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In vitro and *in silico* investingation of antimicrobial activity of essential oils from two *Pastinaca sativa* subspecies

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Introduction

Cultivated parsnip (***Pastinaca sativa* subsp. *sativa*** L., Apiaceae) root is a well-known vegetable, common ingredient of soups, stews, salads, casseroles etc. Besides, its leaves and young shoots can be added to soups and fruits are used as a condiment. Furthermore, young shoots of wild-growing parsnips, e.g. ***P. sativa* subsp. *urens*** (Req. ex Godr.) Čelak., are consumed pickled or in salads or soups and it is considered that their essential oil acts as a natural preservative.

Objective

To investigate and compare the **antimicrobial activity** of the **essential oils** obtained from the **roots**, **leaves**, **stems**, **flowers** and **fruits** of **cultivated *P. sativa* subsp. *sativa*** (**sat**) and **wild-growing *P. sativa* subsp. *urens*** (**ure**) collected in Serbia. Furthermore, the most active essential oil constituents (against the most susceptible microorganisms) were predicted *in silico*.

Methods

Minimum inhibitory concentrations (MICs) and minimum bactericidal/fungicidal concentrations (MBCs/MFCs) of the essential oils (isolated by hydrodistillation using Clevenger-type apparatus) were determined by microdilution method against three ***Candida*** standard strains: ***C. albicans*** ATCC 10231 (***C.a.***), ***C. tropicalis*** (***C.t.***) ATCC 750 and ***C. parapsilosis*** (***C.p.***) ATCC 22019, three *Candida* isolates from oral cavity: ***C. albicans*** 475/15, ***C. krusei*** (***C.k.***) H1/16 and ***C. glabrata*** (***C.g.***) 4/6/15, three Gram-positive bacteria: ***Staphylococcus aureus*** (***S.a.***) ATCC 11632, ***Bacillus cereus*** (***B.c.***) clinical isolate and ***Listeria monocytogenes*** (***L.m.***) NCTC 7973 and three Gram-negative bacteria: ***Escherichia coli*** (***E.co.***) ATCC 25922, ***Salmonella* Typhimurium** (***S.T.***) ATCC 13311 and ***Enterobacter cloacae*** (***E.cl.***) ATCC 35030. Pharmacokinetic properties of the compounds present in at least one oil in the quantity ≥ 1% (determined by GC-FID and GC-MS) were initially evaluated using SwissADME web tool and molecular docking was performed using **AutoDock Vina** 1.1.2 (interactions were visualized using Discovery Studio Visualizer 2019).

Results

All the investigated essential oils of the two *Pastinaca sativa* subspecies were able to reduce the growth of different tested ***Candida*** strains (**MIC range 0.25-2 mg/mL; MFC range 0.5-4 mg/mL**). The most promising activity was observed for both root oils (MIC range 0.25-1 mg/mL; MFC range 0.5-2 mg/mL). Among investigated *Candida* strains, *C. parapsilosis* strain was the most sensitive to these essential oils (MIC range 0.25-1 mg/mL; MFC range 0.5-2 mg/mL). The antibacterial activity of the tested essential oils was lower compared to their anticandidal potential (MIC range 1-4 mg/mL; MBC range 2-8 mg/mL).

P. sativa subsp. urens

Table 1. Anticandidal activity of the essential oils of three investigated *Pastinaca* taxa and ketoconazole (mg/mL)

	Standard strains				Clinical isolates							
	C.p.		***C.t.***		***C.a.***		***C.a.***		***C.k.***		***C.g.***	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
root (sat)	0.25	0.5	1	2	0.5	1	0.5	1	0.5	1	1	2
leaf (sat)	0.5	1	1	2	1	2	1	2	0.5	1	1	2
stem (sat)	0.5	1	1	2	2	4	1	2	1	2	1	2
flower (sat)	0.5	1	1	2	1	2	1	2	1	2	1	2
fruit (sat)	0.5	1	1	2	2	4	1	2	1	2	1	2
root (ure)	0.25	0.5	1	2	0.5	1	0.5	1	1	2	1	2
leaf (ure)	0.5	1	1	2	1	2	1	2	1	2	1	2
stem (ure)	0.5	1	1	2	1	2	1	2	1	2	1	2
flower (ure)	0.5	1	1	2	1	2	1	2	1	2	1	2
fruit (ure)	1	2	1	2	1	2	2	4	1	2	2	4
ketoconazole	0.003	0.006	0.002	0.006	0.002	0.006	0.003	0.006	0.002	0.003	0.002	0.006

Table 2. Antibacterial activity of the essential oils of three investigated *Pastinaca* taxa and antibiotics (mg/mL)

	S.a.		***B.c.***		***L.m.***		***E.co.***		***S.T.***		***E.cl.***	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
root (sat)	4	8	2	4	4	8	2	4	4	8	2	4
leaf (sat)	2	4	2	4	2	4	2	4	4	8	4	8
stem (sat)	2	4	1	2	2	4	2	4	2	4	2	4
flower (sat)	2	4	2	4	2	4	1	2	2	4	2	4
fruit (sat)	4	8	1	2	4	8	2	4	4	8	2	4
root (ure)	2	4	1	2	2	4	1	2	2	4	2	4
leaf (ure)	4	8	1	2	2	4	2	4	4	8	2	4
stem (ure)	2	4	2	4	2	4	2	4	4	8	4	8
flower (ure)	2	4	1	2	2	4	2	4	2	4	2	4
fruit (ure)	2	4	1	2	2	4	2	4	2	4	4	8
streptomycin	0.1	0.2	0.025	0.05	0.15	0.3	0.1	0.2	0.1	0.2	0.025	0.05
ampicillin	0.1	0.15	0.1	0.15	0.15	0.3	0.15	0.2	0.1	0.2	0.1	0.15

Thirty compounds were present in at least one oil in the quantity ≥ 1%. Estimation of pharmacokinetic properties using SwissADME tool suggested that 23 of these compounds are inhibitors of some of the cytochrome P450 system isoenzymes. This fact led to assumption that they could also act against fungal sterol 14*α*-demethylase (CYP51), which is a common target of antifungal drugs (e.g., ketoconazole). Thus, the compounds (3D structures downloaded from PubChem) were docked to the active site of this enzyme (downloaded from Protein Data Bank, PDB code 5TZ1). The highest affinities were predicted for sesquiterpenes **caryophyllene oxide**, **(*E*)-caryophyllene**, **germacrene D**, ***α*-copaene**, ***β*-bourbonene** and ***δ*-cadinene** (**free binding energies from -9.4 to -8.7 kcal/mol**; ketoconazole -11.6 kcal/mol). These compounds were present in somewhat lower quantities in the essential oils (≤ 9.9%). For dominant compounds of the tested essential oils, e.g. myristicin, γ-palmitolactone and octyl butanoate a bit lower affinities were predicted (free binding energies from -7.3 to -5.8 kcal/mol). Tested compounds mostly docked near the heme of the enzyme and formed hydrophobic interactions with the amino acid residues of the active site and heme. According to SwissADME tool, four of five most active compounds have low absorption from gastrointestinal tract and higher skin permeation value, while caryophyllene oxide and three dominant compounds have high absorption and lower skin permeation value (similarly to ketoconazole).

Table 3. Absorption (A), skin permeation (log K_p) and free binding energies (E) to fungal lanosterol 14*α*-demethylase (PDB code 5TZ1) of the compounds present in at least one tested essential oil in the quantity ≥ 1% and ketoconazole

Compound	A	log K_p (cm/s)	E (kcal/mol)	Compound	A	log K_p (cm/s)	E (kcal/mol)
Caryophyllene oxide	High	-5.12	-9.4	Limonene	Low	-3.89	-7.0
(*E*)-Caryophyllene	Low	-4.44	-9.1	Terpinolene	Low	-3.96	-7.0
Germacrene D	Low	-4.18	-8.9	γ-Palmitolactone	High	-3.61	-7.0
α-Copaene	Low	-4.37	-8.8	Apiole	High	-5.70	-6.8
β-Bourbonene	Low	-4.20	-8.7	Lavadulyl acetate	High	-4.94	-6.8
δ-Cadinene	Low	-4.85	-8.7	*β*-Pinene	Low	-4.18	-6.6
α-trans-Bergamotene	Low	-2.97	-8.1	(*E*)-*β*-Ocimene	Low	-4.11	-6.4
(*E*,*E*)-*α*-Farnesene	Low	-3.20	-7.6	Octyl hexanoate	High	-3.84	-6.4
(*E*)-Nerolidol	High	-4.23	-7.4	(*Z*)-*β*-Ocimene	Low	-4.11	-6.3
Myristicin	High	-5.39	-7.3	Octyl butanoate	High	-4.44	-5.8
(*E*)-*β*-Farnesene	Low	-3.27	-7.2	Decyl acetate	High	-4.33	-5.7
(*Z*)-Falcarinol	High	-3.89	-7.1	Hexyl butanoate	High	-5.04	-5.7
Hexahydrofarnesyl acetone	High	-3.00	-7.1	Octyl acetate	High	-4.93	-5.5
p-Cymen-8-ol	High	-5.80	-7.1	*n*-Octanal	High	-5.15	-4.9
2-Phenyl ethyl butanoate	High	-5.25	-7.0	*n*-Octanol	High	-4.96	-4.8
				Ketoconasole	High	-6.46	-11.6

Conclusions

Investigated parsnips represent sources of essential oils and compounds with anticandidal activity.

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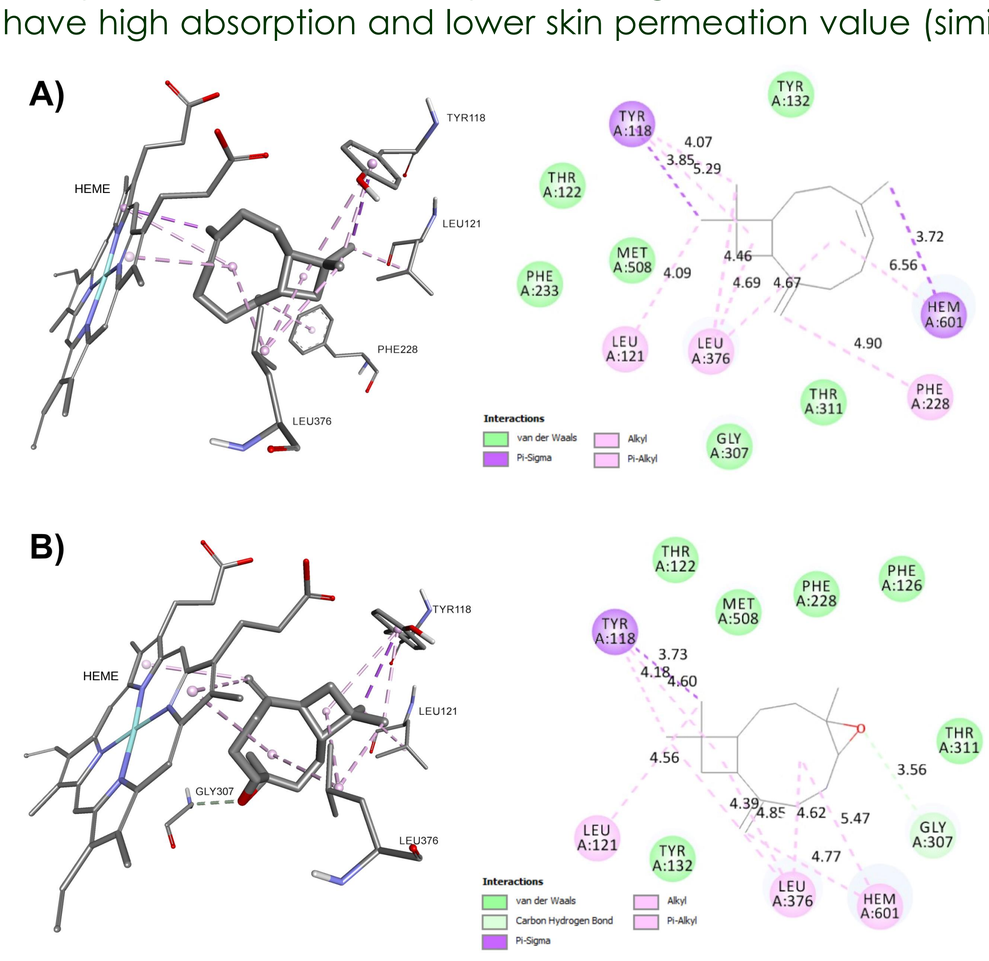

Fig. 1. Best binding modes of **(*E*)-caryophyllene** (**A**) and **caryophyllene oxide** (**B**) to the active site of fungal lanosterol 14*α*-demethylase (PDB code 5TZ1). 3D models are presented on the left and 2D diagrams on the right; different types of interactions are represented with different colors (distances [Å] are noted).