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Potential allelochemicals from the essential oil of *Ruta graveolens*

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Abstract

The essential oil of aerial parts of *Ruta graveolens* was obtained by hydrodistillation with a 0.74% yield on a dry weight basis. Thirty-eight components were identified by GC and GC–MS analyses. 2-Ketones predominated in the essential oil, with undecan-2-one (46.8%) and nonan-2-one (18.8%) as the main constituents. The essential oil and some of its constituents were tested for their allelopathic activity in vitro on radish germination and radicle growth in light and darkness. The essential oil and some of its minor constituents were effective and dose-dependent inhibitors of both the germination and radicle growth; 2-ketones are not active. The possible allelopathic activity of rue essential oil and some its isolated constituents is reported.

Keywords: *Ruta graveolens* L.; Essential oil; Allelochemicals; 2-Ketones; Terpenoids; Germination; Root inhibition; *Raphanus sativus* L.

1. Introduction

Plants have evolved several strategies to interact with other organisms, for self defense, sexual attraction, symbiosis, and development (Rice, 1984; Harborne, 1989). The production and accumulation of secondary metabolites, which inhibit and/or stimulate germination and development of other plants, is an important mechanism in the interactions between plants. Aromatic plants, known to be rich in active principles, can play an important role in plant-plant interactions and constitute a primary source of potential allelochemics (Aliotta et al., 1989; Vokou, 1992).

Our research group is carrying out a series of studies on the possible allelopathic properties of rue, *Ruta graveolens* L. (Rutaceae), a traditional medicinal plant, known to prevent the attacks by fleas and other noxious insects. The plant is also used as a flavouring agent for spirits and foods. In previous papers, we have shown the inhibtion of *in vitro* germination and the radicle growth of radish seeds by an aqueous extract of rue and some of its constituents, in particular furanocoumarin derivatives (Aliotta et al., 1994). These findings prompted us to investigate the potential effect of rue extracts as a herbicide against the germination in the soil of some common weeds. The total extract of rue and some isolated compounds resulted in an active inhibition of weed germination, in particular *Portulaca oleracea* L., causing an irreversible damage to protruded radicles. Some constituents of this extract (furocoumarins and flavonols) also inhibited in vitro germination of several weeds (Aliotta et al., 1995, 1996).

This paper deals with the possible allelopathic effects of the essential oil of the aerial parts of *R. graveolens* and some isolated constituents, investigating the difference in biological activity in light and darkness.

2. Results and discussion

Hydrodistillation of the dry aerial parts of *R. graveolens* furnished 0.74% of a yellowish essential oil, having an intense and penetrating odor. Thirty-eight compounds, representing 97.3% of the oil, were identified. Table 1 shows the percentage composition of the essential oil that is characterized by the great prevalence of 2-ketones. Undecan-2-one (46.8%) and nonan-2-one (18.8%) represent the main constituents of the oil; decan-2-one and tridecan-2-one are also present in considerable amounts, accounting for 2.2% and 2.5%,

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Table 1
Composition of the essential oil from the aerial parts of *Ruta graveolens* L. (%) (the compounds are listed in order of their elution)

Compound	Identification[a]	%
α-Pinene	RI, MS, Co-GC	1.3
Camphene	RI, MS, Co-GC	0.1
β-Pinene	RI, MS, Co-GC	0.2
Heptan-2-one	RI, MS	0.2
p-Cymene	RI,.MS, Co-GC	0.1
Limonene	RI, MS, Co-GC	3.0
1,8-Cineole	Ri, MS, Co-GC	2.9
n-Octanol	RI, MS, Co-GC	0.3
Terpinolene	RI, MS, Co-GC	0.3
Valeric acid	RI, MS, Co-GC	1.6
Non-2-ene	RI, MS	0.1
Nonan-2-one	RI, MS, Co-GC	18.8
Linalol	RI, MS, Co-GC	1.5
Phenyl ethyl alcohol	RI, MS	0.3
Nonan-2-ol	RI, MS	1.5
Octanoic acid	RI, MS, Co-GC	3.4
Methyl salicylate	RI, MS, Co-GC	3.9
Decan-2-one	RI, MS	2.2
Decan-2-ol	RI, MS	0.8
Octyl acetate	Ri, MS	0.1
Undecan-2-one	RI, MS, Co-GC	46.8
Undecan-2-ol	RI, MS	0.2
Dodec-2-ene	RI, MS	0.2
Tridecane	RI, MS	0.1
Decyl-2-acetate	RI, MS	0.1
Dodecan-2-one	RI, MS	0.9
Tridecan-2-one	RI, MS, Co-GC	2.5
α-Copaene	RI, MS	0.1
β-Caryophyllene	RI, MS	0.6
α-Humulene	RI, MS	0.6
δ-Cadinene	RI, MS	0.2
Hexadecane	RI, MS	0.9
α-Eudesmol	RI, MS	0.3
Pentadecan-2-one	RI, MS	0.1
Heptadecane	RI, MS	0.1
Pentadecanol	RI, MS	0.1
Hexadecanol	RI, MS	0.1
Xanthotoxin	RI, MS, Co-GC	0.8

[a] RI, retention index identical to bibliography; Co-GC, retention time identical to authentic compounds; MS, identification based on comparison of mass spectra.

respectively. Terpenoids constitute 11.2% of the oil with α-pinene (1.3%), limonene (3.0%) and 1,8-cineole (2.9%) as the main monoterpene constituents. Sesquiterpenoids accounted for 1.8%. Among other compounds, considerable amounts of valeric acid (1.6%), octanoic acid (3.4%), nonan-2-ol (1.5%) and methyl salicylate (3.9%) were detected. Xanthotoxin (0.8%), a furocoumarin and a well known allelochemical, was also found in the oil. This compositional pattern is in good agreement with literature data (Kubeczka, 1971; Nagel and Reinhard, 1975; Aboutabl et al., 1988; Yacoob et al., 1989).

To evaluate the essential oil's possible allelopathic effects, it was tested in vitro on germination and radical growth of radish seeds, in concentrations from 62.5 to 500 μg/ml, both in light and in darkness. Table 2 shows the effects of rue oil on germination and radical elongation of radish, 120 h after the treatment. The oil inhibited both germination and radical elongation of radish seeds, in a dose-dependent manner, with the effect being significantly more effective in the dark. Germination was inhibited by 60 and 77%, respectively in light and in darkness, at the highest concentration tested (500 μg/ml) and by 30–49% at lowest one (62.5 μg/ml). These findings were confirmed by other experiments, where the effects of substances volatilized from the aerial parts of rue were evaluated. After 120 h, the germination was inhibited by 77% in light and 69% in darkness and radical growth was reduced by 53% (light) and 56% (darkness) (data not shown).

On the basis of the inhibitory activity showed by the essential oil, components present at concentrations of at least in 1%, were tested by the same bioassay, both in light and in darkness, at concentrations of 10^{-3} M. Table 3 shows the data obtained on radish germination and radicle elongation, 120 h after sowing. Germination is totally inhibited by valeric acid, both in light and in darkness. In light, methyl salicylate inhibited germination by 90%, α-pinene by 68%, nonan-2-ol by 65%, octanoic acid by 50% and 1,8-cineole by 48%. In

Table 2
Activity of rue essential oil on germination and radicle elongation of radish, 120 h after sowing

	Germination				Radical length (mm)			
	Light	% Inhibition	Darkness	% Inhibition	Light	% Inhibition	Darkness	% Inhibition
Control	10.0±0.1		9.83±0.1 n.s.		75±0.4		86±0.9 n.s.	
Essential oil								
62.5 μg/ml	7.0±0.8	30	5.0±0.4*	49	77±0.8	+3	59±0.6**	31
125 μg/ml	6.5±0.4	35	3.7±0.2**	62	73±0.9	3	69±0.7**	20
250 μg/ml	4.7±0.5	53	2.5±0.4**	75	47±0.6	37	41±0.5 n.s.	53
500 μg/ml	4.0±0.5	60	2.3±0.1**	77	38±0.3	49	23±0.4*	72

Data are expressed as mean of germinated seeds±S.E.M. and radical length±S.E.M. Each Petri dish contained 10 seeds; each determination was repeated six times. n.s. = not significative. *$P<0.05$. **$P<0.01$.

Table 3
Activity of some components of rue essential oil, at a concentration of 10^{-3} M on germination and radicle elongation of radish, 120 h after sowing

	Germination				Radical length (mm)			
	Light	% Inhibition	Darkness	% Inhibition	Light	% Inhibition	Darkness	% Inhibition
Control	10±0.1		10±0.1 n.s.		78±0.7		83±1.3 n.s.	
Compounds								
1,8-Cineole	5.2±0.3	48	6.0±0.2 n.s.	40	71±0.9	9	91±0.3*	+10
Decan-2-one	6.3±0.2	37	8.3±0.1*	17	54±0.6	31	87±0.9**	+5
Limonene	7.2±0.2	28	8.7±0.1 n.s.	13	61±0.3	22	97±0.9**	+17
Methyl salicylate	1.0±0.1	90	4.3±0.2**	57	51±0.4	35	26±0.3**	69
Nonan-2-ol	3.5±0.3	65	4.2±0.1 n.s.	58	30±0.4	62	20±0.9 n.s.	76
Nonan-2-one	7.0±0.3	30	5.2±0.1 n.s.	48	37±0.2	53	63±0.1**	24
Octanoic acid	5.0±0.1	50	7.3±0.2*	27	67±0.7	14	27±0.3**	68
α-Pinene	3.2±0.1	68	5.2±0.2*	48	47±0.9	40	85±0.6**	+2
Tridecan-2-one	8.8±0.2	12	9.8±0.5 n.s.	2	49±0.3	37	97±0.7**	+17
Undecan-2-one	6.2±0.3	38	9.8±0.9*	2	66±0.5	15	103±0.2**	+24
Valeric acid	0	100	0	100				

Data are expressed as mean of germinated seeds±S.E.M. and radical length±S.E.M. Each Petri dish contained 10 seeds; each determination was repeated six times. n.s. = not significative. *$P<0.05$. **$P<0.01$.

darkness, the major inhibitor of radish germination were nonan-2-ol (58%), methyl salicylate (57%), nonan-2-one (48%), α-pinene (48%) and 1,8-cineole (40%). In light, inhibitions recorded for root elongation ranged from 62% (nonan-2-ol) to 9% (1,8-cineole). In darkness, nonan-2-ol (76%) and methyl salicylate (69%) were the major inhibitors; some compounds, on the contrary, stimulated the radicle elongation.

The more active components of the oil were tested in lower concentrations, from 10^{-4} M to 10^{-6} M; these data are shown in Table 4. At 10^{-4} M, methyl salicylate, valeric acid and 1,8-cineole inhibited germination in light respectively by 73, 37 and 35%. A similar inhibitory pattern was observed in darkness. Radical elongation was also affected in a similar way. The inhibtions were dose-dependent and at lowest tested concentration (10^{-6} M), methyl salicylate and 1,8-cineole retained their inhibitory activity on germination, respectively for 35 and 30% in light and 2-nonanol inhibited radical growth by 35%. The data clearly showed the allelopathic potential of rue volatiles. In fact, both in the assay with chopped leaf aerial parts of the plant and in that with the essential oil obtained by hydrodistillation, radish germination and radical growth were severely and dose-dependently affected.

Our data agree with the literature on inhibitory activity exerted by essential oils on seed germination and radical elongation. There is evidence in the literature about the translocation of essential oils and/or their components in the soil (Halligan, 1975; Rice, 1984; Friedman, 1987; Kholi, 1994), where they remain absorbed and retain their inhibitory activity for several months. Regarding the radish germination, our data revealed more marked inhibitory effects exerted by rue essential oil in darkness (Table 2), while the pure compounds seem to be more inhibitory in light. The more active compounds seem to affect the radicle growth slightly more.

Among the pure compounds, 2 ketones, that dominate in the oil, were inactive at all tested concentrations, while terpenoids, organic acids and the aliphatic alcohol nonan-2-ol showed appreciable and dose-dependent inhibitory acitvity. Our data disagree with Rice (1984), who reported that aliphatic ketones have generally a good inhibitory activity. Moreover, Asplund reported monoterpene ketones to be strong allelochemicals, correlating the toxicity of other monoterpenoids to their ease to being metabolically altered at ketones (Asplund, 1968, 1969).

Valeric acid completely inhibits the seed germination at a concentration of 10^{-3} M, but rapidly looses its activity at lower concentration. This finding is probably due to the acidity of the most concentrated solution tested, although according to Evenari (1949), the acidity seems to contribute to inhibition, but is not its only cause. On the other hand, organic acids are well known inhibitors of seed germination and radicle elongation (Evenari, 1949; Rice, 1984; Mandava, 1985). Salicylic acid is an important allelopathic agent and enzymatic glucosylation as defense mechanism of *Avena sativa* against such inhibitory effects is reported (Balke et al., 1987).

The other active compounds (1,8-cineole and α-pinene) are monoterpenes. Our data showed considerable inhibitory activities for 1,8-cineole and α-pinene. 1,8-Cineole constitutes a potent phytotoxin

Table 4
Activity of some components of rue essential oil, from 10^{-4} M to 10^{-6} M, on germination and radicle elongation of radish, 120 h after sowing

	Germination				Radical length (mm)			
	Light	% Inhibition	Darkness	% Inhibition	Light	% Inhibition	Darkness	% Inhibition
Control	10±0.1		10±0.1		65±0.7		72±0.3 n.s.	
Compounds								
1,8-Cineole								
10^{-4} M	6.5±1.3	35	6.5±0.3 n.s.	35	42±1.3	35	63±1.5*	13
10^{-5} M	7.2±0.5	28	7.8±0.7 n.s.	22	62±1.4	5	77±1.5 n.s.	+7
10^{-6} M	7.0±0.7	30	7.8±0.9 n.s.	22	69±1.2	+6	81±1.7 n.s.	+13
Methyl salicylate								
10^{-4} M	3.7±0.7	73	4.5±0.9 n.s.	55	54±1.5	17	65±1.9 n.s.	10
10^{-5} M	7.2±1.8	28	6.8±0.5 n.s.	32	33±0.9	49	69±1.7**	4
10^{-6} M	6.5±0.7	35	8.5±0.9*	15	71±1.7	+9	71±2.1 n.s.	1
Nonan-2-ol								
10^{-4} M	7.1±0.9	29	7.1±0.3 n.s.	29	29±0.3	55	45±0.6**	38
10^{-5} M	8.3±0.8	17	6.2±0.5 n.s.	38	33±0.4	49	63±0.7**	13
10^{-6} M	9.2±0.5	8	9.1±0.7 n.s.	9	42±0.2	35	67±0.3**	7
Octanoic acid								
10^{-4} M	7.8±0.6	22	7.3±0.6 n.s.	27	41±0.3	37	39±0.4 n.s.	46
10^{-5} M	8.3±0.6	17	8.1±1.6 n.s.	19	53±0.4	19	53±0.3 n.s.	26
10^{-6} M	9.3±0.9	7	8.8±0.7 n.s.	12	65±0.5	0	61±0.4 n.s.	15
α-Pinene								
10^{-4} M	7.5±0.5	25	6.6±0.2 n.s.	34	42±0.4	35	31±0.2 n.s.	57
10^{-5} M	8.1±0.7	19	7.1±0.5 n.s.	29	51±0.4	22	44±0.2 n.s.	39
10^{-6} M	9.3±0.9	7	8.0±0.5 n.s.	20	59±0.3	9	56±0.4 n.s.	22
Valeric acid								
10^{-4} M	6.3±1.0	37	7.1±0.9 n.s.	29	41±0.3	37	51±0.6 n.s.	29
10^{-5} M	6.5±0.3	35	6.2±0.6 n.s.	38	52±1.2	20	63±1.4 n.s.	13
10^{-6} M	8.9±0.7	11	9.1±0.5 n.s.	9	63±2.3	3	71±1.7 n.s.	1

Data are expressed as mean of germinated seeds±S.E.M. and radical length±S.E.M. Each Petri dish contained 10 seeds; each determination was repeated six times. n.s. = not significative. *$P<0.05$. **$P<0.01$.

and is produced by many plant species (e. g. *Salvia* and *Eucalyptus*). This compound is a potent inhibitor of oxygen uptake by mitochondrial suspensions, resulting probably from the gradual penetration of the terpene through the membrane of the mitochondria to the site of action (Muller et al., 1969). Although reported as less active, α-pinene also possesses important allelopathic activities. It has been reported as an allelopathic compound from a number of plants species (Rice, 1984). Our data also confirm the greater inhibitor activity of 1,8-cineole in comparison with that of α-pinene.

The presence of the furanocoumarin xanthotoxin in rue oil, previously isolated as a potent allelopathic constituent from an aqueous extract of the plant (Aliotta et al., 1994, 1996), may be important in explaining the allelopathic activity of the essential oil, whose components probably may act synergistically.

This preliminary in vitro study, showed the contribution of the essential oil to the allelopathic potency of rue and put new questions: if the volatiles and the water-soluble active compounds (Aliotta et al., 1994, 1995, 1996) exert an allelopathic synergistic action, the effectiveness of essential oils and allelochemicals in soil, their relationships with water and minerals and their effects on soil microorganisms.

3. Experimental

3.1. Plant material

Aerial parts of *R. graveolens*, at flowering stage, were collected in September 1995, from the Experimental Institute for Horticulture farm, at Pontecagnano (Salerno). A voucher specimen of the plant (DF000278) is deposited in the herbarium of the School of Pharmacy, University of Salerno.

3.2. Oil isolation and analysis

Aerial parts of the plant (200 g) were air dried and cut into small pieces and then submitted to hydrodistillation for 3 h, according to the standard procedure reported in the European Pharmacopoeia (1975). The oil content was 0.74% (v/w), on a dry weight basis. The oil was analysed by GC and CG–MS. GC analyses were performed using a Perkin-Elmer Sigma-115 gas chromatograph with a data handling system and FID. The analysis was carried out using a DB-1 fused-silica column (30 m × 0.25 mm i.d.; film thickness 0.25 μm). The operating conditions were as follows: injector and detector temperatures, 150 and 280 °C, respectively;

carrier gas, He; oven temperature programme, 5 min isothermal at 40 °C, then at 2 °C/min up to 250 °C and finally held isothermally for 20 min. GC–MS analyses were performed using a Varian Model 3400 gas chromatograph coupled to a Finnigan Mat ion trap detector, equipped with a DB-5 fused-silica column (30 m × 0.25 mm i.d.; film thickness 0.25 μm); ionization voltage 70 eV; electron multiplier energy 2000 V. Gas chromatographic conditions were as given above; transfer line temperature, 295 °C. The identity of oil components was established from their GC retention times (Jennings and Shibamoto, 1980), by comparison of their MS spectra with those reported in literature (Eight Peak Index of Mass Spectra, 1983; Adams, 1989) and by computer matching with the Wiley 5 mass spectra library, as well as, whenever possible, by co-injection with standards available in our laboratories.

3.3. Bioassays

A bioassay based on radish germination and subsequent radicle growth was used to study allelopathic effects of the essential oil of rue and the compounds present in this oil in concentrations up 1%.

Seeds of *Raphanus sativus* L. vc. 'Saxa', collected during 1995, were purchased from Mattei Co., Naples. The seeds were surface-sterilized in 95% ethanol for 15 s and sown in Petri dishes (Ø = 90 mm), containing five layers of Whatman filter paper, impregnated with 7 ml of distilled water (control) or 7 ml of tested solution of the essential oil or the compound at known concentration. Compounds of low solubility in water were dissolved in water–acetone mixture (97.5:2.5). Controls performed with this mixture alone showed no appreciable difference in comparison with controls in water alone. Germination conditions were 20 ± 1 °C, with either continuous light (25 μM photons/m^2/s) or in darkness. Seed germination process was observed directly in Petri dishes, each 8 h, with a stereomicroscope. A seed was considered germinated when the protrusion of the radicle became evident (Bewley and Black, 1985). Effects of radicle elongation were determined by measuring radicle length to the nearest millimeter each 8 h. Each determination was repeated six times, using Petri dishes containing 10 seeds each.

Pure compounds were purchased from Aldrich, Co, Milan.

To evaluate also the volatilization of substances from the aerial parts of rue, another experiment was carried out, using germination chambers, as suggested by Olezsek (1987). Fresh rue aerial parts were chopped into small pieces and placed in germination chambers, constitued of glass jars of 400 cm^2 at the bottom of which were laid Whatman filter paper. Fifty radish seeds were put on the paper, and the paper was dampened with 10 ml of distilled water. Chambers were equipped inside with plastic stands which supported watch glasses, on which 3 g of rue sample was placed. The jars were immediately closed with plastic lids, to avoid the loss of volatiles. The experiments were conducted both in light and in darkness. Jars without rue material constitued the control. The effects on germination and radical elongation of radish seeds were evaluated as described above.

Each determination was repeated six times.

3.4. Statistical analysis

Data are expressed as the mean ± S.E.M. and a *t*-test Student for paired data was performed between light and darkness both for germination and radical length.

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