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Unravelling the phytochemical composition and the pharmacological properties of an optimized extract from the fruit from Prunus mahaleb L.: From traditional liqueur market to the pharmacy shelf

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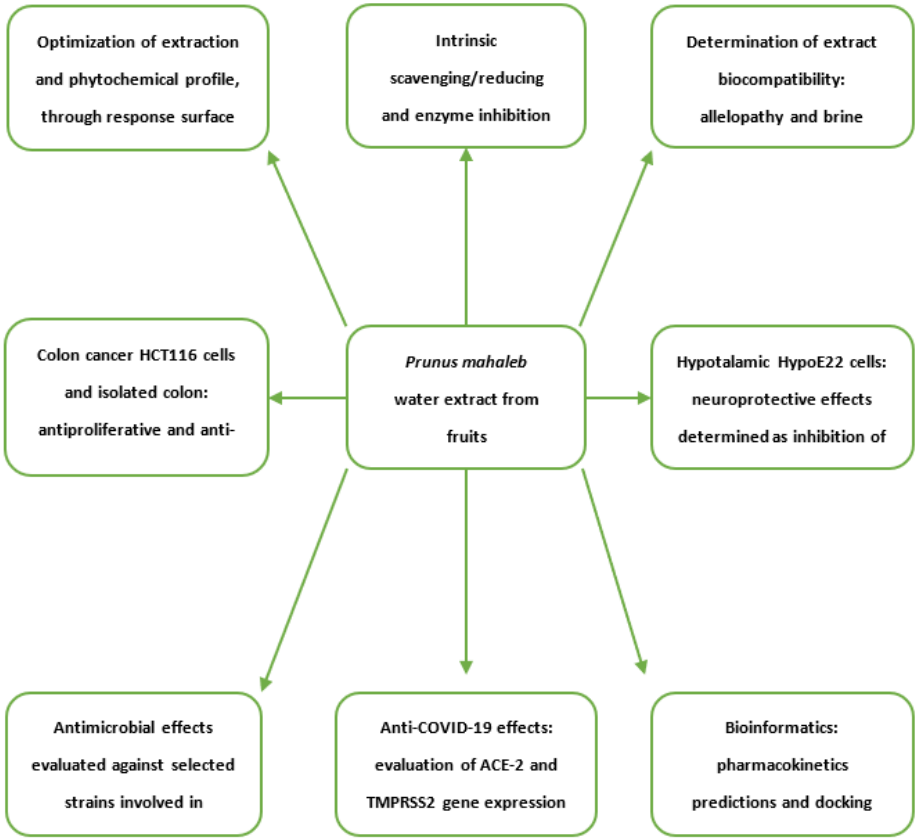


Abstract:

Prunus mahaleb L. fruit has long been used in the production of traditional liqueurs. The fruit also displayed scavenging and reducing activity, in vitro. The present study focused on unravelling peripheral and central protective effects, antimicrobial but also anti-COVID-19 properties exerted by the water extract of *P. mahaleb*. Anti-inflammatory effects were studied in isolated mouse colons exposed to lipopolysaccharide. Neuroprotection, measured as a blunting effect on hydrogen-peroxide-induced dopamine turnover, was investigated in hypothalamic HypoE22 cells. Antimicrobial effects were tested against different Gram+ and Gram- bacterial strains. Whereas anti-COVID-19 activity was studied in lung adenocarcinoma H1299 cells, where the gene expression of ACE2 and TMPRSS2 was measured after extract treatment. The bacteriostatic effects induced on Gram+ and Gram- strains, together with the inhibition of COX-2, TNFα, HIF1α, and VEGFA in the colon, suggest the potential of P. mahaleb water extract in contrasting the clinical symptoms related to ulcerative colitis. The inhibition of the hydrogen peroxide-induced DOPAC/DA ratio indicates promising neuroprotective effects. Finally, the downregulation of the gene expression of ACE2 and TMPRSS2 in H1299 cells, suggests the potential to inhibit SARS-CoV-2 virus entry in the human host. Overall, the results support the valorization of the local cultivation of *P. mahaleb*.

Keywords: *Prunus mahaleb* L.; phenolic profile; protective effects; anti-bacterial effects; anti-COVID-19 effects

Introduction

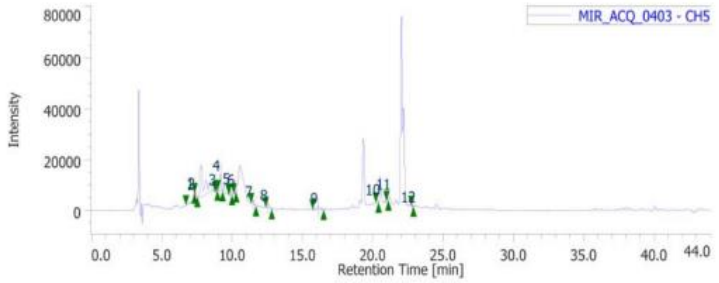
- *Prunus mahaleb* L. fruit has long been used in the production of traditional liqueurs. The fruit also displayed scavenging and reducing activity, in vitro, whereas protective effects induced by fruits were observed in an experimental paradigm of ulcerative colitis. The present study focused on unravelling peripheral and central protective effects, antimicrobial but also anti-COVID-19 properties exerted by the water extract of *P. mahaleb*. Anti-inflammatory effects were studied in isolated mouse colons exposed to lipopolysaccharide. Neuroprotection, measured as blunting effect on hydrogen-peroxide-induced dopamine turnover, was investigated in hypothalamic HypoE22 cells. Antimicrobial effects were tested against different Gram+ and Gram- bacterial strains. Whereas, considering the very recent interest in studying natural compounds and raw extracts as anti-COVID-19 agents, in lung adenocarcinoma H1299 cells, the gene expression of ACE2 and TMPRSS2, deeply involved in the SarsCov-2 entry in the human host, was measured after extract treatment.

Results and discussion

Variables					Experimental Results					
Conditions	Time (min)	Temp (°C)	Ethanol %	Solid/Liquid (g/mL)	TPC	SD	TFC	SD	TTC	SD
1	5	52.5	50	0.01	0.198	0.035	0.044	0.001	0.184	0.005
2	5	52.5	50	0.1	0.429	0.018	0.148	0.003	0.469	0.053
3	60	52.5	50	0.01	0.130	0.003	0.003	0.000	0.127	0.007
4	60	52.5	50	0.1	0.658	0.023	0.197	0.006	0.591	0.032
5	32.5	25	0	0.055	0.612	0.005	0.104	0.001	0.599	0.053
6	32.5	25	100	0.055	0.236	0.011	0.086	0.002	0.206	0.013
7	32.5	80	0	0.055	0.648	0.082	0.096	0.002	0.645	0.048
8	32.5	80	100	0.055	0.263	0.007	0.099	0.003	0.250	0.046
9	32.5	25	50	0.01	0.176	0.003	0.034	0.002	0.175	0.009
10	32.5	25	50	0.1	0.447	0.013	0.119	0.001	0.437	0.024
11	32.5	80	50	0.01	0.122	0.003	0.024	0.002	0.105	0.008
12	32.5	80	50	0.1	0.749	0.082	0.252	0.002	0.589	0.033
13	5	52.5	0	0.055	0.568	0.011	0.099	0.001	0.587	0.060
14	60	52.5	0	0.055	0.597	0.015	0.104	0.003	0.616	0.068
15	5	52.5	100	0.055	0.235	0.012	0.084	0.002	0.208	0.007
16	60	52.5	100	0.055	0.243	0.002	0.084	0.002	0.182	0.006
17	32.5	52.5	0	0.01	0.164	0.002	0.028	0.001	0.147	0.016
18	32.5	52.5	0	0.1	0.769	0.008	0.143	0.002	0.812	0.038
19	32.5	52.5	100	0.01	0.078	0.005	0.019	0.001	0.105	0.034
20	32.5	52.5	100	0.1	0.311	0.017	0.104	0.002	0.248	0.005
21	5	25	50	0.055	0.466	0.008	0.141	0.002	0.354	0.027
22	60	25	50	0.055	0.455	0.012	0.133	0.002	0.291	0.009
23	5	80	50	0.055	0.367	0.041	0.100	0.003	0.368	0.017
24	60	80	50	0.055	0.421	0.019	0.122	0.005	0.394	0.008
25	32.5	52.5	50	0.055	0.374	0.012	0.123	0.005	0.386	0.026
26	32.5	52.5	50	0.055	0.531	0.016	0.161	0.001	0.332	0.012
27	32.5	52.5	50	0.055	0.527	0.030	0.149	0.002	0.313	0.026

Run sequence was conducted randomly. TPC: total polyphenols content expressed as GAE (mg/g); TFC: total flavonoids content expressed as rutin equivalents; TTC: total tannins content expressed as tannic acid equivalents.

RSM



Chromatographic analysis of *Prunus mahaleb* L. phenolic compounds. The chromatographic analysis confirmed the presence of different phytochemicals, namely gallic acid (peak #2), catechin (peak #5), chlorogenic acid (peak #6), epicatechin (peak #7), caffeic acid (peak #8), chicoric acid (peak #9), coumaric acid (peak #10), ferulic acid (peak #11), and rutin (peak #12).

Treatments	DPPH	ABTS	CUPRAC	FRAP	Chelating Ability	PBD
P. mahaleb water extract	1.16 ± 0.01	1.11 ± 0.03	1.68 ± 0.09	0.97 ± 0.05	1.33 ± 0.04	2.76 ± 0.09
TROLOX	0.05 ± 0.01	0.08 ± 0.01	0.11 ± 0.01	0.04 ± 0.01	nt	0.60 ± 0.02
EDTA	nt	nt	nt	nt	0.03±0.01	nt

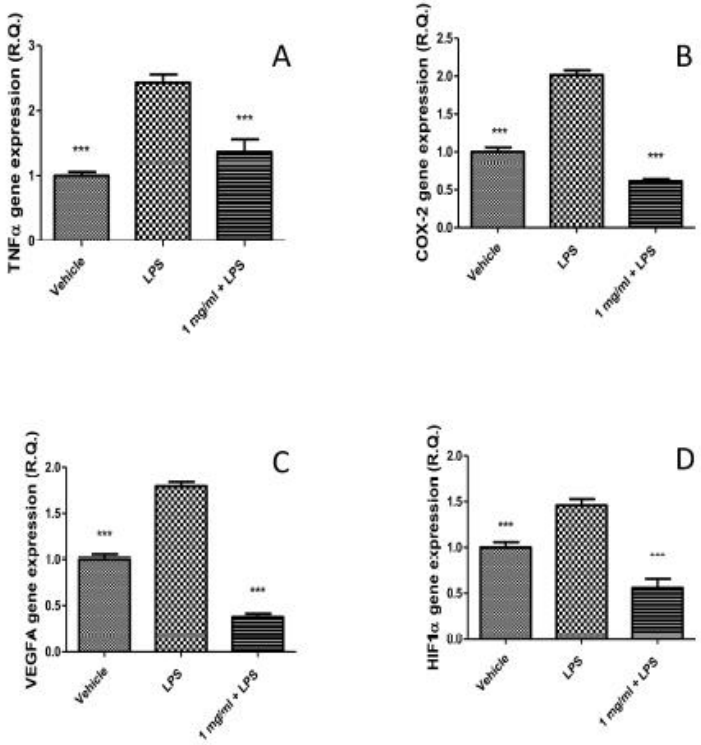
nt: not tested. PBD: Phosphomolybdenum. Values are reported as IC_{50} (mg/mL).

Table 2. Enzyme inhibition properties.

Treatments	AchE	BChE	Tyrosinase	α-Amylase	α-Glucosidase
P. mahaleb water extract	1.53 ± 0.10	1.34 ± 0.05	1.28 ± 0.04	3.44 ± 0.14	1.35 ± 0.04
Galantamine	0.003 ± 0.0001	0.004 ± 0.0001	nt	nt	nt
Kojic acid	nt	nt	0.08 ± 0.01	nt	nt

nt: not tested. Values are reported as IC_{50} (mg/mL).

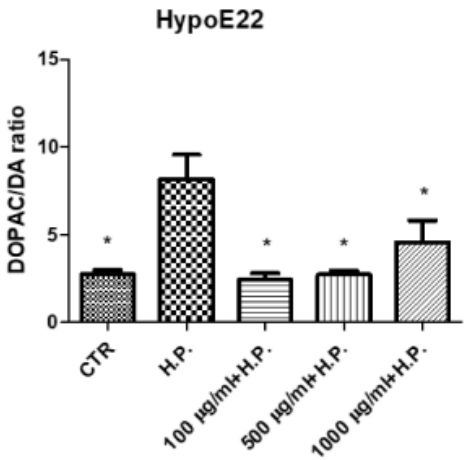
Results and discussion



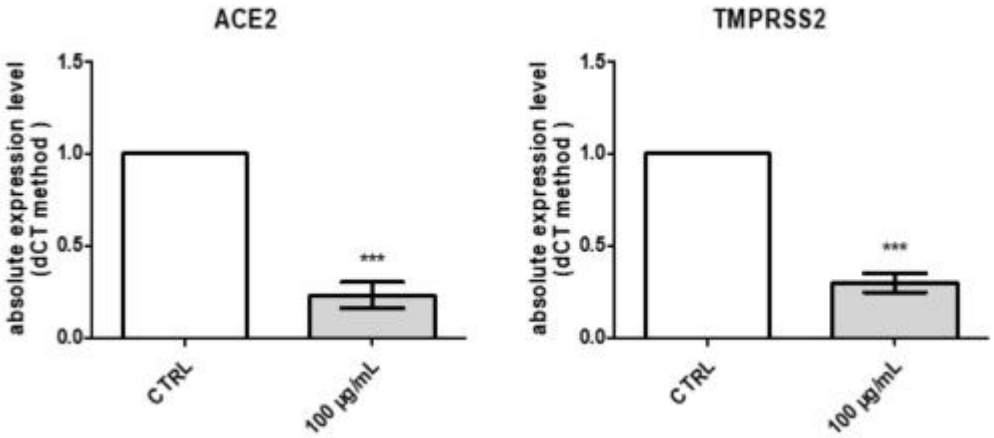
Treatments	Bacterial Strains	MIC (μg/mL)
P. mahaleb water extract	*E. coli* (ATCC 10 536)	31.49 (25–50)
P. mahaleb water extract	*P. aeruginosa* (ATCC 15442)	>200
P. mahaleb water extract	*B. cereus* (ATCC 12826)	>200
Ciprofloxacin	*E. coli* (ATCC 10536)	<0.12
Ciprofloxacin	*P. aeruginosa* (ATCC 15442)	1.23 (1.95–0.98)
Ciprofloxacin	*B. cereus* (ATCC 12826)	0.62 (0.98–0.49)

Inhibitory effects induced by *Prunus mahaleb* L. water extract (1 mg/mL) on LPS-induced upregulation of TNFα (**A**), COX-2 (**B**), VEGFA (**C**), and HIF1α (**D**) gene expression in isolated mouse colon. ANOVA, $p < 0.0001$; *** $p < 0.001$ vs. respective LPS group.

Results and discussion



Inhibitory effects induced by *Prunus mahaleb* L. water extract (100–1000 µg/mL) on hydrogen peroxide (H.P.)-induced DA turnover (DOPAC/DA ratio). ANOVA, $p < 0.001$; * $p < 0.05$ vs. H.P. group.



Inhibitory effects induced by *Prunus mahaleb* L. water extract (100–1000 µg/mL) on hydrogen ACE-2 and TMPRSS-2 gene expression. *** $p < 0.05$ vs. CTRL group.

Conclusions

In conclusion, the present study explored the health potential of the water extract from the fruit of *P. mahaleb*, a wild edible plant that has been used for centuries in the liqueur tradition. The study explored the phytochemical composition in phenolic compounds, finding significant amounts of catechin and chicoric acid that may explain, albeit partially, the observed pharmacological properties, in terms of protective effects against inflammatory and infectious diseases. In this regard, the bacteriostatic effects induced on Gram+ and Gram- strains, together with the inhibition of COX-2, TNFα, HIF1α, and VEGFA suggest the potential of *P. mahaleb* water extract in contrasting the clinical symptoms related to ulcerative colitis. The inhibition of hydrogen peroxide-induced DOPAC/DA ratio, in hypothalamic neurons, indicates promising neuroprotective effects. In view of future in vivo studies to confirm this finding, it is sensitive to highlight the capability of chicoric acid to cross the blood brain barrier and its putative affinity toward MAO-B, which is deeply involved in DA turnover. Finally, but not for importance, there is the ability of the extract to downregulate the gene expression of ACE2 and TMPRSS2 in human adenocarcinoma H1299 cells. As ACE2 and TMPRSS2 are involved in SARS-CoV-2 virus entry in the human host, with the present findings, we hypothesize the inclusion of the present extract in protection devices, such as surgical masks, functioning as physical barriers against COVID-19. Overall, the results of this research point to the valorization of the local cultivation of *P. mahaleb*, an ancient botanical resource with promising health perspectives.

Acknowledgments

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