Andrographis paniculata (Burm. F.) Wall. Ex Nees, Andrographolide, and Andrographolide Analogues as SARS-CoV-2 Antivirals? A Rapid Review

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Abstract
Drug repurposing is commonly employed in the search for potential therapeutic agents. Andrographis paniculata, a medicinal plant commonly used for symptomatic relief of the common cold, and its phytoconstituent andrographolide, have been repeatedly identified as potential antivirals against SARS-CoV-2. In light of new evidence emerging since the onset of the COVID-19 pandemic, this rapid review was conducted to identify and evaluate the current SARS-CoV-2 antiviral evidence for A. paniculata, andrographolide, and andrographolide analogs. A systematic search and screen strategy of electronic databases and gray literature was undertaken to identify relevant primary articles. One target-based in vitro study reported the 3CLpro inhibitory activity of andrographolide as being no better than disulfiram. Another Vero cell-based study reported potential SARS-CoV-2 inhibitory activity for both andrographolide and A. paniculata extract. Eleven in silico studies predicted the binding of andrographolide and its analogs to several key antiviral targets of SARS-CoV-2 including the spike protein-ACE-2 receptor complex, spike protein, ACE-2 receptor, RdRp, 3CLpro, PLpro, and N-protein RNA-binding domain. In conclusion, in silico and in vitro studies collectively suggest multi-pathway targeting SARS-CoV-2 antiviral properties of andrographolide and its analogs, but in vivo data are needed to support these predictions.

Keywords
COVID-19, andrographolide, Andrographis paniculata, antiviral, herbal medicines, bioactivity

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Results and Discussion

Study Inclusion

A total of 139 records were identified through keyword searches on online databases of published journals and gray literature. Of the 139 records, 12 articles are included in this review as presented in the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) flow chart (Figure 1).

Study Demographics

All 12 articles identified and included based on this review’s inclusion and exclusion criteria are on preclinical studies. From the 12 studies, more than half (58.3%) were reported by Indian scientists. All of the Indian studies were computational investigations and analysis. In fact, a majority (83.3%) of the total papers identified were exclusively in silico papers reporting molecular docking and 3D simulation of the interaction of phytochemical compounds to potential therapeutic targets to prevent attachment and replication of SARS-CoV-2. There were only 2 articles reporting in vitro results. No articles covered clinical and in vivo preclinical studies. Although the search was conducted to identify studies investigating both A. paniculata as a plant (in any formulation) as well as in the form of its isolated constituents, it was found that most of the studies were only conducted on the potential bioactive compounds (91.6%), corresponding to the majority study type, which was in silico (Table 1).

Results: Antiviral Evidence Against SARS-CoV-2

In silico. In silico docking and simulation studies revealed andrographolide’s potential to bind with several therapeutic targets that are important for viral attachment to host cells, replication, and production (Table 2). In several studies, various docking methods and software consistently predicted negative binding energy (ie, indicating potential for good binding affinity) for andrographolides and its derivatives when docked against SARS-CoV-2 spike protein-angiotensin converting enzyme (ACE)-2 complex, spike protein, ACE-2 receptor, 3-chymotrypsin-like protease (3CLpro, previously known as SARS-CoV-2 main protease (Mpro)), and RNA dependent RNA polymerase (RdRp). 3CLpro was the most commonly investigated target in 9 out of 11 in silico studies (81.8%).

Negative binding energy was also predicted for andrographolide and its derivatives when docked against SARS-CoV-2 papain-like protease (PLpro) while 9 studies (81.8%) compared these parameters with other phytochemicals, including analogs of...
andrographolide and other bioactive compounds of *A. paniculata*. A qualitative comparison of receptor-ligand activities (andrographolide versus major reference drugs/compounds and other phytochemicals) within each study is summarized and presented in Table 3. In general, andrographolide and its derivatives were predicted to have weaker binding affinities than most standard reference drugs or compounds as well as other phytochemicals. However, andrographolide was simulated to have higher binding affinity towards the human ACE-2 receptor compared to captopril in one study. In this same study, other phytochemicals such as curcumin, nimbin, and piperine were predicted to have better binding affinity than both andrographolide and captopril to the ACE-2 receptor. A detailed comparison of andrographolide against both major reference drugs/compounds and other phytochemicals is included in Supporting information: Supplemental Table S1.

**In vitro.** Two in vitro studies that investigated the antiviral efficacy of *A. paniculata* extract and andrographolide against SARS-CoV-2 were identified and included. Compared to disulfiram (a SARS coronavirus M-pro inhibitor), andrographolide inhibited SARS-CoV-2 3CLpro activity at a higher 50% inhibitory concentration (IC$_{50}$) value (15.05 ± 1.58 mM vs 5.61 ± 0.34 mM), indicating its weaker inhibitory potency. Further mass spectrometric analysis revealed covalent linkage formation between andrographolide and SARS-CoV-2 3CLpro. Another study has showed the potential anti-SARS-CoV-2 activities of *A. paniculata* extract (extract type not mentioned) and andrographolide against a live SARS-CoV-2 virus (phenotypic assay). From a pool of 114 Thai medicinal plant extracts and 8 isolated natural compounds screened, *A. paniculata* extract (IC$_{50}$ = 68.06 µg/mL; 50% cytotoxic concentration (CC$_{50}$) > 100 µg/mL) and andrographolide (IC$_{50}$ = 6.58 µM; CC$_{50}$ = 27.77 µM), were substantially less potent than the most active candidates, *Boesenbergia rotunda* (Roxb.) Schltr. ethanolic extract (IC$_{50}$ = 3.62 µg/mL) and its bioactive compound panduratin A (IC$_{50}$ = 0.81 µM). Assuming a molar mass of 350.4 g/mol for andrographolide, its IC$_{50}$ is calculated as 2.31 µg/mL. This

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**Figure 1.** Preferred reporting items for systematic review and meta-analysis (PRISMA) flow chart of articles inclusion.
lower IC$_{50}$ value of andrographolide (2.31 µg/mL) compared to that of A. paniculata extract (IC$_{50}$ = 68.06 µg/mL) indicates that the former may be more potent than the latter. As the CC$_{50}$ in this study comparing andrographolide and A. paniculata extract was limited to 100 µg/mL, accurate calculations of selective index (ratio of CC$_{50}$/IC50) cannot be made for the purpose of comparison.

**Discussion: Antiviral Evidence Against SARS-CoV-2**

Based on media coverage, andrographolide, a constituent of A. paniculata, appears to be one of the more popular phytochemicals under investigation. After performing a systematic search and screen, this review identified mostly in silico papers that reported on the anti-SARS-CoV-2 effect of andrographolide, instead of the plant. The bulk of studies were conducted by researchers from India where A. paniculata is a common herb used in traditional Ayurvedic medicine. In herbal medicine research, plant extracts may at times have better effects than single isolated phytochemicals due to the high potential for several compounds to interact either synergistically or additively with each other. However, the efficacy of an herbal extract is also dependent on its content of bioactive compounds, which differ by the extraction method used. For example, ethanolic extracts of A. paniculata were reported to yield higher concentrations of andrographolide compared to aqueous extracts. In the case of A. paniculata, one in vitro study reported andrographolide to be potentially more potent than the extract itself. However, details on the extract itself were not reported in this study.

In urgent times, focusing on individual small molecules instead of the whole plant may produce faster results. Historically, the therapeutic agents of medicinal plant origins that have been successfully developed for clinical use have mostly been single compounds such as opioids and the anticancer drug Paclitaxel.

The versatility of andrographolide as a SARS-CoV-2 antiviral is demonstrated by its potential to bind to several important targets at various stages of viral attachment, replication, and host-pathogen interactions. This property may be an important advantage to any potential therapeutic agent being developed. Viral life cycle modeling studies have suggested that effective infection attenuation of repurposed drugs is more likely to be achieved when multiple segments of the viral life cycle are targeted, especially when timely administration of an antiviral in the early phases of infection is challenging in real-life settings.

Molecular dynamics simulation suggests that andrographolide can bind to interfacial regions of both the SARS-CoV-2 spike protein and human ACE-2 receptors. The SARS-CoV-2 spike protein-ACE-2 attachment is one of the most well studied and recognized interactions that constitute the first step of viral invasion into human lung cells. This step is thought to initiate viral membrane fusion, entry, and initiation of downstream inflammatory and immunogenic effects.

Another study predicted that andrographolide and its derivatives bind allosterically to the spike protein S1 subunit of the spike protein-ACE-2 complex, instead of interfacial regions. Therefore, it was suggested that this allosteric binding may alter the conformation of the complex and subsequently interfere with the binding of SARS-CoV-2 to the ACE-2 receptors. This study did not investigate further the dynamics of allosteric modulation and its effects on virus spike protein and human ACE-2 receptor interaction. As the spike protein-ACE-2 interaction is thought to be one of the key targets of anti-SARS-CoV-2 therapeutic candidates, there are existing concerns that administration of potent ACE-2 inhibitors may eventually lead to upregulation of ACE-2 receptors at lung epithelial cell surfaces. Ironically, ACE-2 receptors at lung cell surfaces may have a protective role, especially at severe stages of the infection with overt inflammation. Therefore, the benefits of ACE-2 blocking are still debated. As the involvement of the ACE-2 receptor in multiple physiological functions further complicates its position as a potential therapeutic target, it has been suggested that antivirals targeting multiple pathways of the viral life-cycle may be more effective.

Two papers predicted good binding of andrographolide and its derivatives with RdRp. At present, the first and only antiviral currently approved by the U.S. FDA for the treatment of severe COVID-19, Remdesivir, is an antiviral that is thought to act through inhibition of RdRp, though its benefit in lowering mortality in COVID-19 patients remains to be investigated. Remdesivir is a repurposed broad-spectrum antiviral against RNA viruses, originally developed for Ebola. It is a prodrug, which after a series of transformations, forms a nucleoside triphosphate to be uptaken by the virus's RdRp enzyme, a crucial enzyme responsible for the RNA replication of coronavirus. This intentional but faulty incorporation is thought to be responsible for the viral replication inhibitory
Table 2. Predicted Ligand-Receptor Interactions of Andrographolide and Its Analogs Against Potential SARS-CoV-2 Antiviral Therapeutic Targets Based on Molecular Modeling.

| Target                      | PDB ID | Docking program/simulation | Compounds                                      | Binding energy Δ (kcal mol⁻¹) | Predicted interacting residues | Ref. |
|-----------------------------|--------|----------------------------|------------------------------------------------|-------------------------------|--------------------------------|------|
| A. Viral attachment         |        |                            | Donor ligand                                   |                               |                                |      |
| Spike protein- ACE-2 complex| 6M17   | AutoDock 4.0 Suite         | Andrographolide                                | -9.1                          | H-bonds: Asn33, Arg93, Tyr505  | 29   |
|                             |        |                            |                                                |                               | Alkyl interactions: His34, Pro389 |      |
|                             | 6LZG   | NS                         | Andrographolactone                             | -10.2                         | H-bonds: Lys441                 | 36   |
|                             |        |                            |                                                |                               | Alkyl interactions: Met366, Pro415, His540, Lys541 |      |
|                             |        |                            | Bisandrographolide A                           | -9.6                          | NS²                            |      |
|                             |        |                            | Neoandrographolide                             | -8.6                          | NS²                            |      |
|                             |        |                            | 3-O-β-D-Glucopyranosyl-14,19-dideoxyandrographolide | -8.1                          | NS²                            |      |
|                             |        |                            | Andrographolide                                | -7.2                          | NS²                            |      |
|                             | NA¹    | AutoDock Vina              | Andrographolactone                             | -24.1                         | NS²                            | 34   |
|                             |        |                            | 3-O-β-D-Glucopyranosyl-14,19-dideoxyandrographolide | -23.9                         | NS²                            |      |
|                             |        |                            | Neoandrographolide                             | -13.3                         | H-bonds: Asp333, Tyr368, Asp365, Ala331, Thr330 |      |
|                             |        |                            | Andrographolactone                             | -12.7                         | NS²                            |      |
| Spike protein               | 6VYB   | AutoDock 4.2               | Andrographolide                                | -61.3                         | H-bonds: Tyr28, Phe59           | 32   |
|                             | 6 VXX  | Molegro                    | Andrographolide                                | -98.80                        | H-bonds: Thr761, Gln314         | 33   |
|                             |        | Virtual Docker 3.0          | Andrographolide                                |                                |                                |      |
|                             |        |                            | 3-O-β-D-Glucopyranosyl-14,19-dideoxyandrographolide |                                |                                |      |
| ACE-2 receptor              | 1R42   | AutoDock 4.2               | Andrographolide                                | -68                           | H-bonds: Thr27, Lys26, Gln89, Gla22 | 32   |
|                             |        |                            |                                                |                               | Alkyl interactions: Leu29, Val83, Lys94 |      |
|                             | 1R42   | Molegro                    | Andrographolide                                | -99.354                       | H-bonds: Tyr385, His401, Gla402, Phe400, Arg514 | 33   |
|                             |        | Virtual Docker 3.0          | Andrographolide                                |                                |                                |      |
| BViral replication          | 6LU7   | Dock 6                     | Andrographolide                                | -0.74                         | H-bonds: Gly143, Cys145, Glu166 | 30   |

(Continued)
| Target | PDB ID | Docking program/simulation | Compounds | Binding energy \(^4\) (kcal mol\(^{-1}\)) | Predicted interacting residues | Ref. |
|--------|--------|-----------------------------|------------|---------------------------------|-----------------------------|------|
| 6LU7   | AutoDock Vina | Andrographolide | −6.3 | His41, Leu141, Asn142, Gly143, Cys154, Met165, Glu166, Glh189 | | 31 |
| 6LU7   | AutoDock Vina | Oxoandrographolide | −6.9 | H-bonds: Thr11, Thr292 | | 37 |
| 6LU7   | AutoDock Vina | Andrographolide | −6.5 | H-bonds: Gln107, Thr292 | | 37 |
| 6LU7   | AutoDock Vina 1.1.2 and Chimera 1.1.3.1 | Neoandrographolide | −6.5 | H-bonds: Gka290 | | 37 |
| 6LU7   | AutoDock Vina 1.1.2 and Chimera 1.1.3.1 | 9-Dehydro-17-hydro-andrographolide | −6.1 | H-bonds: Gln107, Gln110 | | 37 |
| 5R82   | AutoDock 4.2 | 3-O-β-D-glucopyranosyl-andrographolide | −8.3 | | NS\(^2\) | 39 |
| 5R82   | AutoDock 4.2 | Andrographolide | −6.8 | | NS\(^2\) | 39 |
| 5R82   | Schrödinger suite 2019-4 | 14-Deoxyandrographolide | −39.62 | H-bonds: Gly143, Thr25 | | 32 |
| 5R82   | Schrödinger suite 2019-4 | Andrographolide | −34.68 | H-bonds: Ser46, Gly143, Thr25 | | 32 |
| 7BUY   | NS | 14-Deoxy-12-hydroxy-andrographolide | −29.36 | | NS\(^2\) | 35 |
| NA\(^1\) | AutoDock Vina | Neoandrographolide | −31.4 | H-bonds: Gly167, Phel41, Leu42, Ser145, His164 | | 34 |
| 7BUY   | NS | Andrographolactone | −8.1 | | NS\(^2\) | 34 |
| 7BUY   | NS | Andrographolide | −7.6 | | NS\(^2\) | 34 |
| 7BUY   | NS | 3-O-β-D-Glucopyranosyl 14,19-dideoxyandrographolide | −7.6 | | NS\(^2\) | 34 |
| NA\(^1\) | AutoDock Vina | Neoandrographolide | −31.4 | | H-bonds: Gly167, Phel41, Leu42, Ser145, His164 | | 34 |
| 14-Deoxyandrographolide | −26.1 | NS\(^2\) | | 38 |
| Andrographolide | −21.7 | NS\(^2\) | | 38 |
| 14-Deoxy-11,12-didehydroandrographolide | −18.9 | NS\(^2\) | | 38 |
| NS | Arguslab 4.0.1 | 14-Deoxy-11,12-didehydroandrographolide | −9.72 | | NS\(^2\) | 38 |
| 14-Deoxyandrographolide | −28.5 | | | 34 |
| Andrographolide | −26.6 | NS\(^2\) | | 34 |
| 14-Deoxy-11,12-didehydroandrographolide | −22.9 | NS\(^2\) | | 34 |
| Andrographolide | −12.7 | NS\(^2\) | | 34 |

(Continued)
| Target                           | PDB ID  | Docking program/simulation | Compounds                                | Binding energy (kcal mol\(^{-1}\)) | Predicted interacting residues | Ref       |
|---------------------------------|---------|----------------------------|-------------------------------------------|-------------------------------------|--------------------------------|-----------|
| RdRp                            | 6M71    | AutoDock Vina              | Oxoandrographolide                         | −7.1                                | NS\(^2\)                          | 37        |
|                                 |         |                            | Neoandrographolide                         | −7.0                                | NS\(^2\)                          |           |
|                                 |         |                            | Andrographolide                            | −6.3                                | NS\(^2\)                          |           |
|                                 |         |                            | Hydro-andrographolide                      | −6.3                                | NS\(^2\)                          |           |
|                                 |         |                            | Isoandrographolide                         | −6.3                                | NS\(^2\)                          |           |
|                                 | NA\(^1\)| AutoDock Vina             | Neoandrographolide                         | −17.1                               | H-bonds: Ser644, Arg438, Asp337, Arg509, Asp508 | 34        |
|                                 |         |                            |                                            |                                     |                                 |           |
| 14-Deoxy-11,12-didehydroandrographolide |         |                            |                                            | −9.8                                | NS\(^2\)                          |           |
| 14-deoxyandrographolide         |         |                            |                                            | −7.8                                | NS\(^2\)                          |           |
| Andrographolide                 |         |                            |                                            | −3.0                                | NS\(^2\)                          |           |
| Nucleocapsid protein binding domain \(^3\) | 6M3M   | NS                         | Bisandrographolide A                       | −10.3                               | H-bonds: Glu137, Leu162, Gly165, Thr166, Alkyl interactions: Ile75, Pro81, Pro163, Leu168, Tyr173 | 36        |
|                                 |         |                            | 3-O-β-D-Glucopyranosyl-14,19-dideoxyandrographolide | −9.8                                | NS\(^2\)                          |           |
|                                 |         |                            | Neoandrographolide                         | −9.4                                | NS\(^2\)                          |           |
|                                 |         |                            | Andrographolide                            | −9.0                                | NS\(^2\)                          |           |
|                                 |         |                            | Andrographolactone                         | −8.8                                | NS\(^2\)                          |           |

Abbreviations: NA = not applicable; NS = not specified; PDB = protein data bank.

\(^1\)Not obtained from database, but was modified from the SARS coronavirus proteins instead to mimic the corresponding SARS-CoV-2 protein, hence a PDB ID is not available.

\(^2\)Details on amino acid residues bond interactions were not extractable from paper.

\(^3\)Targets may have additional functions in modulating host-pathogen interactions.

\(^4\)Negative binding energy denotes potential for binding. A more negative value represents better predicted binding affinity than a less negative value in the same study.
action of Remdesivir. Unlike Remdesivir’s mechanism of action as a prodrug, *in silico* docking suggested that andrographolide and its derivatives bind directly to the RdRp enzyme. Molecular simulation predicted that the analogs

**Figure 2.** Potential SARS-CoV-2 antiviral target sites and mechanisms of actions predicted for andrographolide and its derivatives as indicated by the red boxes: (1) spike protein-ACE-2 complex, (2) spike protein, (3) host cell ACE-2 receptor, (4) PLpro, (5) 3CLpro, (6) RdRp, and (7) nucleotide protein binding domain. The diagram illustrates the overall suggested life cycle of SARS-CoV-2 infecting a human lung cell, including (A) attachment of viral spike protein to ACE-2 receptor, (B) viral membrane fusion via interaction with transmembrane serine protease 2 (TMPRSS2) receptor, (C) viral entry via internalization by host cell, (D) viral replication and transcription complex involving important proteins and enzymes such as RdRp, 3CLpro, PLpro, and nucleocapsid protein, (E) translocation of newly formed non-structural proteins, and (F) viral assembly and release to infect other cells [adapted from Mousavizadeh et al., Romano et al., and V’kovski et al].

**Table 3.** Comparison Summary of Antiviral Receptor-Ligand Activity of Andrographolide With Reference Drugs/Compounds and Other Phytochemicals Against SARS-CoV-2.

| Target                          | Ligand comparators                                                                 | Reference drugs/compounds | Standard inhibitors of target (eg, Captopril, N3, Nafamostat) | Andrographolide analogs | Phytochemicals | Other phytochemicals (eg, from other plants) |
|---------------------------------|-------------------------------------------------------------------------------------|---------------------------|----------------------------------------------------------------|-------------------------|-----------------|---------------------------------------------|
| Spike protein-ACE-2 complex     | Remdesivir                                                                         | L                         | NA                                                              | L                       | H/L            |                              |
| Spike protein                   | Hydroxychloroquine                                                                | L                         | L                                                               | NA                      | L               |                              |
| ACE-2 receptor                  | NA                                                                                 | L                         | L                                                               | NA                      | L               |                              |
| Mpro / 3CLpro                   | NA                                                                                 | L                         | L                                                               | NA                      | L               |                              |
| PLpro                           | NA                                                                                 | H                         | H                                                               | NA                      | L               |                              |
| RdRp                            | NA                                                                                 | H/L                       | H                                                               | L                       | NA              |                              |
| Nucleocapsid protein binding domain | NA                                                                               | L                         | NA                                                              | NA                      | L               |                              |

Abbreviations: H = higher binding affinity reported; H/L = both higher and lower binding affinity reported from different studies; L = lower binding affinity reported; NA = data not available.
neandrographolide and 14-deoxyandrographolide have stronger binding affinity than the parent compound andrographolide, and can potentially bind to and block the nucleotide entry site of RdRp.\textsuperscript{34,37}

Several in silico studies collectively indicated the potential of andrographolide binding to SARS-CoV-2 3CL\textsuperscript{pro},\textsuperscript{30,32,34-39} supported by a target-based in vitro study.\textsuperscript{38} 3CL\textsuperscript{pro} is an important protease enzyme involved in viral genome replication, transcription, translation, and other cellular processes in coronaviruses,\textsuperscript{63,64} though details of its molecular processes in SARS-CoV-2 are yet to be fully elucidated.\textsuperscript{65} Molecular modeling suggested that andrographolide behaves as an electrophilic Michael acceptor inhibitor and binds to the catalytic pocket of SARS-CoV-2, similar to N3, a computer designed potent coronavirus and SARS-CoV-2 3CL\textsuperscript{pro} inhibitor.\textsuperscript{38} Compared to N3, the andrographolide analog 3-O-\beta-D-glucopyranosyl-andrographolide was predicted to exhibit 17% similarities in hydrogen-bonding patterns when docked against the SARS-CoV-2 3CL\textsuperscript{pro}.\textsuperscript{30-32,34-39} Interestingly though, an in silico and in vitro high throughput screening of 10,000 compounds, including natural compounds conducted to identify potent 3CL\textsuperscript{pro} inhibitors did not identify andrographolide or any compound of \textit{A. paniculata} as among the top 6 potential hits and drug candidates.\textsuperscript{66} In the same study, among the 6 potential hits identified, the only natural compound identified was shikonin,\textsuperscript{66} which originates from the roots of \textit{Lithospermum erythrorhizon} Siebold & Zucc.\textsuperscript{67}

Andrographolide and its derivatives were also predicted to bind to 2 lesser investigated therapeutic targets of SARS-CoV-2, PL\textsuperscript{pro} and nucleocapsid protein N-terminal RNA binding domain.\textsuperscript{34,36} Both PL\textsuperscript{pro} and nucleocapsid protein (N-protein) are thought to play multiple roles in viral replication and host-pathogen interactions.\textsuperscript{68} In addition to attenuating viral replication, PL\textsuperscript{pro} inhibition reportedly reduced the virus-induced pathogenic effects in host cells.\textsuperscript{68,69} The structure of the binding domain for the RNA terminal on the SARS-CoV-2 N-protein is one of the most recently elucidated structures.\textsuperscript{70} In SARS-CoV-2, the N-protein is suggested to promote viral transcription via RNA-induced liquid-liquid phase separation to form condensates with RNA and RdRp.\textsuperscript{71} The structure of the SARS-CoV-2 N-protein has been reported to be similar to previously reported coronaviruses, with some differences in surface electrostatic potential, while details of its molecular immunogenic pathways in SARS-CoV-2 infection remain to be established.\textsuperscript{52}

As single compounds, andrographolide and its analogs were generally not predicted to have the best binding affinity when compared to standard drugs like Remdesivir or other phytochemicals such as curcumin (Table 3 and Supporting information: Supplemental Table S1). However, andrographolide and related compounds were predicted to have binding affinity to several key components of the SARS-CoV-2 life cycle and pathogenicity.\textsuperscript{29,31-39} This potential ability to target multiple pathways of viral attachment, replication, and function may provide a unique advantage for andrographolide to be investigated as a versatile antiviral against SARS-CoV-2. When comparison is made between andrographolide and its analogs, the analogs, especially neandrographolide and bisandrographolide, have better predicted binding affinities regardless of target receptor.\textsuperscript{52,34,36,39} Future research to verify these andrographolide analogs as being better SARS-CoV-2 antivirals may be warranted.

There is rising interest in repurposing known small drug molecules as potential therapeutic candidates, especially in emergency situations like the present COVID-19 pandemic. The use of in silico methods and the application of molecular modeling provide a rapid way to screen available compound databases, explore the activities of potential therapeutic candidates through specific algorithms, as well as predict a ligand’s potential to be developed as a medicinal drug.\textsuperscript{73} In addition to general screening of databases that encompass tens of thousands, and at times up to millions of available chemical structures, targeted screening for potential therapeutic candidates based on structural similarities with side chains or structures of known drugs for a particular indication can be performed. In this review, it was observed that andrographolide was identified as a potential ligand that can bind to various SARS-CoV-2 viral targets from different screening methods. General screening on more than ten thousand compounds and targeted screening on selected groups of potential medicinal plants predicted the binding affinities of andrographolide and its analogs, highlighting their potential to be investigated further in vivo. Studies applying structure-based screening which successfully identified andrographolide and its analogs as potential antivirals against SARS-CoV-2 were not found. However, molecular simulation suggested minor similarities in hydrogen bonding of 3-O-\beta-D-glucopyranosyl-andrographolide and N3 to SARS-CoV-2 3CL\textsuperscript{pro}.\textsuperscript{30-32,34-39}

Regardless of the prediction accuracy of an in silico approach, the integration of phenotypic and target-based assays can further substantiate the role of a potential therapeutic candidate in managing a disease.\textsuperscript{74} Despite the high number of in silico articles collectively predicting the anti-SARS-CoV-2 activity of andrographolide, and its analogs as potential antivirals against SARS-CoV-2 were not found. However, molecular simulation suggested minor similarities in hydrogen bonding of 3-O-\beta-D-glucopyranosyl-andrographolide and N3 to SARS-CoV-2 3CL\textsuperscript{pro}.\textsuperscript{30-32,34-39}

From in vivo studies are generally required to conduct further clinical trials.\textsuperscript{75} Specifically for SARS-CoV-2, it has been shown that in vitro antiviral efficacy demonstrated using Vero-cells (similar to the cell models used in one of the in vitro studies included in this review\textsuperscript{43} needs to be validated in primary human airway epithelial cells and in vivo studies.\textsuperscript{76} Therefore, to support these findings, a recent preprint on SARS-CoV-2 antiviral evidence of \textit{A. paniculata} ethanolic extract and andrographolide using human lung epithelial cells was published by the same group of researchers.\textsuperscript{77} In addition to efficacy evidence, pharmacokinetic studies are also crucial to assess and understand the pharmacodynamic potential of a therapeutic candidate. The pharmacokinetic properties, including bioavailability and propensity to bind to plasma proteins such as albumin, will significantly affect the amount of free bioactive
compound available to exert pharmacodynamic effects in the body.\textsuperscript{78} Although several data modeling algorithms were developed to aid in predicting pharmacokinetic properties of a therapeutic agent in silico, their prediction accuracy remains to be optimized.\textsuperscript{79} To assess pharmacokinetic properties better, in vivo studies are still required. Andrographolide is reported to have very low bioavailability in animal studies due to extensive p-glycoprotein metabolism in the gastrointestinal tract.\textsuperscript{80,81} Therefore, efforts to improve the bioavailability of andrographolide should be carried out when considering it as a potential SARS-CoV-2 antiviral. The suggested solutions include nanoemulsions and spray drying.

In addition to their multiple targets on the SARS-CoV-2 viral life cycle, andrographolide and the major bioactive compounds of \textit{A. paniculata} may have additional pleiotropic effects post viral infection due to their reported anti-inflammatory and immunomodulatory properties.\textsuperscript{17,18,84-87} Raised white blood cells and inflammatory markers such as the C-reactive protein and interleukins-1 and 6 were found to be associated with severe SARS-CoV-2 virus infection when compared to mild cases.\textsuperscript{88} In clinical settings, administration of corticosteroids such as dexamethasone in severe COVID-19 patients on mechanical ventilation with acute respiratory distress syndrome and in COVID-19 patients with refractory shock may be considered to help improve short term mortality.\textsuperscript{89} However, there are also concerns on delayed viral clearance and an increase in secondary infections due to immunosuppression.\textsuperscript{90} Therefore, the optimal timing of administration of andrographolide as an immunomodulatory\textsuperscript{84,87} and anti-inflammatory agent\textsuperscript{85,86} should be carefully considered, especially in the context of altering the host immune response towards the SARS-CoV-2 invasion and perpetuating a cytokine storm.\textsuperscript{27,91}

The looming safety concern over andrographolide is primarily due to the premature halt of a phase 1 dose-escalating clinical trial among HIV patients. In that study, one out of 13 patients reported an anaphylactic event, which was not fatal.\textsuperscript{92} That being said, \textit{A. paniculata} products including andrographolide formulated in capsules are sold in certain countries such as Thailand. In 2014, a study in Thailand was conducted to review case reports of adverse reactions associated with \textit{A. paniculata} and its phytochemicals from the Health Product Vigilance Center database. Thirteen hypersensitivity cases, including anaphylactic shock, anaphylactic reaction, and angioedema were identified from a total of 248 \textit{A. paniculata} associated adverse reactions reported over the span of a decade.\textsuperscript{93} In 2015, the Australian Therapeutic Goods Administration (TGA) safety review on \textit{A. paniculata} also suggested potential association between anaphylactic type reactions and \textit{A. paniculata}.\textsuperscript{3} Specific to this safety review conducted by the Australian TGA, anaphylactic type reactions include sudden onset and rapid progression of anaphylaxis symptoms involving the dermatological, cardiovascular, and/or respiratory system, as diagnosed according to established diagnostic criteria by Rüggeberg et al.\textsuperscript{94} Despite this, the causal relationship between anaphylaxis and \textit{A. paniculata} is challenging to establish given the anecdotal nature and limitations of voluntary adverse reaction reports.\textsuperscript{95} Due to such complexities, the Australian TGA could not establish a causal relationship between anaphylaxis and a specific type of extract, compound, or dose.\textsuperscript{3} A preclinical study using active systemic anaphylaxis assay in Guinea pigs reported no clinical signs of anaphylaxis in animals administered with \textit{A. paniculata} methanol extract (25 mg/kg; p.o) and andrographolide (8 mg/kg; p.o) for 5 days. However, andrographolide (0.1, 10 µg/mL) was able to induce the release of allergic mediators in both IgE sensitized and non-IgE sensitized RBL-2H3 cells in the same study.\textsuperscript{89} In view of the risk of hypersensitivity reactions, precautions are warranted for any future trials involving andrographolide and its analogs.

Review Limitations

This rapid review has a few limitations, mainly the lack of in vivo data to support all of the in silico predictions, which, therefore, does not enable us to draw strong conclusions on the SARS-CoV-2 antiviral effects of andrographolide. As only one phenotypic-based in vitro study briefly investigated \textit{A. paniculata} in the form of plant extract, there was insufficient data available to compare meaningfully the efficacy of extracts with the single compound, andrographolide, as well as the potential for synergism between various bioactive phytochemicals present. Although studies of all languages were included, we did not specifically search Chinese journal databases. After screening and selection, only English language articles met all our inclusion criteria. As \textit{A. paniculata} is among one of the listed TCMs for COVID-19 patients in China, there may be a small chance of missing out articles that were not available on international journal platforms during our search period. However, given the limitations and challenges faced in conducting and reporting TCM research,\textsuperscript{97} it is unlikely that any missed data will largely alter the findings of this rapid review.

Conclusions

In conclusion, in silico modeling has predicted the potential of andrographolide and its analogs to exert SARS-CoV-2 antiviral effects by binding to several key targets in SARS-CoV-2 infection, including the spike protein-ACE-2 receptor complex, spike protein, ACE-2 receptor, RdRp, 3CL\textsuperscript{pro}, PI\textsuperscript{pro}, and N-protein RNA-binding domain. The potential SARS-CoV-2 antiviral activities of \textit{A. paniculata} extract and its main active compound, andrographolide, has also been demonstrated in vitro. Based on current evidence, in vitro models with better predictive properties and in vivo studies are needed to confirm the predicted ability of andrographolide and its analogs to target multiple antiviral pathways of viral attachment, replication, and host-pathogen interactions to pave the way for future clinical trials. Exploring analogs of andrographolide as antivirals may be useful as these compounds have been suggested to establish causal relationship between anaphylaxis and specific type of extract, compound, or dose.
have better binding affinities than the parent andrographolide structure.

Materials and Methods

Research Question

This review aimed to address the main research question “What is the available scientific evidence on the antiviral effects and mechanisms of *A. paniculata*, andrographolide, and its analogs against SARS-CoV-2?” Antiviral evidence specifically against SARS-CoV-2 was identified based on the predetermined Population, Intervention, Comparator, Outcome (PICO) elements (Table 4). As pilot search and data analysis resulted in mostly in silico papers, this review was not registered with the International Prospective Register of Systematic Reviews (PROSPERO). As the PRISMA checklist extension for rapid review is still in development, this review was conducted and reported according to the relevant criteria outlined in the PRISMA guidelines and checklist for systematic reviews and meta-analysis.98 (Supporting information: Supplemental Table S2).

Search Strategy and Articles Inclusion

A search using predetermined keywords and synonyms of *A. paniculata* in several major languages (*Andrographis paniculata*, *Justicia latebrosa*, *Justicia paniculata*, akar cerita, bempedu bumi, bidara, obyan xin lian, nilavempu, green chiretta, *Fib Talai Jone*); and andrographolide (andrographolide, andrographis, andropanolide), in combination with synonyms of COVID-19 (COVID-19, COVID19, 2019-nCoV, coronavirus, SARS-CoV-2) and antiviral (antiviral, antivirus) was conducted on multiple electronic databases including MEDLINE, Web of Science, LILAC, Google Scholar, and Cochrane Central. The search was restricted to articles published since January 2020 to the present day of search (January 2021), to reflect the period of the pandemic. No language restrictions were applied. An example of the search strategy is presented in Supporting information: Supplemental Appendix S1. Gray literature search was conducted on the World Health Organisation (WHO), United States Food & Drug Administration (U.S. FDA), and European Medicines Agency (EMA) websites using the keywords ‘*Andrographis paniculata*’ and ‘andrographolide’. A bibliographic manager (EndNote X8.1) was used to manage and deduplicate the search results. Search and article screening for article inclusion was performed by 2 independent investigators while disparities were addressed by a third. Primary articles investigating *A. paniculata*, andrographolide, and its analogs as a single herb or compound, with reported antiviral efficacy evidence against the SARS-CoV-2 at both preclinical and clinical levels were searched to be included.

Data Extraction and Analysis

Data extraction was carried out by 2 independent investigators using a predesigned data extraction table (Supporting information: Supplemental Table S3). Disagreements were reviewed by a third investigator. The main data extracted include study characteristics (author, year, title, country, type of study, objective), methodology (study model, in silico model and program), intervention details (description, dose, frequency, duration), comparator (description, dose, frequency, duration), and outcome (quantitative values, qualitative description, comparison with comparator).

In line with the nature of rapid reviews, in addition to our findings that most were in silico data with different study models, programs, and simulation settings applied; the data obtained were numerically and descriptively analyzed. Numerical descriptive analysis was carried out for type of study, country, and methods leading to the discovery of *A. paniculata*, andrographolides, and its analogs as potential antivirals against SARS-CoV-2. The discovery methods were categorized as (1) general screening, (2) targeted screening, and (3) specific. General screening included studies which identified andrographolides and its derivatives through universal screening on an entire compound database. Targeted screening included studies which identified andrographolides and its derivatives via screening on a pre-defined group of medicinal plants or compounds (eg, plants which were commonly used for common cold and flu). Specific studies were those that solely investigated the efficacy of *A. paniculata*, andrographolides, and its derivatives without comparison with other natural compounds or medicinal plants. Lastly, evidence of efficacy and direct antiviral mechanisms of
action, as well their corresponding methods of investigation were qualitatively and descriptively analyzed.

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