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Original article

Larvicidal potential of *Thuja orientalis* leaves and fruits extracts against *Culex pipiens* (Diptera: Culicidae)

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ABSTRACT

Botanical pesticides targeted to avoid the pesticide resistance to synthetic ones that conventionally affecting ecosystems diversity. The study designed to evaluate the larvicidal potentials of leaves and fruits extracts (methanol, acetone, hexane, and aqueous) from *Thuja orientalis* (Pinales: Cupressaceae) against *Culex pipiens* 3rd instar larvae and to identify the extracts compounds by gas chromatography-mass spectrometry (GC–MS) method of analysis. Leaves and fruits extracts used in concentrations, 25, 50, 100, 200, and 400 ppm against the 3rd larval instar of *Cx. pipiens* in five replicates and larval mortalities recorded after 24 and 48 h post exposure. The larvicidal potentials of extracts showed concentration dependent and varied between leaves and fruits extracts. At 400 ppm concentration, leaves extracts showed 100 % larval mortality except in hexane extract exhibited 98 %. Acetone, methanol, aqueous and hexane leaves extracts recorded LC_{50} values, 58.04, 70.20, 77.19 and 84.25 ppm, respectively. Fruits extracts by hexane and methanol exhibited 100 % larval mortality and LC_{50}, 68.26 and 83.21 ppm, respectively, while, acetone and aqueous fruits extracts showed 98 % and 96 % larval mortality and LC_{50}, 92.81 and 102.97 ppm, respectively. Terpenoids and sesquiterpenoids, fatty acid esters mainly identified in both extracts.

The present study showed that extracts from *Thuja orientalis* leaves and fruits acquired promising larvicidal potential for the control of *Cx. pipiens* larvae with the role of their chemical constituents.

1. Introduction

Mosquitoes considered as a burden to human health by transmitting diseases including malaria, filariasis, dengue, and leishmaniasis (Wilson et al., 2020). *Culex pipiens* mosquitoes is common house mosquito and one of the most widely distributed mosquitoes worldwide due to its adaptation to human environments and its mode of feeding on birds and mammals reflected by its role in transmission of the West Nile virus and other pathogens (Farajollahi et al., 2011) that urged researches of its control.

Vector control is the main way to reduce public concerns about mosquito-borne diseases including filariasis, dengue, malaria, and leishmaniasis (Wilson et al., 2020). Control of mosquito larvae in aquatic phases is an effective method for reducing mosquito-spread (WHO, 2013). Excessive use of synthetic insecticides, with a complete lack of awareness of the strategy of changing the pesticides, led to resistance to pesticides along with environmental pollution and health risks to humans and non-target biota. Therefore, the search for environmentally friendly alternatives as plants or oils rich in secondary metabolites is a recent trend since they are more efficient, less toxic, biodegradable, and capable of insect decrease plant resistance to these natural compounds (Mouden et al., 2017; Ahmed et al., 2021) besides serving as larvicides, adult pesticides, insect repellents and deterrents (Govindarajan et al., 2016) they destroy only the insects they are meant to kill, leaving no residue on food or in the environment.

Thuja orientalis is a dense, evergreen and coniferous tree belongs to the family Cupressaceae growing in Saudi Arabia (Elsharkawy et al., 2017; Elsharkawy and Ali, 2019). In folk medicine *T. orientalis* used as herbal medicine for treatment of psoriasis, amenorrhea, enuresis, rheumatism, cystitis, bronchial catarrh and uterine carcinomas (Srivastava et al., 2012). Studies evaluated different activities of *T. orientalis* like, antimicrobial (Choi et al., 2021), antifungal activity (Caruntu et al., 2020), antioxidant (Nizam and Mushfiq, 2007), anticancer (Elsharkawy et al., 2017),

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anti-inflammatory (Darwish et al., 2021; Shin et al., 2015), hair growth promotion (Zhang et al., 2013).

The present study designed to investigate the larvicidal activities of methanol, hexane and acetone and aqueous extracts from leaves and fruits of *Thuja orientalis* plant against *Cx. pipiens* third instar larvae after exposure to extracts for 24 and 48 h. As well, identification of the chemical composition of the tested extracts by the aid of gas chromatography-mass spectrometry (GC–MS) method of analysis.

2. Materials and methods

2.1. Plants materials

Thuja orientalis L. (Pinales: Cupressaceae) leaves and fruits were obtained from Arar region, at Northern Region of Saudi Arabia, where growing wild in March 2022 as previously recorded (Elsharkawy and Ali, 2019). The plant was identified by a taxonomist, prof. Dr. Yahya Masrahi, from the Department of Biology, Faculty of Science, Jazan University, Saudi Arabia.

2.2. Culex pipiens colony

Mosquitoes (*Cx. pipiens*) were obtained from the Center for Environmental Research and Studies at Jazan University. Rearing was performed under controlled conditions (27 ± 2 °C, relative humidity at 70 % ± 10 %, and 12:12 h light:dark regime). Mosquito larvae were reared in round enamel plates (25 × 20 × 10 cm) filled with 2 L de-chlorinated water and fed with fish food daily. The third instar *Cx. pipiens* larvae were used for the larvicidal examination.

2.3. Plant extracts.

Leaves and fruits were dried in shade for 7 days at laboratory temperatures (27–29 °C). The dried leaves (40 g) and fruits (25 g) were powdered using a commercial electrical stainless-steel blender and extracted for each solvent including methanol, acetone, hexane, and aqueous using Soxhlet apparatus for 6–8 h according to the solvent type. The leaves and fruits extracts were filtered with Whatman number 1 filter paper through a Buchner funnel. The filtrates then dried using a rotary evaporator under vacuum at 40 °C. The plant leaves extract yields 3.7, 3.2, 2.8, and 1.7 g for methanol, acetone, hexane, and aqueous solvents and yields 2.1, 1.5, 2.3 and 1.1 g for plant fruits, respectively.

2.4. Larvicidal assay

The larvicidal activity was determined for the extracts in concentrations that prepared as 25, 50, 100, 200, and 400 ppm based on 1 g/1L (1000 ppm) from each extract stock solution against the 3rd larval instar of *Cx. pipiens* (WHO, 2005). Twenty *Cx. pipiens* third instar larvae of were subjected to each extract concentration in 250 mL glass beakers containing 150 mL de-chlorinated water (aqueous suspension) at 27 ± 2 °C, 70 ± 10 % RH, and a 12:12 h (L/D) photoperiod. Five replicates per concentration per extract and control were conducted. Larval mortalities were recorded after 24 and 48 h of exposure.

2.5. Identification of chemical compounds in extracts by gas chromatography-mass spectrometer.

Extracts from promising plant, *Thuja orientalis* L. were analyzed to investigate their chemical constituents by GC–MS using the Trace GC-TSQ mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) through TG–5MS direct capillary column (30 m × 0.25 mm × 0.25 m thickness of the film) where the column oven temperature initially maintained at 50 °C, then rate was increased 5 °C/min up to 250 °C, for 2 min, and then increased 30 °C/min to 300 °C. The lines for the injector and MS transfer were suspended at 280 °C and 260 °C, respectively, helium was the carrier gas at the rate of 1 mL/min. The solvent delay was 3 min and 2 µl samples were injected automatically using Autosampler AS1310 coupled with GC in the splitless mode. In full scanning mode, electrospray ionization (EI) mass spectra were obtained covering the range 50–650 m/s at an ionization voltage of 70 V. The ion source temperature was fixed at 200 °C. The chemical constituents were identified from the Total Ion Chromatogram (TIC), where the chemical compounds were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral databases.

2.6. Data analysis

The percentage mortalities were determined according to Abbott, (1925). The larval control results did not need correction, as the mortality was less than 5 %, according to the WHO guidelines (WHO, 2005) (no larval control mortality recorded throughout the study). Mortality data from all the replicates were performed using one-way analysis of variance (ANOVA) to find the differences among the activity between each plant extract concentrations using the least significant difference (LSD) test. Also, data from all the replicates were subjected to analysis to determine the larval LC_{50}, LC_{90}, and LC_{95} as well as chi-square values within confidence limits at 95 % (lower confidence limit (LCL) and upper confidence limit (UCL) by using probit analysis and regression between log extract concentration and probit values. Data analysis was performed using SPSS software (IBM SPSS Statistics v22 – 64 bit), and $p < 0.05$ was considered significant.

3. Results

3.1. Larvicidal activity

The data about the larvicidal activities of the tested *Thuja orientalis* leaves extracts against the third instar larvae of *Cx. pipiens* after 24 h are summarized in Table 1, and after 48 h are summarized in Table 2. The analysis of responses of the leaves extracts revealed that the larvicidal activities after 24 h showed 100 % mortalities at 400 ppm of methanol, acetone and aqueous extracts. While hexane leaves extract at 400 ppm showed 98 % larval mortality. Acetone leaves extract showed the highest efficacy by inducing 100 % mortality (LC_{50}, 58.04, LC_{90}, 169.27, and LC_{95}, 229.27 ppm), followed by methanol extract by 100 % mortality (LC_{50}, 70.20, LC_{90}, 221.66, and LC_{95}, 307.07 ppm), followed by aqueous extract by 100 % mortality (LC_{50}, 77.19, LC_{90}, 244.26, and LC_{95}, 338.95 ppm). While, hexane extract showed the least efficacy as compared to others, inducing 98.00 % mortality (LC_{50}, 84.25, LC_{90}, 283.47, and LC_{95}, 399.84 ppm) (Table 1). The larvicidal activities of the leaves extracts after 48 h showed 100 % mortalities at 400 ppm for all tested extracts. Acetone leaves extract showed the highest efficacy (LC_{50}, 43.40, LC_{90}, 102.62, and LC_{95}, 130.98 ppm), followed by methanol extract (LC_{50}, 54.75, LC_{90}, 156.00, and LC_{95}, 209.92 ppm), followed by the aqueous extract (LC_{50}, 63.76, LC_{90}, 198.50, and LC_{95}, 273.89 ppm). Then, hexane showed (LC_{50}, 69.02, LC_{90}, 225.14, and LC_{95}, 314.78 ppm) (Table 2).

The results about the larvicidal activities of the tested *T. orientalis* fruits extracts against the third instar larvae of *Cx. pipiens* after 24 h are summarized in Table 3 and after 48 h are summarized in Table 4. The analysis of fruits extracts data revealed that the

Table 1
The larvicidal effects of leaves extracts of *Thuja orientalis* (Pinales: Cupressaceae) against the third instar larvae of *Culex pipiens* at 24 h post-treatment.

Leaves extract	Conc. ppm	Mortality% (Mean ± SE)	LC_{50} (LCL–UCL.)	LC_{90} (LCL–UCL.)	LC_{95} (LCL–UCL.)	Chi (Sig)	Regression equation	R^2
Methanol	0.0	0.00 ± 0.0[a]	70.20	221.66	307.07	4.417	Y = −4.33 + 2.33*x	0.997
	25	15.00 ± 2.24[b]	(61.92–79.19)	(185.53–278.05)	(248.31–404.83)	(0.220[a])		
	50	35.00 ± 3.16[c]						
	100	61.00 ± 4.30[d]						
	200	86.00 ± 2.45[e]						
	400	100.00 ± 0.00[f]						
Acetone	0.0	0.00 ± 0.0[a]	58.04	169.27	229.27	1.884	Y = −4.68 + 2.65*x	0.997
	25	18.00 ± 2.55[b]	(51.25–65.24)	(143.49–208.94)	(188.22–296.63)	(0.597[a])		
	50	41.00 ± 2.92[c]						
	100	72.00 ± 2.55[d]						
	200	93.00 ± 2.55[e]						
	400	100.00 ± 0.00[f]						
Hexane	0.0	0.00 ± 0.0[a]	84.25	283.47	399.84	3.348	Y = −4.95 + 2.6*x	0.976
	25	12.00 ± 2.00[b]	(74.21–95.43)	(234.18–361.87)	(318.35–537.98)	(0.341[a])		
	50	29.00 ± 3.32[c]						
	100	54.00 ± 2.45[d]						
	200	79.00 ± 2.45[e]						
	400	98.00 ± 1.22[f]						
Aqueous	0.0	0.00 ± 0.0[a]	77.19	244.26	338.95	5.630	Y = −4.32 + 2.27*x	
	25	13.00 ± 2.00[b]	(68.22–87.06)	(204.00–307.18)	(273.22–447.30)	(0.131[a])		0.999
	50	31.00 ± 4.00[c]						
	100	58.00 ± 2.55[d]						
	200	82.00 ± 1.22[e]						
	400	100.00 ± 0.00[f]						

Significance at 0.05 level between different superscripts. (a) In Chi-Square Tests, no heterogeneity factor was used in the calculation of confidence limits because the significance level was greater than 0.05.

Table 2
The larvicidal effects of leaves extracts of *Thuja orientalis* (Pinales: Cupressaceae) against the third instar larvae of *Culex pipiens* at 48 h post-treatment.

Leaves extract	Conc. ppm	Mortality % (Mean ± SE)	LC_{50} (LCL–UCL.)	LC_{90} (LCL–UCL.)	LC_{95} (LCL–UCL.)	Chi (Sig)	Regression equation	R^2
Methanol	0.0	0.00 ± 0.0[a]	54.75	156.00	209.92	2.260	Y = −4.8 + 2.77*x	0.992
	25	20.00 ± 1.58[b]	(48.63–61.48)	(132.59–192.02)	(172.87–270.73)	(0.520[a])		
	50	42.00 ± 4.90[c]						
	100	75.00 ± 4.18[d]						
	200	95.00 ± 3.16[e]						
	400	100.00 ± 0.00[e]						
Acetone	0.0	0.00 ± 0.0[a]	43.40	102.62	130.98	3.112	Y = −5.26 + 3.21*x	0.988
	25	24.00 ± 2.45[b]	(38.72–48.22)	(88.81–123.99)	(110.22–165.22)	(0.375[a])		
	50	53.00 ± 4.06[c]						
	100	89.00 ± 1.87[d]						
	200	100.00 ± 0.00[e]						
	400	100.00 ± 0.00[e]						
Hexane	0.0	0.00 ± 0.0[a]	69.02	225.14	314.78	4.622	Y = −4.15 + 2.24*x	0.999
	25	16.00 ± 1.87[b]	(60.68–78.06)	(187.68–284.10)	(253.15–418.36)	(0.202[a])		
	50	36.00 ± 1.87[c]						
	100	62.00 ± 2.55[d]						
	200	85.00 ± 2.24[e]						
	400	100.00 ± 0.00[f]						
Aqueous	0.0	0.00 ± 0.0[a]	63.76	198.50	273.89	4.041	Y = −4.38 + 2.43*x	
	25	18.00 ± 2.55[b]	(56.17–71.92)	(166.68–248.05)	(222.26–359.70)	(0.257[a])		0.991
	50	37.00 ± 2.00[c]						
	100	65.00 ± 4.18[d]						
	200	90.00 ± 4.18[e]						
	400	100.00 ± 0.00[f]						

Significance at 0.05 level between different superscripts. (a) In Chi-Square Tests, no heterogeneity factor was used in the calculation of confidence limits because the significance level was greater than 0.05.

larvicidal activities after 24 h showed 100 % mortalities at 400 ppm of methanol and hexane extracts. While acetone and aqueous fruits extract at 400 ppm showed 98 % and 96 % larval mortalities, respectively. Hexane fruits extract showed the highest efficacy by inducing 100 % mortality (LC_{50}, 68.26, LC_{90}, 201.48, and LC_{95}, 273.84 ppm), followed by methanol extract by 100 % mortality (LC_{50}, 83.21, LC_{90}, 253.85, and LC_{95}, 348.25 ppm). The acetone extract induced 98 % mortality (LC_{50}, 92.81, LC_{90}, 296.34, and LC_{95}, 411.83 ppm). While, the aqueous extract showed the least efficacy as compared to others, inducing 96 % mortality (LC_{50}, 102.97, LC_{90}, 333.60, and LC_{95}, 465.54 ppm) (Table 3). The larvicidal activities of the fruits extracts after 48 h showed 100 %

Table 3
The larvicidal effects of fruits extracts of *Thuja orientalis* (Pinales: Cupressaceae) against the third instar larvae of *Culex pipiens* at 24 h post-treatment.

Fruit extract	Conc. ppm	Mortality% (Mean ± SE)	LC_{50} (LCL–UCL.)	LC_{90} (LCL–UCL.)	LC_{95} (LCL–UCL.)	Chi (Sig)	Regression equation	R^2
Methanol	0.0	0.00 ± 0.0[a]	83.21	253.85	348.25	5.980	Y = −4.55 + 2.34*x	1.000
	25	10.00 ± 1.58[b]	(73.84–93.61)	(212.80–317.49)	(282.48–456.44)	(0.113[a])		
	50	29.00 ± 2.92[c]						
	100	55.00 ± 4.74[d]						
	200	80.00 ± 3.16[e]						
	400	100.00 ± 0.00[f]						
Acetone	0.0	0.00 ± 0.0[a]	92.81	296.34	411.83	3.894	Y = −5.27 + 2.71*x	0.974
	25	9.00 ± 1.00[b]	(82.18–104.78)	(246.13–375.30)	(330.40–547.84)	(0.273[a])		
	50	25.00 ± 1.58[c]						
	100	51.00 ± 3.32[d]						
	200	76.00 ± 2.92[e]						
	400	98.00 ± 1.22[f]						
Hexane	0.0	0.00 ± 0.0[a]	68.26	201.48	273.84	2.659	Y = −4.68 + 2.54*x	1.000
	25	13.00 ± 2.55[b]	(60.49–76.66)	(170.32–249.29)	(224.24–354.73)	(0.447[a])		
	50	36.00 ± 1.00[c]						
	100	65.00 ± 4.18[d]						
	200	88.00 ± 3.00[e]						
	400	100.00 ± 0.00[f]						
Aqueous	0.0	0.00 ± 0.0[a]	102.97	333.60	465.54	2.596	Y = −5.18 + 2.59*x	0.987
	25	7.00 ± 1.22[b]	(91.14–116.45)	(275.53–425.82)	(371.08–624.83)	(0.458[a])		
	50	23.00 ± 2.00[c]						
	100	46.00 ± 4.30[d]						
	200	73.00 ± 2.00[e]						
	400	96.00 ± 1.87[f]						

Significance at 0.05 level between different superscripts. (a) In Chi-Square Tests, no heterogeneity factor was used in the calculation of confidence limits because the significance level was greater than 0.05.

Table 4
The larvicidal effects of fruits extracts of *Thuja orientalis* (Pinales: Cupressaceae) against the third instar larvae of *Culex pipiens* at 48 h post-treatment.

Fruits extract	Conc. ppm	Mortality% (Mean ± SE)	LC_{50} (LCL–UCL.)	LC_{90} (LCL–UCL.)	LC_{95} (LCL–UCL.)	Chi (Sig)	Regression equation	R^2
Methanol	0.0	0.00 ± 0.0[a]	68.62	205.88	281.12	3.189	Y = −4.56 + 2.47*x	0.999
	25	14.00 ± 2.92[b]	(60.74–77.16)	(173.67–255.45)	(229.59–365.45)	(0.363[a])		
	50	34.00 ± 3.67[c]						
	100	66.00 ± 4.30[d]						
	200	87.00 ± 5.83[e]						
	400	100.00 ± 0.00[e]						
Acetone	0.0	0.00 ± 0.0[a]	76.43	236.40	325.58	5.274	Y = −4.44 + 2.34*x	0.997
	25	13.00 ± 3.00[b]	(67.66–86.07)	(198.09–295.92)	(263.83–427.56)	(0.153[a])		
	50	31.00 ± 4.85[c]						
	100	57.00 ± 4.66[d]						
	200	84.00 ± 4.00[e]						
	400	100.00 ± 0.00[e]						
Hexane	0.0	0.00 ± 0.0[a]	50.60	119.30	152.14	4.305	Y = −5.21 + 3.04*x	0.994
	25	18.00 ± 3.39[b]	(45.43–56.07)	(103.29–143.64)	(128.27–190.61)	(0.230[a])		
	50	45.00 ± 3.54[c]						
	100	82.00 ± 6.04[d]						
	200	100.00 ± 0.00[e]						
	400	100.00 ± 0.00[e]						
Aqueous	0.0	0.00 ± 0.0[a]	81.85	253.48	349.23	6.258	Y = −4.46 + 2.3*x	0.998
	25	11.00 ± 1.87[b]	(72.54–92.17)	(212.04–317.98)	(282.50–459.55)	(0.100[a])		
	50	30.00 ± 2.24[c]						
	100	54.00 ± 2.92[d]						
	200	81.00 ± 3.32[e]						
	400	100.00 ± 0.00[f]						

Significance at 0.05 level between different superscripts. (a) In Chi-Square Tests, no heterogeneity factor was used in the calculation of confidence limits because the significance level was greater than 0.05.

mortalities at 400 ppm for all tested extracts. Hexane extract showed the highest efficacy (LC_{50}, 50.60, LC_{90}, 119.30, and LC_{95}, 152.14 ppm), followed by methanol extract (LC_{50}, 68.62, LC_{90}, 205.88, and LC_{95}, 281.12 ppm), followed by the acetone extract (LC_{50}, 76.43, LC_{90}, 236.40, and LC_{95} (), 325.58 ppm). While, aqueous showed (LC_{50}, 81.85, LC_{90}, 253.48, and LC_{95}, 349.23 ppm) (Table 4).

3.2. Chemical analysis

The GC–MS analysis revealed that the main constituents of the *T. orientalis* leaves extracts were the sesquiterpenoids and terpenoids (Table 5) and (Fig. 1). The main % area for sesquiterpenoids compounds commonly detected in all extracts, were, cedrol (33.03, 35.77, 33.99 and 38.25 %), caryophyllene (18.49, 25.31, 21.68 and

Table 5
Chemical constituents of *Thuja orientalis* leaves methanol, hexane, acetone and aqueous extracts.

No	Molecular formula	Chemical compound	Methanol (%)	Hexan (%)	Acetone (%)	Aqueous (%)	Nature of compound
1	$C_{12}H_{20}O_2$	Bornyl acetate	0.39	–	0.44	–	Bicyclic monoterpenoid
2	$C_{12}H_{20}O_2$	α-Terpinyl acetate	0.48	–	0.36	–	Monoterpene ester monoterpenoid
3	$C_{17}H_{28}O_2$	Elemyl acetate	–	–	0.75	–	Monocyclic monoterpenoid
4	$C_{20}H_{32}O$	Pimara-7,15-dien-3-ol	6.04	–	1.53	0.54	Terpenoid
5	$C_{20}H_{34}O$	Copalol	0.51	–	–	–	Terpenoid
6	$C_{20}H_{30}O$	Sugiol	1.49	–	–	–	Terpenoid
7	$C_{20}H_{30}O$	Totarol	0.52	–	–	–	Terpenoid
8	$C_{20}H_{30}O$	Dehydro-4-epiabietol	–	–	0.73	1.03	Terpenoid
9	$C_{15}H_{24}$	β-Elemene	0.52	–	1.73	–	Sesquiterpenoid
10	$C_{15}H_{24}$	1,7-Di-*epi*-β-cedrene	2.47	–	2.06	–	Sesquiterpenoid
11	$C_{15}H_{24}$	**Caryophyllene**	**18.49**	**25.31**	**21.68**	**20.67**	Sesquiterpenoid
12	$C_{15}H_{24}$	α-Muurolene	0.29	–	1.80	0.31	Sesquiterpenoid
13	$C_{15}H_{24}$	**γ-Elemene**	**0.28**	**1.54**	**0.62**	**1.52**	Sesquiterpenoid
14	$C_{15}H_{24}$	**Humulene**	**10.94**	**15.22**	**12.28**	**12.35**	Sesquiterpenoid
15	$C_{15}H_{24}$	**Cedrene**	**0.39**	**2.66**	**0.60**	**2.33**	Sesquiterpenoid
16	$C_{15}H_{24}$	**Selinene**	**1.27**	**1.18**	**1.32**	**1.59**	Sesquiterpenoid
17	$C_{15}H_{24}$	**Germacrene D**	**2.80**	**4.00**	**3.60**	**3.35**	Sesquiterpenoid
18	$C_{15}H_{24}$	Valencen	1.05	–	1.44	–	Sesquiterpenoid
19	$C_{15}H_{24}$	**γ-Muurolene**	**1.17**	**1.47**	**1.08**	**1.26**	Sesquiterpenoid
20	$C_{15}H_{24}$	**Cadina-1(10),4-diene**	**1.29**	**1.67**	**1.30**	**1.58**	Sesquiterpenoid
21	$C_{15}H_{24}$	Alloaromadendrene	1.11	–	2.11	0.64	Sesquiterpenoid
22	$C_{15}H_{24}$	Cedrenol	–	–	–	0.20	Sesquiterpenoid
23	$C_{15}H_{24}$	Aromandendrene	–	–	–	1.65	Sesquiterpenoid
24	$C_{15}H_{24}O$	**Caryophyllene oxide**	**1.16**	**1.25**	**1.39**	**1.29**	Sesquiterpenoid
25	$C_{15}H_{26}O$	**Allocedrol**	**2.26**	**2.22**	**2.16**	**2.43**	Sesquiterpenoid
26	$C_{15}H_{26}O$	**Cedrol**	**33.03**	**35.77**	**33.99**	**38.25**	Sesquiterpenoid alcohol
27	$C_{15}H_{26}O$	Cedran-8-ol	0.19	–	0.14	–	Sesquiterpenoid
28	$C_{15}H_{26}O$	α -acorenol	1.20	–	1.64	–	Sesquiterpenoid
29	$C_{15}H_{26}O$	**α-Eudesmol**	**1.95**	**1.92**	**2.91**	**2.58**	Cycloeudesmane sesquiterpene
30	$C_{15}H_{24}$	β-Chamigrene	–	1.81	–	–	Sesquiterpenoid
31	$C_{15}H_{24}$	γ-Gurjunene	–	–	0.33	1.10	Sesquiterpenoid
32	$C_{19}H_{32}O_2$	Linolenic acid, methyl ester	–	1.26	–	–	Fatty acid methyl ester
33	$C_{23}H_{34}O2$	***cis*-4,7,10,13,16,19-Docosahexaenoic acid, methyl ester**	**1.22**	**1.31**	**0.29**	**0.97**	very long-chain fatty acid methyl ester
34	$C_{26}H_{40}O_2$	Butyl 4,7,10,13,16,19-docosahexaenoate	3.52	–	1.43	3.00	very long-chain fatty acid
35	$C_{17}H_{18}O_4$	4-Hydroxy-3,3′,4-trimethoxystilbene	3.95				Stilbene polyphenol
36	$C_{20}H_{30}O$	4,14-retro-retinol	–	1.44	1.25	1.25	Retinoid

20.67 %) and humulene (10.94, 15.22, 12.28 and 12.35 %) for methanol, hexane, acetone and aqueous leaves extracts, respectively. Followed by, germacrene D (2.80, 4.00, 3.60 and 3.35 %), allocedrol (2.26, 2.22, 2.16 and 2.43 %), α-Eudesmol (1.95, 1.92, 2.91 and 2.58 %), cadina-1(10),4-diene (1.29, 1.67, 1.30 and 1.58 %), selinene (1.27, 1.18, 1.32 and 1.59 %), γ-muurolene (1.17, 1.47, 1.08 and 1.26 %), caryophyllene oxide (1.16, 1.25, 1.39 and 1.29 %), cedrene (0.39, 2.66, 0.60 and 2.33 %) and γ-elemene (0.28, 1.54, 0.62 and 1.52 %) for methanol, hexane, acetone and aqueous leaves extracts, respectively. Also, fatty acid methyl ester, *cis*-4,7,10,13,16,19-docosahexaenoic acid, methyl ester (1.22, 1.31, 0.29 and 0.97 %) for the same aforementioned extracts, respectively. other compounds detected in three extracts only, pimara-7,15-dien-3-ol (6.04, 1.53 and 0.54 %), α-muurolene (0.29, 1.80 and 0.31 %), alloaromadendrene (1.11, 2.11 and 0.64 %) and butyl 4,7,10,13,16,19-docosahexaenoate (3.52, 1.43 and 3.00 %) where, were detected in methanol, acetone and aqueous leaves extracts, respectively. The retinoid compound 4,14-*retro*-retinol represented by 1.44, 1.25 and 1.25 area % in hexan, acetone and aqueous leaves extracts, respectively. The compounds, Bornyl acetate (0.39 and 0.44 %), α-terpinyl acetate (0.48 and 0.36 %), β-elemene (0.52 and 1.73 %), 1,7-Di-*epi*-β-cedrene (2.47 and 2.06 %), valencen (1.05 and 1.44 %), cedarn-8-ol (0.19 and 0.14 %) and α-acorenol (1.20 and 1.64 %) were recorded in methanol and acetone leaves extracts, respectively. Meanwhile, the terpenoid compound, ehydro-4-epiabietol represented by 0.73 and 1.03 area % and the sesquiterpenoid, γ-gurjunene represented by 0.33 and 1.10 area % in acetone and aqueous leaves extracts, respectively. There are compounds were detected only in the methanol leaves extract as 4-hydroxy-3,3′,4-trimethoxystilbene (3.95 %), sugiol (1.49 %), totarol (0.52 %) and copalol (0.51 %). β-Chamigrene and linolenic acid, methyl ester were detected in hexane leaves extract and represented by 1.81 and 1.26 area %, respectively.

Elemyl acetate compound detected in acetone leaves extract only and represented by 0.75 area %. Cedrenol and aromandendrene compounds detected in aqueous leaves extract only and represented by 0.20 and 1.65 area %, respectively.

The GC–MS analysis revealed that the main constituents of the fruits extracts were represented in Table 6 and (Fig. 2). The main commonly sesquiterpenoids compounds in all extracts were, cedrol (21.26, 25.11, 18.55 and 36.66 %) and caryophyllene (17.06, 19.93, 15.21 and 17.04 %) for methanol, hexane, acetone and aqueous fruits extracts, respectively. Followed by the compounds, humuline (9.05, 10.93, 8.62 and 9.06 %), germacrene D (4.93, 5.76, 5.16 and 4.44 %), elemol (4.20, 4.57, 3.42 and 4.05 %), bornyl acetate (3.14, 3.08, 2.48 and 3.35 %), terpinolene (1.14, 2.75, 1.77 and 1.62 %), α-terpinyl acetate (1.10, 0.93, 1.10 and 1.22 %), cedrene (1.45, 1.36, 1.22 and 1.44 %), allocedrol (1.48, 1.18, 1.26 and 1.84 %), β-elemene (1.15, 1.28, 0.86 and 1.07 %), caryophyllene oxide (1.08, 1.14, 1.04 and 1.62 %), selinene (0.60, 0.59, 0.56 and 0.65 %) for methanol, hexane, acetone and aqueous fruits extracts, respectively. Two compounds were detected in three extracts only, labda-8(20),12,14-triene (4.64, 1.80 and 8.02 %) and trachyloban (0.81, 0.63 and 0.52 %), in methanol, hexan and acetone fruits extracts, respectively. Alloaromadendrene recorded in methanol and acetone fruits extracts by 0.48 and 0.53 area%, respectively. While, γ-elemene detected in methanol, hexane and aqueous extracts by 0.51, 0.53 and 0.42 area %,

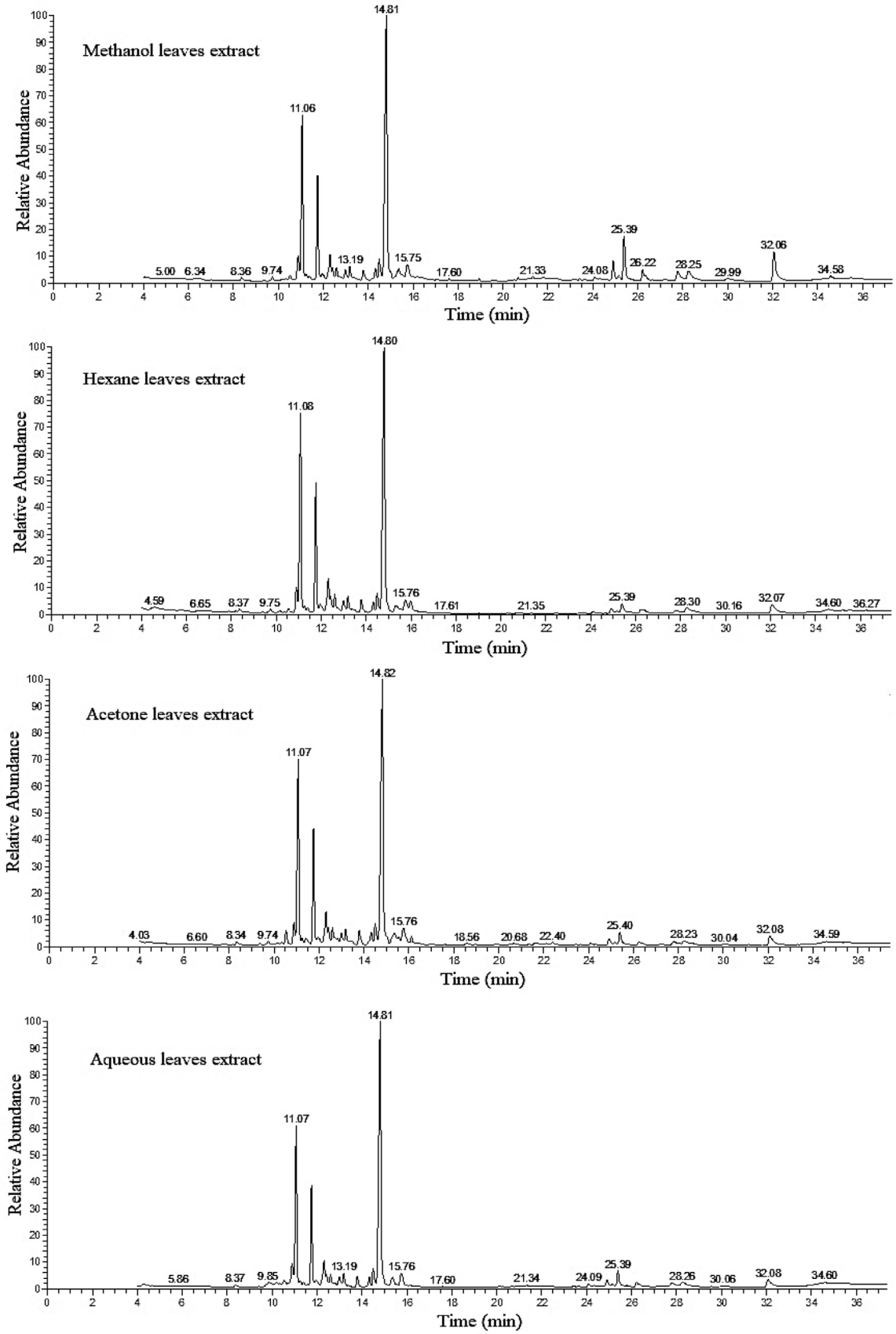

Fig. 1. The TIC chromatograms of leaves, methanol, hexane, acetone and aqueous extracts from *Thuja orientalis* showing chemical constituents separation detected by GC–MS.

Table 6
Chemical constituents of *Thuja orientalis* fruits methanol, hexane, acetone and aqueous extracts.

No	Molecular formula	Chemical compound	Methanol (%)	Hexan (%)	Acetone (%)	Aqueous (%)	Nature of compound
1	$C_{10}H_{16}$	**Terpinolene**	**1.14**	**2.75**	**1.77**	**1.62**	Menthane monoterpenoid
2	$C_{10}H_{16}$	2-Carene	1.00	–	–	–	Bicyclic monoterpenoid
3	$C_{20}H_{32}$	Labda-8(20),12,14-triene	4.64	1.80	8.02		Terpenoid
4	$C_{21}H_{32}O_2$	Labda-8(20) 12 14-triene-19-oic acid methyl ester (z)-	–	2.95	–	–	Terpenoid
5	$C_{21}H_{34}O$	Labda-8(20),14-dien-13-ol, (13S)-	–	–	–	1.62	Terpenoid
6	$C_{20}H_{32}$	(-)-Atisirene	0.42	–	–	–	Terpenoid
7	$C_{20}H_{32}$	Trachyloban	0.81	0.63	0.52	–	Terpenoid
8	$C_{12}H_{20}O_2$	**Bornyl acetate**	**3.14**	**3.08**	**2.48**	**3.35**	Bicyclic monoterpenoid
9	$C_{12}H_{20}O_2$	**α-Terpinyl acetate**	**1.10**	**0.93**	**1.10**	**1.22**	Monoterpene ester
10	$C_{20}H_{28}O_6$	16-Hydroxyingenol	0.45	–	–	–	Terpenoid
11	$C_{20}H_{30}O_2$	Communic Acid	0.93	–	–	–	Terpenoid
12	$C_{20}H_{32}O$	Pimara-7,15-dien-3-ol	–	–	1.46	–	Terpenoid
13	$C_{23}H_{38}O_2Si$	Pimaric acid TMS derivative	–	–	8.43	–	Terpenoid
14	$C_{23}H_{38}O_2Si$	Isopimaric acid TMS ester	–	–	–	–	Terpenoid
15	$C_{20}H_{30}O$	Isopimaral	–	5.64	5.64	–	Terpenoid
16	$C_{20}H_{34}O$	Copalol	–	1.05	–	–	Terpenoid
17	$C_{20}H_{30}O$	Totarol	–	–	–	–	Terpenoid
18	$C_{20}H_{30}O$	Dehydro-4-epiabietol	–	–	–	–	Terpenoid
19	$C_{21}H_{32}O_2$	Isopimaric acid, methyl ester	–	1.00	–	–	Terpenoid
20	$C_{15}H_{24}$	β-Elemene	1.15	1.28	0.86	1.07	Sesquiterpenoid
21	$C_{15}H_{24}$	γ-Elemene	0.51	0.53	–	0.42	Sesquiterpenoid
22	$C_{15}H_{24}$	δ-Elemene	0.68	–	–	–	Sesquiterpenoid
23	$C_{15}H_{24}$	1,7-Di-*epi*-β-cedrene		–	–	–	Sesquiterpenoid
24	$C_{15}H_{24}$	**Caryophyllene**	**17.06**	**19.93**	**15.21**	**17.04**	Sesquiterpenoid
25	$C_{15}H_{24}$	**Humulene**	**9.05**	**10.93**	**8.62**	**9.06**	Sesquiterpenoid
26	$C_{15}H_{24}$	**Cedrene**	**1.45**	**1.36**	**1.22**	**1.44**	Sesquiterpenoid
27	$C_{15}H_{24}$	**Selinene**	**0.60**	**0.59**	**0.56**	**0.65**	Sesquiterpenoid
28	$C_{15}H_{24}$	**Germacrene D**	**4.93**	**5.76**	**5.16**	**4.44**	Sesquiterpenoid
29	$C_{15}H_{24}$	Isogermacrene D	0.31	–	–	–	Sesquiterpenoid
30	$C_{15}H_{24}$	Valencen	–	–	–	1.05	Sesquiterpenoid
31	$C_{15}H_{24}$	Cadina-1(10),4-diene	0.47	–	–	–	Sesquiterpenoid
32	$C_{15}H_{24}$	Alloaromadendrene	0.48	–	0.53	–	Sesquiterpenoid
33	$C_{15}H_{24}$	Chamigrene	–	0.61	–	–	Sesquiterpenoid
34	$C_{20}H_{32}$	Cubenol	–	–	–	1.48	Sesquiterpenoid
35	$C_{15}H_{24}O$	**Caryophyllene oxide**	**1.08**	**1.14**	**1.04**	**1.62**	Sesquiterpenoid
36	$C_{15}H_{26}O$	**Allocedrol**	**1.48**	**1.18**	**1.26**	**1.84**	Sesquiterpenoid
37	$C_{15}H_{26}O$	**Cedrol**	**21.26**	**25.11**	**18.55**	**36.66**	Sesquiterpenoid alcohol
38	$C_{15}H_{26}O$	α-Eudesmol	0.64	–	–	–	Cycloeudesmane sesquiterpene
39	$C_{15}H_{26}O$	β-Eudesmol	–	0.80	–	0.70	Cycloeudesmane sesquiterpene
40	$C_{15}H_{26}O$	**Elemol**	**4.20**	**4.57**	**3.42**	**4.05**	Sesquiterpenoid
41	$C_{17}H_{18}O_4$	1-Heptatriacotanol	0.95	–	–	–	Fatty alcohol
42	$C_{18}H_{34}O_2$	Oleic Acid	–	2.02	0.76	–	Fatty acid
43	$C_{18}H_{30}O_2$	10-Heptadecen-8-ynoic acid, methyl ester,(E)-	–	–	–	0.49	Fatty acid methyl ester
44	$C_{19}H_{34}O_6$	Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester	0.76	–	–	0.42	Lauric fatty acid ester with hydroxypropanediyl diacetate
45	$C_{19}H_{30}O_2$	13,16-Octadecadiynoic acid, methyl ester	3.24	–	–	–	Fatty acid methyl ester
46	$C_{19}H_{32}O_2$	6,9,12-Octadecatrienoic acid, methyl ester		–	–	0.62	Fatty acid methyl ester
47	$C_{21}H_{34}O_2$	Arachidonic acid methyl ester	0.58	–	–	1.13	Fatty acid methyl ester
48	$C_{21}H_{32}O_2$	*cis*-5,8,11,14,17-Eicosapentaenoic acid, methyl ester	7.11	–	–	3.48	Fatty acid methyl ester
49	$C_{21}H_{36}O2$	Linolenic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (Z,Z,Z)-	0.29	–	–	–	Fatty acid ethyl ester
50	$C_{23}H_{36}O_2$	7,10,13,16,19-docosapentaenoic acid, methyl ester	–	–	–	0.81	Fatty acid ethyl ester
51	$C_{22}H_{32}O_2$	Doconexent	1.42	–	–	–	very long-chain fatty acid
52	$C_{23}H_{34}O2$	*cis*-4,7,10,13,16,19-Docosahexaenoic acid	0.84	–	–	–	very long-chain fatty acid
53	$C_{25}H_{40}O2$	i-Propyl 7,10,13,16,19-docosapentaenoate	2.26	–	–	2.26	very long-chain fatty acid
54	$C_{23}H_{38}O2$	6,9,12,15-Docosatetraenoic acid, methyl ester	0.63	–	–	1.89	very long-chain fatty acid methyl ester
55	$C_{21}H_{36}O_4$	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	0.59	–	–	1.24	1-monoglyceride derives from α-linolenic acid
56	$C_{21}H_{38}O_2Si$	α-Linolenic acid, TMS derivative	–	–	12.23	–	Fatty acid trimethyl ester
57	$C_{23}H_{38}O_2Si$	Eicosapentaenoic acid, TMS derivative	–	–	1.81	–	Fatty acid trimethyl ester
58	$C_{21}H_{34}$	Androst-5-ene, 4,4-dimethyl-, (13à)-	1.85	–	–	–	Steroid
59	$C_{21}H_{34}O_2$	Androstan-17-one, 3-ethyl-3-hydroxy-, (5à)-	–	0.60	–	–	Steroid
60	$C_{27}H_{42}O_3$	Pseduosarsasapogenin-5,20-dien	0.54				Steroidal saponin
61	$C_{17}H_{18}O_4$	4-Hydroxy-3,3′,4-trimethoxystilbene					Stilbene polyphenol
62	$C_{20}H_{30}O$	4,14-retro-retinol		1.45	1.71		Retinoid
63	$C_{20}H_{28}O_6$	Dotriacontane		0.45			Alkane

respectively. Isopimaral and olic acid detected in hexan and acetone extracts by 5.64, 5.64 and 2.02 and 0.76 area %, respectively. *cis*-5,8,11,14,17-eicosapentaenoic acid, methyl ester represented by 7.11 and 3.48 area %, i-Propyl 7,10,13,16,19-docosa

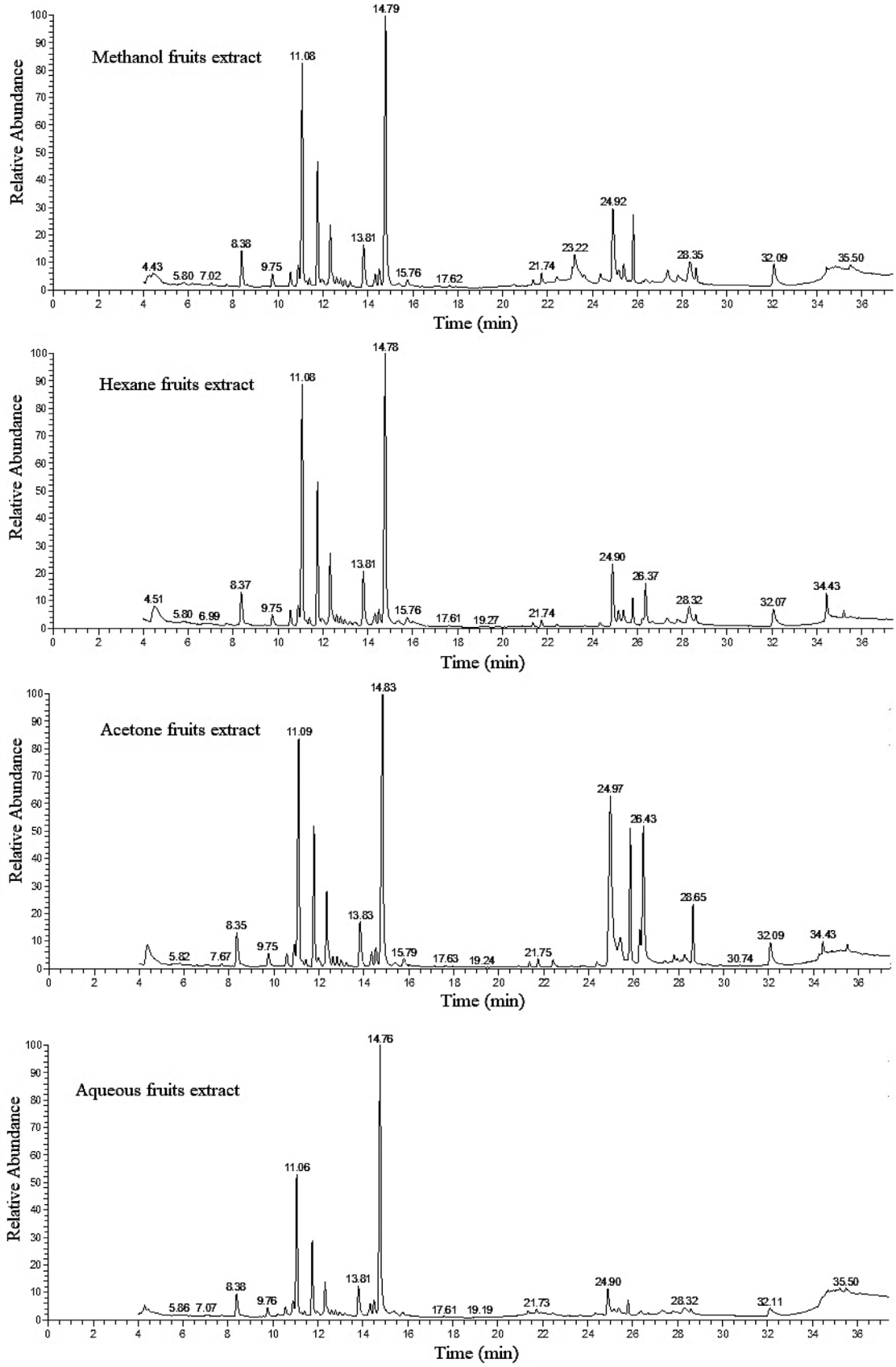

Fig. 2. The TIC chromatograms of fruits, methanol, hexane, acetone and aqueous extracts from *Thuja orientalis* showing chemical constituents separation detected by GC–MS.

pentaenoate represented by 2.26 and 2.26 area %, arachidonic acid methyl ester represented by 0.58 and 1.13 area %, 6,9,12,15-docosatetraenoic acid, methyl ester represented by 0.63 and 1.89 area %, dodecanoic acid, 2,3-bis(acetyloxy)propyl ester represented by 0.76 and 0.42 area %, and in methanol and aqueous extracts, respectively. β-Eudesmol compound detected in hexane and aqueous fruits extracts and represented by by 0.80 and 0.70 area %, respectively.

There are 15 compounds detected only in methanol fruits extract only which were, 13,16-octadecadiynoic acid, methyl ester (3.24 %), Androst-5-ene, 4,4-dimethyl-, (13à)- (1.85), doconexent (1.42 %), 2-Carene (1.00 %), 1-heptatriacotanol (0.95 %), communic acid (0.93 %), *cis*-4,7,10,13,16,19-docosahexaenoic acid (0.84 %), δ-eIemene (0.68 %), α-eudesmol (0.64 %), Pseduosarsasapogenin-5,20-dien (0.54 %), Cadina-1(10),4-diene (0.47 %), 16-hydroxyingenol (0.45), (-)-Atisirene (0.42 %), isogermacrene D (0.31 %), linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Z,Z,Z)-(0.29 %). Six compounds detected only in hexane fruits extract which were, labda-8(20) 12 14-triene-19-oic acid methyl ester (z)-(2.95 %), copalol (1.05 %), isopimaric acid, methyl ester (1.00 %), chamigrene (0.61 %), Androstan-17-one, 3-ethyl-3-hydroxy-, (5à)-(0.60 %) and Dotriacontane (0.45 %). Four compounds detected only in acetone fruits extract were, α-linolenic acid, TMS derivative (12.23 %), pimaric acid TMS derivative (8.43 %), eicosapentaenoic acid, TMS derivative (1.81 %) and pimara-7,15-dien-3-ol (1.46 %). While, in aqueous fruits extract there are six compounds detected which were, labda-8(20),14-dien-13-ol, (13S)- (1.62 %), valencen (1.05 %), cubenol (1.48 %), 7,10,13,16,19-docosapentaenoic acid, methyl ester (0.81 %), 6,9,12-octadecatrienoic acid, methyl ester (0.62 %) and 10-heptadecen-8-ynoic acid, methyl ester,(E)- (0.49 %) (Table 6) and (Fig. 2).

4. Discussion

Generally the study reviled larvicidal activities of *T. orientalis* leaves and fruits extracts in a concentration dependent manner whatever the activities varied between leaves and fruits tested extracts. Leaves extracts ordered according to larval toxicity as acetone > methanol > aqueous > hexane. While, fruits extracts ordered as hexane > methanol > acetone > aqueous. Collectively acetone *T. orientalis* leaves extract showed throughout the tested extracts, the most effective larvicidal activity against *C. pipiens* larvae (100 % mortality) and (LC_{50}, 58.04 ppm LC_{90}, 169.27 ppm LC_{50}, 229.27 ppm) at concentration 400 ppm.

Different Plant extracts previously tested for their larvicidal activities against mosquitoes, El-Sheikh et al. (2012) tested the larval toxicity of ethanol, acetone and petroleum ether extracts of *Tribulus terrestris* leaves, against the third instar larvae *Ae. aegypti* and predicted dose dependent larvicidal activities for the tested extracts in addition to variation of larvicidal activities related to solvent used. Where, crude plant extracts showed previously more efficiency in controlling mosquitoes over the purified compounds toxicity, which in line with the present study results (Ghosh et al., 2012). Another study estimated larval toxicity of leaves aqueous extracts in three tested concentrations from *Ricinus communis* L. (0.06, 0.12 and 0.2 g/l), *Daphne gnidium* L (0.09, 0.18 and 0.3 g/l) and *Thymus vulgaris* L. (0.0225, 0.045 and 0.09 g/l), against early instar larvae of *Cx. pipiens* and *Cs. Longiareolata* and showed in line with the present study results that the larval mortality increased with exposure times (24, 48 and 72 h), and the LC_{50} recorded values decreased in the same manner (Dahchar et al., 2016).

The chemical compounds detected in the extracts in line with that detected in previous studies, however, there were differences in the amount or number of the main components (Elsharkawy et al., 2017; Guleria et al., 2008; Nickavar et al., 2003; Ololade et al., 2014; Sanei-Dehkordi et al., 2018).

The common sesquiterpenoids highly represented as % areas in the chromatograms of all tested leaves and fruits extracts were, Cedrol, Caryophyllene, Humulene, Germacrene D and Elemol (common only in fruits extracts). The medium common ones were, γ-Elemene, Cedrene, Selinene, γ-Muurolene, Cadina-1(10),4-diene, Caryophyllene oxide, Allocedrol, α-Eudesmol and the fatty acid methyl ester, *cis*-4,7,10,13,16,19-Docosahexaenoic acid, methyl ester in leaves extracts and bornyl acetate, α-Terpinyl acetate, Cedrene, β-Elemene, Selinene and Caryophyllene oxide were medium common in fruits extracts. The observed larvicidal activities of the tested extracts may related to their chemical constituents synergistic actions, either the major or minor ones that may impact on the predicted larvicidal activities (Huong et al., 2020).

The essential oil *P. orientalis* L. (Family Cupressaceae) oil showed insecticide and molluscicidal activity (Hashemi and Safavi 2012; Ju-Hyun et al., 2005; Lei, et al., 2010). *Thuja orientalis* previously acquired cytotoxic principles and contained terpenoids including pimaric and isopimaric acids, fatty acids, aliphatic compounds like alkanes and bioflavonoids (Mehta et al., 1999). The essential oil extracted from *T. orientalis* leaves predicted larvicidal activity against *Anopheles stephensi* and *Culex pipiens* late third or young 4th instar larvae, where carene and cedrol were from the main constituents of the extract (Sanei-Dehkordi et al., 2018). Also extracts predicted diterpenes and labdane-type diterpenes in line with the previously recorded (Kim et al 2012; Kim et al., 2013). Cedrol the main constituent in the tested extracts was postulated as alternative to conventional synthetic acaricides for the black-legged ticks, Ixodes scapularis Say which is a human vector causing disease (Eller et al., 2014). Caryophyllene oxide and germacrene D individual compounds previously showed potent larvicidal activities against *A. anthropophagus* (Zhu and Tian, 2013). Also β-elemene and α-humulene showed larvicidal activities against A. subpictus, *Ae albopictus*, and *Cx. tritaeniorhynchus* (Govindarajan and Benelli, 2016). Jeon et al. (2005) showed that essential oils extracted from *P. orientalis* had strong activities on mosquito larvae *Cx. pipiens* and *Ae. aegypti* recording 100 % mortality at 400 ppm. Furthermore, Sanei-Dehkordi et al. (2018) verified the high efficiency of *P. orientalis* oil against *A. stephensi* (LC_{50}, 11.67 ppm) and *Cx. pipiens* (LC_{50}, 18.60 ppm). Ethanol and acetone leaves extracts of *T. orientalis* previously evaluated larvicidal activities against mosquitoes recording LC_{50}, 13.10 and 200.87 ppm after 24 h and 9.02 and 127.53 ppm after 48 h, against *A. stephensi* third instar larvae, respectively. Besides recording against *C. quinquefasciatus* third instar larvae, LC_{50}, 22.74 and 69.03 ppm after 24 h and 16.72 and 51.14 ppm after 48 h, respectively (Sharma et al., 2005).

In addition and in accordance with the present results behavior, hexane plant parts and seeds extracts of *Physalis angulate, Peganum harmala*, *Tecrium polium* and *Thymus vulgaris* were evaluated for their larvicidal potentials against the fourth instar larvae of *Culex pipiens molestus*, and showed that mortality increased with exposure times (24 and 48 h) and the LC_{50} recorded values decreased with exposure times (Mekhlif and Muhammad, 2021). A study tested larvicidal activities leaves oils extracts against *A. aegypti* and recorded that the main components in leaves of *G. blepharophylla* was the caryophyllene oxide, while, in *G. friesiana* were α-, β- and γ-eudesmols and in *G. hispida,* was (E)-caryophyllene. According to the predicted results oil extracted from *G. friesiana* recorded the best larvicidal effect against *A. aegypti* and hypothesized that sesquiterpenes in oils can reflect more controlling activity as compared to monoterpenes (Aciole et al., 2011). The aforementioned study results may declare the predicted advantage of acetone leaves extract results where the extract acquired highest content of eudesmol as compared to other extracts.

5. Conclusion

The leaves and fruits methanol, hexane, acetone and aqueous extracts of *T. orientalis* reviled high larvicidal potential at 400 ppm concentration against the third instar larvae of *Cx. pipiens* with the advantage of leaves acetone extract that predicted the lowest effective larval toxicity concentration. Further studies about the mode of the larvicidal action of the extracts should be under investigation.

Authorship contribution

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Ethics approval and consent to participate

Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2022.102396.

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