Available online at www.scholarsresearchlibrary.com

Scholars Research Library

J. Nat. Prod. Plant Resour., 2014, 4 (3):39-43
(http://scholarsresearchlibrary.com/archive.html)

Journal of Natural Product and Plant Resource
Scholars Research Library

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Essential oil composition of *Rhus cotinus* and its antioxidant activity

Subhash C. Joshi[1] and C. S. Mathela

[1]Jubilant Life Sciences Ltd, R & D-I, C-26, Sector 59, Noida, UP, India
Department of Chemistry, Kumaun University, Nainital, Uttarakhand, India

---

ABSTRACT

The essential oil composition of Rhus cotinus L. (syn. Cotinus coggygria Scop.; Family: Anacardiaceae), was analyzed by GC and GC-MS. The in vitro antioxidant activity was assessed by β-carotene bleaching test, reducing power, DPPH radical scavenging and inhibition of lipid peroxidation methods. A total of 30 compounds were identified with the dominance of monoterpenes (65.9%) viz. β-Pinene, camphene, limonene, α-pinene and p-cymene followed by sesquiterpene hydrocarbons (20.6%). The oil exhibited antioxidant activity by inhibiting β-carotene bleaching (56.4 ± 1.88%) and by scavenging DPPH free radical (IC_{50} = 720 ± 0.10 μg mL^{-1}).

Keywords: Monoterpenes, β-carotene bleaching, DPPH free radical scavenging, lipid peroxidation, reducing power.

INTRODUCTION

Rhus L., a woody genus belonging to the Anacardiaceae, is a deciduous and multibranched shrub. The wood is durable, hard, tough, and used for making fences and posts. Some species contain high levels of bioflavonoids in leaves, bark and roots, making them important for medicinal purposes while sour and astringent fruits of many species are used for making beaverages [1,2].

Rhus cotinus L. (syn *Cotinus coggygria* Scop.) is widely distributed from southern Europe, the Mediterranean, Moldova, and Caucasus to central China and Himalayas [3-5]. In folk medicine, *C. coggygria* is routinely used in Turkey as an antiseptic, anti-inflammatory, antimicrobial, and antihaemorragic agent in wound healing [6], as well as for countering diarrhea, paradontosis, gastric and duodenal ulcers [7]. A yellow to orange dye is obtained from roots and stem. The leaves and bark are good source of tannins [8]. Earlier, cardanols and dammarane triterpenoids were reported from *R. thyrsiflora* and *R. javanica,* respectively. Limonene, nonanol, (*Z*)-2-decenal, β-caryophyllene, patchouline and polyphenolic compounds have been reported from *Rhus coriaria* while triterpenoids were reported from *R. semialata* and *R. alata*. [9-14]. Activity-guided isolation of antioxidative compounds of *Cotinus coggygria* extract has been previously reported [15]. Limonene, (*Z*)-β-ocimene and (*E*)-β-ocimene were reported from the essential oil of *Rhus* species from Turkey along with antibacterial and antifungal activities of the essential oil [6, 16]. α-Pinene, limonene, and β-pinene were found to be the major constituents in the essential oil of the Bulgarian *Rhus* species [17]. Recently antioxidant activity of the methanolic extract of *C. coggygria* has been reported from Pakistan [18].

Antioxidant and antibacterial properties of the essential oils have recently been of great interest in both research and food industry because of their possible use as natural additives to replace synthetic antioxidants. Literature survey revealed no report on the essential oil composition and antioxidant activity of the *Rhus cotinus* L from Himalayan regions so far.

MATERIALS AND METHODS

Oil extraction

The plant was collected from Pithoragarh district (1800 m) of Uttarakhand in September 2010, identified at Botanical survey of India (BSI Dehradun) and a voucher specimen was submitted to the Phytochemistry laboratory of Kumaun University (No. Chem/RC/10/01). Fresh aerial parts (2.0 kg) were subjected to steam distillation. The distillate saturated with NaCl was extracted with *n*-hexane and dichloromethane. The organic phase was dried over anhydrous Na_2SO_4 and the solvent was distilled off in a thin film rotary vacuum evaporator at 30^oC to yield the essential oil.

GC and GC-MS analysis

The oil was analyzed by using a Nucon 5765 gas chromatograph (Rtx-5 column, 30 m × 0.32 mm, FID; New Delhi, India), split ratio 1: 48, N_2 flow of 4 kg/cm^2 and on Thermo Quest Trace GC 2000 interfaced with Finnigan MAT Polaris Q Ion Trap Mass spectrometer (Milan, Italy) fitted with a Rtx-5 (Restek Corp.) fused silica capillary column (30 m × 0.25 mm; 0.25 µm film coating) fabricated with stainless steel. The column temperature was programmed 60^oC -210^oC at 3^oC /min using helium as carrier gas at 1.0 mL min^{-1}. The injector temperature was 210^oC, injection size 0.1 µL prepared in hexane, split ratio 1:40. MS were taken at 70 eV with a mass range of 40-450 amu. The identification was done on the basis of retention index (RI) calculated using alkane standards (heptane to *n*-pentacosane), MS Library search (NIST & WILEY) and by comparing with the MS literature data [19].

DPPH free radicals scavenging activity

The DPPH free radicals scavenging activity was determined [20]. Percent inhibition of DPPH (I%) was calculated as I % = (A_C – A_S/ A_C) × 100 where, A_C is the absorbance of the control (containing 0.1 mL of methanol except the test sample), and A_S is the absorbance of the test sample [21]. The inhibitory concentration IC_{50} was estimated and calculated as described in the literarure [22]. IC_{50} value is the concentration of the sample required to scavenge 50% DPPH free radicals and was calculated from a calibration curve by linear regression.

β-Carotene bleaching assay

Antioxidant activity (AOA%) was determined by β-carotene bleaching assay by using standard method of Emmons and Peterson [23]. β-Carotene (2.0 mg) was dissolved in 40 ml of $CHCl_3$ and its 6.0 mL was added to 40 µL linoleic acid and 400 µL Tween 40. After removing $CHCl_3$ under reduced pressure, 100 mL of oxygenated water was added and mixed properly to obtain a stable emulsion. Emulsion (3.0 mL) was mixed with 40 µL of sample and incubated for 1 h at 50^oC. The absorbance was recorded at 0 min and after 60 min of incubation at 470 nm. Antioxidant activity was expressed as percent inhibition relative to control after a 60 min incubation period and calculated by AOA% = (D_C – D_S/D_C) × 100 where D_C = degradation rate of control and D_S = degradation rate of the sample [21].

Estimation of reducing power (RP)

Reducing power was determined using ferric reducing–antioxidant power assay taking quercetin as standard [24]. Different aliquots of the sample maintained to 1ml, followed by the addition of 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1% w/v potassium ferricyanide in each reaction mixture thus obtained were incubated at 50^oC for 20 min. After incubation, reaction was terminated by addition of 2.5 mL of 10% w/v trichloroacetic acid solution; 2.5 mL of above solution from each reaction was diluted with equal amount of distilled water. Aliquot of 0.5 mL $FeCl_3$ (0.1%) was added to each and absorbance was recorded after 10 min at 700 nm. Reducing power was expressed as ascorbic acid equivalent (1 m Mol = 1 ASE).

Lipid peroxidation inhibition (LPO)

Rats were fasted overnight and sacrificed by cervical dislocation, dissected and abdominal cavity was perfused with 0.9% saline. Whole liver was taken out and weighted amount of liver processed to get 10% homogenate in cold phosphate buffer saline (pH 7.4). The degree of lipid peroxidation was assayed by estimating the thiobarbituric acid-reactive substances (TBARS). Different concentrations of oils were added to 1 mL liver homogenate. Liver

peroxidation was initiated by adding 100 µL of 15m Mol $FeSO_4$ solution to liver homogenate. After 30 min incubation at 37°C, 100 µL of this reaction mixture was taken in a tube containing 1.5 mL of 10% TCA. After 10 min tubes were centrifuged and supernatant was mixed with 1.5 mL of 0.67% TBA in 50% acetic acid. The mixture was heated in a water bath for 30 min. The intensity of coloured complex formed was measured at 532 nm. The percentage of inhibition of lipid peroxidation was calculated by comparing with those of control.

Statistical analysis

Tests were carried out in triplicates and the results were calculated as mean ± SD.

RESULTS AND DISCUSSION

Essential oil composition

The essential oil composition of the aerial parts of *R. cotinus* from Kumaon Himalaya, analyzed by GC and GC-MS are shown in Table-1. A total of 30 compounds were identified. The dominant presence of monoterpene hydrocarbons (65.9%) was observed followed by sesquiterpene hydrocarbons (20.6%). Oxygenated monoterpenes (5.8%) and oxygenated sesquiterpenes (4.7%) were less abundant in the essential oil. The dominant presence of monoterpenes was noticed with β-Pinene (30.6%), camphene (13.6%), limonene (12.4%), α-pinene (5.2%) and *p*-cymene (4.6%). Oxygenated monoterpenes constituted 1, 8-cineole (1.3%) and terpin-4-ol (2.8%) as representative constituents followed by linalool (0.2%) and α-terpenol (0.3%). Among sesquiterpene hydrocarbons bicyclogermacrene (12.6%), β-caryophyllene (4.4%) and germacrene D (2.0%) were found as major constituents. Oxygenated sesquiterpenes were found in relatively less amount with the minute presence of *epi*-α-cadinol and β-eudesmol (1.0% each).

Table – 1, Terpenoid composition of the leaf essential oil of *Rhus cotinus* aerial parts

S. No.	Compounds	LRI	% FID	Mode of identification*
1	α-thujene	931	0.1	a, b
2	α-pinene	941	5.2	a, b
3	camphene	955	13.6	a, b
4	β-pinene	982	30.6	a, b
5	α-terpinene	1020	0.1	a, b
6	*p*-cymene	1029	4.6	a, b
7	limonene	1034	12.4	a, b
8	1,8-cineole	1038	1.3	a, b
9	terpinolene	1089	0.3	a, b
10	linalool	1101	0.2	a, b
11	terpin-4-ol	1180	2.8	a, b
12	α-terpineol	1192	0.3	a, b
13	bornyl acetate	1285	0.2	a, b
14	δ-elemene	1341	0.2	a, b
15	α-copaene	1379	0.1	a, b
16	β-caryophyllene	1420	4.4	a, b
17	γ-gurjurene	1435	0.1	a, b
18	α-hummulene	1457	0.1	a, b
19	germacrene D	1482	2.0	a, b
20	bicyclogermacrene	1494	12.6	a, b
21	*epi*-cubebol	1497	0.7	a, b
22	γ-cadinene	1516	1.1	a, b
23	germacren D-4-ol	1578	0.5	a, b
24	spathulenol	1579	0.4	a, b
25	caryophyllene oxide	1584	0.3	a, b
26	hummulene epoxide	1606	0.2	a, b
27	*epi*-α-cadinol	1643	1.0	a, b
28	cubebol	1645	0.4	a, b
29	β-eudesmol	1652	1.0	a, b
30	α-cadinol	1655	0.2	a, b
	Total		**97.0%**	
	Monoterpene hydrocarbons		65.9%	
	Oxygenated monoterpenes		5.8%	
	Sesquiterpene hydrocarbons		20.6%	
	Oxygenated sesquiterpenes		4.7%	

**a=Linear retention index, b=GC-MS*

Antioxidant activity

The antioxidant activity of the essential oil of the aerial parts of *R. cotinus* was evaluated by four methods *viz*. β-carotene bleaching assay, reducing power, DPPH radical scavenging and lipid peroxidation. The results of the antioxidant activity are shown in Table-2. The essential oil exhibited significant antioxidant power by inhibition of β-carotene bleaching (56.4 ± 1.88%) and showed a direct role in trapping free radicals which is comparable to the reference standard BHT (56.20 ± 3.15%). The reducing power of the essential oil showed its potential as electron donor to scavange the free radicals (2.29 ± 0.60 ASE mL^{-1}). Free radical (DPPH) scavenging activity of the essential oil was evaluated against quercetin as reference standard and it was found to be 720 ± 0.10 μg mL^{-1}. The inhibition of lipid peroxidation showed that the essential oil inhibited TBARS (thiobarbituric acid reactive substances) formation upto 1480 ± 0.72 μg mL^{-1}. Antioxidant activity of compounds obtained from the extract of *C. coggygria* was previously reported [15]. In the present study, antioxidant activity was noticed in spite of having low percentage of oxygenated monoterpenoids (5.8%, Table 1) which shows that the presence of these compounds is not obligatory for this activity. However, several *Rhus* species contain bioflavonoids in leaves, bark, roots and fruits [1,2]. Therefore the antioxidant activity of the essential oil of *R. cotinus* could be attributed to the synergetic effect of mixture of mono and sesquiterpene hydrocarbons along with oxygenated sesquiterpenoids.

Table 2, Antioxidant activity of essential oil of *Rhus cotinus*

Sample	DPPH (IC_{50} μg mL^{-1})	AOA %	Reducing Power (ASE mL^{-1})	LPO (IC_{50} μg mL^{-1})
Essential oil	720 ± 0.10	56.40 ± 1.88	2.29 ± 0.60	1480 ± 0.72
*BHT	nd	56.20 ± 3.15	nd	nd
*Quercetin	35 ± 0.02	nd	0.52 ± 0.09	89 ± 0.04

**Standard; quercetin, butylated hydroxy toluene (BHT); nd= Not determined*

CONCLUSION

The essential oil composition of the aerial parts of *Rhus cotinus*, dominated by monoterpenes was found to exhibit significant antioxidant activity by inhibiting β-carotene bleaching, ferric reducing antioxidant power and by scavenging DPPH free radical. Owing to its significant protective features exhibited in antioxidant activity tests, further studies can be done on *Rhus species* in order to obtain more information regarding the practical effectiveness of these oils in *in vivo* studies.

Acknowledgements

The author (SCJ) is grateful to Department of Science and Technology (DST) New Delhi for fellowship and to A. R. Verma (NBRI, Lucknow) for antioxidant activity support.

REFERENCES

[1] S Matic; S Stanic; D Bogojevic; S Solujic; N Grdovic; M Vidakovic; M Mihailovic. *Genet Mol Biol*, **2011**, 34, 298.
[2] S Prakash; JV Staden. *Genet Resour Crop Evol*, **2007**, 54, 7.
[3] BL Gupta. *Forest flora of Chakrata, Dehradun and Saharanpur*. Forest Research Institute Press, 19**4**5.
[4] BD Naithani. *Flora of Chamoli*. Botanical Survey of India, Calcutta, **1984**,1, pp.133.
[5] M Novakovic; I Vuckovic; P Janackovic; M Sokovic; V Tesevic; S Milosavljevic. *J Serb Chem Soc*, **2007**, 72, 104.
[6] B Demirci; F Demirci; KH Baser. *Flavour Frag Jour* **2003**, 18, 43.
[7] D Ivanova; D Gerova; T Chervenkov; T Yankova. *J Ethnopharmacol*, **2005**, 96, 145.
[8] G Perkin. *J Chem Soc Trans*, **1898**, 73, 1016.
[9] M Parveen; UK Nizam; B Achari; PK Dutta. *Curr Sci*, **1989**, 58, 1326.
[10] N Parveen; MP Singh; NU Khan; B Achari; MK Logani. *Phytochemistry*, **1991**, 30, 2415.
[11] G Schmaus; EJ Brunke. Proceedings of an international conference "Essential oils for perfumery and flavours", Turkey, **1990**, 178.
[12] S Kurucu; M Koyuncu; A Guvenc; KHC Baser; T Ozek. *J Essent Oil Res*, **1993**, 5, 481.
[13] IS Lee; HR Oh; KS Ahn; HK Lee. *Chem Pharm Bull*, **2001**, 49, 1024.
[14] K Franke; M Masaoud; J Schmidt. *Planta Med*, **2001**, 67, 477.

[15] HE Westenburg; KJ Lee; SK Lee; HHS Fong; RB van Breemen; JM Pezzuto; AD Kinghorn. *J Nat Prod*, **2000**, 63, 1696.
[16] M Digrak; MH Alma; A Ilcim. *Pharm Biol*, **2001**, 39, 346.
[17] ET Tsankova; AS Dyulgerov; BK Milenkov. *J Essent Oil Res*, **1993**, 5, 205.
[18] T Riaz; MA Abbasi; A Rehman; K Rubab; T Shahzadi; M Ajaib; KM Khan. *Pak J Pharm Sci*, **2012**, 25, 679.
[19] RP Adams. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*. Allured Publishing Corporation, Carol Stream, Illinois, **2007**.
[20] GC Yen; PD Duh. *J Agr Food Chem*, **1994**, 42, 629.
[21] SC Joshi; AR Verma; CS Mathela. *Food Chem Toxicol*, **2010**, 48, 37.
[22] GT Kroyer. *Innov Food Sci Emerg*, **2004**, 5,101-105.
[23] CL Emmons; DM Peterson. *Cereal Chem*, **1999**, 76, 902.
[24] P Apati; K Szentmihalyi; Sz T Kristo; I Papp; P Vinkler; E Szoke; A Kery. *J Pharm Biomed. Anal*. **2003**, 32, 1045.