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Short report

# Antimicrobial and cytotoxic activity of *Ruta graveolens*

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**Abstract**

The methanol, petroleum ether, ethyl acetate and water–methanol extracts of *Ruta graveolens* were found to possess antimicrobial and cytotoxic activities.


*Keywords:* *Ruta graveolens*; Antimicrobial activity; Cytotoxic activity

## 1. Plant

*Ruta graveolens* L. (Rutaceae), aerial parts cultivated in the region of Gorna Orjahovitza, collected in 1996 and purchased from Bilkocoop Ltd. (Sofia, Bulgaria).

## 2. Uses in traditional medicine

Antiseptic, stimulant, emmenagogue, abortifacient, used against rheumatic pain, hysteria, worms, colics, atonic, amenorrhea and menorrhagia [1,2].

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## 3. Previously isolated classes of compounds

Coumarins [1], terpenoids [3], alkaloids [4,5], flavonoids [6], aliphatic acids and ketones [3].

## 4. New-isolated constituents

Rutarin [7], isorutarin [7], rutin [4] and isorhamnetin-3-*O*-rutinoside [8], cnidioside A [9], methylcnidioside A [10] cnidioside B (**1**) [9] and its methyl ester (**2**) [11] (Fig. 1).

*Cnidioside B* (**1**), $^{1}$H-NMR (250 MHz, $CD_3OD$): $\delta$ 7.58 (1H, *d*, *J* 2.2 Hz, H-3), 7.03 (1H, *s*, H-4), 6.63 (1H, *d*, *J* 2.2 Hz, H-2), 4.93 (1H, *d*, *J* 7.3 Hz, H-1′Glc), 4.02 (3H, *s*, OMe-7), 3.68 (2H, *dd*, *J* 12.3, 2.3, $CH_2$-6′), 3.56 (2H, *dd*, *J* 12.2, 5.0, $CH_2$-6′), 3.1–3.35 (4H, *m*, H-2′-H-4′), 3.02 (2H, *t*, *J* 7.8 Hz, $CH_2$-8), 2.47 (2H, *m*, $CH_2$-9). $^{13}$C-NMR (62.89 MHz, $CD_3OD$): $\delta$ 146.9 (C-3), 146.5 (C-7a), 144.6 (C-6), 138.8 (C-7), 133.6 (C-3a), 127.0 (C-5), 115.6 (C-4), 107.6 (C-2), 105.5 (C-1′), 78.3 (C-3′), 77.9 (C-5′), 75.8 (C-2′), 71.4 (C-4′), 62.3 (C-6′), 61.4 (7-OMe), 39.1 (C-9), 28.4 (C-8).

## 5. Tested material

Total MeOH extract (yield 14.5%). This extract was subjected to solvent–solvent partition to give the petroleum ether, EtOAc and $H_2O$/MeOH extracts (yields: 4.4, 7.6 and 58.7%, respectively).

## 6. Studied activity

Antibacterial and antifungal activities by the modified disk diffusion method [12]. Cytotoxicity by *Artemia salina* lethality test (Brine shrimp assay) using caffeic acid phenethyl ester (CAPE) as active reference substance [13].

## 7. Used microorganisms

Listed in Table 1 (antimicrobial) and Table 2 (cytotoxicity).

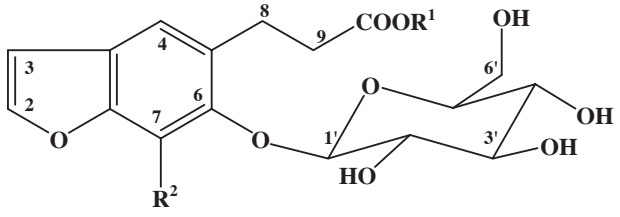


Fig. 1. Cnidioside B (**1**) and its methyl ester (**2**). (**1**) $R^1$=H; $R^2$=OMe. (**2**) $R^1$=$CH_3$; $R^2$=OMe.

Table 1
Antibacterial and antifungal activity of the *R. graveolens* extracts[a]

| Extracts | *St. a.*[b] | *St. e.*[c] | *Str. p.*[d] | *C. d.*[e] | *L. m.*[f] | *B. s.*[g] | *E. c.*[h] | *C. a.*[i] |
|---|---|---|---|---|---|---|---|---|
| | Zone of inhibition diameter (mm) | | | | | | | |
| MeOH | 23.3 ± 0.6 | – | 20.0 ± 0.0 | – | 17.7 ± 0.6 | 16.0 ± 0.0 | – | – |
| Petroleum ether | 23.6 ± 0.6 | – | 20.3 ± 0.6 | – | 22.7 ± 1.2 | 14.3 ± 0.6 | – | – |
| EtOAc | 23.0 ± 0.0 | 13.3 ± 1.2 | 24.7 ± 1.2 | – | 19.3 ± 1.2 | 22.7 ± 1.2 | – | – |
| $H_2O$/MeOH | – | – | 18.0 ± 0.0 | – | 16.0 ± 0.0 | 14.0 ± 0.0 | – | – |
| Streptomycin[j] | 28.0 ± 0.1 | 28.0 ± 0.1 | 28.0 ± 0.1 | 28.0 ± 0.1 | 28.0 ± 0.1 | 28.0 ± 0.1 | – | – |

–=no activity (diameter of the inhibitory zone less than 10 mm means absence of activity).
[a] Results are the mean of three replications; 0.5 mg/disk.
[b] *Staphylococcus aureus* 209.
[c] *Staphylococcus epidermidis* 1093.
[d] *Streptococcus pyogenes* 15346.
[e] *Corynebacterium diphtheriae* 39179.
[f] *Listeria monocytogenes* $C_{12}$.
[g] *Bacillus subtilis* 1A95.
[h] *Escherichia coli* $WF^{+}$.
[i] *Candida albicans* 562.
[j] Tested at 0.1 mg/disk.

## 8. Results and conclusions

All the extracts showed no activity against the Gram (−) strain *Escherichia coli* and the fungus *Candida albicans*. However, they exhibited inhibitory effects and a clear selectivity towards the studied Gram (+) microorganisms. The data (Table 1) demonstrate a good antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Listeria monocytogenes* and *Bacillus subtilis* and no inhibition of *Corynebacterium diphtheriae*. Only the EtOAc extract inhibits the growth of *Staphylococcus epidermidis*. The results (Table 2) reveal a strong cytotoxic activity for the total MeOH, petroleum ether and EtOAc extracts and a moderate one for the $H_2O$/MeOH extract.

Table 2
Cytotoxic activity of the *R. graveolens* extracts

| Extracts | % Death | | | | $LC_{50}$[a] ± S.D.[b] (μg/ml) |
|---|---|---|---|---|---|
| | 1000 μg/ml | 100 μg/ml | 10 μg/ml | 1 μg/ml | |
| MeOH | 100 | 100 | 88 | 79 | 0.18 ± 0.08 |
| Petroleum ether | 100 | 100 | 100 | 92 | < 0.10 |
| EtOAc | 100 | 100 | 100 | 92 | < 0.10 |
| $H_2O$/MeOH | 100 | 96 | 71 | 54 | 0.80 ± 0.67 |
| CAPE[c] | 100 | 100 | 83 | 63 | 0.45 ± 0.05 |

[a] Lethal concentration for 50% of *Artemia salina* nauplii.
[b] Mean of three measurements (10 nauplii per concentration plus control in one measurement; dead nauplii were counted).
[c] Caffeic acid phenethyl ester as active reference.

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