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Study of Chemical composition, antibacterial and antioxidant activities of *Rapistrum rugosum* L. essential oils from flowers, leaves, and stems

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Abstract: The chemical composition of the essential oils of three organs (flowers, leaves and stems) from *Rapistrum rugosum* (L.) All., was studied and screened for their possible antibacterial and antioxidant properties. According to the GC and GC/ MS analysis, 28 (represent 92.5% of the total oil composition), 23 (93.9%), and 38 compounds (94.3%) were identified from flowers, leaves and stems, respectively. The major compound in the leaves essential oil was found to be pentadecanal (55.3%) followed by hexahydrofarnesylacetone (8.4%) and tetradecanoic acid (5.7%).

While hexahydrofarnesylacetone (17.2%) followed by 1-pentadecanol (8.3%), tetradecanoic acid (5.9%), *(E)*-β-damascenone (5.7%) were the major compounds of the flowers oil. Tetradecanoic acid (13.1%), hexahydrofarnesylacetone (10.1%), dodecanoic acid (10.0%), isobornyl acetate (7.6%), 5-methylthiopentyl isothiocyanate (6.7 %) and *(E)*-β-ionone (6.2%) were the main constituents for the leaves oil.

The flowers essential oil exhibited the higher activity in each antioxidant system (ABTS, DPPH and β-Carotene–linoleic acid methods) with an important value for DPPH assay (PI = 69.88 ± 0.02%). Furthermore, the isolated oils were tested against five Gram-positive and Gram-negative bacteria. It was found that the oil of all organs exhibited interesting antibacterial activities against Gram-positive bacteria, comparable to those of Gentamicin, which was used as positive control.

Keywords: *Rapistrum rugosum*, essential oil, chemical composition, antioxidant, antibacterial.

Introduction

Plants and herbal extracts have found important position in modern medicine cabinet, due to their content in natural medicinal chemicals. Their secondary metabolites represent a large reservoir of structural moieties which work together exhibiting a wide range of biological activities[1]. Efforts have been made in many regions of the world to identify plants having medicinal properties effective against various modern diseases. Many plants have been used for different purposes, such as food, drugs and perfumery[2].

Essential oils and extracts obtained from many plants have recently gained popularity and scientific interest. They have been screened for their potential uses as alternative remedies for the treatment of many infections and preservation of foods from the toxic effects of oxidants[3-5]. The crucifer family or *Brassicaceae* is an economically important family for its many food and oilseed crops, as well as for containing many important ornamental plants and noxious weeds[6]. Moreover, the consumption of cruciferous vegetables has been associated with a reduced risk of cancer of the lung, stomach, breast, prostate, pancreas, colon and rectum, which has been attributed to its isothiocyanate contents[7,8]. Tunisia, located in the Mediterranean area, has a large number of medicinal and aromatic species. More than 500 species out of 2103 (approximately 25% of the total flora) are considered for therapeutic use[9]. *Rapistrum rugosum* (L.) All. (*R. rugosum*), belonging to the *Brassicaceae* (cruciferous) family locally named as

''lebsen'', is a wild plant common to Libya, Algeria, Tunisia and Mediterranean regions[10]. The cruciferous plant family contains 338 genera and 3350 species that are distributed worldwide[11]. A previous study has shown that Rapistrum rugosum extracts contains active constituents which possess antioxidant, anti-acetylcholinesterase and cytotoxic activities[12,13]. To the best of our knowledge, the antioxidant and antibacterial activities of essential oil of *R. rugosum* L. (Brassicaceae) have not been studied hitherto. Therefore, the aim of this study is to determine the chemical composition of the essential oil from *R. rugosum* L. and evaluate their *in vitro* antioxidant and antibacterial properties.

Materials and methods

Plant material

The aerial parts of *R. rugosum* L. (8 Kg), were collected at the flowering stage in February 2012 in the area of Kasserin, in Midwest of Tunisia (35°16′N latitude, 8°83′E longitude, altitude 656 m above sea, average T° = 8.4°C, average rain fall = 13.6 mm; February 2012). A voucher specimen (Rr-110) was deposited in the Medicinal Chemistry and Natural Products Team, Laboratory of Heterocyclic Chemistry, Natural Products and Reactivity at the Faculty of Science of Monastir, Tunisia. The fresh flowers, leaves and stems were divided into small pieces and weighed before the isolation of the volatile

compounds. The species was identified by Dr. Fethia HARZALLAH-SKHIRI, High Institute of Biotechnology of Monastir, Tunisia.

Isolation of the essential oil

Fresh aerial parts (leaves, stems and flowers) of *R. rugosum* L. (300 g + 1.5 liters of distilled water) were placed in a Clevenger hydrodistillation for 4 hours. Each essential oil was collected by decantation by addition of hexane, evaporation, dried over Na_2SO_4, weighed and stored in sealed glass vials at 4-5˚C until analysis.

Chemical analysis of essential oils

Analytical GC

The GC/ FID analysis of the oils was carried out on a HP 5890-series II equipped with flame ionization detectors (FID), attached to HP-5 (30m x 0.25 mm ID, 0.52 µm film thickness) fused silica capillary column. Carrier gas (hydrogen) flow rate was 1.2 mL/min, temperature oven was programmed from 50˚C (1 min) to 280˚C at 5˚C/min (1 min), injector and detector temperatures were 250˚C and 280˚C, respectively, the volume injected was 0.1 µL of a 1% solution of hexane. The identification of the components was performed by comparison of their retention times with those of pure authentic samples and by means of their Linear Retention Indices (L.R.I) relative

to the series of *n*-hydrocarbons. The percentage by mass of the various chemical constituents of the *R. rugosum* L. oil is given on the relative surface area of the peaks (GC-FID).

Analytical GC/ MS

The same analytical conditions as those mentioned for GC/FID were employed for GC/MS analysis, along with a HP-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 µm), equipped with a Varian CP-3800 gas-chromatograph and Varian Saturn 2000 ion trap mass detector. The injector and transfer line temperatures were 220 and 240˚C, respectively, oven temperature programmed was linearly programmed from 60˚C to 240˚C at 3˚C/min, helium was used as carrier gas at rate of 1 mL/ min, the injection was 0.2 µL (10% hexane solution), split ratio was 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra built up from pure substances and components of known essential oils and MS literature data[14-29]. Moreover, the molecular weights of all the identified substances were confirmed by GC/ CIMS, using MeOH as CI ionizing gas.

Antibacterial activity

Microorganisms

Five bacteria make part of two Gram positive (*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212), with three Gram negative bacteria (*Escherichia coli* ATCC 25922, *Acinetobacter sp* and *Pseudomonas aeruginosa* ATCC 27853) were used.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC values for the antibacterial screening were determined with the broth dilution method (microdilution using 96-well microplates) following the procedure described by Cintia, et.al, 2007[30]. All samples were prepared at a concentration of 10 mg/mL by dissolution of the oils in 10% DMSO. The final concentrations of the plant samples tested ranged from 10 to 0.015 mg/mL. The MIC of each sample was defined as the lowest concentration of oil that inhibited either the bacterial growth, after incubation at 37˚C for 18 to 24 h. The MBC was determined by subculture on blood agar at 37˚C for 18 to 24 h. Gentamicin was used as positive control against the bacterial strains. All measurements were performed in triplicate.

Antioxidant activity

Scavenging effect on DPPH

The hydrogen atoms or electrons donation ability of the corresponding samples were measured from the bleaching of purple colored methanol solution of DPPH[31]. The effect of essential oils on DPPH radical was estimated according to Hatano, et.al, 1988[32]. 0.5 mL of each sample, with a concentration of 1 mg/mL and BHT (Butylated hydroxytoluene) was mixed with the same volume of DPP methanolic solution. The mixture was shaken vigorously and allowed standing for 30 min in darkness Inhibition of free radical DPPH in percent (I%) was calculated in the following way:

$I\% = 100 \times (A_{Control} - A_{Sample})/A_{Control}$

And at a temperature of 25˚C; the absorbance of the resulting solution was measured at 520 nm with a UV spectrophotometer. All measurements were performed in triplicate. A mixture of 0.5 mL of DPPH solution and 0.5 mL of methanol was taken as a control.

Where, $A_{Control}$ is the absorbance of the control reaction (containing all reagents except the test compound), and A_{Sample} is the absorbance of the tested sample.

β-Carotene-linoleic acid method

In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [33]. β-Carotene bleaching inhibition of *R. rugosum* L. essential oils according to the method of Ikram, et.al,2009[34]. Briefly, 2 mL of β-carotene solution (1.5 mg β-carotene/2.5 mL chloroform) were added to 20 µL of linoleic acid and 200 µL of Tween-20. The chloroform was removed at 40˚C under vacuum using a rotary evaporator. Immediately, 50 mL of distilled water were added to the dried mixture to form a β-carotene-linoleic acid emulsion. In order to determine the β-carotene bleaching activity of the essential oil, 5 mL of emulsion were added to 500 µL of essential oils in concentration of 1 mg/mL. The mixtures were incubated in a water bath at 50˚C for 60 min and the absorption of the reaction mixtures was read at 470 nm. All measurements were performed in triplicate.

The antioxidant activity (AA) of the essential oils was calculated by using the following equation:

AA% = (β-carotene content after 2h assay / initial β-carotene content)*100.

ABTS radical scavenging activity assay

Antiradical activity was done by using the $ABTS^{\cdot+}$ free radical decolorization assay developed by Re, et.al., 1999, with some modifications. Briefly, the preformed radical monocation

of ABTS was generated by reacting ABTS solution (7 mM) with 2.45 mM $K_2S_2O_8$. The mixture was allowed to stand for 15 hours in the dark at room temperature[35]. The solution was diluted with methanol to obtain the absorbance of 0.7 ± 0.2 units at 734 nm. Samples were separately dissolved in methanol to yield the concentration of 1mg/mL. In order to measure the antioxidant activity of samples and BHT, 10 µL of each one at various concentrations was added to 990 µL of diluted $ABTS^{\cdot+}$. The absorbance was measured spectrophotometrically at 734 nm after 20 min. All measurements were performed in triplicate. The percentage decrease of absorbance at 734 nm was calculated for each point and the antioxidant capacity of the test samples was expressed as percent inhibition (%). The percentage scavenging of ABTS was calculated by the following formula:

$$\text{Scavenging activity (\%)} = [(A_o - A_x)]/ A_o \times 100$$

A_x and A_o were the absorbance at 734 nm of samples with and without essential oil, respectively.

Results and discussion

Chemical composition of the essential oils

Three samples were analysed by GC and GC-MS. The yields (w/w) of the different oil samples of *R. rugosum*, reported in Table 1, ranged from 0.0061% to 0.23%, with the

highest yield for the flowers oil (0.23%) and the lowest yield for the leaves oil (0.0061%).

A quantitative and qualitative variation between the oils from flowers, leaves and stems was apparent. 53 components were identified in flowers, leaves and stems representing 92.5%, 93.9% and 94.3% of the total essential oil, respectively.

The Non-terpene derivatives such as aldehydes, ketones, esters and acids were dominant in all essential oils (33.6-69.6%) followed by sulfur and/or nitrogen compounds, apocarotenoids and oxygenated sesquiterpenes.

Comparing the three essential oils, in the flowers oil, apocarotenoids formed 28.7% followed by sulfur and nitrogen compounds (16.8%), oxygenated sesquiterpenes (8.3%) and oxygenated monoterpenes (3.5%), while in the leaves, apocarotenoids formed 15.1% followed by oxygenated sesquiterpenes (7.2%), the amounts of the sulfur and nitrogen compounds and oxygenated monoterpenes were 0.5%. On the other hand, in the stems, apocarotenoids formed 22.1% followed by sulfur and nitrogen compounds (12.2%), oxygenated monoterpenes (10.5%) and oxygenated sesquiterpenes (7.2%).

28 Compounds were identified in flowers essential oil representing 92.5% of the total oil composition. The major component was hexahydrofarnesylacetone (17.2%) followed by 1-pentadecanol (8.3%), tetradecanoic acid (5.9%), *(E)*-β-

damascenone (5.7%), dimethyl tetrasulfide (4.5%), α-acorenol (3.7%), pentadecanal (3.6%), berteroin (3.6%) and *(E)*-β-ionone (3.5%). On the other hand, the representative compounds on the flowers found relatively low percentages were dodecanoic acid (2.7%), dimethyl trisulfide (2.6%), erucin (2.5%), *trans*-3,5-dimethyl-1,2,4-trithiolane (2.1%), 1,10-di-*epi*-cubenol (1.8%), eugenol (1.6%), 1,2,3-trithiane (1.5%), 10-*epi*-g-eudesmol (1.5%), methyl carvacrol (1.4%) and α-cadinol (1.3%).

23 Compounds were identified in leaves essential oils representing 93.9% of the total oil composition. The major compound in the leaves was pentadecanal (55.3%) followed by hexahydrofarnesylacetone (8.4%), tetradecanoic acid (5.7%), dodecanoic acid (3.9%), *(E)*-β-ionone (2.6%), β-cyclocitral (2.2%), 1-tetradecanol (2.1%), and some other compounds were present only in minor amounts such as 1,10-di-*epi*-cubenol (1.5%), 10-*epi*-g-eudesmol (1.5%), α-cadinol (1.4%) and *(Z)*-jasmone (1.4%).

In stems essential oil, 38 components representing 94.3% of the total oil composition were revealed by this analysis. This oil was dominated by tetradecanoic acid (13.1%), hexahydrofarnesylacetone (10.1%), dodecanoic acid (10.0%), isobornyl acetate (7.6%), 5-methylthiopentyl isothiocyanate (6.7 %) and *(E)*-β-ionone (6.2%). Some of other representative compounds in the stems oil were present in moderate amounts

such as nonanal (4.3%), decanoic acid (3.5%), berteroin (3.4%), *(E)*-geranylacetone (2.2%), β-cyclocitral (2.0%), caryophyllene oxide (2.0%), and some other compounds were detected in minor amounts such as camphor (1.8%) and 1,10-di-*epi*-cubenol (1.7%).

Four compounds that occur in the three oils of leaves, stems, and flowers of *R. rugosum* plant which are *(E)*-β-ionone, dodecanoic acid, tetradecanoic acid and hexahydrofarnesylacetone with significant percentages.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The *in vitro* antimicrobial activity of the essential oils from *R. rugosum* against five bacteria species, selected as representative of the classes of Gram (+) and Gram (–). The antimicrobial activity of the essential oils against the microorganisms employed was qualitatively and quantitatively assessed by the MIC and MBC values. MIC and MBC results (Table 2) indicate that the *R. rugosum* oils had different levels of activity against the microorganisms. We found that the activity of the essential oils depends on their concentrations and the strain of tested bacteria. The inhibitory properties of the oils were observed within a range of concentrations from 2.5 to 10 mg/mL. In liquid medium the essential oils were active against all the bacteria.

MIC and MBC values indicate that the essential oils of *R. rugosum* were efficient against all tested bacteria with MIC and MBC value (2.5 mg/mL and 5 mg/mL, respectively) against Gram-positive bacteria and MIC value were 10 mg/ml against Gram-negative bacteria.

Antimicrobial activity of essential oil is preservative one of the most examined features, important for both food preservation and control of human and animal diseases of microbial origin. These observations may be attributed to the nature of biologically active components. Indeed, various chemical compounds have direct activity against many species of bacteria such as terpenes and a variety of aliphatic hydrocarbon (alcohols, aldehydes and ketones). Therefore, a rank of activity has been proposed as follows phenols > aldehydes > ketones > alcohols > esters > hydrocarbons[36]. However, essential oils consisting of numerous components and other major and/or minor compounds) possibly producing a synergistic effect between other components may affect antibacterial activity[37,38].

The antimicrobial activity of the essential oils of *R. rugosum* from flowers and stems against the tested microorganisms could be attributed to the presence of percentages of nitrogen compounds and isothiocyanate appreciated for their antibacterial potentials[39]. It has been demonstrated that the volatile samples containing glucosinolate degradationd products were evaluated for antimicrobial activity using the

disc diffusion method with calculated minimum inhibitory concentrations (MIC) and expressed a wide range of growth inhibition activity against both Gram-positive and Gram-negative bacteria and fungi. The minimum inhibitory concentrations varied between 0.008 and 0.115 mg/mL[40]. On the other hand the major components of the stem essential oil such as fatty acids, hexahydrofarnesylacetone and ionones are reported to have moderate antimicrobial activities[41,42].

Antioxidant activity

DPPH radical-scavenging activity

Essential oils of *R. rugosum* were subjected to screening for their possible DPPH radical-scavenging activities (Table 3). The effect of antioxidant on DPPH radical-scavenging was thought to be due to their hydrogen donating ability of some constituent of the tested essential oils. When a solution of DPPH is mixed with that of a substance, it can generate a hydrogen atom. This results in the reduced form of DPPH (non-radical) with the loss of the violet color. DPPH scavenging activity is usually presented by PI value. *R. rugosum* essential oils were able to effectively reduce the stable free radical DPPH with an PI values ranging from 69.88% to 49.72%, compared with the standard BHT (PI = 94.7%) (Table 3). For comparative purposes the flowers essential oil showed

stronger DPPH radical-scavenging activity (69.88 %) than that of stems and leaves (62.56 % and 49.72 %, respectively).

β-Carotene bleaching method

In this assay, antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation. Hence, the free radical linoleic acid attacks the highly unsaturated β-carotene, and the presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system. Table 3 shows the antioxidant activity of the essential oils and BHT as measured by the bleaching of the β-carotene-linoleate system. The results showed that the essential oils of *R. rugosum* exhibited moderate antioxidant activity (AA = 39.52 %) compared to that of BHT (AA = 92±0.04%). By comparing the antioxidant activity measured by the three different methods and the relationships between the chemical composition and antioxidant activity, it is seen that *R. rugosum* essential oil possesses a moderate capacity to prevent lipid peroxidation, which can be ascribed to the low content of sulfur and/or nitrogen and phenolic components in the three oils[43].

Radical cation $ABTS^{+\bullet}$ scavenging activity

The ABTS method gives a measure of the antioxidant activity of essential oils by determining the reduction of the radical cation as the percentage of inhibition (PI) of absorbance at 734 nm. Re et al. reported that the decolorization of the $ABTS^{\cdot+}$ cation reflects the capacity of an antioxidant to donate electrons or hydrogen atoms in order to inactivate this radical species[35].

Table 3 shows the antioxidant activity of all essential oils of *R. rugosum*. An average PI of $ABTS^{\bullet+}$ in the presence of the flowers essential oil was observed showing their moderate scavenging ability (PI = 42.18 ± 0.092%). The antioxidant activity of stems and leaves essential oils (PI = 26.87 ± 0.188% and PI = 24.87 ± 0.188, respectively) is less important compared to that of BHT (PI = 95.2 ± 0.6%). In general, the antioxidative effectiveness of essential oil depends on the content of phenolic compounds and the reaction activity of the phenol towards the chain-carrying peroxyl radicals and on the stability of the phenoxyl radical formed in the reaction[44].

In additions this observation is certainly associated with the low content of phenolic, isothyocyanate and nitrogen constituents in the three investigated oils[45]. Moreover, it is known that the synergistic or antagonistic effect of a compound present in minor percentage in a mixture has to be considered as well[46].

Conclusion

Essential oil of different parts of (stems, leaves and flowers) *R. rugosum* plant were extracted, analyzed, investigated and identified for their activities,such as antimicrobial and antioxidant. The results of the present study indicate that the non-terpene derivatives such as aldehydes, ketones, esters and acids were dominant in all essential oils (33.6-69.6%) followed by sulfur and/or nitrogen compounds, apocarotenoids and oxygenated sesquiterpenes. *R. rugosum* essential oils showed moderate free radical scavenging activity but displayed high antibacterial activity against gram-positive bacteria.

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Table 1. Chemical composition of essential oil from different parts of *R. rugosum*

Nº.	Compounds (%)	L.r.i.[a]	Flowers[b]	Leaves[b]	Stems[b]	Identification	RI[c]
1	dimethyl trisulfide	974	2.6		0.5	GC-MS, RI	978 [18]
2	hexanoic acid	983			0 .7	GC-MS, RI	986 [19]
3	1-octanol	1071		0.5	1.2	GC-MS, RI	1070 [20]
4	*(E,E)*-3,5-octadien-2-one	1096			0.7	GC-MS, RI	1099 [19]
5	Nonanal	1104		0.9	4.3	GC-MS, RI	1108 [19]
6	*trans*-3,5-dimethyl-1,2,4-trithiolane	1141	2.1		0.6	GC-MS, RI	1135 [21]
7	Camphor	1145			1.8	GC-MS, RI	1143 [20]
8	Borneol	1167			1.1	GC-MS, RI	1165 [20]
9	1-nonanol	1172			0.6	GC-MS, RI	1175 [19]
10	octanoic acid	1175			0.7	GC-MS, RI	1179 [19]
11	Decanal	1206			1.2	GC-MS, RI	1209 [19]
12	dimethyl tetrasulfide	1210	4.5		1.0	GC-MS, RI	1217 [18]
13	1,2,3-trithiane	1213	1.5			GC-MS, RI	1217 [18]
14	β-cyclocitral	1222		2.2	2.0	GC-MS, RI	1217 [22]

15	methyl carvacrol	1244	1.4			GC-MS, RI	1241 [22]
16	nonanoic acid	1276			1.6	GC-MS, RI	1279 [23]
17	isobornyl acetate	1287	2.1		**7.6**[d]	GC-MS, RI	1285 [20]
18	2-undecanone	1293	2.2			GC-MS, RI	1297 [19]
19	methyl decanoate	1328	2.4			GC-MS, RI	1322 [24]
20	dehydro-*ar*-ionene	1352			0.9	GC-MS, RI	1352 [19]
21	eugenol	1359	1.6	0.6		GC-MS, RI	1358 [22]
22	decanoic acid	1375			3.5	GC-MS, RI	1379 [23]
23	β-maaliene	1381			0.9	GC-MS, RI	1380 [22]
24	*(E)*-β-damascenone	1383	**5.7**	0.6	0.7	GC-MS, RI	1382 [20]
25	β-elemene	1392		0.5		GC-MS, RI	1391 [22]
26	*(Z)*-jasmone	1396		1.2		GC-MS, RI	1396 [20]
27	dodecanal	1409			0.6	GC-MS, RI	1412 [23]
28	*(E)*-β-damascone	1412		0.8		GC-MS, RI	1411 [20]
29	erucin	1431	2.5			GC-MS, RI	1431 [25]
30	*(E)*-geranylacetone	1455	2.3	0.5	2.2	GC-MS, RI	1448 [22]

31	*(E)*-β-ionone	1486	3.5	2.6	**6.2**	GC-MS, RI	1486 [25]
32	Tridecanal	1508			0.7	GC-MS, RI	1509 [23]
33	berteroin	1521	3.6	0.5	3.4	GC-MS, RI	1521 [25]
34	methyl dodecanoate	1527	2.9			GC-MS, RI	1522 [24]
35	5-methylthiopentyl isothiocyanate	1531			6.7	GC-MS, RI	1531 [25]
36	dodecanoic acid	1569	2.7	3.9	**10.0**	GC-MS, RI	1573 [23]
37	*(Z)*-3-hexenyl benzoate	1571	1.5			GC-MS, RI	1569 [24]
38	caryophyllene oxide	1582			2.0	GC-MS, RI	1582 [26]
39	viridiflorol	1591		0.5		GC-MS, RI	1590 [27]
40	1,10-di-*epi*-cubenol	1616	1.8	1.5	1.7	GC-MS, RI	1616 [27]
41	10-*epi*-g-eudesmol	1622	1.5	1.0	0.6	GC-MS, RI	1622 [20]
42	α-acorenol	1631	3.7	1.5	0.9	GC-MS, RI	1632 [27]
43	T-cadinol	1641		0.7	0.6	GC-MS, RI	1641 [26]
44	α-cadinol	1654	1.3	1.4	0.5	GC-MS, RI	1654 [26]
45	1-tetradecanol	1675		2.1	1.2	GC-MS, RI	1676 [23]
46	α-bisabolol	1684		1.0	0.9	GC-MS, RI	1681 [27]

47	*n*-heptadecane	1700	1.3		0.6	GC-MS, RI	1695 [27]
48	pentadecanal	1716	3.6	**55.3**	0.7	GC-MS, RI	1716 [27]
49	metyl tetradecanoate	1727	1.4			GC-MS, RI	1725 [27]
50	tetradecanoic acid	1765	**5.9**	**5.7**	**13.1**	GC-MS, RI	1767 [23]
51	1-pentadecanol	1780	**8.3**			GC-MS, RI	1780 [27]
52	*n*-octadecane	1800	1.4			GC-MS, RI	1800 [20]
53	hexahydrofarnesylacetone	1843	**17.2**	**8.4**	**10.1**	GC-MS, RI	1843 [24]
	Number of compounds identified		**28**	**23**	**38**		
	Oxygenated monoterpenes		3.5	0.0	10.5		
	Sesquiterpene hydrocarbons		0.0	0.5	0.9		
	Oxygenated sesquiterpenes		8.3	7.6	7.2		
	Apocarotenoids		28.7	15.1	22.1		
	Sulfur and/or nitrogen compounds		16.8	0.5	12.2		
	Phenylpropanoids		1.6	0.6	0.0		
	Non-terpene derivatives		33.6	69.6	41.4		
	Yields		0.023	0.0061	0.016		

Total identified	92.5	93.9	94.3	

[a]L.R.I: Linear retention index relative to n-alkanes on fused silica capillary column *HP-5*

[b] Content (%): Relative percentage calculated by GC/ FID on an apolar capillary column *HP-5*.

[c] Retention index values obtained on column *HP-5* and reported in literature

[d] Bold type indicates major component.

Table 2. Antibacterial Activity of the Essential Oils Isolated from the Stems, Leaves and Flowers of *R.rugosum*

Microorganisms	Leaves		Flowers		Stems		GM[a](µg/mL)
	MIC	MBC	MIC	MBC	MIC	MBC	MBC
Gram positive bacteria							
Staphylococcus aureus ATCC 25923	2.5	5	2.5	5	2.5	5	15.62
Enterococcus faecalis ATCC29212	2.5	5	2.5	5	2.5	5	7.81
Gram negative bacteria							
Escherichia coli ATCC 25922	10	>10	10	>10	10	>10	3.90
Pseudomonas aeruginosa ATCC 27950	10	>10	10	>10	10	>10	500
Acinetobacter baumaniie	10	>10	10	>10	10	>10	nd[b]

[a]CMI and CMB (mg/mL)

[b]GM: Gentamycin

[c]Nd: Not determined

Table 3. Antioxidant activities of *R.rugosum* essential oil on DPPH, ABTS and β-carotene/linoleic acid test.

	PI of DPPH scavenging (1 mg/mL)	PI of ABTS scavenging (1 mg/mL) Time at 20 min	AA of β-carotene/linoleic acid test (1mg/mL)
Flowers	69.88 ± 0.02	42.18 ± 0.09	39.52 ± 0.01
Stems	62.56 ± 0.01	24.87 ± 0.18	23.92 ± 0.01
Leaves	49.72 ± 0.01	26.87 ± 0.18	18.96 ± 0.04
BHT	94.7 ± 0.01[d]	95.2 ± 0.6	92 ± 0.04

Tables:

Table 1. Chemical composition of essential oil from different parts of *R. rugosum*

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