Propolis effects in periodontal disease seem to affect coronavirus disease: a meta-analysis

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Abstract: This meta-analysis aimed to investigate the effects of propolis on the severity of coronavirus disease symptoms by reducing periodontal disease. PubMed, EMBASE, SciELO, Web of Science, and SCOPUS databases were systematically searched. Studies have been conducted analyzing propolis's effects on COVID-19 and periodontitis. The study was conducted according to the PRISMA statement and registered in PROSPERO. Risk of Bias (RoB) assessment and meta-analysis of clinical studies were performed (Review Manager 5, Cochrane). The certainty of the evidence was assessed using GradePro (GDT). Studies have shown propolis flavonoids inhibit viral replication in several DNA and RNA viruses, including coronaviruses. Propolis components have an aminopeptidase inhibitor activity that can inhibit the main proteases of SARS viruses and seem to inhibit protein spikes, which are sites of most mutations in SARS-CoV strains. The meta-analysis showed favorable results with the use of propolis on probing depth (95%CI: 0.92; $p < 0.001$), clinical attachment level (95%CI: 1.48; $p < 0.001$), gingival index (95%CI: 0.14; $p = 0.03$), plaque index (95%CI: 0.11; $p = 0.23$), and blending on probing (95%CI: 0.39; $p < 0.001$). The antibacterial activity of propolis could be mediated through its direct action on microorganisms or the stimulation of the immune system, activating natural defenses. Thus, propolis inhibits the replication of SARS-CoV-2 as well as its bacterial activity. Treatment with propolis improves general health and facilitates the activation of the immune system against coronavirus.

Keywords: COVID-19; Propolis; Periodontal Diseases; Periodontitis.

Introduction

Coronavirus disease (COVID-19) is an infectious disease caused by a novel coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which affects the respiratory and hematological systems. SARS-CoV-2 is a single-stranded RNA virus with an envelope similar to that of coronaviruses, causing severe acute respiratory syndrome and Middle East respiratory syndrome, which mainly causes respiratory and enteric diseases. The primary clinical symptoms of COVID-19 are fever, dry cough, sore throat, body pain, diarrhea, anosmia, and ageusia.[1]

In the absence of specific drugs to treat COVID-19, there is an urgent need to find alternative approaches to prevent and control the spread of the virus. Bioactive substances, such as propolis, may play an essential role in combating COVID-19. Studies have shown that the pathogenesis of COVID-19 can be minimized by propolis components that act on the functional activity of the main proteases of SARS-CoV-2.[2,3]

Propolis is a balsamic and resinous product containing different plant parts and molecules secreted by bees.[4,5] It is composed of a mixture of 50% vegetable resins, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% other organic substances, including polyphenols, flavonoids, amino acids, minerals, ethanol, vitamin A, vitamin B complexes, and vitamin E. The chemical composition, aroma, and color of propolis vary according to geographic region and can be found in several types. The most common are green, red, and brown ones.[4,5]

The biological activity of propolis is attributed to various chemical constituents that block or reduce the chance of viral entry into the host cells.[4,5] However, the precise mechanism underlying this antiviral activity remains unknown. Among the proposed hypotheses, the one that stands out suggests that propolis destroys the external envelope of the virus, thereby inhibiting its entry into cells and interrupting viral replication.[6] Propolis has anti-inflammatory, antiviral, antioxidant, and immunoregulatory effects. Its components have an inhibitory effect on angiotensin-converting enzyme 2 (ACE2), transmembrane protease, serine 2 (TMPRSS2), main protease (M^{PRO}), and serine/threonine-protein kinase PAK 1 (PAK1) signaling pathways, all of which could contribute to reducing the pathophysiological consequences of COVID-19.[7,8]

A recent study showed an association between the severity of COVID-19 and periodontal inflammation since the latter causes an increase in markers such as D-dimer, white blood cell count, and C-reactive protein level, which are also related to the severity of COVID-19.[9] Regardless of its association with chronic non-communicable diseases, periodontal disease has been shown to affect systemic health, especially cardiovascular disease, diabetes, and hypertension.[10-13] Periodontitis is one of the most prevalent oral inflammatory diseases.[14] It is characterized as a multifactorial chronic inflammatory disease associated with biofilm dysbiosis and the progressive destruction of dental insertion.[15,16]

Currently, there are no antiviral therapeutic drugs available for COVID-19 in humans. Thus, in the absence of specific antiviral agents against COVID-19, the reuse of drugs previously used to reduce viral infectivity and replication and improve the immune system has become an alternative that should be investigated. Thus, the present study aimed to highlight the role of propolis in reducing the severity of COVID-19 symptoms by reducing periodontal disease.

Methodology

This systematic review was reported according to Cochrane, the National Health Service Centre for Reviews and Dissemination,[17] and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA statement 2020) guidelines.[18] The study protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO-CRD42021233759). The eligibility criteria for the participants, interventions, comparisons, outcomes, and study design were defined in accordance with the PRISMA statement. The following questions were raised in this systematic review: a) Does propolis affect the treatment of periodontal diseases? b) Does propolis exert an antiviral action against SARS-CoV-2? c) Can treating periodontal disease with propolis positively influence COVID-19 patients?

This study presented a systematic review of the effect of propolis on periodontitis and considered a comprehensive review of the impact of propolis on coronavirus. These two searches were necessary because of the lack of studies that addressed the direct relationship between periodontal disease and COVID-19, using propolis as a therapeutic agent.

Search strategy and selection criteria

PubMed, EMBASE, Web of Science, SciELO, and SCOPUS databases were systematically searched using the following critical medical subject headings terms using a two-search strategy: ("Propolis" AND ["Periodontal disease" OR "Periodontitis"]) and ("Propolis" AND ["COVID-19" OR "SARS-CoV-2"]).

Furthermore, a manual search was performed, and reference lists and previous systematic reviews were reviewed to identify other potentially relevant studies. The final search was conducted on September 28, 2021.

According to our selection criteria, we included only studies published in peer-reviewed journals. The studies were divided into two domains: the first considered the relationship between periodontal disease and propolis, and the second considered the relationship between COVID-19 and propolis. In the first domain, we included case reports, case series, and clinical studies that investigated the treatment of periodontal disease using propolis. For the second domain, we included all studies that investigated the relationship between COVID-19 and propolis because of the small number of published studies on this subject. Duplicate publications and articles that did not meet the objectives of the systematic review were excluded. Figures 1 and 2 outline the procedure used in the literature search.

Data extraction

Two investigators participated in each phase of the review and independently screened the titles and

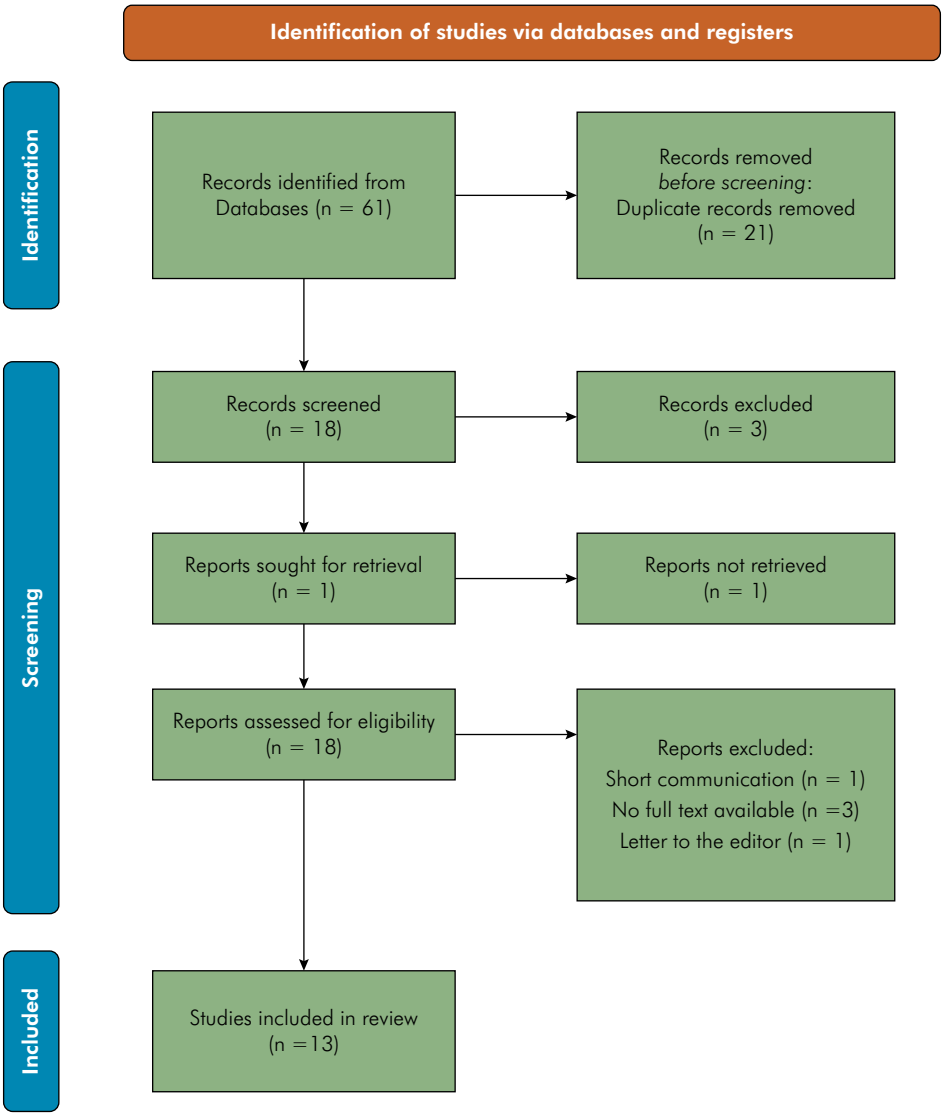

Figure 1. Prisma 2020 Flow Diagram for Propolis and COVID-19 search.

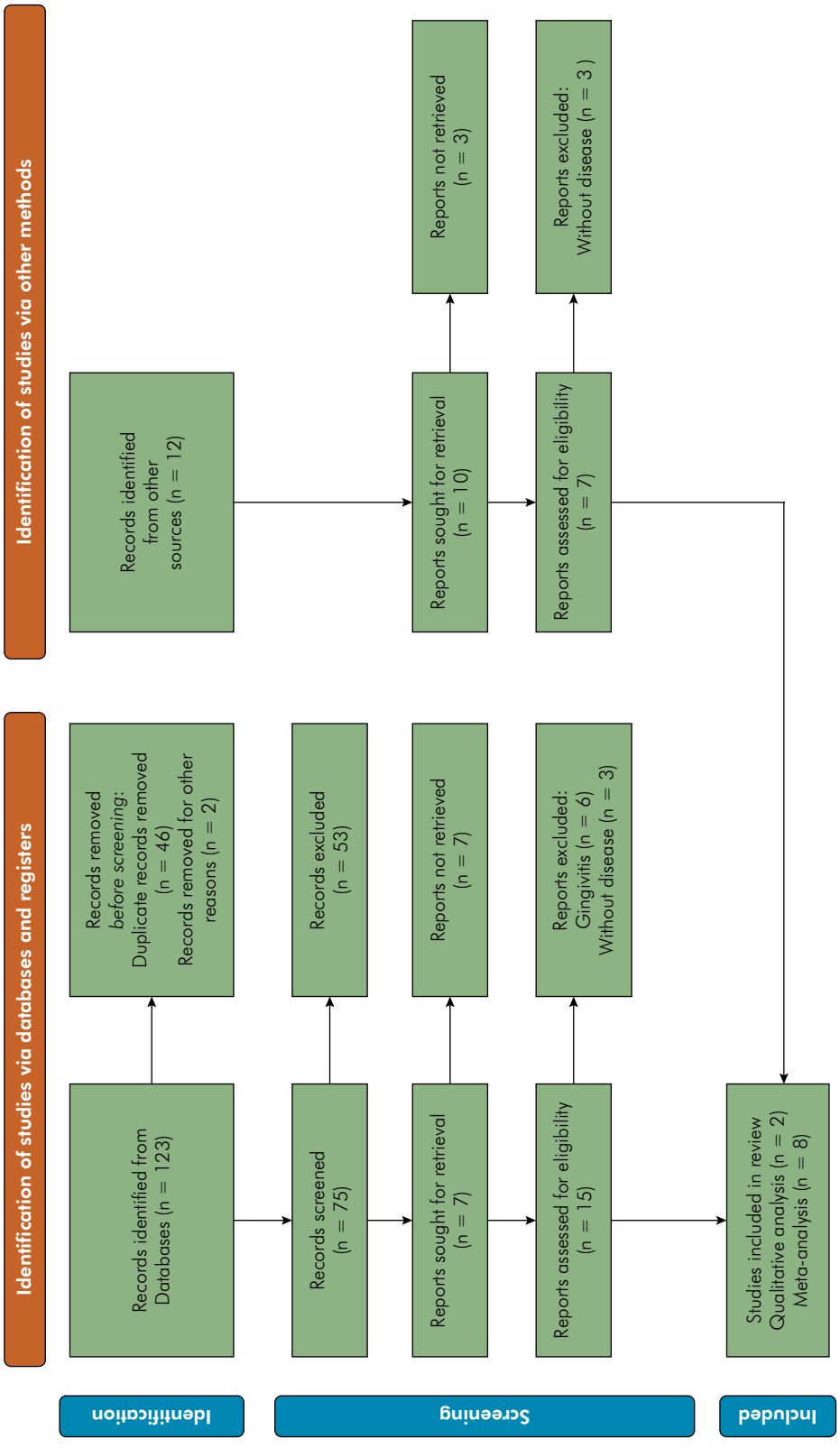

Figure 2. Prisma 2020 Flow Diagram for Propolis and periodontitis search.

abstracts of the articles listed in the search based on our inclusion criteria. The investigators screened full-text reports to determine whether they met the inclusion criteria. Any discrepancies between reviewers were resolved through discussion and consensus, and a senior investigator reviewed the final results. The following data were extracted from the included articles: a) study design, sample (size, case definition, and age), intervention, control groups, methodology evaluation, and results; and b) study information, propolis components, properties, and mechanism of action.

Quality appraisal and risk of bias

The selected randomized and non-randomized clinical trials were appraised using appropriate tools for quality and risk of bias assessments for each study design. Non-randomized studies were assessed using the Methodological Index for Non-Randomized Studies (MINORS),[19] and randomized clinical trials were evaluated using the Jadad scale.[20]

The Jadad quality scale considers five items, each of which may vary in score from 0 to 5. Studies with scores ≤ 2 points were considered to be of poor methodological quality, whereas those with a score of ≥ 2 were considered good quality. The MINORS consider eight items for non-comparative studies and 12 for comparative studies. These items can be scored from 0 to 2 points each, with maximum total scores of 16 and 24 points for the comparative and non-comparative studies, respectively.

The Revised Cochrane risk-of-bias tool for randomized trials (RoB-2)[21] and the risk of bias in non-randomized studies[22] were used to assess the risk of bias (ROB) in randomized and non-randomized studies, respectively. The Review Manager (RevMan) software (Cochrane Collaboration) was used to create the ROB graph and summary. The results were categorized as: a) low risk of bias, b) unclear risk of bias, and c) high risk of bias. The certainty of the evidence was performed on GradePro (GDT) based on the study design, risk of bias, inconsistency, indirectness, imprecision, other covariates (confounding and publication bias), and the results of the study (number of patients and Confidence Interval, CI). Certainty was classified as high, moderate, low, or very low.

Summary measures

Periodontal disease × propolis studies were categorized based on sample characterization, exposure, comparators, and outcomes. For COVID-19 studies, the highlights in each study were selected, grouped according to similarity, and presented in the form of a table. The data on clinical periodontal parameters were analyzed and grouped by two researchers and tabulated by one researcher. Another researcher reviewed the tabulated data, thereby decreasing the likelihood of disagreement.

Data synthesis and meta-analysis

The meta-analysis was performed using the Cochrane Collaboration software Review Manager (RevMan) version 5.2. The effects of propolis use in patients with periodontitis were estimated using the mean differences and 95% confidence intervals (CIs) for each clinical parameter. Statistical heterogeneity was assessed using the chi-squared test ($p < 0.1$) and the calculation of the I2 statistic. We considered an I2 value > 75% to indicate significant heterogeneity across the studies.[23] A random-effects model was adopted for all meta-analyses to reduce potential heterogeneity because the studies had different characteristics, sample sizes, and group data. A funnel plot was used for all the analyses.

Results

Database search

The process used for selecting studies in the systematic review is outlined in Figures 1 and 2. We identified 61 articles related to ("Propolis" AND ["COVID-19" OR "SARS-CoV-2"]) using this strategy to search all the scientific databases. After removing unrelated and duplicate articles, 18 cases were assessed for eligibility, and 13 articles were identified as eligible for qualitative analysis. Similarly, we identified 123 studies related to ("Propolis" AND ["Periodontal disease" OR "Periodontitis"]) and

12 from other sources. After removing duplicates and unrelated articles, 22 cases were assessed for eligibility, and 10 studies were identified as eligible for qualitative analysis. Eight of the ten final studies were included in the quantitative analysis.

Sample characteristics

Clinical studies assessing the effect of propolis on periodontitis and studies reporting its impact on COVID-19 symptoms were considered for analysis. Nine randomized clinical trials and one non-randomized clinical trial (n = 328 patients) demonstrated propolis's effectiveness in treating periodontitis. Thirteen studies reported the mechanism of action of propolis against coronaviruses. The characteristics of the studies are presented in Tables 1 and 2.

Propolis components, properties, and mechanism of action

The properties of the propolis components and their mechanisms of action against SARS-CoV-2 and COVID-19 are summarized in Table 2.

Clinical outcomes of periodontal treatment with propolis

The periodontal outcomes assessed after propolis treatment are shown in Table 3. Five clinical parameters presented in the articles were grouped and analyzed for meta-analysis (Figure 3a-e). Probing pocket depth (PPD) data were grouped from eight studies, Clinical Attachment Level (CAL) data were grouped from seven studies, Gingival Index (GI) data were grouped from five studies, and Plaque Index (PI) and Blending on Probing (BOP) data were grouped from four studies. None of the other parameters could be grouped for quantitative analysis by the authors. Clinical parameters were reported to improve following propolis therapy in all studies included in our review.

Probing pocket depth (PPD)

Studies analyzed PPD data after treatment with (n = 132) or without (n = 134) propolis for periodontitis. The observed mean difference ranged from 0.15 to 2.53. The results were based on the random-effects model was -0.92 (random; 95%CI: -1.57, -0.26; Figure 3a). According to the Q-test, the true outcomes appeared to be heterogeneous ($Tau^2 = 0.76$, $Chi^2 = 92.35$, df = 7 [$p < 0.00001$], $I^2 = 92$ %). (Figure 3a).

Clinical attachment level (CAL)

Studies analyzed clinical attachment level data after treatment with (n = 115) or without (n = 117) propolis for periodontitis. The observed mean difference ranged from 0.15 to 3.67. The results based on the random-effects model were -1.48 (random; 95%CI: -2.40, -0.55; Figure 3b). According to the Q-test, the true outcomes appeared to be heterogeneous ($Tau^2 = 1.42$, $Chi^2 = 153.7$, df = 6 [$p < 0.00001$], $I^2 = 96$ %). (Figure 3b).

Gingival index (GI)

Studies analyzed gingival index level data after treatment with (n=94) and without (n = 96) propolis for periodontitis. The observed mean difference ranged from 0.10 to 0.29. The results based on the random-effects model were -0.14 (random; 95%CI: -0,30, 0.01; Figure 3c). According to the Q-test, true outcomes appeared to have low heterogeneity ($Tau^2 = 0.02$, $Chi^2 = 10.43$, df = 4 ($p < 0.03$), $I^2 = 62$ %). (Figure 3c).

Plaque index (PI)

Studies analyzed plaque index level data after treatment with (n=74) and without (n = 76) propolis for periodontitis. The observed mean difference ranged from 0.00 to 0.28. The results based on the random-effects model was -0.11 (random; 95%CI: -0,26, 0.03; Figure 3d). According to the Q-test, the true outcomes appeared homogeneous ($Tau^2 = 0.01$; $Chi^2 = 4.27$; df = 3 ($p < 0.23$); $I^2 = 30$ %) (Figure 3d).

Blending on probing (BOP)

Studies analyzed the blending of probing data after treatment with (n = 67) and without (n = 67) propolis for periodontitis. The observed mean difference ranged from 0.05 to 2.22. The results were based on the random-effects model was -0.39 (random; 95%CI: -0.73, -0.05; Figure 3e). According to the Q-test, the true outcomes appeared to be heterogeneous ($Tau^2 = 0.08$, $Chi^2 = 20.67$, df = 3 [$p < 0.0001$], $I^2 = 85$ %). (Figure 3e).

Table 1. Sample and intervention characterization for clinical studies assessing propolis effects in periodontitis treatment.

Study	Design	Total (group)	Age (range)	Intervention	Control	Quality assessment
El-Sharkawy et al., 2016[24]	Randomized Clinical Trial	Patients with Moderate to severe chronic periodontitis 50 (n=24;26)	38–63	Scaling and root planning + capsule 400 mg propolis	Scaling and root planning + capsule placebo	2 (JADAD)
Kirti et al., 2017[25]	Randomized Clinical Trial	Patients with Chronic Periodontitis 45 (n=15)	30–55	Scaling and root planning followed by subgingival irrigation with Propolis Platinum Scaling and root planning followed by subgingival irrigation with PerioGard®	Scaling and root planning followed by subgingival irrigation with Normal Saline	5 (JADAD)
Kumar et al., 2015[26]	Randomized Clinical Trial	Patients with Chronic Periodontitis 40 (n=20)	35–55	Commercial toothpaste with propolis	Commercial toothpaste with aloe-vera	3 (JADAD)
Nakao et al., 2020[27]	Randomized Clinical Trial	Patients with Chronic Periodontitis 24 (n=6)	–	Propolis Curry leaf Minocycline	Placebo 1% ethanol	5 (JADAD)
Pundir et al., 2017[28]	Randomized Clinical Trial	Patients with Chronic Periodontitis 30 (n=15)	25–55	20% propolis hydroalcoholic solution, 24 h after scaling and root planning	Scaling and root planning	4 (JADAD)
Sanghani et al., 2014[29]	Randomized Clinical Trial	Patients with Chronic Periodontitis 40 (n=20)	25–50	Scaling and root planning followed by subgingival placement of Indian propolis	Scaling and root planning	4 (JADAD)
Shohdy et al., 2020[30]	Randomized Clinical Trial	Patients with Chronic Periodontitis 30 (n=10)	-	Propolis and chitosan polymer gel Propolis and polyox polymer gel	Non surgical therapy	3 (JADAD)
Sparabombe et al., 2019[31]	Randomized Clinical Trial	Patients with severe or moderate periodontitis 34 (n=17)	20–65	Mouthwash containing Propolis resin extract (1:3), Plantago lanceolata leaves extract (1:10), Salvia officinalis leaves extract (1:1) and 1.75% of essential oils from Salvia officinalis, Syzygium aromaticum buds, Mentha piperita leaves, Commiphora myrrha oleoresin and Pistacia lentiscus oleoresin	Aqueous mouthwash	3 (JADAD)
Sreedhar et al., 2017[32]	Randomized Clinical Trial	Patients with Chronic Periodontitis 15 (split mouth)	35–53	Irrigation with 3mL of 30% Propolis solution per tooth	Irrigation with 3mL of distilled water per tooth	3 (JADAD)
Gebara et al., 2003[33]	Clinical trial	Patients with Chronic Periodontitis 20 (n=10)	25–57	Scaling and root planning + Irrigation with 3ml of a propolis hydro alcoholic solution (20% propolis extract) Scaling and root planning + Irrigation with 3ml of a placebo solution (containing 14% ethanol-propolis	Scaling and root planning	19 (MINORS)

Table 2. Properties and mechanisms of Propolis components against SARS-CoV-2 and COVID-19.

Study	Properties	Components	Mechanism
Al Naggar et al., 2020[34]	Antiviral Antiseptic Anti-inflammatory Antioxidant Immunomodulatory Anti-fungal Anti-bacterial	CAPE, artepilin C, chrysin, caempferol and quercetin	It strengthens the cell wall and leaves the internal environment aseptic, interfering with viral maturation and replication; and mitigating the exaggerated inflammatory response, especially the cytokine storm associated with COVID-19 infection.
Bachevski et al., 2020[35]	Antiviral	CAPE, quercetin, kaempferol, artepelin C and chrysin	Propolis acts on enveloped viruses. CAPE acts on PAK1 and can be useful in stopping / inhibiting coronavirus-induced fibrosis in the lungs.
Berretta et al., 2020[7]	Antiviral Anti-inflammatory Immunomodulatory Antioxidant Anticancer	Phytochemicals of propolis such as caffeic acid, quercetin and myricetin	Inhibitory effect on ACE2, TMPRSS2 and PAK1 signaling pathways. Immunoregulation of pro-inflammatory cytokines, including reduction of IL-6, IL-1 beta and TNF-α, proving antiviral activity to COVID-19.
Fiorini et al., 2021[36]	Antiviral	Quercetin	Potential to inhibit the functional activity of ACE-2 receptor in SARS-CoV-2
Keflie and Biesalski, 2020[37]	Antiviral Anti-inflammatory	Bioactive substancies	Promising effects in interrupting transmission, reducing susceptibility and improving the severity of SARS-CoV, MERS-CoV and other viral infections.
Khayrani et al., 2021[38]	Antiviral	Glicosperin A, broussoflavonol F, sulabiroinas A, (2S) -5,7-dihydroxy-4'-methoxy-8-prenylflavanone and isorhamnetina.	Inhibition of the converting activity of angiotensin-2 (ACE-2), a SARS-CoV-2 receptor in the human body, preventing replication of the virus.
Kumar et al., 2020[39]	Antiviral Antimicrobial	CAPE	Inhibition of TMPRSS2 (protein S activator of several strains of SARS-CoV) and reduction of the SARS-COV-2.
Kumar et al., 2021[2]	Antiviral Antimicrobial	CAPE	CAPE is able to inhibit M^{pro} (highly conserved SARS-COV-2 protein), inhibiting the functional activity of the SARS-CoV-2 protease.
Lima et al., 2020[40]	Antiviral Immunomodulatory Anti-inflammatory Antioxidant	Phenolic compounds such as galangin, chrysin, p-cumaric acid, kaempferol and quercetin	Blocking / reducing the entry of the virus into host cells, with the ability to stimulate the immune system and antiviral effect in the treatment of COVID-19
Maruta and He, 2020[8]	Antiviral Antibacterial	CAPE and Artepelin C	PAK1 blockade. Propolis components have the capacity to stimulate the immune system, block coronavirus-induced fibrosis of the lungs and have an antiviral effect in the treatment of COVID-19.
Refaat et al., 2021[41]	Antiviral	Flavonoid components of Egyptian propolis	The liposomal formula of propolis showed an inhibitory effect against the protease COVID-3CL and the protein spike, inhibiting viral replication.
Sahlan et al., 2021[42]	Antiviral	Glicosperin A, broussoflavonol F, sulabiroinas A, (2S) -5,7-dihydroxy-4'-methoxy-8-prenylflavanone and isorhamnetina,	Propolis glycosperin A and broussoflavonol F can inhibit the enzyme activity of the main SARS-CoV-2 protease, blocking viral replication.
Shahinozzaman et al., 2020[43]	Antioxidant Antiviral Anti-inflammatory Anticancer Immunomodulatory	Artepillin C	Blocking PAK1 (COVID-19 protein kinase), has anti-inflammatory properties and can also increase immunity against COVID-19 by inhibiting PAK1

Table 3. Assessment methods and outcomes of intervention with propolis in patients with periodontal disease.

Study	Evaluation	Results
El-Sharkawy et al., 2016[24]	Probing Depth (PD); Clinical attachment level (CAL); Eastman interdental bleeding index (EIBI); Gingival index (GI); Plaque Index (PI).	GI, PI, and EIBI: no statistically significant differences at 3 and 6 months between groups; PD and CAL: statistically improved results in propolis group when compared with control at 3 and 6 months.
Kirti et al., 2017[25]	Pocket Probing Depth (PPD); Relative Attachment Level (RAL); Sulcular Blending Index (SBI); Plaque Index (PI); Gingival Index (GI)	Intragroup comparison: baseline to 6 weeks, statistically significant for all groups in all parameters; PI and GI: non-significant difference between groups; SBI and RAL: statistically significance was found when comparing propolis and Periogard groups with the control group; PPD: statistically significant difference with better results in propolis group compared to others; Microbial colony count: significantly higher percentage reduction in propolis group comparing to control. No difference between Propolis and Periogard group.
Kumar et al., 2015[26]	Plaque score (PS); Gingival score (GS); Bleeding score (BS); Probing pocket depth (PPD); Clinical attachment level (CAL)	PS, GS, BS, PPD and CAL: statistically significant reduction in Propolis when compared to Aloe-vera group; *Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola:* statistically significant reduction in propolis group.
Nakao et al., 2020[27]	Plaque control record (PCR); Tooth mobility (TM); Probing pocket depth (PPD); Clinical attachment level (CAL); Bleeding on probing (BOP).	CAL: improved in the propolis and minocycline groups, as compared to the placebo, but with no statistically significant difference; PPD: statistically significant improvement in the propolis group as compared to the placebo; Bacterial number: decrease in the propolis group for *P. gingivalis, T. forsythia and P. intermedia*.
Pundir et al., 2017[28]	Gingival index (GI); Plaque index (PI); Modified sulcus bleeding index; Probing pocket depth (PPD); Clinical attachment level (CAL).	PI, GI, BOP, PPD, and CAL: statistically significant improvement in propolis group; *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia* and *P. gingivalis:* statistically significantly greater reduction in the test group when compared with the control group.
Sanghani et al., 2014[29]	Gingival index (GI); Sulcus bleeding Index (SBI); Probing pocket depth (PPD); Clinical attachment level (CAL)	GI, SBI, PPD and CAL: statistically significant decrease in propolis group; Microbial count of *P. gingivalis, P. intermedia* and *Fusobacterium nucleatum:* Decrease for both propolis and control groups.
Shohdy et al., 2020[30]	Plaque index (PI); Probing pocket depth (PPD); Clinical attachment level (CAL)	PI and CAL: statistically significant difference between baseline and final follow-up for both treatment groups with propolis. PPD: no difference in groups for the overall results.
Sparabombe et al., 2019[31]	Full mouth bleeding score (FMBS); Full mouth plaque score (FMPS); Probing depth (PD); Clinical attachment level (CAL).	FMBS and FMPS: propolis showed a significant decrease comparing with the control group; PD and CAL: no significant difference between groups.
Sreedhar et al., 2017[32]	Plaque Index (PI); Modified Gingival Index (MGI); Blending on Probing (BOP); Pocket probing depth (PPD); Clinical attachment level (CAL).	PI and MGI: slight improvement in propolis group compared to placebo group; BOP, PPD and CAL: significant decrease in propolis comparing with placebo group;
Gebara et al., 2003[23]	Plaque index (PI); Gingival index (GI); Pocket probing depth (PPD); Bleeding upon probing (BOP); Clinical attachment level (CAL)	Viable counts of anaerobic bacteria and *P. gingivalis:* significant decrease in propolis group compared with others; PPD: significant decrease was observed in propolis group compared with others; GI, PI and CAL: no significant difference between groups.

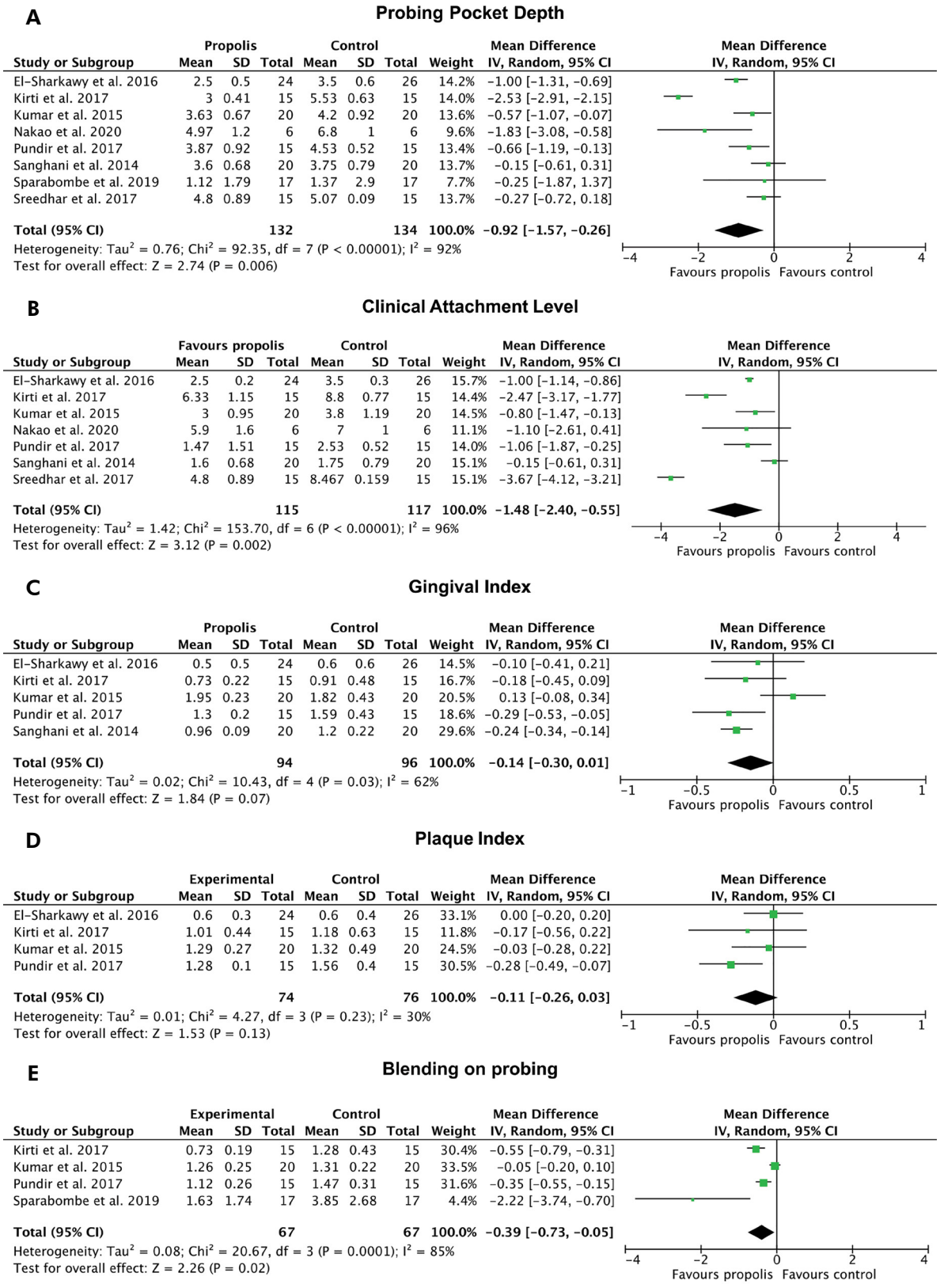

Figure 3. Forest plots for clinical parameters with and without propolis treatment. a) Pocket probing depth; b) Clinical attachment level; c) Gingival index; d) Plaque index and; e) Blending on probing.

Quality assessment and risk of bias

All clinical studies were analyzed using specific scales for quality and ROB assessments. One randomized clinical trial scored 2 (poor quality) on the Jadad scale. All other studies scored between 3 and 5. A summary of the ROB in each study is presented in Figure 4. The studies obtained an overall bias percentage for the five domains evaluated, as shown in Figure 5.

The non-randomized clinical trial scored 19 on the MINORS scale to evaluate comparative studies (maximum score = 24). The results of the ROB assessment are shown in Figure 6.

RCT studies included in the meta-analysis were considered and evaluated for the certainty of evidence. The certainty grade was evaluated as "High" for the BOP parameter and "Moderate" for PPD, GI, PI, and CAL (Figure 7).

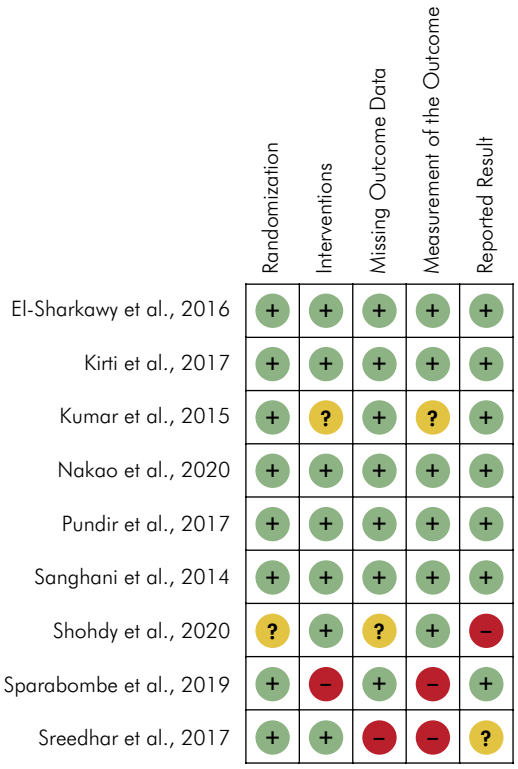

Figure 4. Summary Risk of bias of Randomized Clinical Trial studies.

Discussion

This systematic review and meta-analysis aimed to study the relationship between propolis treatment and periodontal disease and its possible systemic effects, leading to a decrease in the risk of COVID-19 severity. According to the results of studies on propolis, there is evidence that it may be a possible herbal agent against coronavirus. The effect of propolis on periodontitis also affects systemic health, supporting the improvement of the patient's immunity and reducing inflammation, thus reducing the possibility of the severity of COVID-19.

COVID-19 is associated with increased levels of activated pro-inflammatory chemokines and cytokines that lead to the development of atypical pneumonia with rapid respiratory impairment and lung failure.[44] COVID-19 is characterized by a connection between viral peak protein S (spike) and angiotensin-converting enzyme 2 (ACE2).

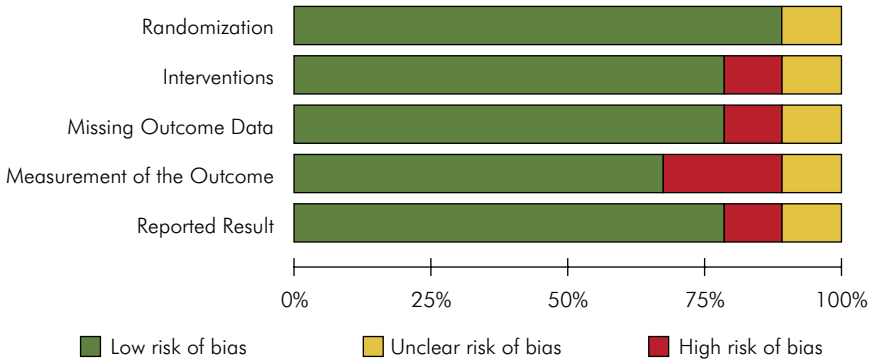

Figure 5. Risk of Bias graph of Randomized Clinical Trials.

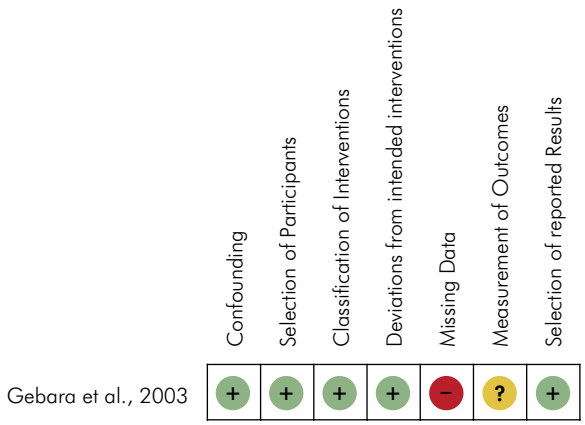

Figure 6. Summary Risk of bias of non-randomized clinical study.

ACE2 is a protein in the human body that facilitates virus entry into cells and is an essential receptor for SARS-CoV-2. Activation of the spike protein is mediated by proteases, such as TMPRSS2, which are critical in viral infection.[7,34,45] After entry and subsequent endocytosis, coronavirus infection causes the positive regulation of PAK1. The main protease of SARS-CoV-2 coronavirus, M^{PRO} (cysteine enzyme), is essential for the processing of polyproteins by the coronavirus and its life cycle. Therefore, inhibition of the active site of this enzyme is of great relevance for the discovery of effective drugs. The inhibition of ACE2, TMPRSS2, PAK1, and M^{PRO} are important targets for treating COVID-19.[7,8]

Furthermore, propolis can be beneficial and efficient against new strains of COVID-19. Mutations in SARS-CoV strains usually occur in the spike protein.[46,47] The omicron variant presents many mutations in this virus protein, causing genetic changes that affect virus characteristics such as transmissibility, disease severity, immune escape, and diagnostic and therapeutic escape.[46].Thus, treatment with propolis can improve the prognosis of individuals infected with this and other strains.

Propolis can be used as a complementary treatment for patients with COVID-19. It has a potential impact on the replication of SARS-CoV-2, either by direct antiviral effects, anti-inflammatory effects promoting immunoregulation of pro-inflammatory cytokines, or indirect effects owing to its immunomodulatory effect on the host immune system and interference with the host inflammatory response. The antiviral effects of these compounds are mediated by inhibition of viral transmission to other cells, inhibition of viral propagation, and destruction of the external envelope of the virus.[7,34,45]

The biological activity of propolis is attributed to various chemical constituents, such as caffeic acid phenethyl ester (CAPE), flavonoids, quercetin, kaempferol, artepillin C, phenolic acid ester, chrysin, galangin, apigenin, pinobanksin 5-methyl ether, pinobanksin.[5]

CAPE is a bioactive component of propolis that may inhibit the functional activities of M^{PRO} and PAK1.[7,8] Artepillin C, a prenylated derivative of p-coumaric acid, is one of the main phenolic compounds found in Brazilian green propolis. It inhibits PAK1 activity, has anti-inflammatory properties, and can increase immunity against COVID-19 by inhibiting PAK1, which is responsible for suppressing the host immune system.[36,43]

Propolis components such as CAPE, rutin, chrysin, and myricetin affect ACE2 receptors.[7,37] Rutin and CAPE affect the protease COVID-3CL and spike protein.[41] Proteases are potential targets for inhibition of COVID-19 replication. Kaempferol is involved in the inhibition of TMPRSS2.[7,37] Quercetin and vitamin C have shown aminopeptidase inhibitory activities that can interrupt the main proteases of SARS-CoV-2.[35,36]

Propolis produced by the bee Tetragonula *sapiens* inhibits the conversion of ACE2 (the SARS-CoV-2 receptor in the human body). Its compounds, sulabiroin A, isorhamnetin, (2S) -5,7-dihydroxy-4-methoxy-8-prenylflavanone, and in particular, glyasperin A and broussoflavonol F, exhibit the ability to inhibit SARS-CoV-2 main protease activity.[38,42]

Some of the propolis extracts' flavonoid and caffeic acid components can act on the microbial membrane or cell wall, causing functional and structural damage to the microorganisms.[48] In addition, they can effectively modulate cytokines and inflammatory mediators, thereby inhibiting the production of prostaglandins and transforming growth factor-ß.[49]

These components have also been shown to be effective in treating periodontitis. Previous

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Propolis	Conventional treatment	Relative (95% CI)	Absolute (95% CI)		
PPD												
8	randomised trials	not serious	not serious	not serious	extremely serious[a]	strong association all plausible residual confounding would reduce the demonstrated effect	132	134	-	MD **0.92 lower** (1.57 lower to 0.26 lower)	⨁⨁⨁◯ Moderate	CRITICAL
GI												
5	randomised trials	not serious	not serious	not serious	very serious[b]	all plausible residual confounding would reduce the demonstrated effect	94	96	-	MD **0.14 lower** (0.3 lower to 0.01 higher)	⨁⨁⨁◯ Moderate	CRITICAL
PI												
4	randomised trials	not serious	not serious	serious[c]	not serious	none	74	76	-	MD **0.11 lower** (0.26 lower to 0.03 higher)	⨁⨁⨁◯ Moderate	IMPORTANT
CAL												
7	randomised trials	not serious	not serious	not serious	extremely serious[d]	strong association all plausible residual confounding would reduce the demonstrated effect	115	117	-	MD **1.48 lower** (2.4 lower to 0.55 lower)	⨁⨁⨁◯ Moderate	CRITICAL
BOP												
4	randomised trials	not serious	not serious	not serious	very serious[e]	strong association all plausible residual confounding would reduce the demonstrated effect	67	67	-	MD **0.39 lower** (0.73 lower to 0.05 lower)	⨁⨁⨁⨁ High	IMPORTANT

CI: confidence interval; **MD:** mean difference

Explanations

a. I2 = 92%
b. I2= 62%
c. This clinical parameter assesses the % of plaque, which is a indirect parameter related to periodontitis (antimicrobial effect of propolis)
d. I2 = 96%
e. I2=85%

Figure 7. Certainty of evidence assessment.

studies have reported a decrease in the levels of Porphyromonas *gingivalis*, Prevotella *intermedia*, and Fusobacterium *nucleatum* in patients with periodontitis treated with propolis.[26,31] In addition to its antimicrobial effects against *P. gingivalis*, artepillin-C, a component of propolis, also exerts an inflammatory effect.[50]

Previous systematic reviews concluded that treating periodontitis with propolis is safe and combined with conventional therapy may enhance the clinical parameters compared with non-surgical treatment alone or with placebo.[51,52] Assunção et al.[51] excluded studies comprising patients with systemic disease. This reduces the power of propolis analysis in patients' systemic health.

This systematic review and meta-analysis analyzed studies that clinically evaluated patients with periodontitis. Eight studies classified patients according to the chronicity of the disease,[25-30,32,33] whereas two[24,31] classified patients according to the severity of the disease (moderate or severe). This may have led to a bias in evaluating the studies included in our review.

Despite these possible biases, the certainty of evidence in our analyses showed moderate certainty for PPD, GI, PI, and CAL and high certainty for BOP. These results are related to the type of parameter used for evaluation, which is directly linked to the reduction in inflammation in periodontal tissues. For the RCTs RoB, the selected studies had more than 50% on the "measurement of the outcome" domain and more than 75% low risk of bias in the other four evaluated domains. For the non-RCT study, only one domain (missing data) was categorized as high RoB, and the others (measurement of outcomes) were categorized as unclear RoB. In this way, we can consider that, overall, the selected studies were evaluated as having low RoB or unclear RoB. The controlled study design positively influenced the quality of the results.

However, all the analyzed studies showed favorable results for using propolis compared with placebo or conventional treatments as control groups. The meta-analysis showed statistical significance for PPD (95%CI: 0.92; $p < 0.001$), CAL (95%CI: 1.48; $p < 0.001$), GI (95%CI: 0.14; $p = 0.03$), and BOP (95%CI: 0.39; $p < 0.001$). The PI results (95%CI :0.11; $p = 0.23$) did not present statistically significant differences, but they similarly favored treatment with propolis. These data support the hypothesis that this treatment is an effective option for patients with periodontitis.

Individuals with diabetes and periodontitis may have positive regulation of ACE2 expression, making it a favorable environment for SARS-CoV-2 entry into the cell through ACE2 receptors.[53] Treatment of periodontal disease has been shown to positively influence glycemia in patients with diabetes.[3] The control of glycemic levels throughout periodontal treatment is of great relevance, as diabetes is one of the most prevalent comorbidities in patients with severe COVID-19.

Propolis has shown positive results as an adjunctive treatment for periodontitis compared with traditional therapy. Likewise, various components of propolis have shown antiviral effects against SARS-CoV-2. The antiviral effect of propolis, in combination with a reduction in the levels of periodontal inflammation, can lead to an improvement in general health and, consequently, a decrease in the risk of severe COVID-19.

Conclusions

The results of our meta-analysis suggest that propolis is a low-cost treatment option that affects SARS-CoV-2 protease through basic treatments in patients with periodontitis. Several components of propolis have anti-inflammatory and immunoregulatory activities, including the inhibition of PAK1, ACE2, M^{PRO}, and TMPRSS2. In addition to this direct relationship, the reduction of comorbidities such as hypertension and diabetes by periodontal treatment is a major factor for improvement in general health and, thus, a reduction in the severity of COVID-19.

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