Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(4): 326-331
Received: 23-05-2019
Accepted: 25-06-2019

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Chemical composition and biological activities of the essential oil of *Tanacetum sinaicum* Del. grown in Egypt

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Abstract
Tanacetum sinaicum, family Asteraceae is a wild plant indigenous to Egypt and grows on the rocky slopes of Sinai. Because of the large variation observed between the previous reports concerning the essential oil constituents. The present work aimed to perform a detailed investigation of the chemical composition of essential oil from *T. sinaicum*. Furthermore, the present study represents the first report about cytotoxic and antioxidant activities of essential oil of this plant. The essential oil was subjected to GC-MS analysis which resulted in identification of 41 constituents representing 85.81% of its total composition. The biological activity of the oil showed moderate cytotoxic activity against human hepatocellular carcinoma, colon carcinoma and human lung cancer cell lines. In addition, the antioxidant activity was investigated using the DPPH free radical assay and the results indicated that the oil has strong antioxidant activity. Furthermore, the insecticidal activity on *culex pipiens* mosquitoes was measured using the immersion method, the oil showed high mortality percentages for the larval, pupal and adult stages. The antimicrobial results indicate that the oil has a positive activity against the gram negative bacteria *Proteus vulgaris* and gram positive bacteria *Bacillus subtilis*. Also, it has some activity against *Escherichia coli* and *Staphylococcus aureus*.

Keywords: *Tanacetum sinaicum*, essential oil, cytotoxicity, antioxidant, insecticidal, antimicrobial

1. Introduction
Tanacetum sinaicum Del. is a wild plant distributed on rocky slopes in Sinai, Egypt, with discoid, yellow heads having only tubular florets and bipinnatifid woolly leaves [1]. This plant is rich in important pharmacologically active secondary metabolites such as essential oils [2], sesquiterpene lactones [3] and flavonoids [4].
Traditional uses of this plant include treatment of fevers, migraines, stomach ailments, bronchitis and arthritis [5-6]. Antiviral [7] and anti-inflammatory activity [8-9] have been reported. Also, *T. sinaicum* oil was reported to have strong *in vitro* activity against *E. coli*, *Bacillus subtilis* and *Candida albicans* [2].
There are few studies have addressed the chemical composition of essential oil of *T. sinaicum* aerial parts. An early study on the essential oil of the flower heads of *T. sinaicum* [10] reported only 12 compounds.
The essential oil of the aerial parts was analyzed and nineteen compounds were identified by [11]. Also, the oil as well as the *n*-hexane-ether extract of the aerial parts were analyzed and 30 components were identified by [2].
There are large variations observed between the previous reports concerning the essential oil constituents from *T. sinaicum*. So, the aim of the present work was to perform a more detailed investigation of the chemical composition of essential oil from *T. sinaicum* growing wild in Egypt using GC-MS. Furthermore, the present study represents the first report about cytotoxic and antioxidant activities of essential oil of this plant.

2. Materials and Methods
2.1 Plant materials
The plant material of *Tanacetum sinaicum* Del. (*Syn. Tanacetum santolinoides* (DC.) *Feinbrun & Fertig*, *Chrysanthemum sinaicum* Del.) Was collected from Rocky Mountains of Wadi Elarbaeen, St. Catherine, and Sinai Peninsula, Egypt in May 2014. The plant was identified and verified by Dr. Azza El-Hadidy (Professor of plant taxonomy, The Herbarium of Department of Botany, Faculty of Science, Cairo University). A voucher specimen is deposited in the Department of Pharmacognosy, Zagazig University, Egypt.

2.2 Essential oil isolation

The air-dried aerial parts (100 g) were packed in a 2 L stoppered flask with distilled water (800 ml) and subjected to hydrodistillation using a Clevenger apparatus for 5 hrs. The separated pale yellow oil was dehydrated using anhydrous sodium sulphate and stored in sealed vials at 4 °C before chemical investigation and biological study. The percentage of the essential oil yield was 1.2% V/W.

2.3 GC-MS analysis

The GC-MS analysis of the essential oil were carried out at the Central Agricultural Pesticide Laboratory (CAPL), Cairo, Egypt; by Agilent 6890 gas chromatograph with fused silica capillary column PAS-5 ms (30 mm × 0.25 um film thickness). The carrier gas was helium with1 ml/ min flow rate. The sample injection size was 1 µl. Oven temperature program started at 45 ºC for 2 min., then elevated to 280 ºC at rate of 8 ºC/min and kept at 280 ºC for 2 min. The injector temperature was adjusted at 250 ºC while the detector temperature was at 280 ºC. Mass spectrometry detector was used, scanning from m/z 50 to 500, EI 70 ev. A mixture of aliphatic hydrocarbons (C6–C24) diluted in hexane was injected under the above mentioned temperature program to calculate the retention indices (as Kovats indices) of each extracted compound. Identification of the compounds was based on the comparison of their retention indices (RI) with those reported by Adams (2007), NIST 08 libraries and literature and comparison of their EI–mass spectra with the NIST 05 (National Institute of Standards and Technology) and Wiley library spectra. The relative percentages of the individual components were calculated based on GC peak area which was obtained by dividing the area of each component by the total area of all isolated components.

2.4 Cytotoxic activity

Human lung cancer (A-549), human hepatocellular carcinoma (HepG-2) and colon carcinoma (HCT-116) cell lines obtained from VACSERA tissue culture unit were used to evaluate the cytotoxic effect of the essential oil using MTT cell viability assay. Cells were routinely cultured in DMEM (Dulbeco's Modified Eagle's Medium), which was supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% L-glutamine, HEPES (4-(2-hydroxyethyl)-1- piperazine ethane sulfonic acid) buffer and 50 µg/ml gentamycin (Sigma/Aldrich, USA). The essential oil of the wild plant dissolved in dimethyl sulphoxide (DMSO) was tested. The viability percentage was plotted against the oil concentrations and the 50% cell viability (IC_{50}) was calculated from the curve [12-13]. All experiments were repeated three times.

2.5 Antioxidant Assay

The antioxidant activity of the essential oil was determined at the Regional Center for Mycology and Biotechnology (RCMB) at Al- Azhar University by the DPPH free radical scavenging assay in triplicate and average values were considered. Freshly prepared (0.004% w/v) methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was prepared and stored at 10 ºC in the dark. Methanol solution of the tested oil was prepared. A aliquot of the methanol solution (40 µL) was added to DPPH solution (3 ml). Absorbance measurements were recorded immediately with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min intervals until the absorbance stabilized (16 min). The absorbance of the DPPH radical without antioxidant (control) and the reference compound ascorbic acid were also measured. The percentage inhibition (PI) of the DPPH radical was calculated according to the formula: PI = [{(*AC- AT*)/ *AC*} x 100] (1)**,** Where *AC* = Absorbance of the control at t = 0 min and *AT* = absorbance of the sample + DPPH at t = 16 min [14].

2.6 Insecticidal activity

Mosquitoes used in this study were *Culex pipiens*. They were collected from Abu Rawash, Giza governorate, Egypt, then they were reared for several generations in the insectariums of medical entomology at the Department of Zoology, Faculty of Sciences, Al-Azhar University, Egypt, under controlled conditions. The insecticidal activity was measured using the immersion method as reported previously [15]. *Culex pipiens* larvae (2^{nd} instar) were treated with essential oil of *T. sinaicum* (at100 µg/ml) in plastic cup. The control tubes were maintained as tap water (10 ml) free from the oil. Each treatment was performed using 30 larvae and repeated for three times. The numbers of dead and live larvae were counted 48 h after treatment. The mortality percent was determined as follows: Mortality (%) = (Number of dead larva in treatment group - Number of dead larva in control group)/ Number of total tested larva × 100.

2.7 Antimicrobial activity

Antibacterial and antifungal activities of the essential oil were determined using the well diffusion method [16]. The sample was dissolved in dimethyl sulfoxide (DMSO) (Oxoid laboratories, UK) at concentration of 1mg/ml. The tested organisms were subcultured on nutrient agar medium for bacteria and Sabouraud dextrose agar (Oxoid laboratories, UK) for fungi. The microorganisms used in this study were *Aspergillus fumigatus* (RCMB 02568) and *Candida albicans (RCMB 005003)* as fungi; *Streptococcus Pneumonia* (RCMB 010010) and *Bacillus subtilis* (RCMB 010067) as Gram positive bacteria; *Pseudomonas aeruginosa* (RCMB 010043), *Escherichia coli* (RCMB 010052) as Gram negative bacteria (RCMB at Antimicrobial Unit, Al-Azhar University). Gentamicin was used as a positive control against Gram positive and Gram negative bacteria. Ketoconazole was used as a positive control for fungi.

The plates were done in triplicate. Bacterial cultures were incubated at 37 °C for 24 h, while the fungal cultures were incubated at 37 °C for 2-7 days. Antibacterial and antifungal activities were determined by measuring the diameter of the inhibition zone formed around the well (mm). Results were expressed in mean zone of inhibition in mm ± standard deviation (SD) beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms. Minimal inhibitory concentration (MIC) was also determined.

3. Results and Discussion

3.1 GC-MS analysis

The volatile constituents of the essential oil were analyzed by GC-MS under the same conditions, and their chemical composition is shown in Table 1. Analyses of the GC-MS chromatograms (Fig.1) resulted in identification of 41 compounds, accounting for 85.81% of the total oil composition.

The oil of the aerial parts consists mainly of oxygenated monoterpenes representing 67.33% including trans- thujone (21.36%) as a major compound, followed by trans-2,7-dimethyl-4,6-octadien-2-ol (15.67%), cis- thujone (7.83%),

yomogi alcohol (6.45%), artemisia alcohol (4.75%) and artemisia ketone (4.42%). The only detected monoterpene hydrocarbon was santolina triene (1.09%). On the other hand, the oxygenated sesquiterpenes (7.55%) included globulol (0.30%), isospathulenol (1.54%), torreyol (1.61%), t-Muurolol (1.54%), β–eudesmol (2.05%) and valerenol (0.51%). The sesquiterpene hydrocarbons (4.23%) detected were bicycloelemene (0.21%), α -copaene (0.35%), β-cubebene (0.79%), β copaene (0.17%), α-amorphene (0.29%), γ murrolene (0.33%), germacrene-D (1.41%), aromadendrene (0.19%), β-cadinene (0.15%) and α-murrolene.

Comparing our results with those reported in literature we noticed similarities in the essential oil composition reported by [2] such as santolina triene, Yomogi alcohol, cis-thujone, trans-thujone, trans-pinocarveol, terpinen-4-ol, β–Eudesmol, artemisia ketone, p-cymen-7-ol and γ-Murrolene. Also, as in the case with many essential oils, differences in the essential oil composition may be detected depending on the chemotypicity, geographic location, harvesting period, and storage time of the samples [17]. So this may be the reason that compounds as globulol, isospathulenol, torreyol, bicycloelemene, α -copaene, β-cubebene, β copaene, α-amorphene and valerenol have not been detected before in the oil of *T. sinaicum*.

This is the first report of the occurrence of the amide pellitorine in the genus *Tanaceum*. Pellitorine is one of the many compounds known for fungicidal, larvicidal and insecticidal activities [18]. It was isolated before from *Piper tuberculatum* as an insecticidal compound active against the velvet bean caterpillar [19]. It has also been isolated from several *Zanthoxylum* species as a bioactive compound against several insect pests [20]. Insecticidal activities of pellitorine has been studied against mosquitoes [21]. Presence of pellitorine (2.33%), may be responsible for the high insecticidal activity of the essential oil of *T. sinaicum* reported before by [11].

Table 1: Chemical composition of the volatile oil of *T. sinaicum* aerial parts

No.	Component [a]	RI [b]	MWT	Mass fragments *m/z*	% [c]
1	*Santolina triene*	0901	136	136, 121, 107, 93, 79, 67, 55, 41, 27	1.09
2	*Yomogi alcohol*	0997	154	154, 139, 128.1, 111, 104, 97, 85, 77, 67, 59, 51, 43	6.45
3	*Trans-2,7-Dimethyl-4,6-octadien-2-ol*	1048	154	154, 139, 121, 96, 81, 59,43, 18	15.67
4	Artemisia ketone	1063	152	152, 137, 122, 109, 83, 69, 55, 39, 26	4.42
5	*Artemisia alcohol*	1091	154	154, 139, 121, 105, 95, 85, 67, 55, 41	4.75
6	*cis- thujone*	1107	152	152, 137, 124, 110, 95, 81, 67, 54, 41	7.83
7	*trans- thujone*	1116	152	152, 137, 137, 124, 110, 95, 81, 67, 54, 41	21.36
8	*α Phellandrine epoxide*	1180	152	152, 134, 119, 109, 92, 81, 70, 55, 41, 27, 15	1.04
9	*Trans pinocarveol*	1152	152	152, 134, 119, 109, 92, 83, 70, 41, 55, 27,15	0.34
10	*Lavandulol*	1163	154	154, 136, 123, 111, 103, 93, 81, 61, 69, 53, 41, 29, 15	0.24
11	*L-4-terpineneol*	1188	154	154, 136, 121, 111, 103, 93, 81, 71, 63, 55, 43, 27	1.04
12	*Estragole= (methyl chavicole)*	1199	148	148, 133, 121, 105, 91, 77, 51	0.38
13	*Myrtenol*	1202	152	152, 134, 119, 108, 91, 79, 67, 55, 41	0.32
14	*4.α.- Hydroxyachipendol*	1229	170	155, 137, 123, 109, 94, 82, 67, 55, 43	1.52
15	*Lavandulyl acetate*	1282	196	154, 136, 121, 107, 93, 80, 69, 53, 43	0.23
16	*Sabinyl acetate*	1296	194	233, 205, 169, 147, 119, 91, 71, 43	1.09
17	*p-cymen-7-ol (cuminol)*	1288	150	135, 119, 105,91, 79, 60, 41	0.10
18	*Bicycloelemene*	1329	204	204, 189, 161, 147, 136, 121, 107, 93, 79, 67, 55, 41, 29	0.21
19	*Ethylhydrocinnamate*	1353	178	178, 149, 133, 119, 104, 91, 77, 65, 51, 39, 29, 15	0.15
20	*α -copaene*	1379	204	204, 189, 175, 161, 147, 133, 119, 105, 91, 77, 69, 55, 41	0.35
21	*Cis - Jasmone*	1385	164	164, 149, 135, 122, 110, 91, 79, 67, 55	0.49
22	β-cubebene	1387	204	204, 189, 176, 161, 148, 133, 119, 105, 91, 79, 69, 55, 43	0.79
23	*β copaene*	1446	204	204, 189, 176, 161, 148, 133, 119, 105, 91, 79, 69, 55, 41, 27	0.17
24	*α-Amorphene*	1456	204	189, 175, 161, 147, 133, 119, 105, 91, 81, 67, 55, 41, 29	0.29
25	*Massoia lactone*	1458	168	168, 150, 139, 122, 108, 97, 81, 68, 55, 41, 29	0.55
26	*γ-Murrolene*	1480	204	204, 161, 133, 119, 105, 91, 79, 69, 55, 41, 27	0.33
27	*Germacrene-D*	1473	204	204, 161, 147, 133, 119, 105, 91, 81, 67, 55, 41, 29	1.41
28	Isoamyl phenylacetate	1476	206	206, 191, 163, 149, 136, 119, 105, 91, 70, 55, 43, 29, 15	0.40
29	*Aromadendrene*	1493	204	204, 189, 175, 161, 147, 133, 119, 105, 93, 81, 69, 55, 41, 29	0.19
30	*α-Muurolene*	1506	204	161, 147, 133, 119, 105, 91, 79, 67, 55, 41, 27	0.34
31	*β.-Cadinene*	1516	204	204, 189, 161, 147, 133, 120, 105, 91, 79, 67, 55, 41, 27	0.15
32	*Myristicin*	1526	192	192, 177, 165, 147, 131, 119, 105, 91, 77, 65, 53, 39, 27	1.84
33	*Globulol*	1601	222	222, 204, 189, 175, 161, 147, 133, 121, 107, 93, 81, 67, 55, 43	0.30
34	*Isospathulenol*	1624	220	205, 187, 173, 159, 147, 131, 131, 119, 105, 91, 79, 79, 67, 55, 43	1.54
35	*Torreyol*	1633	222	204, 189, 176, 161, 148, 133, 119, 105, 91, 79, 67, 55, 41	1.61
36	t-Muurolol	1644	222	222, 204, 189, 161, 149, 139, 121	1.54
37	*β - Eudesmol*	1647	222	222, 204, 189, 176, 164, 149, 135, 122, 108, 93, 81, 67, 59, 41, 18	2.05
38	*n- Heptadecane*	1695	240	240, 211, 197, 183, 169, 155, 141, 127, 113, 99, 85, 71, 57, 43, 29,	0.21
39	*Valerenol*	1739	220	220, 205, 187, 173, 159, 145, 131, 118, 105, 91, 77, 55, 41, 27	0.51
40	*Pellitorine* (*N-Isobutyl-2,4-decadienamid*))	1925	223	223, 203, 180, 151, 131 110, 96, 81, 57, 41	2.33
41	Phytol	1969	296	296, 278, 263, 249, 230, 210, 196, 182, 165, 151, 137, 123, 109, 95, 82, 71, 57, 43, 29, 15	0.19
% of	Monoterpene hydrocarbons	1.09			
	Oxygenated monoterpenes	67.33			
	Sesquiterpene hydrocarbons	4.23			
	Oxygenated sesquiterpenes	7.55			

	Others	5.61
% of Total identified compounds		85.81

[a] Compounds are listed in order of their elution from a PAS-5ms column. [b] RI, linear retention indices on PAS-5ms column, experimentally determined using homologous series of n-alkanes (C8–C24). [C] Relative percentage.

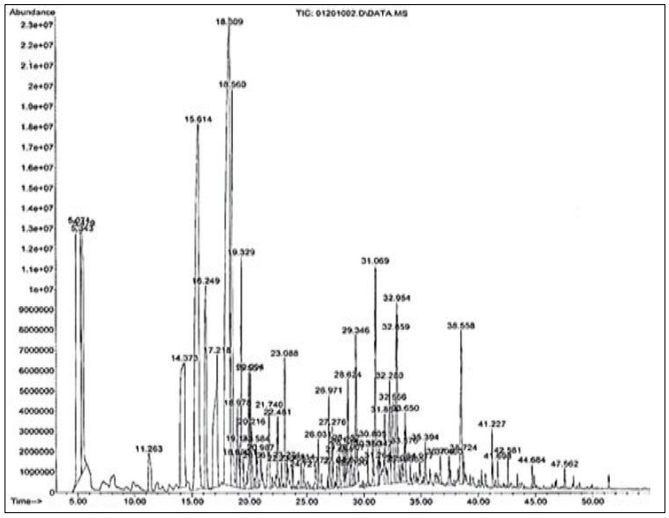

Fig 1: Gas chromatogram of the essential oil of the aerial part of *T. sinaicum. Del*

3.2 Cytotoxic activity

There are only few studies reported the cytotoxic activity of *T. sinaicum* extracts using the brine shrimp eggs method. It was found that the activity was due to the isolated sesquiterpene lactones [3], for example, sesquiterpene lactone tanacetonic acid [22]. To our knowledge, the present work is the first report on investigation of cytotoxicity of essential oil of *T. sinaicum* against different cancer cell lines using MTT assay. Cytotoxicity of the essential oil of the wild plant (Fig.2) was evaluated against *human lung cancer* (A-549), *human hepatocellular carcinoma* (HepG-2) and *colon carcinoma* (HCT-116) cell lines using MTT cell viability assay. The IC_{50} values of the oil against Hep-G2, HCT-116 and A-549 (Table 2 & Fig.3) were found to be 51, 61.3 and 76.1µg/ml; respectively. This means that the oil showed a moderate cytotoxic activity against all the tested cell lines, but Hep-G2 cell line was the most sensitive one.

Table 2: Half-maximal inhibitory concentration (IC_{50}) by µg/ml of volatile oil of *T. sinaicum* against Hep-G2, HCT-116 and A-549 cell line cells

	Hep-G2	**HCT-116**	**A-549**
Cisplatin	3.67	2.43	0.95
Oil	*51 ± 2.9*	*61.3 ± 1.8*	*76.1 ±3.4*

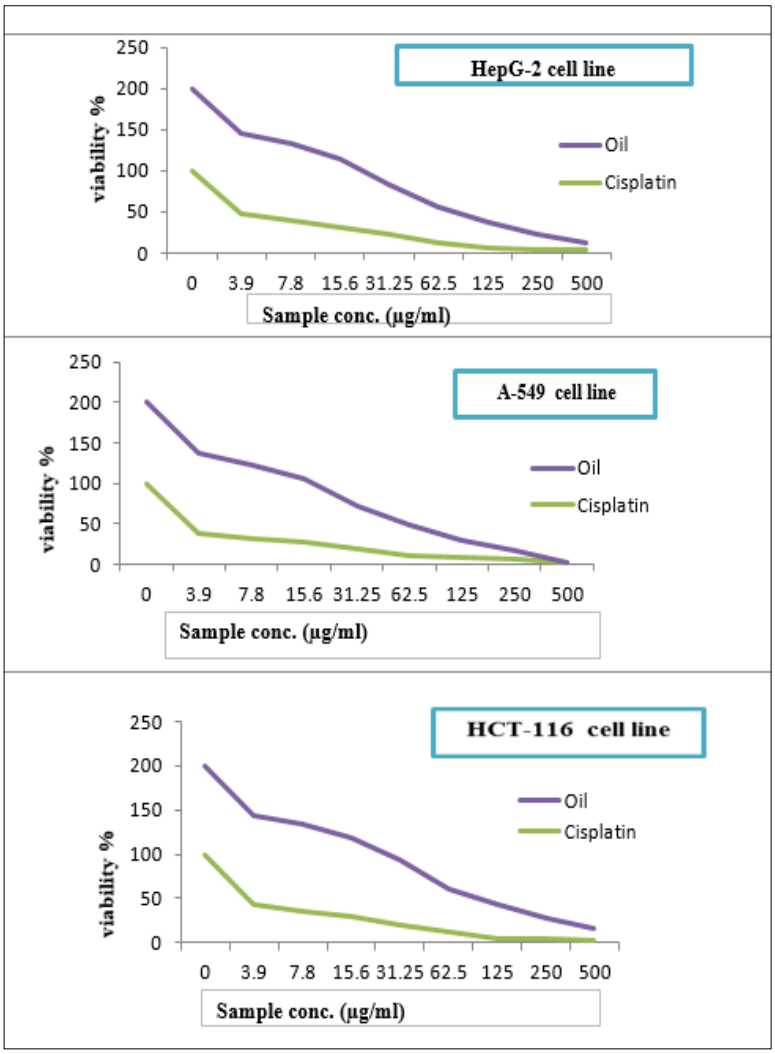

Fig 2: Inhibitory activity of the essential oil of the aerial part of *T. sinaicum. Del* and the standard cisplatin against A: Hep-G2; B: HCT-116 and C: A-549 cell line cells.

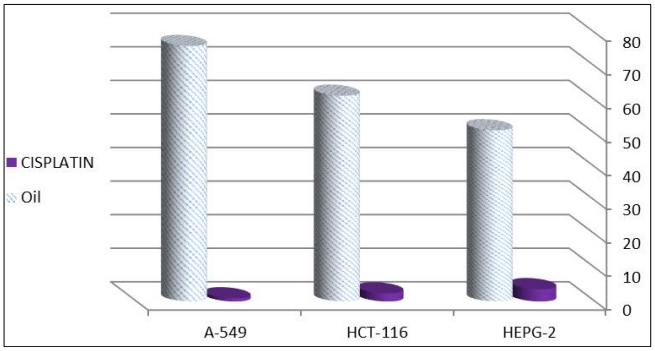

Fig 3: Half-maximal inhibitory concentration (IC_{50}) of the essential oil of the aerial part of *T. sinaicum* and the standard cisplatin

3.3 Anti-oxidant activity

The antioxidant activity of the essential oil of *T. sinaicum* using the DPPH free radical assay was investigated. The SC_{50} value of the oil and standard antioxidant (L-ascorbic acid) were found to be 7.63 and 7.7 µg/ml; respectively. This means that the scavenging activity of the oil was equal to the standard antioxidant which indicates that the oil has very strong antioxidant activity (Fig. 4 & 5). The antioxidant efficiency of essential of *T. sinaicum* may be attributed mainly to the synergic effect of its components. Since the majority of authors agree that terpenoids with phenolic groups are the most active antioxidant principles in essential oils [23-24], the antioxidant activity of *T. sinaicum* oil could be attributed to the presence of methyl chavicole, cymen-7-ol, ethylhydrocinnamate. Moreover, presence of the monocyclic aliphatic L-4-terpineneol (1.04%) and the bicyclic sabinyl acetate (1.09%) may be responsible for this strong antioxidant activity as reported by [25]. Recently there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in reducing oxidative stress-induced tissue injury, So our essential oil stand out as a natural antioxidant and, further investigations are needed to replace synthetic antioxidants.

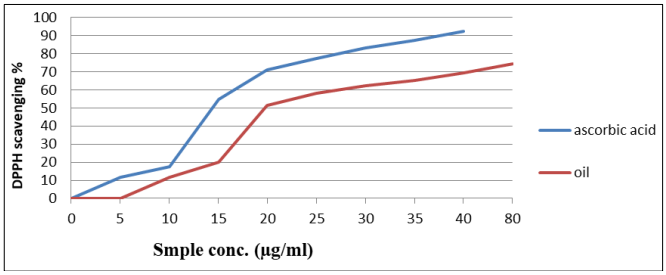

Fig 4: DPPH scavenging capacity of the oil of the essential oil of the aerial part of *T. sinaicum. Del* and the standard ascorbic acid.

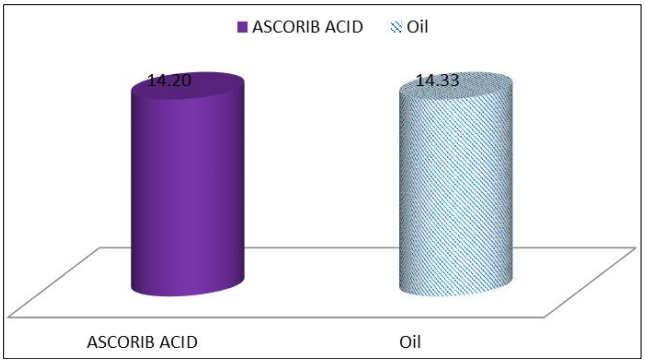

Fig 5: DPPH radical scavenging activity SC_{50} % (µg/mL) of the essential oil of the aerial part of *T. sinaicum. Del* and the standard ascorbic acid.

3.4 Insecticidal activity

The essential oil of *T. sinaicum* showed high mortality percentages 72.8, 50 and 94.6% for the larval, pupal and adult stages; respectively (Fig. 6).

The insecticidal constituents of many plant extracts and essential oils are the monoterpenoids, which are the major components of the essential oil [26]. One of the most active insecticidal monoterpenoids is the α-thujone [27] which is one of the major components detected in the oil in a percentag of 7.83%.

There are numerous reports on the insecticidal activity of terpinen-4-ol (1.04%) which has been detected in the oil of *T. sinaicum* [26]. Also, the amide pellitorine which is detected in a percentages of 2.33% was previously reported as one of the many compounds known for fungicidal, larvicidal and insecticidal activities [18].

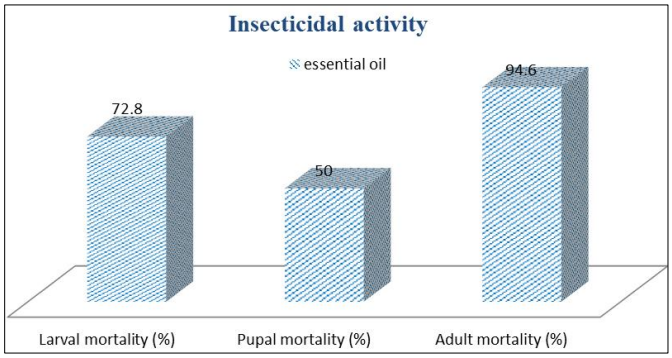

Fig 6: Mortality (%) of the essential oil of *T. sinaicum. Del* against *culex pipiens* mosquito

3.5 Antimicrobial activity

Results of the antibacterial and antifungal activities of the essential oil of *T. sinaicum* plant are shown in Table (3).

Results indicate that the oil has a good activity against the gram negative bacteria *Proteus vulgaris* (MIC 0.625 mg/ml) and gram positive bacteria *Bacillus subtilis* (MIC 0.625 mg/ml). Also, it has some activity against *Escherichia coli* (MIC 1.25 mg/ml) and *Staphylococcus aureus* (MIC 2.5 mg/ml). Also, the essential oil showed no antifungal activity against *Aspergillus fumigatus* and *Candida albicans*.

Table 3: Mean zone of inhibition in mm produced on a range of pathogenic microorganisms. The sample was tested at 10 mg/ml concentration

Sample code Tested microorganisms	Oil	Control
FUNGI		*Ketoconazole*
Aspergillus fumigatus (RCMB 002008)	NA	17
Candida albicans RCMB 005003 (1) ATCC 10231	NA	20
Gram Positive Bacteria		*Gentamycin*
Staphylococcus aureus (RCMB010010)	13	24
Bacillus subtilis RCMB 015 (1) NRRL B-543	8	15
Gram Negatvie Bacteria		*Gentamycin*
Escherichia coli (RCMB 010052) ATCC 25955	6	17
Proteus vulgaris RCMB 004 (1) ATCC 13315	15	25

*NA: No activity

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