Can propolis and their compounds be efficacy in the treatment of coronavirus disease 2019 (COVID-19)? A systematic review

A própolis e seus compostos podem ser eficazes no tratamento da doença do coronavírus 2019 (COVID-19)? Uma revisão sistemática

¿Pueden los propóleos y sus compuestos ser efectivos en el tratamiento de la enfermedad por Coronavirus 2019 (COVID-19)? Una revisión sistemática

Abstract

Despite the advancement of vaccination and the reduction in the number of deaths, there is still the emergence of new variants, such as the omicron of SARS-CoV-2 (COVID-19). In this sense, new natural antiviral therapies are highly explored. One of these products, propolis, have shown promising results against COVID-19, including the inhibition of the binding between the coronavirus and ACE2. This systematic review aimed to gather a summary of scientific evidence existing on the effective of the therapeutic use of propolis and their components in the treatment of COVID-19. The protocol for the present systematic review was registered on the PROSPERO (CRD42021267016). In this study, we analyzed 185 articles, selecting 13 of them. Some phenolic compounds and flavonoids, such as artepillin C, hesperetin, CAPE and rutin, were widely cited, as they have great potential for binding with the molecular targets of SARS-CoV-2. Some clinical studies that evaluated the effects of propolis against COVID-19 were included, and confirmed the effectiveness of propolis and its components. The results of this review demonstrate the effectiveness of using propolis and their components in the treatment of COVID-19 due to its antiviral activities. Additionally, the anti-inflammatory and immunomodulatory properties can help to patients with COVID-19.

Keywords: SARS-CoV-2; Rutin; Quercetin; CAPE; Systematic Review; Health teaching.
Resumo

Palavras-chave: SARS-CoV-2; Rutina; Quercetina; CAPE; Revisão Sistemática; Ensino em saúde.

Resumen
A pesar del avance de la vacunación y la reducción en el número de muertes, todavía está surgiendo nuevas variantes, como el omicrón SARS-CoV-2 (COVID-19). En este sentido, las nuevas terapias antivirales naturales están muy exploradas. Uno de esos productos, el propóleo, ha mostrado resultados prometedores contra el COVID-19, incluida la inhibición del vínculo entre el coronavirus y la ACE2. Esta revisión sistemática tuvo como objetivo recopilar un resumen de la evidencia científica existente sobre la efectividad del uso terapéutico del propóleo y sus componentes en el tratamiento de la COVID-19. El protocolo de la presente revisión sistemática se registró en PROSPERO (CRD42021267016). En este estudio analizamos 185 artículos, seleccionando 13 de ellos. Algunos compuestos fenólicos y flavonoides, como la artepilina C, la hesperetina, la CAPE y la rutina, fueron ampliamente citados, ya que tienen un gran potencial para unirse a los objetivos moleculares del SARS-CoV-2. Se incluyeron algunos estudios clínicos que evaluaron los efectos del propóleo contra el COVID-19 y confirmaron la efectividad del propóleo y sus componentes. Los resultados de esta revisión demuestran la efectividad del uso de propóleos y sus componentes en el tratamiento de la COVID-19 debido a sus actividades antivirales. Además, las propiedades antiinflamatorias e imunomoduladoras pueden ayudar a los pacientes con COVID-19.

Palabras clave: SARS-CoV-2; Rutina; Quercetina; CAPE; Revisión Sistemática; Enseñanza en salud.

Graphical abstract

Source: Authors.
1. Introduction

Despite the advancement of vaccination and the reduction in the number of deaths, there is still the emergence of new variants, such as omicron of SARS-CoV-2 (COVID-19). In this sense, the rapid evaluation of possible resistance to anti-viral therapies and vaccines is highly required. However, data on the efficacy of available therapeutic agents and vaccines is clearly insufficient (Droždżal et al., 2021; Yao et al., 2020).

SARS-CoV-2 infection is initiated by binding of a viral protein spike to the angiotensin II-converting enzyme receptor (ACE2) on the surface of the host cell, fusing with the cell membrane and releasing viral RNA. Activation of viral proteins is mediated by proteases, such as transmembrane serine protease 2 (TMPRSS2) (Hoffmann et al., 2020). After infection, the new coronavirus is able to positively regulate p21-activated kinase 1 (PAK1), which mediates several critical mortality factors, such as inflammation and pulmonary fibrosis. Increased levels of PAK1 can also suppress the adaptive immune response and thus facilitate viral replication (Maruta & He, 2020). SARS-CoV-1 infection is associated with increased levels of activated pro-inflammatory chemokines and cytokines leading to the development of atypical pneumonia, with rapid respiratory impairment and lung failure (Ding et al., 2004).

Natural products are among the options explored, as many of them have shown the capability of affecting various metabolic pathways in humans and microorganisms, with anti-inflammatory, antiviral and immunomodulatory activities. One of these products, propolis, a resinous compound produced by bees with a variable composition influenced by environmental factors (Bachevski et al., 2020), have shown promising results against COVID-19 (Berretta et al., 2020), including the inhibition of the binding between the coronavirus and ACE2 (Khayrani et al., 2021).

Quercetin, one of the major flavonoids found in propolis, was able to inhibit the protease enzymes from the SARS-CoV-1 and MERS-CoV viruses in vitro (Rocha et al., 2013). In addition, as coronaviruses modulate the cellular unfolded protein response (UPR) to complete their replication cycle, quercetin may interfere with this pathway (Polansky & Lori, 2020).

Another promising pharmacological approach to propolis in the treatment of COVID-19 is to target downstream effectors, such as p21-activated kinases (PAKs). Caffeic acid phenethyl ester (CAPE) demonstrated the potential to block viral infection and negatively regulate ras related C3 botulinum toxic substrate (RAC). The practical aspect of those results is that propolis may be a useful drug to prevent coronavirus-induced lung fibrosis (Maruta & He, 2020).

Recent reviews have highlighted the potential benefits of propolis overall and specifically against COVID-19. Ripari et al. (2021) reviewed in vitro and preclinical trials to substantiate anti-inflammatory, immunomodulatory and antiviral activities. Berretta et al. (2020) reviewed the use of propolis against SARS-CoV-2 and highlighted previous in silico and in vitro studies of its antiviral and immunomodulatory properties showing, however, no results from clinical trials. Arentz et al. (2021) performed a rapid review of clinical studies on the effects of honeybee products on respiratory tract diseases, including COVID-19. However, there was 1 article in COVID-19. Yosri et al. (2021) performed a review of in silico, preclinical and clinical applications of propolis on respiratory tract diseases, including COVID-19. However, these authors were published before the results of clinical trials using propolis against COVID-19, cited in our review.

Propolis has also demonstrated an antiviral effect, in vivo and in vitro studies, against the influenza virus (Shimizu et al., 2008), human rhinovirus (Kwon et al., 2020) and human respiratory diseases (Takeshita et al., 2013). This antiviral activity is associated with the presence of phenolic compounds, which are able to block or reduce the adsorption and entry of the virus in host cells (Kwon et al., 2020; Lima et al., 2021). Furthermore, propolis is a stimulant of the adaptive immune system, which can reinforce its prophylactic antiviral effect (Babaei et al., 2016; Lima et al., 2021). Governa et al. (2019) reported that propolis was able to reduce the key protein (neuraminidase) for the entry of the H1N1 virus into cells, and can be an important antiviral agent. Finally, Wang et al. (2021) reported that the propolis ameliorates pulmonary fibrosis through Akt activation and regulates the protein expression of PPARγ (Kao et al., 2013).
Kwon et al. (2020) showed a superior antiviral activity of some propolis compounds (kaempferol, quercetin, chrysin and luteolin) in relation to ribavirin against rhinoviruses, which cause 50% of common colds across the globe. In addition, kaempferol and p-coumaric acid were able to reduce the levels of RNA replication when administered within 4 h after inoculation of the virus.

The present systematic review had the guiding question: “Is the use of propolis and their components are efficient in the treatment of COVID-19?” Thus, this systematic review aimed to gather a summary of scientific evidence existing between December 2019 and April 1, 2021, to confirm the effectiveness of propolis and their compounds in the treatment of COVID-19.

2. Methodology

The present systematic review had the guiding question: “Is the use of propolis and its components are efficacy in the treatment of COVID-19?”. To search for the answer, it was decided to develop a systematic literature review. The protocol for the present systematic review was registered on the International Prospective Register of Systematic Reviews (PROSPERO; https://www.crd.york.ac.uk/PROSPERO, protocol number CRD42021267016) (Sobrinho et al., 2021a). This study was conducted and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations (Moher et al., 2015).

2.1 Search Strategy

We conducted a search for articles using the databases Cochrane Central Register of Controlled Trials (CENTRAL), Lilacs, PubMed, Scielo, Scholar Google, Scopus and Web of Science, type of document or language. The dates of search and the search criteria by databases are described in Table S1. All supplementary data was available on Mendeley Data (https://doi.org/10.17632/tcyrmp43f6) (Sobrinho et al., 2021b). The last search was completed on April 1, 2021 (dez/2021 to April/2021).

2.2 Selection of Relevant Articles

Articles that evaluated the use of propolis and their components in the treatment of COVID-19 in clinical trials, in vivo, in vitro and in silico studies were included. Papers published in Spanish, English and Portuguese were considered eligible. Reviews, case reports, protocol of study, clinical trials protocols, editorials and studies published in summary form and articles that were inaccessible, even after attempts to contact the corresponding author, were excluded.

2.3 Selection of Studies and Data extraction

Duplicate studies were excluded using reference managers (Mendeley Desktop® and JabRef). The extracted citations were imported into the Rayyan systematic review (https://rayyan.qcri.org/) (Ouzzani et al., 2016) for inclusion or exclusion based on the defined criteria.

The eligibility process was conducted in two separate stages. First, two researchers (BCA, and IRM or RCSS) independently screened all non-duplicate articles and excluded non-relevant articles based on title and/or abstract. A final list was agreed on, with discrepancies resolved by a third researcher (JMDA, CFO or BJMS). Second, full-text versions of the studies selected from stage 1 screening were downloaded and independently assessed for eligibility by the two researchers. Any discrepancies were resolved by a third researcher. Final decisions of stage 1 regarding inclusion of studies in the review were made by consensus, and the agreement between the two reviewers was analyzed by using the Kappa Test (Landis & Koch, 1977).

The collected data were author, year of publication, location of the study, composition of the extracts, type of study, objective of the study, results found and limitations. In silico studies were collected based on data related to binding affinity,
compounds, anti-covid-19 effect (in vitro assay), protein and/or receptor evaluated, methodological approach, software and model evaluation. In clinical studies, the numbers of participants, parameters evaluated and anti-covid-19 effect were collected.

2.4 Risk of Bias of included clinical studies

The quality of the studies was evaluated using the Jadad Scale (Jadad et al., 1996). In addition, the risk of bias for each reference included in the systematic-review was assessed using the Cochrane Risk of Bias Tool (J. Higgins & Green, 2015).

3. Results and Discussion

3.1 Search strategy

The initial research identified 185 records. First, duplicate records were removed (n = 95), excluded by reading the title and/or abstract (n = 71; kappa test = 0.43±0.09) and excluded after reading the articles (n = 6). Finally, 13 articles were selected. Figure 1 reports the PRISMA flowchart detailing the screening process.

3.2 Characteristics of studies included

The 13 reports found in the search strategy step, 11 have in silico studies, which differ in the type of propolis extract, their compounds and/or target proteins. In addition, among the five clinical trials records found in the ClinicalTrial.gov database, only two responded to contact with the publication submission and/or main results. Both clinical trials (Kosari et al., 2021; Silveira et al., 2021) scored as low quality on the Jadad scale and low risk of bias in the Cochrane Risk of Bias tool. The relevant results and characteristics of the studies are described in Table 1, 2 and 3.

3.3 Results of the Systematic review

3.3.1 In silico studies

These studies were carried out with different approaches of docking, virtual screening and molecular dynamics. Table S2 summarizes the major relevant results and findings obtained from these studies, while Table S3 contains all extracted data. This section highlighted the specific interaction of propolis and their components on the targets of SARS-CoV-2. Molecular docking is a suitable computational tool to describe the affinity between a ligand and biomacromolecule; and its respective pharmacophoric pose into the binding site (Maia et al., 2020; Meng et al., 2012). In other words, docking has two tasks to solve, the first is the determination of each intramolecular interaction, resulting in an affinity measure; the second one, is the determination of the correct pose of ligand into the binding site, which docking methods have to be able to reproduce the conformation crystallographic ligand of a specific molecular target. The obtention of affinity values, described by binding energy or scores, have permitted the ranking of a set of ligands. Thus, only the most promising compounds are selected and addressed to experimental work. In the case of all methods described by this review, the lowest values indicate a high affinity for the molecular target. Noteworthy that different force fields can reach different results due their own parametrization process. Consequently, they cannot be compared directly due to the difference in the order of magnitude. The docking process is beyond the scope of this review; however, it can be clarified.
Figure 1. PRISMA Flowchart reporting the study screening process.

3.3.2 Interaction of propolis components with main protease (M_{3CL})

3CL-protease (M_{3CL}) is the enzyme responsible for processing viral polyproteins (Refaat et al., 2021) and its inhibition can block viral replication (Sahlan et al., 2021). Propolis components were selected using the Lipinski’s rule of five (RO5) (Lipinski et al., 2012) and evaluated for their potential inhibition of M_{3CL}, comparing those components with the crystallographic
ligand of α-ketoamide 13b (PDB ID: 6Y2F). The results of this study suggested that broussoflavonol F, glyasperin A and sulabiroin A are able to interact with the catalytic sites of M\(^{\text{pro}}\) and bind with 75%, 63% and 44% similarity, respectively, in comparison with the crystallographic ligand (Sahlan et al., 2021). In another study conducted in India, 4 out of 6 compounds (CAPE, caffeic acid, chrysin and galagin) showed affinity by the receptor suggesting a potential to inhibit M\(^{\text{pro}}\) from SARS-CoV-2 (Hashem, 2020). However, none of these studies were evaluated properly using tools such as redocking, roc curve, molecular dynamics or preclinical and clinical studies, so there is no guarantee that they obtained false positive results.

All the 10 components evaluated showed less binding affinity than remdesivir. Interestingly, rutin and CAPE showed greater binding affinity than hydroxychloroquine, and rutin showed the best binding affinity among propolis compounds (Refaat et al., 2021).

Kumar et al. (2020) demonstrated the CAPE inhibitory potential for M\(^{\text{pro}}\) of SARS-CoV-2 with results similar to its tested crystallographic inhibitor (N3). These results showed CAPE can be a lead compound to develop a new drug for the treatment of COVID-19 (Kumar, Dhanjal, Kaul, et al., 2020). Noteworthy, CAPE has a Michael acceptor moiety, which can react with others coronavirus proteases as described.

### 3.3.3 Interaction of propolis compounds with TMPRSS2

The potential inhibitor of TMPRSS2 for CAPE was slightly greater than its known inhibitor (camostat mesylate). (Kumar, Dhanjal, Bhargava, et al., 2020) In other words, Michael acceptor moiety of CAPE can interact with different proteases showing a non-selective compound.

### 3.3.4 Interaction of propolis compounds with ACE2

Khayrani et al. (2021) evaluated the potential of propolis compounds to modulate ACE2 and thus inhibit receptor binding with SARS-CoV-2. The results showed that five compounds (glucosperin A, broussoflavonol F, sulabiroins A, (2)-5,7-dihydroxy-4’-methoxy-8-prenylflavonone A isorhamnetin) has the potential to inhibit the binding of the virus to ACE2. The docking scores obtained are more favorable than a tested potent inhibitor (MLN-4760). However, taking into account the similarity of binding, two compounds (isorhamnetin and glucosperin A) were considered to have the greatest potential (Khayrani et al., 2021). As described previously, the docking methodologies should reproduce the pose of crystallographic ligand. Thus, the first approach is called redocking. Redocking consists in the removal of crystallographic ligand of the binding site following its docking. This redocking process shows: i) the binding energy of crystallographic ligands; ii) if the docking parameters are suitable; and iii) the docking methodology can reproduce the crystallographic pose of the ligand. Thus, the determination of root means square deviation (RMSD) between the atomic position of crystallographic ligand and redocking ligand should be less than 2.0 Å (Maia et al., 2020; Meng et al., 2012). Thus, even though the redocking process was carried out by these papers, the RMSD was not described, beyond others evaluation methods, such as roc curve or molecular dynamics simulations.

Güler et al. (2020) also evaluated the ability of flavonoids present in propolis to bind to ACE2 receptors. According to the docking analysis, it was demonstrated that rutin, myricetin, CAPE, hesperetin and pinocembrin showed the best potential for inhibition compared to the natural inhibitor (MLN-4760). Therefore, it is considered that rutin can compete with SARS-CoV-2
Table 1. Relevant results of *in silico* and *in vitro* studies on effectiveness of propolis from different regions of the world in the treatment of COVID-19. Papers collected in dez/2021 to April/2021.

<table>
<thead>
<tr>
<th>Propolis source</th>
<th>Country</th>
<th>Type of study</th>
<th>Objective</th>
<th>Outcome</th>
<th>Limitation</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatolian propolis</td>
<td>Turkey</td>
<td><em>In silico</em> and <em>in vitro</em></td>
<td>To evaluate whether the ethanolic extracts of propolis from Anatolia inhibit the COVID-19 virus in terms of binding of the S1 spike protein and the ACE2 receptor in both <em>in vitro</em> and <em>in silico</em> studies.</td>
<td>• Pinocembrine, chrysin, caffeic acid phenethyl ester and hesperetin had very low free binding energies to the ACE2 receptor and the SARS-CoV-2 RBD peak protein. • These four flavonoids were also found to have greater binding potential than hydroxychloroquine. • Hesperetin is the best inhibitor of spike protein of SARS-CoV-2 and ACE2, has the lowest IC₅₀ value (16.88 mM), pinocembrine and CAPE were followed. • Pinocembrine had a high inhibitory effect in the <em>in silico</em> study, while hesperetin was more active in the <em>in vitro</em> study.</td>
<td>• More detailed studies are needed.</td>
<td>Gülér et al. (2020)</td>
</tr>
<tr>
<td>Brazilian green propolis</td>
<td>Canada</td>
<td><em>In silico</em></td>
<td>Find molecules containing amino acid substituents that mimic the SLiM responsible for recruiting host PPA2-B56.</td>
<td>• LxxLxE-like motifs from CoV-2 allowed us to find a small molecule called Artepillin C, which is known to have anti-inflammatory activity.</td>
<td>• Clinical studies are needed.</td>
<td>Maaroufi (2020)</td>
</tr>
<tr>
<td>Egyptian propolis</td>
<td>Egypt</td>
<td><em>In silico</em></td>
<td>Comparative analysis of chemical composition between samples collected in different locations in Egypt with analysis concomitant with anti-COVID-19 activity.</td>
<td>• The results of docking studies showed that most compounds had promising binding scores.</td>
<td>• Some Egyptian propolis compounds are excellent candidates for combating COVID-19.</td>
<td>Elwakil et al. (2021)</td>
</tr>
<tr>
<td>Egyptian propolis</td>
<td>Egypt</td>
<td><em>In silico</em> and <em>in vitro.</em></td>
<td>Develop liposomal formulation optimized to enhance the antiviral activity of propolis against COVID-19.</td>
<td>• All Egyptian propolis flavonoid compounds have binding affinity to the M⁰ and spike protein compared to Avigan, hydroxychloroquine and Remdesivir. • Liposomal formulation can guarantee delivery to target cells.</td>
<td>• Clinical studies are needed to estimate the effectiveness of the formulation.</td>
<td>Refaat et al. (2021)</td>
</tr>
<tr>
<td>Propolis (PT Nano Herballtama Internasional)</td>
<td>Indonesia</td>
<td><em>In silico</em></td>
<td>Investigate the interaction of the active molecular compounds of propolis against the main protease and protein spike of SARS-CoV-2 by molecular docking approach.</td>
<td>• The candidate to inhibit M⁰ of SARS-CoV-2 was methylphiphogonone A, 3’-methoxyididine and genistin. • Neohlavaisoflavone, methylphiphogonone A, 3’-methoxyididine and genistin) have lower binding affinity energy than pravastatin (control), making these compounds candidates for spike protein inhibition.</td>
<td>• More <em>in vivo</em> studies are needed.</td>
<td>Harisna et al. (2021)</td>
</tr>
<tr>
<td>Sudawesi propolis</td>
<td>Indonesia</td>
<td><em>In silico</em></td>
<td>Evaluate the potency of Sulawesi’s propolis compounds as ACE2 inhibitors.</td>
<td>• Glicosperina B, bromosflavonol F, salalibrianas A, (2S)-5,7-dihydroxy-4’-methoxy-8-prenyllavananol, and isorhamnetina are potential to inhibit the binding of ACE-2 and SARS-CoV-2.</td>
<td>• Clinical studies are needed.</td>
<td>Khayrani et al. (2021)</td>
</tr>
<tr>
<td>Sudawesi propolis</td>
<td>Indonesia</td>
<td><em>In silico</em></td>
<td>Analyze molecular interactions between selected compounds of propolis Sulawesi produced by <em>Tetragonula sapiens</em> and the M⁰ of SARS-CoV-2.</td>
<td>• Brousse flavanol F, glucosperin A and salalibrianas A are able to bind to the M⁰ of SARS-CoV-2.</td>
<td>• Studies are needed to assess the potency and safety of bromosflavonol F and glucosperin A</td>
<td>Sahlan et al. (2021)</td>
</tr>
<tr>
<td>Turkish propolis</td>
<td>Turkey</td>
<td><em>In silico</em></td>
<td>Calculate the inhibition constants of some flavonoids, one of the active ingredients of propolis from Anatolia, to the enzyme ACE2 by molecular modeling with a positive control</td>
<td>• The high binding constants for ACE2 receptors and flavanones in the propolis ethanolic extract make it a good competitive inhibitor and natural protector of agents for the treatment of COVID-19.</td>
<td>• This study should be supported by further studies <em>in vivo</em>.</td>
<td>Gülér et al. (2021)</td>
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</tbody>
</table>

NI: Not informed; ACE2: Angiotensin II-converting enzyme receptor; TMPRSS2: Transmembrane serine protease 2; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019; CAPE: Caffeic acid phenethyl ester; M⁰: main protein. Source: Authors.
Table 2. Relevant results of in silico studies on effectiveness of compounds of propolis from different regions of the world in the treatment of COVID-19. Papers collected in Dez/2021 to April/2021.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Country</th>
<th>Objective</th>
<th>Outcome</th>
<th>Limitation</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-phenyllactic acid, CAPE, caffeic acid, chrysin, galangin and lumichrome</td>
<td>Egypt</td>
<td>To evaluate the activity of six active compounds of bee product and propolis to inhibit the main protease of COVID-19</td>
<td>• CAPE, Chrysin, Galangin and Lumichrome are linked with good glide scores and can inhibit the M&lt;sub&gt;pro&lt;/sub&gt; and virus replication of COVID-19.</td>
<td>• More in vivo studies are necessary</td>
<td>Hashem et al. (2020)</td>
</tr>
<tr>
<td>CAPE</td>
<td>India</td>
<td>Examine the inhibitory potential of three natural compounds CAPE for TMPRSS2.</td>
<td>• CAPE have the same binding affinity for TMPRSS2 as Camostat mesylate.</td>
<td>• Clinical studies are needed.</td>
<td>Kumar, Dhanjal, Bhargava, et al. (2020)</td>
</tr>
<tr>
<td>CAPE</td>
<td>India</td>
<td>Examine the potential for binding CAPE to M&lt;sub&gt;pro&lt;/sub&gt; of SARS-CoV-2</td>
<td>• Strong possibility that CAPE have an inhibitory potential for the M&lt;sub&gt;pro&lt;/sub&gt; of SARS-CoV-2</td>
<td>• Studies are need experimental validation and clinical studies</td>
<td>Kumar, Dhanjal, Kaul, et al. (2020)</td>
</tr>
</tbody>
</table>

NI: Not informed; ACE2: Angiotensin II-converting enzyme receptor; TMPRSS2: Transmembrane serine protease 2; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019; CAPE: Caffeic acid phenethyl ester; M<sub>pro</sub>: main protein. Source: Authors.


<table>
<thead>
<tr>
<th>Propolis source</th>
<th>Country</th>
<th>Sample and design</th>
<th>Objective</th>
<th>Outcome</th>
<th>Limitation</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazilian green propolis</td>
<td>Brazil</td>
<td>80 individuals used propolis. Single-center, open-label, randomized and controlled trial</td>
<td>Verify the effectiveness of Brazilian green propolis (EPP-AF®) as an adjuvant treatment in patients hospitalized with COVID-19.</td>
<td>• Reduction in the hospitalization time.</td>
<td>• This trial was open.</td>
<td>Silveira et al. (2021)</td>
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<td></td>
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<td>• Possibly, administration early in the disease course would have an even greater benefit in reducing the disease’s impact. There was also renal protection in COVID-19 patients.</td>
<td>• The patients were followed for only a short period</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>• Propolis can affect various disease mechanisms that are relevant to SARS-CoV-2 infection.</td>
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<td></td>
</tr>
<tr>
<td>NI</td>
<td>Iran</td>
<td>25 individuals used propolis plus Hyoscyamus niger L. in randomized and controlled-placebo clinical trial.</td>
<td>Evaluate the effect of a syrup formulation in patients with clinical symptoms of acute respiratory syndrome with suspected COVID-19.</td>
<td>• The results of this study showed that the symptoms of COVID-19 were reduced by the administration of extract of Hyoscyamus niger L. plus propolis.</td>
<td>• This study includes the period until the onset of symptoms of the disease is uncontrolled, and the speed of its progression can be variable.</td>
<td>Kosari et al. (2021)</td>
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<td></td>
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<td></td>
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<td>• It is uncertain whether the treatment would be effective in hospitalized patients.</td>
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</table>

for the binding site of ACE2 and can prevent or delay the entry of SARS-CoV-2 into the cell. Thus, rutin and other flavonoids have prophylactic capacity as inhibitors and competitors of ACE2 (Güler et al., 2020).

### 3.3.5 Interaction of the compound Artepillin C with eukaryotic protein phosphatase 2A (PP2A)

Artepillin C could be a potential drug to exert immunomodulatory activity and reduce the inflammation caused by COVID-19. Maaroufi (2020) analyzed the interactions between PP2A, involved in the regulation of pro-inflammatory responses during infections by pathogens, of SARS-CoV-2 and the compound artepillin C, which is found exclusively in Brazilian green propolis (Marcucci et al., 2001; Park et al., 2004). The results of molecular docking demonstrated that artepillin C could compete with the virus to bind to PP2A-B56 (Maaroufi, 2020). This suggests, according to the anti-inflammatory effect of artepillin C, that it could activate, in vivo, PP2A-B56 (Maaroufi, 2020).

### 3.3.6 Interaction of the components and spike protein of SARS-CoV-2

All propolis components had reasonable binding affinity against spike protein when compared to avigan, hydroxychloroquine and remdesivir. In addition, rutin had the highest affinity among propolis compounds, suggesting it as a lead compound for further ligand optimization cycle (Refaat et al., 2021).

### 3.3.7 Anti-COVID-19 effects

Elwakil et al. (2021) carried out a molecular docking study to predict the binding affinity of compounds from different extracts of Egyptian propolis to RNA-dependent RNA polymerase, spike protein S1 and Mpro. Most of the tested fractions had promising binding scores in relation to the reference antiviral drugs (lopinavir and umifenovir). Güler et al. (2020) showed that four compounds (pinocembrin, chrysin, CAPE and hesperetin) showed very low binding energies to the ACE2 receptor and the SARS-CoV-2 RBD peak protein. These four flavonoids were also found to have higher affinity than hydroxychloroquine, used as a standard ligand, which was described COVID-19 drug.

Finally, Harisna et al. (2021) investigated the molecular interaction of the bioactive compounds of propolis against Mpro and the spike protein SARS-CoV-2 by molecular docking. The main compounds with the greatest potential to inhibit Mpro from the new coronavirus were methylphiopogonone A, 3’-methoxy daidzein and genistin. As for the spike protein, 4 compounds (neoblavaisoflavone, methylofiopogonone A, 3’-methoxy daidzein and genistin) showed less binding affinity energy than pravastatin (control). However, only neoblavaisoflavone and methylphiopogonone A are able to be absorbed by the gastrointestinal tract.

### 3.3.8 In vitro evaluation

Güler et al. (2021) tested five different concentrations of hesperetin, CAPE and pinocebrine by the ELISA assay. The half maximal inhibitory concentration (IC₅₀) value of commercial propolis was 3 times higher than the sample prepared manually. Hesperetin is the best inhibitor against SARS-CoV-2 spike protein and ACE2 receptor, which has the lowest IC₅₀ value (16.88 mmol/L), followed by pinocembrin and CAPE. In addition, pinocembrin had a high affinity by molecular target according to in silico studies, while hesperetin was more active in the in vivo study.

Refaat et al. (2021) also evaluated the potential for inhibition of Mpro by propolis components in vitro. The results showed that the propolis extract had a good inhibitory effect against Mpro (IC₅₀ = 2.452±0.11 µg/mL).
3.3.9 Clinical trials

Silveira et al. (2021) conducted a study using standardized propolis extract in hospitalized adult patients with COVID-19. As a result, although not relevant to the need for oxygen supplementation, showed a significant improvement in the clinical status of patients, categorized as a reduction in the length of hospital stay. The group treated with a dose of 800 mg/day showed a median of 6 days (5-11), followed by the group that used a dose of 400 mg/day of 7 days (5-12), compared with the control group with a median of 12 days (8-16) for standard treatment alone. The addition of oral propolis to standard care procedures was safe. No patient had propolis treatment interrupted due to side effects (Silveira et al., 2021).

Kosari et al. (2021) also evaluated the therapeutic effects of propolis plus Hyoscyamus niger L. in patients with acute respiratory syndrome suspected to COVID-19. As a result, after 6 days of intervention, shows a decreasing trend in the clinical symptoms of COVID-19. This finding shows the effectiveness of propolis for the patient’s clinical improvement (Kosari et al., 2021).

Fiorini et al. (2021) showed a case study with improved significantly of patient’s general clinical condition, and she recovered, with an negative RT-PCR test result, after consuming three times of a standardized dose of propolis for 12 days in a 52-year-old woman who tested positive for COVID-19 and with mild symptoms.

3.4 Safety of propolis use

Crude propolis is not suitable for human consumption, because it is highly viscous and hydrophobic. However, its 60-80% dilution in ethyl alcohol results in an extract rich in most of its bioactive polyphenols (Ali & Kunugi, 2020; Güler et al., 2020).

Despite the absence of adverse effects in animals and humans, few cases of allergy and contact dermatitis related to the use of propolis were described (Oryan et al., 2018). The removal of allergens from propolis may be necessary to avoid possible adverse effects (Aliboni et al., 2011). The contact dermatitis with propolis is less frequent in children than adults (Francuzik et al., 2019). Reactions may occur due to some allergens, such as 1,1-dimethyl-salicylate, benzyl and benzyl-cinnamate (Pereira & Bártolo, 2016).

This substance has no cytotoxic and genotoxic effect (Cardoso et al., 2016; Conti et al., 2016; Rocha et al., 2013). In addition, these compounds have possible genoprotective effects, reducing damage to DNA in mice (Kumari et al., 2017). Fikri et al. (2019) showed that a low dose of propolis during the gestation of mice did not alter the fetal development parameters.

Silveira et al. (2019) and Fukuda et al. (2015) reported that the use of long-term propolis is safe and well tolerated (226.8 mg/day for two months) both by diabetic and non-diabetic patients, reducing proteinuria, lipids, total creatinine and bilirubin (2019). Moreover, there were no changes in the liver, in fasting glycemia and lipids after administration of propolis in patients infected with HIV (Ripari et al., 2021).

3.5 Anti-inflammatory property and COVID-19

The activation of PAK1 can cause pulmonary fibrosis (Maruta & He, 2020), which is an aggravating factor in patients with COVID-19. Xu et al. (2005) demonstrated that CAPE can inactivate RAC and, consequently, inhibit PAK1. In addition, PAK1 contributes to the suppression of B and T cells, and a normal immune response of patients (Maruta & He, 2020). In addition, several types of flavonoids and prenylated phenylpropanoids, such as baccharin, drupanin and artepillin C, can inactivate PAK1, which the last one is a selectively inhibitor (Messerli et al., 2009).

CAPE is a potent inhibitor of NF-kB activation in myelo-monocytic cells (Shimizu et al., 2011). Propolis can modulate the function of different cells of the adaptive and innate immune system, such as macrophages, neutrophils, natural killer (NK) cells and lymphocytes, increasing their activity and mechanisms to fight infectious agents (Sforcin, 2007, 2016). Propolis
stimulated the generation of reactive oxygen species (ROS), the expression of toll-like receptor (TLR)-2 and TLR-4, production of pro-inflammatory cytokines and bactericidal and fungicidal activity, suggesting that propolis may activate mechanisms involved in the death of microorganisms (Bachiega et al., 2012; Orsatti et al., 2010).

In in vivo and in vitro studies, propolis was able to inhibit the production of interleukin (IL) -17, IL-1β, interferon gamma (IFN-γ), IL-2, IL-6, IL-10 (Okamoto et al., 2012; Ripari et al., 2021), in addition to increasing the polarization of Th17 to CD4⁺Foxp³ cells (Piñeros et al., 2020). Propolis was able to reduce inflammation, decrease mucus production, the total count of immune, eosinophilic cells and macrophages in bronchoalveolar fluid, IL-5, and the gene expression of IL-13 in the lungs (Piñeros et al., 2020). Governa et al. (2019) described, in an in vitro study against the H1N1 virus, that propolis was able to stimulate pro-inflammatory cytokines (IL-6 and IL-1β).

Moreover, Bufálo et al. (2014) demonstrated that propolis was able to exert an immunomodulatory effect on the expression of cell receptors, production of cytokines and fungicidal activity of human monocytes. Propolis modulates the maturation and function of DCS and can be useful in the early stages of the immune response (Conti et al., 2016).

Naringin exhibited the highest binding affinity (-9.8 kcal/mol), higher than dexamethasone (-7.9 kcal/mol), a potent anti-inflammatory used to treat patients with COVID-19 in a critical state (Jain et al., 2021). Quercetin was superior to kaempferol, myricetin and synthetic indole-chalcone of -7.8 kcal/mol (Vijayakumar et al., 2020). In addition, rutin has higher binding affinity than hydroxychloroquine, while all other dissipated compounds with higher binding affinity than Avigan (Refaat et al., 2021).

Artepillin C was able to interact via hydrogen bonds, with a higher binding affinity than that of the protein S peptide, suggesting that artepillin C could compete with SARS-CoV-2 to bind to PP2A-B56, and can inhibit acute inflammation associated with COVID-19 (Maaroufi, 2020).

Artepillin C showed inhibitory effect on the binding of LxxlxE-like motifs, which exist in S1 and S2 subunits of S protein, to PP2A-B56 of host cells. Artepillin C can regulate cell function and provide protection against the SARS-CoV-2 induced cytokine storm (Ali & Kunugi, 2021; Maaroufi, 2020). Likewise, the inhibition of deubiquitinating (DUB) activity of papain-like protease (Plpro) by rutin, both in vitro as in silico, is likely to alter the inflammatory activity of this enzyme (Pitsillou et al., 2020).

In summary, propolis and their components have a potent immunomodulatory effect, which can be observed as an inhibitory or stimulatory activity, affecting various cells and components of the immune/inflammatory response, such as adhesion and transmigration of neutrophils, cytokines, chemokines, C-reactive protein, PGE2 and signaling pathways. As propolis is capable of exerting pro and anti-inflammatory activities, it is suggested that clinical trials could be carried out to investigate the effectiveness of propolis and their components in individuals with COVID-19.

### 3.6 Effect of propolis and their components in the treatment of COVID-19

In silico studies suggest that the use of propolis’s flavonoids may be a viable therapeutic option in the treatment of COVID-19 or can be used as lead compounds for ligand optimization. Since they can cleavage of protein S by host cell proteases, for example, TMPRSS2 (Kumar, Dhanjal, Bhargava, et al., 2020), binding of protein spike to cell surface receptors, such as ACE2 (Güler et al., 2020; Refaat et al., 2021), inhibiting protein spike (Jain et al., 2021; Refaat et al., 2021) and/or binding of protein spike to the inflammatory in PP2A-B56 (Maaroufi, 2020). In addition to interfering with predicted non-structural proteins (NSPs) of SARS-CoV-2, in order to prevent viral replication (da Silva et al., 2020). Therefore, these flavonoids can decrease the chances of the virus entering the host cells and decreasing the viral load, as well as the inflammatory reaction after infection (Ali & Kunugi, 2021).
Kumar et al. (2020) observed that the CAPE has affinity to TMPRSS2 involved interactions of hydrogen bonds with two main amino acid residues, in addition to pi-pi interactions with other residues. The inhibitory effect of the propolis compound was better than withaferin, withanine and camostat mesylate, which all had a hydrogen bonding interaction with only one residue.

Luteolin expressed a binding affinity (-10.1 kcal/mol) higher than hydroxychloroquine (-7.7 kcal/mol), camostat mesylate (-9.0 kcal/mol) and remdesivir (-10.0 kcal/mol) (Shawan et al., 2021). Another study showed that rutin, CAPE, myricetin, quercetin, pinocembrin and hesperetin expressed a binding affinity for ACE2 higher than the reference molecule (MLN-4760) (Güler et al., 2020).

Preliminary results address an inhibitory activity of flavonoids on NSPs of SARS-CoV-2 (da Silva et al., 2020; Hashem, 2020; Refaat et al., 2021). CAPE, chrysin, caffeic acid and galangin bound to NSPs with high binding affinity (Hashem, 2020).

Rutin had a stronger binding affinity with NSPs than hydroxychloroquine (Refaat et al., 2021). Likewise, rutin formed stable intramolecular bonds with M\textsuperscript{PP} in a high affinity, comparable to that of teaflavine-3-3 (Shivanika et al., 2020). Rutin, comparable to remdesivir, showed the higher affinity with catalytic sites through hydrogen bonds and electrostatic interactions (Arora et al., 2020). In addition, it showed higher inhibitory potential to papain-like protease (PLpro) (Pitsillou et al., 2021).

Da Silva et al. (2020) showed that the affinity of nicotiflorin and rutin binding to protease was close to that of the positive control, while its affinity for RNA-dependent RNA polymerase (RdRp) was better than teaflavine. The binding affinity of rutin metabolites to 3-chymotrypsin like protease (3CLpro) was better than rutin, and its binding to RdRp was also high, while nicotiflorin derivatives expressed the highest binding affinity of all kaempferol glucuronides to RdRp was high. Kaempferol and quercetin were the least potent inhibitors of 3CLpro and RdRp among nicotiflorin and rutin derivatives. Most of the inhibitory effects of all compounds involved hydrogen bonds and pi interactions with 3CLpro and RdRp protein residues (da Silva et al., 2020). Vijayakumar et al. (2020) showed that several flavonoids and synthetic indole chalcones into the active sites of M\textsuperscript{PP}, quercetin was the second-best inhibitor candidate (-9.2 kcal/mol) following C23 indole-chalcone (-10.4 kcal/mol).

Among the 22 compounds found in Indonesian propolis, broussoflavonol F and glucosperin A had a higher affinity for binding to M\textsuperscript{PP} than the affinity for potent β-coronavirus inhibitors (Sahlan et al., 2021). A derivative of podophyllotoxin compounds known as sulabiroin A inhibited M\textsuperscript{PP} through a hydrophobic interaction with His41. In general, flavonoids can inhibit NSPs of SARS-CoV-2 and decrease viral replication (da Silva et al., 2020; Dewi et al., 2021; Hashem, 2020; Pitsillou et al., 2021; Refaat et al., 2021; Sahlan et al., 2021).

In an in vitro study, Vero E6 cells were infected with SARS-CoV-2 and treated with naringin. Naringin treatment inhibited SARS-CoV-2 infection and improved the cytopathic effects of the virus compared to control. In addition, naringin exhibited a strong inhibition of human coronavirus replication (Clementi et al., 2021). The rutin showed inhibitory activity of PLpro (Pitsillou et al., 2021) and DUB activity (Pitsillou et al., 2020). Propolis extract and propolis liposome were able to inhibit the enzymatic activity of 3CLpro. Moreover, beyond propolis liposome could be more active than propolis extract, it had an inhibitory effect similar to remdesivir as well (Ali & Kunugi, 2021).

Docking methods can exhibit variability in their ability to reproduce crystallographic pose, indicating that the results on the binding affinity of these methods may not be directly compared (Perola et al., 2004). However, similar data were found among the in silico studies, suggesting that ellagic acid, p-coumaric acid, CAPE, kaempferol, naringin, luteolin, rutin, quercetin and naringin may be inhibitors of the molecular targets of SARS-CoV-2 (Ali & Kunugi, 2021; Shaldam et al., 2021). Rutin showed the highest potential for inhibition of protein spike, ACE2 and several NSPs of the virus that caused COVID-19 (da Silva et al., 2020; Pitsillou et al., 2021; Refaat et al., 2021).

The effect of rutin was stronger than that of avigan and hydroxychloroquine (Arora et al., 2020; Refaat et al., 2021). Likewise, the inhibitory effect of luteolin against NSPCs was comparable to camostat mesylate and remdesivir (Shawan et al., 2021).
CAPE was able to inhibit TMPRSS2, ACE2 and Mpro (Güler et al., 2020; Hashem, 2020; Refaat et al., 2021), while quercetin inhibited protein S and Mpro in an affinity higher than avigan (Refaat et al., 2021). Rutin had a considerably higher binding affinity than quercetin against RdRp and Mpro of SARS-CoV-2 (da Silva et al., 2020). In addition, naringin inhibited spike protein (Jain et al., 2021), decreased viral load and cytopathic effects in Vero E6 cells (Ali & Kunugi, 2021; Clementi et al., 2021).

Likewise, clinical trials report early viral elimination, faster symptom recovery and reduced hospital stay in patients with COVID-19 who receive whole propolis extracts (Silveira et al., 2021).

Vardhan and Sahoo (2020) in in silico study, reported that limonene was the most active compound against the relevant targets (RdRp, ACE2 and peak glycoproteins), but quercetin and kaempferol also obtained high docking scores. Kaempferol can also inhibit TMPRSS2 (Da et al., 2019), potentially interacting with ACE2, RdRp and peak glycoprotein. As shown through in silico studies, Osés et al. (2020) observed a strong inhibition of ACE2 (higher than 90%) of various types of propolis. The best results were with catechin and p-coumaric acid.

3.7 Limitations and prospects

It is important to note that we have included two preprints, which have not been subjected to any form of peer review. In some cases, essential information was missing, such as the nature of the interactions of flavonoids with receptors, amino acid residues and residues involved in the interaction of artepillin C with PP2A, beyond property evaluations of several docking methods. In vitro, in vivo and clinical trials are very few and, therefore, more elaborate studies are needed to confirm the efficacy of propolis in patients with COVID-19. It was not possible to perform a meta-analysis of clinical trials because clinical trials are underway.

In general, the methodologies of Structure Based Drug Design (SBDS) are the first tools which have applied into the drug development context. These tools have the principal advantage of decreasing the cost of the process of development as a whole by their practicality and low computational cost (except for molecular dynamics simulations). Thus, these methodologies can search for hits in large data collections of ligands, useful to enrich the high-throughput screening (HTS) campaign, beyond to describe the main forces for intermolecular recognition between molecular targets and ligands. These findings address experimental, semi-synthesis, synthesis total and biological assays studies. On the other hand, as disadvantage, several approximations are applied in the models, beginning with the limitations of molecular mechanics methods, which require an extensive parametrization process. In other words, docking, the principal SBDS method used, is structure dependent, which there is no such tool robust enough to cover all enzymatic diversity. Consequently, evaluation methods have been required to avoid the obtention of false positive results. Finally, propolis has the advantage of natural products, with several bioactive compounds in its composition, requiring further steps of drug development such as ligand optimization.

4. Final Considerations

Brazilian propolis was the only one mentioned in clinical studies that aim and prove the effectiveness of its benefits against COVID-19. Anatolian propolis and Egyptian were tested in vitro. In the docking studies, the compounds present in different types of propolis were evaluated, mainly Egyptian and Sulawesi propolis. It was not possible to make a comparison between the types of propolis. Thus, it is suggested that particularities of studies be carried out with the different propolis, both in pre-clinical and clinical studies, in order to evaluate which shows the best results.
In *sìlico* studies of the propolis showed potential anti-inflammatory, immunoregulatory and anti-COVID-19 effects, including PAK-1 inhibition. In addition, binding to ACE2, one of the main routes of infection for SARS-CoV-2, can be inhibited by propolis. Propolis compounds, rutin and CAPE specially, demonstrated a strong interaction with ACE2 and TMPRSS2.

In clinical studies, one study demonstrated the effectiveness of propolis in reducing the length of stay in hospitalized patients, while the other showed an improvement in the patient’s clinical condition. In addition to these observed effects, there were no reports of adverse events in the clinical studies found.

The return to normal life, but with the risk of contamination by COVID-19, brings the need for drugs that can prevent and treat this disease. In this way, the findings of this systematic review demonstrate that, collectively, there is scientific evidence to confirm that propolis is effective in treating COVID-19. Health professionals should recommend and encourage evidence-based pharmacological treatment. The incentive to health must also follow official guidelines, as well as promote these practices with patients, avoiding self-medication.

The authors suggest that future research involving propolis and its components in COVID-19 be directed towards randomized controlled clinical trials, in addition to comparing the different types of propolis.

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