

Activity profiling of essential oils and aromatic extracts on SARS-CoV-2 key enzymes

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Two of the very well characterized and promising drug targets are the main protease (M^{pro}, 3CL^{pro}) and the papain-like protease (PL^{pro}), which have a key role in viral replication and transcription. Coronaviral M^{pro} and PL^{pro} inhibitors have been designed and assessed over the years, and Michael acceptor inhibitors, ebsele, and its derivatives can be promising drug leads. In the present study [1], we wanted to address the question of whether common flavor and fragrance materials are inhibitors or have the potential to be a source for effective inhibitors of the SARS-CoV-2 main and papain-like proteases. 400 samples of essential oils, aromatic extracts, and F&F raw materials of various origins were screened for their inhibitory properties on SARS-CoV-2 M^{pro} and PL^{pro}. Screening (spectrofluorimetric enzymatic assay) was carried out with the employment of fluorogenic substrates designed by our group in previous works on SARS-CoV-2 proteases [2,3]

Most of the activity of the tested samples was attributed to the inhibition of the SARS-CoV-2 main protease. M^{pro} is the primary protease of the virus, the papain-like protease has a regulatory role, and both of them are essential for the processing of the polyprotein. In addition, the PL^{pro} has an important role to play in counteracting the innate immunity of the host cell. Ultimately, the best inhibitor would be the one that has high activities against one or both enzymes. There were only two examples (out of 400 tested) of such high activities – turmeric oleoresin (89% M^{pro} and 64% PL^{pro} inhibition) and petitgrain mandarin essential oil (85% M^{pro} and 100% PL^{pro} inhibition). The essential oil isolated from mandarin leaves was the most potent in the preliminary screening studies. Petitgrain mandarin essential oil was fractionated on kugelrohr, three fractions and distillation residue were analyzed chromatographically, and their activity on both SARS-CoV-2 proteases was assessed (Table 1).

		Area [%]					
		EO	Fr. 1	Fr. 2	Fr. 3	Res.	DMA
1	α-pinene	2.19	4.07	0.7	0.03	0.0	-
2	β-pinene	2.18	4.36	1.2	0.03	0.0	-
3	p-cymene	14.7	27.5	20.2	0.6	0.06	-
4	limonene	8.26	16.4	9.9	0.2	0.03	-
5	γ-terpinene	17.9	33.5	27.1	0.8	0.05	-
6	DMA	43.5	7.0	33.8	93.0	92.7	100
M ^{pro} inh. [%]		85	3	62	100	100	100
PL ^{pro} inh. [%]		100	79	100	100	100	100

Table 1. Discovery of the most active constituents of the petitgrain mandarin (*Citrus reticulata* blanco var. Mandarin) oil against SARS-CoV-2 M^{pro} and PL^{pro}.

The detailed inhibition properties of the petitgrain mandarin essential oil and natural dimethyl anthranilate were assessed (Table 2). As could be suspected from the fractionation studies, PM oil and DMA are significantly stronger inhibitors of SARS-CoV-2 papain-like protease.

	IC ₅₀	
	PM oil [μg/mL]	DMA [μg/mL] ([μM])
SARS-CoV-2 M ^{pro}	>50	>50 (>300)
SARS-CoV-2 PL ^{pro}	22.9±4.6	5.20±1.22 (31.5±7.4)

Table 2. Inhibition parameters of the petitgrain mandarin (*Citrus reticulata* blanco var. Mandarin) oil and dimethyl anthranilate against SARS-CoV-2 M^{pro} and PL^{pro}

[1] Strub, D. *et al.*, PREPRINT (Version 1) available at Research Square, **2021**

[2] Rut, W. *et al.*, *Nature Chemical Biology*, **2020**, *17*, 222–228

[3] Rut, W. *et al.*, *Science Advances*, **2020**, *6*, eabd4596