Received: 14 February 2022 | Revised: 21 March 2022 | Accepted: 25 March 2022

DOI: 10.1002/ptr.7463

WILEY

LETTER TO THE EDITOR

In vitro antiviral activity against SARS-CoV-2 of common herbal medicinal extracts and their bioactive compounds

To the Editor,

The coronavirus SARS-CoV-2 spread all around the world, leading to an unprecedented pandemic. Up to date, some effective treatments have been highlighted to treat the symptoms, but not to cure the COVID-19 disease. At least, some well-known medicines have been proposed, but have rapidly become the subject of some controversy, such as hydroxychloroquine combined or not azithromycin (Maisonnasse et al., 2020; Okour, Al-Kofahi, & Austin, 2020) and ivermectin (Zaidi & Dehgani-Mobaraki, 2022). As no treatments were considered neither safe nor efficient enough, new coronavirus vaccines obtained conditional marketing authorisation. The public health crisis of COVID-19 led to countries around the world promoting massive vaccination programs. However, one can observe that the results are not fulfilling the hopes and expectations concerning virus transmission (Franco-Paredes, 2022), while the data concerning pharmacovigilance are still in process. In this context, research should focus on the hindrances of implementing cost-effective and safe drugs, as well as preventive health products. Plants extracts and natural isolated compounds which are already used as medicines, dietary supplements or preventive health products, can offer a cost-effective way to fight against COVID-19, especially since they have proven to be safe to use. In accordance with this strategy, we have then evaluated different plants and extracts for their antiviral activity against SARS-CoV-2 strain. The selection of plant species included in this antiviral screening was based on either their use in common winter ailments, on the previous demonstration of activity against other coronaviruses, or on the suggested activity of one of their main metabolites against specific targets of SARS-CoV-2, as highlighted in several studies published at the beginning of the pandemic. All selected plants are well-known plants that are frequently used in phytotherapy.

Plant materials have been kindly provided by the Belgian pharmaceutical laboratory Tilman SA, as herbal raw materials or commercially standardized extract, when available. The following plant material has been evaluated: *Aesculus hippocastanum* L. (*semen*, dry extract), *Camellia sinensis* (L.) Kuntze (*folium*), *Cinnamomum verum* J. Presl. (*cortex*), *Curcuma longa* L. (*rhizoma*, dry extract), *Echinaceae purpurea* (L.) Moench. (*herba*, dry extract), *Eucalyptus globulus* Labill. (*folium*), *Filipendula ulmaria* (L.) maxim. (*flos*), *Glycyrrhiza glabra* L. (*radix*), *Gingko biloba* L. (*folium*, dry extract), *Mentha piperita* L. (*folium*), *Pelargonium sidoides* DC. (*radix*, dry extract), *Rhodiola rosea* L. (*rhizoma et radix*, dry extract), *Rheum officinale* Baillon. (*radix*), *Sambucus nigra* L. (*flos*), *Salvia officinalis* L. (*folium*), *Thymus vulgaris* L. (*folium*) and *Zingiber officinale* Roscoe. (*rhizoma*, dry extract).

For raw herbal materials, ethanol crude extracts were prepared by maceration of powdered plant with EtOH at room temperature. A 10 g of dried powder was used for 100 ml of EtOH. The solvent of the resulting filtrates was evaporated at low temperature (<40°C) under reduced pressure and dissolved in DMSO. Commercially manufactured extracts were readily dissolved in DMSO for the assay. Cells culture and virus preparation were performed as previously described by our team (Ledoux et al., 2022). The antiviral and cytotoxic experiments were performed in triplicate in 96-well plates, as previously reported (Ledoux et al., 2022). On day one, cells grown in 10 cm standard tissue culture dishes (Falcon, Becton-Dickinson) were trypsinized and counted using a Cedex XS cell analyser (Roche Innovatis). A 100 µl of cell suspension containing 2,000 Vero E6 viable cells was seeded in a 96-well tissue culture plate (Falcon, Becton-Dickinson) and grown overnight. On day two, 50 µl of extracts (or pure compound) were added to the wells of the 96-well plate containing confluent cells with a final range of concentration from 50 to 1.5 µg/ml in duplicate. A 50 µl of virus suspension or virus-free medium was added, respectively, to determine the antiviral activity or the cytotoxicity. The antiviral activity was established regarding the minimal concentration, which was able to protect the cells from the viral cytopathic effects, observed through a microscope, compared to the untreated control on day five post-infection.

Regarding the 17 plants investigated, one extract showed weak antiviral activity: *M. piperita*, which prevented the virus-induced cytopathic effects at a concentration of 50 µg/ml without cytotoxic activities against Vero E6 cells. Eight extracts demonstrated interesting activity, with a protective effect at a concentration of 25 µg/ml: *A. hippocastanum*, *E. purpurea*, *F. ulmaria*, *G. biloba*, *G. glabra*, *P. sidoides*, *R. rosea*, *T. vulgaris*. Two extracts demonstrated very interesting activity, with a protective effect at a concentration of 12.5 µg/ml: *E. globulus* and *S. officinalis*. One demonstrated a promising activity with a protective at a concentration of 6.25 µg/ml: *C. sinensis*, and one demonstrated a very promising activity at a concentration of 3.125 µg/ml: *C. longa* (Table 1).

Regarding this high potential, the most important bioactive compound from *C. longa*, as well as its metabolites have been investigated: curcumin, tetrahydrocurcumin and hexahydrocurcumin. Curcumin induced a protective effect at a concentration between 1.56 and 3.125 µg/ml (4.2–8.5 µM), so there is no doubt that curcumin is the main active compound responsible for the antiviral activity of the tested *C. longa* extract. Furthermore, its two main metabolites,

TABLE 1 Effects of medicinal herbal species on SARS-CoV-2

Species	Part of the plant	Batch	Chemical informations available in the certificate of analysis	Minimal extract concentration inducing a protection against virus-induced cytopathic effect
Aesculus hippocastanum	*Semen*, dry extract	25,303	19.43% of anhydrous aescin	25 μg/ml
Camellia sinensis	*Folium*	24,605	3.0% of caffeine	6.25 μg/ml
Cinnamomum verum	*Cortex*	24,447	16.56 ml per kg of essential oil	>50 μg/ml
Curcuma longa	*Rhizoma*, dry extract	25,892	90.3% of curcumin	3.125 μg/ml
Echinaceae purpurea	*Herba*, dry extract	24,930	6.44% of cichoric and caftaric acids	25 μg/ml
Eucalyptus globulus	*Folium*	25,276	29 ml per kg of essential oil	12.5 μg/ml
Filipendula ulmaria	*Flos*	25,754	0.3% of essential oil	25 μg/ml
Gingko biloba	*Folium*, dry extract	25,783	27.0% of flavonoids and 6.24% of terpene lactones	25 μg/ml
Glycyrrhiza glabra	*Radix*	23,794	4.4% of glycyrrhizic acid	25 μg/ml
Mentha piperita	*Folium*	25,986	29.8 ml per kg of essential oil	50 μg/ml
Pelargonium sidoides	*Radix*, dry extract	25,373	ND	25 μg/ml
Rheum officinale	*Radix*	25,950	ND	>50 μg/ml
Rhodiola rosea	*Rhizoma et radix*, dry extract	24,235	2.53% of total rosavins expressed as rosarin, rosavin and rosin	25 μg/ml
Salvia officinalis	*Folium*	25,466	27 ml per kg of essential oil	12.5 μg/ml
Sambucus nigra	*Flos*	25,807	ND	>50 μg/ml
Thymus vulgaris	*Folium*	25,274	15.6 ml per kg of essential oil (containing 70.7% of thymol and carvacrol)	25 μg/ml
Zingiber officinale	*Rhizoma*, dry extract	25,853	9.4% of gingerol derivatives	>50 μg/ml

Abbreviation: ND, not determined.

tetrahydrocurcumin and hexahydrocurcumin were also both active against the virus in vitro, with a protective effect at a concentration of 6.25 μg/ml (16.8 and 16.7 μM, respectively), which is very encouraging for potential use in human. These results are in accordance with literature in which the anti-SARS-CoV-2 potential of curcumin is highlighted (Soni et al., 2020; Zahedipour et al., 2020). Indeed, curcumin demonstrated some interesting effects, such as inhibiting the entry of the virus into the cell, preventing the encapsulation of the virus, a possible inhibitory effect on viral protease, as well as potentially modulating different cellular signalling pathways (Zahedipour et al., 2020). More recently, an open-label nonrandomized clinical trial was conducted with oral nano-curcumin formulation and highlighted its efficacy to fasten the resolution time of COVID-19 symptoms, improving oxygenation and reducing hospital stay time (Saber-Moghaddam et al., 2021).

Some *C. longa* extracts have been commercialized alone, or even in combination with other products such as vitamin D and quercetin in some dietary supplements. The combination of *Curcuma* extract with anti-SARS-CoV flavonoids, such as quercetin (Derosa, Maffioli, D'Angelo, & Di Pierro, 2021) or naringenin (Tutunchi, Naeini, Ostadrahimi, & Hosseinzadeh-Attar, 2020) could be very interesting in the worldwide fight against COVID-19. Considering these data, we recommend deeply investigating *C. longa* extract, which can be combined with quercetin or naringenin, as a new cost-effective and safe treatment—or at least as an adjuvant through, clinical trials to help in the fight against this unprecedented pandemic.

ACKNOWLEDGEMENTS

The authors thank the Belgian pharmaceutical laboratory Tilman SA for giving us the plant materials. This work was supported by the Fondation Hospitalo Universitaire Léon Fredericq (FHULF, University of Liège).

FUNDING INFORMATION

Fonds Léon Fredericq, Grant/Award Number: 2020-2021 - 113 - Sub. F/E; Fondation Hospitalo Universitaire Léon Fredericq.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Kristi Leka[1,2,3]
Carla Hamann[1]
Pauline Desdemoustier[2]
Michel Frédérich[1]
Mutien-Marie Garigliany[3]
Allison Ledoux[1]

[1]*Laboratory of Pharmacognosy, Center of Interdisciplinary Research on Medicines (CIRM), University of Liège, Liège, Belgium*

[2]*Center of Interdisciplinary Research on Medicines (CIRM), University of Liège, Liège, Belgium*

[3]*Laboratory of Veterinary Pathology, Fundamental and Applied Research for Animals & Health (FARAH), University of Liège, Liège, Belgium*

Correspondence

Allison Ledoux, University of Liège, Sart Tilman, B-4000 Liège, Belgium.

Email: allison.ledoux@uliege.be

ORCID

Allison Ledoux https://orcid.org/0000-0002-4052-6336

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