Comparison of the insecticidal effectiveness of synthetic and natural myristicin against housefly (Musca domestica L.) and oriental cockroach (Blatta orientalis)

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Introduction

Parsley (*Petroselinum crispum*) is one of herbs the most extensively used as a condiment for garnishing and seasoning, but also in perfumery, and in a larger extent in folk medicine. It is a biennial plant belonging to *Apiaceae* family (Simon and Quinn, 1988) [11]. Parts used in therapeutics are ripe fruits (seeds), root and aboveground parts of the plant. The curiosity is that parsley is a toxin for most of birds' species, nevertheless it is useful in healing various animals afflictions, for example foot rot suffered by goats and sheeps. In human medicine, parsley seeds are used as a remedy for many different ailments. In addition to its recognized role as a stomachic, carminative and laxative, the herb has also a powerful diuretic properties (Kreydiyyeh and Usta, 2001; Kreydiyyeh, Usta, Kaouk and Sadi, 2001) [5, 6]. The mentioned activities are the result of ingredients present in essential oil from parsley. It contains mainly apiol (chemical name IUPAC: 1-allyl-2,5-dimethoxy-3,4-methylenedioxobenzene) and myristicin (chemical name IUPAC: 6-allyl-4-methoxy-1,3-benzodioxole). The average contents of these substances in plants oscillate between 0.1% in root, 0.3% in leaves and from 2% to 7% in seeds (Simon and Quinn, 1988; Krukowska and Gałązka, 2006) [7, 11]. Due to the fact that both apiol and myristicin are toxic for insects, they are treated as natural insecticides (Fuhremann and Lichtenstein, 1979; Lichtenstein and Casida, 1963; Lichtenstein et al., 1974) [1, 8, 9]..

It is worth to note that myristicin occurs also in essential oil from nutmeg and other seasonings plants like dill or parsnip (Huopalahti and Linko, 1983; Stahl, 1981) [3, 12]. Myristicin also shows psychoactive properties, but its content is very low in plants enumerated for this purpose (Jakovljevic, Raskovic, Popovic and Sabo, 2002) [4].

In presented work, comparative tests of insecticidal activity of synthetic and natural myristicin against two bioindicators: housefly and oriental cockroach were conducted.

Materials and methods

2.1. Myristicin

Synthetic myristicin (purity 92.3% by GC) was obtained using the procedure, according to Rao, Seshadri et al. (1949), starting from eugenol.

The natural myristicin was separated from the concentrated MeOH-EtOAc extracts of the grinded biological materials – seeds and dried leaves of parsley grown in our greenhouse according to hints presented by Gruszecki (2005) [2]. The following methods were used: 1. column chromatography (CC) on silica gel (preliminary method for the extract enrichment in myristicin), and then 2. preparative thin layer chromatography (PTLC) on glass plates covered with silica gel. In both cases, the mixture of hexane-ethyl acetate was used as eluent, in proportions 1:0, 95:5, *v/v* for CC and 95:5, *v/v* for PTLC. Finally, from the extract of grinded parsley seed 70 mg of myristicin (purity by GC 94.2%) was obtained, and from the extract of grinded leaves – 48 mg of myristicin (purity by GC 83.7%). These samples, as well as synthetic myristicin, were used in the biological tests.

2.2. Chemicals for synthesis

Eugenol, hexamethylenetetramine, pyridin, dibromomethane were bought from Sigma Aldrich. Silica gel for CC 0.063-0.200 mm, glass plates (used here for PTLC) and aluminium plates covered with silica gel 60F254 for TLC were from Merck. The other applied chemicals and solvents were from POCh.

2.3. GC and HPLC conditions for myristicin evaluation

GC: Varian 3400 chromatograph with flame-ionization detector (FID), universal injector "on column"; column DB-1, length 30 m, diameter 0.53 mm, film thickness 1.5 μm; analysis conditions: injector temp. 180°C, detector temp. 230°C, programme 70°C (3min.), temp. increases 5°C/min., final temp. 200°C (5 min.); the samples were dissolved in ethyl acetate (concn. ~3 mg/cm^3).

HPLC: Shimadzu chromatograph, UV detector, valve Rheodyne (20μL), column C18 Nucleosil 150/4.6, mobile phase a cetonitril – water 1.2 :1 + ortophosphoric acid 0.01, flow 1.2 cm^3/min.

2.4. Bioindicators

Biological tests of insecticidal efficiency were conducted against insects from own breeding – housefly (*Musca domestica L.*) and oriental cockroach (*Blatta orientalis*).

2.5. Procedures for biological activity evaluation

Biological tests of insecticidal activity of myristicin were made by using exposition method with filter paper on Petri dishes. Concentrations of synthetic myristicin in acetone solutions were made in geometric progression: for housefly was used a multiplier 0.5 and for cockroach it was equal to 0.7. Solutions were pipetted on Petri dishes, 1 cm^3 of each concentration to every dish. After evaporation of the solvent, insects were put on the dishes. Tests were conducted in three repetitions for every concentration. At the same time control tests were performed – acetone control: dishes with pure acetone (solvent) and the normal control: dishes with no chemical (clean filter paper). Mortality counting was performed after 24 and 48 h of insects exposure. The results were analyzed by using a statistic method called Finne's with Swaroop's simplification. The LC_{50} and LC_{95} values were computed.

Biological tests of natural myristicin were conducted similarly. First concentrations were close to LC_{95} value from tests of synthetic myristicin, but when it turned out to be too low and expected activity was not observed, the concentrations were increased.

Results and discussion

3.1. Synthesis

The synthesis of myristicin, following the procedure described by Rao and Seshadri (1949) [10] is presented in Figure 1.

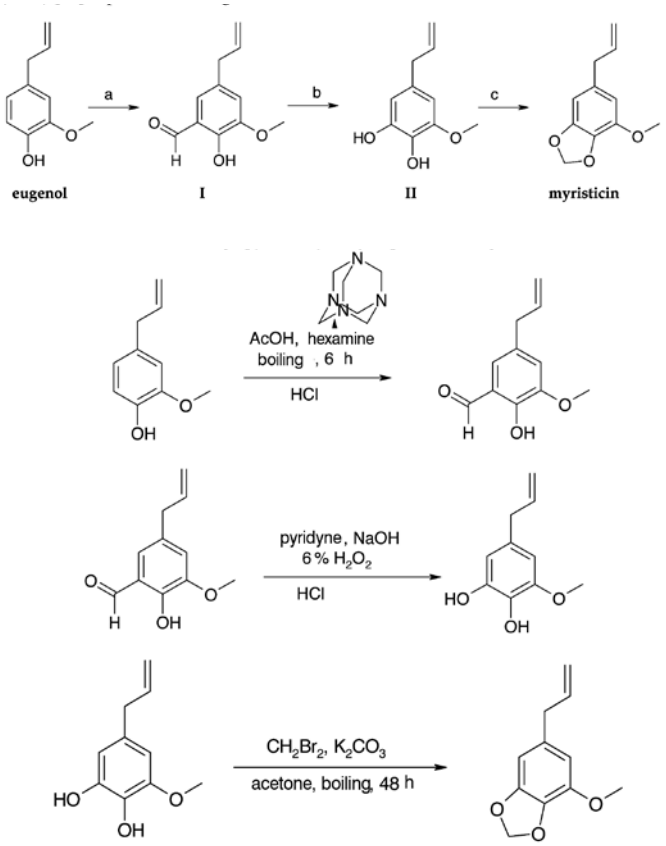

Fig. 1. Synthesis of myristicin according to Rao, Seshadri et al. (1949): a) AcOH, hexamethylenetetramine, reflux 6 h, HCl; b) pyridine, NaOH, 6% H_2O_2, HCl; c) CH_2Br_2, acetone, reflux 48 h

In the first step of synthesis, the aromatic ring of eugenol was substituted by formyl group (Sommelet reaction) to form intermediate compound I. Next, in the subsequently performed decarboxylation and oxidation, the respective catechol II was obtained. In the third step, the dioxolane ring closure and myristicin formation was done by means of Williamson ether synthesis from II with dibromomethane, in basic conditions.

Although none of the described process steps was optimized, the structure of each intermediate obtained, as well as the final product, was confirmed by the spectral methods (GC/EI MS, FT IR), and myristicin also by ^{1}H NMR. The resulted synthetic myristicin was purified by CC on silica gel (eluent hexane-ethyl acetate 1:0, 95:5, *v/v*) to obtain pure fraction of 92.3% (internal normalization, GC).

The quantitative HPLC analysis for the EtOAC-MeOH extracts from dried leaves and seeds of parsley were also performed, in order to estimate the content of myristicin in these two materials. In the studies, the synthetic myristicin was used as the reference standard. On the basis of the results it was estimated that the leaves content 0.08, and the seeds 5.03wt% of myristicin.

3.2. Biological tests

The results are shown in tables below. Table I shows LC_{50} and LC_{95} values and the average for synthetic myristicin according to four tests conducted against oriental cockroach. Scores proclaim that acetonic solution which contains 0.083% of myristicin killed a half of tested population of oriental cockroach. 95% of this population were killed by 0.130% concentration (graphic depiction of the activity – Fig. 2).

Table I

Activity of synthetic myristicin against oriental cockroach

Test number	Oriental cockroach	
	LC_{50}, %	LC_{95}, %
1	0.077	0.105
2	0.068	0.131
3	0.082	0.144
4	0.103	0.139
Average	0.083	0.130

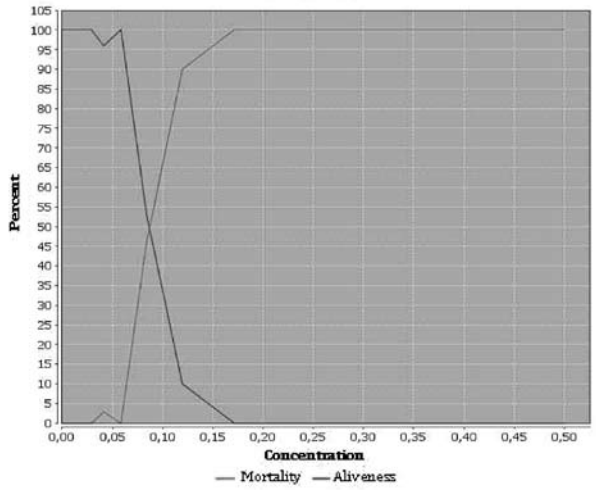

Fig. 2. Mortality of oriental cockroach – activity of synthetic myristicin

Table 2 shows results for tests conducted against housefly. Scores proclaim that half of the tested insects treated with 0.002% concentration has died. Acetone solution which contains 0.015% of myristicin killed 95% of tested population (Fig. 3).

Table 2

Activity of synthetic myristicin against housefly

Test number	Housefly	
	LC_{50}, %	LC_{95}, %
1	0.001	0.011
2	0.003	0.028
3	0.001	0.005
4	0.001	0.004
5	0.005	0.025
Average	0.002	0.015

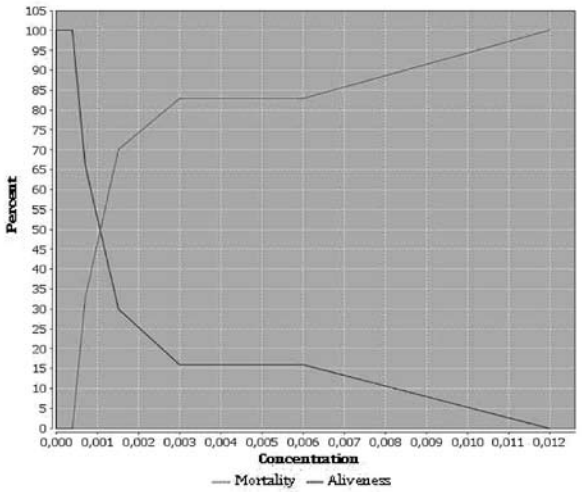

Fig. 3. Mortality of housefly – activity of synthetic myristicin

Table 3 shows results for natural myristicin obtained from parsley seeds. Concentrations used in the test was determined on a basis of a mean LC_{95} value for synthetic myristicin. However, the concentrations were found to be too low and therefore expected activity was not observed. Application of higher concentrations was needed. Results for next tests with higher concentrations of myristicin from parsley seeds and leaves are shown in Tables 4 and 5.

Table 3

Activity of natural myristicin from seeds against housefly and oriental cockroach

Housefly		Oriental cockroach	
concentration, %	mortality, %	concentration, %	mortality, %
0.02	3.3	0.20	13.3
0.01	0	0.10	0
0.005	3.3	0.05	0

Table 4

Efficiency of higher concentrations of natural myristicin from seeds against housefly and oriental cockroach

Housefly		Oriental cockroach	
concentration, %	mortality, %	concentration, %	mortality, %
0.10	100	1.00	100
0.05	100	0.50	80

Table 5

Efficiency of higher concentrations of natural myristicin from leaves against housefly and oriental cockroach

Housefly		Oriental cockroach	
concentration, %	mortality, %	concentration, %	mortality, %
0.10	100	1.00	100
0.05	100	0.50	100

Table 6 is a comparison of efficient concentrations (more than 90% of mortality) of synthetic and natural myristicin obtained from two sources (seeds and leaves).

Table 6

Comparison of efficient concentrations of synthetic and natural myristicin

Bio-indicator	Synthetic myristicin, %	Myristicin from seeds, %	Myristicin from leaves, %
Housefly	0.015	0.05	0.05
Oriental cockroach	0.13	0.5	0.5

Conclusions

The effect of presented research was determination of LC_{50} and LC_{95} values for synthetic myristicin and comparison of the efficiency of synthetic and natural myristicin, extracted from parsley leaves and seeds. For oriental cockroach the results were consistent and repeatable, but for housefly there was two different ranges of activity. It means that LC_{50} and LC_{95} values were higher or lower, but always adequate towards each other. Once the scope of efficient concentrations has increased in value and accordingly both LC_{50} and LC_{95} were determined as higher. During another tests it has decreased and therefore the lower LC values were noted as a result. It might have depended from insects condition in different tests or from temperature. Strong activity against insects' nervous system was supposed.

Synthetic myristicin turned out to be more biologically active against tested bioindicators than natural myristicin obtained from parsley seeds and leaves. It might be an effect of variety contents and types of pollutions that could enhance or suppress substance's activity. In general all tested substances have demonstrated better efficiency against a housefly.

Literature

1. Fuhremann T.W. & Lichtenstein E.P.: *Insecticide. Toxicity and Degradation in Houseflies as Affected by Naturally Occurring Food Plant Components*. Journal of Agricultural and Food Chemistry 1979, **27**, 87–91.
2. Gruszecki R.: *Parsley – the vegetable hard to culivate*. Hasło Ogrodnicze 2005, 4, 120–123.
3. Huopalahti R. & Linko R.R.: *Composition and Content of Aroma Compounds in Dill, Anethum graveolens L., at Three Different Growth Stages*. Journal of Agricultural and Food Chemistry 1983, **31**, 331–333.
4. Jakovljevic V., Raskovic A., Popovic M. & Sabo J.: *The effect of celery and parsley juices on pharmacodynamic activity of drugs involving cytochrome P450 in their metabolism*. European Journal of Drug Metabolism and Pharmacokinetics 2002, 27(3), 153–156.
5. Kreydiyyeh S.I. & Usta J.: *Diuretic effect and mechanism of action of parsley*. Journal of Ethnopharmacology 2001, **79**, 353–357.
6. Kreydiyyeh S.I., Usta J., Kaouk I. & Al-Sadi R.: *The mechanism underlying the laxative properties of parsley extract*. Phytomedicine 2001, **8**(5), 382–388.
7. Krukowska A. & Gałązka I.: *Essential oil composition of the parsley seed of cultivars marketem In Poland*. Flavour and Fragrance Journal 2006, 21, 143–147.
8. Lichtenstein E.P. and others: *Insecticidal and Sinergistic Components Isolated from Dill Plants*. Journal of Agricultural and Food Chemistry 1974, **22**, 658–664.
9. Lichtenstein E.P. & Casida J.E.: *Myristicin, an Insecticide and Synergistic Occuring Naturally in the Edible Parts off Parsnip*. Journal of Agricultural and Food Chemistry 1963, **11**, 410–415.
10. Rao, Seshadri: *Synthesis of Myristicin and Elemicin from Eugenol*. Proceed. Indian Academy of Sciences 1949, Sect. A. 30, 114–119. http://www.erowid.org/archive/rhodium/chemistry/eugenol2myristicin.html access date:
11. Simon J.E. & Quinn J.: *Characterization of Essential Oil of Parsley*. Journal of Agricultural and Food Chemistry 1988, 36, 467–472.
12. Stahl E.: *Variation of Myristicin Content In Cultivated Parsnip Roots* (*Pastinaca sativa* ssp. *sativa* var. *hortensis*). Journal of Agricultural and Food Chemistry 1981, **29**, 890–892.

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