GC-MS analysis and biological activity of hydroalcholic extracts and essential oil of Rhus typhina L. wood (Anacardiaceae) in comparison with leaves and fruits.

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GC-MS analysis and biological activity of hydroalcholic extracts and essentiasl oils of *Rhus typhina* L. wood (Anacardiaceae) in comparison with leaves and fruits.

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Keywords: R*hus typhina* L., EO, hydroalcoholic extract, wood, antioxidant activity, antimicrobial activity, GC-MS

Abstract

Hydroalcoholic extract and essential oils (EO) of branches, leaves and fruits of *Rhus typhina* L., were characterized by GC-MS. The hydroalcoholic extracts (branches 68.30 mg/g, leaves 35.82 mg/g and fruits 257.76 mg/g), showed different compositions dominated by gallic acid (33.46%) in branches, its precursor 1-cyclohexane-3,4,5-hydroxy-carboxylic acid (20.55%) in leaves and malic acid (89.15%) in fruits.

EO yields were 210 µg/g for branches with δ-cadinene as main component (22.00%), followed by fruits with 132 µg/g (β-pinene main component 32.2%) and leaves with phenylacetaldehyde as major component (54µg/g, 40.13%).

Total phenolic content (TPC) was highest in branches hydroalcoholic extracts (5.87µg $_{GAE}$/mL), while the maximal TPC of EO was observed in leaves (17.71µg $_{GAE}$/mL).

The highest value of radical scavenging activity (DPPH test) was detected in both leaves hydroalcoholic extracts and EO.

The branches EO i*n vitro* antimicrobial activity was strong against *C. albicans* (Ø>35mm, MIC 0.16 µg/mL) and negligible against *E. coli*. The leaves and fruits EO showed strong activity against *C. albicans* and intermediate activity against *Escherichia coli*.

1. Introduction

Sumac is the name of the genus *Rhus* that includes more than 250 individual species of plants in the family *Anacardiaceae* (USDA, 2007 Germplasm Resources Information Network), growing mostly in wild areas in temperate and tropical regions.

R. typhina, staghorn sumac (Chinese sumac) is a shrub found in North America and Europe; it develops spontaneously in marginal areas of Northern Italy and is extensively cultivated in Northwest China to obtain its red fruits. They are used for the production of a traditional beverage named ‘sumacade’, sumac iced tea or Rhus juice (Peterson, 1977; Kossah et al., 2010). However also other parts of this plant are considered a source of a variety of nutritionally and medicinally useful chemical components such as essential aminoacids, unsaturated fatty acids, vitamins and organic acids (Wang and Zhu, 2017). Concerning the plant wood, only the biological activities of extracts from the species *R. verniciflua* were investigated (Kitts and Lim, 2001), while the work of Islambekov et al. (1994) on *R. glabra* and *R. typhina* stems extracts and of Antal et al. (2010) on *R. cotinus* L. wood determined the structure of some of its components.

EO are the object of continuous studies for their antibacteric, antimicrobial, antiviral, antimicotic and antioxidant properties (Beretta et al., 2011; Gelmini et al., 2015) but to the best of our knowledge no information on hydroalcoholic extracts of branches of *R. typhina* and on EO of

branches and fruits is available. Hence, the aim of the present study was to determine the composition and bioactivity (antioxidant and antimicrobial activity) of hydroalcoholic extracts and EO of its branches with a GC-MS based metabolomic approach and to compare them with those of leaves and fruits.

2. Results and discussion

2.1. Chemical composition of hydroalcoholic extracts of differeht vegetative organs of R. thyphina

2.1.1 Hydroalcoholic extracts of branches

The chromatographic profile of branches hydroalcoholic extracts is shown in Fig. S1A with 40 peaks of identified substances, accounting for 96.07% of the total area (with RT>8 min to exclude peaks generated by the derivatizing agent). Compound identifications (Table S1) revealed four main classes: (I) phenolic acids such as gallic acid $C_7H_6O_5$ (33.78%, RT=31.06 min, main peak 61), (II) sugars and polyalcohols of which arabitol $C_5H_{12}O_5$ (8.26%, RT=24.97min) was the main peak 27, (III) flavonoids, identified in the range RT= 47-51 min and recognized all as catechins (total relative amount 8.4%), as previously found in the aqueous extract of leaves of *R. coriaria* (Regazzoni et al. 2013) and (IV) other constituents of relevant nutraceutical interest as lactic acid (1.93%, RT= 8.48 min).

2.1.2 Hydroalcoholic extract of leaves

The chemical composition of leaves hydroalcoholic extracts (RT range 13-60 min, Table S1, Fig S1B) indicated the presence of (I) phenolic acids dominated by 3,4,5-trihydroxy-1-cyclohexane-carboxylic acid ($C_7H_{12}O_5$, 20.55%, RT=27.52 min, peak 41), (II) sugars and polyalcohols (29.54%) and (III) fatty acids and their derivatives detected mainly in the range RT=43- 48 min (total amount 28.54 %).

2.1.3 Hydroalcoholic extract of fruits

The fruits hydroalcohlic extract (Fig. S1C) exhibited very strong quantitative and qualitative differences compared to those of branches and leaves (Table S1). Its composition was largely dominated by malic acid (89.15%, RT= 18.93 min, peak 20), citric acid (RT = 27.64 min, 1.95%, peak 42) and gallic acid, 1.73%, peak 61.

2.2. *Chemical composition of EO of different vegetative organs of R. typhina.*

2.2.1 Branches EO

The GC-MS profile of branches EO is shown in Fig. S2A and Table S2. The main compounds detected in the 25-33 min RT range were essentially non oxygenated sesquiterpenes (76.52% of the total peak area) and δ-cadinene (22.07%, RT = 31.49 min, peak 113), γ-cadinene (9.91%, RT = 31.22 min, peak 112), γ-muurolene (8.91%, RT=29.65 min, peak 99), aromandendrene (5.96%, peak 94). Oxygenated sesquiterpenoids (amounting to 6.50%) appeared at RT>33 min: among them there is τ-muurolol (1.94%, peak 140).

2.2.2 Leaves EO

The phytochemical composition of leaves EO were in good accordance with those of Bestmann et al. (1988) from both the qualitative and quantitative (0.005% w/w) point of view. The chemical profile of Fig. 2B appeared completely different from that of branches, with oxygenated derivatives amounting to 86.42% and with aldehydes accounting for 60.96 % of the total peaks area (Table S2). Phenylacetaldehyde (40.13%, RT=11.11 min, peak 27), a fragrance and flavour agent with pheromone properties (Youlian et al., 2009) was the major peak with other main pheromone-aldehydes furfural (5.54%, RT = 4.28 min) and 2-hexenal (5.22%, RT= 4.66 min). In the 3-20 min RT range a set of oxygenated monoterpenoids appeared (19.40%), while at 20 <RT<37 min sesquiterpenes and sesquiterpenoids were detected in minor quantity (totally 8.75%).

2.2.3 Fruits EO

The chemical composition of fruits EO was characterized by a high content of non oxygenated derivatives (89.52%) as shown in Table S2. In Fig. S2C the most abundant compounds were four monoterpenes enclosed in the 5-11 min RT range: β-pinene (32.26%, RT = 8.28 min, peak 16), α-pinene (14.95%, RT=6.77 min, peak 11), β-*cis*-O-cimene (11.42%, RT= 9.47 min, peak 21) and D-limonene (8.01%, RT=10.32 min, peak 23). A second group of compounds in the 25-32 min RT range consists of sesquiterpenes and accounts for 21.79% of the total chromatogram area. The main components were γ- cadinene (2.53%, RT=31.16 min, peak 112), δ-cadinene (6.82%, RT=31.37 min peak 113) and γ-muurolene (2.10%, RT= 29.66 min, peak 99). Little amounts of compounds with 8,11,13-abietatriene skeleton are also detected: they are responsible for *in vitro* anti inflammatory activity *vs* leukotriene B4 formation (Pferschy-Wenzig et al. 2008).

2.3. *Antiradical activity and TPC of hydroalcoholic extracts of R. thyphina*

2.3.1 Antiradical activity (DPPH test)

The DPPH IC_{50} of branches hydroalcoholic extract was 2.41±0.08 μg_{ext}/mL, value intermediate between those of leaves (1.11±0.03 μg_{ext}/mL) and fruits (3.80±0.12 μg_{ext}/mL). These results were comparable to IC_{50} reported by Šavikin et al. (2009) who investigated the antiradical activities of *R. cotinus* methanol extracts of leaves and fruits, whose values are respectively 2.6±0.4 μg_{ext}/mL and 3.8±0.5 μg_{ext}/mL. Simić et al. (2008) determined SC_{50}= 1.7 μg_{ext}/mL (ethyl acetate) in the same fraction of dried young shoots. To the best of our knowledge, the antiradical activity of the hydroalcoholic extracts of *R. typhina* branches is reported for the first time in this study.

2.3.2 Total phenol content Fast Blue BB test (TPC-BB)

Branches hydroalcoholic extract showed a TPC-BBcontent of 5.87±0.19 mg_{GAE}/g, a value higher than that of fruits and leaves extracts (1.70 ± 0.05 mg_{GAE}/g and 1.22±0.06 mg_{GAE}/g respectively). These results are in good agreement with the GC-MS quantitative determination of GAE of hydroalcoholic extracts of branches (6.81± 0.13% w/w), fruits (1.91±0.06% w/w) and leaves (0.77±0.04% w/w) (Pearson's product-moment correlation coefficient R= 0.996, $p< 0.05$).

2.3.3 Total Phenol Content Folin- Ciocolteau method (TPC-FC)

According to the TPC-BB results, the TPC-FC of *R. thyphina* branches hydroalcoholic extract was higher than that of leaves and fruits (86.00 mg_{GAE}/g vs. 34.26 mg_{GAE}/g and 6.22 mg_{GAE}/g, respectively). The differences of these data in comparison to those reported in literature can be attributed to different factors such as plant genetics, growing conditions, harvesting time and method of extraction and quantification (Itidel et al., 2013).

2.4. Antiradical activity, TPC and antimicrobial activity of EO of R. thyphina

2.4.1 EO antiradical activity

R. typhina leaves and fruits EO showed greater DPPH scavenging capacity compared to that of branches, as indicated by their respective IC_{50} values of 2.29±0.10 µL/mL (leaves), 2.54±0.06 µL/mL (fruits) and 5.80±0.18 µL/mL (branches).

2.4.2 EO TPC-BB test

The EO from leaves showed the highest TPC value, amounting to 17.71±0.82 mg_{GAE}/mL_{EO}, followed by that of fruits (10.75±0.45 mg_{GAE}/mL_{EO}) and that of branches (4.72±0.22

mg_{GAE}/mL_{EO}). In EO a good correlation between TPC values and DPPH scavenging activity observed (R= 0.877; P<0.05), suggests that the reducing components are those responsible also for the EO antiradical activity.

2.4.3 EO antimicrobial activity

The antimicrobial activity of *R. typhina* EO is reported in the present study for the first time (Table 1), being reported in literature only data of antimicrobial activity of *R. cotinus* EO (Milošević et al., 2008). All tested EO (branches, leaves and fruits) showed good *in vitro* microbial inhibition against *C. albicans* (inhibition zone 22.6<d<35 mm vs. *S. montana* EO positive control, MIC 0.02 mg/mL). Furthermore, EO from leaves and fruits, but not from branches (inhibition zone 0<d<17.5 mm, MIC=0.64 mg/mL) were active against *E. coli* ATCC (inibition zone 17.6<d<22.5 mm, MIC=0.064 mg/mL).

3. Conclusions

In this study, the extensive fingerprinting and characterization of Italian (Northern Italy) *R. thyphina* L. branches hydroalcoholic extract and EO were reported for the first time, and the results compared with those of its leaves and fruits.

The analyses were performed using a GC-MS methodology that allowed for the single-run, simultaneous detection of several classes of analytes, from oligosaccharides to flavonoids, organic acids, glycerides, highlighting huge compositional differences among the plant vegetative organs. Branches hydroalcohlic extracts of *R. typhina* showed the highest content of gallic acid and the major value of TPC, while leaves extract was characterized mainly by sugars, fatty acids and their derivatives, and by a lower antioxidant activity. The fruits extract was strongly dominated by organic bicarboxylic acids (malic acid89.15%) and showed a TPC similar to that of leaves.

Among the great number of identified structures of nutraceutical interest, the high content of gallic acid in branches may be exploited in pharmaceutical industry as antioxidant agent while malic acid in the hydroalcoholic extract of fruits may be suitable for the confectionary industry (*i.e.* biscuits, candy and fruit-based preparations) as savoury agent (tartness), as stabilizer and as preservative ingredient. The presence of bi- and tri-carboxylic acids belonging to the Krebs cycle (succinic, fumaric, citric, isocitric acids) can explain its use for the preparation of tonic beverages with health promoting activity.

Significant differences were observed in the chemical composition of EO of branches in respect of that of leaves and fruits. The former is dominated by cyclic sesquiterpenes and low TPC while that of leaves EO by aldehydes with strong antiradical/antioxidant activity. Fruits EO most

abundant compounds are monoterpenes (β-pinene 32.2%) accompanied by sesquiterpenes with low TPC.

Tests of antimicrobial activity on EO of all aerial parts of this medicinal plant found that they are endowed with strong antimicrobial activity against the infections of the saprophytic fungus *C. albicans* while only leaves and fruits have satisfying activity against the Gram negative bacteria *E. coli*.

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Declaration

The autors declaire no conflict of interest

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Table 1. Antimicrobial activity of *R. typhina* EO determined by disk diffusion assay (+: 0<d<17.5 mm (1); ++: 17.6<d<22.5 mm; +++: 22.6<d<35 mm) and by microdilution assay (mg_{EO}/mL). Positive control: *Satureja montana* EO. d: inhibition area diameter. d.: not detectable.

	Disk diffusion assay				*Microdilution assay*			
	Branches	Leaves	Fruits	*S. montana*	Branches	Leaves	Fruits	*S. montana*
C. albicans ATCC	+++	+++	+++	+++	0.16	0.08	0.08	0.02
E. coli ATCC	+	++	++	++	d.	0.64	0.64	0.02

EXPERIMENTAL

GC-MS analysis and biological activity of hydroalcholic extracts and EO of *Rhus typhina* L. wood (Anacardiaceae) in comparison with leaves and fruits.

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Materials and methods

Plant material

Branches, leaves and fruits of *R. typhina* were collected by E. Arlandini in different periods of the year in Northern Italy (Piedmont and Lombardy regions) in the following localities: Isola S. Antonio (Alessandria) lat. 45°02'0" N; long. 8°51'0" E, elevation: 76 m a.s.l.; Sale (Alessandria) lat. 44°58'54" N; long. 8°48'37" E, elevation: 83 m a.s.l.; Lomello (Pavia) lat. 45°07'12" N, long. 8°47'46" E, elevation: 93 m a.s.l.

Chemicals

All the following reagents and solvents used were of analytical grade: gallic acid; 2,2-diphenyl-1-picrylhydrazyl (DPPH); N,O-bis[trimethylsilyltrifluoroacetamide] (BSTFA); Fast Blue BB; dichloromethane; formic acid; methanol; ethanol; ethylacetate; acetonitrile; pyridine, acetic acid, sodium hydroxide; sodium acetate-tri-hydrate (Sigma-Aldrich, Milan, Italy). Folin-Ciocalteu reagent was purchased from Fluka (Switzerland). Ultra-pure water for reagents preparation was produced using a Milli-Q System (Millipore, Milan, Italy).

Moisture Determination

The moisture of the plant material was determined in triplicate independent determinations on 35 g of each specimen in a thermostated oven (Nüve FN-500, Turkey) at 105°C until constant weight. The moisture % content was 10.47±0.7 % for branches, 56.85±1.5 % for leaves and 41.98±0.9 % for fruits.

Hydroalcholic extraction and derivatization

The hydroalcoholic extraction of branches, leaves and fruits was accomplished on 5.0 g of powdered material in 100 mL of EtOH/H_2O=45:55 under stirring for 24 hours at room temperature. After filtration, the solvent was evaporated under reduced pressure and 5.0 mg of each dry extract were submitted to derivatization with 140 µL of BSTFA, 60 µL of pyridine and 200 µL of ethyl acetate at 70°C for 3 hours. The mixtures were then diluted 1:10 with ethyl acetate and 1.0 µL injected into the GC-MS apparatus.

EO extraction

EOs were obtained by steam distillation for three hours of three aliquots of powdered branches, each of about 124 g (379.00 g total amount), of six aliquots of powdered leaves of about 123 g (738.75 g total weight) and seven aliquots o powdered fruits each of about 145 g (1012.50 g total weight). The hydrodistillation apparatus had a boiler flask of 1000 mL filled with 800 mL of water; the length of the column containing the extractable material was 40 cm. The hydrolats resulting from each operation (around 60 mL) were extracted with 40 mL of dichloromethane (three times) in an extraction funnel, the organic fractions were pooled and the solvent evaporated under reduced pressure. The EOs- obtained were diluted (1:1000) in ethyl acetate and 1.0 µL of these solutions analysed by GC-MS.

GC-MS

GC-MS analyses were performed using a Bruker Scion SQ instrument (Bruker Daltonics, Macerata, Italy), equipped with a Varian Factor Four capillary column (VF-5 MS, 30 m; 0.25 mm i.d., film thickness 0.25 µm) coupled with a single quadrupole (SQ) detector. For the hydroalcoholic extracts, the oven temperature was initially set at 60°C (hold time 3 min), with a gradient from 60 to 120°C (8.0°C/min, hold 1 min), 120-280°C (4°C/min, hold 1.5 min), 280°C-330°C (10 °C/min, hold 2 min). Total run time was 60 min. For EO analyses the temperature gradient was setted according to Gelmini et al., 2015. Injector temperature 250°C, hold 20 min. Column flow 1.00 mL/min. Carrier gas helium 5.5; ionization energy 70 eV. The split/splitless ratio was set to 1:30 after 45 s. Peaks were identified by matching their mass spectra with those of the commercial library NIST mass spectral database (vers. 2.0, 2011) and with those of owned commercial standards. The percentage compositions of the constituents were obtained by normalization of the peak areas.

Antioxidant activity

Antiradical activity (DPPH test)

Radical scavenging activity was determined with the DPPH test according to Beretta et al. (2011). Briefly, a final test solution of 3.0 mL was obtained by adding 0.5 mL of DPPH in ethanol (500 µM) and 0.1 mL of ethanol containing different quantities of hydroalcoholic extracts to a mixture formed by 1 mL of acetate buffer and 1.4 mL of ethanol. The solutions were shaken and than incubated at 25 °C in the dark for 90 min. The absorbance of unreacted DPPH was read against a sample blank at λ = 517 nm (Varian Cary 50 Bio spectrophotometer). The scavenging activity was reported as IC_{50} expressed as µg of hydroalcoholic extract/mL of final solution (μg_{ext}/mL) or as µL of EO/mL of final solution, required to quench the DPPH by 50%.

Total phenol content (Fast Blue BB test, TPC-BB)

TPC was determined using the method reported by Medina (2011), with minor modifications. This assay was based on the reaction of 4-amino-2,5diethoxybenzanilide diazotated zinc double salt with phenols to give colored azo-complexes. The test was done adding 0.1 mL of Fast Blue BB reagent (0.1% w/v in methanol) and 0.1 mL of 5% NaOH to a methanol sample solution (1.0 mg/mL). After keeping the solutions in the dark at room temperature for 90 min, the absorbances were read at λ=420 nm. TPC values were calculated by comparison with a calibration curve built using methanol solution of gallic acid at different concentrations (0-500 µg/mL); y = 121522610470,35 x + 3682836246,23; R^2=0.9952.

The GC-MS quantitation was obtained by comparison of the peaks area with that of a calibration curve of silanized gallic acid (y = 1,291E+11x, R^2= 0.989).

Results were expressed as mg of gallic acid equivalents/mL of EO (mg_{GAE}/mL_{EO}) or mg of gallic acid equivalents/g of plant dry material (mg_{GAE}/g).

Total content of phenol/reducing substances (Folin Ciocoltea test, TPC-FC)

The total content of reducing substances was determined according to the Folin Ciocolteau method with minor modifications (Beretta et al., 2005, 2011). The hydroalcoholic extracts (50 mg) of branches, leaves and fruits of *R. typhina* were dissolved in 1.0 ml of EtOH/H_2O 45:55 vol/vol and sonicated at maximal power for 2 minutes. 5.0 µl of each solution was diluted to 1.0 ml with the Folin-Ciocolteau reagent diluted 1:10 with MilliQ water. The solutions were vortexed for 2 min and then incubated for 20 min in the dark at room temperature. The spectrophotometric absorbances were measured at λ = 750 nm. All determinations were done in triplicate. The results were expressed as mg of gallic acid equivalents/g of plant dry material

(mg_{GAE}/g). The GAE was calculated by comparison with a calibration curve plotted with a diluted stock solution (1mg/mL) of gallic acid in EtOH/H_20=45/55.

Antimicrobial activity

The EO antimicrobial activity was determined by disk diffusion test using a series of Kirby-Bauer antibiogram tests (Microbiologics Inc. USA) as previously reported (Bauer et al.1966, Hudzicki et al. 2009). Briefly, yeast isolates (*C. albicans*) grew in CHROMAgar and *E. coli* in McConkey agar without salts. Microorganisms were incubated overnight at 37 °C under controlled atmosphere. Inocula were prepared diluting the overnight cultures in saline solution to 1.0×10^8 CFU/mL. Petri dishes then were inoculated with 1.0 mL of this suspension. EO (13 µL) were added to 13 mm diameter sterile blank filter disk and placed on the culture medium surface. The agar plates were incubated at 37°C for 18-24 h (*E. coli*) or 18-48 h (*C. albicans*). After incubation, the diameter (d) of the inhibition zones were measured (mm). Results were expressed as follows: resistant (R); 0 <d<17.5 mm (1); 17.6<d<22.5 mm, (2); 22.6<d<35 mm (3). An authentic EO from *Satureja montana* was used as positive control.

MICs were determined by the broth microdilution method using 96-well microplates modified according to recommendations for the assessment of the antimicrobial potential of natural products (Cos et al., 2006). Serial dilutions (100 µL) of each extract were distributed into the plate and diluted in the Muller Hilton Broth making concentrations ranging from 0.02 to 5.12 µg/mL. Thereafter, the plates were inoculated with the respective microorganism suspension to make a final density 5×10^5 CFU/mL for bacteria and 1.5×10^3 CFU/mL for yeast, respectively. Plates were then incubated at 37 °C for 24 h (48 h for *C. albicans*). Microorganism growth was measured in terms of turbidity recorded at 405 nm.

The MIC was expressed as the lowest concentration that showed ≥80% inhibition of microbial growth compared to an extract-free growth control.

Table S1. GC-MS analysis of *R. typhina* branches, leaves and fruits hydroalcoholic extracts.

Peak #	RT (min)	KI	Substance	Relative content (%)		
				Branches	Leaves	Fruits
1	5.26	-	No match	-	-	0.06
2	5.659	-	No Match	-	-	0.11
3	6.168	686	Ethylbis(TMS)amine	-	0.56	0.19
4	6.283	-	No Match	-	-	0.02
5	6.335	-	No Match	-	-	0.01
6	6.464	-	Bis(TMS)carbodiimide	-	-	0.07
7	6.638	n.a.	Bis(TMS)carbonate	-	-	0.17
8	7.749	-	No Match	-	-	0.05
9	8.082	824	Propyleneglicol,bis-TMS	0.21	-	-
10	8.495	915	Lactic acid, bis-TMS	1.93	-	0.15
11	12.995	1273	Phosphoric acid, tris(TMS) ester	-	-	1.01
12	13.039	1108	Glycerol, tris(TMS) ether	4.07	2.06	-
13	13.727	1252	Proline, di-TMS	-	-	0.04
14	14.005	1178	Maleic acid, diTMS	-	-	0.09
15	14.283	1170	Succinic acid, di-TMS	-	-	0.08
16	14.499	1199	Glyceric acid, tri-TMS	-	-	0.03
17	15.171	1178	Fumaric acid, di-TMS	-	-	0.85
18	17.358	1332	DL-Malic acid, bis(TMS) ester	-	-	0.09
19	17.776	1317	D-Erythronic acid γ-lactone, bis(TMS) ether	-	-	0.28
20	18.932	1390	Malic acid,tris-TMS	0.26	-	89.15
21	19.287	1491	L-Threitol,tetrakis (TMS)ether	0.19	-	-

22	20.768	1518	2,3,4-Trihydroxybutyric acid, tetra-TMS	-	0.16	-
23	20.77	-	No match	0.06	-	-
24	23.06	1651	Lyxose, tetra-(TMS)-ether	-	-	0.01
25	24.334	1746	Ribitol, 5TMS	0.72	-	0.05
26	24.604	1326	d-Erythrotetrofuranose,tris-O-(TMS)	1.01	-	-
27	24.976	1746	Arabitol,pentakis(TMS)ether	8.26	-	-
28	25.459	-	No Match	-	0.04	-
29	25.888	1446	2-Deoxy-1,3,4-tris-O-(TMS)pentopyranose	-	0.16	0.03
30	25.892	-	No Match	0.17		
31	26.03	-	No Match	-	0.04	0.02
32	26.485	2029	D-(-)-Tagatofuranose, pentakis(TMS) ether (isomer 2)	-	0.12	-
33	26.495	-	No Match	0.23	-	-
34	26.592	-	No Match	0.14	-	-
35	26.643	-	No Match	-	0.13	-
36	26.647	1928	Methyl 2,3,4,6-tetrakis-O-(TMS)hexopyranoside	-	-	0.03
37	27.147	2049	D-(-)-Tagatofuranose, pentakis(TMS) ether (isomer 1)	-	4.53	-
38	27.153	1882	Glucofuranoside, methyl 2,3,5,6-tetrakis-O-(TMS)	0.64	-	0.25
39	27.167	2029	Sorbofuranose,pentakis(TMS)ether	4.00	-	-
40	27.361	2049	D-(-)-Fructopyranose, pentakis(TMS) ether (isomer 2)	4.01	9.89	0.50
41	27.516	1904	3,4,5-Tris(TMS)-1-cyclohexene-1-carboxylic acid, TMS ester	3.20	20.55	0.25
42	27.641	1944	Citric acid, tetra-TMS	-	-	1.95
43	27.739	1900	Isocitric acid, tetra-TMS	-	-	0.11
44	27.74	1928	Glycoside, α-methyl-tertrakis-O-(TMS)-	0.20	0.12	-
45	28.246	1843	1,5-Anhydro-D-sorbitol, tetrakis(TMS) ether	6.42	2.93	0.32

46	28.492	2126	Isocitric acid lactone, bis(tert-butyldimethylsilyl) ester	0.78	5.10	-
47	28.676	1788	TMS myristate	0.22	2.00	-
48	28.878	-	No Match	-	1.67	0.34
49	28.906	1928	Mannopyranoside,methyl 2,3,4,6-tetrakis-O-TMS	0.81	-	-
50	29.349	1970	Mannopyranose, pentakis(TMS) ether	0.59	2.35	-
51	29.538	1981	Gulonic acid, 1,4-lactone,(4TMS)	0.09	-	-
52	29.35	1692	D-Xylopyranose, 1,2,3,4-tetrakis-O-(TMS)-	-	-	0.08
53	29.61	1970	Talopyranose, pentakis(TMS) ether	0.43	0.60	0.03
54	29.818	1577	Levoglucosan, tris(TMS)-	4.10	1.26	0.10
55	30.295	1735	2-Deoxy-arabino-hexonic acid, 1,4-lactone, tris-O-TMS	-	-	0.02
56	30.156	2173	D-Galactose,2,3,4,5,6-pentakis-O-TMS-O-methyloxyme	0.51		
57	30.307	-	No Match	1.81		
58	30.358	-	No Match	-	0.29	-
59	30.456	-	No Match	-	0.10	-
60	30.605	2053	3,4,5-Trihydroxybenzoic acid ethyl ester, tris (O-TMS)-	7.61	1.36	0.51
61	31.06	2063	Gallic acid, tetraTMS	33.78	4.29	1.73
62	31.628	2194	Inositol, 1,2,3,4,5,6-hexakis-O-(TMS)-,scyllo-	0.17	4.69	0.08
63	31.838	-	No Match	0.12		
64	32.097	1991	D-Allofuranose, pentakis(TMS) ether	-	0.71	-
65	33.411	n.a.	Palmitic acid TMS ester	0.74		
66	34.835	1618	2-Deoxy-erythro-pentonic acid, tetrakis-TMS	-	1.02	0.01
67	33.913	2194	Myoinositol TMS	-	0.08	-
68	34.835	1.618	2-Deoxy-erythro-pentonic acid, tetrakis-TMS		1.02	0.01
69	35.406	-	No Match	-	-	0.06
70	35.507	-	No Match	-	-	0.11

71	36.493	-	No Match	0.08		
72	37.166	2194	Oleic acid,TMSester	0.18		
73	37.767	2186	Stearic acid, TMS ester	-	0.62	-
74	40.793	2382	Myristic acid, 2,3-bis(trimethylsiloxy)propyl ester	-	0.04	-
75	43.883	2581	2-Monopalmitoylglycerol TMS ether	0.13	1.50	0.02
76	44.017	2461	4-[(TMS)oxy]butyl palmitate	0.19	8.75	-
77	44.476	2581	1-Monopalmitin TMS ether	0.41	4.82	0.21
78	44.779	-	No Match	0.27		
79	46.549	-	No Match	0.17		
80	46.735	-	No Match	0.11		
81	47.157	-	No Match	0.24	-	-
82	47.363	2780	2-Monostearin TMS ether	0.13	2.81	0.02
83	47.631	-	No Match	-	6.52	-
84	47.914	2780	Stearic acid, 2,3-bis(trimethylsiloxy)propyl ester	-	7.85	014
85	47.959	3227	Catechine,penta-TMS-ether,(2R-trans)	1.59	-	-
86	48.415	-	No Match	0.08	-	-
87	48.529	3227	Catechine,penta-TMS-ether,(2R-cis)	2.19	-	-
88	50.103	2861	Catechine	3.55	-	-
89	50.279	2861	Catechine	0.70	-	-
90	50.422	n.a.	TMSether,methyl ester of p-Hydroxymandelic acid	0.83	-	-
91	50.983	2861	Catechine	0.37		
92	51.782	-	No Match	-	0.05	-
93	51.806	-	No Match	0.05	-	-
94	51.957	-	No Match	-	-	0.14
95	52.305	3323	Aucubin, hexakis(TMS) ether	-	-	0.03
96	53.059	-	No Match	-	-	0.06
97	53.51	-	No Match	0.22		
98	53.93	2397	Anthraquinone, 1,2-dihydroxy, bis-TMS	0.15	0.10	0.03

99	57.407	2417	Arachidonic acid TMS ester	0.70	-	-
100	58.515	-	No match	-	0.10	-

Table S2. GC-MS analysis of *R. typhina* branches, leaves and fruits EOs.

Peak #	RT (min)	Kovats Index	Substance	Relative content (%)		
				Branches	Leaves	Fruits
1	3.314	790	Acetylacetone	0.06	-	-
2	3.559	806	Hexanal	0.18	0.74	-
3	4.007	732	Amylene dichloride	0.03	-	-
4	4.220	976	Maleic anhydride	-	0.20	-
5	4.272	831	Furfural	0.29	5.54	1.84
6	4.664	814	2-Hexenal	-	5.22	-
7	4.715	-	No Match	0.16	-	-
8	4.960	860	n-Hexanol	0.08	-	-
9	5.236	-	No Match	0.03	-	-
10	6.203	856	Methional	-	0.30	-
11	6.769	948	α-Pinene	0.19	0.68	14.95
12	7.338	943	Camphene	-	-	0.20
13	7.831	913	2-Heptenal	-	-	0.07
14	8.069	920	Furfural, 5-methyl-	-	1.16	-
15	8.143	948	3-Carene		-	0.42
16	8.276	943	β-Pinene	0.19	-	32.26
17	8.428	969	1-Octen-3-ol	0.18	-	-
18	8.600	938	Sulcatone	0.69	0.59	-
19	8.737	958	β-Myrcene	-	-	0.28
20	9.073	-	No Match	0.06	-	-
21	9.479	976	β-cis-Ocimene	-	-	11.42
22	10.162	1042	p-Cymene	0.04	-	0.09
23	10.30	1018	Limonene	0.36	-	8.01
24	10.397	1212	2-Decenal	-	0.33	-
25	10.398	1059	Eucalyptol	0.17	-	-
26	10.884	-	No Match	0.07	0.76	-
27	11.117	1081	Phenylacetaldehyde	0.90	40.13	-
28	11.517	998	γ-Terpinene	0.01	-	0.14
29	12.060	1164	cis-Linalool oxide	0.07	2.29	0.27
30	12.714	1052	Terpinolene	-	-	1.29
31	12.761	1164	trans-Linalool oxide	0.10	1.46	-
32	13.35	1082	β-Linalool	0.62	2.12	0.19
33	13.578	1072	Hotrienol	-	3.63	-
34	13.628	1104	Nonanal	0.18	-	0.38
35	13.829	-	No Match	0.12	0.18	-
36	14.261	1138	Fenchol	-	-	0.03
37	15.224	1131	trans-Pinocarveol	-	-	0.33
38	15.492	1136	cis-Verbenol	-	-	0.07

39	15.551	1110	4-Oxoisophorone	-	0.51	-
40	15.639	-	No Match	0.09	0.59	-
41	16.538	-	No Match	-	0.35	-
42	16.603	1138	endo-Borneol	0.10	1.32	0.25
43	16.824	1109	Isopinocamphone	-	-	0.12
44	17.036	1137	L-Terpinen-4-ol	0.31	0.75	0.32
45	17.170	-	No Match	-	0.32	-
46	17.443	1191	Myrtenol	0.09	-	0.08
47	17.48	1197	p-Cymen-8-ol	-	0.66	-
48	17.671	1143	α-Terpineol	0.88	2.06	1.84
49	17.847	1172	Estragole	1.18	-	-
50	17.895	1186	Safranal	-	3.14	-
51	18.166	-	No Match	-	0.05	-
52	18.251	1119	Verbenone	0.21	2.05	0.14
53	18.49	-	No Match	-	0.27	-
54	18.769	1204	β-Cyclocitral	-	1.04	-
55	18.874	1206	cis-Carveol	0.02	-	-
56	18.932	1230	6,6-Dimethylcycloocta-2,4-dienone	-	0.55	-
57	19.176	-	No Match	-	0.69	-
58	19.647	1212	Pulegone	0.10	0.82	-
59	20.024	1190	Carvone	0.13	-	-
60	20.205	1212	α-Ionene	-	0.23	-
61	20.801	1212	2-Decenal	-	-	0.43
62	21.009	-	No Match	-	0.09	-
63	21.350	1487	1-Methyl-9-(1-methylethylidene)bicyclo[3.3.1]nonan-2-one	-	0.39	-
64	21.583	1277	Bornyl acetate	-	-	0.60
65	22.177	1262	Thymol	0.15	-	-
66	22.585	1262	Carvacrol	0.03	-	-
67	22.764	1286	Undecanal	-	-	0,11
68	23.376	-	No Match	-	0.22	-
69	23.486	1339	Sativene	0.05	0.91	-
70	25.459	1221	α-Copaene	0.10	-	0.05
71	24.672	1396	Dehydro-ar-ionene	-	0.93	-
72	25.167	1221	Ylangene	0.54	-	0.16
73	25.459	1344	α-Cubebene	4.09	0.26	1.52
74	25.814	1398	α-Cedrene	0.04	-	-
75	26.359	1474	α-Chamigrene	0.08	-	-
76	26.443	1410	3-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-propenal	-	3.36	-
77	26.559	-	No Match	0.05	-	-
78	27.078	1402	Lauraldehyde	-	-	0.42
79	27.273	1494	β-Caryophyllene	1.21	0.21	1.35
80	27.397	-	No Match	0.13	-	-
81	27.487	1398	Cedr-8(15)-ene	0.25	-	-

82	27.6	1386	Alloaromadendrene	0.17	-	-
83	27.735	1515	Germacrene D	0.43	-	-
84	27.753	1403	β-Gurjunene	-	-	0.11
85	27.824	1440	4-(2,4,4-Trimethyl-1,5-cyclohexadien-1-yl)-3-buten-2-one	-	0.26	-
86	27.925	1416	cis-Thujopsene	1.04	-	0.13
87	28.07	1492	Elixene	0.68	-	-
88	28.379	-	No Match	0.09	-	-
89	28.503	1490	δ-Guaiene	0.72	-	-
90	28.585	1420	Geranyl acetone	0.61	0.48	-
91	28.741	1440	β-Famesene	0.55	-	-
92	28.793	1597	Humulene	-	-	0.27
93	28.983	1461	γ-Gurjunene	-	-	1.39
94	28.993	1386	Aromandendrene	5.96	-	0.11
95	29.053	1440	3,4-Dehydro-β-ionone	-	1.74	-
96	29.248	1403	Cedr-8-ene	0.23	-	-
97	29.484	29,484	Isoledene	1.21	-	-
98	29.494	1440	β-Cadinene	-	-	0.40
99	29.657	1459	γ-Muurolene	8.91	0.60	2.10
100	29.793	1493	1-Isopropyl-4,7-dimethyl-1,2,4a,5,6,8a-hexahydronaphthalene	1.08	-	0.44
101	29.902	-	No Match	0.23	0.41	-
102	30.024	1416	cis-Thujopsene	-	0.27	-
103	28.503	1508	δ-Guaiene	0.42	-	-
104	30.185	1495	α-Selinene	-	-	0.02
105	30.322	1458	α-Farnesene	4.14	-	-
106	30.327	1424	4,11,11-Trimethyl-8-methylenebicyclo[7.2.0] undec-3-ene	-	-	0.71
107	30.504	1481	δ-Selinene	-	-	0.31
108	30.506	1580	β-Cadinene	1.01	-	0.17
109	30.626	1580	α-Muurolene	5.81	0.33	1.39
110	30.753	1403	α-Longipinene	0.23	-	-
111	30.993	1556	Cuparene	1.57	-	0.15
112	31.226	1435	γ-Cadinene	9.91	0.77	2.53
113	31.494	1469	δ-Cadinene	22.07	2.15	6.82
114	31.602	1537	trans-Calamenene	2.62	0.93	0.21
115	31.995	1440	1,2,3,4,4a,7-Hexahydro-1,6-dimethyl-4-(1-methylethyl)-naphthalene	1.28	-	0.37
116	32.113	1474	Valencene	-	-	0.30
117	32.117	1440	α-Cadinene	1.56	-	-
118	32.142	-	No Match	-	0.29	-
119	32.312	1517	α-Calacorene	1.72	-	0.18
120	33.073	1564	Nerolidol	0.92		0.07
121	33.414	1462	Aromadendrene oxide-(2)	0.09	-	-

122	33.726	1484	4-epi-Cubedol	-	-	0.05
123	33.732	1462	Aromadendrene oxide-(1)	0.06	-	-
124	33.915	1507	Caryophyllene oxide	0.10	0.32	0.04
125	34.107	1530	Ledol	0.22		0.03
126	34.418	1530	Viridiflorol	0.12	-	-
127	34.475	1580	Lauric acid, ethyl ester	-	-	0.08
128	34.532	n.a.	Tricyclo[3.1.0.0(2,4)]hexane, 3,6-diethyl-3,6-dimethyl-, trans-	0.49	-	0.06
129	34.781	1530	Globulol	0.16	-	-
130	34.863	1593	β-Eudesmol	0.11	-	-
131	35.507	1584	Cubedol	0.28	-	-
132	35.235	-	No Match	0.22	-	-
133	35.328	-	No Match	0.15	-	-
134	35.507	1484	Cubenol	1.14	-	-
135	35.688	1514	α-Gurjunene	-	0.37	-
136	35.706	-	No Match	0.16	-	-
137	35.800	-	No Match	0.02	-	-
138	35.933	1580	T-Cadinol	1.10	-	0.15
139	35.99	1580	α-Cadinol	1.35	0.48	0.30
140	36.287	1580	T-MuurolоI	1.94	0.39	0.28
141	36.051	1580	δ-Cadinol	0.42	-	-
142	36.653	1536	Spathulenol	0.04	-	-
143	36.748	1706	Cadalene	0.20	-	-
144	36.831	-	No Match	0.36	-	0.04
145	36.999	1625	α-Bisabolol	0.33	-	-
146	37.215	1612	Hexadecane	0.20	-	-
147	37.651	1702	Hexadecylene oxide	-	0.85	-
148	38.731	-	No Match	-	0.34	-
149	38.996	2109	n-Heneicosane	0.06	-	-
150	39.061	-	No Match	0.07	-	-
151	39.661	1754	Hexahydrofarnesyl acetone	0.04	0.29	-
152	40.394	2407	Tetracosane	0.02	-	-
153	40.594	1902	Farnesyl acetone	0.50	-	-
154	41.529	2139	Abieta-8,11,13-trien-18-al	-	-	0.21
155	41.732	1978	Manoyl oxide	-	-	0.17
156	41.781		No Match	-	-	0.08
157	41.975	1931	18-Norabieta-8,11,13-triene	-	-	0.38
158	42.393	2004	Abieta-8(14),9(11),12-triene	-	0.21	0.14
159	42.626		No Match	-	0.14	-
160	42.760	2072	Cembrene	-	-	0.08
161	42.800	2045	Phytol	-	1.28	-

Fig. S1. GC-MS chromatographic profile of *R. typhina* L. hydroalcoholic extracts pf (A) branches, (B) leaves and (C) fruits. Main peaks are numbered according to assignments in Table S1.

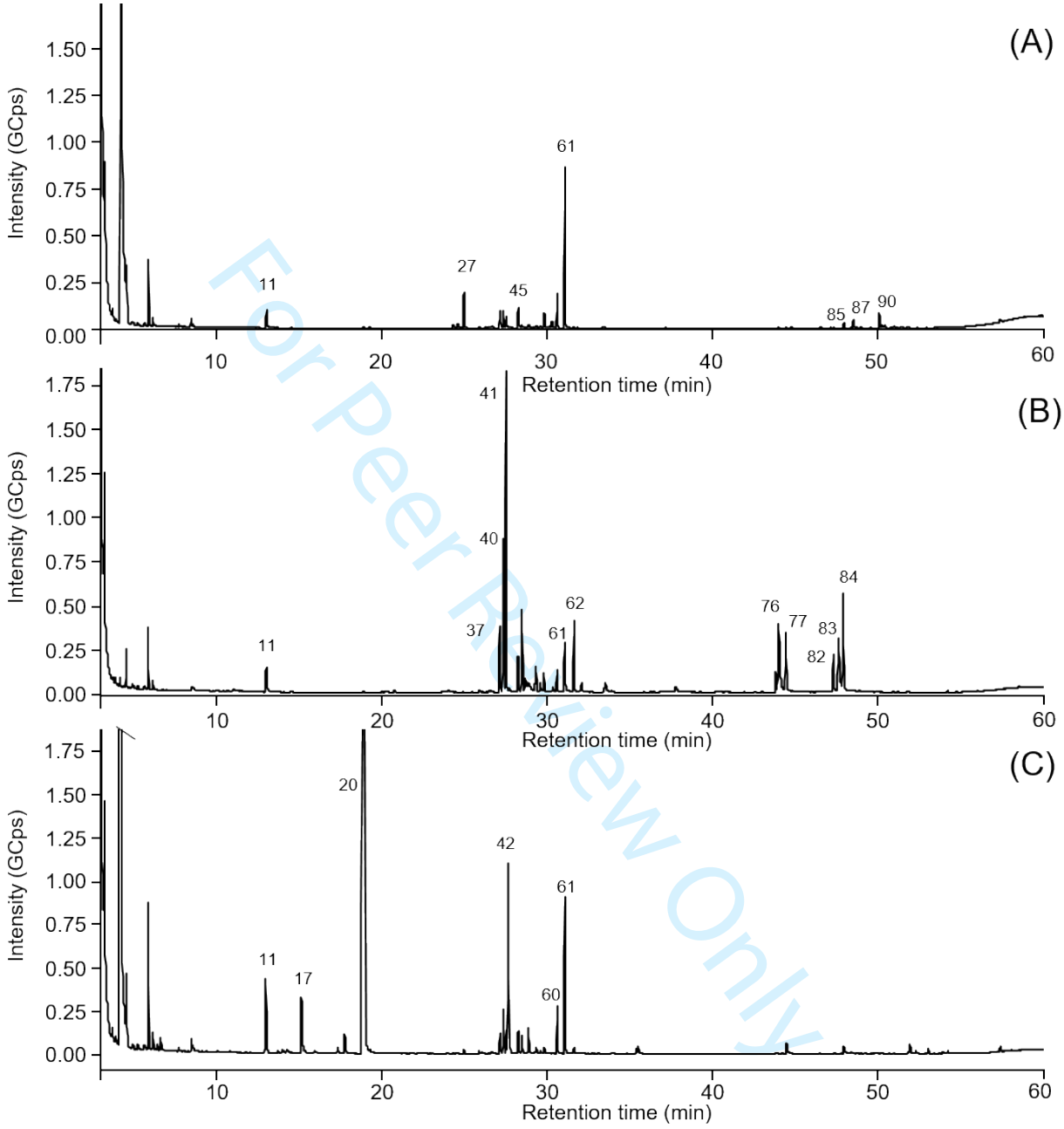

Fig. S2. GC-MS chromatographic profile of *R. typhina* L. (A) branches, (B) leaves and (C) fruits EOs. Main peaks are numbered according to assignments in Table S2.