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# The Role of Olive Tree Polyphenols In The Prevention of COVID-19: A Scoping Review Part 2

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**Abstract**

The recent COVID-19 pandemic caused by SARS-CoV-2 affected hundreds of millions of people and caused millions of deaths. There are few effective medications against SARS-CoV-2, and several studies attempted to make drugs based on natural components, such as olive leaves. Olive leaves are rich in polyphenolic compounds, which were proposed as a viable co-therapy supplement to treat and improve clinical symptoms in COVID-19 patients. Polyphenols have renown anti-inflammatory and multitarget antiviral effects on several virus families, which could be among the reasons of the beneficial effects of the Mediterranean diet against COVID-19. This scoping review is focused on the effect of olive tree polyphenols as a natural remedy to inhibit SARS-CoV-2, mainly discussing their influence on the process of viral entry into host cells by endocytosis. ***Clin Ter 2023; 174 Suppl. 2 (6):149-153 doi: 10.7417/CT.2023.2481***

**Key words:** *SARS-CoV2, COVID-19 pandemic, polyphenols, antiviral, olive tree*

## Introduction

In December 2019, a new coronavirus was identified in the city of Wuhan, China, in patients who had severe unexplained pneumonia (1). In February 2020, the World Health Organization (WHO) assigned the name of COVID-19 to designate the disease caused by this virus, initially called nCoV-2019 and then SARS-CoV-2 by the International Committee on Taxonomy of Viruses (2). After SARS-CoV-1 (China, 2002) and MERS-CoV (Arabian Peninsula, 2012), both responsible for fatal respiratory distress syndromes, this is the third global health threat linked to a coronavirus in less than twenty years (3).

SARS-CoV-2, like SARS-CoV-1, uses angiotensin-converting enzyme 2 (ACE2) as its main cell receptor in order to enter the host cell (4). Its incubation lasts about five days, leading in 70% of infected patients to respiratory symptoms like cough, fever, or dyspnea (5). After eight to ten days from the first symptoms, in some patients the viral infection is followed by an unsuitable immune reaction and inflammatory syndrome, marked by the worsening of respiratory symptoms (6). This dysimmune phase, called cytokine storm, can be associated with a coagulopathy, a life-threatening condition (7).

Currently, COVID-19 infection is treated with several medications, none of which can effectively cure the disease, but only alleviate the symptoms (8), (9). An effective drug should target the receptor ACE2, preventing SARS-CoV-2 from entering the cells, while also improving the host's immune system. Considering their widespread antiviral action, olive tree polyphenols have been proposed for SARS-CoV-2 treatment. Indeed, olive leaves extracts contain many polyphenolic components, such as hydroxytyrosol (HT), having antioxidant, anti-inflammatory, and antiviral properties (10)–(13).

The goal of this scoping review is to highlight the role of polyphenols in modulating inflammation and their antiviral effects against COVID19, which might help researches to find new insights for treatment to decrease viral infectivity and to fight the SARS-CoV-2 pandemic.

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## Materials and Methods

### *Literature Scoping Review and Inclusion Criteria*

The scoping review section followed PRISMA guidelines for scoping reviews (14). Our search included original and review articles published by MAGI laboratories focusing on SARS-CoV-2 and the effects of olive polyphenols and HT.

The articles employed had to be written in English and published in the period 2019-2022. Congress abstracts, manuscripts not written in English, and studies that were not relevant to the topic of the present manuscript were excluded.

### *Literature Search*

PubMed database was searched to retrieve articles published from 2019 to 2022 that satisfied the inclusion criteria. The search keywords were: (MAGI(Affiliation) AND COVID-19 OR SARS-CoV-2 OR polyphenols OR hydroxytyrosol). In addition, the reference lists produced by the automated research were scanned manually to find further relevant scientific article.

### *Study Selection*

All the resulting articles were assessed independently for eligibility by authors who evaluated titles and abstracts, according to the above inclusion criteria. Once a paper was found eligible, its references were screened to find new papers.

## Results

Based on the literature review search criteria, 16 manuscripts published from 2019 to 2022 by the MAGI laboratories were found. Only 9 pertained to the topic of the current review and were thus read completely and discussed in the current manuscript. Their reference list was scanned for retrieving any other relevant scientific article.

The following sections will highlight the main findings of the included articles. Indeed, the antiviral and immunomodulatory activity of olive polyphenols, such as HT, and of α-cyclodextrin will be discussed. Moreover, the results of studies already performed, and the possible advantages of additional in vitro and in vivo studies or clinical trials studying the effects of natural molecules in fighting SARS-CoV-2 infection will also be discussed in this manuscript.

### *Polyphenols and Viral Infectivity*

Viral infectivity depends on the interactions between components of the host cell plasma membrane and the virus envelope. Coronaviruses are a class of viruses with a long single positive RNA molecule and a lipid envelope, which requires a plasma membrane fusion process, mediated by endocytosis, a mechanism in which cholesterol and lipid rafts play a fundamental role. Identifying molecular targets that could block and inhibit the virus entry into the cell is important to decrease the viral activity of SARS-COV-2 (15, 16).

Baglivo et al. (17) presented a qualitative review on the importance of membrane molecules in coronavirus infectivity, and proposed their role as potential targets for lowering SARS-COV-2 infectivity. The authors of the study have focused on the involvement of lipid structures—such as cholesterol and lipid rafts—in the endocytosis-mediated process of viral attachment and cell infection. Moreover, they discussed a variety of naturally occurring compounds, including cyclodextrin and sterols, that can decrease the infectiousness of a variety of viruses, including members of the coronavirus family, by interfering with their lipid-dependent attachment to human host cells.

Natural compounds that may inhibit viral entry into host cells by endocytosis were subsequently reviewed by Kiani et al. (18). The authors showed that in recent years natural remedies for viral infections have become more significant, and that many natural substances, such as phytosterols, polyphenols, flavonoids, citrus, galangal, curcuma, and hydroxytyrosol (HT), are being studied to determine whether they may suppress SARS-CoV-2.

### *The Antiviral Activity of Hydroxytyrosol and α-Cyclodextrin and the Molecular Docking Studies to Evaluate Their Interaction with SARS-Cov-2*

HT is a natural molecule, having anti-inflammatory, anti-tumor, antiviral, antibacterial, and antifungal properties, that can be extracted from olive leaves and fruits (19). HT also reduces the serum lipids in mice fed with high-cholesterol diets, indirectly modifying the composition of their plasma membrane (18). Furthermore, HT improves endothelial dysfunction, decreases oxidative stress, and is neuro- and cardio-protective. Due to all these biological properties, HT is currently one of the most actively investigated natural phenols, with a great pharmacological potential (19).

α-cyclodextrin is a natural molecule, produced by bacteria that can deplete sphingolipids and phospholipids from cell membranes. Indeed, cyclodextrins have been exploited by many researchers to replace membrane leaflets with exogenous lipids. Moreover, α-cyclodextrin can reduce serum concentration of phospholipids, reducing viral endocytosis processes (20).

Considering all the properties of HT and α-cyclodextrin, it has been decided to study them in silico, in order to determine their interactions with lipid-raft-mediated endocytosis of SARS-CoV-2. In two articles by Paolacci et al. (20) and Ergoren et al. (21), the authors firstly reviewed the role and interactions of HT and α-cyclodextrin in lipid-raft-mediated endocytosis of SARS-CoV-2. Then, thanks to in silico studies, they demonstrated that α-cyclodextrin and HT interact with the viral spike protein and its host cell receptor ACE2, suggesting an impact on the SARS-CoV-2 endocytosis process.

HT and α-cyclodextrin were finally tested in vitro and in vivo for their anti-SARS-CoV-2 properties. In the study by Paolacci et al (22), in vitro and clinical studies on the efficacy of α-cyclodextrin and HT against SARS-CoV-2 infection were examined. Both the in vitro analysis (performed on Vero E6 cells, Caco2, and human fibroblast cell lines) and

the clinical studies, which recruited 149 volunteers and 76 controls, proved that HT and α-cyclodextrin increase defenses against SARS-CoV-2 infection and reduce the synthesis of viral particles.

Apart from SARS-CoV-2, HT and α-cyclodextrin were tested for preventing the proliferation of oral pathogens due to prolonged face mask use. HT and α-cyclodextrin proved to reduce the growth of bacteria and fungi and also halitosis and gingival and mouth inflammation in several volunteers. Thus, it can be deduced that it is safe and beneficial to use α-cyclodextrin and HT to lessen the bacterial and fungal load brought on by frequent use of face masks (23).

### *HT α-Cyclodextrin as Oral Spray to Fight SARS-CoV-2 Infection*

A study by Paolacci et al (24) presented an observational study to evaluate the safety profile of the "Endovir Stop" spray. The authors proposed a mouth spray that might stop SARS-CoV-2 endocytosis, based on HT and α-cyclodextrin. The spray was tested on 87 healthy subjects; also, the cytotoxicity and antioxidant capacity of the spray was evaluated in vitro. From the results, the spray is not cytotoxic and it has a good antioxidant capability. Moreover, the clinical tests on healthy volunteers revealed that there were no adverse effects and no medication interactions while receiving therapy, proving the safety of "Endovir Stop" spray.

The effectiveness of an oral spray containing α-cyclodextrin and HT against SARS-CoV-2 transmission was tested in a pilot study carried out by Ergoren et al (21). They recruited 50 healthy volunteers at a higher risk of SARS-CoV-2 infection from Northern Cyprus and 6 individuals that tested positive for SARS-CoV-2. Despite being at a greater risk of infection than the general population, the 50 healthy volunteers did not test positive for SARS-CoV-2 after receiving the spray for two weeks. Interestingly, despite the viral load being larger in the treated individuals than in the untreated patients who became negative after ten days, 2 of the cohort's 6 positive patients went from positive to negative within five days. Moreover, they made an in silico prediction to evaluate the interactions of HT and α-cyclodextrin with proteins involved in SARS-CoV-2 endocytosis. They discovered potential interactions between HT and α-cyclodextrin and the human cell proteins Spike, ACE2, and TMPRSS2. To conclude their pilot study, they mentioned that their findings suggested a potential contribution of HT and α-cyclodextrin in strengthening immune defenses against SARS-CoV-2.

### *The Role of Polyphenols in Treating Post-COVID Syndrome*

A proportion of COVID-19 patients experience post-COVID fatigue, persistent enervating symptoms and post-exertional neuroimmune exhaustion similar to those observed in SARS patients. This condition was consequently named "post-COVID syndrome" (PCS) (25). PCS is characterized by persistent multi-organ damage due to severe inflammatory responses, oxygen deprivation, thrombotic microangiopathy, and venous thromboembolism (26). However, there are currently few reports on the mechanisms underlying PCS, and it remains extremely difficult to understand why some people recover quickly while others develop the syndrome. In certain cases, prolonged illness seems to be linked to older age and multiple chronic medical conditions (27).

In a study by Naureen et al. (28), the authors reviewed data on post-COVID syndrome, in order to emphasize its etiological cause and the dietary regimens and supplements that might lessen or remove the associated chronic fatigue, gastrointestinal problems, and ongoing inflammatory responses. The authors selected acetyl L-carnitine, HT, and vitamins B, C, and D as possible natural molecules that show great potential as dietary supplements for the treatment of post-COVID syndrome. Thus, they begun a pilot observational trial, evaluating how HT, acetyl L-carnitine, and vitamins B, C, and D affected individuals who, despite recovering from COVID-19, were still experiencing post-COVID syndrome, obtaining encouraging findings.

## Conclusion

Few medications and vaccinations are currently on the market for COVID-19 treatment, and scientific research is now working on finding new effective molecules. The main goal of this scoping review is to present studies involved in finding potential molecular targets and natural molecules to stop the spread and interrupt SARS-CoV-2 transmission. Early on, during a coronavirus infection, endocytosis occurs, and it is directly connected to viral contagiousness. The effects of inhibitors, such as cyclodextrin and phytosterols, as well as naturally occurring inhibitors like flavonoids, α- cyclodextrin and HT, are examined. Various molecular targets implicated in this process, including ACE2 receptors, lipid rafts, and proteases, are investigated. In particular, HT appear to hold great potential for the development of COVID-19 treatment approaches.

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*Conflicts of interest statement*

Authors declare no conflict of interest.

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*Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) Checklist*

| **Title** | **1** | **Identify the report as a scoping review.** | **1** |
|---|---|---|---|
| **ABSTRACT** | | | |
| Structured summary | 2 | Provide a structured summary that includes (as applicable): background, objectives, eligibility criteria, sources of evidence, charting methods, results, and conclusions that relate to the review questions and objectives. | 1 |
| **INTRODUCTION** | | | |
| Rationale | 3 | Describe the rationale for the review in the context of what is already known. Explain why the review questions/objectives lend themselves to a scoping review approach. | 2 |
| Objectives | 4 | Provide an explicit statement of the questions and objectives being addressed with reference to their key elements (e.g., population or participants, concepts, and context) or other relevant key elements used to conceptualize the review questions and/or objectives. | 2 |
| **METHODS** | | | |
| Protocol and registration | 5 | Indicate whether a review protocol exists; state if and where it can be accessed (e.g., a Web address); and if available, provide registration information, including the registration number. | N.A. |
| Eligibility criteria | 6 | Specify characteristics of the sources of evidence used as eligibility criteria (e.g., years considered, language, and publication status), and provide a rationale. | 2-3 |
| Information sources* | 7 | Describe all information sources in the search (e.g., databases with dates of coverage and contact with authors to identify additional sources), as well as the date the most recent search was executed. | 2-3 |
| Search | 8 | Present the full electronic search strategy for at least 1 database, including any limits used, such that it could be repeated. | 2-3 |
| Selection of sources of evidence† | 9 | State the process for selecting sources of evidence (i.e., screening and eligibility) included in the scoping review. | 2-3 |
| Data charting process‡ | 10 | Describe the methods of charting data from the included sources of evidence (e.g., calibrated forms or forms that have been tested by the team before their use, and whether data charting was done independently or in duplicate) and any processes for obtaining and confirming data from investigators. | N.A. |
| Data items | 11 | List and define all variables for which data were sought and any assumptions and simplifications made. | 2-3 |
| Critical appraisal of individual sources of evidence§ | 12 | If done, provide a rationale for conducting a critical appraisal of included sources of evidence; describe the methods used and how this information was used in any data synthesis (if appropriate). | N.A. |
| Synthesis of results | 13 | Describe the methods of handling and summarizing the data that were charted. | 2-3 |
| **RESULTS** | | | |
| Selection of sources of evidence | 14 | Give numbers of sources of evidence screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally using a flow diagram. | 3-5 |
| Characteristics of sources of evidence | 15 | For each source of evidence, present characteristics for which data were charted and provide the citations. | 3-5 |
| Critical appraisal within sources of evidence | 16 | If done, present data on critical appraisal of included sources of evidence (see item 12). | N.A. |
| Results of individual sources of evidence | 17 | For each included source of evidence, present the relevant data that were charted that relate to the review questions and objectives. | 3-5 |
| Synthesis of results | 18 | Summarize and/or present the charting results as they relate to the review questions and objectives. | 3-5 |
| **DISCUSSION** | | | |
| Summary of evidence | 19 | Summarize the main results (including an overview of concepts, themes, and types of evidence available), link to the review questions and objectives, and consider the relevance to key groups. | 3-5 |
| Limitations | 20 | Discuss the limitations of the scoping review process. | 5 |
| Conclusions | 21 | Provide a general interpretation of the results with respect to the review questions and objectives, as well as potential implications and/or next steps. | 5 |
| **FUNDING** | | | |
| Funding | 22 | Describe sources of funding for the included sources of evidence, as well as sources of funding for the scoping review. Describe the role of the funders of the scoping review. | 5 |

JBI = Joanna Briggs Institute; PRISMA-ScR = Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews.
* Where sources of evidence (see second footnote) are compiled from, such as bibliographic databases, social media platforms, and Web sites.
† A more inclusive/heterogeneous term used to account for the different types of evidence or data sources (e.g., quantitative and/or qualitative research, expert opinion, and policy documents) that may be eligible in a scoping review as opposed to only studies. This is not to be confused with information sources (see first footnote).
‡ The frameworks by Arksey and O'Malley (6) and Levac and colleagues (7) and the JBI guidance (4, 5) refer to the process of data extraction in a scoping review as data charting.
§ The process of systematically examining research evidence to assess its validity, results, and relevance before using it to inform a decision. This term is used for items 12 and 19 instead of "risk of bias" (which is more applicable to systematic reviews of interventions) to include and acknowledge the various sources of evidence that may be used in a scoping review (e.g., quantitative and/or qualitative research, expert opinion, and policy document).

From: Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, et al. PRISMA Extension for Scoping Reviews (PRISMAScR): Checklist and Explanation. Ann Intern Med. 2018;169:467–473. doi: 10.7326/M18-0850.