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ABSTRACT

This study aims to investigate the quality, nutritional value and therapeutic properties of essential oil of the *Eruca sativa Mill*. Our study evaluated the nutritional value of essential oil of the *E. sativa* by locating the content of moisture, carbohydrates, vitamins, mineral (nutritional and toxic) elements and fatty acids. Also, determined the quality of *E. sativa* essential oil by physicochemical parameters. Total phenolic content (TPC) and total flavonoids content (TFC) were determined. This study also evaluated the *in vitro* antimicrobial activities by two methods. The yield of the essential oil extracted (w/w) was 0.3% and chemically analyzed. The results of the study show that the oil has a high nutritional value that proved to be particularly rich in mineral nutrients and fatty acids. The results indicated that essential oil of *E. sativa* has higher resistance to autoxidation, higher quality, longer shelf life, suitable for use as salad, chocolate or cooking oil and edible. The results showed that essential oil of the *E. sativa* has significant antimicrobial activities against the microorganisms, including seven human pathogenic bacteria showed resistance to some antibiotics.

Keywords: *Eruca sativa*, nutritional value, total phenolic and antimicrobial activities

1. Introduction

Aromatic plants are most widely used as a source of traditional herbal medicine and their formulations are vital with respect to nutrient, antioxidant and medicinal values. **(Sharma et al., 2021)**. They are

containing especially biological activity compounds **(Messaoudi et al., 2021)**. Because essential oil possessed variously biological properties including antioxidant, antimicrobial, antiviral, antitumor, fragrance, insect attraction, antiseptic, skin penetration enhancer, fungicidal, insecticidal and herbicidal, they are widely employed in food preservation, agriculture, skin therapeutic, aromatherapy, and medicine fields **(Nguyen et al., 2018)**. *E. sativa* is aromatic and medicinal plant also known as herb or spice. *E. sativa* belongs to the Brassicaceae family is a wild plant common to Mediterranean regions and in Europe average. *E. sativa* plant has been used as a food ingredient, especially in a salad. *E. sativa* plant contain a wide range of health-promoting phytochemical compounds such as glucosinolates, cardiac glycosides, polyphenols, flavonoid, phenolics, alkaloids, saponins, tannins, ascorbic acid, and essential oil **(Alqasoumi et al., 2009; Helana et al., 2011; KUTLU et al., 2021; Marwat et al., 2016; Nurzyńska-Wierdak, 2015; Zhu et al., 2021)**. In Egypt and many regions of the world, *E. sativa* production has been significantly increased for the high demand for volatile oils for the pharmaceutical purpose **(El-Fadaly et al., 2017)**. Utilization of plant essential oils (EOs) became steadily important in scientific research and industrial applications, including nutritional, pharmaceutical and cosmetic uses, primarily due to the oils' various potent biological activities, including antimicrobial and anti-inflammatory activities **(Tsai et al., 2011)**. Recent studies of the EO of the *E. sativa* species have shown that they have important biological activities including, antioxidant, antimicrobial and antidiabetic **(Hichri et al., 2019)**. EO from the leaves of *E. sativa* was found to contain isothiocyanate **(Miyazawa et al., 2002)** display antifungal, anti-carcinogenic, Anti-inflammatory and antimicrobials activities **(Alvarez et al., 2015; Anubhuti et al., 2016; Prieto et al., 2019; Troncoso-Rojas and Tiznado-Hernández, 2014)**. The antimicrobial activity of EOs is known for many centuries. In the last years, a large number of EOs and their constituents were investigated for their antimicrobial activities against different bacteria and fungi strains **(Serban et al., 2011)**. The EO of *E. sativa* exhibited antimicrobial activity that shown potent inhibitory effects against tested Gram-positive and Gram-negative bacteria **(Hichri et al., 2019)**. The concern over the use of EOs as antimicrobial agents has increased, due to an emergent microbial resistance towards conventional synthetic antimicrobial preservatives. EOs are widely used in medicine, cosmetic industries and in the food industry, where they are used for preservation and increased shelf life of stored food instead of synthetic chemicals charged to be cytotoxic **(Frassinetti et al., 2011)**. Therefore, There is a steady and increasing interest in the development of safe and effective natural food

preservatives **(Bag and Chattopadhyay, 2015).** Despite the abundant information available on several EOs, the investigation dealing with EO of the *E. sativa* has been limited. Moreover, to the best of our knowledge, there are no reports available on the essential oil of the *E. sativa* 's shelf life and use as a functional food, nutritional value. Also, the lack of reports available on the essential oils of the *E. sativa* as an antimicrobial activity. Therefore, the objective of this work was to evaluate the quality, nutritional and therapeutic properties including in vitro antimicrobial activities of essential oils of the *E. sativa*

2. Material and methods

2.1 Plant: *E. sativa* fresh were obtained from local markets at Sharkia Government.

2.2 Extraction of essential oil: the essential oil was extracted by steam distillation of fresh plant (100g) using a Clevenger-type apparatus for 3 h for extract of essential oil. It was extracted according to the standard procedure described in the European Pharmacopoeia (2004). Oil was collected, dried over anhydrous sodium sulphate, weighed and stored in sealed glass vials. Yield was calculated based on dried weight of the sample.

2.3 Nutritional value analysis:

2.3.1Moisture content: moisture content of *E. sativa* essential oils were determined according to A.O.C.S. Recommended Practice Ca 2d-25 **(AOCS, 1998)**.

2.3.2Total carbohydrates: total carbohydrates content in *E. sativa* oil was estimated using phenol sulphuric acid according to **(Nielsen, 2010)**

2.3.3 Vitamins: HPLC was used to identify and determine water or fat soluble vitamins in *E. sativa* essential oil. The separation was carried out using Column C18 Inertsil (4.6x250mm, 5µm). Mobile phase: methanol (100%) for fat soluble vitamins and 0.85 g Hexane sulphonic acid sodium salt in 1000 mL water and adjust pH to 3.00 with orthophosphoric acid. water soluble vitamins flow rate was 1 mL/min. The mode of elution was Isocratic for fat soluble and gradient for water soluble. Column temperature was at 25ºC. The wavelength was 210 nm for fat soluble, 260 nm for water soluble. 180 mg were accurately weighted and dissolved in 1 mL DMSO, sonicate for 15 min then filter then 10 µL was injected into column. Standard, Water soluble vitamins: Mixture of 7 vitamins (Nicotinic acid, Nicotinamide, Pyridoxine, Folic acid, Thiamine, Riboflavin, Vitamins B12 and C) in conc. of 25 µg/mL was prepared then filter then 10µL was injected. Fat soluble vitamins: Mixture of 3 vitamins (D3, E, A) in conc. of 10µg/mL was prepared, filtered then 10µL was injected.

2.3.3 Determination of minerals

2.3.3.1 Phosphorus content: The determination of Phosphorus (P) was carried out using ES 4185/2005 according to **ISO IEC 17025 (2017).**

2.3.3.2 Calcium content: Calcium content was estimated by use **ISO 5491/2005** according to **ISO IEC 17025 (2017).**

2.3.3.3 Magnesium, Iron, Manganese, Zinc, Copper, Sodium, Potassium, Cadmium, Arsenic, Aluminium, Selenium, Nickel, Chromium and Cobalt: Mineral contents were determined using flame atomic absorption spectroscopy (A.A.S) using the method of **(Chen and Teo, 2001)**.

2.3.4 Fatty Acid (FA): FA constituents of *E. sativa* oil were determined by gas chromatography–mass spectrometry (GC-MS) analysis according to **(Abd El-Kareem et al., 2016)**. Briefly, The FA constituents of *E. sativa* oil were performed using Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m × 0.25 mm × 0.25 µm film thickness). Column temperature was initially held at 50°C then increased by 50°C /min to 250°C hold for 2 *min*. increased to the final temperature 300°C hold for 2 min. The injector and MS transfer line temperatures were kept at 270, 260°C, respectively. Helium used as a carrier gas at a constant flow rate of 1.0 mL/min. 1.0 µL *E. sativa* oil was injected automatically using Auto sampler AS1300 coupled with GC in the split mode. EI mass spectra collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source temperature was at 200°C. The constituents were identified by comparison of their mass spectra with those of WILEY 09 and NIST 14 mass spectral database.

2.4 physicochemical parameters analysis

The determinations of physicochemical parameters of *E. sativa* essential oil for acid value, iodine value, saponification value and peroxide value are carried out according to the methods of **(AOCS, 1998)**. The peroxide, acidity, iodine and saponification values are the major characterization parameters for *E. sativa* essential oils quality.

2.4.1Peroxide value (PV): PV is a test for susceptibility of *E. sativa* essential oil to oxidative rancidity. Peroxide value of *E. sativa* essential oil was determined according to A.O.C.S. Recommended Practice Cd 8-53 **(AOCS, 1998)**.

2.4.2Acid values (AV): AV is a test for the measurement of free fatty acids (FFT) present in *E. sativa* essential oil. Acid values of *E. sativa* essential oil was determined according to A.O.C.S. Recommended Practice Cd 3d-63 **(AOCS, 1998).**

2.4.3Iodine Value (IV): IV is a measure to determine the amount of unsaturation in *E. sativa* essential oil. IV of *E. sativa* essential oil was

determined according to A.O.C.S. Recommended Practice Cd 1c-85 **(AOCS, 1998).**

2.4.4Saponification value (SV): SV is a measure of the average molecular weight or chain length of all fatty acids in the *E. sativa* essential oil as triglycerides. SV of the *E. sativa* essential oil was determined according to A.O.C.S. Recommended Practice Cd 3c-91 and Cd 3b-76 **(AOCS, 1998)**.

2.5 Quantitative estimation of phenolic and flavonoids compounds

2.5.1Estimation of total phenolic content (TPC): Total phenolic content in *E. sativa* oil was estimated by use of Folin-Ciocalteu method according to **(Singleton et al., 1999)**. Briefly; 180 µg of *E. sativa* oil was accurately weighted and dissolved in 1.0 mL DMSO, sonicate for 15 *min*, filter then, 500µL was injected into test tube containing 1.0 mL of Folin-Ciocalteu reagent. After 3 min, 1.0 mL of sodium carbonate was added and incubated in dark at room temp for 2 hours. After the incubation, the absorbance was measured at 765 nm. Gallic acid was used as standard to calculate the standard curve.

2.5.2Estimation of total flavonoids content (TFC): According to method of **(Zhishen et al., 1999)**, with some modifications, total flavonoids content was determined using Aluminum chloride colorimetric assay. Briefly, 180 µg of *E. sativa* oil was dissolved in 1.0 mL DMSO then 1.0 mL of the sample was injected into test tube containing 4.0 mL of distilled water. 300 µL sodium nitrite was added. After 5 min, 300µL aluminum chloride. After 6 min, 2 mL of sodium hydroxide was added. The mixture incubated in dark at room temp for 90 min. After incubation, the absorbance was measured at 510 nm. Quercetin was used as standard to calculate the standard curve.

2.6 Evaluation the *in Vitro* Antimicrobial Activity:

2.6.1Microorganisms: Essential oil of *E. sativa* was tested as antibacterial and antifungal on the following microorganisms, four gram-positive bacteria [*Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (ATCC 14579), *Enterococcus faecalis* (ATCC 25175) and *Staphylococcus aureus* (ATCC 6538)]. Four gram-negative bacteria [*Escherichia coli* (ATCC 8739), *Neisseria gonorrhoeae* (ATCC 19424), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhimrium* (ATCC 14028)]. Four fungi [*Aspergillus flavus* (ATCC 9643), *Aspergillus niger* (ATCC 16404), *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 7310)]. Kanamycin was used as standard with gram-positive bacteria, Ampicillin with gram-negative bacteria, Gentamicin with *Pseudomonas aeruginosa* and Amphotericin (B) with fungi. DMSO was used as control.

2.6.2 Antimicrobial assay: The antimicrobial activity and susceptibility tests were carried out according to Recommendations National

Committee For Clinical laboratory Standards **(Jones, 1984)**. Screening tests concerning the inhibition zones were carried out done using two methods as follows:

Disc diffusion method: antibacterial and antifungal activity of the essential oil *E. sativa* was evaluated according to the method described by **(Zaidan et al., 2005)**, with slight modifications. Sample was prepared by dissolving 2mL of oil in 1mL DMSO. The discs were autoclaved and each disc was infused with 100 µL of extract. One hundred microliters of each microorganism suspension was inoculated onto the MHA. Microorganism spread all over the agar surface by using sterile swab. The tested essential oil disc transferred on the surface of MHA and inoculated with microorganism: bacterial strain at 35%°C for 48 hours, fungi incubated at 25%°C for 3 days and yeast incubated at 30%°C for 48 hours. After the incubation period, measured the diameter of the growth inhibition zones.

Well diffusion method: well diffusion method on Mueller-Hinton agar (MHA) method according to **(Baskaran et al., 2012).** Agar was used for the growth of the above mentioned microorganisms and placed in sterile Petri plates. Agar plates were prepared under sterile conditions. In all these plates, 6 mm in diameter are cut using a sterile cork borer and the agar disc is removed from all plates. Sample was prepared by dissolving 2mL of oil in 1mL DMSO. In 96 wells plate (diameter=6mm) were filled with 100µL of the test sample (essential oil of *E. sativa*). One hundred microliters of each microorganism suspension was inoculated onto agar plates. Bacterial strains were incubated at 35%°C for 48 hours, fungi (incubated at 25%°C for 3 days and yeast at 30%°C for 48 hours. After the incubation period is completed, measured the diameter of the growth inhibition zones around each well.

2.6.3 Determination of Minimum Inhibitory Concentration (MIC): MIC was used to define *in vitro* levels of susceptibility or resistance of oil *E. sativa* on specific bacterial strains to apply as antibiotic. The MIC values were determined using broth dilution method according to **(Al-Talib et al., 2016)** with some modifications. In a sterile 96-well microplate, a stock solution of the oil was prepared in DMSO. The concentrations of the tested oil ranged from 5 to 640 µL/mL by twofold serial concentration of the oil as (5, 10, 20, 40, 80, 160, 240, 320 and 640 µL/mL). This test was performed in sterile bottles which were loaded with 100µL of the tested oil concentration into each bottle. Bacterial inoculum (100 µL) containing 5×105 CFU of each bacteria was added to each bottle. All bottles were incubated for 24 *h* at 37°C. After 24 *h* of incubation, 100 µL of each mixture was pipetted and inoculated on nutrient agar and again bottles were incubated for 24 *h* at 37°C. The MIC

value was taken as the lowest concentration of the oil in the plate that showed no growth of the tested bacterium after incubation. The lowest concentration which showed no visible bacterial growth and no turbidity in bottle was considered as MIC

2.7 Statistical analyses

To determine statistically significant differences between samples, two-factor analysis of variance (One- way ANOVA) and t-tests by program SPSS (17.0) were used and followed by and comparison of the means of sample by Duncan's multiple range test. The differences were considered statistically significant with $P < 0.05$. Graphs were performed using GraphPad Prism 8.0.

3. Results and discussion

3.1 Yield of essential oil of *E. sativa*

The essential oil was obtained from aerial parts of fresh *E. sativa* by steam distillation. The yield of essential oil (W/W) was 0.3%. Aerial parts of fresh *E. sativa* had the highest value of essential oil yield comparing to **(Miyazawa et al., 2002)** that the yield of the essential oil from aerial parts of fresh *E. sativa* was found to be 0.007% and the yield of the essential oil was found to be 0.028% according to **Omri Hichri et al., (2016**).

3.2 Sensory properties of essential oil of *E. sativa*

Oil extracted from the aerial parts of fresh *E. sativa* is pale yellow. Essential oil of *E. sativa* has a strong pungent odor. According to **(Bennett et al., 2002)** the strong pungent odor in the essential oil of *E. sativa* is due to glucosinolates compounds and their hydrolysis products (isothiocyanates, thio-cyanates, sulphates and nitriles). Also, the essential oil of *E. sativa* has a pungent taste.

3.3 Nutritional value of *E. sativa* essential oil

The nutritional value of *E. sativa* essential oil was determined by chemical assay to the percentage of moisture, carbohydrates, minerals, vitamins and fatty acids in the *E. sativa* essential oils and shown in table (2). Moisture content for *E. sativa* oil was found to be 0.63%, total carbohydrate (2.50%). The results of the study proved that the *E. sativa* oil is especially rich in elements nutrients. The results have shown that fifteen elements, including macro- and micro-elements were detected, Mg (6.85 mg/mL), Zn (5.28 mg/mL), Cd (0.87 mg/mL), As (2.21 mg/mL), Cu (7.48 mg/mL), Al (0.10 mg/mL), Fe (4.14 mg/mL), K (16.75 mg/mL), Na (5,10 mg/mL), Ni (0.00 mg/mL), Se (4.65 mg/mL), Cr (1.83 mg/mL), Co (0.01 mg/mL), Mn (0.26 mg/mL), Ca (0.2418 mg/mL) and P (1.758mg/mL). According to HPLC chromatogram, no water- or fat-soluble vitamins were identified in the *E. sativa* oil as shown in the figure (1). Fatty acids content was 3.6% w/w of the essential oil and overall 22

fatty acids were identified by GC-MS and illustrated in table (1). The major constituent was Erucic acid (29.91%) followed by Oleic acid (18.57%), *cis*-11-eicosenoic acid, methyl ester (12.11) and linoleic acid (8.51%).

Data of our study shown that the oil has a high nutritional value that proved to be particularly low moisture content, rich in mineral nutrients and fatty acids. The low moisture content is due to drying the *E. sativa* oil with anhydrous sodium sulphate after extraction. The results of the current study suggest that the rate of lipid oxidation will be low and therefore the peroxide value will be low due to the low moisture content. Also, the results explain that the low moisture content increases the quality of the *E. sativa* oil and the storage period. Fifteen elements, including macro- and micro-elements (Mg, Zn, Cd, As, Cu, Al, Fe, K, Na, Se, Cr, Co, Mn, Ca and P), were detected. Minerals have key roles in our body to do necessary functions such as building strong bones and transmitting nerve impulses for healthy and lengthy life **(Gharibzahedi and Jafari, 2017).** Selenium is a trace element, has the ability to counteract oxidative damage that act as antioxidants by scavenging free radicals and can be obtained through food components. Studies showed that selenium can inhibit the carcinogenesis process acted as a cancer preventive agent **(Bennett et al., 2012; Morry et al., 2017)**. Zinc plays an essential role in gene expression, in the regulation of cellular growth and differentiation. It participates as a cofactor in over 200 enzymes and involved in the metabolism of carbohydrates, proteins and nucleic acids. It also plays an important role in male reproductive function and in the immune system. One of the most important functions of Zn is related to its antioxidant role **(Alegría-Torán et al., 2015)**. The data of **(Abdelsalaam et al., 2019)** refer to Zn, Cu act a potent antimicrobial agent toward the *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus flavus* and *Candida albicans*. Magnesium is an essential mineral for glucose metabolism. Magnesium deficiency is known to be associated with hypertension, insulin resistance, and endothelial dysfunction, common risk factors that contribute to the progression of CKD. Lower magnesium levels are associated with an increased risk of both incident Chronic Kidney Disease and progression to end-stage kidney disease. The potential protective effect of magnesium on the progression of Chronic Kidney Disease may be partly derived from its counteracting property against phosphate toxicity **(Sakaguchi et al., 2018)**. Also, 22 fatty acids were identified by GC-MS. Erucic acid (EA) is an omega-9 monounsaturated fatty acid present in Brassicaceae plants **(Altinoz et al., 2021)**. EA functions as a direct and potent antioxidant **(Altinoz, 2022).** Consumption of food rich in EA has been found to have negative effects

on health and EA has been classified a natural toxicant. For consumer protection, maximum levels of EA in food were established in western countries and introduced limit values were set in form of maximum levels from 2% to 5% of erucic acid with a tolerable daily intake (TDI) of 7.5 mg/kg body weight erucic acid **(Vetter et al., 2020)**. Oleic acid (OA) is monounsaturated fatty acid (MUFA). Evidences in the last years have showed the positive effects of OA on various tissues in human health and disease and also have showed that OA rarely has negative impacts. OA presents different properties in treatment and prevention of different types of disorders such as cardiovascular, decreasing of the myocardial infarction rate, platelet aggregation and secretion of TXA2, plus reduce of the systolic blood pressure and LDL cholesterol was decreased. The several studies which were investigating the efficacy of the OA on the tumor tissue proved that OA blocked the action of HER-2 / neu oncogene that led to breast cancer and have shown that oleic acid is responsible for cytotoxicity **(Jung et al., 2016; Karacor and Cam, 2015; Sales-Campos et al., 2013).** OA has been shown in numerous reports to inhibit cellular proliferation and action mechanisms as anti-tumor agent in different cancer types like breast cancer by regulating HER2 gene expression, human esophageal cells (HEC) by activating tumor suppressor genes, colorectal cancer and tongue squamous cell carcinoma (TSCC) by enhancing autophagy and apoptosis via inhibiting the Akt/mTOR signaling pathway **(Carrillo Pérez et al., 2012a; Jiang et al., 2017; Moon et al., 2014)**. In addition, OA was demonstrated to induce beneficial anti-inflammatory effects on autoimmune diseases that the results showed the treatment inflammatory cells with OA inhibits NO production **(Carrillo Pérez et al., 2012b).** OA was demonstrated great anti-inflammatory efficacy action mechanisms in the different body organs eye, lung, skin, liver, blood vessels, and intestine **(Farag and Gad, 2022).** Another study demonstrated that OA exerted excellent anti-oxidative stress activity in vivo **(Wei et al., 2016).** Studies have been shown the efficacy of the oleic acid as antibacterial, Staphylococcus aureus that exposed to OA showed decreased survival within 5 min**.** Linoleic acid (LA) is the polyunsaturated fatty acid (PUFA). The antimicrobial actions of PUFA in particular, linoleic acid has been verified according to **(Das, 2018),** it was suggested that PUFA: linoleic has function as anti-bacterial, anti-fungal, anti-parasitic, anti-viral and immunomodulation.

According to the results of present study, *E. sativa* oil has high nutritional value and multiple active ingredients. The current study suggests that *E. sativa* oil has a great contribution in raising the total nutritional value of *E. sativa* plant that the nutritional value of a product

depends on the nutrients. Consumers in developed countries may be interested in including *E. sativa* oil in their diets.

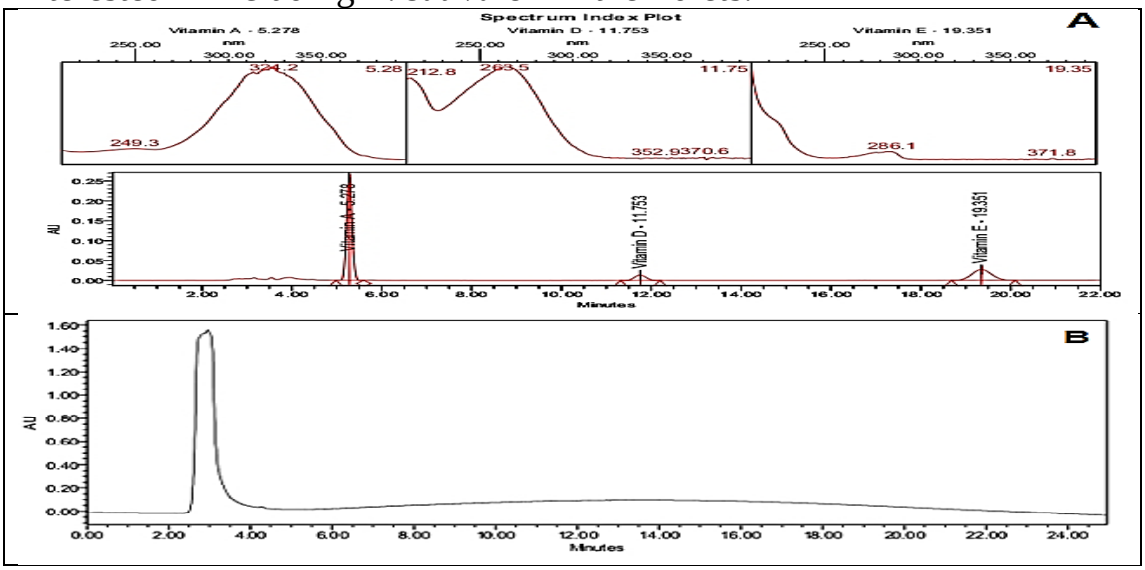


Figure (1). HPLC chromatogram: A) mixture standard fat soluble vitamins and B) sample fat soluble vitamins.

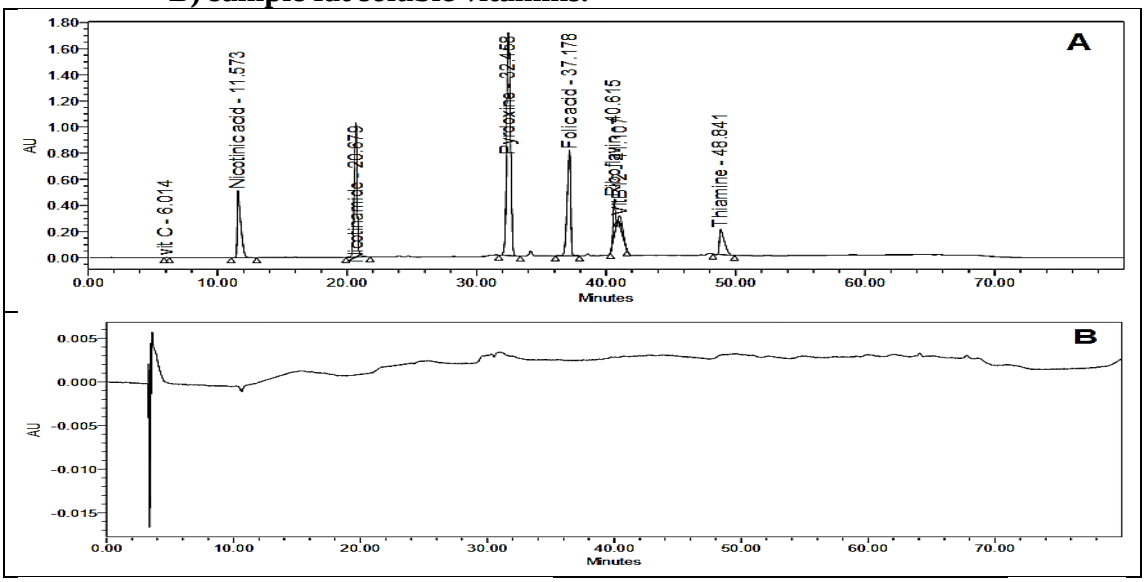


Figure (2). HPLC chromatogram: A) mixture standard water soluble vitamins and B) sample water soluble vitamins

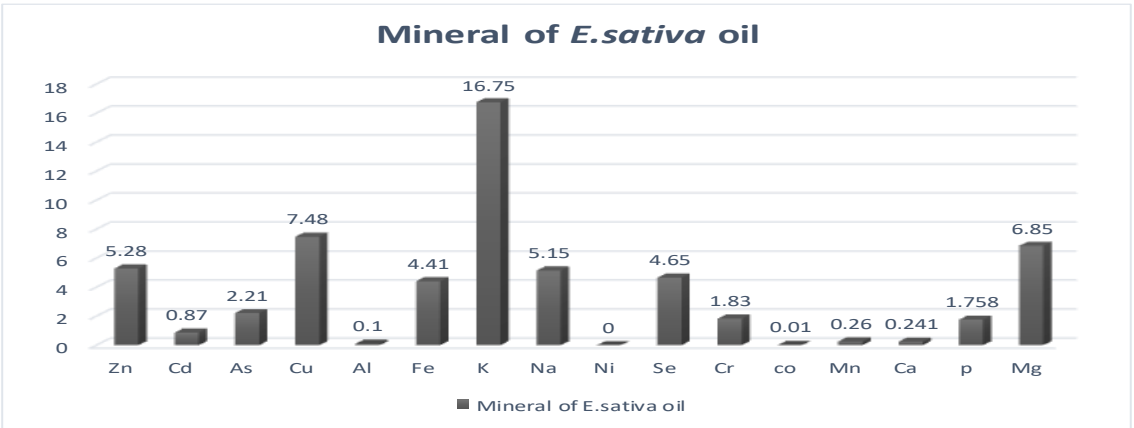


Figure (3): macro- and micro- minerals content of *E. sativa* oil

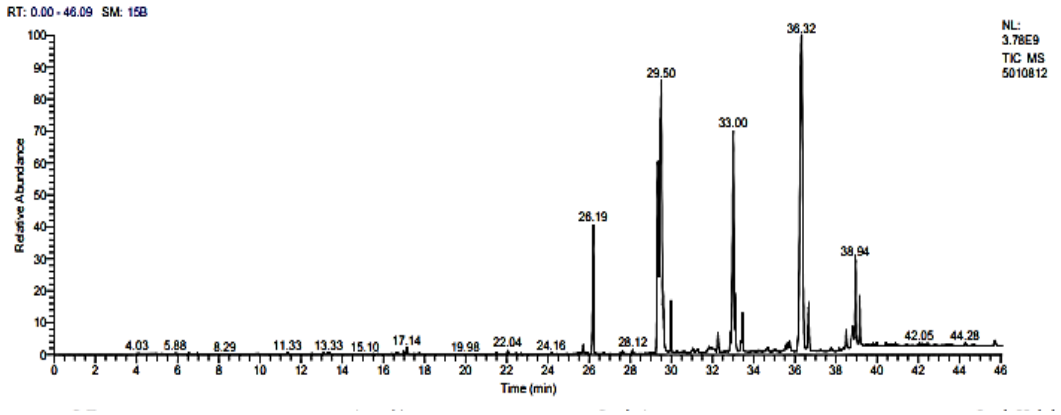


Figure (4): GC-MS spectroscopy of profile of FA constituents

Table (1). Fatty acid profile of EOs obtained from the aerial parts of fresh *E. sativa*, as obtained from GC–MS analysis

No.	FA constituents	RT	Area %	No.	FA constituents	RT	Area %
1	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-	25.72	0.40	12	Eicosanoic acid, methyl ester	33.45	1.84
2	Hexadecanoic acid, methyl ester (Palmitic acid)	26.19	6.36	13	Cyclopropanedecanoic acid, 2-octyl-,methyl ester	34.69	0.23
3	HEPTADECANOIC ACID, METHYL ESTER	28.12	0.19	14	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)- (Linolenin)	35.64	0.43
4	9,12-Octadecadienoic acid (Z,Z)-,methyl ester (Linoleic acid)	29.34	8.51	15	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Olein, 1-mono-)	35.75	0.51
5	9-Octadecenoic acid (Z)-, methyl ester (Oleic acid)	29.51	18.57	16	Methyl (Z)-13-docosenoate (Erucic acid)	36.32	29.91
6	11-Octadecenoic acid, methyl ester	29.59	1.40	17	Docosanoic acid, methyl ester (Behenic acid, methyl ester)	36.67	2.23
7	8,11-OCTADECADIENOIC ACID,METHYL ESTER	31.03	0.31	18	22-Tricosenoic acid	37.77	0.21
8	cis-11,14-Eicosadienoic acid, methyl ester	32.86	0.69	19	Hexadecanoic acid, octadecyl ester	38.50	0.89
9	cis-11-Eicosenoic acid, methyl ester	33.00	12.11	20	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Olein, 2-mono-)	38.80	1.21
10	cis-13-Eicosenoic acid, methyl ester	33.10	2.42	21	15-Tetracosenoic acid, methyl ester, (Z)- (Methyl nervonate)	38.94	3.49
11	Octadecanoic acid, 9,10-dihydroxy-,methyl ester	33.37	0.46	22	Tetracosanoic acid, methyl ester	39.15	2.00

Table (2). Nutritional value of essential oils obtained from the aerial parts of fresh *E. sativa*

Nutritional value of essential oils of *E. sativa*			
Moisture content	0.63%	**Mineral elements**	
Carbohydrate	2.50%.	Mg	6.85 mg/ml
Water and fat soluble vitamins		Zn	5.28 mg/ml
fat soluble vitamins		Cd	0.87 mg/ml
VitA	No identified	As	2.21 mg/ml
Vit E	No identified	Cu	7.48 mg/ml
VitD3	No identified	Al	0.10 mg/ml
Water soluble vitamins		Fe	4.14 mg/ml
Nicotinic acid	No identified	K	16.75 mg/ml
Nicotinamide	No identified	Na	5.1 mg/ml
Pyridoxine	No identified	Ni	0.00 mg/ml
Folic acid	No identified	Se	4.65 mg/ml
Thiamine	No identified	Cr	1.83 mg/ml
Riboflavin	No identified	Co	0.01 mg/ml
Vit b12	No identified	Mn	0.26 mg/ml
Vit C	No identified		
FA constituents: *E. sativa* oil contain 22 FA constituents and shown in the table (3)		Ca	0.241 mg/ml
		P	1.758 mg/ml

3.4 Physicochemical parameters of *E. sativa* essential oil

The quality of oil was analyzed by evaluating their physicochemical properties such as peroxide, acid, iodine and saponification values as shown in. In order to access quality these properties are very important parameters.

Peroxide value (PV) of *E. sativa* oils was found to be 0.003 mg/g oil. The results showed that the number of peroxides formed by oxidation in the oil is negligible value and less than 10mg/g as according to study of **(Pearson, 1976a)** that Peroxide value should be less than10 mg/g in the oil and as any increase in this value (20mg/g oil or above) lead to rancidity of oil. Rancidity is a significant quality. Rancidity is an important quality attribute perceived by consumers, and at intense levels results in product rejection. PV is often selected as an indicator of oxidative rancidity. FFA and PV values were estimated as indicators of quality assessing **(Christopoulos and Tsantili, 2015)**. This result shows

that the percentage of peroxides formed in the *E. sativa* oil is very few, not significant and the oils will not go rancid easily. Our study suggested that the main reason for the low value of peroxide essential oil of *E. sativa* attributed to presence of natural antioxidants in the oil such as phytochemical, flavonoids and phenolic compounds. The oil contains a large amount of flavonoids and a moderate amount of phenolic compounds, as shown in figure. The quantity of total flavonoids was found to be 22.7 mg/g oil and total phenolic compounds 0.96 mg/g oil. These compounds work to prevent the oxidation of the double bond in unsaturated fatty acids and thus reduce the formation of peroxides, which reduces the susceptibility to rancidity and thus gives the oil a longer storage period. In this work, low value of peroxide clearly indicated that essential oil of *E. sativa* has higher resistance to autoxidation (prevent rancid), higher quality longer and shelf life.

Acid value (AV) of *E. sativa* oils was found to be 2.67 KOH mg/g oil. The low AV indicates the lack of free fatty acids (FFT) present in the *E. sativa* oils, resulting from the hydrolysis of triglycerides (TG) by lipolytic enzymes and oxidation **(Aremu et al., 2015)**. This result is consistent with GC- MS data of FA profile that the fatty acids were shown in the form of esters and not in the form of free. Also, the study suggests that the reason for the low acid value of essential oil of *E. sativa* may be attributed to presence flavonoids compounds which inhibit hydrolysis of triglycerides into free fatty acids and glycerol by inhibiting lipolytic enzymes that was mentioned in a report of **(Nagai et al., 2018)**. The essential oil of *E. sativa* contains a large amount of flavonoids, as have been shown in our study. The low acid value indicates that the essential oil of *E. sativa* is suitable for use as salad or cooking oil (edible).

Iodine value (IV) of *E. sativa* oil was found to be 133.63 mg/100g oil. This high value indicates an increase in the amount of unsaturation in the oil and more double bonds present in *E. sativa* oil. A higher iodine value indicates an increase in the number of double bonds and the oil becoming more susceptible to oxidative rancidity. According to report of **(Otunola et al., 2009)** that the presence of oleic and linoleic acids in the oil could account for the high iodine value, and is also an indication that the shelf life of the oil may be short. However, the results of the current study showed that *E. sativa* oil contains abundant amount of flavonoids and a moderate amount of phenolic compounds which work to prevent the oxidation of the double bond in unsaturated fatty acids and thus, reduces the susceptibility to rancidity and thus, gives the *E. sativa* oil shelf life for a longer period of time.

Saponification value (SV) of *E. sativa* oils was found to be 78.2 mg KOH /g oil. However, this value less than 198 mg/KOH/g set by the international codex standard for edible oils **(Pearson, 1976b)**. The results of the saponification value show that *E. sativa* oil contains a high amount of fatty acids with a low molecular weight present in *E. sativa* oil as triglycerides. According to **(Aremu et al., 2015)** that the high value of saponification enhances the quality of *E. sativa* oils because high values of saponification indicate the presence of triglycerides with a lower molecular weight per gram of *E. sativa* oils. Also the high saponification value makes the *E. sativa* oil edible. Several studies indicated that the low saponification value of oils might contain high proportion of higher fatty acid and can be regarded as non-edible oils

Table (3). Physicochemical parameters of essential oil of *E. sativa*

Physicochemical parameters	
Peroxide value (PV)	**0.003 mg/g oil**
Acid value (AV)	**2.67 KOH mg/g oil**
Iodine value (IV)	**133.63 mg/100g oil**
Saponification value (SV)	**78.2 mg KOH /g oil**

3.5 Estimation of total phenolic content (TPC) and total flavonoids content (TFC):

Polyphenols is one of the major classes of compounds and having at least one phenol group in their structure, present in the plants and their extracts. These compounds are divided into various subclasses based on their chemical structures, like phenolic acids, flavonoids, tannins, coumarins, quinones, lignans, stilbenes and curcuminoids **(Mutha et al., 2021)**.

TPC and TFC were determined. The results of the current study showed that the oil contains a moderate amount of TPC that was found to be 0.96±0.01 mg/mL and contains an abundant amount of TFC was found to be 22.7±1.97 mg/ml as shown in figure (5).

Many studies proved that the daily intake of food with a higher levels of flavonoids may have the potential to decrease and resist the risk of certain cancers species, such as colon, breast and pancreatic cancers and oxidative stability **(Tohidi et al., 2017)**. Phenolic and flavonoids compounds play a significant role in preventing chronic diseases by retarding the oxidative degradation **(Gharibi et al., 2015)**. Both of flavonoids and phenolic components are presented in nutrients and herbal medicines and have been reported on their effective anticancer, antibacterial, protective agents of cardiovascular, anti-inflammation, immune system promoting, skin protection from UV radiation, and food industrials application **(Tungmunnithum et al., 2018)**.

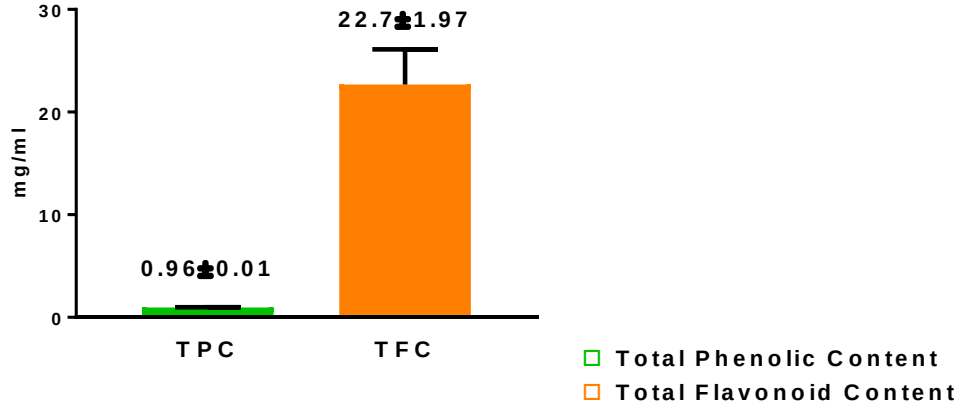


Figure (5): total phenolic and total flavonoids contents of *E. sativa* oil

3.6 *In Vitro* Antimicrobial Activity

Through the disc diffusion method and agar well diffusion method assay, the inhibition is measured by a diameter of inhibition zone (clear zone) formed around disk saturated with tested oil.

Results of the antimicrobial effects of *E. sativa* essential oil against different bacteria and fungi were summarized in table (4) and seen in figure (9،10). The study shown that the solvent DMSO used to solubilize the tested oil was suitable for testing antimicrobial activity and it has not effect on the test results. The results of the study explained according to the figure (8), that DMSO has not any anti-bacterial and anti-fungal activity against microorganisms tested as it has not inhibition zone value (no inhibitory effect)

The data for the inhibition zones (mm) of various micro-organisms of present study indicate that EO of *E. sativa* did not have any antifungal activity by using disc diffusion method and agar well diffusion method. The tested oil did not show inhibitory effect on fungi species tested as seen in table (4) figure (9,10). According to disc diffusion method as seen in figure (9), tested oil did not have any antibacterial activity against tested gram-positive bacteria and gram-negative bacteria.

The data for the inhibition zones (mm) of various micro-organisms by using agar well diffusion method indicated as seen in table (4) and figure (10) that EO of *E. sativa* have high antibacterial activity against most gram-negative bacteria (*E.coli*, *N.gonorrhoeae* and *S.typhimrium*). The inhibition zone values of *E. sativa* oil were (*E.coli* $21.33^{b}\pm1.15$), (*N.gonorrhoeae* $20.33^{b}\pm0.58$) and (*S.typhimrium* $21.33^{b}\pm2.3$) close to inhibition zone values of standard which were (*E.coli* $25^{c}\pm0.0$), (*N.gonorrhoeae* $26^{c}\pm0.0$) and (*S.typhimrium* $26^{c}\pm0.0$). EO of *E. sativa* have moderate antibacterial activity against all gram-positive bacteria Compared to standard. The inhibition zone values of oil were (*B.subtilis* $11.67^{b}\pm1.15$), (*B.cereus* $16^{b}\pm1.73$), (*E.faecalis* $11.67^{b}\pm0.58$) and

(*S.aureus*14.67^{b}±1.15) but the standard were (*B.subtilis* 26c±0.0), (*B.cereus* 28c±0.0), (*E.faecalis* 25 ^{c}±0.0) and (*S.aureus* 24^{c}±0.0).

The results of our study indicated that agar well diffusion method was more efficient than disc diffusion method with clear inhibition zone of tested oil against bacteria although using the same concentration of oil and bacterial suspension avoid prejudice. Our study confirms that agar well diffusion was the most convenient method to test the oil and define in vitro levels of resistance of it on bacterial strains. Study of **(Cui et al., 2021)** proved that well diffusion method was a rapid and effective method to screen in vitro levels of susceptibility of essential oils. Results study of **(Al-Talib et al., 2016)** suggested that the poor performance of disc method could be attributed to low diffusion of the extract on the agar surface.

Data of MIC values against tested bacterial pathogens are seen in figure (7). Lowest MIC value of the oil was 160 µg/mL that showed no growth of the tested bacterium was against gram-negative bacteria *S.typhimrium* followed by *E.coli* (240µg/ml). Highest MIC value of the oil was 640 µg/mL against gram-positive bacteria *B.cereus* and *S.aureus*. Data of MIC values of this study were significantly lower than the values mentioned in **(Hichri et al., 2019)** that MIC value of the oil was 5000 µg/mL against gram-positive bacteria *S.aureus* and MIC value of the oil 10000 µg/mL against gram-negative bacteria *E.coli*. In current study, antimicrobial activity experiment of *E. sativa* essential oil significantly exceeded previously mentioned and reported in **(Omri Hichri et al., 2016)**.

According to the data of **(Machado et al., 2005)**, EO with MIC values lower than 10 µg/mL were considered to have excellent antibacterial activity, values between 10 and 100 µg/mL were considered good, values between 100 and 500 µg/mL were considered moderate, values between 500 and 1000 µg/mL were considered low and for MIC values above 1000 µg/mL, the samples were considered inactive. In our study, all the species of gram-negative bacteria evaluated presented moderate inhibitory activity, with an MIC variation of 100–400 µg/mL for the bacteria tested and the species of gram-negative bacteria with an MIC variation of 500 and 1000 µg/mL for the bacteria tested presented low inhibitory activity.

Results of our study proved that *E. sativa* oil had more antibacterial activity against gram-negative bacteria than gram-positive bacteria. The data show that gram positives bacteria were more resistant to *E. sativa* oil than gram negative bacteria. These results contradict the results in **(Hichri et al., 2019; Omri Hichri et al., 2016)** reported that *E. sativa* oil had more antibacterial activity against gram positive bacteria than gram

negative bacteria and mentioned that this is a general observation derived from studies with essential oils from many other species. Possibly this may be attributed to the fact that Gram negative bacteria possess an outer membrane which acts as a barrier which prevents or decreases the penetration of numerous antimicrobials. Lack of the outer membrane in Gram-positive bacteria, makes it more vulnerable to damaging molecules and this leads to the leakage of their cytoplasm contents **(Shohayeb et al., 2014)**. The current study showed that gram- negative bacteria were more susceptible than gram- positive bacteria to tested *E. sativa* oil and suggests that the *E. sativa* oil has the ability to bypass the outer membrane of Gram-negative bacteria and was able to penetrate through it.

The incidence of microbial infectious diseases and their hitches constantly increasing, mostly due to microbial drug resistance to antimicrobial agents. Microorganisms have developed resistance against several antimicrobial agents. In particular, the most significant bacteria, *S. aureus* and *E. coli* have become resilient to drugs **(Nieto-Maldonado et al., 2022)**. Also, studies carried out in *Salmonella* shown These bacteria was resistance to antibiotics, including tetracycline, nalidixic acid, sulfamethoxazole, strteptomycin, ciprofloxacine, trimethoprim, chloramphenicol and gentamicin **(Serwecińska, 2020)**. Therefore, many researchers have started to study novel antibiotics due to bacterial resistance and toxicity of synthetic drugs **(Moravej et al., 2018)** especially, natural products that derived from medicinal plants and represents the safest, effective and natural alternative for treating many disease-causing effects of infectious pathogens **(Algabr et al., 2022)**. Looking at the results of this study of inhibition zone (mm) and MIC values, *E. sativa* oil showed more significant antibacterial activity against most tested bacteria especially, *E.coli*, *S.typhimrium* and *Salmonella* that these bacteria showed resistance to some of the aforementioned antibiotics.

This study suggests that higher antibacterial activity on tested bacteria of *E. sativa* oil due to secondary metabolites content of *E. sativa* oil such as flavonoids, phenolic and terpenoids. Our study showed that the oil contains a moderate amount of total phenolic (0.96±0.01 mg/mL) and contains an abundant amount of total flavonoids 22.7±1.97mg/mL. Also contains a greater amount of terpenoids according to **(Miyazawa et al., 2002)**. Flavonoids, phenolic acids, terpenoids are secondary metabolites have greater antimicrobial properties. Numerous studies have shown that the antimicrobial activity of these active compounds have the following potential: to promote cell wall disruption and lysis, inhibit cell wall construction, inhibit biofilm formation, inhibit microbial DNA

replication, inhibit energy synthesis, and inhibit or reduce microbial toxin production. In addition, these compounds may prevent antibacterial resistance (**Mickymaray, 2019**).

Table (4). Inhibition zone of *E. sativa* essential oil, standard and DMSO on bacterial and fungus tested by using two methods

Inhibition zone diameter (mm / Sample)												
Sample	Bacterial species								Fungal species			
	Gram – positive bacteria				Gram – negative bacteria							
	Bacillus subtilis	*Bacillus cereus*	*Enterococcus faecalis*	*Staphylococcus aureus*	*Escherichia coli*	*Neisseria gonorrhoeae*	*Pseudomonas aeruginosa*	*Salmonella typhimrium*	*Aspergillus flavus*	*Aspergillus niger*	*Candida albicans*	*Saccharomyces cerevisiae*
Standard	$26^{c} \pm 0.0$	$28^{c} \pm 0.0$	$25^{c} \pm 0.0$	$24^{c} \pm 0.0$	$25^{c} \pm 0.0$	$26^{c} \pm 0.0$	28	$26^{c} \pm 0.0$	17	15	21	19
DMSO	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0	0.0^{a}	0.0	0.0	0.0	0.0
Tested oil by disc diffusion	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0	0.0^{a}	0.0	0.0	0.0	0.0
Tested oil by well diffusion	$11.67^{b} \pm 1.15$	$16^{b} \pm 1.73$	$11.67^{b} \pm 0.58$	$14.67^{b} \pm 1.15$	$21.33^{b} \pm 1.15$	$20.33^{b} \pm 0.58$	0.0	$21.33^{b} \pm 2.3$	0.0	0.0	0.0	0.0

Data shows mean ± Standard Deviation, n = 3. Each treatment was carried out with three replicates. The data was statistically analyzed using One- way ANOVA followed by comparison of the means of sample by Duncan's multiple range test at $P < 0.05$

ATCC: American Type Culture Collection, Rockville, MD

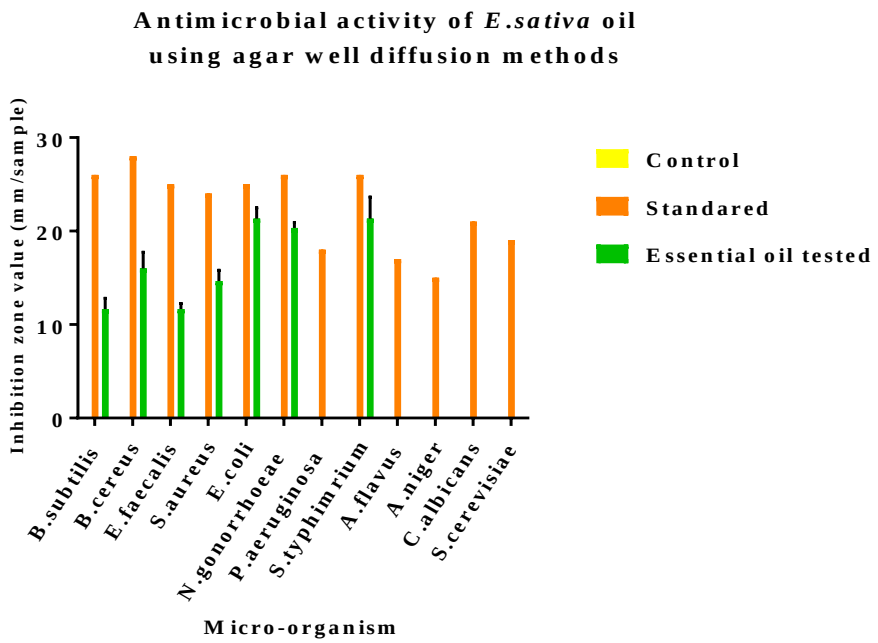


Figure (6). Inhibition zone value of *E.sativa* oil using agar well diffusion methods.

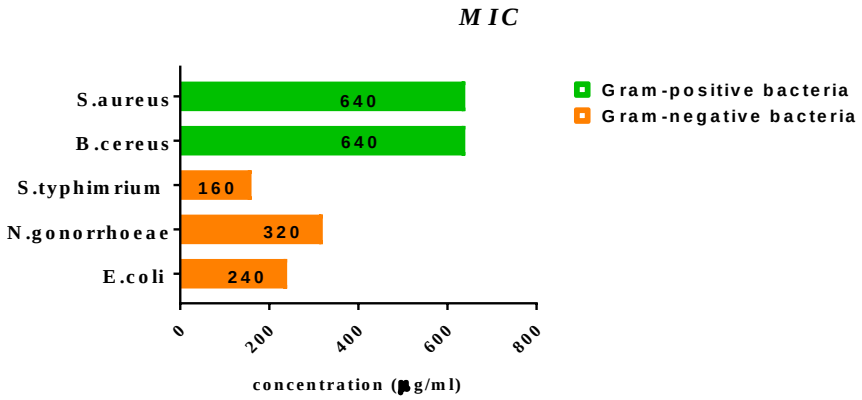


Figure (7). MIC values of *E. sativa* essential oil against tested bacterial pathogens

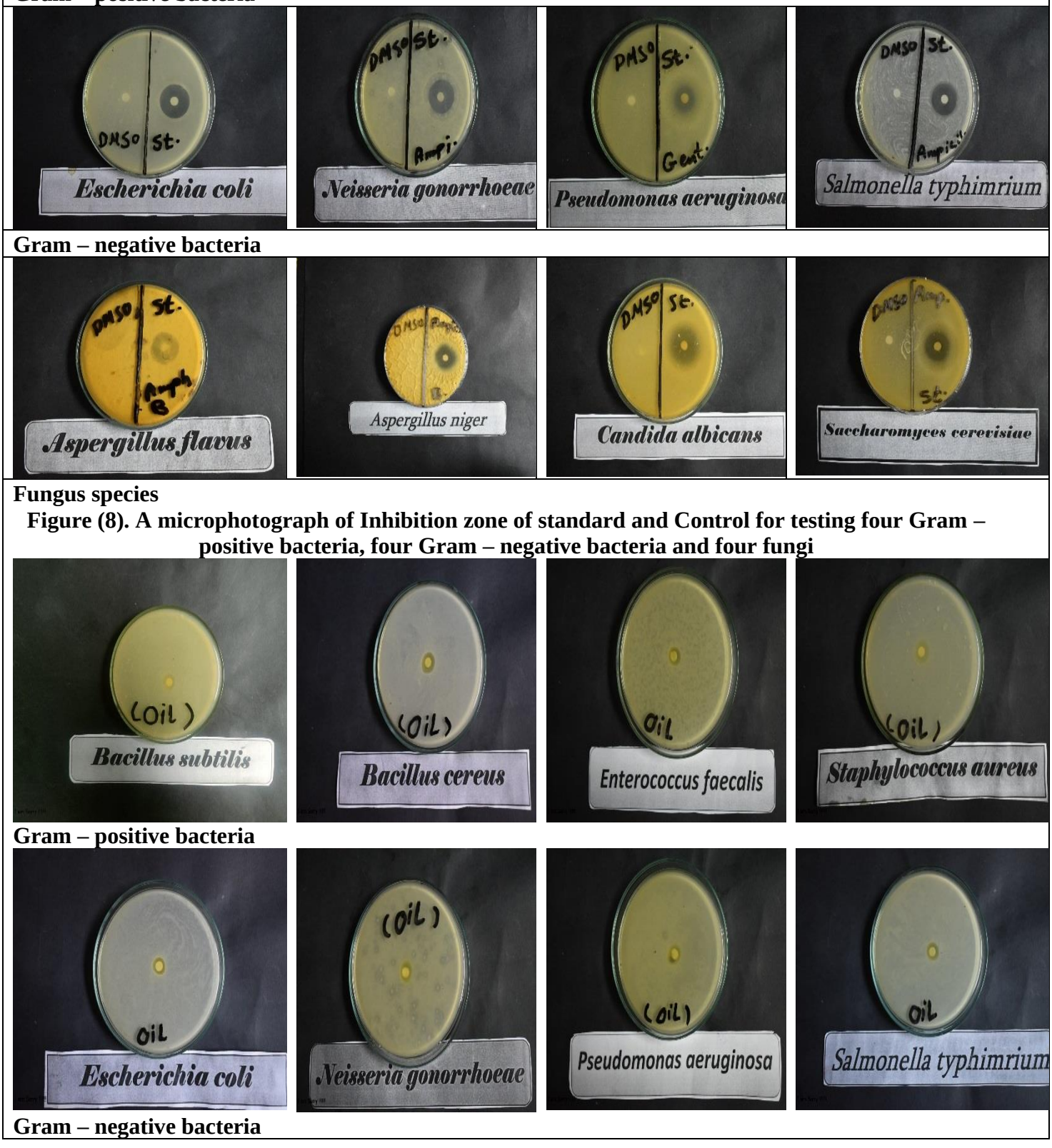
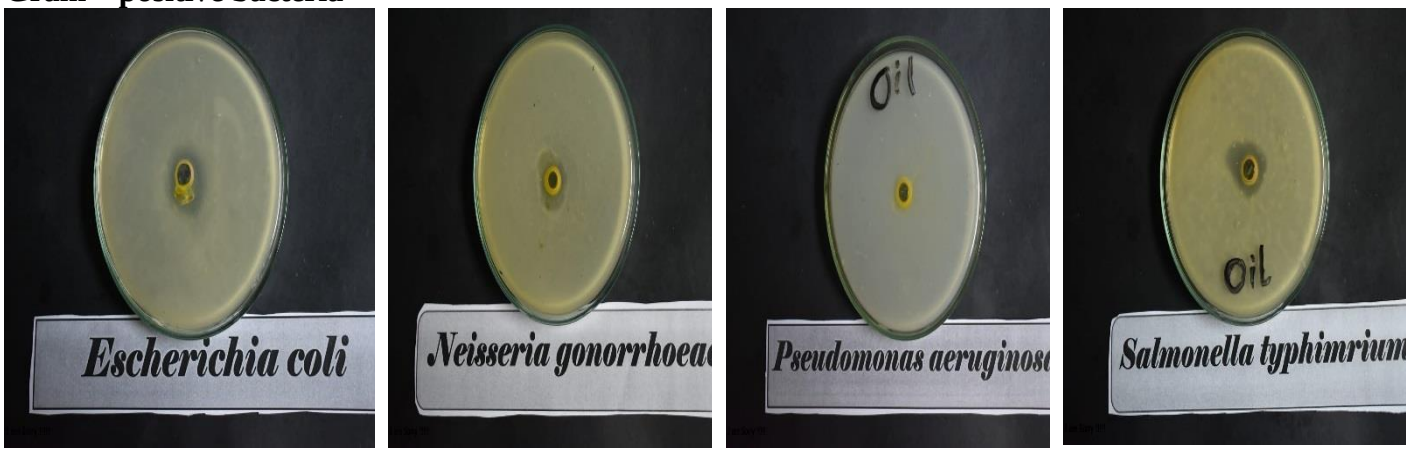


Figure (8). A microphotograph of Inhibition zone of standard and Control for testing four Gram – positive bacteria, four Gram – negative bacteria and four fungi

Fungus species

Figure (9). A microphotograph of Inhibition zone of *E. sativa* essential oil for testing four Gram – positive bacteria, four Gram – negative bacteria and four fungi by using disc diffusion methods.

Gram – positive bacteria



Gram – negative bacteria

Fungus species

Figure (10). A microphotograph of Inhibition zone of *E. sativa* essential oil for testing four Gram– positive bacteria, four Gram – negative bacteria and four fungi by using well diffusion methods.

4. Conclusion

The obtained analytical data on composition and functional properties of *E. sativa* essential oil suggest that it deserves further consideration and investigation as a potential new multi-purpose product for nutritional, industrial, and pharmaceutical uses. *E. sativa* oil seems to be a good source of minerals, fatty acids and bioactive compounds. The high linoleic and oleic acid contents and the considerable amounts of natural antioxidants such as phenolic and flavonoids content make this essential oil nutritious and capable of being conserved safely for a long time and using as function food where some food products will be fortified with *E. sativa* essential oil, a sensory evaluation of these products will be conducted, and their validity period will be studied and presented in the next research, God willing.

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المستخلص

تهدف هذه الدراسة إلى التحقق من الجودة والقيمة الغذائية والخصائص العلاجية لزيت الجرجير العطري. قيمت دراستنا القيمة الغذائية لزيت الجرجير العطري من خلال تحديد محتوى الرطوبة والكربوهيدرات والفيتامينات والعناصر المعدنية (الغذائية والسامة) والأحماض الدهنية. أيضًا، تم تحديد جودة الزيت من خلال الاختبارات الكيميائية. تم تحديد محتوى الفينول ومحتوى الفلافونويد للزيت. قيمت هذه الدراسة أيضًا نشاط الزيت كمضاد للميكروبات من خلال اختبارين مختلفين. كان محصول الزيت العطري المستخلص (وزن / وزن) ٠.٣٪ وتم تحليله كيميائياً. تظهر نتائج الدراسة أن للزيت قيمة غذائية عالية ثبت أنها غنية بشكل خاص بالعناصر الغذائية والأحماض الدهنية. أشارت النتائج إلى أن الزيت العطري لديه مقاومة أعلى للأكسدة ، وجودة أعلى ، ومدة صلاحية أطول ، وصالح للأكل ومناسب للاستخدام في السلطة وصوصات الشيكولاته أو زيت للطبخ. أظهرت النتائج أن الزيت ذات فعالية كبيرة كمضاد بكتيريا ،بما في ذلك سبعة أنواع من البكتيريا المسببة للأمراض البشرية والتي أظهرت مقاومة ضد بعض المضادات الحيوية.