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# Antimicrobial Activity of Undecan-x-ones (x = 2–4)

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Abstract

As a continuation of our research on the biological activity of undecan-x-ones (x = 2–4), their antimicrobial activity towards bacteria *Escherichia coli* and *Bacillus subtilis*, yeast *Candida mycoderma* and mould *Aspergillus niger,* was investigated. The population viability of the tested microbial strains in the presence of undecan-x-ones was determined by the impedimetric and agar disc diffusion methods. Undecan-x-ones showed low antibacterial activity towards both Gram-positive and Gram-negative bacteria. Undecan-2-one and undecan-3-one exhibited high activity towards *C. mycoderma*. All undecan-x-ones expressed the strongest effect on *A. niger*. The tests have proven that due to high fungistatic activity undecan-x-ones can be used to aid stabilization of food and cosmetic matrices.

Key words: antimicrobial activity, essential oil components, fungistatic activity, ketones, undecan-x-one

## Introduction

Essential oils, and specifically their effect on humans, as well as their fungicidal, bactericidal and even virucidal properties, have been the subject of many studies. The results have been documented for a large number of oils (Brud and Chrząszcz, 1998; Chrząszcz, 1998ab). Essential oils are usually mixtures of dozens of chemical compounds and their biological properties depend on the synergy effect of the individual components (Chrząszcz, 1998a; Ochi *et al*., 2005). Large quantities of natural raw materials are required to produce essential oils, and the production process is fairly expensive. When the high prices of essential oils are considered, the following question arises: what is the antimicrobial activity of their individual components? Moreover, it is important to determine if synthetic compounds can replace those from natural sources.

Some oil components can be obtained in a relatively simple and efficient way by organic synthesis and they are commonly recognized as nature-identical. This group also includes aliphatic undecan-x-ones (x = 2–4), the components of essential oils and extracts of exotic plants. Undecan-2-one is the most common undecan-x-one within the plant kingdom. A significant amount of undecan-2-one occurs in the oils obtained from the plants which belong to the *Rutaceae* family (Lawless, 1999). Depending on the species, cultivation conditions and part of the rue plant, essential oils contain from several to dozens percent of this ketone. The essential oil from *Ruta chalepensis* L. growing in Turkey contains 66.5% undecan-2-one (Hüsnü Can Baser *et al.*, 1996), in Iran 66.0–68.0% (Rustaiyan *et al*., 2002), and that from *Ruta montana* L. even 84.2% (Hüsnü Can Baser *et al.*, 1996). Chinese *Ruta graveolens* L. contains 67.0% of this ketone (Lawrence and Reynolds, 1998), the one growing in Cuba 48.7% (Pino *et al*., 1997), and in Malaysia 30.7% (Yaacob and Abdullah, 1989). Large quantities, amounting to 54.3% of undecan-2-one, were isolated from the leaves of *Zanthoxylum pinnatum* (*Rutaceae*) (Brophy *et al.*, 2000), from the fruits and leaves of Brazilian *Siparuna guianensis* (Aubl.) (31.7–32.5%) (Fischer *et al*., 2005), and from the roots of *Philodendron acutatum* Scott. (12.7%) (Viana *et al*., 2002).

Undecan-2-one was also found in the leaves (4.0–7.0%) and fruits (*ca.* 5.0%) of *Pistacia lentiscus* L. growing in Sardinia (Cougiu *et al*., 2002), and in the bark (52–58%) of Indian *Glycosmis pentaphylla* (Ahmed *et al*., 2000). The essential oil from *Cymbopogon schoenanthus* growing in desert areas of Thar contains 14.8% (Shahi and Tava, 1993), and the oil

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produced in Nepal from the fruits of *Cinnamonium glaucescent* contains 3.6% of undecan-2-one (Lawrence and Reynolds, 1997). A small amount, about 1% of undecan-2-one, has been obtained from many other plants, *e.g.* in the essential oils from ginger (Lawrence and Reynolds, 1991a), Chinese rose *Rosa rugosa* (Lawrence and Reynolds, 1991b), Turkish *Salvia blepharochlaena* (Demirci *et al*., 2003), and in the flowers and fruits of *Litsea monopetala* (Roxb.) (Choudhury *et al*., 1997).

Other metameric undecanones are more rarely encountered in nature. Undecan-3-one occurs in the amount of 0.6% in brown sea algae *Dictyopteris membranacea,* which comes from the French Coast of the Mediterranean Sea (Boland and Müller, 1987; Trehan *et al*., 1997). It was found that undecan-3-one is a product of wood-decay processes caused by the fungus *Fomitopsis pinicola* (Rösecke *et al*., 2000). It also is a trail pheromone of African ants *Decophylla longinod* (Boland and Müller, 1987; Trehan *et al*., 1997; Rösecke *et al*., 2000).

Undecan-4-one occurs in concentration 0.25% in the essential oil from the plant *Cymbopogon nardus* (Boland and Müller, 1987).

Undecan-x-ones (x = 2–4) are precious because of their very pleasant, durable floral and fruity odors (Djerassi, 1994; Gibka and Gliński, 2008). Undecan-2-one characterized by a fruity-floral odor with an orange-herbal note is a component of many fragrant compounds and food flavorants (Burdock, 2002). The antimicrobial activity of these ketones has not yet been investigated. They are expected to stabilize microflora and hence act as both an odoriser and preservative in food and cosmetic products.

A simple, ecological and efficient method for the synthesis of undecan-x-ones (x = 2–4) was developed (Gliński and Gibka, 2004). Next, their activity towards Gram-positive bacteria *Bacillus subtilis*, Gram-negative bacteria *Escherichia coli*, yeast *Candida mycoderma* and mould *Aspergillus niger* was established.

## Experimental

### Materials and Methods

**Undecan-x-ones.** The experimental materials were undecan-2-one, undecan-3-one and undecan-4-one, obtained in the catalytic ketonization of carboxylic acids according to the procedure previously described (Gibka and Gliński, 2006).

**Microorganisms.** Bacteria *Bacillus subtilis* ATCC 6633 and *Escherichia coli* ATCC 8793; yeast *Candida mycoderma* ŁOCK 0008 and mould *Aspergillus niger* ŁOCK 0436 were used in the experiments. The microorganisms originated from the ATCC Collection and the Pure Culture Collection of the Institute of Fermentation Technology and Microbiology, Łódź Technical University ŁOCK 105. Double passaging activated the microorganisms: bacteria on TSB medium (Trypticase Soy Broth) Oxoid, UK (*B. subtilis* temperature 30°C, 48 h; *E. coli* temperature 37°C, 48 h), yeast and mould on Sabouraud Agar, bioMerieux, Poland (temperature 28°C, 72h).

**Determination of antimicrobial activity of undecan-x-ones**: The antimicrobial activity of undecan-x-ones was determined by the impedimetric method using a Bactometer M64 System (bioMerieux, Poland). The suspension of tested microbial cells in physiological salt solution (0.85% NaCl) was standardized to the density of about $10^7$ CFU/ml. Each well of the impedimeter module was filled with 0.1 ml of the cell suspension, 1, 5, 10, 20 or 30 µl of undecan-x-one and completed to 1 ml volume with GPM medium (bioMerieux, Poland) for bacteria and YMM medium (bioMerieux, Poland) for fungi. A positive control sample was a suspension of microorganisms in the medium without undecan-x-ones. A negative control was the culture of bacteria and fungi with the addition of novobiocin (0.5 µg/ml) and cycloheximide (0.2 µg/ml), respectively. The samples were incubated for 72 h at temperatures optimal for the growth of individual microorganisms, as described in the strain activation procedure. After incubation in the bactometer, the microorganism's viability was controlled by a surface culture on the PCA medium (Plate Count Agar, bioMerieux, Poland). The plates were incubated for 3 days in the case of bacteria and yeasts and for 5 days for mould at temperatures optimal for the growth of the particular microorganisms.

Minimal Inhibitory Concentration (MIC) was assigned as the lowest concentration inhibiting the growth of microorganisms in the bactometer at parallel growth on the PCA plates. Minimal Bactericidal Concentration (MBC) or Minimal Fungicidal Concentration (MFC) was the lowest undecan-x-one concentration at which no microbial growth was observed either in the bactometer wells or on the PCA plates.

To compare the impedimetric method with classical one recommended by CLSI (Clinical Laboratory Standards Institute), the antimicrobial activity of undecan-x-ones was also determined by the agar disc diffusion method. 10, 20 or 30 µl/ml undecan-x-ones were applied on sterile paper discs of 6 mm in diameter (Whatman No 40, Britania). The discs were placed on the surface of the inoculated TSB agar medium and Sabouraud Agar for bacteria and fungi respectively. Cell suspensions of microorganisms for inoculation were prepared as described above and in amount of 0.1 ml of a particular microorganism transferred onto the agar medium. Petri dishes were kept at 4°C for 2 hours, and then incubated at temperatures optimal for

their growth for 72 hours and the zones of inhibition were measured. Novobiocin (0.5 µg/ml) and cycloheximide (0.2 µg/ml) served as controls. The undecan-x-ones activity was classified by the diameter of the inhibition zones as follows: inactive for diameter less then 8 mm, moderately active for diameter 9–14 mm, active for diameter 15–19 mm and highly active for diameter larger than 20 mm (Ponce *et al*., 2003).

**Statistical analysis of results.** Results were analyzed using a 3-way ANOVA at the confidence level of $p<0.05$. Results of the population viability were presented as an arithmetic mean of three determinations with standard deviation not exceeding 0.2 logarithmic units.

Each assay of the agar disc diffusion method was performed by duplication in two separate experimental runs and the results were presented as a mean with standard deviation.

Table I
Antimicrobial activity of undecan-x-ones presented as MIC (Minimal Inhibitory Concentration) in µl/ml.

| Microorganism | undecan-2-one | undecan-3-one | undecan-4-one |
|---|---|---|---|
| *B. subtilis* | >30 | >30 | >30 |
| *E. coli* | >30 | >30 | >30 |
| *C. mycoderma* | 20 | 20 | >30 |
| *A. niger* | 1 | 5 | 5 |

Table II
Antimicrobial activity of undecan-x-ones presented as MBC/MFC (Minimal Bactericidal/Fungicidal Concentration) in µl/ml.

| Microorganism | undecan-2-one | undecan-3-one | undecan-4-one |
|---|---|---|---|
| *B. subtilis* | >30 | >30 | >30 |
| *E. coli* | >30 | >30 | >30 |
| *C. mycoderma* | 30 | 30 | >30 |
| *A. niger* | 20 | 10 | 20 |

## Results

Undecan-3-one and undecan-4-one at the concentration of up to 30 µl/ml had practically no effect on *B. subtilis*. Undecan-2-one appeared to be much more active. However concentrations of 20 and 30 µl/ml of undecan-2-one decreased the population the most, by 2.86 and 5.47 logarithmic units per ml respectively, the MIC and MBC values were not determined (Tables I and II, Fig. 1A). The tested undecan-x-ones showed low activity against *E. coli*. No statistically significant ($p<0.05$) differences in the population in the presence of undecan-2-one and undecan-3-one were observed. The increase in the undecan-4-one concentration from 1 to 10 µl/ml resulted in a gradual reduction of viable *E. coli* cells to 1.90 logarithmic units per ml. A subsequent increase in undecan-4-one doses did not cause further changes in the population viability (Fig. 1B). The results of the impedimetric method were in agreement with those of the agar disc diffusion method and according to the latest (Ponce *et al*., 2003) undecan-3-one and undecan-4-one were classified as inactive against *B. subtilis*. Undecan-4-one at the concentrations of 10, 20 and 30 µl/ml expressed moderate activity against *E. coli* (Table III).

The activities of undecan-x-ones towards yeast *C. mycoderma* were similar (no statistically significant differences, $p<0.05$), when the compounds were added at concentrations of 1 and 5 µl/ml. At the concentration of 20 µl/ml, undecan-2-one and undecan-3-one inhibited the growth of the yeast population completely (MIC), (Fig. 2A, Table I). The concentration of 30 µl/ml of both of these compounds was found to be lethal for *C. mycoderma* (MFC) (Tab. II). Undecan-4-one showed lower activity, and at the

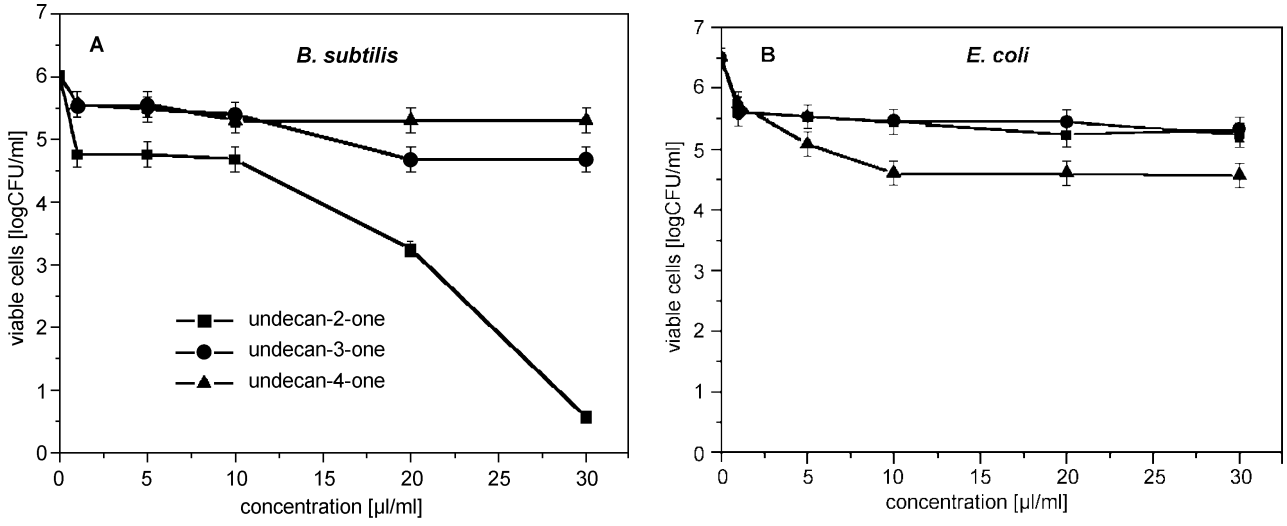


Fig. 1. The effect of undecan-x-ones on bacteria *B. subtilis* (A) and *E. coli* (B).

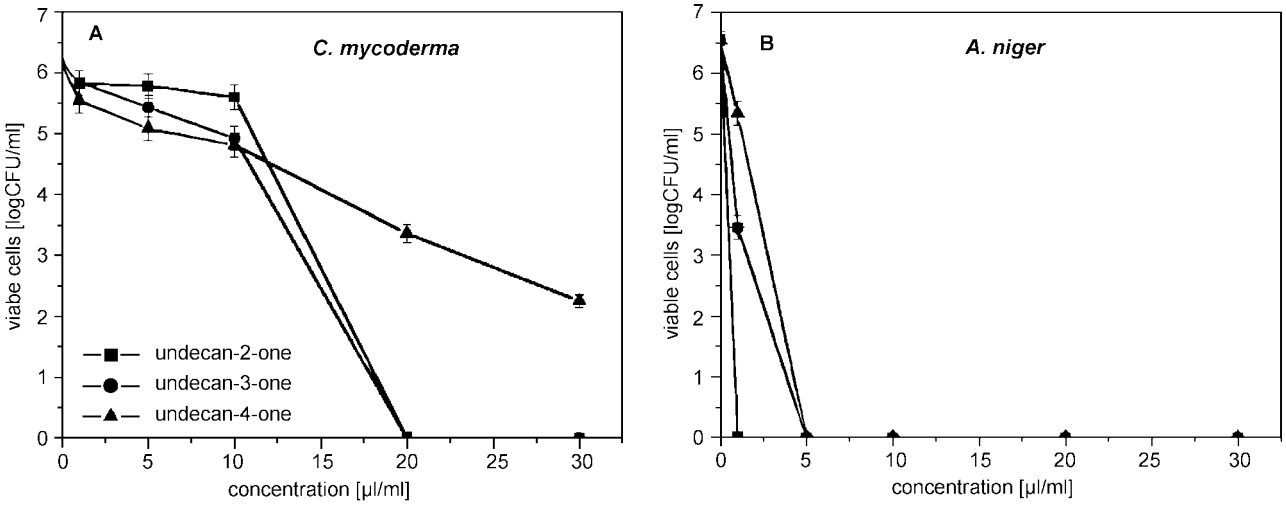


Fig. 2. The effect of undecan-x-ones on yeast *C. mycoderma* (A) and mould *A. niger* (B).

highest tested concentration it caused a reduction of the yeast cell number by 3.89 logarithmic units per ml but on the basis of the results of the agar disc diffusion method (Table III) it was classified as active.

High activity of undecan-x-ones was found in the case of *A. niger* (Fig. 2B), which was also confirmed by the agar disc diffusion method (Table III). Already at the concentration of 1 µl/ml undecan-2-one had an inhibitory effect on this mould (MIC). However, it showed a cidal effect (MFC) only at the 20-fold higher concentration. The Minimal Inhibitory Concentration for undecan-3-one and undecan-4-one was determined to be 5 µl/ml, whereas the concentrations of the cidal effect of these compounds differed, being 10 and 20 µl/ml, respectively (Tables I and II).

## Discussion

Undecan-x-ones revealed low antibacterial activity against both tested Gram-positive and Gram-negative bacteria. In the tested range of pure compound concentrations MIC and MBC values were not determined for *B. subtilis* and *E. coli*.

Due to the lack of literature references concerning studies on the antimicrobial activity of undecan-x-ones, their activity can be referred mainly to plant extracts containing these compounds. Although the plant extracts containing significant quantities of undecan-x-ones were selected for comparison, a complex quality of the extracts and synergy or antagonistic interactions of their constituents (Rhiannon, 2002) should be taken into account.

Extracts from the leaves of *Ruta graveolens* did not show activity towards *E. coli* (Valsaraj *et al*., 1997; Ali-Shtayeh *et al*., 1998; Ojala *et al*., 2000; Alzoreky and Nakahara, 2003; Ivanova *et al*., 2005), just like their main component, undecan-2-one. A search for the mechanism of antibacterial action of ketones showed a quick recovery of *E. coli* after 1-hour moderate blocking effect of undecan-2-one (Együd, 1967), which explained its inactivity also in our testing. At the same time, some studies show low activity of the preparations from *R. graveolens* towards *B. subtilis* (Valsaraj *et al*., 1997; Ojala *et al*., 2000; Alzoreky and Nakahara, 2003), which is in agreement with our results for undecan-2-one. A similar effect was found for extracts from the leaves of *R. chalepensis*

Table III
Zones of growth inhibition of tested microorganisms by undecan-x-ones.

| Microorganism | Inhibition zone diameter (mm)[a] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | undecan-2-one | | | undecan-3-one | | | undecan-4-one | | | novobiocin | cyclo-heximide |
| | 10[b] | 20[b] | 30[b] | 10[b] | 20[b] | 30[b] | 10[b] | 20[b] | 30[b] | 0.5[c] | 0.2[c] |
| *B. subtilis* | 11 ± 1 | 15 ± 1 | 18 ± 1 | 8 ± 0 | 8 ± 1 | 8 ± 0 | 7 ± 0 | 7 ± 0 | 7 ± 0 | 20 ± 1 | – |
| *E. coli* | 8 ± 1 | 8 ± 0 | 8 ± 0 | 8 ± 0 | 8 ± 1 | 8 ± 1 | 11 ± 1 | 12 ± 1 | 12 ± 1 | 24 ± 2 | – |
| *C. mycoderma* | 9 ± 0 | 28 ± 2 | 32 ± 3 | 10 ± 1 | 29 ± 2 | 35 ± 2 | 10 ± 0 | 18 ± 2 | 19 ± 2 | – | 19 ± 1 |
| *A. niger* | 32 ± 3 | 34 ± 2 | 41 ± 3 | 29 ± 2 | 32 ± 3 | 38 ± 3 | 18 ± 2 | 26 ± 2 | 32 ± 1 | – | 28 ± 2 |

Each assay was performed by duplication in two separate experimental runs.
[a] includes diameter of disc (6 mm); [b] compound concentration in µl/ml; [c] compound concentration in µg/ml; – not determined

(Ali-Shtayeh *et al*., 1998; Alzoreky and Nakahara, 2003; Al-Bakri and Afifi, 2007).

Undecan-2-one and undecan-3-one were characterized by high activity towards yeast *C. mycoderma*, as opposed to methanol extracts from the leaves of *R. graveolens* that do not act on members of *Candida* genus, *Candida albicans* (Ojala *et al*., 2000) species. However, the potential activity of undecan-2-one was previously established based on the activity determination of ethanol extracts from the leaves of *R. chalepensis* and *Pistacia lentiscus* towards *C. albicans* (Ali-Shtayeh *et al*., 1998).

Studies on the biological activity of plant extracts and oils refer mainly to bacteria, so there are few literature references concerning moulds. In our studies all tested undecan-x-ones expressed the strongest effect on the mould *A. niger*. Undecan-2-one was noted to be particularly active, which was confirmed in previous studies of *Commiphora rostata* resin components (McDowell *et al*., 1988). Data available in the literature referring to the extract from the leaves of *R. graveolens* (Ojala *et al*., 2000) do not confirm our results, which could be attributed to the lower concentration of undecan-2-one in this extract.

**Conclusions.** Although all undecan-x-ones are characterized by low antibacterial activity, they act efficiently against fungi. Proven high activity against tested yeast and mould species indicates their potential application as components of a preservative system for the stabilization of food and cosmetic matrices.

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