

Essential oils from *Zanthoxylum fagara* Wild Lime, *Ruta chalepensis* L. and *Thymus vulgaris* L.: Composition and activity against *Aedes aegypti* larvae

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Abstract: The dengue virus is transmitted by *Aedes aegypti*. Several plants are used to control this mosquito. In the present study the chemical composition of the essential oils of *Ruta chalepensis*, *Zanthoxylum fagara* and *Thymus vulgaris* were analyzed, and their activities against larvae of two *A. aegypti* populations were evaluated. The major compounds found in *T. vulgaris* were thymol and *o*-cymene at 39.8% and 30.5%, respectively, with the major components being oxygenated monoterpenes and monoterpene hydrocarbons at 55.5% and 40.4%, respectively. For *Z. fagara*, the major compounds were sylvestrene and E-caryophyllene at 25.3% and 23.6%, respectively, with the major components being sesquiterpene and monoterpene hydrocarbons at 51.1% and 37.5%, respectively. Ketones were the predominant group of compounds found in *R. chalepensis*, with the major components being 2-undecanone and 2-nonanone at 43.7% and 35.4%, respectively. Essential oils from *T. vulgaris*, *Z. fagara* and *R. chalepensis* showed activity against larvae of the *A. aegypti* New Orleans strain, producing median lethal concentrations (LC₅₀) of 2.14, 27.57 and 2.69 µg/mL, respectively, at 24 h. LC₅₀ values produced against larvae of a local *A. aegypti* population in Nuevo Leon, México, were 25.37, 60.42 and 20.13 µg/mL, respectively, at 24 h.

Keywords: *Aedes aegypti*, *Zanthoxylum fagara*, *Ruta chalepensis*, *Thymus vulgaris*, essential oils.

INTRODUCTION

Dengue and dengue hemorrhagic fever are viral diseases transmitted by *Aedes aegypti*. They are important public health problems in tropical and subtropical regions of the world, being endemic in almost 100 countries. The World Health Organization (2012) estimates that 50 to 100 million dengue infections occur each year. México is an endemic country, where 26,276 cases of dengue fever and 11,614 cases of dengue hemorrhagic fever were reported in 2013 (Secretaría de Salud, 2013).

In its Global Strategy for Prevention and Control of Dengue 2012-2020, the WHO recommends effective epidemiological and entomological surveillance, prevention, case management with existing health systems, vaccination and vector control measures. These factors should complement each other to achieve a reduction in disease development, making control of dengue ecologically sustainable and profitable (The World Health Organization 2012).

Control of *A. aegypti* larvae using phytoinsecticides offers attractive alternative for combating the spread of dengue virus. In a previous study, our group evaluated the activity of eleven plant extracts against *A. aegypti* larvae. The

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results showed that extracts of *Zanthoxylum fagara* (uña de gato), *Ruta chalepensis* (Ruda), and *Thymus vulgaris* (Tomillo) were the most active (C de la Torre-Rodríguez, 2013). In the current study, was analyzed the composition and larvicidal activity of essential oils of *Z. fagara*, *R. chalepensis*, and *T. vulgaris*.

MATERIALS AND METHODS

Plant material: *R. chalepensis*, *Z. fagara*, and *T. vulgaris* plants were purchased fresh at the local market in the city of Monterrey in November 2010. They were harvested in Santiago, Nuevo León, México. Specimens were deposited at the herbarium of the Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León.

Essential oils

Fresh ground plant materials were individually hydrodistilled in a Clevenger-type apparatus (100g/L water) for 4h. The oils obtained were dried with anhydrous Na₂SO₄, weighed, and stored at 4°C.

GC analysis

GC analysis was carried out on a GC Perkin Elmer Auto system XL equipped with a flame ionization detector and an HP-5MS column (30m × 0.25mm i.d., 0.25mm film thickness). Helium (99.999%) was used as carrier gas at a flow rate of 0.5mL/min. Injector and detector

temperatures were set at 220° and 290°C, respectively. The oven temperature was programmed as follows: 35°C for 9 min, increased from 35° to 150°C at 3°C/min, held at 150°C for 10min, increased to 250°C at 10°C/min, increased at 3°C/min to 270°C and held at 270°C for 10 min. Samples were injected using the split less mode with an injection volume of 2µL (an oil solution, 2mg/mL in methylene chloride). Percentage composition was calculated using the peak normalization method assuming equal detector responses for all compounds.

GC/MS analysis

GC/MS was performed using an Agilent Technologies 6890N gas chromatograph equipped with an HP-5MS column (30m × 0.25mm i.d., 0.25mm film thickness) and a 5973 INERT selective mass spectrometer. The carrier gas was Helium (99.999%) at a flow rate of 0.5mL/min, and the ionization energy was 70 eV. Data acquisition was performed in scan mode. The ionization source temperature was 230°C, quadrupole temperature was 150°C and injector temperature was 220°C. The oven temperature was programmed as above. Samples were injected using the split less mode with an injection volume of 2µL (an oil solution, 2mg/mL in methylene chloride). Components were identified by comparing retention indices relative to C8-C20 *n*-alkanes, and mass spectra were compared with mass spectra from the US National Institute of Standards and Technology (NIST) library and reference data (Adams, 2001).

Larval bioassay

The larval bioassay was previously published by the Centers for Disease Control and Prevention (2002). Briefly, essential oils were dissolved in DMSO and diluted to concentrations ranging from 1 to 70µg/ml, each in 100mL chlorine-free water. Larvae of the susceptible *A. aegypti* strain New Orleans were kindly donated by Dr. Adriana E. Flores (Laboratory of Entomology, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León). Each experiment used 20 larvae at each concentration and was performed in triplicate, giving a total of 60 larvae at each concentration. An equal number of controls were prepared simultaneously. Control included water, solvent, or temephos (a commonly used larvicide) at concentrations of 0.001-0.050µg/mL. Mortality and survival were recorded after 12, 18 and 24 h of exposure. The results were expressed as the mean percentage of larval mortality at each concentration according to the Abbott formula (Abbott, 1925). Dead larvae were identified as those that failed to move after probing with a needle. Test containers were maintained at 25±2°C with a photoperiod of 12:12 (L: D) h. All data on larval mortality obtained using essential oils were subjected to Probit analysis using SPSS software (SPSS version 8.0 for Windows, Chicago, IL, USA) to determine the LC₅₀. The LC₅₀ values obtained at different exposure times for each type of essential oil were subjected to

analysis of variance (ANOVA). No significant difference was found using α value of 0.05, with a P value of 0.9597.

RESULTS

All three essential oils were analyzed by gas chromatography-flame ionization detection and gas chromatography-mass spectrometry to identify their components. The chromatograms of the essential oils of *Z. fagara*, *R. chalepensis*, and *T. vulgaris* are presented in fig. 1.

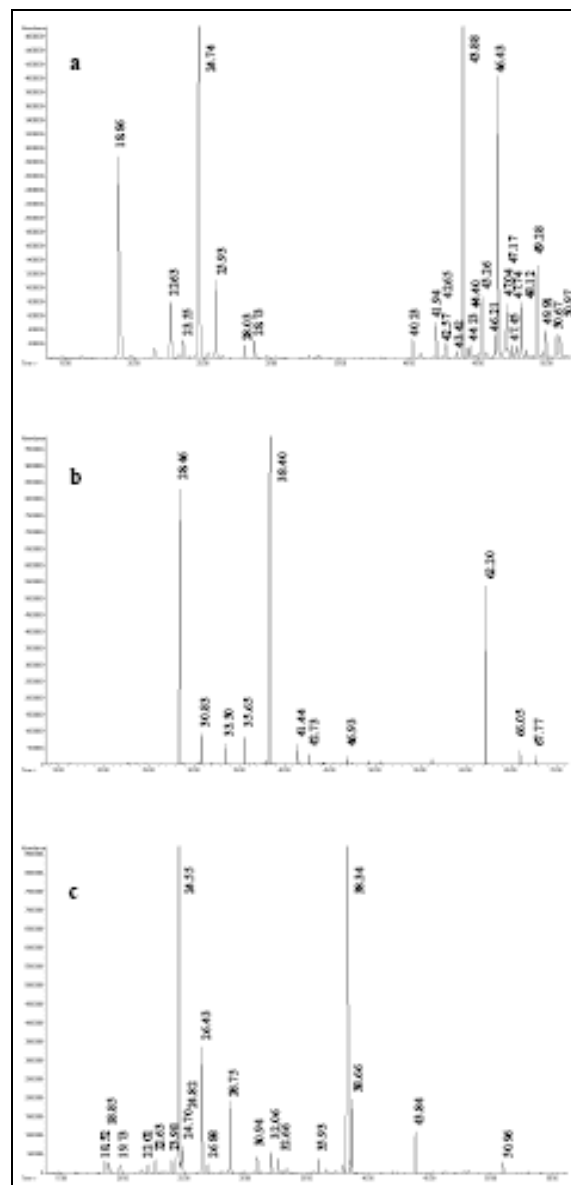


Fig. 1: GC-MS chromatograms of (a) *Z. fagara*, (b) *R. chalepensis*, and (c) *T. vulgaris*.

In *Z. fagara* essential oil 27 compounds were detected and 26 were identified (table 1). The most abundant compounds were Sylvestrene (25.3%) and E-caryophyllene (23.6%).

Table 1: Chemical composition of essential oils from *Z. fagara*, *R. chalepensis* and *T. vulgaris* (% relative to peak area)

Composition	Rt ^a	Rt ^b	<i>Zanthoxylum fagara</i>		<i>Ruta chalepensis</i>		<i>Thymus vulgaris</i>	
			Rt	%	Rt	%	Rt	%
Monoterpene hydrocarbons								
α -Thujene	924	924	--	--	--	--	18.52	0.6
α -Pinene	929	932	18.86	5.2	--	--	18.85	0.6
Camphene	943	946	--	--	--	--	19.73	0.5
Myrcene	990	988	22.63	3.3	--	--	22.63	1.4
δ -3-Carene	1005	1008	23.55	0.7	--	--	--	--
α -Terpinene	1013	1014	--	--	--	--	23.98	0.6
<i>o</i> -Cymene	1023	1022	--	--	--	--	24.55	30.5
Sylvestrene	1026	1025	24.74	25.3	--	--	24.70	0.7
β -E-Ocimene	1048	1044	25.93	2.6	--	--	--	--
γ -Terpinene	1057	1054	--	--	--	--	26.43	5.5
Terpinolene	1086	1086	28.03	0.4	--	--	--	--
Total				37.5		0.0		40.4
Oxygenated monoterpenes								
1,8-Cineole	1028	1026	--	--	--	--	24.82	1.9
<i>cis</i> -Sabinene hydrate	1065	1065	--	--	--	--	26.88	0.7
<i>trans</i> -Sabinene hydrate	1099	1098	28.73	1.5	--	--	28.75	5.6
Camphor	1142	1141	--	--	--	--	30.94	0.5
Borneol	1164	1165	--	--	--	--	32.06	2.0
Terpinen-4-ol	1175	1174	--	--	--	--	32.66	0.9
Carvacrol methyl ether	1243	1241	--	--	--	--	35.93	0.6
Thymol	1294	1289	--	--	--	--	38.34	39.8
Carvacrol	1301	1298	--	--	--	--	38.66	3.5
Total				1.5		0.0		55.5
Sesquiterpene hydrocarbons								
δ -Elemene	1336	1335	40.23	0.5	--	--	--	--
α -Copaene	1375	1374	41.94	1.6	--	--	--	--
Isolongifolene	1390	1389	42.57	0.4	--	--	--	--
β -Elemene	1391	1389	42.65	0.7	--	--	--	--
α -Gurjunene	1409	1409	43.42	0.3	--	--	--	--
E-Caryophyllene	1420	1417	43.88	23.6	--	--	43.84	2.4
β -Copaene	1429	1430	44.23	0.2	--	--	--	--
γ -Elemene	1433	1434	44.40	0.1	--	--	--	--
α -Humulene	1454	1452	45.26	3.2	--	--	--	--
γ -muurolene	1477	1478	46.21	1.1	--	--	--	--
Germacrene D	1482	1480	46.43	11.7	--	--	--	--
Bicyclogermacrene	1497	1500	47.04	4.4	--	--	--	--
α -Muurolene	1500	1500	47.17	0.1	--	--	--	--
E,E- α -Farnesene	1506	1505	47.45	0.4	--	--	--	--
γ -Cadinene	1512	1513	47.74	0.1	--	--	--	--
δ -Cadinene	1520	1522	48.12	2.7	--	--	--	--
Total				51.1		0.0		2.4
Oxygenated sesquiterpenes								
Hedycaryol	1545	1546	49.28	4.9	--	--	--	--
E-Nerolidol	1558	1561	49.91	2.0	--	--	--	--
Not identified	1574		50.67	1.2	--	--	--	--
Caryophyllene oxide	1580	1582	50.97	2.0	--	--	50.96	0.1
Total				10.1		0.0		0.1
Others								
1-Octen-3-ol	980	974	--	--	--	--	22.02	1.4
2-Nonanone	1092	1087	--	--	20.46	35.4	--	--
Geijerene	1139	1138	--	--	30.83	1.4	--	--
2-Decanone	1192	1190	--	--	33.50	2.7	--	--
Cyclopropanecarboxylic acid, dodecyl ester	1237	NR	--	--	35.65	3.4	--	--
2-Undecanone	1295	1293	--	--	38.40	43.7	--	--
2-Methylundecanal	1364	1365	--	--	41.44	2.5	--	--
2-Dodecanone	1393	1388	--	--	42.73	1.4	--	--
2-tridecanone	1494	1495	--	--	46.93	1.1	--	--
Not identified	1811	--	--	--	62.20	6.8	--	--
Not identified	NC	--	--	--	66.05	1.0	--	--
Not identified	NC	--	--	--	67.77	0.5	--	--
Total				0.0		100.00		1.4

Table 2: Median lethal concentration (LC₅₀; µg/mL) of essential oils from *Z. fagara*, *R. chalepensis*, and *T. vulgaris* against third- to fourth-instar *A. aegypti* larvae

<i>A. aegypti</i> strain population	Plant	12 h		18 h		24 h	
		LC ₅₀	CI	LC ₅₀	CI	LC ₅₀	CI
New Orleans	<i>T. vulgaris</i>	3.50	3.12-4.01	2.89	2.54-3.32	2.14	1.81-2.47
	<i>Z. fagara</i>	36.13	33.51-40.13	30.17	31.87-58.60	27.57	26.10-28.99
	<i>R. chalepensis</i>	4.28	3.61-5.51	2.92	2.55-3.37	2.69	2.33-3.10
	Temephos	0.0077	0.0068-0.0089	0.0059	0.0050-0.0071	0.0038	0.0032-0.0045
Local population	<i>T. vulgaris</i>	29.12	27.63-30.65	26.56	27.90-52.12	25.37	23.80-26.76
	<i>Z. fagara</i>	65.23	62.08-70.86	62.42	59.72-66.17	60.42	57.69-63.53
	<i>R. chalepensis</i>	25.01	22.25-29.57	22.72	20.24-26.40	20.13	18.08-22.62
	Temephos	0.0077	0.0062-0.0090	0.0066	0.0049-0.0079	0.0052	0.0037-0.0064

P values < 0.05 were considered significant. CI, 95 % confidence interval; n = 3; temephos = larvicide positive control.

In essential oil of *R. chalepensis* eleven compounds were detected, and eight were identified (table 1). 2-Undecanone (43.7%) and 2-nonanone (35.4%) were the major components.

In essential oil of *T. vulgaris* twenty compounds were detected and identified (table 1), with the major compounds being thymol (39.8%) and *o*-cymene (30.5%).

The three essential oils displayed larvicidal activity (table 2). The essential oil of *Z. fagara* showed activity against both strains New Orleans and local population, the LC₅₀ values at 24 hours were 27.57 µg/mL and 60.42 µg/mL respectively. For *R. chalepensis* essential oil the LC₅₀ values within 24 hours were 2.69 and 20.13 µg/mL against the New Orleans and local strains, respectively. The LC₅₀ values from *T. vulgaris* essential oil were 1.81 and 23.80 µg/mL, at 24 hours for New Orleans and local populations, respectively.

DISCUSIÓN

In *Z. fagara* essential oil, sesquiterpene (51.1%) and monoterpene hydrocarbons (37.5%) were the major components (table 1). The main compounds were Sylvestrene (25.3%) and E-caryophyllene (23.6%).

These results were different qualitatively and quantitatively from those reported by Setzer *et al.* (2005), who found citronellol (26.1%), geraniol (15.3%), citronellal (11.3%), and geranial (11.6%) to be the major compounds in leaf oils. Interestingly, the composition profile of our *Z. fagara* essential oil is similar that of essential oil from *Z. fagara* fruit reported by Prieto *et al.* (2011).

In essential oil of *R. chalepensis*, ketones were determined to be the predominant compounds group. 2-Undecanone (43.7%) and 2-nonanone (35.4%) were the major components. This composition analysis was consistent with results obtained by Ali *et al.* (2013) and

Conti *et al.* (2013). In addition, these compounds have been reported as major components in *Ruta graveolens* (Naguib *et al.*, 2007).

In essential oil of *T. vulgaris*, oxygenated monoterpenes (55.5%) and monoterpene hydrocarbons (40.4%) were the major components, the main compounds being thymol (39.8%) and *o*-cymene (30.5%). The composition profile was similar to one reported previously, except for changes in ratios. Rota *et al.* (2008) reported the major components thymol and *o*-cymene at 57.7% and 26%, respectively. The observed differences in composition between our results and previous reports may be due to the isolation of different chemotypes, use of different collection times and/or differences in pedological and climate conditions at plant collection sites (Pavela, 2009).

Larvicidal activity of all three essential oils was determined through the LC₅₀ at different times in two distinct populations of *A. aegypti*: The New Orleans wild strain and a local population obtained at Enramada in Guadalupe, Nuevo León, México (table 2). We found that the New Orleans strain was more sensitive than the local population to all three essential oils. In addition, the oils of *R. chalepensis* and *T. vulgaris* had the highest larvicidal activity in both populations.

Z. fagara essential oil had the lowest larvicidal activity of the three oils tested. However, no previous report indicated that any essential oil from this plant had any larvicidal activity against *A. aegypti*.

Several groups have reported biological activities for essential oils from *R. chalepensis*. Conti *et al.* (2013) reported activity against *A. albopictus* larvae, with LC₅₀ values ranging from 33.18 to 35.6 µg/mL. In our study, the essential oil of *R. chalepensis* had the greatest larvicidal activity among the plants tested. Within 24 hours, the LC₅₀ values were 2.69 and 20.13 µg/mL against the New Orleans and local strains, respectively. The results obtained from the local population were consistent with

Ali *et al.* (2013), who established an LC₅₀ of 22.2µg/mL. This oil had a completely different composition from the other two oils analyzed, with its major components being 2-undecanone and 2-nonanone. Ali *et al.* (2013) evaluated the activity of these two pure compounds against larvae of *A. aegypti* and reported LC₅₀ values of 14.37µg/mL and 106.9µg/mL, respectively.

Several studies on essential oils of *T. vulgaris* have reported different activities against insects, including larvicidal, repellent and oviposition deterrent activity against some species of *Culex*, *Anopheles* and *Aedes* (Pavela, 2009). In the present investigation, the essential oil of *T. vulgaris* had antilarval activity with LC₅₀ values of 1.81 and 23.80µg/mL for larvae from the New Orleans and local populations, respectively, at 24 hours (table 2). Among its major components, the oil contained thymol (37%) and carvacrol (3.5%) (table 1), both of which have activity against *A. albopictus* larvae with LC₅₀ values of 0.9µl/100mL for pure compounds (Young-Uk *et al.*, 2012).

CONCLUSION

While the larvicidal activities determined for these three essential oils are not comparable in strength with the current larvicide of choice (temephos), these oils can be used for and contribute to the control of *A. aegypti*. Above all, these essential oils are of natural origins and are environmentally friendly. Remarkably, this is the first time that the activity of *Z. fagara* oil is demonstrated against *A. aegypti* larvae.

REFERENCES

- Abbott WS (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, **18**: 265-267.
- Adams RP (2001). Identification of essential oil components by gas chromatography / quadrupole mass spectroscopy. 4th Edition. Allured Publishing Corporation, Carol Stream, Illinois, USA. pp.100-561.
- Ali A, Demirci B, Kiyan HT, Bernier UR, Tsikolia M, Wedge DE, Khan IA, Baser KHC and Tabanca N (2013). Biting Deterrence, Repellency and larvicidal activity of *Ruta chalepensis* (Sapindales: Rutaceae) essential oil and its major individual constituents against mosquitoes. *J. Med. Entomol.*, **50**: 1267-1274.
- C de la Torre-Rodríguez Y, Martínez-Estrada FR, Flores-Suarez AE, Waskman N and Salazar Aranda R (2013). Larvicidal and cytotoxic activities of extracts from 11 native plants from northeastern Mexico. *J. Med. Entomol.*, **50**: 310-313.
- Centers for Disease Control and Prevention (CDC) (2002). Evaluating Mosquitoes for Insecticide Resistance Web-Based Instruction <http://www.cdc.gov/incidod/wbt/resistance/assay/larval/index.htm>, consulted on 27/01/2009.
- Conti B, Leonardi M, Pistelli L, Profeti R, Ouerghemmi I and Benelli G (2013). Larvicidal and repellent activity of essential oils from wild and cultivated *Ruta chalepensis* L. (Rutaceae) against *Aedes albopictus* Skuse (Diptera: Culicidae), an arbovirus vector. *Parasitol. Res.*, **112**: 991-999.
- Naguib YN, Hussein MS, El-Sherbeny SE, Khalil MY and Lazari D (2007). Response of *Ruta graveolens* L. to sowing dates and foliar micronutrients. *J. App. Sci. Res.*, **3**: 1534-1543.
- Pavela R (2009). Larvicidal property of essential oils against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Ind. Crop. Prod.*, **30**: 311-315.
- Prieto JA, Patiño OJ, Delgado WA, Moreno JP and Cuca LE (2011). Chemical composition, insecticidal and antifungal activities of fruit essential oils of three Colombian *Zanthoxylum* species. *Chil. J. Agr. Res.*, **71**: 73-82.
- Rota MC, Herrera A, Martínez RM, Sotomayor JA and Jordán M J (2008). Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. *Food Control*, **19**: 681-687.
- Secretaría de Salud (2013). Panorama Epidemiológico de Fiebre por Dengue y Fiebre Hemorrágica por Dengue. Semana epidemiológica 40, (www.epidemiologia.salud.gob.mx/doctos/panodengue/PANORAMAS_2013/Pano_dengue_sem40_sem2013.pdf), consulted on 14/07/2014.
- Setzer W, Noletto J, Lawton R and Haber W (2005). Leaf essential oil composition of five *Zanthoxylum* species from Monteverde, Costa Rica. *Mol. Divers*, **9**: 3-13.
- World Health Organization (2012). Global Strategy for Dengue Prevention and Control 2012-2020. WHO reference number: WHO/HTM/NTD/VEM/2012.5.
- Young-Uk P, Hyun-Na K and Gil-Hah K (2012). Chemical composition, larvicidal action and adult repellency of *Thymus magnus* against *Aedes albopictus*. *J. Am. Mosquito Contr.*, **28**: 192-198.