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IN VITRO ANTIVIRAL ACTIVITY AGAINST SARS-COV-2 OF 28 STRYCHNOS EXTRACTS

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To the Editor,

By the end of December 2019, an increasing number of patients diagnosed with pneumonia and respiratory failure of unknown origin in the Chinese city of Wuhan, lead to the discovery of the 2019 novel Coronavirus. Up to date, the therapy of the SARS-CoV-2 is limited to treat the symptoms and not to cure the Covid-19 disease (like Tamiflu for influenza), for this reason, the discovery of new antivirals is crucial. Natural products are an important source of antiviral compounds and therefore are extensively studied (Kim, 2021).

Broad studies in our laboratory on Asian, African and Brazilian *Strychnos* species have been conducted, and have led to the isolation of numerous alkaloidic compounds. These plants have been evaluated for their antimalarial and cytotoxic potentials (Beaufay et al., 2018; Frédérich et al., 2002; Frédérich, Hayette, Tits, De Mol, & Angenot, 1999; Philippe et al., 2005). Various *Strychnos* species contained in our herbarium are known to be endowed with interesting biological activity. As far as we know, there is limited information available about the antiviral potentials of *Strychnos* species in the literature. *S. pseudoquina* is a Brazilian *Strychnos*, which demonstrated in vitro anti-herpes activity (Boff et al., 2016). Interestingly, the activity was attributed to a strychnobiflavone. More recently, a docking study presented that different scaffolds from *S. nux-vomica* (demethoxyguiaflavine and strychnoflavine) could interact with the M^{pro} protease of the SARS-CoV-2 (Kumar, Parasuraman, Murahari, & Chandramohan, 2021). We have proceeded with an antiviral screening, in order to highlight the *Strychnos* worth investigating. These *Strychnos* species came from different continents, mainly from Africa. They were collected and identified several years ago, as described in the material part of previous studies performed by our group (Angenot et al., 1990; Philippe et al., 2005). The voucher specimen of each species is available at the National Botanical Garden of Meise (Belgium) and/or at the Laboratory of Pharmacognosy from the University of Liège (Belgium) or at the herbarium CGMS at Federal University de Mato Grosso do Sul. The 28 *Strychnos* species evaluated were S. *angolensis*, *S. boonei*, *S. brasiliensis*, *S. camptoneura*, *S. congolana*, *S. densiflora*, *S*. elaeocarpa, S. gossweileri, S. *henningsii*, *S. icaja*, *S*. ignatii, S. *innocua, S. johnsonii, S. longicaudata, S. malacoclados, S. malchairii, S. mattogrossensis*, *S. mellodora*, *S. nux-vomica*, *S. phaeotricha*, *S. potatorum*, *S. pseudoquina*, *S. scheffleri*, *S. spinosa*, *S. staudtii*, *S. tchibangensis*, *S. usambarensis,* and *S. variabilis.*

Methanol crude extracts were obtained by fast pressurized solvent extraction using a Speed Extractor E-914 (Büchi). The speed extractor was programmed for three cycles of 15 min at 30°C, at a pressure of 100 bars. 10 g of dried powder were used for 100 ml of MeOH. The solvent of the resulting filtrates was evaporated at low temperature (<40°C) under pressure. Strychnogucine B and strychnobrasiline, two previously isolated compounds, were dissolved in DMSO for the assay. This assay was performed according to the protocol described by Shi-you Li et al. (2005), with some modifications. Vero E6 cell lines (ATCC CRL-1586) were maintained in high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 2% (vol/vol) fetal bovine serum, 100 IU/ml penicillin, 100 µg/ml streptomycin, 1 mM sodium pyruvate and 2 mM glutamine. All culture reagents were purchased from Invitrogen. Cultures were maintained in humidified tissue culture incubators (Hera cell, Thermo Scientific) at 37°C with 5% CO_2 and 95% air. All cultures were

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mycoplasma free as confirmed by the MycoAlert detection kit (Lonza). SARS-CoV-2 strain BetaCov/Belgium/Sart-Tilman/2020/1 (Misset et al., 2020) was cultivated on Vero cells. SARS-CoV-2 stocks used for the assays were stored at -80°C. Virus titers were determined using the Tissue Culture Infectious Dose 50 ($TCID_{50}$) method. Experiments were performed in triplicate in 96-well plates. On day-1 cells grown in 10 cm standard tissue culture dishes (Falcon, Becton-Dickinson) were trypsinized and counted using a Cedex XS cell analyzer (Roche Innovatis). 100 µl of cell suspension containing 2,000 Vero E6 viable cells was added manually to each well of a 96-well tissue culture plate (Falcon, Becton-Dickinson) and grown overnight. On day-2, 50 µl of extracts (or pure compound) were added in 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. 50 µl of virus suspension or virusfree medium were 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 with a microscope, compared to the untreated control on day-5 post-infection.

Four *Strychnos*' extracts showed weak antiviral activity: *S. malacoclados, S. congolana, S. densiflora,* and *S. johnsonii,* which were able to protect the cells from the virus at a concentration of 50 µg/ml without cytotoxic activities against Vero E6 cells. Two demonstrated interesting activity, with a protective effect at a dose under 20 µg/ml: *S. icaja* and *S. mellodora,* and one demonstrated a promising activity with a protective effect under 10 µg/ml: S. *mattogrossensis.* Regarding this high antiviral potential, the IC_{50} of the crude extract was determined and evaluated at 2.05 ± 0.50 µg/ml.

S. mellodora is an East-African species, known to contain alkaloids, particularly glucoalkaloids, and possesses antiplasmodial activity (Philippe et al., 2005; Tits et al., 1996). *S. icaja* is an African species used as an arrow poison and to treat malaria in Congo, Cameroun and Gabon. Previous studies in our laboratory highlighted the presence of different alkaloids, like isosungucine, strychnogucine B and strychnobaillonine and their antiplasmodial and antimalarial activities (Beaufay et al., 2018; Tchinda et al., 2014). *S. mattogrossensis* is a South American species growing in the tropical forest of the Amazon. The root, stem bark and twigs of this plant have been investigated and it is known to contain alkaloids, such as mattogrossine, strychnobrasiline, 12-hydroxy-11-methoxystrychnobrasiline (Angenot et al., 1990), and more recently 12-hydroxy-10,11-dimethoxystrychnobrasiline (Belem-Pinheiro, Couceiro, Imbiriba da Rocha, Monte, & Villar, 2002). As far as it could be established, no bioactivity for this plant has been highlighted. A previous strychnos' antiplasmodial screening performed by our group on *Plasmodium* Sp. investigated the root of *S. mattogrossensis* and did not demonstrate any activity (Frédérich et al., 2002). Interestingly, neither strychnogucine B (from *S. icaja)* nor strychnobrasiline (from *S. mattogrossensis*) demonstrated antiviral activity. In the literature, one *strychnos* possesses antiviral activity in vitro (*S. pseudoquina*-against herpes virus), it contains the bioactive strychnobiflavone, a flavonoid (Boff et al., 2016). Even if this *strychnos* is not active on our model, it could be interesting to not only focus on alkaloids for further examinations.

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S. mattogrossensis is the most promising one for in-depth research. For the first time, the leaves of this plant demonstrated biological activity on SARS-CoV-2. Our group started to study this resource 30 years ago and put it aside because of its lack of activity against malaria. These findings thus show that *S. mattogrossensis* can still have a bright future.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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