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Acaricidal activity of five essential oils against *Rhipicephalus annulatus* ticks and their GC-MS analyses

Abeer Moawad[1], Elham Amin[2]*, Waleed Arafa[3], Khaled Hussein[3], Khaled Hassan[4], Asmaa Owis[1,5], Hayam Ahmed[1]

[1]Pharmacognosy Department, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt.
[2]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Qassim University, Buraydah, Saudi Arabia.
[3]Department of Veterinary Medicine, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt.
[4]Animal Health Research institute, Beni-Suef, Egypt.
[5]Pharmacognosy Department, Faculty of Pharmacy, Heliopolis University, Cairo, Egypt.

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ABSTRACT

Rhipicephalus annulatus is regarded as the most common tick in transmitting cattle disease. Chemical acaricides resistance and toxicity have directed research on the use of essential plant oils which have great potential for pest management for safe food production. Therefore, the current study was designed to test the larvicidal activity of essential oils (EOs) of five medicinal plants; cilantro leaves, orange leaves, *Tagetes* flower, geranium herb, and sweet basil herb against *R. annulatus* larvae using the larval packet test. Analysis of EO chemical composition using gas chromatography-mass spectrometry (GC-MS) was also carried out to justify the assigned activities. Cilantro, orange, and *Tagetes* EOs showed 100% larval mortality with lethal concerntrations that kills 50% (LC_{50}) of 1.46%, 0.88%, and 2.94%, respectively. Geranium and sweet basil herbs EOs showed 96.33% ± 3.18% and 92.33% ± 1.45% larval mortality with LC_{50} of 5.28% and 7.20%, respectively. Major compounds were identified by GC-MS as follows, [(E)-dodecenal (49.72%), decanal (21.47%)] from cilantro EO, [methyl methanthranilate (63.45%), γ-terpinene (18.54%)] from orange EO, [trans-*β*-ocimene (24.93%) and isoartemisia ketone (8.84%)] in *Tagetes* EO, [*β*-citronellol (41.83%), citronellyl formate (10.41%), geraniol (9.47%)] from geranium EO, and [*β*-Linalool (55.63%), 1,8-cineole (9.66%)] from sweet basil. Our results showed the potential of EOs as eco-friendly and economic acaricides for tick control.

INTRODUCTION

Ticks are the most prevalent cattle ectoparasites worldwide, especially in tropical and subtropical areas [1]. In Egypt, *Rhipicephalus annulatus* is the most common cattle-infesting tick. Tick-borne diseases, babesiosis, and anaplasmosis present serious constraints to animal productivity of particularly exotic cattle breeds and their crosses. Chemical acaricides are used extensively to control the ticks. Incorrect dilution, application methods, and extensive pressure on a particular compound are the main factors that accelerate acaricide resistance [2]. Synthetic pyrethroid and deltamethrin resistance was recorded in *Rhipicephalus microplus* and *R. annulatus*, respectively, in many countries [3–5]. In addition, chemical acaricides have toxic effects on nontarget species besides meat and milk residues; therefore, there is a massive need to develop eco-friendly effective acaricides [6–8]. For many years, plant EOs have been studied widely as one of the natural acaricides [9]. EOs are composed of terpenoids, monoterpenoids (C_{10}), and sesquiterpenoids (C_{15}). Terpenoid hydrocarbons are hydrophobic, a property associated with protein deactivation and enzyme inhibition activities especially acetylcholinesteras which is the target of many chemical acaricides [10]. In comparison with chemical products, EOs have advantages such as low toxicity to livestock and safety to the environment. Among reported natural acaricides from EOs, thymol and eucalyptus oils were found effective in managing deltamethrin-resistant *R. annulatus* infestation in cattle [11–13].

*Corresponding Author
Elham Amin, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Qassim University, Buraydah, Saudi Arabia.
E-mail: elham_bns @ yahoo.com

In continuation of our search for natural acaricides, larvicidal activity of EOs from five medicinal plants, namely, *Coriandrum sativum* leaves (cilantro, **1**), *Citrus aurantium* leaves (orange, **2**), *Tagetes erecta flowers* (**3**), *Pelargonium graveolens* herb (geranium, **4**), and *Ocimum basilicum* leaves (sweet basil, **5**), were evaluated against *R. annulatus* larvae using larval packet test (LPT). Gas chromatography-mass spectrometry (GC-MS) analysis was performed for the EOs to identify their major components and rationalize their activity.

MATERIALS AND METHODS

Essential oils (EOs)

Five EOs were purchased from a volatile oil factory, in Beni-Suef Governorate, Egypt. The oils were *C. sativum* leaves (cilantro, **1**), *C. aurantium* leaves (orange, **2**), *T. erecta* flowers (Tagetes, **3**), *P. graveolens* herb (geranium, **4**), and *O. basilicum* leaves (sweet basil, **5**). Different concentrations of EOs (10%, 5%, 2.5%, and 1.25%) were prepared in ethanol (95% in water) as a diluent. EOs lethal effects on *R. annulatus* larvae were evaluated by calculating the concentrations that kill 99% (LC_{99}), and lethal concentrations that kill 50% (LC_{50}).

GC-MS analysis

Mass spectra were recorded using Shimadzu GCMS-QP2010 (Tokyo, Japan) Column (Rtx5MS fused, 30 m × 0.25 mm id × 0.25 µm film thickness) (Restek, USA) equipped with a split–splitless injector was used at 45°C isothermal temperature at for 2 minutes then programmed to 300°C at a rate of 5°C/minute kept isothermal at 300°C for 5 minutes. The injector temperature was 250°C. The helium carrier gas flow rate was 1.41 ml/minute. All the mass spectra were recorded applying the following conditions: (equipment current) filament emission current, 60 mA; ionization voltage, 70 eV; and ion source, 200°C. Diluted samples (1% v/v) were injected with split mode (split ratio 1: 15). The identification of components was based on the National Institute of Standards and Technology library attached to the GC-MS instrument. Compounds were recognized by relating their peak retention indices and mass spectral fragmentation patterns to those of the known compounds available in the library.

Acaricidal activity LPT

Larvicidal activities were evaluated by using the modified larval packet technique (LPT) as previously described [14]. Various concentrations of the tested EOs were prepared. Approximately 100 7-day-old larvae were placed on the center of 7 × 7 cm filter papers then 100 µl of the test solutions were added then enclosed to form packets with clips. Control groups were treated with ethanol (95%). There were five replicates for each concentration. Finally, mortality was determined after packet incubation at 27°C–28°C and 80%–90% relative humidity for 24 hours.

Statistics

For the acaricidal study, statistical analysis of data was performed using Statistical Package for Social Science [SPSS for Windows (IBM), version 22, Chicago, USA] to determine if variables differed between treatments. Analysis of variance tests and subsequent Duncan's multiple range tests were applied to determine the differences between means. Results were presented as means. Probability values ($p < 0.05$) were considered significant. The effective concentration (LD_{50}) with a 95% confidence interval was calculated (SPSS version 22).

RESULTS AND DISCUSSION

The acaricidal efficacy of EOs was assessed by estimating *R. annulatus* larvae mortality percentage (Table 1) where the acaricidal activity of cilantro, orange leaf, and *Tagetes* flower EOs against *R. annulatus* is reported here for the first time. LC_{99} indicates the lethal concentrations that kill 99% of larvae; the smaller the concentration the more potent the EO. EO from cilantro is considered the most potent among the tested oils, (LC_{99} = 2.77 µg/ml), followed by orange leaf (3.78 µg/ml), *Tagetes* flower (6.05 µg/ml), geranium herb (10.07 µg/ml), and finally sweet basil (13.46 µg/ml). Major components in each EO were determined using GC-MS (Tables 2–6, Figs. 1,2 and 3) and compared with the reported data.

Acaricidal activity and chemical composition of cilantro leaf EO

LPT showed 100% mortality at 5% and 10% concentrations with LC_{50} of 1.46 and LC_{99} of 2.77 µg/ml. 2.5% concentration still showing potent activity (96.67% ± 1.67% larval mortality) GC-MS analysis of cilantro EO (Table 2, Fig. 2) showed the major components detected were 2(E)-

Table 1. Mean mortality percentage, lethal concentrations (LC_{50} and LC_{99}) of the tested oils against *R. annulatus* larvae.

Oil	Concentration	Mean ± SE	LC_{50}	LC_{99}	Slope ± S E
Cilantro oil	10%	100.00 ± 0.00	1.46	2.77	1.7 ± 0.12
	5%	100.00 ± 0.00			
	2.50%	96.67 ± 1.67			
	1.25%	35.00 ± 2.89			
Orange oil	10%	100.00 ± 0.00	0.88	3.78	0.8 ± 0.09
	5%	100.00 ± 0.00)			
	2.50%	90.00 ± 0.00			
	1.25%	61.67 ± 1.67			
Tagetes oil	10%	100.00 ± 0.00	2.94	6.05	0.75 ± 0.04
	5%	96.00 ± 3.06			
	2.50%	28.33 ± 4.41			
	1.25%	15.00 ± 2.89			
Geranium oil	10%	96.33 ± 3.18	5.28	10.07	0.48 ± 0.02
	5%	56.67 ± 6.67			
	2.50%	3.33 ± 1.67			
	1.25%	1.67 ± 1.67			
Sweet basil oil	10%	92.33 ± 1.45	7.20	13.46	0.37 ± 0.01
	5%	6.67 ± 1.67			
	2.50%	6.67 ± 1.67			
	1.25%	6.67 ± 1.67			

Table 2. Major constituents of cilantro (*C. sativum*) leaves EO, family Apiaceae, were analyzed by GC-MS.

No	R_t	Compound name	Chemical class	%	Mol. formula	Base *m/z*	MI peaks
1.	12.49	β-Linalool	Acyclic monoterpenoid	**9.08**	$C_{10}H_{18}O$	71.00	154
2.	15.38	*cis*-4-Decenal	Aldehyde	0.47	$C_{10}H_{18}O$	55.00	154
3.	15.48	4-Dodecene	Alkenes	1.41	$C_{12}H_{24}$	55.05	168
4.	15.74	Decanal	Aldehyde	**21.47**	$C_{10}H_{20}O$	43.05	156
5.	15.85	Dodecanal	Aldehyde	1.28	$C_{12}H_{24}O$	41.00	184
6.	17.47	2(E)-Decenal	Aldehyde	**49.72**	$C_{10}H_{18}O$	43.05	154
7.	17.64	trans-2-Decenol	Alcohol	0.70	$C_{10}H_{20}O$	57.00	156
8.	17.70	1-Decanol	Alcohol	0.82	$C_{10}H_{20}O$	55.00	158
9.	18.69	Hendecanaldehyde	Aldehyde	1.03	$C_{11}H_{22}O$	43.00	170
10.	20.28	2-Undecenal	Aldehyde	3.02	$C_{11}H_{20}O$	41.00	168
11.	21.49	Palmitaldehyde	Aldehyde	0.81	$C_{16}H_{32}O$	43.00	240
12.	23.02	2-Dodecenal	Aldehyde	**5.98**	$C_{12}H_{22}O$	41.00	182
13.	28.06	(E)-2-Tridecenal	Aldehyde	1.55	$C_{13}H_{24}O$	41.00	196
Terpenes				9.08			
Aldehyde				85.33			
Alcohol				1.52			
Hydrocarbon				1.41			
Total identified				**97.34**			

Bold values indicate constituents with the highest concentration.

Table 3. Major constituents of orange (*C. aurantium*) leaves EO, family Rutaceae, were analyzed by GC-MS.

No	R_t	Compound name	Chemical class	%	Mol. formula	Base *m/z*	MI peaks
1.	7.17	*α*-Thujene	Bicyclic monoterpene	0.57	$C_{10}H_{16}$	93.00	136
2.	7.36	*α*-Pinene	Bicyclic monoterpene	1.69	$C_{10}H_{16}$	93.00	136
3.	8.65	*β*-Pinene	Bicyclic monoterpene	1.74	$C_{10}H_{16}$	93.05	136
4.	10.13	*p*-Cymene	Aromatic monoterpene	4.25	$C_{10}H_{14}$	119.05	134
5.	10.25	D-Limonene	Cyclic monoterpene	**7.23**	$C_{10}H_{16}$	68.00	136
6.	11.22	γ- Terpinene	Cyclic monoterpene	**18.64**	$C_{10}H_{16}$	93.05	136
7.	21.73	Methyl methanthranilate	Ester	**63.45**	$C_9H_{11}NO_2$	105.05	165
8.	21.89	Caryophyllene	Bicyclic sesquiterpene	1.48	$C_{15}H_{24}$	41.00	204
Ester				63.45			
Sesquiterpene				1.48			
Monoterpene				34.12			
Total identified				99.05			

Bold values indicate constituents with the highest concentration.

decenal (49.72%), decanal (21.47%), β-linalool (9.08%), and 2-dodecenal (5.98%) and its GC-MS data agree to some extent with the data reported by Silva *et al.* [15] on leaf EO that showed the same major constituent but with different percentages; 32.23% 2(E)-decenal, 13.97% linalool, 7.51% (E)-2-dodecenal, and 6.56% (E)-2-tetradecenal. In addition, Shavandi *et al.* [16] reported that 2(E)-decenal (19.6%), 1-decanol (26.0%), E-2-tetradecenal (7.0%), decanal (6.6%), and E-2-dodecenal (5.4%) percentage, respectively, while Delaquis and Stanich [17] reported that linalool (25.9%) and (E)-2-decenal (20.2%) are the most abundant component followed by decanal (8.4%) and (E)-2-decenol (7.9%).

Coriander leaf EO acaricidal activity against *R. annulatus* is reported here for the first time and it showed potent activity. The oil also has 100% acaricidal activity against red mite *Dermanyssus gallinae* De Geer [18], 100% nematicidal activity (2 mg/ml) against *Bursaphelenchus xylophilus* [19], and 100% *Tribolium castaneum* egg mortality at 20 mg/ml (96 hours exposure) and 90% repellent activity to the adults at 12 mg/ml [20].

Acaricidal activity and chemical composition of orange leaf EO

LPT showed 100% mortality at 5% and 10% concentrations as cilantro with LC_{50} of 0.88 and LC_{99} of 3.78 µg/ml. 2.5% concentration still showing potent activity (90.00% ± 0.0% larval mortality). GC-MS analysis of orange leaf EO (Table 3, Fig. 2) showed the major components

Table 4. Major constituents of the *Tagetes* (*T. erecta*) flower EO, family Astraceae, were analyzed by GC-MS.

No	R_t	Compound name	Chemical class	%	Mol. formula	Base *m/z*	MI peaks
1.	8.56	(+)-Sabinene	Bicyclic monoterpene	0.60	$C_{10}H_{16}$	93.05	136
2.	10.25	D-Limonene	Cyclic monoterpene	**6.68**	$C_{10}H_{16}$	68.00	136
3.	10.56	trans-*β*-Ocimene	Cyclic monoterpene	**24.93**	$C_{10}H_{16}$	93.00	136
4.	13.39	Allo-Ocimene	Cyclic monoterpene	0.53	$C_{10}H_{16}$	121.00	136
5.	14.17	trans-tagetone	Acyclic monoterpenoid	2.79	$C_{10}H_{16}O$	95.00	152
6.	21.89	Caryophyllene	Bicyclic sesquiterpene	1.06	$C_{15}H_{24}$	41.00	204
7.	22.70	α-Humulene	Monocyclic sesquiterpene	0.51	$C_{15}H_{24}$	93.00	204
8.	23.91	Germacrene B	Monocyclic sesquiterpene	0.61	$C_{15}H_{24}$	121.05	204
9.	35.64	(2-methyl-5-propan-2-ylphenyl) 3,5,5-trimethylhexanoate	Ester	0.53	$C_{19}H_{30}O_2$	150.10	290
10.	36.41	Piperitenone	Cyclic monoterpenoid	1.23	$C_{10}H_{14}O$	150.05	150
11.	37.44	2,6-di-tert-butyl-4-methylphenyl 2-methylcyclopropanecarboxylate	Ester	4.33	$C_{20}H_{30}O_2$	83.00	302
12.	39.17	Isoartemisia ketone	Acyclic monoterpenoid	**8.84**	$C_{10}H_{16}O$	83.00	152
Monoterpene				45.6			
Sesquiterpene				2.18			
Ester				4.86			
Total identified				**52.64**			

Bold values indicate constituents with the highest concentration.

Table 5. Major constituents of the geranium (*P. graveolens*) herb EO, family Geraniaceae, were analyzed by GC-MS.

No	R_t	Compound name	Chemical class	%	Mol. formula	Base *m/z*	MI peaks
1.	12.49	*β*-Linalool	Acyclic monoterpenoid	3.01	$C_{10}H_{18}O$	71.00	154
2.	12.81	trans-rose oxide	Monoterpenoid	1.75	$C_{10}H_{18}O$	139.10	154
3.	14.49	D-isomenthone	Monoterpenoids	**8.89**	$C_{10}H_{18}O$	112.05	154
4.	16.49	*β*-Citronellol	Monoterpenoids	**41.83**	$C_{10}H_{20}O$	41.00	156
5.	17.23	cis -Geraniol	Monoterpenoids	**9.47**	$C_{10}H_{18}O$	69.00	154
6.	17.77	Citronellyl formate	Fatty alcohol esters	**10.41**	$C_{11}H_{20}O_2$	41.00	184
7.	18.56	Geraniol formate	Fatty alcohol esters	1.91	$C_{11}H_{18}O_2$	69.00	182
8.	21.89	Caryophyllene	Bicyclic sesquiterpene	2.15	$C_{15}H_{24}$	41.00	204
9.	23.50	Germacrene D	Monocyclic sesquiterpene	1.41	$C_{15}H_{24}$	161.05	204
10.	24.56	δ-Cadinene,	Bicyclic sesquiterpenes	2.34	$C_{15}H_{24}$	159.10	204
11.	27.03	8-epi-γ-eudesmol	Bicyclic sesquiterpenoid	4.75	$C_{15}H_{26}O$	189.10	222
Monoterpene				64.95			
Sesquiterpene				10.65			
Fatty alcohol esters				12.32			
Total identified				87.92			

Bold values indicate constituents with the highest concentration.

detected were methyl methanthranilate (63.45%), γ-terpinene (18.64%), and D-limonene (7.23%). GC-MS data showed different compositional patterns compared to the previous studies except for the percentage of D-limonene. Khalid *et al.* [21] studied the effects of geographical locations of Egypt on EO composition from leaves and flowers and showed that plant source has significant variation in orange EO composition where sabinene (33.8%–44.9%) and terpinen-4-ol (15.6%–22.6%), where the major constituents followed by Δ-3-carene (9.3%–12.4%) and limonene (5.5%%–8.3%). In another study, major components of leaf EO were terpinen-4-ol (14.1%), limonene (10.18%), β-pinene (8.73%), and *trans*-sabinene hydrate (8.21%) [22].

d-Limonene and peel oil from different *Citrus* species were reported to have acaricidal activity against *R. microplus,* related species to *R. annulatus* where peel oil from *Citrus maxima* (mature and immature fruits) and *Citrus reticulata* exhibited two times stronger acaricidal than *d*-limonene on female tick. *Citrus sinensis*, *C. maxima* (mature and immature fruits), *Citrus hystrix, Citrus suncris*, and *C. reticulata*

Table 6. Major constituents determined by GC-MS analysis of sweet basil leaves EO (*O. basilicum*).

No	R_t	Compound name	Chemical class	%	Mol. formula	Base *m/z*	MI peaks
1.	10.32	1,8-Cineole	Bicyclic monoterpenoid	**9.66**	$C_{10}H_{18}O$	43.00	154
2.	12.49	*β*-Linalool	Acyclic monoterpenoid	**55.63**	$C_{10}H_{18}O$	71.00	154
3.	18.10	bornyl acetate	Fatty alcohol esters	1.32	$C_{12}H_{20}O_2$	95.05	196
4.	20.19	Eugenol	Phenols	2.60	$C_{10}H_{12}O_2$	164.05	164
5.	21.08	β-Elemene	Monocyclicsesquiterpene	3.01	$C_{15}H_{24}$	93.05	204
6.	22.24	α-Bergamotene	Bicyclicsesquiterpene	**6.50**	$C_{15}H_{24}$	93.05	204
7.	22.34	α.-Guaiene	Bicyclic sesquiterpene	0.93	$C_{15}H_{24}$	105.05	204
8.	22.70	α-Humulene	Monocyclic sesquiterpene	0.99	$C_{15}H_{24}$	93.00	204
9.	23.50	Germacrene D	Monocyclic sesquiterpene	**4.09**	$C_{15}H_{24}$	161.05	204
10.	24.13	α.-bulnesene	Bicyclic sesquiterpene	1.78	$C_{15}H_{24}$	107.05	204
11.	24.34	γ.-Muurolene	Bicyclic sesquiterpene	2.81	$C_{15}H_{24}$	161.10	204
12.	27.48	tau.cadinol.	Bicyclic sesquiterpenoid	2.60	$C_{15}H_{26}O$	161.10	222
Monoterpene				65.29			
Sesquiterpene				22.71			
Phenols				2.6			
Fatty alcohol esters				1.32			
Total identified				91.92			

Bold values indicate constituents with the highest concentration.

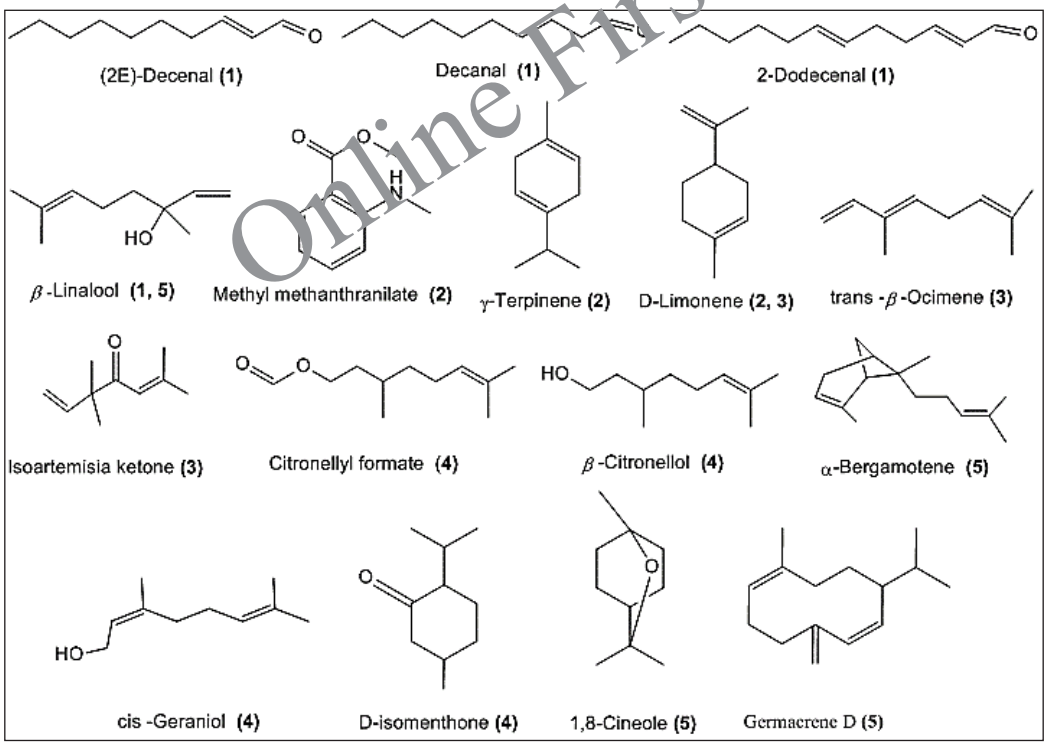

Figure 1. Chemical structure of major compounds identified in the EO of (1) cilantro leaves, (2) orange leaves, (3) *Tagetes* flower, (4) geranium herb, and (5) sweet basil herb. The number between brackets indicates the plant source.

exhibited 1.5 times more larvicidal activity than *d*-limonene [23]. Depending on the acaricidal activity of *d*-limonene, orange oil activity may be attributed to the synergistic action of its constituents also minor components may contribute to the activity.

Acaricidal activity and chemical composition of *Tagetes* flower EO

LPT showed 100% mortality at 10% concentration and about 96.00% at 5% concentration with LC_{50} of 2.94 and

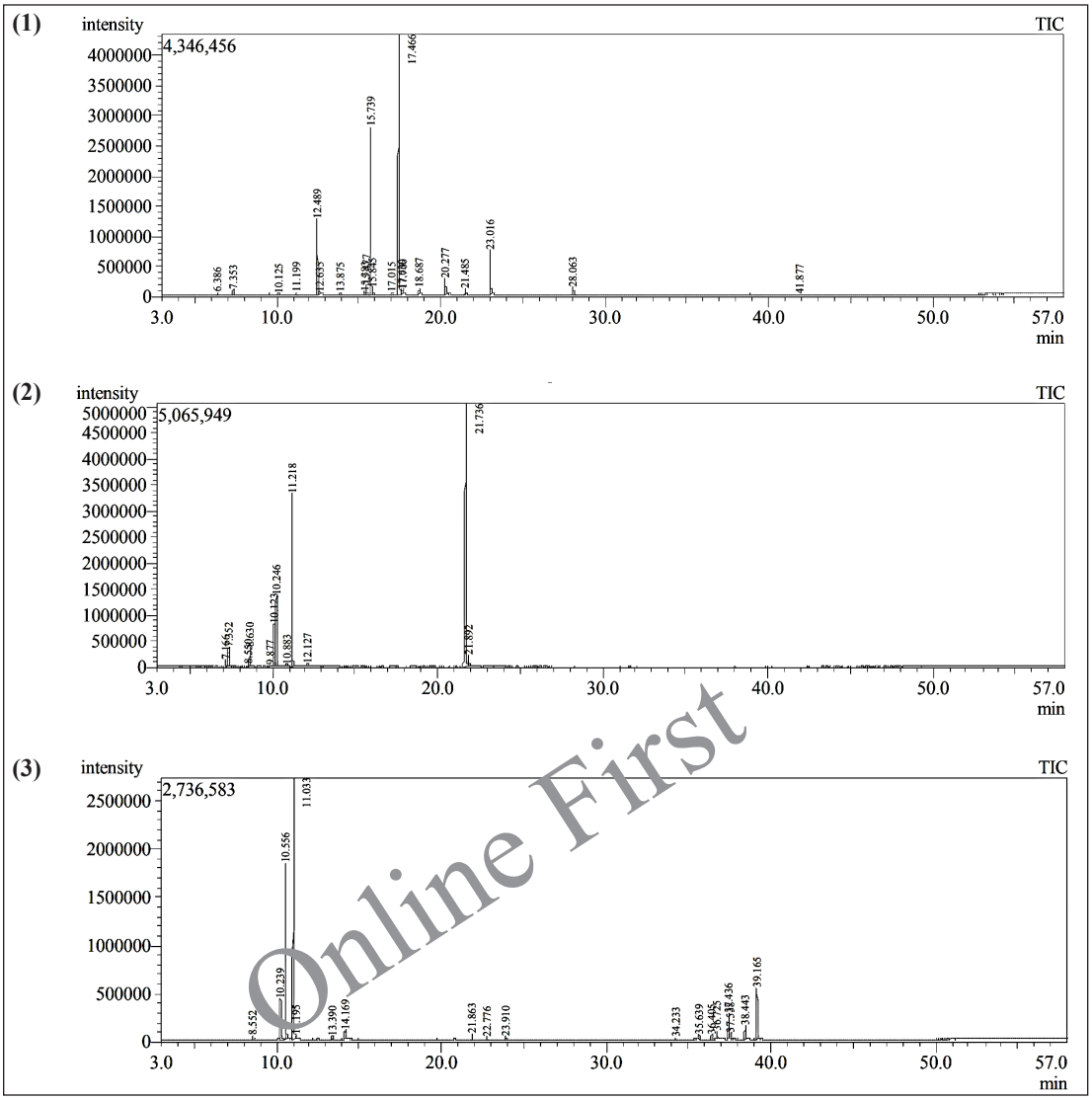

Figure 2. GC-MS chromatogram of the EO of (1) cilantro leaves, (2) orange leaves, and (3) *Tagetes* flower.

LC_{99} of 6.05 µg/ml, while 2.5% showed low larval mortality % (28.33 ± 4.41). Our chemical investigation of *T. erecta* flower EO, showed that the major constituents were *trans*-β-ocimene (24.93%), D-limonene (6.68%), and isoartemisia ketone (8.84%) (Table 4, Fig. 2). EO showed variation in its main components according to plant source. Reported EO composition of fresh flowers collected in México were piperetone (19.2%), b-caryophyllene (15.2%), and (E)-ocimene (13.7%), and also limonene (11.7%) was detected [24], and that is collected from Italy were piperitone 28.9%, terpinolene 5.8%, phyllene 3.8%, and limonene 3.5% [25], while flowers collected from Nigeria, characterized by the presence of 1, 8-cineole (23.1%) as the major constituents followed by α-pinene (11.8%), α-terpineol (10.7%), and piperitone (8.0%) [26].

The Brazilian *T. erecta* L. leaves essential showed schistosomicidal effects after 24 hours against *Schistosoma mansoni* with 50 µg/ml minimum inhibitory concentration and parasites death and coupled pairs separation at 100 µg/ml after 24 hours [27]. In addition, EO was reported to have antifungal activity against *Aspergillus terreus* and *Colletotrichum falcatum* [28].

Acaricidal activity and chemical composition of geranium herb EO

LPT showed 96.33% mortality at 10% concentration and 56.67 at 5% concentration with LC_{50} of 5.28 and LC_{99} of 10.07 µg/ml, while 2.5% concentration showed very low larval mortality % (3.33 ± 1.67). GC-MS analysis of geranium oil (*P. graveolens*, family Geraniaceae) revealed the presence of β-citronellol (41.83%), citronellyl formate (10.41%), geraniol (9.47%), D-isomenthone (8.89%), β-linalool (3.01%) (Table 5, Fig. 3). These major GC-MS detected components were consistent with the previous studies [29] but with different percentages, namely, β-citronellol (44.5%), geraniol (13.7%), citronellyl formate (7.3%), β-linalool (3.9%), and D-isomenthone (3.5%).

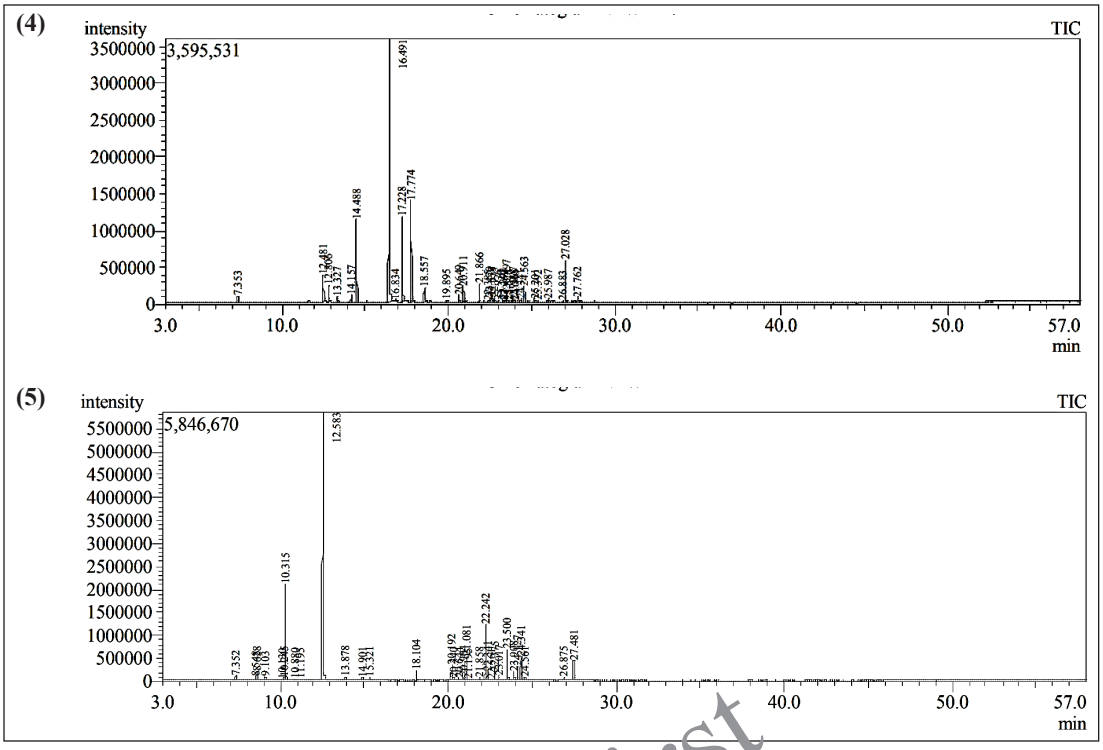

Figure 3. GC-MS chromatogram of the EO of (4) geranium herb and (5) sweet basil herb.

EO of geranium leaves was reported to have acaricidal activity against different stages of *R. annulatus*, where LC of geranium EO, its nanoemulsion, and its combination with the sesame oil against the adult ticks were 7.53%, 5.60%, and 1.91%, respectively, and against larvae were 3.435%, 1.688%, and 0.944%, respectively. In addition, *in-vivo* tested nanoemulsion and geranium-sesame oil combination showed a significant reduction in tick burden 3 weeks post application to 87.97% and 74.83%, respectively [30]. In addition, geranium EO inhibited the oviposition of *R. microplu*s by 97% at 10% concentration [31].

Acaricidal activity and chemical composition of sweet basil EO

LPT showed 92.33% mortality at 10% concentration with LC_{50} of 7.2 and LC_{99} of 13.46 µg/ml, while 5% other concentrations were inactive (only 6.67% ± 1.67% larval mortality). GC-MS analysis of sweet basil oil (*O. basilicum*, family; Lamiaceae) revealed that β-Linalool (55.63%) was the major component in addition to 1,8-cineole (9.66%), α-bergamotene (6.50%), and germacrene D (4.09%) (Table 6, Fig. 3). Reported data on sweet basil leaf oil presented that the major compounds detected were linalool (30.61%) and estragole (20.04%), followed by a nearly equal percentage of *α*-farnesene, eugenol, and 1,8-cineole [32]. In addition, GC-MS analysis of EO extracted from three varieties of basil showed that the most common compounds detected in *var. Nu Far* were linalool (52.2%), estragole (18.2%), and sabinene (6.71%), and in *var. Jolina* were linalool (43.9%), eugenol (11.2%), and α-bergamotene (9.19%), and *var.* in *Aroma* were linalool (48.2%), sabinene (8.99%), and eugenol (8.71%) [33]. Linalool was the dominant component in the reported data which is in agreement with our chemical study of the oil.

Aboelhadid *et al.* [34] studied the larvicidal and repellent efficacy of the oil and its nanocomposite (*O. basilicum* EO/layered double hydroxide) against *R. annulatus* and its results showed 100% larval mortality by the oil at 300 µl/ml and by nanocomposite at 200 µl/ml. Oil and nanocomposite have 100% adult mortality, prevent egg deposition and eggs hatching at a dose of 300 µl/ml [34], but oil did not show any acaricidal activity on 10-day-old *R. microplus* larvae [32], while eugenol which was detected as a minor compound (2.60%) in our study reported having 100% acaricidal activity at 2% against *R. microplus* larvae [35]. Oil has been widely investigated against other insects and showed a high lethal effect on adult mosquitoes (93%–95%) [36], *Eutetranychus orientalis* (Klein), eggs number reduction with 100–87.5 oviposition deterrence indices at 2%–0.5% against *Tetranychus urticae* (Koch) and *E. orientalis* mites [37]; also, methanol extract of the leaves and flowers have insecticidal activity on larvae of the Egyptian cottonworm (*Spodoptera littoralis)* with 1.7 µg/ml lC_{50} [38] and yellow fever mosquito (*Aedes aegypti*) with 3.7%–5.1% LC_{50} for I–IV instar larvae and 5.449% LC_{50} of pupae [39].

CONCLUSION

Our results recommended the use of EOs of five medicinal plants; cilantro leaves, orange leaves, *Tagetes* flower, geranium herb, and sweet basil herb as environment-friendly acaricides for *R. annulatus* control tick control. EO from cilantro is considered the most potent among the tested oils; (LC_{99} = 2.77 µg/ml), followed by orange leaf (3.78 µg/ml), *Tagetes* flower (6.05 µg/ml), geranium herb (10.07 µg/ml), and finally sweet

basil (13.46 µg/ml). The acaricidal activity of cilantro, orange leaf, and *Tagetes* flower EOs against *R. annulatus* are reported here for the first time. The high mortality percentage caused by these oils will shed light on natural alternatives for tick control which will have both economic and environmental impact and encourage us to pursue more future work to get pharmaceutical products.

LIST OF ABBREVIATIONS

EOs: essential oils; GC-MS: gas chromatography-mass spectrometry; LC_{50}: lethal concentrations that kill 50%; LC_{99}: lethal concentrations that kill 99%; LPT: larval packet test; NIST: National Institute of Standards and Technology; SE: standard error.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data. A.M., H. A. and W. A. choose the oils and designed the experiments. H. A. E. A. and A.O. analyzed the oils and interpreted the data, K.H. and K.H. collected the ticks and grew the larvae. W. A, K. H. and K. H. made the larvicidal activity, analyzed the data and made the statistical analysis. All authors were involved in writing, review and editing and gave the final approval of the version to be published.

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The authors declare that they have no conflict of interest.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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