

Chemical composition and antibacterial activities of *Rhus tripartita* essential oils from Algeria

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Abstract. Benlembarek K, Lograda T, Ramdani M, Figueredo G, Chalard P. 2021. Chemical composition and antibacterial activities of *Rhus tripartita* essential oils from Algeria. *Biodiversitas* 22: 480-490. The yield of *Rhus tripartita* essential oils in Algeria is low with an average of 0.16 ± 0.017 . The chemical composition of the oils was determined by GC-MS analysis. Seventy-eight compounds were identified with an average of $92.3 \pm 26.4\%$ in the total oil. The α -pinene ($58.72 \pm 14.45\%$), Δ^3 -carene ($5.21 \pm 6.89\%$), caryophyllene-Z ($4.55 \pm 4.68\%$) and β -pinene ($3.07 \pm 1.85\%$) are the major components of the oils of *R. tripartita* populations. The results reveal a notable difference between the chemical composition of essential oils from male and female populations. The oils of this species are composed of four chemotypes, the Δ^3 -carene, α -pinene; limonene chemotype characterizes the male populations of Tamanrasset and Boussaada. The α -pinene - Δ^3 -carene - caryophyllene-Z chemotype characterizes the male populations of M'sila and Mergueb. The female M'sila population is characterized by the α -pinene - Δ^3 -carene - Δ -cadinene chemotype. The remaining populations show the α -pinene - β -pinene - caryophyllene-Z chemotype. The evaluation of antibacterial activities reveals that *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* are not sensitive to the essential oils of *R. tripartita*. In contrast, *Enterococcus faecalis* is the most sensitive to the oils.

Keywords: Algeria, essential oils, chemotypes, antibacterial activities, *Rhus tripartita*

INTRODUCTION

The *Rhus* (*Anacardiaceae*) consists of approximately 200 species distributed in different temperate zones (Giovanelli et al. 2017). In Algeria, the genus *Rhus* is represented by three species, *Rhus tripartita*, *R. coriaria*, and *R. pentaphylla* (Quézel and Santa 1962). The *Rhus tripartita* or *Searsia tripartita* (Ucria) Moffett is located in arid and semi-arid North Africa (Quézel and Santa 1962) and in the steppes of Western Asia (Idm'hand et al. 2019).

Rhus tripartita is widely used in traditional and modern medicine (Lee et al. 2010; Wu et al. 2013). It is used in southern Algeria to treat diabetes, bloating, fever, kidney disease, skin lesions, diarrhea and several gut diseases (Hadjadj et al. 2015). The fruits of *Rhus tripartita* are eaten fresh or mixed with water, honey, or milk; the leaves are also used as animal feed; the barks and roots are used in crafts to dye leather, wool and silk (Floch 1983; Ben Mahmoud et al. 2015). The products of secondary metabolism of *R. tripartita*, in particular the phenolic compounds, have anti-carcinogenic, anti-thrombotic and anti-inflammatory (Chung et al. 2010; Ben Barka et al. 2019), anti-nephritic, antimicrobial (Abbassi et al. 2012) activities; antioxidants and preventive properties of breast cancer (Wang et al. 2005; Amin et al. 2007; Shahat et al. 2016).

The chemical composition of the genus *Rhus* is not much studied. The essential oils of *R. coriaria* from Sicily present cembrene and β -caryophyllene as the majority

products of the leaves, while the flowers are characterized by the presence of α -pinene and tridecanoic acid (Ridel et al. 2017); the same results were obtained in Iran by Zhaleh et al. (2018). In a similar study in Turkey, the chemical profile of *R. coriaria* populations showed that the main components were α -pinene, β -caryophyllene and cembrene (Brunke et al. 1993). Palestinian, Jordanian, and Egyptian sumac (*R. coriaria*) are characterized by α -pinene, naphthalene and cymene-O, respectively (Farag et al. 2018). Work on the chemical composition of the leaves of *R. typhina* has shown that monoterpenes are in the majority (Bestmann et al. 1988). The analysis of essential oils from the leaves and flowers of *R. mysorensis* allowed the estimation of the abundance of α -pinene, limonene, sabinene and β -eudesmol (Srivastava et al. 2005).

The ethanolic extracts, from the aerial parts of *R. tripartita* from the Bechar region (Algeria), showed a weak antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter cloacae* and *Pseudomonas aeruginosa* (Bereksi et al. 2018). While the chloroform extracts from Tunisian populations presented a strong antibacterial activity against *S. aureus* and strong antifungal activity against the yeast *Candida albicans* (Abbasi and Hani 2011).

The *R. tripartita* leaves in Libya showed, that both ethanolic extracts and chloroformic solvents, have a low inhibitory effect against *E. coli* and *P. aeruginosa* (Habibi et al. 2015). On the other hand, the studies by Tlili et al.

(2019) show significant activity of leaf extracts against *Listeria monocytogenes*, *S. epidermidis*, and *S. aureus* (MRS); but they did not show any significant activity against Gram-negative bacteria. The hydro-methanolic extract of stems, roots and leaves of *R. tripartita* showed strong antimicrobial activity against *L. monocytogene* and *Bacillus cereus* (Benbarka et al. 2019).

The evaluation of the antibacterial activity of *R. tripartita* alcoholic extracts in Egypt showed significant antibacterial activity against *Staphylococcus aureus* and *S. aureus* (MRSA) (Abd El-Salam 2015). A similar study by Njoroge and Opiyo (2019) in Kenya shows that compounds from the root bark of *R. natalensis* exhibited moderate antibacterial and antifungal activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Penicillium notatum*, and *Aspergillus niger*. Zhaleh et al. (2018), by studying the antibacterial activity of essential oils of *Rhus coriaria* in Iran, showed that this species could be used as an antibiotic to treat bacterial infections.

The aim of this study is to analyze the essential oils of *Rhus tripartita* populations in Algeria, to compare the chemical composition of essential oils of populations, identify the chemotypes of this species and to study the antibacterial activities of different populations.

MATERIALS AND METHODS

Plant materials

The *Rhus tripartita* is an easily recognizable shrub due to its thorny branches and its small leaves, cut into three

folioles. It is a dioecious plant with small clusters of yellowish-white flowers. The fruit is a small drupe 3-5 mm in diameter with a single seed inside having a spherical shape and a red to black color (Quézel and Santa 1962; Furth 1985).

The aerial parts of *R. tripartita* were collected in the flowering stage from seven localities in East Algeria (Figure 1) in February and March 2020. The species *R. tripartita* is dioecious; from each station, both male and female populations were sampled. Geographic coordinates of the stations are listed in Table 1.

Table 1. Geographical coordinates of sampled stations

Localities	Populations	Long. E	Lat. N	Alt. (m)
M'sila	1. M'sila F	4°35'04"	35°49'	622
	M'sila M	4°35'04"	35°49'	619
	2. Mergueb F	3°57'22"	35°35'51"	630
	Mergueb M	3°57'22"	35°35'51"	628
	3. Maïtar F	4°06'42"	35°14'11"	610
	Maïtar M	4°06'42"	35°14'11"	604
	4. Boussaada F	4°09'52"	35°11'37"	596
	Boussaada M	4°09'52"	35°11'37"	590
Biskra	5. Ksaïate M	4°42'08"	34°49'18"	784
	6. Chaïba F	4°56'38"	34°49'39"	417
	Chaïba M	4°56'38"	34°49'39"	416
Tamanrasset	7. Tamanrasset	5°28'98"	24°24'17"	1140

Note: F = female population; M = male population

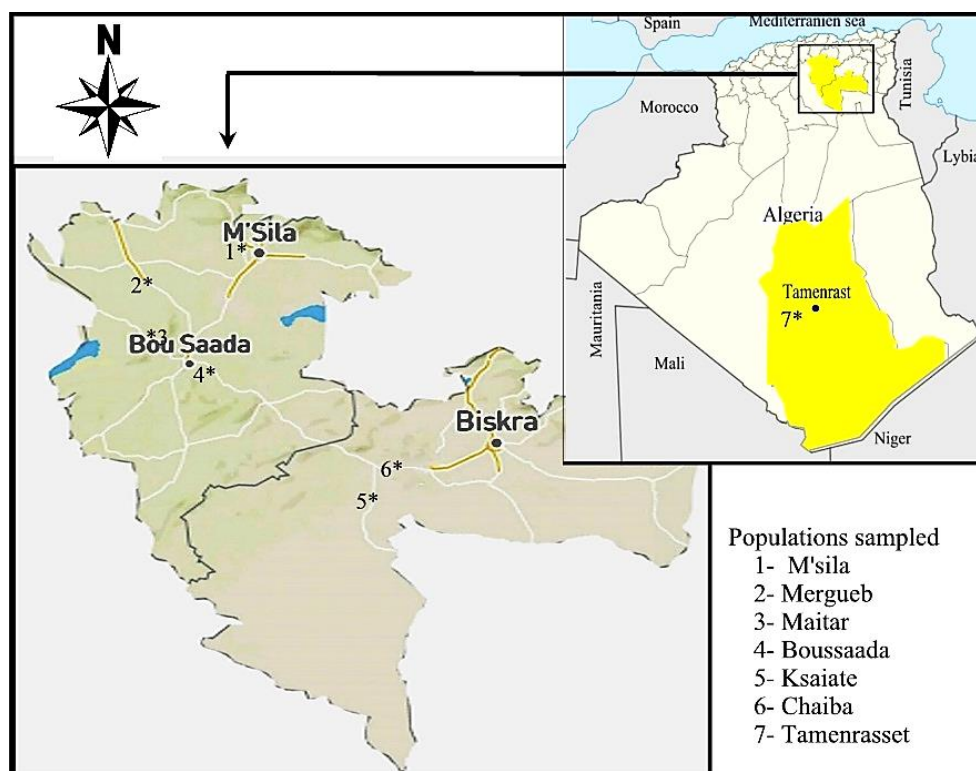


Figure 1. Populations of *Rhus tripartita* sampled in Algeria

The essential oils extraction

The air-dried materials were subjected to hydro-distillation for 3h using a Clevenger apparatus type. Voucher specimens were deposited in the herbarium of the Department of Biology and Ecology, Setif-1 University, Algeria (Identification of samples was assured by botanist from the Research Institute). The oil obtained was collected and dried over anhydrous sodium sulfate, and stored in screw-capped glass vials in a refrigerator at 4-5°C prior to analysis. The Essential oils yield was calculated by the following formula:

$$\text{Yield} = \frac{\text{Mass of essential oil}}{\text{Mass of plant matter}} \times 100$$

Essential oils analysis

The essential oils were analyzed using a Hewlett-Packard gas chromatograph (CG/FID 7890), coupled to a gas chromatograph (CG/MS 7890/5975C), equipped with a Column Apolar: DB5 MS: 40 m 0.18 mm 0.18 µm and programming from 50°C for 5min at rate of 5°C/min up to 300°C. Helium was used as the carrier gas (1.0 ml/min); injected in the split mode (1:30), injector and detector temperature of 280°C with split 1/100. The mass spectrometer worked in the EI mode at 70 eV; electron multiplier, 2500 V; ion source temperature, 180°C; the MS data were acquired in the scan mode in the m/z range of 33-450. The identification of the components was based on the comparison of their mass spectra with those of NIST mass spectral library (Masada 1976; NIST 2002) and those described by Adams, and the comparison of their retention indices either with those of authentic compounds or with literature values (Adams 2007).

Antibacterial activity assessment

The in-vitro evaluation of antimicrobial activity of *Rhus tripartita* essential oils was performed by the disc diffusion method, against three Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 35659 and *Pseudomonas aeruginosa* ATCC 27853); and two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, and *Enterococcus faecalis* ATCC 51299). These bacteria were obtained from the Pasteur Institute of Algiers and M'sila, the applied microbiology laboratory of Setif University and Setif Hospital. The bacterial inoculums were prepared from overnight broth culture in physiological saline (0.8% NaCl) to obtain an optical density ranged from 0.08 to 0.1 at 625 nm. Muller Hinton agar (MHA) was poured in Petri dishes, solidified, and surface dried before bacteria inoculation. Sterile discs (6 mm) were placed on inoculated MHA, filled with 10 µl of stock solution of essential oils or diluted essential oils (1/1, 1/2 and 1/3 v:v of DMSO). DMSO was used as a negative control and the antibiotics Colistin, Cefotaxime, Gentamicin and Imipenem were used as positive control. The bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs. The Petri dishes were incubated at 37°C for 18 to 24h aerobically. All the tests were performed in triplicate, and the means were calculated as results. The sensitivity to essential oils was classified by diameter of inhibition halos as follows:

not sensitive (-) for diameter less than 8 mm; sensitive (+) for diameter 9-14 mm; very sensitive (++) for diameter 15-19 mm and extremely sensitive (+++) for diameter larger than 20 mm (Ponce et al. 2003).

Statistical analysis

The data were first, subjected to the Principal Components Analysis (PCA) to examine the relationships among the terpenes compounds and identify the possible structure of the populations. Cluster analysis (UPGMA) was carried out on the original variables and on the Manhattan distance matrix to seek hierarchical associations among the populations. Cluster analyzes were performed using Statistica v10 software. The data of antibacterial activity were expressed as mean ± SD. The significant results were analyzed by the three-way ANOVA and post-hoc tests using a CoStat v 6.4 software package. Differences with P < 0.05 were considered significant.

Desirability test

A desirability test is a tool for evaluating the effect of factors or variables, which can predict the effect of each factor of the design. The scales range from zero (most unfavorable/undesirable values) to one (most desirable values).

RESULTS AND DISCUSSION

The essential oil, of *Rhus tripartita*, isolated by hydrodistillation from the aerial parts, were obtained in low yield ($0.16 \pm 0.02\%$) (v / w). This yield is important in the altitudes that range between 596m and 628m, for against its value decreases in the lower and higher altitudes (Figure 2). The regression curve shows a small increase in efficiency from low altitudes to high altitudes.

The composition of the chemical profile of Algerian populations of the *Rhus tripartita* is obtained by gas chromatography/mass spectrometry (GC-MS). The analysis of the chemical composition of essential oils, allowed us to identify seventy-eight components with an average of $92.31 \pm 26.36\%$ of the total oil. The compounds, identified in this oil and their relative abundance, are presented in their order of appearance (Table 2).

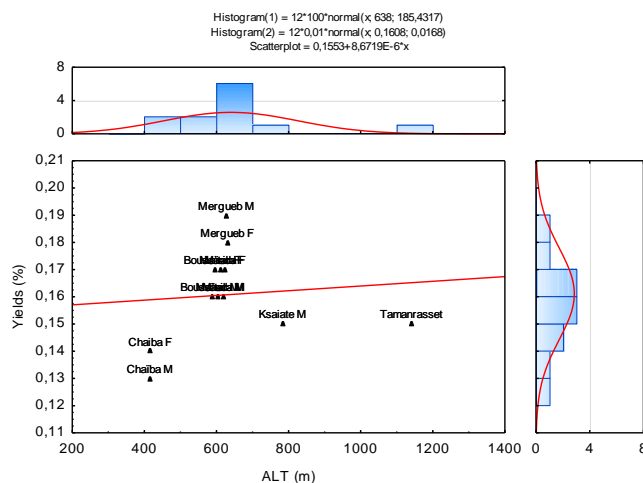


Figure 2. Yields in essential oils of *Rhus tripartita*

Table 2. Chemical composition of *Rhus tripartita* essential oils in Algeria

Stations		Tamanrasset	M'sila		Mergueb		Maitar		Ksaïate		Chaïba		Boussaâda		Average	SD
Populations	IK		M	F	M	F	M	F	M	M	F	M	F			
Nb of components		32	54	52	41	42	47	47	63	43	47	53	53	48	8	
Yields		0.15	0.16	0.17	0.19	0.18	0.16	0.17	0.15	0.13	0.14	0.16	0.17	0.16	0.02	
Total %		96.8	99.6	97.9	99.9	99.9	99.9	99.5	99.5	99.8	99.9	99.4	99.8	92.3	26.4	
Tricyclene	921	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.2	0.0	0.0	0.1	0.1	0.1	
α-thujene	927	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.4	0.0	0.0	0.1	0.1	
α-pinene	934	35.8	34.6	54.8	43.5	64.4	65.6	69.7	67.6	66.4	78.4	52.0	71.9	58.7	14.5	
α-fenchene	950	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	
Camphene	953	0.8	0.8	0.7	1.1	1.6	0.4	1.8	0.8	1.7	0.5	0.3	1.1	1.0	0.5	
Sabinene	973	0.0	0.0	0.1	0.3	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.1	
β-pinene	980	2.5	1.3	1.6	3.0	4.0	0.7	6.4	3.4	6.4	1.8	1.3	4.6	3.1	1.9	
Myrcene	989	2.4	0.5	1.5	7.7	5.3	0.7	1.0	2.4	2.7	2.8	3.3	2.1	2.7	2.1	
Δ2-carene	999	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	
α-phellandrene	1008	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.2	0.2	0.1	0.1	0.1	
Δ3-carene	1009	22.3	6.2	8.3	7.1	0.4	0.0	0.0	0.4	0.0	0.0	15.3	2.4	5.2	7.2	
α-terpinene	1017	1.5	0.1	0.2	0.2	0.1	0.1	0.0	0.3	0.6	0.4	1.0	0.1	0.4	0.4	
Cymene-ortho	1026	0.6	0.3	0.3	0.6	0.5	0.5	0.3	0.5	0.6	2.1	0.4	0.2	0.5	0.5	
Limonene	1031	7.2	2.2	2.1	2.3	1.3	0.8	1.1	1.6	2.3	2.0	6.0	2.2	2.6	2.0	
β-phellandrene	1032	0.4	0.2	0.2	0.3	0.3	0.3	0.3	0.6	0.6	0.5	0.8	0.5	0.4	0.2	
β-ocimene-Z	1035	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.1	
β-ocimene-E	1046	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.2	0.4	0.8	0.2	1.1	0.2	0.4	
δ-terpinene	1058	1.1	0.0	0.0	0.2	0.3	0.4	0.0	1.2	2.8	2.5	0.6	0.1	0.8	1.0	
Terpinolene	1086	11.4	1.6	1.5	1.7	0.3	0.1	0.0	0.5	0.6	0.6	9.4	1.3	2.4	3.8	
Cymenene-para	1093	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	
Linalool alcool	1098	0.0	0.2	0.1	0.0	0.0	0.3	0.2	0.1	0.2	0.1	0.1	0.2	0.1	0.1	
Nonanal-n	1101	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	
Fenchol	1125	0.0	0.1	0.8	0.0	0.0	0.0	0.0	0.2	0.2	0.5	0.1	0.3	0.2	0.3	
α-campholenal	1133	0.0	0.0	0.9	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.3	
Sabinol trans	1148	0.0	0.1	0.0	0.0	0.0	0.2	1.1	0.0	0.0	0.0	0.0	0.0	0.1	0.3	
Pinocarvone	1172	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Isoborneol	1182	0.0	0.2	0.1	0.0	0.1	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.1	0.1	
Terpinen-4-ol	1184	0.0	0.5	0.2	0.0	0.2	0.4	0.3	0.0	0.1	0.0	0.0	0.1	0.2	0.2	
α-terpineol	1186	0.0	0.1	0.1	0.3	0.0	0.0	0.0	0.2	0.4	0.2	0.2	0.4	0.2	0.2	
Cymene 8-ol-para	1197	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Myrtenol	1206	0.0	0.5	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	
Isoborneol formate	1212	0.0	0.3	0.7	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	
Bornyl acetate	1288	0.6	1.1	0.0	1.6	1.2	0.0	1.5	0.4	1.4	0.2	0.2	0.4	0.7	0.6	
Pinocarvyl acetate	1297	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	
Δ-elemene	1354	0.0	0.4	0.6	0.7	0.3	0.8	0.6	0.2	0.6	0.2	0.2	0.3	0.4	0.3	
α-ylangene	1377	0.2	0.2	0.3	0.1	0.2	0.1	0.2	0.2	0.0	0.0	0.0	0.1	0.1	0.1	
α-copaene	1382	0.0	0.9	1.9	1.2	0.9	0.9	1.2	0.6	0.2	0.2	0.2	0.3	0.7	0.6	
β-bourbonene	1391	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	
β-elemene	1394	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.3	0.0	0.1	0.1	0.2	
Longifolene	1425	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Caryophyllene-Z	1433	2.8	17.2	1.3	7.0	3.1	10.5	0.8	4.0	3.7	0.4	1.8	1.9	4.6	4.9	
δ-elemene	1434	0.0	0.0	0.2	0.0	0.1	0.1	0.2	0.7	0.2	1.1	0.2	0.4	0.3	0.3	
Aromadendrene	1448	0.4	0.0	0.2	0.1	0.2	0.0	0.2	0.3	0.0	0.0	0.0	0.0	0.1	0.1	
α-humulene	1465	0.4	3.1	0.6	1.3	0.7	1.6	0.3	0.7	0.6	0.2	0.4	0.4	0.9	0.8	
Cadina-1 (6)-4- diene-T	1479	0.0	0.0	0.4	0.2	0.2	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.1	
δ-muurolene	1481	0.0	2.0	2.9	1.7	1.8	1.4	2.1	0.6	0.4	0.3	0.6	0.3	1.2	0.9	
α-amorphene	1485	0.0	0.5	0.5	0.4	0.3	0.3	0.3	0.1	0.0	0.0	0.1	0.1	0.2	0.2	
Germacrene-D	1489	0.2	0.3	0.4	0.5	0.2	0.7	0.2	3.1	0.0	0.1	0.3	1.2	0.6	0.8	
δ-cadinene	1498	0.1	1.0	0.2	1.0	1.2	1.0	1.3	0.0	0.2	0.0	0.2	0.2	0.5	0.5	
Muuro-4 (14)5- diene-T	1501	0.2	0.1	0.9	0.1	0.0	0.0	0.0	0.6	0.2	0.1	0.2	0.2	0.2	0.3	
δ-amorphene	1502	0.0	1.2	0.9	0.8	0.9	0.6	0.7	0.0	0.0	0.0	0.0	0.0	0.4	0.5	
α-muurolene	1505	0.9	1.3	1.1	0.8	0.7	0.7	0.7	0.6	0.3	0.2	0.2	0.3	0.6	0.4	
Δ-amorphene	1509	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.1	
Δ-cadinene	1520	2.5	2.2	5.3	3.2	3.2	2.4	2.5	0.3	0.7	0.3	0.8	0.6	2.0	1.5	
γ-bisabolene- E)	1524	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.1	0.4	

Calamenene cis	1529	0.0	0.7	0.9	0.5	0.5	0.4	0.9	0.2	0.0	0.1	0.1	0.1	0.4	0.3
Cadina-1,4-D-T	1539	0.3	0.3	0.5	0.3	0.3	0.3	0.1	0.2	0.2	0.1	0.1	0.1	0.2	0.1
α -cadinene	1543	0.1	2.5	0.4	1.1	0.8	0.2	0.3	0.3	0.0	0.0	0.0	0.1	0.5	0.7
Dauca-4 (11),7-diene-T	1546	0.2	0.0	0.0	0.0	0.0	0.4	0.3	0.0	0.5	0.0	0.2	0.0	0.1	0.2
α -calacorene	1549	0.0	2.3	0.5	1.1	0.8	0.5	0.4	0.0	0.0	0.0	0.0	0.0	0.5	0.7
Selina-3,7 (11)-diene	1552	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.6	0.0	0.2	0.1	0.1	0.2
Germacrene-B	1570	0.0	0.6	0.2	0.2	0.0	0.3	0.1	0.6	0.2	0.8	0.2	0.3	0.3	0.3
Hexenyl benzoate-3Z	1574	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Spathulenol	1587	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.1	0.0	0.1
Caryophyllene oxide	1593	0.0	3.9	0.6	3.5	0.7	2.0	0.4	0.4	0.3	0.1	0.1	0.2	1.0	1.3
Globulol	1596	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Salvia 4 (14)en 1-one	1609	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Humulene epoxide II	1620	0.0	0.5	0.3	0.4	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.1	0.1	0.2
α -eudesmol-5-7-epi	1634	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Cubenol-1-epi	1636	0.0	0.6	0.3	0.0	0.3	0.0	0.5	0.3	0.0	0.1	0.1	0.2	0.2	0.2
β -muurola 4 (10) (14)dien-ol	1641	0.0	0.2	0.6	1.5	0.4	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.4
δ -eudesmol	1642	0.0	1.2	0.0	0.3	0.2	0.3	0.0	0.3	0.4	0.3	0.2	0.2	0.3	0.3
α -muurolol	1650	0.0	0.2	1.1	0.0	0.0	0.0	0.2	0.4	0.0	0.2	0.2	0.4	0.2	0.3
Muurolol epi	1652	0.2	0.5	0.0	0.3	0.0	0.4	0.2	0.1	0.0	0.0	0.0	0.0	0.1	0.2
Desmethoxyencecalin	1660	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	1.0	0.1	0.3
α -eudesmol-5-7-epi	1668	0.0	4.0	0.0	1.7	1.4	1.8	0.0	1.0	2.2	0.6	0.8	0.9	1.2	1.2
Calamenene 10-ol	1673	0.0	0.5	0.5	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.1	0.2
Germacrene-4 (15)5,10 (14)-T	1677	0.0	0.2	0.0	0.0	0.0	0.6	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.2

Chemical classes

Hydrocarbon monoterpenes	86.6	48.0	71.4	67.9	78.8	69.6	81	79.9	85.6	93.2	91.2	87.9	78.4	12.7
Oxygenated monoterpenes	0.8	2.9	2.9	1.8	1.6	1.5	3.3	0.9	2.4	0.9	0.6	1.4	1.8	0.9
Hydrocarbon sesquiterpenes	9.1	36.8	20.2	22.5	16.4	23.1	13.6	15.5	8.6	4.4	6.2	7.4	15.3	9.3
Oxygenated sesquiterpenes	0.4	11.9	3.4	7.8	3.2	5.6	1.9	2.9	3.2	1.3	1.4	2.1	3.8	3.3
Aldehyde	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1
Ester	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Other	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	1.0	0.1	0.3

Note: F = female population; M = male population

With an average of $58.72 \pm 14.45\%$, the α -pinene is the major component in all populations of *R. tripartita* studied; followed by Δ^3 -carene ($5.21 \pm 6.89\%$), caryophyllene-Z ($4.55 \pm 4.68\%$), β -pinene ($3.07 \pm 1.85\%$), myrcene ($2.69 \pm 1.96\%$), limonene ($2.59 \pm 1.88\%$), terpinolene ($2.40 \pm 3.64\%$) and Δ -cadinene ($2.02 \pm 1.45\%$). The terpenes composition of *R. tripartita* essential oils presents certain heterogeneity. The level of α -pinene in the populations of Tamanrasset male, M'sila male and Mergueb male is less than 50%, unlike in the other populations the rate varies between 52.02 and 71.88%.

The Δ^3 -carene, limonene and terpinolene are present with a significant percentage in Boussaada (male) and Tamanrasset populations. Male populations from M'sila, Maïtar and Mergueb show a high percentage of caryophyllene-Z. The highest percentage of Δ^3 -cadinene is observed in the female M'sila population with a rate of 5.33%; the remaining populations contain rates varying between 0.33% and 3.24%.

The essential oils of *Rhus tripartita* is rich in hydrocarbon monoterpenes and sesquiterpenes. The hydrocarbon monoterpenes are more abundant in the female populations, however; the male populations contain high levels of hydrocarbon sesquiterpenes.

The hydrocarbon monoterpenes are abundant in all populations with an average of $78.39 \pm 12.65\%$ (Figure 3), particularly in female populations. On the other hand male population's present high levels of hydrocarbon

sesquiterpenes.

The female Chaïba population reveals the highest value of hydrocarbon monoterpenes (93.22%), on the other hand, the lowest value was found in the samples of the male M'sila population with 47.98%. Hydrocarbon sesquiterpenes are present with an average of $15.32 \pm 9.3\%$. The aldehydes, esters and other components are present with low values in all the populations studied.

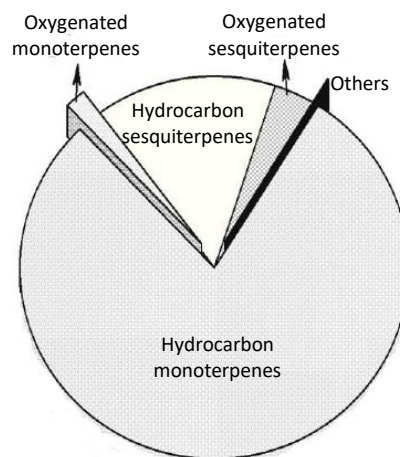


Figure 3. Chemical classes' distribution of essential oils of *Rhus tripartita*

The analysis of essential oils shows a difference between the chemical composition of male and female *R. tripartita* populations. The female populations are rich in α -pinene (54.80-78.43%) and β -pinene (1.62-6.40%), while the male populations show high levels of limonene (0.76-6.03%), terpinolene (0.14-9.39%) and caryophyllene (Z) (1.80-17.19%). The statistical analysis (Box whisker) of the chemical composition of *Rhus tripartita* essential oils reveals a significant variability of the components; α -pinene with an average $58.72 \pm 14.45\%$, followed by Δ^3 -carene, caryophyllene-Z and terpinolene (Figure 4).

The three-dimensional spatial projection of populations based on the three main axes from the ACP shows that the populations studied are dispersed and remote from each other (Figure 5). They do not gather in homogeneous groups. We notice that certain populations are well individualized like the populations of Tamanrasset and Boussaada male.

The analysis of UPGMA clusters, based on the distance of linkage, indicates the presence of chemical variability between the populations studied. The analysis separated the twelve populations of *R. tripartita* into two main clusters (Figure 6).

The first cluster includes male populations characterized by the presence of low levels of α -pinene and high rate of Δ^3 -carene, limonene and caryophyllene-Z. It is divided into two groups, Tamanrasset and Boussaada male populations which are characterized by a very high rate of Δ^3 -carene (15.30 to 22.3%), α -pinene (35.84 to 52.02%), limonene (6.03-7.23%) and terpinolene (9.39-11.41%). The second group is formed by the male populations of M'sila and Mergueb, which are characterized by α -pinene (34.57% - 43.50%), Δ^3 -carene (6.24% - 7.13%), and caryophyllene-Z (6.99% - 17.19%).

The second cluster, rich in α -pinene, is subdivided into two groups; the female M'sila population, characterized by α -pinene (54.80%), Δ^3 -carene (8.34%) and Δ -cadinene (5.33%), represents the first group. The second group,

formed mainly by female populations, is characterized by a high level of α -pinene (64.40-78.43%) associated with β -pinene (0.65-6.40%), caryophyllene-Z (0.37-10.50%) and myrcene (0.68-5.34%).

The chemical analysis and statistical analysis have enabled us to identify four chemotypes in the essential oils of our populations (Table 3). The first chemotype with Δ^3 -carene α -pinene - limonene characterizes the male populations of Tamanrasset and Boussaada. The second chemotype with α -pinene - Δ^3 -carene - caryophyllene-Z symbolised the male populations of M'sila and Mergueb. The female M'sila population is characterized by the α -pinene - Δ^3 -carene - Δ -cadinene chemotype. The remaining populations are characterized by the α -pinene - β -pinene - caryophyllene-Z chemotype.

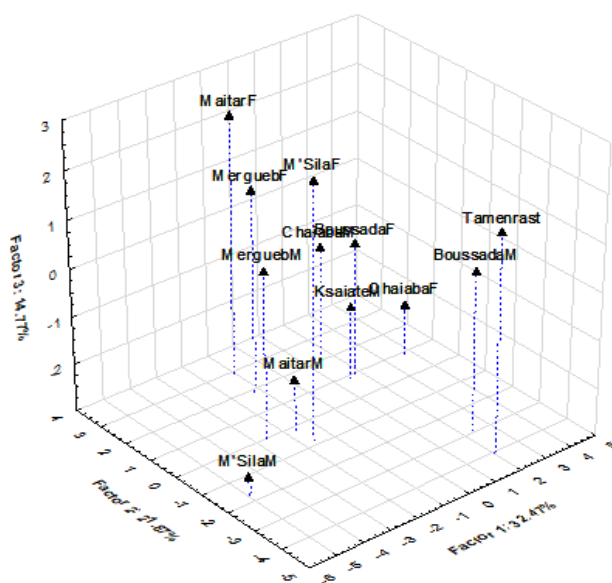


Figure 5. Spatial projection of the *Rhus tripartita* populations

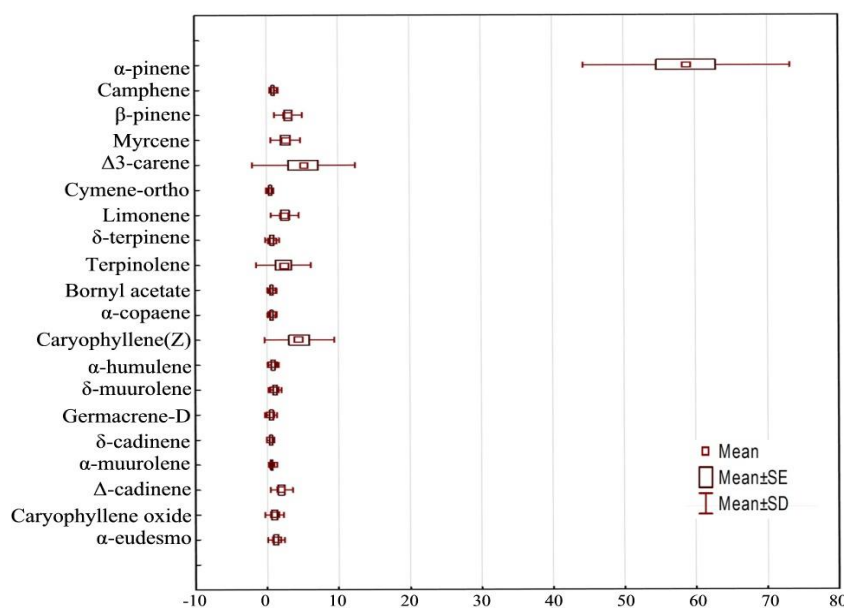


Figure 4. Chemical components variation of *Rhus tripartita* essential oils

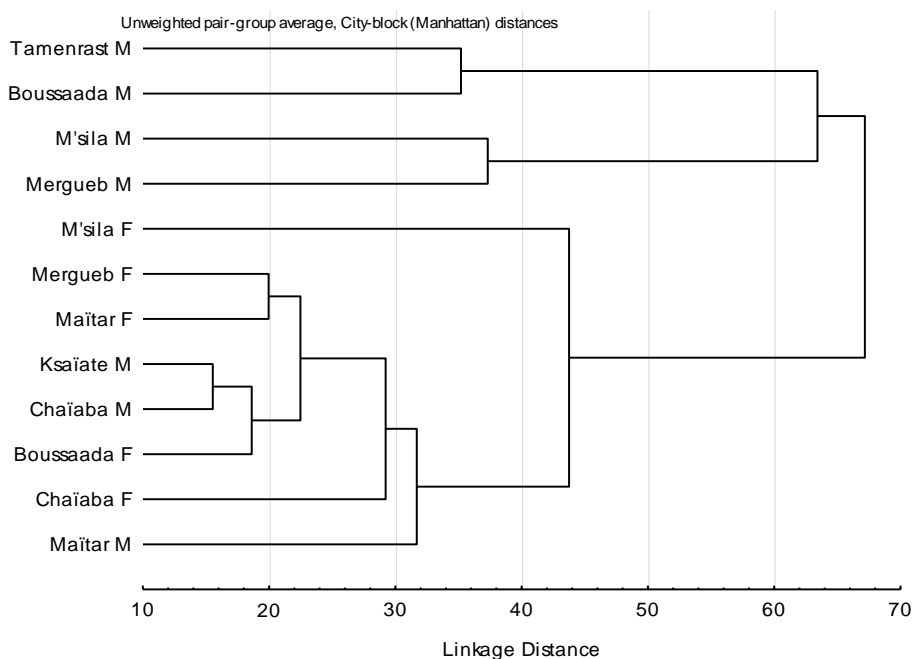


Figure 6. UPGMA cluster of populations of *Rhus tripartita* in Algeria

Table 3. Chemotypes of *Rhus tripartita*

Chemotypes	Populations
1 Δ^3 -carene, α -pinene, limonene	Tamanrasset and Boussaada male
2 α -pinene - Δ^3 -carene - caryophyllene-Z	M'sila and Mergueb male
3 α -pinene - Δ^3 -carene - Δ -cadinene	M'sila female
4 α -pinene - β -pinene - caryophyllene-Z	Boussaada female, Mergueb female, Maïtar (male and female), Ksaïate (male and female), Chaïba (male and female)

The antibacterial activity of *Rhus tripartita* essential oils was estimated using the disk diffusion method. After 24 hours of incubation at 37°C, the antibacterial activity was assessed by measuring the diameters of inhibition zone (Table 4). Antibacterial tests show that *S. aureus* and *E. coli* bacteria are sensitive to the antibiotics used, while *E. faecalis*, *P. mirabilis* and *P. aeruginosa* are resistant to Colistin. The most resistant bacterial strains to the essential oils of *R. tripartita* are *S. aureus* and *E. coli*. Pure essential oils are the most effective against *E. faecalis* and *P. aeruginosa*. The inhibition of bacterial growth is highly significant; especially in the oil from the Boussaâda male population, whose diameter of inhibition is 55.33 ± 1.52 mm.

The essential oils from the populations of Tamanrasset, M'sila, Ksaïate and Chaïba female showed low activity against *P. mirabilis* with an average zone of inhibition of 10.66 ± 1.5 mm, while the other populations did not affect the growth of this bacteria.

The *Rhus tripartita* essential oils from M'sila female population exhibits a low antimicrobial activity against the strains tested with an average inhibition diameter of 12.76 mm, although the diameter of inhibition is the largest in the test series. The essential oils from the female population of Mergueb shows the lowest zone of inhibition with a diameter of 11.02 mm (Table 6).

The antibiotics used in this study were able to inhibit bacterial growth better than *R. tripartita* oils (Table 7). The diameter of inhibition of the essential oils of *R. tripartita* was lower than that of antibiotics with diameters of inhibition inferiors of 4.93 mm. Therefore, these results are not significant in inhibiting the growth of bacteria tested.

The three-way analysis of variance revealed a very significant difference in the diameters of the inhibitory zones among the essential oils. The statistical analysis showed that the Main Effects (Populations, doses, bacterial species, and their interactions are highly significant ($P < 0.001$) (Table 5).

The bacteria tested are not sensitive to the essential oils of *R. tripartita* (Table 8). The results reveal that *S. aureus*, *E. coli*, *P. mirabilis* and *P. aeruginosa* are the most not sensitive to the essential oils of *R. tripartita*. In contrast, *E. faecalis* is the most sensitive to oil.

The desirability profile of *Rhus tripartita* essential oils against the bacteria tested had a prediction value of 0.246 (Figure 7). The predictive value of essential oils was low. The doses of the oils used in this study have low activity against the bacteria tested, with values not much higher than the predicted value. The desirability test showed that the bacterial species used in this study have values greater than the predicted value, which confirms that these bacteria are resistant to the essential oils of *Rhus tripartita*.

Table 4. Inhibition diameter zone (mm) of *R. tripartita* essential oils

[illegible]

Table 5. Main effects and interactions of *Rhus tripartita* essential oils

Sources	df	MS	F	P
Main effects				
Populations	11	41.49	97.45	.0000 ***
Bacteria	4	612.41	1438.43	.0000 ***
Doses	6	20744.71	48725.15	.0000 ***
Interaction				
Populations* Bacteria	44	56.15	131.90	.0000 ***
Populations * Doses	66	24.91	58.52	.0000 ***
Bacteria * Doses	24	1719.57	4038.94	.0000 ***
Populations* Bacteria * Doses	264	28.93	67.96	.0000 ***

Table 6 The effectiveness of *Rhus tripartita* essential oils from several sampling locations

Rank	Populations	Mean	n	Non-significant ranges
1	M'sila F	12.76	105	a
2	Boussaâda M	12.47	105	b
3	Chaiba F	12.45	105	b
4	Tamenrasset	12.26	105	c
5	Chaiba M	11.82	105	d
6	Mergueb M	11.81	105	d
7	Ksaïate F	11.73	105	d
8	Boussaâda F	11.45	105	e
9	Maitar M	11.20	105	f
10	M'sila M	11.10	105	f
11	Maitar F	11.07	105	f
12	Mergueb F	11.02	105	f

Note: LSD 0.05 = 0.1767

Table 7. Effect of oil dilutions on the inhibitory zone of bacteria tested

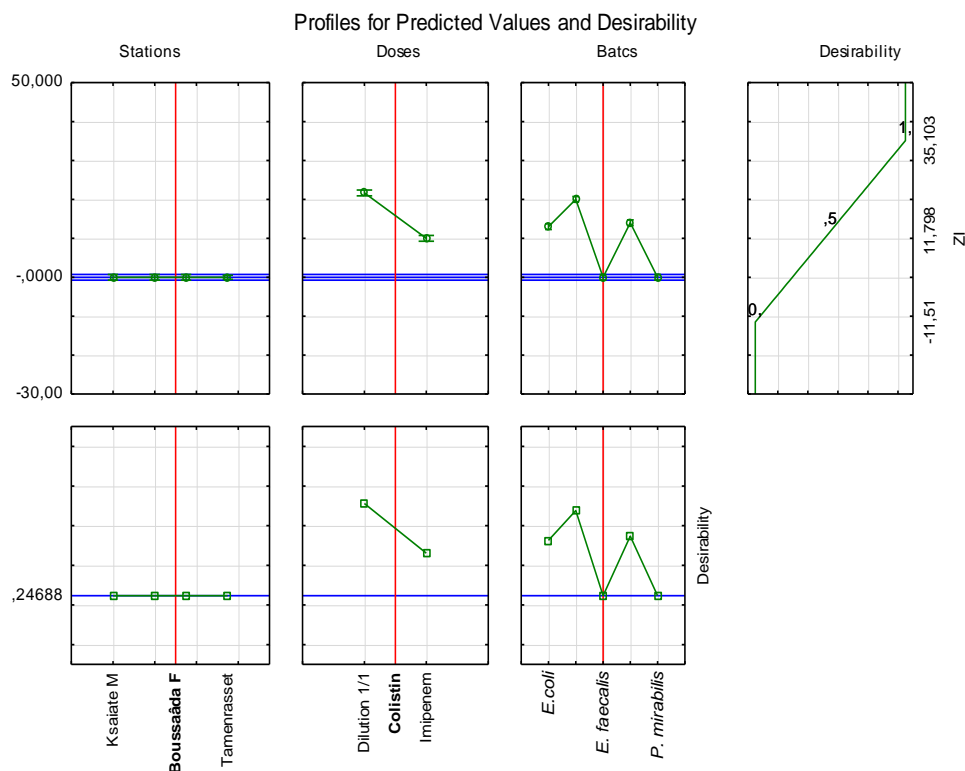
Rank	Doses	Mean	n	Non-significant ranges	S*
1	Gentamicin	26.43	195	a	+++
2	Cefotaxime	26	195	b	+++
3	Imipenem	11.6	195	c	+
4	Colistin	9.4	195	d	+
5	1	4.93	195	e	-
6	1/2	2.59	195	f	-
7	1/3	1.63	195	g	-

Note: LSD 0.05 = 0.129; * S= (+++) extremely sensitive, (+) sensitive, (-) not sensitive

Table 8. Effect of oil dilutions on the inhibitory zone of bacteria tested

Rank	Bacteria	Mean	n	Non-significant ranges
1	<i>S. aureus</i>	14.09	273	a
2	<i>E. coli</i>	12.43	273	b
3	<i>P. mirabilis</i>	11.36	273	c
4	<i>P. aeruginosa</i>	10.04	273	d
5	<i>E. faecalis</i>	10.06	273	e

Note: LSD 0.05 = 0.109

**Figure 7.** Profiles for predicted values and desirability for the inhibition zones induced by *Rhus tripartita* essential oils against bacteria

Discussion

The essential oils yield of the *Rhus tripartita* populations in Algeria is low (0.16%). This yield represents some similarity with the yield of *R. coriaria*, which varies between 0.04 and 0.19% (Morshedlooa et al. 2017). The essential oils yield variation is influenced by several environmental factors, such as drought and salinity (Aghaei et al. 2013; Sangwan et al. 2001); as well as the harvest after flowering, where 70% of essential oils are evaporated in the air (Salle and Pelletier 1991).

The results of the chemical composition of the essential oils of *R. tripartita* in Algeria show a certain resemblance to other *Rhus* species, especially in the major components, α -pinene, Δ^3 -carene, caryophyllene-Z, limonene, and Δ^3 -cadinene. Egyptian sumac is rich in cymene-o, β -ocimene and limonene, while Jordanian and Palestinian *Rhus* specimens are rich in α -pinene and naphthalene (Frag et al. 2018).

The essential oils of *R. coriaria* from Turkey are characterized by α -pinene, limonene, nonanol, β -caryophyllene, α -terpineol and carvacrol (Kurucu et al. 1993). The chemical composition analysis of essential oils of *R. coriaria* by Brunke et al. (1993) in Turkey, showed the dominance of hydrocarbon monoterpenes (α -pinene and limonene), followed by hydrocarbon sesquiterpenes (β -caryophyllene) and oxygenated sesquiterpenes (caryophyllene oxide and β -caryophyllene alcohol).

The analysis of flowers and leaves oils of *Rhus coriaria* of Italy showed that hydrocarbon sesquiterpenes are dominant in leaves, while hydrocarbon monoterpenes are major constituents of the flowers (Reidel et al. 2017), on the other hand, the essential oils of *Rhus coriaria* fruit in Sicily presented an abundance of non-terpene compounds (Giovannelli et al. 2017). Zhu et al. (2007) made the same findings on the chemical composition of *Rhus typhina* leaves in China. This variation in chemical composition is caused by variations in habitat, climatic conditions, as well as sampling conditions and genetic factors (Morshedlooa et al. 2017). The richness of *R. tripartita* in hydrocarbon monoterpenes, as well as aromatic monoterpenes such as limonene, γ -terpinene, and terpinolene explains the characteristic therapeutic properties of this species (Deschepper 2017; Thormar 2011).

The chemotypes of *R. tripartita*, identified in our study, are based on two major components (α -pinene and Δ^3 -carene), followed by limonene and caryophyllene-Z, while the chemotypes identified in the Iranian populations of *R. coriaria* are characterized by caryophyllene-E, α -pinene and cembrene (Morshedlooa et al. 2017). The results of the antibacterial activity showed that the bacteria tested are moderately sensitive to the *R. tripartita* essential oils. The bacteria *E. faecalis* and *P. aeruginosa* are the most sensitive to the oil, while *P. mirabilis*, *S. aureus* and *E. coli* are the most resistant to oils from all populations.

Our results are in agreement with the work of Bereksi et al. (2019), which showed that extracts from the aerial parts of *R. tripartita* have weak antibacterial activity against *S. aureus* and *E. coli*. On the other hand, the results of Tlili et al. (2019) confirm that the methanolic extracts of *R.*

tripartita have a weak activity on Gram-negative bacteria. Chloroform extracts of *R. tripartita* have significant activity against *S. aureus* and moderate activity against *E. coli* (Abbasi and Hani 2012), while methanolic extracts from the roots, stems and leaves of *R. tripartita* have significant antibacterial activity against *P. aeruginosa* (Ben Barka et al. 2019).

In conclusion, the analysis of *Rhus tripartita* essential oils by GC/MS, allowed us to identify seventy-eight compounds of which α -pinene is the major component, along with Δ^3 -carene and caryophyllene-Z. The results reveal a notable difference between the chemical composition of essential oils of male populations and female populations. In this study, four chemotypes were identified in *R. tripartita*. The essential oils of *R. tripartita* shows moderate activity against the bacterial strains tested. *E. faecalis* and *P. aeruginosa* are the most sensitive to oils, while *S. aureus*, *E. coli* and *P. mirabilis* are the most resistant.

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