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Original article

Chemical variability in essential oils isolated from roots, stems, leaves and flowers of three *Ruta* species growing in Morocco

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ABSTRACT

In this study, the essential oils (EOs) of separated organs (roots, leaves, stems and flowers) of *Ruta chalapensis* L., *Ruta gravealens* L., and *Ruta montana* L. are characterized by GC/MS analysis and the intra-species variations of the chemical composition of essential oils from three Morocco species were investigated using statistical analysis (principal component analysis (PCA) and cluster analysis (CA)). Correlations between the EO chemical compositions, organs and species were discussed. The results obtained show that a mixture of ketones generally characterizes the EOs of the different organs of three species studied, which 2-undecanone reach 74,36%. Nevertheless, it was observed specific significant disparities between species but also within the same species, especially for presence of chalepensin, geijerene and elemol.
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1. Introduction

About 700 spontaneous species, in temperate and warm regions, make up the Rutaceae family. The *Ruta* genus encompasses about sixty species, some of which are found around the Mediterranean (Hammiche and Azzouz, 2013). In Morocco, the *Ruta* genus is represented by four species: *Ruta tuberculata* Forsk., *Ruta montana* L., *Ruta gravealens* L., and *Ruta chalepensis* L., they are designated by the same vernacular names fidjel in Arabic and aourmi in Berber (Bellakhdar, 1997). These last three species are the most diffused and are morphologically poorly differentiated and were probably interchangeably used during Antiquity (Pollio et al., 2008). All are perennial plants with yellow flowers, characterized by a strong smell, fetid, nausea, due to an essential oil contained in huge dry pockets. They were already known in ancient Greece and studied by Hippocrates, five centuries before our era, then by Dioscoride, in the first century. The "herb of grace", which intervened during Catholic rites as a means of spraying holy water, was the first plant introduced into the American continent (Hammiche and Azzouz, 2013).

Essential oils (sometimes called vegetable essence) are generally complex combinations containing many unique compounds, which are produced in all aromatic plants and trees through three pathways (mevalonic acid (MEV), 2-C-methyl-D-erythritol-4-phosphate (MEP) and shikimate pathway). Terpenoids are produced by two biosynthetic pathways: the MEV that occurs in the cytoplasm, and the MEP, which occurs in the plastids. Phenylpropanoids are derived from the shikimate pathway (Luz et al., 2020). Therefore, when referring about constituents of essential oils; there are two distinct chemical classes: terpenoids and phenylpropanoids. Terpenoids are the most common and abundant, in addition, they are extremely variable, showing different carbon skeletons and a wide variety of oxygenated derivatives including alcohols, esters, aldehydes, ketones, ethers, peroxides and phenols. Phenylpropanoids are the least common group of aromatic compounds and when these compounds are present, they impart a specific smell and flavor to plants (Zuzarte and Salgueiro, 2015).

The applications of essential oils are always oriented by its composition. Nowadays in the world intensively developed research with use of essential oils from plants with their healthy effects (Bakkali et al., 2008; Barão Paixão and Freire de Carvalho, 2021; Elshafie and Camele, 2017; Karakaya et al., 2020, 2019; Ložienė et al., 2018; Luz et al., 2020; Matias et al., 2016). Hence, the importance of studying the composition of essential oils because each of

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its constituents contributes their beneficial or harmful effects (Haddouchi et al., 2013). Beyond the benefit provided by essential oils, we can, however, realize that there were risks which, in some cases, could lead to toxic effects (Barão Paixão and Freire de Carvalho, 2021; Elshafie and Camele, 2017; Posadzki et al., 2012).

Analysis of the composition of EOs of several *Ruta* species indicates that a mixture of ketones, generally characterizes them; which 2-undécanone, 2-nonanone and 2-dodécanone are its main constituents. Nevertheless, variations and the existence of significant specific disparities can occur between species but also within the same species (Khadhri et al., 2014). 2-undécanone was reported as a promising antifungal and antibacterial compound (Benali et al., 2020).

In folk medicine, *Ruta* species represents several therapeutic properties for the treatment of many diseases involved in public health problems, such as infections, neurological diseases, cardiovascular and reproductive system disorders, menstrual disorders, skin inflammations, cramps, earache and headache (Coimbra et al., 2020; Orlanda and Nascimento, 2015; Pollio et al., 2008). Pharmacological trials have proven their as anthelmintic, antioxidants, antiparasitics, anti-inflammatory, and-rheumatic, antifebrile, antiulcer, anti-diabetic, antidepressants, anti-diarrheic, and antimicrobial properties (Attia et al., 2018; Haddouchi et al., 2013; Khlifi et al., 2013; Orlanda and Nascimento, 2015).

To the best of our knowledge the chemical composition of EOs of separated organs (roots, leaves, stems and flowers) from *Ruta chalapensis* L., *Ruta gravealens* L., and *Ruta montana* L. has not been studied previously, see all cited references of results and discussion section, also (Al-shuneigat et al., 2015; Bennaoum et al., 2017; Ghazghazi et al., 2015; Haddouchi et al., 2013; Hammami et al., 2015; Semerdjieva et al., 2019; Yosra et al., 2019). Therefore, in this case, the investigation seems to be an interesting case of study. In this study, the EOs of separated organs (roots, leaves, stems and flowers) from three Moroccan species (*Ruta chalapensis* L., *Ruta gravealens* L., and *Ruta montana* L.) were characterized by GC/MS analysis and the intra-species variations of the chemical composition of EOs were investigated using statistical analysis PCA and CA.

2. Material and methods

2.1. Plant material

The roots, stems, leaves and flowers of *Ruta* species (Rutaceae family) were collected in the morning between 9:00–11:00 am (during the flowering stage: Mai) from two different regions of Morocco. *Ruta chalepensis* and *Ruta montana* were collected from Moulay Idriss Zerhoun (33° 50′ 50,4636′′ N, 5° 19′ 0,3972′′ W) and *Ruta gravealens* from Meknes (33° 51′ 57,6468′′ N, 5° 32′ 05,8164′′ W). Botanical identification was carried out by Prof. Nadia Belahbib, Laboratory of Botany, Biotechnology and Plant Protection, Faculty of Sciences, University Ibn Tofail, BP 133, 14,000 Kenitra, Morocco. Plant materials were air-dried in the shade at room temperature for 10 days and then extracted separately using hydrodistillation method for 4 h in Clevenger type apparatus. EOs were stored in the dark vials and stored frozen at 4 °C, until analysed.

The extracted EOs were weighed in order to calculate their extraction yield and evaluated from 3 extractions. It is expressed as a percentage and calculated by the following formula:

$$EO\% = \frac{W_2}{W_1} \times 100$$

With: W_1: Weight of the dry matter of the plant in g
W_2: Weight of EO in g.

2.2. Gas chromatographic-Mass spectral analysis

The analysis process of each essential oil was carried out using Perkin Elmer Clarus SQ 8C Gas chromatograph coupled with a mass spectrometer (GC/MS), equipped with a Rxi-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm). The carrier gas was helium (He: 1 mL/min. Temperature program: For the first 2 min the oven temperature was kept at 40 °C and then increased at a rate of 4 °C/min until reached a temperature of 180 °C and from 180 to 300 at a rate of 20 °C/min and then held isothermally at 300 °C for 2 min. As regards the split injection was conducted with a ratio split of 1/20, the injected volume: 1 μL. Injector and detector temperature were held at 220 °C. Ion source temperature: 200 °C; energy ionization: 70 eV; electron ionization mass spectra were acquired oven the mass ranges 40–450 Da. The chemical components of *Ruta* species essential oils were identified by their Kovats retention indexes, as well as the mass spectra with those on the stored in NIST library-version 2014. For the Kovats index were estimated using to the retention time of the homologous series of n-alkanes (C_8-C_{20}) under the same operating conditions.

2.3. Statistical studies

Statistical analyses of the chemical compositions were performed using the XLSTAT (version 2015) statistical software; Principal Component Analysis (PCA) and Cluster Analysis (CA) were used to evaluate the chemical composition variability between the EOs obtained from different plant organs and the three *Ruta* species.

Indeed, the PCA provides the data for the diagrams in which the objects (EO samples) and variables (EO components) are plotted while canonical analysis informs a classification tree in which the objects (the sampling species) are gathered. The CA produced a dendrogram (tree) using Ward's method of Hierarchical Clustering, based on the Euclidean distance between pairs of EO samples.

3. Results and discussion

3.1. Yields of essential oils from Ruta plants

The essential oil yields from the Moroccan samples shown difference between analyzed plant organs and species. In the organs from the *Ruta chalapensis*, the yield ranged from 0.06% in roots, 0.22% in stems, 3.37% in leaves and 3.86% in flowers. As regards to the *Ruta gravealens* EOs, the yield was 0.07% in roots, 0.17% in stems, 0.84% in leaves and 1.13% in flowers, while in the *Ruta montana* EOs, the yield was 0.08% in roots, 0.09% in stems, 0.85% in leaves and 2.24% in flowers.

It is apparent through observing the extraction yields presented in Table 1, they differ significantly ($p < 0.05$) on the one hand, according to the organs, on the other hand, the species. The flower EOs gives the height yield an average of 2.41%, while the root EOs gives the lowest yield (0.07% on average).

Previous work has shown that the yield of *Ruta* plants differs depending from which plant organs the EOs were extracted, the species and the origin of the plant population. For example, from a Tunisian population the leaf and flower EOs of *Ruta chalapensis* yielded 0.25% and 1.75% (Akkari et al., 2020), while the leaf EO from Palestinian yield ranged between 0.66%−1.6% to (Jaradat et al., 2017). The yield of *Ruta graveolens* leaf and flower EOs obtained from Egypt was 0.36% and 0.21%, respectively (Attia et al., 2018). The essential oil yield from *Ruta gravealens* leaf was 0.1% v/w. The leaf and stem EOs of *Ruta montana* from Tunisia (Khadhri et al., 2014) showed a same yield 0.66%.

Table 1
Essential oil yields (%) in different organs of *Ruta* species.[a]

	Yields% (w/w)			
	Roots	Stems	Leaves	Flowers
Ruta chalapensis	0,06 ± 0.00[c]	0,22 ± 0.01[a]	3,37 ± 0.04[a]	3,86 ± 0.02[a]
Ruta gravealens	0,07 ± 0.00[b]	0,17 ± 0.02[a]	0,84 ± 0.02[b]	1,13 ± 0.04[c]
Ruta montana	0,08 ± 0.00[a]	0,09 ± 0.00[b]	0,85 ± 0.00[b]	2,24 ± 0.02[b]
Means Yields%	0,07 ± 0.00	0,16 ± 0.00	1,86 ± 0.01	2,41 ± 0.01

[a] Mean values ±standard deviations of triplicate determinations are reported; Means with different letters in the columns are significantly different ($p < 0.05$).

3.2. Chemical composition of essential oils

The chemical composition of the EOs from different organs of *Ruta chalapensis*, *Ruta gravealens*, and *Ruta montana* was reported in Table 2. In total, 27 volatile components were identified and listed based on their Kovats indices. The number of identified components varies and depends both on the plant organ and species.

The most abundant compound in the EOs from stems, leaves and flowers of *Ruta chalapensis* were 2-undecanone, which ranged from 28.69 to 32.42%, 2-dodecanone 11.17–16.17% and 2-decanone 10.36–16.20%, while the pentadecane-2,4-dione was the most abundant compound found in the root EO, with levels of 13.59%, followed by geijerene (12,21%), chalapensin (11.59%) and 2-undecanone (10.76%).

For *Ruta gravealens*, the main constituents of the root EO are chalepensin (36.41%), elemol (20.61%) and 2-undecanone (18.43%), whereas its stem EO is characterized by the dominance of 2-undecanone (26.54%) while the leaf and fruit EOs showed a predominance of 2-undecanone (22.44% and 31.07%, respectively) and 2-nonanone (13.94% and 24%).

As regards to the root EO of *Ruta montana*, the chalepensin, pentadecane-2,4-dione and elemol are the dominant compounds (29.78%, 25.12% and 17.19%, respectively), the major components detected in the stem EO were 2-undecanone (48.64%) and 2-undecanol (14.15%), the 2-undecanone was the major compound of the leaf and fruit EOs (74,36% and 40,61%, respectively), we also note the abundant of undecan-2-yl acetate (22.16%) and 2-undecanol (20.38%).

It can be concluded from the results obtained that a mixture of ketones generally characterizes the essential oils of the different parts of three species studied, namely: 2-undecanone, 2-dodecanone, 2-decanone, pentadecane-2,4-dione and 2-nonanone. Nevertheless, there are specific significant disparities between species but also within the same species, such as compounds elemol, geijerene and chalepensin.

Previous studies have studied the chemical composition of the EOs obtained from the aerial parts of *Ruta chalapensis*, *Ruta gravealens* and *Ruta montana*. All these studies clearly showed that the EOs from of three species characterized by high contents of 2-undecanone compound. Table 3 describes the aforementioned results in detail.

However, studies on the EOs from different organs (leaf, stem, fruit, or flower) of the three species have also shown that there is a qualitative and quantitative difference in their chemical composition. Thus, it should be noted that the 2-undecanone compound is always present with high contents in all the EOs studied.

According to (Krayni et al., 2018), the chemical composition of the EOs obtained from the leaves, stems and fruits of *Ruta*

Table 2
Composition (%) of the essential oils from roots, stems, leaves and flowers of *Ruta* plants.

	Compounds	IK	*Ruta chalepensis*				*Ruta gravealens*				*Ruta montana*			
			R	S	L	F	R	S	L	F	R	S	L	F
1	α-Pinene	930	2,78	3,39	1,66	1,67	–	0,92	–	–	–	tr	0,68	–
2	β-Terpinene	969	0,47	–	0,46	–	–	–	–	–	tr	–	–	–
3	β-Myrcene	988	0,32	–	tr	–	–	1,28	0,44	2,18	–	–	–	tr
4	*p*-Cymene	1020	0,85	–	tr	–	–	–	–	–	–	–	–	–
5	Limonene	1024	1,92	–	1,63	1,00	–	0,62	–	–	–	tr	–	–
6	Eucalyptol	1027	2,07	3,29	5,97	3,64	–	–	–	–	–	–	–	–
7	2-Nonanone	1089	0,04	4,58	5,21	5,01	tr	8,60	13,94	24,00	–	–	8,64	1,77
8	Linanool	1096	0,60	tr	0,73	0,80	–	–	–	–	–	–	–	–
9	2-Nonanol	1098	–	–	–	–	–	2,03	1,43	–	tr	–	–	–
10	Nonanal	1100	0,22	3,28	2,41	2,75	–	tr	–	–	–	–	tr	–
11	Geijerene	1140	12,21	3,91	–	–	8,90	9,90	3,72	–	2,22	–	–	tr
12	2-Decanone	1191	2,28	10,36	16,20	13,39	–	8,27	9,23	10,74	tr	5,91	7,74	2,96
13	Nonan-2-yl, acetate	1235	–	–	–	–	–	6,81	10,12	2,81	tr	–	–	–
14	2-Undecanone	1294	10,76	32,42	30,04	28,60	18,43	26,54	22,44	31,07	4,29	48,64	74,36	40,61
15	2-Undecanol	1296	1,15	3,19	2,35	2,76	tr	1,99	tr	–	tr	14,15	4,72	20,38
16	Undecanal-2-methyl-	1352	0,95	9,63	8,15	8,13	–	8,42	7,47	8,43	–	–	–	–
17	2-Dodecanone	1377	1,94	15,09	16,17	11,17	tr	7,60	6,97	5,53	–	3,27	2,76	–
18	Caryophellene	1408	–	–	–	–	–	–	–	–	–	3,46	–	2,89
19	Undecan-2-yl acetate	1417	9,54	–	tr	1,49	8,39	0,27	8,61	tr	6,34	9,96	tr	22,16
20	*trans*-β-Ionone	1486	tr	–	–	–	6,17	–	–	–	9,46	–	–	–
21	2-Tridecanone	1494	7,27	6,79	7,81	15,64	–	6,24	5,36	8,31	–	2,77	–	3,32
22	Elemol	1546	6,79	tr	–	–	20,61	3,20	1,41	tr	17,19	tr	–	tr
23	Tridecane-2,4-dione	1577	8,94	–	–	–	tr	tr	–	–	4,13	5,97	–	4,13
24	Pentadecane-2,4-dione	1770	13,59	–	–	–	0,26	1,63	–	–	25,12	–	–	–
25	Palmitic acid	1959	1,87	–	–	–	–	–	–	–	–	2,09	–	–
26	(*Z*)-Phytol	2116	–	–	–	tr	–	–	2,05	–	–	–	–	0,91
27	Chalepensin	2198	11,59	2,99	0,75	1,23	36,41	4,48	5,80	6,02	29,78	2,81	tr	–
Total identified (%)		99,06	98,91	99,10	99,09	99,18	98,77	98,99	99,10	98,52	99,04	98,90	99,11	

R: Roots; S: Stems; L: Leaves; F: Flowers; tr: trace (<0.2); –: Not detected.

Table 3
Chemical comparison of *Ruta* plants EOs from the literature of different world's countries.

Ruta Plants	Organes	Predominant compounds	Countries	References
Ruta chalepensis	Aerial parts	2-undecanone (36.42%), 2-acetoxytridecane (25.42%)	Algeria	(Terkmane et al., 2017)
Ruta chalepensis		2-nonanone (41.7%) 2-undecanone (40.1%).	Chile	(Tampe et al., 2016)
Ruta graveolens		2-undecanone (43.66%), 2-nonanone (16.09%)	India	(Reddy and Al-rajab, 2016)
Ruta graveolens		2-undecanone (47.21%) 2-nonanone (39.17%).	Brazil	(Orlanda and Nascimento, 2015)
Ruta graveolens		2-undecanone (56.92%) 2-nonanone (23.62%)	Tunis	(Yosra et al., 2019)
Ruta montana		2-undecanone (63.97%)	Morocco	(Benali et al., 2020)
Ruta montana		2-undecanone (67%) 2-decanone (9%)	Algeria	(Drioueche et al., 2020)
Ruta montana		2-undecanone (27.2–81.7%) 2-nonanone (1.9–39.5%)	Algeria (7 locations)	(Mohammedi et al., 2020)
Ruta montana		2-undecanone (86.77%)	Tunis	(Yosra et al., 2019)

chalepensis from Tunisia show that the 2-undecanone is the most predominant compound (23.0–58.4%) and 2-nonanone (16.7–23.3%). In another study conducted by (Akkari et al., 2020), were found that the leaf and flower EOs of this species are dominated by 2-undecanone with high contents (85.94% and 89.89%, respectively). As shown to (Jaradat et al., 2017), 2-undecanone (44.31%) and 2-nonanone (43.02%) were found to be the main chemical group in the leaf EO from Palestinian (Jenin region), while the leaf EOs from the regions of Hebron and Jerusalem were characterized by the dominance of linalyl acetate 29.51 34.21%, followed by β-linalool 26.78–31.78%, 2-undecanone 14.99–7.66% and 2-nonanone 14.29–8.15%. *Ruta graveolens* leaf and flower EOs obtained from Egypt (Attia et al., 2018), the major identified compounds, were 2-undecanone (50.06% and 74.80%, respectively) and 2-nonanone (25.14% and 7.44%). The leaf and stem EOs of *Ruta montana* from Tunisia (Khadhri et al., 2014) showed a predominance of 2-undecanone (52.20% and 44.9%, respectively) and 2-nonanone (13.50% and 5.80%).

3.3. Chemical variability

PCA and CA were used to identify the possible relationships between EO compounds and the three species of the *Ruta* plants. The CA (Fig. 1) for organ essential oils (Roots (R), Stems (S), Leaves (L), and flowers (F)) makes it possible to distinguish 3 groups of samples. The first group (I) consists of six EO samples, the second group (II) contains three EO samples and the third group (III) three EO samples.

The PCA (Fig. 2) confirms the results of the CA and highlights the main quantitative differences between the 3 groups of chemical composition. The two-dimensional axial system of analysis identified three groups of *Ruta* species based on the chemical composition of their essential oils (Fig. 2). The first two principal axes represented 51.11% of the total variance, the first axis (29.72% of the total variance) and the second axis (21.39% of the total variance).

The discriminating compounds for the first group are α-Pinene (C1), β-Terpinene (C2), Limonene (C5), Eucalyptol (C6), Linanool (C8), Nonanal (C10), 2-Decanone (C12), Undecanal-2-methyl-

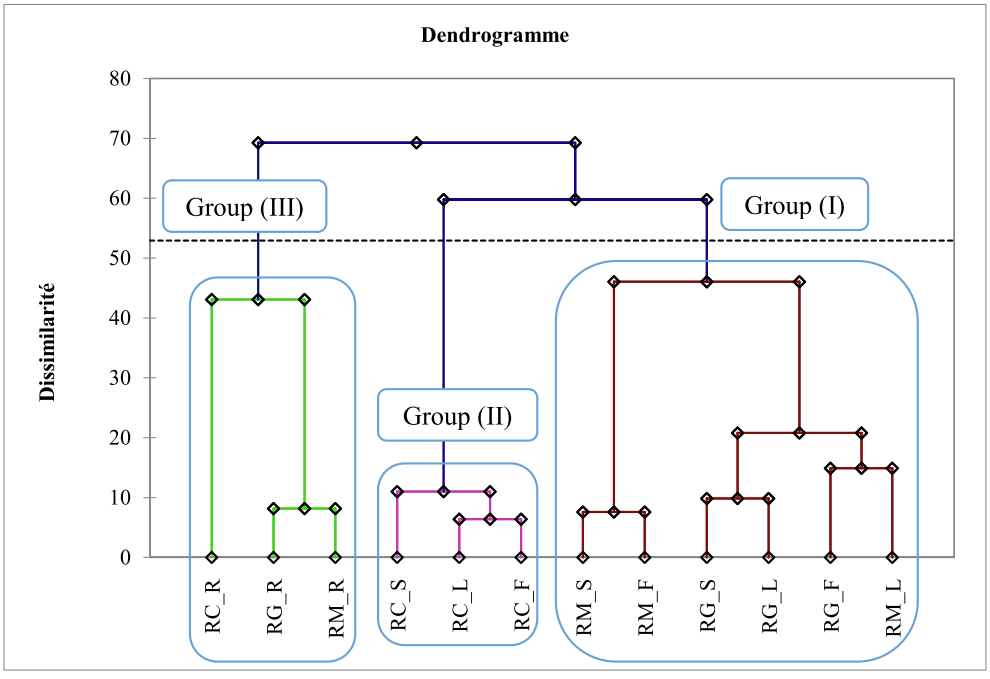

Fig. 1. Cluster Analysis of chemical compositions of Ruta species from Morocco. RG: *Ruta gravelons*; RM: *Ruta montana*; RC: *Ruta chalepensis*; R: Roots, S: Stems; L: Leaves; F: Flowers.

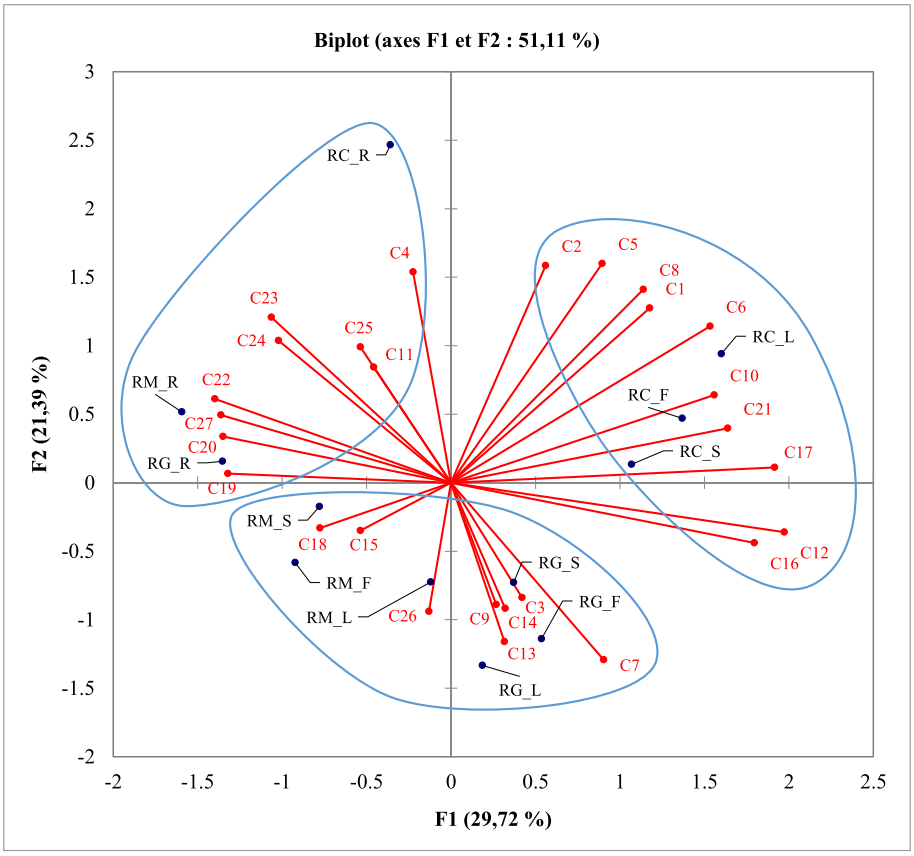

Fig. 2. PCA analysis performed on all essential oil compounds of the analyzed *Ruta* species. Mainly correlated compounds: α-pinene (C1), β-terpinene (C2), β-myrcene (C3), *p*-cymene (4), limonene (C5), eucalyptol (C6), 2-nonanone (C7), linanool (C8), nonanol (C9), nonanal (C10), 2-geijerene (C11), 2-decanone (C12), nonan-2-yl, acetate (C13), 2-undecanone (C14), 2-undecanol (C15), undecanal-2-methyl- (C16), 2-dodecanone (C17), caryophellene (C18), undecan-2-yl acetate (C19), *trans*-β-ionone (C20), 2-tridecanone (C21), elemol (C22), tridecane-2,4-dione (C23), pentadecane-2,4-dione (C24), palmitic acid (C25), (*Z*)-phytol (C26), and chalepensin (C27). RG: *Ruta gravelons*; RM: *Ruta montana*; RC: *Ruta chalepensis*; R: Roots, S: Stems; L: Leaves; F: Flowers.

(C16), 2-Dodecanone (C17), and 2-Tridecanone (C21). The discriminating compounds for the second group are β-Myrcene (C3), 2-Nonanone (C7), 2-Nonanol (C9), Nonan-2-yl, acetate (C13), 2-Undecanone (C14), 2-Undecanol (C15), Caryophellene (C18), and (*Z*)-Phytol (C26). However, the discriminating compounds for the third group are *p*-Cymene (4), Geijerene (C11), Undecan-2-yl acetate (C19), *trans*-β-Ionone (C20), Elemol (C22), Tridecane-2,4-dione (C23), Pentadecane-2,4-dione (C24), Palmitic acid (C25), and Chalepensin (C27).

4. Conclusions

In this work, we study the chemical composition of the three *Ruta* species essential oils growing in Morocco such as *Ruta* chalpensis, *Ruta gravelons* and *Ruta montana*. Results showed differences in the composition and yields according to the part used and species. Different parts of three species are rich in ketones. The use of ketone oils are very beneficial therapeutically, but they should be handled with great care. Hence, the importance of studying the composition of essential oils because each of its constituents contributes their beneficial or harmful effects. However, in spite of considerable data on the several therapeutic properties of essential oils and their constituents of *Ruta* species. Surprisingly few therapeutic and products based on plant essential oils have appeared in the market place. Further studies are required to determine the cost, applicability, safety and phytotoxicity of these essential oils as therapeutic agents, before any application in the pharmaceutical industry.

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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