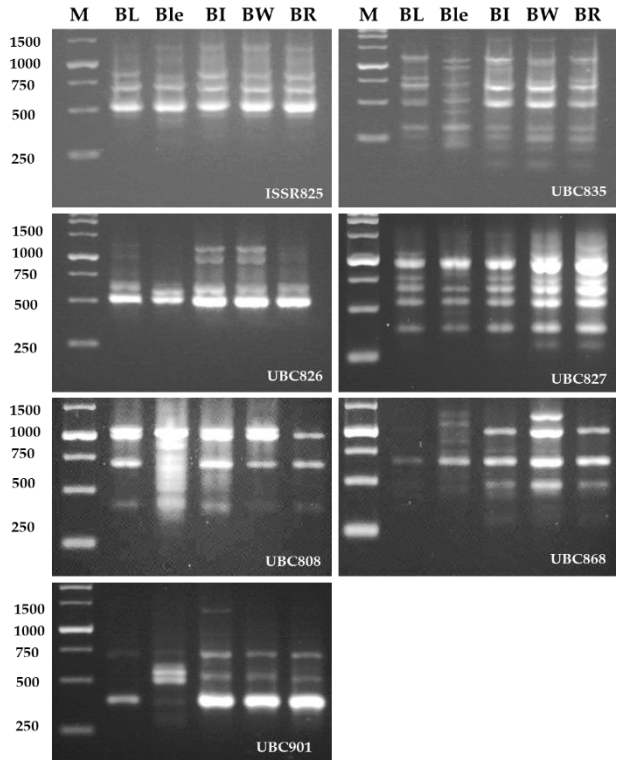

Fig. 7: Electrophoretic banding patterns of ISSR primers produced from the studied (*Ocimum basilicum* L.) cultivars. ISSR-PCR amplification using primers ISSR825, UBC835, UBC826, UBC827, UBC808, UBC868, and UBC901, respectively. M = 1 kbp DNA Ladder

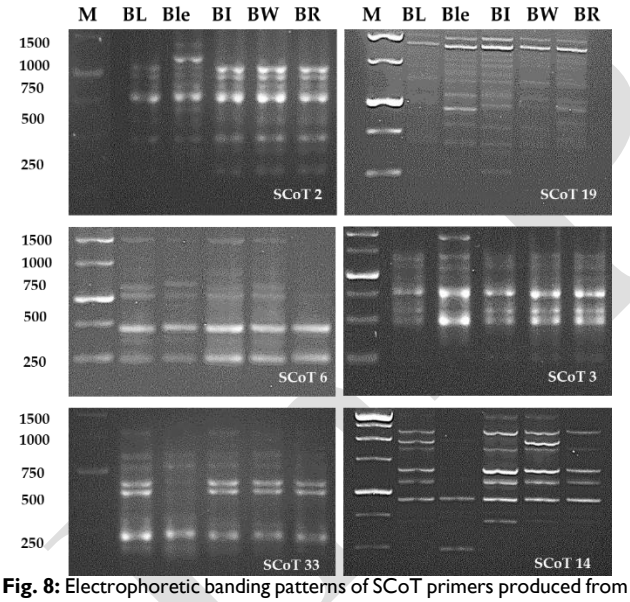

Fig. 8: Electrophoretic banding patterns of SCoT primers produced from the studied (*Ocimum basilicum* L.) cultivars. SCoT-PCR amplification using primers SCoT2, SCoT19, SCoT6, SCoT3, SCoT-14, and SCoT33, respectively. M = 1 kbp DNA Ladder

The genetic Euclidean distances among the *Ocimum basilicum* L. cultivars based on the total numbers of the seven ISSR and six SCoT-PCR products are shown in Table (S7). The data revealed that the widest distance was observed between BI and BLe (7.42), followed by between BR and BLe (7.35). However, the nearest genetic distance was detected between BR and BW (4.59), followed by BR and BI (4.80).

The constructed dendrogram of the studied *Ocimum bacilicum* L. cultivars based on the total numbers of the seven ISSR and six SCoT-PCR products is given in Figure

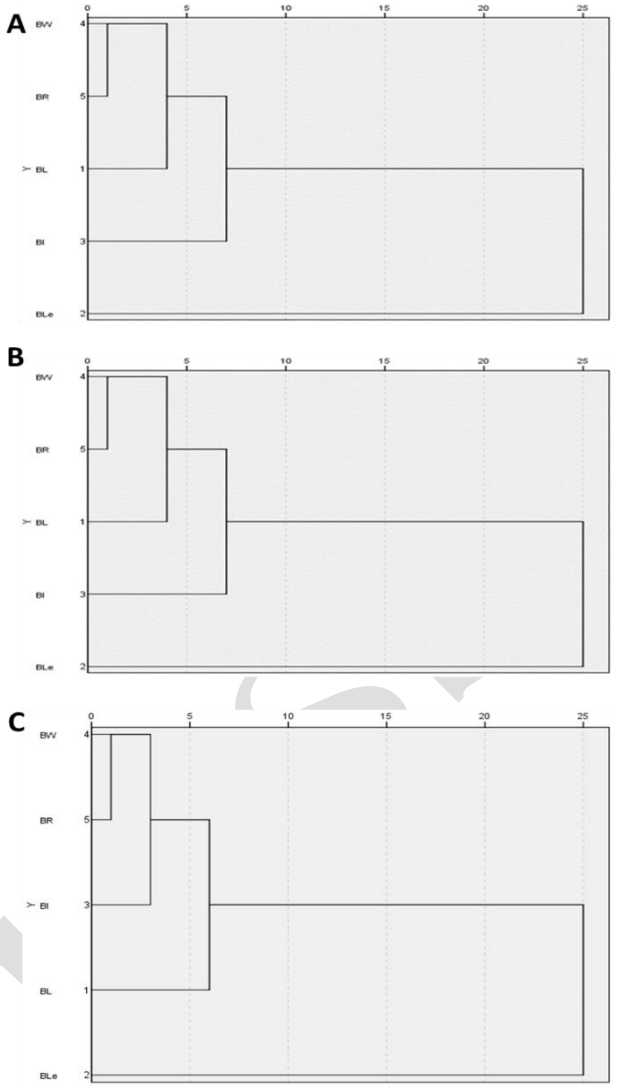

Fig. 9: Linkage dendrogram of studied (*Ocimum basilicum L.*) cultivars based on total numbers of the (A) seven ISSR- PCR products, (B) six Scot- PCR products, (C) ISSR and SCoT- PCR products.

(9C). The cut-off point is 10 Euclidean distances, and the *Ocimum bacilicum* L. cultivars are grouped into two clusters. The first cluster (I), the largest one, cluster II, comprised the BLe cultivar. I divided the largest cluster into two sub-clusters. Subcluster I is the largest sub-cluster. Sub-cluster II comprised (BL) forming a single sub-cluster by itself. Sub-clusters I consist of two groups: Group One comprised BR and BW, and group two comprised BI.

DISCUSSION

Unfortunately, contemporary agricultural methods predominantly reliant on monocropping have led to the depletion of genetic diversity in our rural environments (Patel *et al*., 2020). Hence, it is crucial to implement innovative strategies to restore genetic and species diversity in our farming systems (Brito *et al*., 2021). Urgent policy measures are necessary to preserve genetic diversity, manage agrobiodiversity, and meet present and future generations' food, nutrition, fodder, fuel, and therapeutic needs (Heckelman *et al*., 2022). Furthermore, utilizing local and indigenous expertise to preserve plant genetic variety to benefit humanity and the environment is imperative.

ISSR has a randomly amplified marker but a SCoT gene-targeted marker. Such markers possess many advantages, such as simplicity of use, low cost, and using small amounts of studied plant materials (Gupta *et al*., 2021). Twelve ISSR primers amplified 238 bands, showing 234 polymorphism bands in the *Ocimum* species (Hanumanthaiah *et al*., 2020). Moreover, Kumari *et al*. (2020) revealed that ten primers of ISSR amplified 66 bands ranging from 200-2000bp. Furthermore, Gupta *et al*. (2021) investigated six species of *Ocimum* using three ISSR primers to study their genetic divergence and stated that only three primers amplified 209 scorable bands. Patel *et al*. (2015) reported that the polymorphism % of ISSR primers in *Ocimum basilicum* L was 98.17, which disagrees with our results.

Bakhtiar *et al*. (2024) detected genetic diversity and Euclidean distances among some *Ocimum bacilicum* L using ISSR- PCR products, which agreed with the obtained results. Aghaei *et al*. (2012) manifested that the mean similarity index was 0.735 with the same 12 ISSR primers on 50 genotypes of *O. basilicum*. But, Lal (2014) reported a low mean similarity index (0.39). Patel *et al*. (2015) declared that clustering analysis of seventeen genotypes grouped into six main clusters, But Ibrahim *et al*. (2013) studied 37 basil accessions from different species (*Ocimum basilicum*, *O. americanum*, O. *gratissimum* and *O. tenuiflorum*). Eleven genotypes of *Ocimum basilicum* were grouped into two groups (nine accessions in Group I and two in Group III. Also, Gupta *et al*. (2021) showed two major clustering groups, cluster A and Cluster B. Cluster A comprised of O. *polystachyon, O. sanctum, O. americanum, O. basillicum, O. gratissimum,* and Cluster B comprised of *O. viride*.

SCoT markers were proven highly effective in differentiating among closely related basil cultivars, therefore serving as a significant tool for investigating the genetic links among various basil accessions. By analyzing SCoT profiles in basil plants, we may discern genetic clusters and ascertain the level of relatedness across various Ocimum species variants (Gupta *et al*., 2021). This data can provide valuable guidance for breeding operations, conservation initiatives, and creating novel cultivars with specific desirable characteristics (Akbari *et al*., 2019). An analysis was conducted on Egyptian basil landraces' genetic diversity and polymorphism using eleven SCoT primers. The study revealed a significant genetic variety across the five basil cultivars, exhibiting a broad spectrum of polymorphism variance. This suggests the presence of genetic variation. The SCoT primers' phylogenetic analysis offers valuable information on the genetic diversity and connections among various basil landraces (Gupta *et al*., 2021). This data can contribute to the formulation of conservation and breeding tactics that can protect the genetic assets of this significant crop in light of worldwide climate change and other potential dangers.

Gupta *et al*. (Gupta *et al*., 2021) showed large genetic diversity and similarity between the accessions of Ocimum. Patel *et al*. (Patel *et al*., 2015) stated that the similarity index between 17 *Ocimum* genotypes based on ISSR-PCR products ranges from (0.13–0.96), which indicates higher genetic variations. Bakhtiar *et al*. (2024) detected genetic diversity and Euclidean distances among some *Ocimum bacilicum* L using ISSR- PCR products, which agreed with the result. Gupta *et al.* (Gupta *et al*., 2021) showed Two major clustering groups. Patel *et al*. (2020) declared that clustering analysis of seventeen genotypes grouped into six main clusters.

Genetic diversity is crucial for understanding the various characteristics of plants, such as their appearance, function, chemical makeup, and genetic composition (Nonić and Šijačić-Nikolić, 2021). This knowledge allows us to develop effective strategies for improving existing crop plants by enhancing their desirable traits. It also enables us to breed better-adapted crops to future climate conditions (Bailey-Serres *et al*., 2019; Sedeek *et al*., 2019). The chemical composition of five different basil cultivars correlated with their genetic structure. This study involved the identification of 24 compounds in five samples of each cultivar of *Ocimum* sp. essential oils. Several of these compounds were shown to be useful as cultivar identifiers.

This is in agreement with Mahmoud *et al*. (2022), who reported that a significant portion of the total volatiles quantified were derived from Linalool, methyl cinnamate, 1,8-cineole, and eugenol in different cultivars of *O.basilicum*. Our experimental findings were consistent with those reported by (Ladwani *et al*., 2018; Perveen *et al*., 2020).

The quantity and quality of the components vary, though, and these variations may be attributable to hereditary and environmental influences. A growing need for alternative therapeutic agents devoid of severe adverse effects has necessitated the widespread application of natural plant compounds to treat various diseases. The global market has witnessed a surge in the demand for natural product research due to its challenging therapeutic attributes (Piras *et al*., 2018). Furthermore, research has indicated that the escalating demand is significantly influenced by the therapeutic chemical composition and biological activity of natural products, such as essential oils (EOs), extracts, and other plant-based products, which possess surplus medicinal values (Sakkas and Papadopoulou, 2017). EOs derived from plants possessing multifunctional properties have been utilized to prevent and treat various ailments since ancient times (Shiwakoti *et al*., 2017; Varga *et al*., 2017). Essential oils (EOs) are rudimentary metabolites derived from aromatic plants. According to studies, extracted EO samples typically contain 20%–60% bioactive compounds, with two or three main phytoconstituents comprising 20–70% of the sample (Tiwari *et al*., 2023).

In this study, we extracted the essential oils of *Ocimum forsskaolii* benth; the content of the essential oil profile consisted of Bader *et al*. (2023), who found the primary constituents of DEO were methyl eugenol, Eugenol, Linalool, germacrene D and β-caryophyllene with contents between (2.57-55.65%) %.

Also, Elansary and Mahmoud (2015) found the main compounds in the *O. basilicum* oil were methyl cinnamate (43.8%) and chavicol methyl ether (39.1%). Furthermore, Ahmed *et al.* (2019) detected the EOs in two types of basil using GC/MS; the predominant and common components of both purple and green types oils were Linalool (44.37%; 46.24%), estragole (20.05%; 13.26%), trans ethyl cinnamate (15.05%; 0.45%), 1,8-cineole (9.28%; 3.28%), and α-cardinal (1.38%; 3.10%). Perveen *et al.* (2020) extracted the

Table 9: Polymorphism detected by ISSR primers over all the studied (*Ocimum basilicum L.*) cultivars.

No	Primers	No. of scorable bands	Number of Monomorphic bands	Number of Polymorphic bands	Number of unique bands	Polymorphism (%)
1	ISSR825	9	4	3	2	55.56%
2	UBC835	13	5	6	2	61.54%
3	UBC826	5	2	3	-	60%
4	UBC827	9	4	5	-	55.56%
5	UBC808	7	1	2	4	85.72%
6	UBC868	9	1	5	3	88.89%
7	UBC901	8	1	2	5	87.5%
Total		60	18	26	16	70%

Table 10: Polymorphism detected by Scot primers over all the studied (*Ocimum basilicum L.*) cultivars.

No	Primers	No. of scorable bands	Number of Monomorphic bands	Number of Polymorphic bands	Number of unique bands	Polymorphism (%)
1	SCoT 2	10	5	2	3	50%
2	SCoT 19	8	3	4	1	62.5%
3	SCoT 6	7	3	3	1	57.14%
4	SCoT 3	9	3	5	1	66.67%
5	SCoT 14	9	3	6	-	66.67%
6	SCoT 33	11	3	6	2	72.72%
Total		54	20	26	8	63%

chemical compounds of volatile oil from *Ocimum basilicum* L obtained by hydro-distilled and analyzed by gas chromatography. The main compounds were methyl chavicol (38.2 %) and Linalool (28.7 %).

The considerable content of EOs in basil cultivars possess various biological activities where the obtained results were consistent with other findings Antonescu *et al*. (2019), Nguyen *et al*. (2021), and Teofilović *et al*. (2021) stated that the volatile oil extracted from *Ocimum basilicum* by distillation steam and a Clevenger-type device, that have antioxidant activity determined by DPPH and linoleic acid peroxidation. The findings demonstrated that essential oils have antioxidant characteristics and revealed that the basil leaf extract exhibited a substantial antioxidant capacity, as evidenced by its IC50 of 285.36 µg/mL. This study demonstrates that basil has the potential to be a valuable source of phenolic chemicals. Also, the extract shows promise as a medicinal antioxidant (Do *et al*., 2020).

The genus *Ocimum*, which is part of the family Lamiaceae, has been extensively utilized in traditional medicine to treat inflammation, diarrhea, chronic diarrhea, and insect bites. The study aims to assess the phytochemical composition and pharmacological effects of different *Ocimum* spp. i.e., *O. basilicum* (OB), *O. canum* (OC), *O. gratissimum* (OG), *O. tenuiflorum* (OT), *O. kilimandscharicum* (OK), and *O. citriodorum* (OXC) extracts. The OB extract had a significantly higher amount of polyphenols (246.2 mg GAE/g) than other extracts. Regarding antioxidant activity, the OG extract showed considerable DPPH scavenging activity (14.73 µg/mL). The OB and OG extracts were the most active (Anusmitha *et al*., 2022).

Furthermore, Tshilanda *et al*. (2016) determined the antioxidant potential of the basil plant using the DPPH free radical scavenging activity method. The essential oil had antioxidant properties, with an IC50 of 1180 µg/mL. The methanolic and ethyl acetate extracts showed higher antioxidant activity than essential oil, with IC50 values of 25 and 85 µg/mL, respectively. The essential oil was less active than methanol and ethyl acetate extracts regarding IC50 values. The most active extract was methanol crude extract. The ability of non-polar extracts to scavenge radicals was minimal; hence, IC50 values could not be determined**.** Also, Teofilovic *et al*. (2021) estimated the antioxidant properties of basil (*Ocimum basilicum* L.) extracts that inhibit DPPH radicals with IC50 20 µg/ml; this activity because of the considerable content of the total flavonoids from 40 mg/g and total phenolic of 65 mg/g.

The ethanolic extract of basil leaves has a wide range of antioxidant activity, suggesting that the plant could be a natural source of antioxidants, potentially lowering oxidative stress and providing health benefits. Ultimately, the plant could be used in the pharmaceutical and food industries. Also, Nguyen *et al*. (2021) determined the scavenging activity and phenolic content of ethanolic and aqueous extracts of sweet basil. The results indicated the superiority of ethanolic extract with IC50 of 91 µg/mL and 85 µg/mL, respectively, and the phenolic content was 29 mg/g.

The antimicrobial activity of basil essential oil affects the development of three different kinds of bacteria. According to the survey findings, essential oils have a strong antibacterial effect on all Gram (+) and Gram (-) bacteria strains and the fungus C. albicans. The capacity of an essential oil constituent to permeate the cell walls of a bacteria or fungus is directly correlated with how soluble they are in water. Therefore, the solubility of essential oils in the phospholipid bilayer of cell membranes accounts for their antibacterial activity (Knobloch *et al*., 1989)**.** According to some research, Linalool may contribute to its antibacterial effect by acting as a solvent-dehydrating or protein-denaturing agent (Zhou and Liu, 2024). The antibacterial properties of monoterpene alcohols, such as Linalool, nerol, citronellol, and geraniol, have also been found to be more potent than their antifungal properties**.** Alterations in the permeability of the damaged cell membrane result in leakage of potassium, proteins, and nucleic acids from inside the bacterial cell, making the cell membrane unstable and inhibiting the proliferation of the bacterial cells (Fachriyah *et al*., 2022). Consistent with the current study, the essential oil showed superior antibacterial activity against gram-positive bacteria (S. aureus) and moderate activity against gram-negative

bacteria (E. coli), as evaluated by the agar diffusion method. The growth of bacteria was only faintly suppressed by essential oil at low concentrations (5µl). Numerous literature studies have shown that several *Ocimum basilicum* essential oil components have antibacterial properties (Ababutain, 2019; Rezzoug *et al*., 2019; Zhakipbekov *et al*., 2024).

Essential oils exhibit similar mechanisms of action against fungi and bacteria. The antifungal activity of basil essential oil is primarily attributed to its key components. Research has demonstrated that specific compounds within essential oil mixtures, like eugenol, Linalool, and methyl chavicol, can work together or individually to disrupt cell membranes and interfere with various cellular functions, including energy production. These compounds can disrupt proton pumps, reduce membrane potential, and deplete ATP, all contributing to antifungal effects. Additionally, basil essential oil compounds can cause the coagulation of cellular contents, leakage of cytoplasm, and, ultimately, cell death through apoptosis or necrosis (Mkaddem Mounira *et al*., 2022)

Recent studies have delved into the potential anticancer properties of basil (*Ocimum basilicum* L.), focusing on its various components. Aburjai *et al*. (2020) examined the anticancer effects of basil leaf volatile oil against breast (MCF7 and MDA-MB-231) and brain cancer (U-87 MG) cell lines. The oil demonstrated significant cytotoxicity, with IC50 values ranging from 320.4 to 432.3 µg/ml. Also, Eid *et al*. (Eid *et al*., 2023) indicated basil essential oil demonstrated potent anticancer activity against MCF-7 (80.35 µg/ml) compared to Doxorubicin. Our research supports the long-standing use of this medicinal herb as a supplementary and alternative medicine**.** Additionally, our findings are consistent with earlier research that found that an extract of *Ocimum basilicum* leaves suppressed the multiplication of tumor cells (Aburjai *et al*., 2020; Alkhateeb *et al*., 2021). Leaf essential oils demonstrated anti-proliferative solid effects.

Lanave *et al*. (2024) investigated the ability of five commercially available essential oils (EOs) to inactivate BVDV, a significant cattle pathogen. Although BVDV is not the same as HCV, it can be used as a model to study antiviral agents due to similarities in their replication process (DeWald *et al*., 2020). Unlike some HCV cell culture systems, BVDV allows researchers to examine both the early and late stages of viral replication. While more advanced HCV cell culture systems exist, studying antiviral compounds against BVDV can still provide valuable insights for developing new treatments against HCV. Targeting host enzymes involved in BVDV replication may also be effective against HCV (Ma *et al*., 2022).

Conclusions: This study aimed to comprehensively characterize five basil cultivars based on their biochemical and molecular profiles, focusing on enhancing their potential as natural remedies. The research delved into these cultivars' antioxidant, antiviral, anticancer, antibacterial, and antifungal properties. Significant variations were observed in the phytochemical composition of the five cultivars, including phenolic compounds, flavonoids, and essential oils. These compounds are known to possess potent biological activities, contributing to the overall therapeutic potential of basil. The Genetic analysis revealed distinct molecular profiles among the cultivars, suggesting genetic diversity within the species; this diversity may explain the observed variations in bioactive compound content and biological activities. The Biological Activities reveled that all five cultivars exhibited promising antioxidant, antiviral, anticancer, antibacterial, and antifungal properties; However, the extent of these activities varied among cultivars, highlighting the importance of cultivar selection for specific applications. The findings of this study provide valuable insights into the potential of basil cultivars as natural sources of bioactive compounds. These cultivars can be further explored to develop novel pharmaceutical and nutraceutical products. Additionally, the genetic diversity identified in this study offers opportunities for breeding programs to develop cultivars with enhanced therapeutic properties. Further research is needed to elucidate the mechanisms underlying the observed biological activities. Also, clinical trials are essential to evaluate the safety and efficacy of basil extracts in human subjects. Finally, developing standardized methods for cultivating, harvesting, and extracting basil can ensure consistent quality and potency of bioactive compounds.

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