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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF MEDICINALLY USEFUL ESSENTIAL OIL FROM THE INFLORESCENCE OF *EUPHORBIA HELIOSCOPIA* L. GROWN IN EGYPT

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Keywords:

Euphorbia helioscopia, Essential oil, GC/MS, Antibacterial activity

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ABSTRACT: The genus Euphorbia has about 2000 members making it one of the largest ones among the flowering plants. *Euphorbia helioscopia* L. As the chemical composition of the essential oil from *Euphorbia helioscopia* developed in Egypt has not yet been explored, along these lines the present to extricate the essential oil and investigate the oil chemical composition and antimicrobial activity. Essential oil from the whole inflorescence of the flowering plant, *Euphorbia helioscopia* Linn. was extracted by hydrodistillation method. The chemical composition of the obtained volatile oil was investigated by GC-MS. The major components include thymol (48.36%), Caryophyllene (23.57%), carvacrol (6.70%), caffeic acid (6.48%) and caryophyllene oxide (5.58). Minor components as translongipinocarveol (2.77%) and DL-malic acid (1.9%). Screening antimicrobial activity of the isolated volatile oil against four bacteria showed that it was active only against *Bacillus subtilis* with an inhibition zone of 11 mm ± 0.25 and MIC value 17 mg /ml. Gentamycin was utilized as a positive antibacterial control. The data of this study might be considered as the first identification of the chemical composition of the essential oil of the plant species grown in Egypt. Essential oils may have a moderate potential as a natural medicine for microbial infections against *Bacillus subtilis*.

INTRODUCTION: Euphorbiaceae is a vast plant family with 300 genera and 5000 species. The genus Euphorbia is the biggest genus in F. Euphorbiaceae. The genus has around 2,000 members making it one of the biggest genera of flowering plants. All govern latex and have unique flower structures. The plant is local to calm districts however has likewise adjusted to sub-tropical regions everywhere throughout the world.

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In Egypt, the plant is basic in the Nile Delta and Upper Egypt [1]. The plant, *Euphorbia helioscopia* Linn. subsp. helioscopia, is known as sun spurge or wolf's milk as an English name. The plant has great therapeutic significance, often used to treat ascites, edema, pulmonary tuberculosis, tinea and cervical tuberculous lymphaden [2]. Leaves and stems are used as febrifuge and vermifuge. The oil from the seeds has purgative properties while the roots are used as anthelmintic [3].

Uzair M *et al.*, (2009) reported that on mixing the seeds with roasted pepper, the mixture could be used in the treatment of cholera [4]. The plant contains diterpenes, flavonoids, tannins, polyphenols, glycosides, lipids and volatile oils [3]. Ethanolic extract of *E. helioscopia* amongst

medicinal plants, collected from Islamabad and the Murree region of Pakistan was studied for insulin secretagogue activity and was found to be active at 10 µg/mL ($p<0.05$) [3]. Other plant biological activities as vasodepressor [5], anti-allergic [6] and were reported. Also, the plant extract was found to have considerable antiviral activity [7].

Three extracts of *E. helioscopia* namely petroleum ether, dichloromethane, and methanol, were tested for their antifungal activity against *Cladosporium cucumerinum* and found to be devoid of antifungal activity [8]. Dichloromethane and methanol extracts of the aerial parts of *E. helioscopia* were also tested against *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani*, and *Candida glabrata*. Among them, dichloromethane extract showed 90% Inhibition against *Fusarium solani*. Antifungal activity of the dichloromethane extract of the aerial parts of the plant was also confirmed by Uzair [4].

Different plant extracts of the Iranian species was active against *Bacillus anthracis* and inactive against *Klebsiella pneumoniae, Proteus vulgaris, Shigella sonei, Vilorio cholera, Escherichia coli, Staphylococcus aureus* and *Salmonella paratyphi* A.

Assessment of the growth inhibitory effects of *Euphorbia helioscopia* L. extracts and fractions of the alcoholic extract on different five human cancer cell lines were performed and found to possess anticancer activity and could be a chemopreventive agent against human cancer [9]. Allelopathic impact of the water extracts of various plant organs and infested soil of *E. helioscopia* L. on the seed germination and seedling development of wheat and lentil was reported [10].

A study on *E. helioscopia* species grown in Saudi Arabia amongst other local plants investigated the plant nutritional value and explored the lipoidal plant profile. While the main components of essential oil of Saudi Arabia species were reported as elemol and β-eudesmol [11].

Analysis of volatile steam oil obtained from the inflorescence of *E. helioscopia* grown in Greece resulted in the identification and quantification of 40 constituents (94.3%). The major compounds were phytol (21.2%), trans-Caryophyllene (10.0%) and docosanoic acid methyl ester (8.1%) [12]. The chemical composition of the essential oil of the whole flowering herb of *Euphorbia helioscopia* grown in Egypt has not been identified yet. Thus this work aimed at identifying the plant essential oil composition and at investigating the oil antimicrobial activity.

MATERIALS AND METHODS:

Plant Material: The whole flowering and fruiting plant of *Euphorbia helioscopia* Linn. was collected from its natural habitats from wheat farms in El-Gharbia governorate in Egypt in April 2017. The plant was identified by Prof. Dr. Kamal Shaltout, Department of Botany, Faculty of Science, Tanta University, Tanta, Egypt. A voucher specimen was deposited (with accession no. EH 202) in Herbarium of Faculty of Science, Tanta University, Tanta, Egypt.

Essential oil Extraction: The aerial parts of the plant were air dried with high care to keep the seeds. The air-dried powder (200 gm) whole inflorescence was communicated and at once subjected to hydrodistillation using Clevenger -type distillation apparatus for 3 h. The resulted extracted oil was then protected from light and stored at - 4 ºC till GC/MS analysis.

Antimicrobial Activity: Antimicrobial activity assay using a cut plug method as described previously [13]. Two Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 11921 and *Salmonella typhimurium* isolates), two Gram-positive bacteria *Staphylococcus aureus* ATCC 6538) and *Bacillus subtilis* ATCC were selected for antibacterial activity assay. Gentamycin was used as a positive antibacterial control.

Cut plug method recorded by Pridham *et al.*, [14] was employed to determine the antimicrobial activity of the essential oil. Three replicates were made for each test, and all plates were incubated at 32 °C for 24 h. Then the average diameters of the inhibition zones were recorded in millimeters (mm) and compared for all plates.

MIC Determination of the Essential Oil against the Most Susceptible Microorganism: Half-fold serial dilutions were made for an obtained essential oil to prepare concentrations of 6.25, 12.5, 25, 50 and 100 mg/mL in distilled water. Distilled water

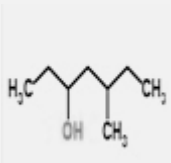
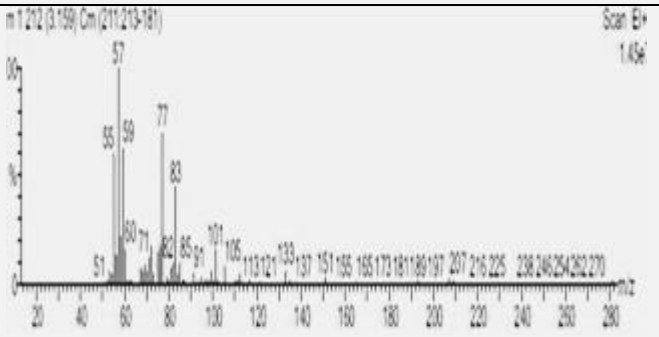
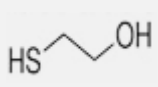
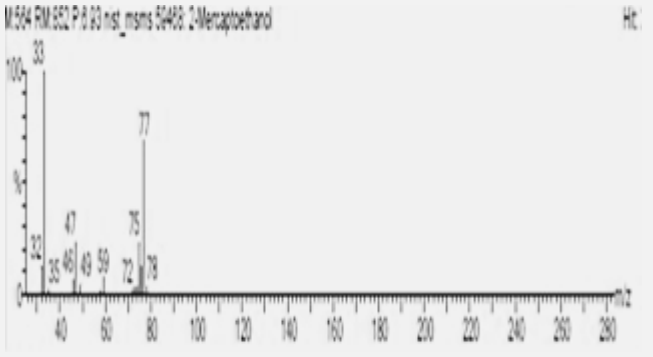
was considered as a negative control. A previously prepared (0.5 mL of about 106 cells/mL) pure spore suspension of the most susceptible microorganism was mixed with 9.5 mL of each concentration in sterile test tubes, incubated at 32 °C for 24 h; then the optical density of growth was measured spectrophotometry at 620 nm for each incubated mixture. Quantification of the growth-inhibiting effect was made by determining the percentage of the surviving cells that represented percent optical density [15].

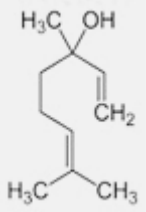
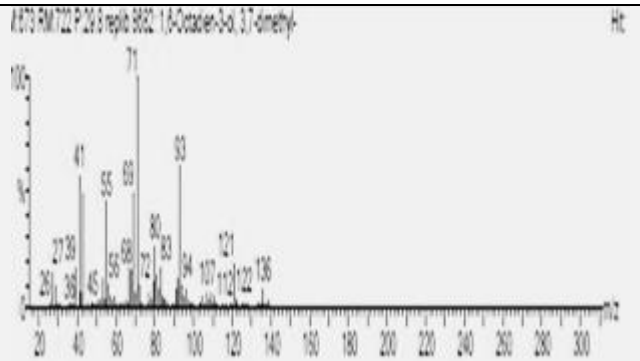
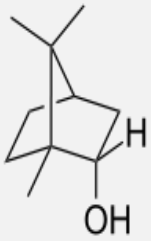
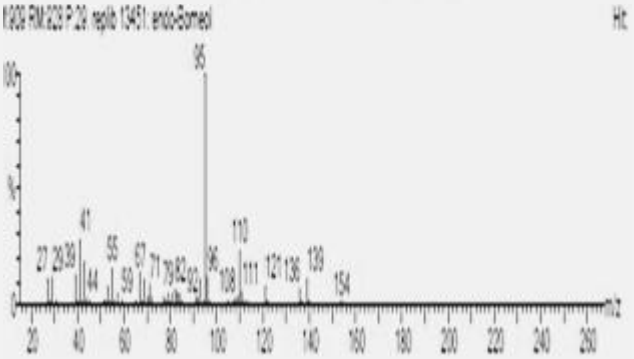
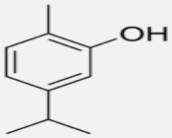
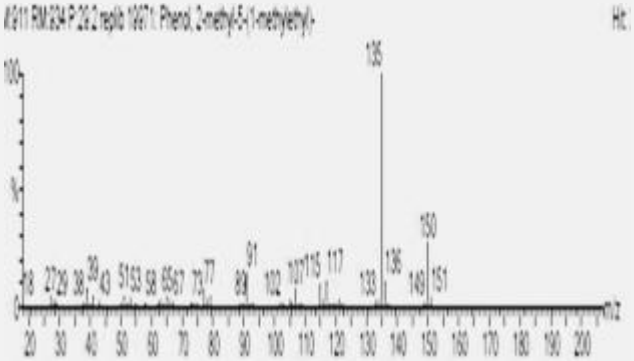
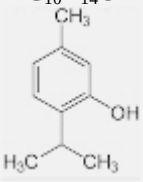
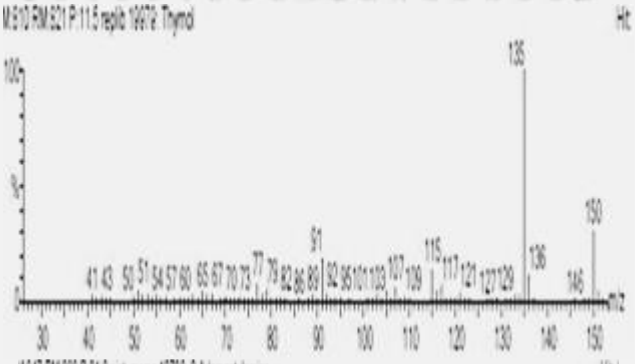
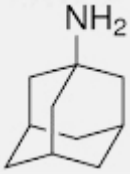
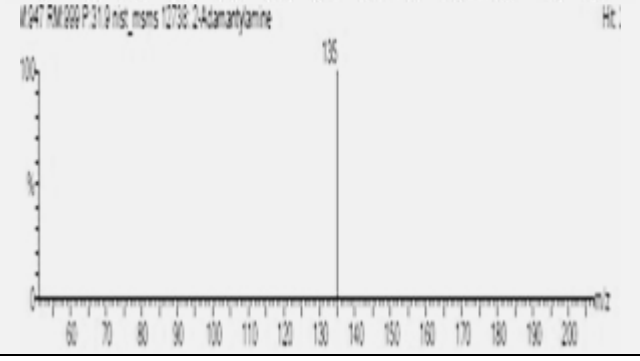
Antibacterial activity was repeated three times. Inhibition zone was expressed as mean ± standard deviation (SD). MIC against the susceptible organism was determined.

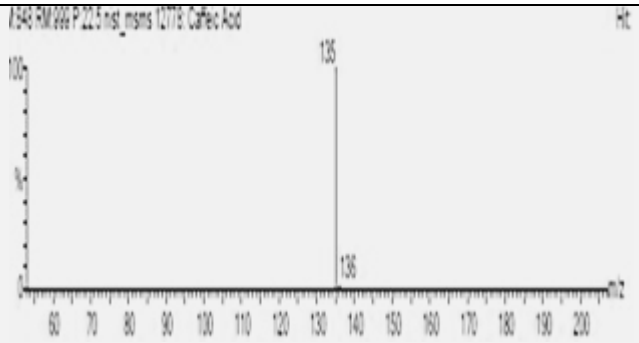
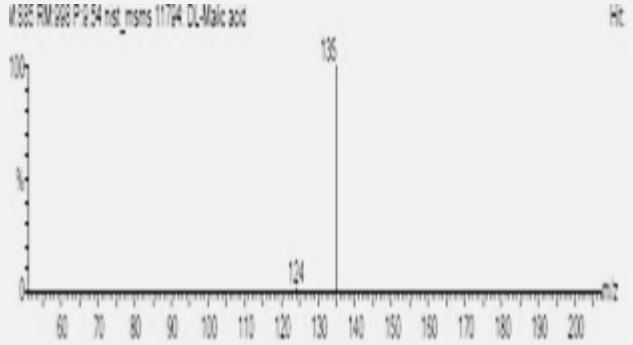
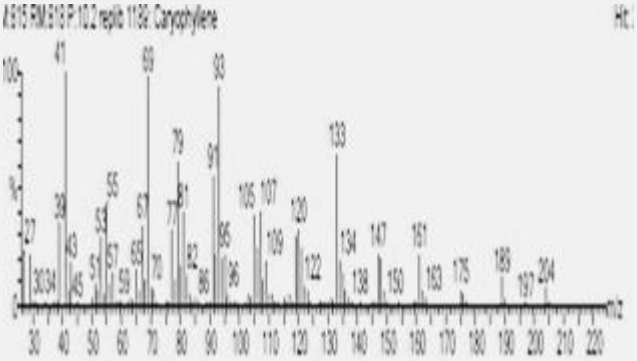
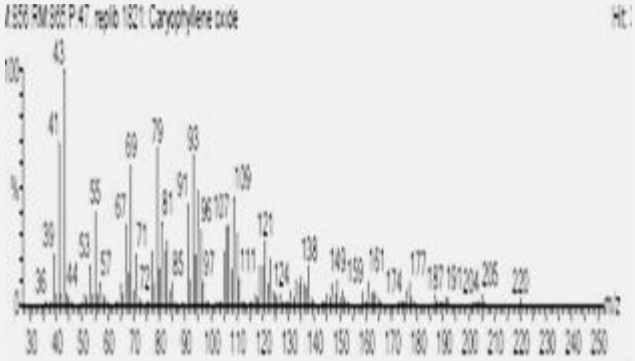
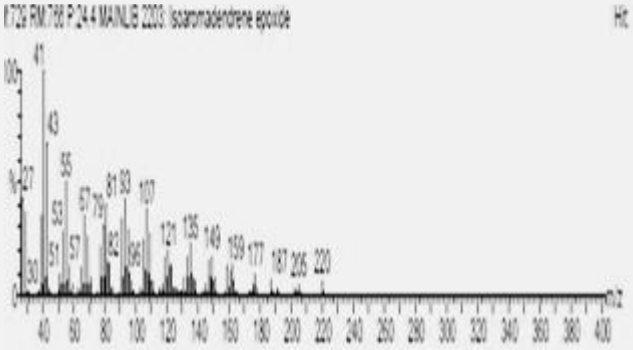
RESULTS: The yield of the essential oil obtained was 0.18v/w. The composition of the essential oil was identified and quantified by GC/MS. Eighteen constituents from *E. helioscopia* were identified and quantified. These compounds and their corresponding mass spectra, as well as their retention times, are given in **Table 1**. The major component is the monohydric phenol, thymol with concentration 48.36% followed by the sesquiterpene, Carophyllene with a concentration of 23.57%. Other major constituents included carvacrol (6.70%) and caffeic acid (6.48%). In general, the volatile oil composition can be classified as phenols (thymol and carvacrol) with total percentage composition of 55.06%. Sesquiterpenes represented by caryophyllene was identified in a concentration of 23.57%. Oxygenated sesquiterpenes as caryophyllene oxide, isoaromadendrene epoxide, and translongipino carveol, were represented in the volatile oil by a total concentration of 9.03%. Organic acids (caffeic acid and DL-malic acid) add up to 8.39% to the total constituents.

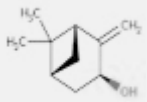
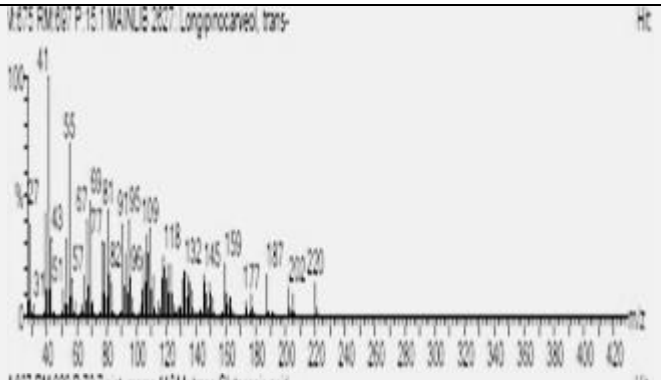
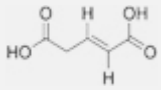
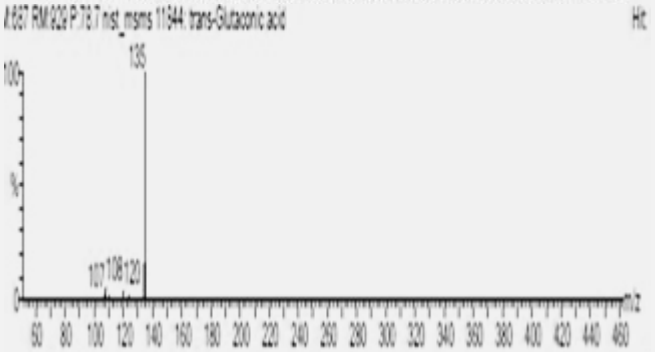
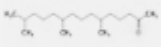
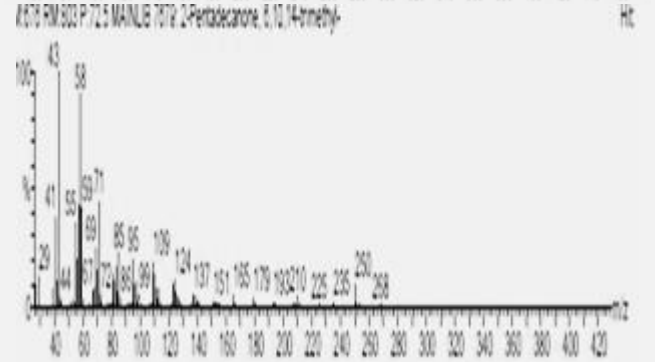
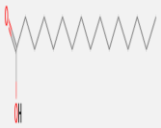
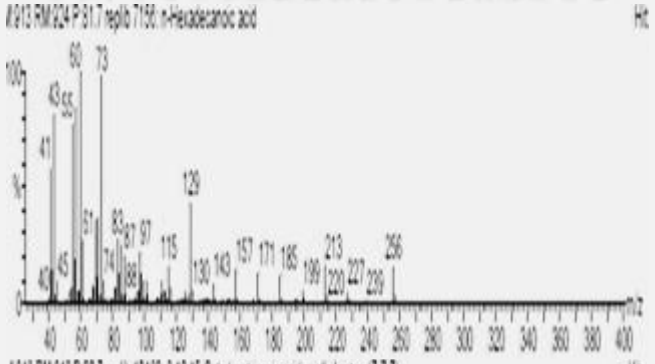
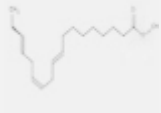
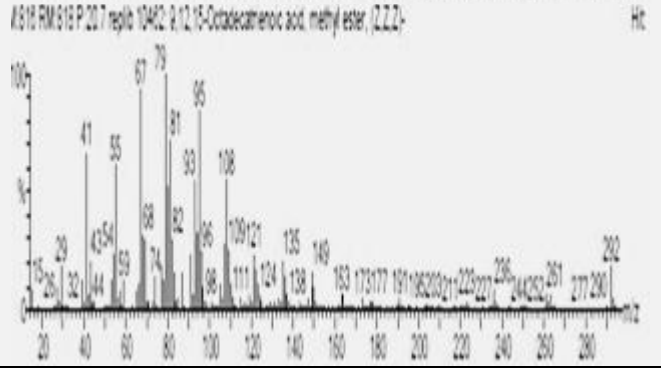
Alcohols (linalool and endoborneol) represented only 0.82 % of the total oil composition. The remaining detected compounds are fatty acids namely transglutaconic acid, palmitic acid and oleic acid with a total percentage of 0.84%. Methylated Fatty acids namely 2-pentadecane 6, 10, 14-trimethyl and methyl-linolenate were represented by 0.30 percent concentration. The biological amine; 2-adamantanamine is represented by a percent concentration of 1.08.

TABLE 1: GC/ MS RESULTS OF VOLATILE OIL OF *EUPHORBIA HELIOSCOPIA* L.

S. no.	Retention time (min)	Percent of total	Compound & Molecular Formula	Structure & Mass Spectrum
1	3.159	0.1	3-Heptanol, 5-methyl- $C_8H_{18}O$	 
2	3.709	0.07	2-Mercaptoethanol $HOCH_2CH_2SH$	 

3	4.319	0.18	1,6-Octadien-3-ol, 3,7-dimethyl- $C_{10}H_{18}O$ (Linalol) 	
4	5.670	0.64	Endoborneol $C_{10}H_{18}O$ 	
5	8.846	6.70	Phenol, 2-methyl-5-(1-methylethyl)- $\mid C_{10}H_{14}O$ Carvacrol 	
6	9.917	48.36	Thymol $C_{10}H_{14}O$ 	
7	11.802	1.08	2-Adamantylamine $C_{10}H_{17}N$ 	

8	11.827	6.476	Caffeic Acid $C_9H_8O_4$	
9	12.463	1.912	DL-Malic acid $C_4H_6O_5$	
10	12.768	23.57	Caryophyllene $C_{15}H_{24}$	
11	17.625	5.583	Caryophyllene oxide $C_{15}H_{24}O$	
12	19.415	0.675	Isoaromadendrene epoxide C15H24O	

13	19.801	2.766	Longipinocarveol, trans- C15H24O 	
14	22.517	0.291	trans-Glutaconic acid C5H6O4 	
15	23.602	0.182	2-Pentadecanone, 6,10,14-trimethyl- C18H36O 	
16	26.588	0.398	n-Hexadecanoic acid C16H32O2 (palmitic acid) 	
17	29.084	0.119	Methyl linolenate C19H32O2 	

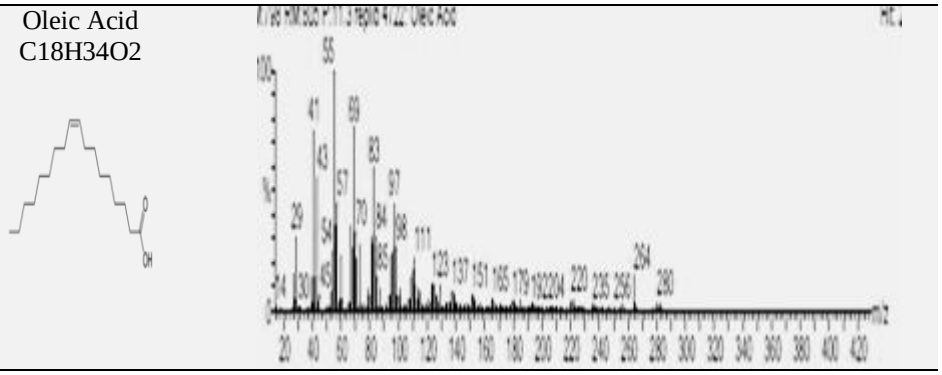
18	30.015	0.148	Oleic Acid C18H34O2	

TABLE 2: MIC OF VOLATILE OIL ISOLATED FROM *E. HELIOSCOPIA* AGAINST THE SENSITIVE MICROORGANISM *BACILLUS SUBTILIS*

Active constituent	Percentage of surviving cells (% Optical density) Concentration (mg/ml)						Microorganism
	0.0	**6.25**	**12.5**	**25**	**50**	**100**	
Volatile oil	100	72	36	32	31	32	*Bacillus subtilis*

Essential oil isolated from *E. helioscopia* showed antibacterial activity against the Gram-positive bacteria, *Bacillus subtilis* with MIC value 17 mg/ ml. Inhibition zone of the volatile oil was 11 mm ± 25 while as inhibition zone of gentamycin against *Bacillus subtilis* was 10 mm. The other tested microbes were resistant against this volatile oil.

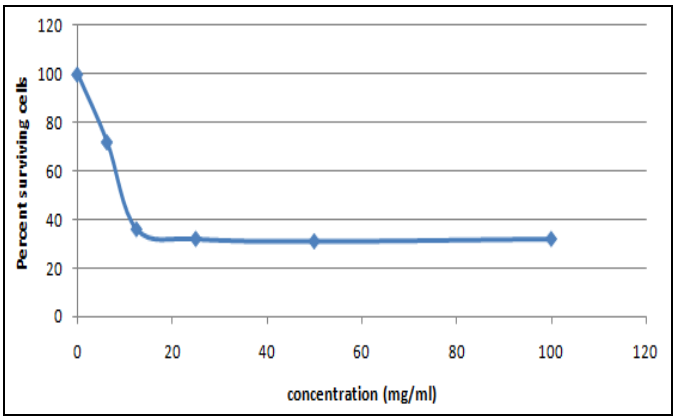


FIG. 1: GROWTH INHIBITION OF THE ESSENTIAL OF *EUPHORBIA HELIOSCOPIA* L. AGAINST *BACILLUS SUBTILIS*

DISCUSSION: Essential oil composition of *Euphorbia helioscopia* herb changes with various geographical sources. Looking at the oil composition collected from the Nile Delta of Egypt with those from Saudi Arabia and Iran brought about distinguishing proof of various constituents. This study recognized the significant constituents of the oil extricated from Egyptian plant as thymol and caryophyllene. While as the major compounds essential oil of Saudi Arabia species was represented as elemol and β-eudesmol [11]. Major oil constituents of the plant gathered from Greece were phytol, trans-Caryophyllene and docosanoic corrosive methyl ester [12].

Carvacrol that constitutes 6.70% of the essential oil was reported to possess antioxidant activity [16]. Caffeic acid is an organic compound found in the plant as a key intermediate in the biosynthesis of lignin which composes biomass plant component. The other organic bicarboxylic acid in the oil, malic acid is used as a food additive.

The antibacterial activity of the essential oil against *Bacillus subtilis* may be related to its composition of phenols especially thymol that comprises 48.36%. This study can presume that some obtrusive field weeds, for example, *Euphorbia Helioscopia* that is normally developed in Egypt on wheat and pea might be advantageous plants to be proposed for culture as a therapeutic plant.

CONCLUSION: The present work was designed to identify the chemical composition of the essential oil from the inflorescence of the plant *Euphorbia helioscopia* grown in Egypt. Also, this work was aimed at testing for possible antimicrobial activity of the isolated oil. GC–MS analysis of the isolated oil resulted in the identification of thymol and caryophyllene as the major components in the oil. The oil showed antimicrobial activity against *Bacillus subtilis*. This may be due to its high percentage of phenolic content. Further investigation of the plant is recommended to clarify its phytochemical constituents and also for possible pharmacological effects as the Egyptian plant is rarely worked on.

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CONFLICT OF INTEREST: The author declares no conflict of interest.

REFERENCES:

1. Batanouny KH, Stichler W and Ziegler H: Photosynthetic pathways and ecological distribution of Euphorbia Species in Egypt. Oecologia 1991; 87(4): 565-96.
2. Feng WS, Gao L and Zheng XK: A new lathyrane diterpene glycoside from *Euphorbia helioscopia* L. Chinese Chemical Letters 2010; 21: 191-93.
3. Abou-El-Hamd HM, Mohamed-Elamir F, Hegazy and Mahmoud FM: International Journal of Phyto-pharmacology 2012; 3(1): 78-90.
4. Uzair M, Loothar BA and Choudhary BA: Biological screening of *Euphorbia helioscopia* L: Pak J Pharm Sci 2009; 22(2): 184-86.
5. Barla A, Biraman H and Kultur S: Secondary metabolites from *Euphorbia helioscopia* and their Vasodepressor activity. Turk J Chem 2006; 30: 325-32.
6. Saleem U and Ahmad B: *Euphorbia helioscopia*: A review on pharmacological activities. Pharmacologyonline 2016; 16(1): 1-6.
7. Ramezani M, Behravan J and Arab M: Antiviral activity of *Euphorbia helioscopia* extract. Journal of Biological Sciences 2008; 8(4): 809-13.
8. Chaudhry BA, Janbaz KH and Uzair M: Biological studies of Conyza and Euphorbia species. J Res Sci 2001; 12(1): 85-88.
9. Wang ZY, Liu HP and Zhang YC: Anticancer potential of *Euphorbia helioscopia* L. extracts against human cancer cells. The anatomical record 2012, 295: 223-33.
10. Tanveer A, Rehman A and Mansoor M: Allelopathic potential of *Euphorbia helioscopia* L. against wheat (*Triticuma estivum* L.), chickpea (*Cicerari etinum* L.) and lentil (*Lens culinaris* Medic.); Turk J Agric 2010; 34: 75-81.
11. Awaad, AS, Alothman, MR and Zain, YM: Comparative nutritional value and antimicrobial activities between three Euphorbia species growing in Saudi Arabia. Saudi Pharmaceutical Journal 2017; 25(8): 1226-30.
12. Fokialakis N, Melliou E and Magiatis P: Composition of the steam volatiles of six Euphorbia spp. from Greece. Flavour and Fragrance Journal 2003; 18: 39-42.
13. Beltagy AM: Phytochemical attributes and antimicrobial activity of green tea and rosemary leaves: a comparative study. World Journal of Pharmaceutical Research 2016; 5(6): 1882-96.
14. Balouiri M, Sadiki M and Saad-Koraichi I: Methods for *in-vitro* evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis 2016; 6(2): 71-79
15. Campbell J: High-throughput assessment of bacterial growth inhibition by optical density measurements. Curr Protoc Chem Biol 2011; 3(3): 1001-15.
16. Samarghandian S, Farkhondeh T, Samini F and Borji A: Protective effects of carvacrol against oxidative stress induced by chronic stress in rat's brain, liver, and kidney. Biochem Res Int 2016; 26: 45237.

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