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Exploring the Antiviral Activity of Flavonoids from *Strychnos variabilis* Leaves against SARS-CoV-2

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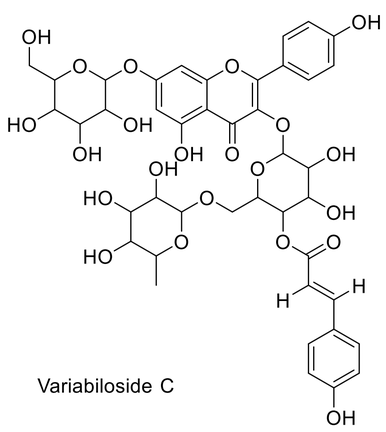
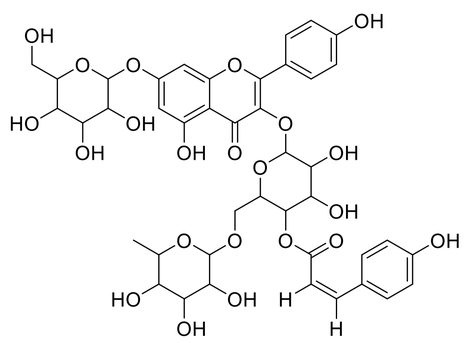
Introduction

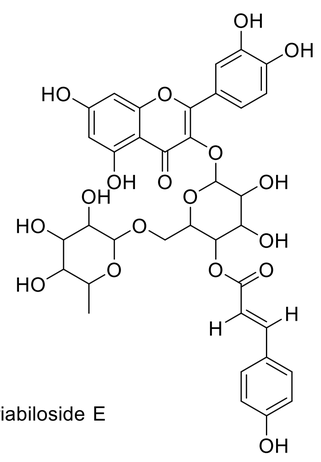
❖ *Strychnos variabilis* De Wild. is a small deciduous tree primarily found in and around Brazzaville and Kinshasa, situated on both sides of the Congo River in Africa (1). Although the leaves contain only minimal amounts of indoline alkaloids from the retuline series (2-3), our laboratory successfully identified and isolated numerous rare flavonoids, including flavonol glycosides, from these leaves over three decades ago (4-6). Flavonoids are renowned for their antiviral activity, particularly when present in glycosidic form, as it enhances their solubility and effectiveness compared to their aglycone counterparts (7,8).

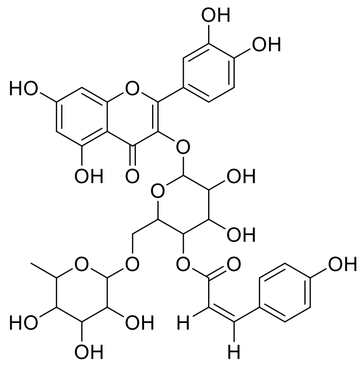
Objective

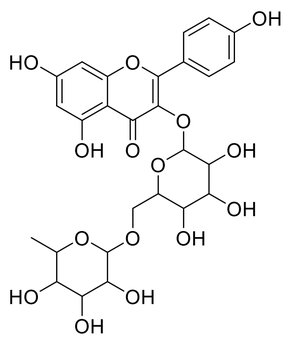
The objective of our present study is to investigate the potential antiviral activity of *S. variabili*s leaves and their isolated flavonoids against SARS-CoV-2.


Variabiloside C


Variabiloside D


Variabiloside E


Variabiloside F


Kaempferol 3-robinobioside

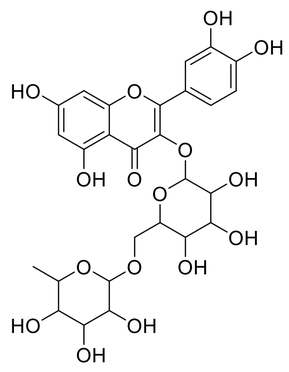

Quercetin 3-robinobioside

Figure 1: Structure of variabilosides C-F, kaempferol 3-robinobioside and quercetin 3-robinobioside.

Methods

❖ Vero E6 cell lines (ATCC CRL-1586) were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 2% fetal bovine serum, 100 IU/ml penicillin, 100 µg/ml streptomycin, 1 mM sodium pyruvate, and 2 mM glutamine. The cell cultures were maintained in humidified tissue culture incubators at 37°C with 5% CO2 and 95% air. The absence of mycoplasma contamination was confirmed using the MycoAlert detection kit (Lonza).

❖ For the experiments, the SARS-CoV-2 strain BetaCov/Belgium/Sart-Tilman/2020/1 was cultivated on Vero cells, and virus titers were determined using the Tissue Culture Infectious Dose 50 (TCID50) method. The experiments were performed in triplicate in 96-well plates. On day-1, Vero E6 cells were trypsinized, counted using a Cedex XS cell analyzer (Roche Innovatis), and 10,000 viable cells were added manually to each well of a 96-well tissue culture plate. The cells were then incubated overnight. On day-2, extracts or pure compounds were added in duplicate to the wells of the 96-well plate containing confluent cells, with concentrations ranging from 50 to 1.5 µg/ml. Additionally, 50 µl of virus suspension or virus-free medium were added to determine the antiviral activity or the cytotoxicity, respectively.

❖ The antiviral activity was established by microscopic analysis regarding the minimal concentration which was able to protect the cells from the viral cytopathic effects, compared to the untreated control on day-5 post-infection.

Results & Discussion

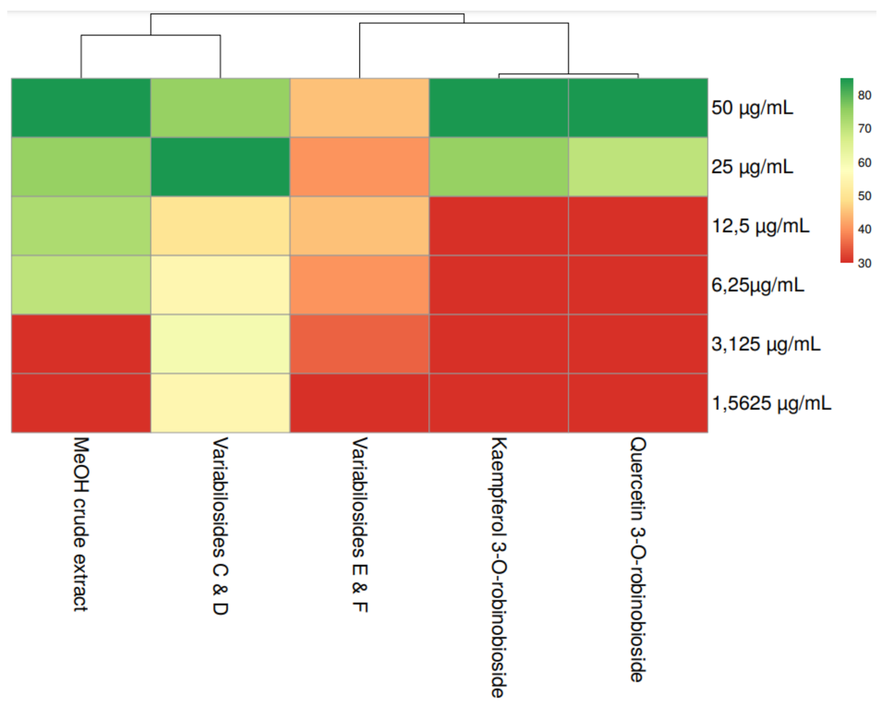


Figure 2: Heat map showing the percentage of living cells on day 5 post-infection of variabilosides C-F and the crude extract, with clustering of compounds/extract (red = low rate of living cells – green = high rate of living cells expressed in %). Columns are clustered using Euclidean distances and Ward method.

❖ The dendrograms generated from our analysis reveal that the activity of the crude extract can be primarily attributed to the presence of variabilosides C and D. These compounds exhibit a high protection rate, demonstrating their significant contribution to the observed activity.

❖ Additionally, kaempferol 3-O-robinobioside and quercetine 3-O-robinobioside were found to moderately participate in the activity, with equivalent impact. Their activity highlights their role in the overall effectiveness of the extract.

❖ On the other hand, variabilosides E and F, which differ from C and D only in the genin (quercetine instead of kaempferol), exhibited very low activity levels.

Conclusions

❖Variabilosides C and D have been identified as the primary contributors to the observed antiviral activity, exhibiting a high protection rate and significantly influencing the extract's effectiveness.

❖Conversely, variabilosides E and F, which differ only in the genin (quercetine instead of kaempferol), exhibited very low activity levels.

❖Interestingly, both kaempferol 3-O-robinobioside and quercetine 3-O-robinobioside displayed equivalent activity levels, despite the difference in their genin (kaempferol vs. quercetine).

❖The unexpected findings regarding the comparable activity of kaempferol 3-O-robinobioside and quercetine 3-O-robinobioside, as well as the contrasting activity levels of variabilosides E and F compared to C and D emphasize the complexity of the structure-activity relationship in natural compounds. Further investigations are needed to unravel the underlying mechanisms responsible for these intriguing observations.

❖These findings not only contribute to our understanding of the chemical composition of *S. variabilis* leaves but also provide insights into the potential therapeutic applications of the identified rare flavonoid compounds. Future research can delve into elucidating the specific molecular interactions and exploring potential synergistic effects of these compounds for further advancements in natural product-based drug discovery.

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In loving memory of Dr. Thierry Brasseur, whose relentless efforts in isolating these compounds years ago were truly remarkable. It is with great appreciation that we acknowledge how this specific flavonoid continues to unveil new interests and perspectives, a testament to Dr. Brasseur's foresight and dedication.

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