In-vitro Antiviral Activity of Natural Products against Coronavirus Strains: A Systemic Review

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Systematic Review

Keywords: Coronavirus, Natural Products, Antiviral Activity, Coronavirus Strains

DOI: https://doi.org/10.21203/rs.3.rs-63691/v1

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Abstract

Coronavirus is a non-segmented, positive-sense RNA genome belonging to the family coronaviridae in the order Nidovirales. Coronavirus infections have created serious threats in the last couple of decades and recently claiming the death of thousands of humans. Natural products provide a valuable and powerful resource of chemical compounds alkaloids, tannins, caffeine, bioterin, actinophine, etc. displaying antiviral properties. The data was reviewed from various databases or search engines: PubMed, Science Direct, MedLine, Google Scholar, and Biomed central for published articles. The data inclusion criteria was natural products and their isolated and different synthetic compounds. Data duplication and titles or contents that do not meet the inclusion criteria and Reports on antiviral activities of natural products or their derivatives against other than CoV strains were excluded. We encountered 49 plants and 19 compound chemically defined natural molecules reported in the literature, which have evaluated for potent antiviral activity against different coronavirus strains. The listed plants and their compounds in this review are highly potent with promising results against coronavirus. These can be further screened for invasive tests and used for making different formulations or may be polyherbal formulations considering its safety profile and toxicity.

1. Introduction

Coronavirus (diameters of approximately 125 nm) is a non-segmented, positive-sense RNA genome belonging to the family coronaviridae in the order Nidovirales [1, 2]. The genome packed inside a helical capsid formed by the nucleocapsid protein (N) and further surrounded by an envelope. The viral envelope consists of four main structural proteins, i.e., spike protein (S) responsible for the formation of structure and attachment to the host receptor. The membrane protein (M) responsible for giving the virion its shape helping to bind to the nucleocapsid, envelope protein (E) responsible for assembly, and release of the virus, which are required for pathogenesis and nucleocapsid protein (N) responsible for replication [3-5]. These four proteins are encoded within the 3' end of the viral genome. Some coronavirus also encodes an envelope-associated Hemagglutinin esterase protein that enhances spike protein to mediate cell entry and virus spread through the mucosa. Coronavirus can be classified into four genera, which are alpha, beta, gamma, and delta. Among them, alpha and beta infect mammals, whereas gamma infects avian species, and delta infects mammalian and avians both. The S-protein–receptor collaboration is the essential determinant for a coronavirus to contaminate a host animal category and oversees the infection's tissue tropism. Diverse coronaviruses use peptidases as their cell receptor. It is indistinct why peptidases are utilized, as passage happens even without the enzymatic area of these proteins. Numerous α-coronaviruses use aminopeptidase (APN) as their receptor, SARS-CoV and HCoV-NL63 use angiotensin-changing over enzyme 2 (ACE2) as their receptor, MHV enters through CEACAM1, and as of late recognized MERS-CoV ties to dipeptidyl-peptidase 4 (DPP4) to pick up section into the human cell [6-10].

The first coronavirus was discovered in 1930 when the infectious bronchitis virus caused an acute respiratory tract infection of domesticated chicken. In 1940, two more animal coronaviruses, mouse hepatitis virus (MHV) [11, 12], and transmissible gastroenteritis virus isolated. Similarly, the first human coronavirus was identified in 1960 in the form of common cold among human beings. A study carried out in Canada in 2001 showed that more than 500 patients present with flu-like symptoms, on virological analyses, 3.6% of those cases were positive for the HCoV-NL63 strain by polymerase chain reaction [13]. Until 2002, coronavirus was considered a relatively simple, nonfatal virus; however, an outbreak in 2002-2003 in Guangdong province in China, which result in spread to many other countries, caused severe acute respiratory syndrome (SARS-CoV) and high mortality rate in over 1000 patient [14]. Since 2012 middle-east respiratory syndrome coronavirus (MERS-CoV) has infected more than 1700 people with a fatality rate of nearly 36% [15]. Since 2013, the porcine epidemic diarrhoea coronavirus (PEDV) has swept throughout the united states, causing an almost 100% fatality rate in piglets in less than a year [16]. At the end of 2019, in Wuhan province of China, a novel coronavirus, i.e., COVID-19 outbreak, killed more than eighteen hundred and infected over seventy thousand individuals within the first fifty days of the epidemic [17, 18]. In the past, SARS-CoV (2003) infected 8098 individuals with a mortality rate of 9%, across 26 countries in the world whereas novel coronavirus (COVID-19) affected 4218212 individuals with a mortality rate of 3.4% across 116 countries, till the date of this writing which shows transmission rate of SARS-CoV-2 is higher than SARS-CoV. The reason behind it could be genetic recombination event at S protein in the RBD region of SARS-CoV-2, which may have enhanced its transmission ability [19].

The evolution of this virus demonstrates that coronavirus is not a stable virus and can adapt to the new environment through mutation and recombination with relative ease. Hence coronavirus are programmed to alter host range and tissue tropism efficiently to become more virulent, even lethal to human and animal by causing widespread respiratory, GI and CNS diseases in human and another animal. So, mutating this virus's mutating behaviour is becoming a great topic of research among drug developers, researchers, and scientists [20]. After the outbreak of MERS-CoV, SARS-CoV, and other respiratory like diseases bring high mortality and incidence of occurrence, which make it essential public health and economic issue due to which effective prevention is required. There are no specific vaccines or drugs or any formulations that can treat or cure novel coronavirus, and the researcher starts studying the alternative method by comparing the efficacy of the natural product. Resources against the various strains of these viruses with the standard one and emerging viral replication lead to the development and search of a distinct form of solutions from the natural product for drug discovery.

Plants have been the major source of many powerful drugs worldwide, and humans have been using it to heal different illnesses since prehistoric times. Thus, plants are considered the most important source of modern medicines that possess various therapeutic effects [21]. About 25 % of
the medicines used worldwide are derived from plant sources [22]. The phytochemicals or metabolites (primary or secondary) are responsible for various pharmacological activities [23, 24]. Its variation within the plant species confers the specificity in its therapeutic effects [25]. It has always been the challenge and opportunity for researchers to identify the phytochemicals responsible for the particular effects. The emergence of antiviral agents' importance from natural sources requires more research to develop more drugs to treat viral infection. Thus, we need to apply antiviral phyto-constituents within medication therapy to achieve an increased pharmacological response. Herbal medicine is a promoting subject in medicine, and of course, we have to increase our knowledge about them. Therefore, in this review, an effort has been made to provide information about the medicinal plant that possesses antiviral activity against different coronavirus strains.

2. Results And Discussion

In common use today, many phytochemicals are associated with health benefits. Natural products have been the primary source of commercial medicines and drug leads until now. A recent survey revealed that 61% of the 877 drugs introduced worldwide could be traced to or inspired by natural products, out of roughly 350,000 species of plants believed to exist, one-third of those yet to be discovered [26]. The search for antiviral materials from plants is inadequate compared to the investigation of the antimicrobial properties. Preliminary studies have shown that plants have an optimistic antiviral activity in vitro and in vivo [27]. However, the same plants can have different antiviral activities against RNA or DNA viruses, regardless of whether they are coated or not, and even against different types or strains of viruses [28, 29]. Many viral infectious diseases still cause high mortality. Although antiviral chemotherapy has made great strides, antivirals are still mandatory. The appearance of drug-resistant viruses during treatment poses a potential difficulty for effective therapy. New viral pathogens can also be discovered. Biologically active substances of plant origin have long been recognized as viral inhibitors. These antiviral compounds can be extracted from sources such as higher plants, which, for many reasons, have been discovered much less than the traditional ones [30].

There is a great need for readily available antiviral drugs at a reasonable cost with the least side effects. From now on, traditional drugs need to be investigated as new antivirals because many of these old drugs, which contain various plant metabolites, have strong antiviral activities [31]. Research into the antiviral potential of plants began in 1952, and 12 of the 288 plants are effective against influenza. Various screening studies have been conducted in recent years to determine the antiviral efficacy of natural products using in vitro and in vivo tests [32]. The fall of the SARS CoV and MERS CoV highlight the inadequacy of available treatment for life-threatening zoonotic CoV infection in humans. Still, there is no specific drug or vaccine that has been available for its treatment. The FDA (Food Drug Administration) has approved various drugs that inhibit entry and replication of MERS-CoV, SARS-CoV, or another human coronavirus in multiple cell lines; still, various plants and their compounds are on investigation for the search of the antiviral agent against coronavirus strain in this pandemic condition [33].

Table 1: Plants showing antiviral activity against different coronavirus strains
| S.N | Plant source        | Families       | Parts used | Culture cells | Virus/strain | Method applied         | Compounds responsible                        | Results                                                                 | Conclusion                                                                                   | Ref. |
|-----|---------------------|----------------|------------|---------------|--------------|------------------------|-----------------------------------------------|---------------------------------------------------------------------------------------------|-----------------------------------------------|------|
| 1   | *Allium porrum*     | Alliaceae      | ND         | Vero cell ATCC line | SARS-CoV Frankfurt 1 strain | CPE based Antiviral assay | Mannose specific lectin | Most potent against the SARS-CoV induced CPE with EC50 (8.45µg/ml) and SI (222) | ND | Probably interfering with the glycans on the spike protein during virus entry and virus release | [34] |
| 2   | *Urtica dioica*     | Urticaceae     | ND         | Vero cell ATCC line | SARS-CoV Frankfurt 1 strain | CPE based Antiviral assay | N-acetyl glucosamine - specific lectin | Markedly active against the SARS-CoV with EC50 (1.3±0.1µg/ml), CC50 (100) and SI (>77) | ND | Probably interfering with the glycans on the spike protein during virus entry and virus release | [34] |
| 3   | *Hippophae rhamnoides* hybrid | Amaranthaceae | ND         | Vero cell ATCC line | SARS-CoV Frankfurt 1 strain | CPE based Antiviral assay, Virus entry assay | Mannose specific lectin | Shows marked inhibition against SARS-CoV with EC50 (1.7±0.3µg/ml) and CC50 (100), SI (>59) | ND | Probably interfering with the glycans on the spike protein during virus entry and virus release | [34] |
| 4   | *Nicotiana tabacum* | Solanacea      | ND         | Vero cell ATCC line | SARS-CoV Frankfurt 1 strain | CPE based Antiviral assay | N-acetyl glucosamine - specific lectin | Markedly active against the SARS-CoV with EC50 (2.3±2.8 µg/ml) and CC50 (100), SI (>31.3) | ND | Probably interfering with the glycans on the spike protein during virus entry and virus release | [34] |
| 5   | *Laurus nobilis*    | Lauraceae      | Berry      | Vero cell      | SARS-CoV         | Cytotoxicity assay Antiviral assay | ND | Strong antiviral activity against SARS-CoV with IC50=120±2µg/ml and SI value (4.2) | Where positive control glycyrrhizin shown IC50 (641µg/ml with SI value (1.2) | ND | [35] |
| 6   | *Thuja orientalis*  | Cupressaceae   | Fruit      | Vero cell      | SARS-CoV         | Cytotoxicity Antiviral assay | ND | Certain activity against SARS-CoV with IC50=130µg/ml and SI value (3.8) | Where positive control glycyrrhizin shown IC50 (641µg/ml with SI value (1.2) | ND | [35] |
| 7   | *Calophyllum blanci* | Guttiferae     | Roots      | MRC-S         | HCoV 229DE      | CPE based Antiviral assay | Blanco xanthome | Potential candidate for the treatment of coronavirus infection with EC50,3µg/ml | Quercetin D (IC50 0.02µg/ml) | ND | [36] |
| 8   | *Broussonetia papyrifera* | Moraceae      | Roots      | E. coliBL21 HIT competent cells | MERS-CoV 3Cl Pro | Antiviral assay by Protease inhibition method | Broussonetin A (against MERS-CoV 3Cl Pro) | Showed effective IC50=27.9µm against MERS-CoV 3Cl Pro Showed most potent IC50=39.5µm against MERS-CoV PL pro | Quercetin shows IC50 (52.7µm) against SARS-CoV 3CL pro | May be prey group form strong hydrophobic interaction with enzyme | [37] |
| 9   | *Paulownia tomentosa* | Paulowniaceae  | Fruits     | *E. coli*     | SARS-CoV PI pro | Protease inhibition assay(flurogenic assay) | Compound(1-12) flavonoids | Showed inhibition of PI pro in a dose dependent manner with IC50 range between 5.0 and 14.4µm | ND | Compound having dilhydro-2H-pyran group shows better inhibition and may be all this compound bind allotrophic site | [38] |
| **No.** | **Genus** | **Family** | **Organ** | **Cell line** | **Assay Type** | **Inhibitory Effect** |
| --- | --- | --- | --- | --- | --- | --- |
| 10 | *Pseudaloe corylifolia* | Fabaceae | Seeds | BL21(DE3) E. coli | SARS-CoV PLpro | Protease inhibition assay | All this compound showed inhibitory action against protease enzyme with IC₅₀ value of Bavachinin (38.4±2.4), neobavaisoflavone(18.3±1.1), isobavachalcone(7.3±0.8) methyl 2kavachalcone(101±1.2) psoralidin(4.2±1.0), corylifol A(32.3±3.2) when compared with control |
| 11 | *Juniperus oxycedrus* | Cupressaceae | Berry | Vero cell | SARS-CoV | Cytotoxicity assay, Antiviral assay | Certain activity against SARS-CoV with IC₅₀ of 270µg/ml and SI value (3.7), TC₅₀ (1000±1.7) Where positive control glycyrrhizin shown IC₅₀ 641µg/ml with SI value (1.2) |
| 12 | *Sambus formosana* | Adoxaceae | Stem | LLC-MK2 cells HCoV- NL63 | Virucidal assay, Attachment assay, Plaque assay | Caffeic acid (most prominent result) | Extract show antiviral activity with IC₅₀ (1.75µg/ml) for virus yield, IC₅₀ (4.67µg/ml) for plaque formation, IC₅₀ (15.75µg/ml) for virus attachment |
| 13 | *Lycoris radiata* | Amaryllidaceae | Stem cortex | Vero E6 / HEP2 line BJ001 BJ006 | CPE/MTS assay | Lycorine SI= 370 (against BJ001 strain and SI=422) for BJ006 strain by extract Lycorine showed SI value >900 ( this data is sufficient to show this compound have antiviral activity against SARS-CoV/ |
| 14 | *Artemisia annua* | Asteraceae | Whole plant | Vero E6 / HEP2 line BJ001 BJ006 | CPE/MTS assay | ND | S1= 31 (against BJ001) SI=27 against BJ006 That shows it have certain amount of antiviral activity against SARS-CoV strain in vero cells |
| 15 | *Pyrrosia lingua* | Polypodiaceae | Leaf | Vero E6 / HEP2 line BJ001 BJ006 | CPE/MTS assay | ND | S1= 55 (against BJ001) SI=59 ( against BJ006) That shows it have certain amount of antiviral activity against SARS-CoV strain in vero cells |
| 16 | *Lindera aggregata* | Lauraceae | Root | Vero E6 / HEP2 line BJ001 BJ006 | CPE/MTS assay | ND | S1= 16 (against BJ001) SI=17 against BJ006 That shows it Have certain amount of antiviral activity against SARS-CoV strain in vero cells |
| 17 | *Isatis indigotica* | Brassicaceae | Root | Vero cells | SARS-CoV 3CL pro | CPE/MTS assay | Sinigrin With IC₅₀ = 217µg/ml was more efficient in blocking the cleavage processing of the ND |

**Notes:**
- The IC₅₀ values for psoralen as control >150 against SARS-CoV PLpro enzyme.
- Compound Isobavachalcone and psoraladin showed reversible mixed type of mechanism for inhibition of enzyme and coumestrol (compound 5) group of compound show most potent inhibitor against enzyme.
- Caffeic acid may interfere the binding interaction of HCoV-NL63 with heparan sulfate proteoglycans and ACE2 receptor on the cell surface.
- Lycorine showed SI value (>151) for BJ001 and SI value (>170) for BJ006
- May be by interacting with expressed viral protein / antigen
- Interferon α showed SI value (>151) for BJ001 and SI value (>170) for BJ006 Can be moderate antiviral activities against this strain of virus
- Interferon a showed SI value (>151) for BJ001 and SI value (>170) for BJ006

**References:**
- [39]
- [35]
- [40]
- [41]
- [41]
- [41]
| No. | Species                  | Family       | Extract | Cells/vero cells | Virus     | Assay                      | Inhibitory effect of extract on enzyme | SI value                                                                 | Antiviral activity                                                                                      |
|-----|-------------------------|--------------|---------|------------------|-----------|----------------------------|----------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| 18  | Torreya nucifera        | Taxaceae     | ND      | DBT cells/vero | MHV-A59/PEDV | Cell viability assay,     | 19.4±7.7µg/ml                          | 0.9±0.1 and PEDV production in dose dependent manner | 3Cl pro in cell based assay where also aloe emodin and hesperetin inhibit 3Cl pro inhibitory activity |
| 19  | Cimicifuga rhizoma      | Ranunculaceae | ND      | DBT cells/vero | MHV-A59/PEDV | Cell viability assay,     | 13.0±1.4µg/m                          | 0.8±0.0 and PEDV production in dose dependent manner | May be due to the inhibition of RNA dependent RNA polymerase                                    |
| 20  | Meliae cortex           | Meliaceae    | ND      | DBT cells/vero | MHV-A59/PEDV | Cell viability assay,     | 2.0±0.5µg/m                           | PEDV production in dose dependent manner |
| 21  | Coptidis rhizoma        | Cibotiacae   | ND      | DBT cells/vero | MHV-A59/PEDV | Cell viability assay,     | 21.7±1.9µg/ml                          | 0.9±0.0 and PEDV production in dose dependent manner | May be due to the inhibition of RNA dependent RNA polymerase                                    |
| 22  | Phellodendron cortex    | Rutaceae     | ND      | DBT cells/vero | MHV-A59/PEDV | Cell viability assay,     | 27.5±1.1µg/ml                          | 0.8±0.0 and PEDV production in dose dependent manner | May be due to the inhibition of RNA dependent RNA polymerase                                    |
| 23  | Sophora subprostrata radix | Fabaceae   | ND      | DBT cells/vero | MHV-A59/PEDV | Cell viability assay,     | 188.9                                 | 0.8±0.0 and PEDV production in dose dependent manner | 3Cl pro inhibitory activity may be due to inducing COX2 expression through the activation of ERK and P38 or ERK alone |
| 24  | Torilis fructus         | Apiaceae     | ND      | DBT cells/vero cell line | MHV-A59/PEDV | Plaque assay, viability assay | 195.6                                 | 0.8±0.0 and PEDV production in dose dependent manner | 3Cl pro inhibitory activity may be due to inducing COX2 expression through the activation of ERK and P38 or ERK alone |
| 25  | Acanthopanax curcas     | Araliaceae   | ND      | DBT cells/vero cell line | MHV-A59/PEDV | Plaque assay, cell viability assay | 188.9                                 | 0.8±0.0 and PEDV production in dose dependent manner | 3Cl pro inhibitory activity may be due to inducing COX2 expression through the activation of ERK and P38 or ERK alone |
| 26  | Sophora radix           | Fabaceae     | ND      | DBT cells/vero cell line | MHV-A59/PEDV | Plaque assay, viability assay | 61.5                                  | 0.8±0.0 and PEDV production in dose dependent manner | 3Cl pro inhibitory activity may be due to inducing COX2 expression through the activation of ERK and P38 or ERK alone |
| No. | Plant Name | Family | Part Used | Cell Line | Assay 1 | Assay 2 | Assay 3 | Assay 4 | Assay 5 | Activity |
|-----|------------|--------|-----------|-----------|---------|---------|---------|---------|---------|----------|
| 27  | Sanguisorba radix | Rosaceae | DBT cells/vero cell line | MHV-A59/PEDV | Plaque assay, cell viability assay | ND | Extract reduced intracellular viral protein (45%) and production and replication of virus with EC50 = 0.8 ± 0.2 µg/ml and SI value 696.0 | | Ribavirin showed inhibitory action against MHV-A59 strain with EC50 = 17.5 ± 2.9 µg/ml and SI value 61.5 | Reduce CoV production partly as a result of decreased in protein synthesis |
| 28  | Gentiana scabra | Gentianaceae | Vero E6 cells | SARS-CoV 3CL pro | CPE assay, cell proliferation assay, viral replication assay, MTT assay (cell based assay), protease inhibition assay | ND | In GSH fraction, inhibitory cytopathogenic effect at 25 to 200 µg/ml | Inhibition of viral replication EC50 = 8.70 µg/ml at concentration 0.1-10 µg/ml with SI (>57.5) | Inhibition of 3CL protease enzyme at IC50 (>50 µg/ml) with biological safety for host cell in concentration 10 to 500 µg/ml | SARS-CoV replication inhibition shown by Valinomycin with SI value (41.4) | |
| 29  | Dioscorea batatas | Dioscoreaceae | Vero E6 cells | SARS-CoV 3CL pro | CPE assay, cell proliferation assay, viral replication assay, MTT assay (cell based assay), protease inhibition assay | ND | In DBM fraction, inhibitory cytopathogenic effect at 25 to 200 µg/ml | Inhibition of Viral replication EC50 = 8.706 µg/ml at concentration 0.1-10 µg/ml with SI (>62) Considerable inhibitory of SARS-CoV protease activity at IC50 value 442±2µg/ml, with biological safety for host cell in concentration 10 to 500 µg/ml | SARS-CoV replication inhibition shown by Valinomycin with SI value (41.4) | |
| 30  | Cassia tora | Fabaceae | Vero E6 cells | SARS-CoV 3CL pro | CPE assay, cell proliferation assay, viral replication assay, MTT assay (cell based assay), protease inhibition assay | ND | In CTH fraction, inhibitory cytopathogenic effect at 25 to 200 µg/ml | Inhibition of Viral replication EC50 = 8.70 µg/ml at concentration 0.1-10 µg/ml with SI (>59.3) Inhibitory effect on 3CL protease enzyme with IC50 (>50 µg/ml) with biological safety for host cell in concentration 10 to 500 µg/ml | SARS-CoV replication inhibition shown by Valinomycin with SI value (41.4) | |
| 31  | Taxillus chinensis | Lurantiaceae | Vero E6 cells | SARS-CoV 3CL pro | CPE assay, cell proliferation assay, viral replication assay, MTT assay (cell based assay), protease inhibition assay | ND | In TCH fraction, inhibitory cytopathogenic effect at 25 to 200 µg/ml | Inhibition of Viral replication EC50 = 5.3 µg/ml at concentration 0.1-10 µg/ml with SI (>92.8) Inhibitory effect on 3CL protease enzyme with IC50 (>50 µg/ml), with biological safety for host cell in concentration 10 to 500 µg/ml | SARS-CoV replication inhibition shown by Valinomycin with SI value (41.4) | |
| No. | Species                  | Family       | Part         | Assay Type | IC50 | Activity Description                                                                 |
|-----|--------------------------|--------------|--------------|------------|------|--------------------------------------------------------------------------------------|
| 32  | *Cibotium barometz*      | Cibotiaceae  | Rhizomes     | CPE assay, cell proliferation inhibition assay   | ND   | In CBE /CBM fraction, Inhibitory of cytopathogenic effect at 25 to 200µg/ml SI (59.4) for CBE shows anti-SARS-CoV activity IC50 (39µg/ml) for CBM shows inhibitory effect SARS-CoV 3CL protease activity, with biological safe for host cell in concentration 10 to 500µg/ml |
| 33  | *Toona sinensis*         | Meliaceae    | Leaf         | Antiviral assay, cell proliferation assay, MTT assay( Cell based assay) | ND   | Inhibitory of cytopathogenic effect at 25 to 200µg/ml SI (>59.4) for CBE shows anti-SARS-CoV activity IC50 (39µg/ml) for CBM shows inhibitory effect SARS-CoV 3CL protease activity, with biological safe for host cell in concentration 10 to 500µg/ml |
| 34  | *Camellia japonica*      | Theaceae     | Flower       | PEDV       | ND   | Inhibitory effect of Azauridine on PEDV replication with SI value(14.30±1.24) May be by reducing the RNA level associated with GP6 nucleocapsid ,GP2 spike and GPS protein responsible for PEDV replication |
| 35  | *Saposhnikovia divaricata* | Apiaceae    | Radix        | PEDV       | ND   | Inhibitory effect on gene encoding PEDV GP6 nucleocapsid GP2 spike ,GPS membrane protein |
| 36  | *Dryopteris crassirhizoma* | Dryopteridaceae | Rhizomes    | PEDV       | ND   | Inhibitory effect on gene encoding PEDV GP6 nucleocapsid GP2 spike ,GPS membrane protein |
| 37  | *Rheum palmatum*         | Polygonaceae | Leaves       | SARS-CoV 3CL pro inhibition assay through determination of enzyme velocity represented by absorption assay | ND   | Inhibiting the interaction of SARS-CoV S protein and ACE2 in dose dependent manner an maximum result is 96% inhibition with IC50 =13.76±0.003 at 100µg/ml |
| 38  | *Houttuynia corda*       | Saururaceae  | Whole parts  | SARS-CoV 3CL pro, protein based fluorescence assay,ELISA | ND   | Extract shows inhibiting effect in decrease in fluorescence ratio of the substrate implying that it could inactivate the SARS-CoV 3CL protease enzyme at conc. 200µg/ml and increased CD4+ cell after 48hr and CD8+ cell after 24hr and IL -2 after 72hr in dose dependent manner |

[46] [47] [48] [49] [50] [51]
| Plant Name          | Family               | Part     | Cell Line | Assay Method                          | Result                                                                 | Immune Response                                                                 |
|---------------------|----------------------|----------|-----------|--------------------------------------|------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| **39 Euphoria nortilis** | Euphorbiaceae       | Leaves  | MRC-5 cells | HCoV-229E XTT assay to determine cell viability | This three compound showed 132.4%, 111.0%, 109% cell survival through inhibition of virus respectively | Actinomycin shows 69.5% cell survival against HCoV-229E strain                     |
| **40 Black tea/puer tea** | Theaceae             | Leaves  | vero cell  | SARS-CoV 3Cl protease inhibition assay | 50% inhibition of 3Cl protease enzyme activity encoded by SARS-CoV at concentration <10 μM | N-ethylmaleimide showed inhibition of protease activity against SARS-CoV 3CL |
| **41 Prunus serrulata** | Rosaceae             | Cherry   | vero, ATCC CCL-81 | PEDV CPE assay | Extract showed highest anti-PEDV with 50% CPE inhibition at 1.85µg/ml conc. | Ribavirin, interferon α inhibit 50% PEDV replication at conc.: 4.1µg/ml and 9µg/ml respectively |
| **42 Nigella sativa** | Ranunculaceae        | Seeds    | Murine fibroblast LR7 | MTT assay, Time of addition assay, ELISA, Calcium conc. assay, Viral load assay | ND | ND |
| **43 Galanthus nivalis** | Amaryllidaceae       | FCWF-4   | P-CoV      | MTT assay, CPE assay | ND | ND |
| **44 Rosa nutkana**   | Rosaceae             | Whole part | MDBK   | IBV Antiviral assay | Extract showed antiviral effect against enteric coronavirus | ND |
| **45 Alstonias scholaris** | Apocynaceae         | ND     | vero | IBV Plague assay, Antiviral assay | Pretreatment with AS1 could inhibit plaque formation in dose-dependent manner with IC₅₀(35µg at 50µm retain the inhibition activity(38% inhibition) | ND |
| **46 Sambucus nigra** | Adoxaceae            | Fruit    | Vero     | IBV Plague assay, cytotoxicity assay | ND | ND |
magnitude in dose dependent manner

|   |   |   |   |   |
|---|---|---|---|---|
| 47 | Chamaecyparis obtusa | Cupressaceae | Heart wood | Vero E6 |
|   |   |   | SARS-CoV 3CL | CPE assay, Inhibition of viral replication assay(ELISA), protease inhibition assay |
|   |   |   |   | 8β-hydroxyabieta 8(11),13-diene-12one (5) |
|   |   |   |   | Pinusolidic acid (9) |
|   |   |   |   | Savinin (16) |
|   |   |   |   | Compound 5, 9 showed high inhibition against SARS-CoV induced CPE with SI value \(>510\), 89.8 against SARS-CoV replication |
|   |   |   |   | and compound 16 showed potent inhibition activity against SARS-CoV 3CLpro with IC_{50} (25um) |
|   |   |   |   | Positive control niclosamide showed high inhibition against CPE induced by virus with SI value \(>221\) against virus replication and IC_{50} (40) against protease activity encoded by SARS-CoV |
|   |   |   |   | ND | [60] |

| 48 | Juniperus formosana | Cupressaceae | Heart wood | Vero E6 |
|   |   |   | SARS-CoV 3CL | CPE assay, Inhibition of viral replication assay(ELISA), protease inhibition assay |
|   |   |   |   | 3β,12, diacetoxyabieta-6,8,11,13 tetra(8) |
|   |   |   |   | Butulonic acid(14) |
|   |   |   |   | Compound 8, 14 showed high inhibition against SARS-CoV induced CPE with SI value 193, 180 respectively against SARS-CoV replication. |
|   |   |   |   | Positive control niclosamide showed high inhibition against CPE induced by virus with SI value \(>221\) against virus replication and IC_{50} (40) against protease activity encoded by SARS-CoV |
|   |   |   |   | ND | [60] |

| 49 | Cryptomerica japonica | Cupressaceae | Heart wood | Vero E6 |
|   |   |   | SARS-CoV 3CL | CPE assay, Inhibition of viral replication assay(ELISA), protease inhibition assay |
|   |   |   |   | 7β-hydroxydeoxycryptojaponol(6) |
|   |   |   |   | Compound 4 showed high inhibition against SARS-CoV induced CPE with SI value 111 against SARS-CoV replication |
|   |   |   |   | Positive control niclosamide showed high inhibition against CPE induced by virus with SI value \(>221\) against virus replication and IC_{50} (40) against protease activity encoded by SARS-CoV |
|   |   |   |   | Another positive control bitulinic acid showed IC_{50} (10um) against protease activity |
|   |   |   |   | ND | [60] |

IC_{50}: concentration required to inhibit 50% of virus growth, TC_{50}: drug concentration that reduces the cell growth by 50% (cellular toxicity), CC_{50}: concentration required for the reduction of cell viability by 50%, SI: Selectivity Index, ND: Not Defined

Table 2: Compounds showing antiviral property against different coronavirus strains
| SN | Compound | Source | Culture cell | Virus/ strain | Method | Result | Conclusion | References |
|----|----------|--------|--------------|---------------|--------|--------|------------|------------|
| 1  | Salisaponin B2 | Sigma Chemical (St. Louis, MO, USA) | MRC-5 cell | SARS-CoV | XTP assay, Attachment assay/penetration assay | At concentration 0.25-2.5µmol/l showed strongest activity with EC50 =1.7±0.1µmol/l and SI =221.9 | May be due to interference in the early stage of viral replication, absorption, and penetration of the virus | [61] |
| 2  | Glycyrrhizin | ND | Vero cells | SARS-CoV | FFM-1, FFM-2 | Most effective when given at early stages of viral replication and during and after adsorption with SI =>67, show potent inhibition of viral replication | May be by affecting cellular signaling pathway or by inducing production of nitrous oxide which inhibit viral replication | [62] |
| 3  | Tannic acid | Microsource Discovery Systems, Inc., Gaylord, CT | E-coli | SARS-CoV 3CL pro | Protease inhibition assay, flurogenic substrate peptide assay | Found to have inhibitory activity against 3CL pro encoded by SARS-CoV with IC50 =3µm | Gallate group may be responsible for inhibitory activity against target strain | [53] |
| 4  | 3-isothiocyanin-3'3'-digallate | Microsource Discovery Systems, Inc., Gaylord, CT | E-coli | SARS-CoV 3CL pro | Protease inhibition assay, flurogenic substrate peptide assay | Found to have inhibitory activity against 3CL pro encoded by SARS-CoV with IC50=7µm | Gallate group may be responsible for inhibitory activity against target strain | [53] |
| 5  | Theaflavin -3',3'-gallicate (TF2B) | Microsource Discovery Systems, Inc., Gaylord, CT | E-coli | SARS-CoV 3CL pro | Protease inhibition assay, flurogenic substrate peptide assay | Found to have inhibitory activity against 3CL pro encoded by SARS-CoV with IC50=9.5µm | Gallate group may be responsible for inhibitory activity against target strain | [53] |
| 6  | Myristin | Chromadex | MCF10A | SARS CoV helicase, nsp13 | FRET DS , DNA unwinding assay | Inhibit the ATPase activity of nsp13 by more than 90% at concentration 10µm | By interfering with ATP/ADP binding pocket of the SARS-CoV helicase protein | [63] |
| 7  | Scutellarein | Scutellaria baicalensis | MCF10A | SARS CoV helicase, nsp13 | FRET DS , DNA unwinding assay | Inhibit SARS CoV helicase with IC50=0.8±0.19µm | By interfering with ATPase activity of the SARS-CoV helicase protein | [63] |
| 8  | Tetrandrine | Wuhan ChemFaces Biochemical Co., Ltd. (Wuhan, China) | MRC-5 human lung cells | HCoV-OC43 | CPE/MTS assay | Anti HCoV-OC43 in dose and time dependent manner with SI=40 with IC50 = 0.33±0.03 | Inhibit virus induced cell death at the early stage of virus infection and suppressed the replication of virus and inhibited viral S and N protein expression | [64] |
| 9  | Fangchinoline | Wuhan ChemFaces Biochemical Co., Ltd. (Wuhan, China) | MRC-5 human lung cells | HCoV-OC43 | CPE/MTS assay | Anti HCoV-OC43 in dose and time dependent manner with SI=11 with IC50 =1.01±0.07µm | Inhibit virus induced cell death at the early stage of virus infection and suppressed the replication of virus and inhibited viral S and N protein expression | [64] |
| 10 | Cepharanthine | Wuhan ChemFaces Biochemical Co., Ltd. (Wuhan, China) | MRC-5 human lung cells | HCoV-OC43 | CPE/MTS assay | Anti HCoV-OC43 in dose and time dependent manner with SI >13 with IC50 =0.83±0.07µm | Inhibit virus induced cell death at the early stage of virus infection and suppressed the replication of virus and inhibited viral S and N protein expression | [64] |
| 11 | Quercetin | Sigma-Aldrich | Picia pastoris GS 11 | 3CL pro SARS-CoV | FRET inhibition assay, Docking analysis | 82% inhibition of SARS-CoV 3CL pro activity with IC50 = 73±4µm | Numerous hydrophilic and H-bonds interaction with amino acid residues in the active site pocket of 3CL pro and inhibiting the activity. | [65] |
| 12 | Epigallocatechin gallate | Sigma-Aldrich | Picia pastoris GS 11 | 3CL pro SARS-CoV | FRET inhibition assay, Docking analysis | 85% inhibition of SARS-CoV 3CL pro activity with IC50 = 73±2µm | Numerous hydrophilic and H-bonds interaction with amino acid residues in the active site pocket of 3CL pro and inhibiting the activity. | [65] |
| 13 | Gallicotin | Sigma-Aldrich | Picia pastoris GS 11 | 3CL pro SARS-CoV | FRET inhibition assay, Docking analysis | 91% inhibition of SARS-CoV 3CL pro activity with IC50 =47±0.9 | Numerous hydrophilic and H-bonds interaction with amino acid residues in the active site pocket of 3CL pro and inhibiting the activity. | [65] |
| 14 | Herbacetin | ND | ND | 3CL pro MERS-CoV | Antiviral assay, tryptophan based | Block enzymatic activity of MERS-CoV 3CL pro with IC50 =40.59µm | Inhibition of main viral proteases and thus nullify a process of virus peptides | [66] |
| 15 | Isobavachalcone | ND | ND | 3CL pro MERS-CoV | Antiviral assay, tryptophan based | Block enzymatic activity of MERS-CoV 3CL pro with IC50 =35.85µm | Inhibition of main viral proteases and thus nullify a process of virus peptides | [66] |
| 16 | Quercetin-3-B-D-glucoside | ND | ND | 3CL pro MERS-CoV | Antiviral assay, tryptophan based | Block enzymatic activity of MERS-CoV 3CL pro with IC50 =37.03µm | Inhibition of main viral proteases and thus nullify a process of virus peptides | [66] |
| 17 | Hulihexoside | ND | ND | 3CL pro MERS-CoV | Antiviral assay, tryptophan based | Block enzymatic activity of MERS-CoV 3CL pro with IC50 =67.0µm | Inhibition of main viral proteases and thus nullify a process of virus peptides | [66] |
In this review, as per the data available on the various parts of the plants and compounds from different articles published, they showed that they possess antiviral activity against coronavirus strains (human coronavirus and non-human coronavirus) in the mild, moderate and strong condition. Through in-vitro tests on various parts of the plant (leaves, flower, aerial parts, rhizomes, and fruit) and different compound bought from the market/ pharmaceutical/chemical company. Among various techniques used for the detection of viral inhibition, cytopathic reduction assay was the most common technique for in-vitro analysis in the antiviral study. We know that medicinal plants are the good sources of various phytochemical compounds that provide the basis for the development of new antiviral agents against different virus strains. The WHO (world health organization) has an estimated 80% of the world population fulfil their healthcare needs from phytomedicinal sources. The mechanism of the antiviral potential of plant extract or various compounds varies among the different strains of the virus. Some phytochemical compounds target viral envelope, some membrane protein, some of them focus ion channel, some inhibit the virus’s attachment to the host cell, and some inhibit CPE (cytopathogenic effect) on host cells/plagues formation or ion concentration intracellularly.

The present review showed that the above mention plant and compound (Table 1 and Table 2) containing bioactive substances have some amount of promising antiviral activity. Among the listed plants, the most prominent and potent effect shown by Allium porum, Urtica dioica, Lycoris radiata, Juniperus formosa and Cryptomeria japonica against SARS-CoV with SI value 222, >77, >900, >180 and >111 respectively as compared with standard mentioned by [69]. Similarly, Sophorae radix was found to have the highest inhibition activity against MHV -A59 coronavirus with SI value 696 compared with other compounds. Plant and for SARS-CoV 3 CL proenzyme Dioscorea batatas showed the most effective result Cibotium barometz, Cassia tora with SI value >62, >59.4, >59.3 respectively.

Calophyllum Blancoi, Torilis fructus, Acanthopanacis cortex, Sophorae Radix, Allium porum, Urtica dioica and Nicotiana tabacum were found to have 50% maximum potent activity against coronavirus species. At low concentration 3µg/ml, 0.6µg/ml, 0.9±0.1 µg/ml, 0.8±0.2µg/ml, 0.45µg/ml, 1.3±0.1µg/ml and 1.7±0.3µg/ml respectively which have a comparable result to the standard drug used for the coronavirus strain according to [69]. Taxillus chinesis showed a similar result to the standard one to inhibit replicating the virus with EC50 5.3µg/ml. Similarly, plants showing the highest IC50 activity at lower concentrations are Brosnoeti papyrifera (IC50=3.7µm), Paulownia tomentosa (IC50=5-14.4µm), Torreya nucifera (IC50=8.3µg/ml) against SARS-CoV enzyme. Sambus formosana showed the most potent IC50 value activity against plaque formation (IC50=1.75) and for virus attachment (4.67µg/ml) in host cells infected with HCoV-NL63 strain of the virus. Torilis fructus, Acanthopanacis cortex showed the highest value for reducing intracellular viral mRNA by 93% and 90%, respectively. Euphoria nerifolia was showed >100% cell survival through inhibition of virus activity against coronavirus when compared with standard.

A compound like Saikosaponin B2 was found to have maximum effective anti virus against Human coronavirus in a dose-dependent and time-dependent manner with SI (221.9) and EC50 =1.7±0.1µmol/l, Isobavachalcole against 3CL pro-MERS CoV with IC50 (35.8µm) and Gallocatechin gallate against 3CL pro-SARS CoV with IC50 (47±0.9um).

We encountered 49 plants and 19 compound chemically defined natural molecules reported in the literature, which have evaluated for potent antiviral activity against different coronavirus strains. The active compounds, which have been isolated and identified, belong to the classes of alkaloids, terpenoids, xanthones,), flavonoids, steroid, lipids, oxygen benzenoids, carbohydrates, lignans, proteins, coumarins, phenylpropanoids, polyphenols, resin, glycosides, etc. These natural metabolites act as a key for antiviral activity.

### 3. Methodology

A search was conducted in the following databases or search engines: PubMed, Science Direct, MedLine, Google Scholar, and Biomed central for published articles. The keyword ‘coronavirus’ was paired with ‘natural products’, ‘medicinal plants’, ‘phytochemicals’, ‘alkaloids’, ‘glycosides’, ‘flavonoids’, ‘saponins’, ‘terpenes’, ‘monoterpenes’, ‘diterpenes’, ‘sesquiterpenes’, ‘triterpenes’, ‘terpenoids’, ‘tannins’, ‘saponins’, ‘phenols’, ‘polyphenols’, ‘herbal drugs’, ‘crude extracts’, or ‘synthetic derivatives of natural products’ to obtain published records till May 2020. No language restriction was imposed. Obtained records in this study were included and excluded based on the following criteria. The inclusion data criteria included

1. Studies involving crude extract, fraction, or their preparation of plants acting against CoV strains.
2. Studies related to derivatives of natural products (e.g., isolated compounds) and chemicals or biochemicals acting against CoV strains.
3. Studies with natural product inspired synthetic derivatives acting against CoV strains.

The exclusion data criteria included: (a) Data duplication and titles or contents that do not meet the inclusion criteria, (b) Reports on antiviral activities of natural products or their derivatives against other than CoV strains.

4. Conclusion

Natural products provide a valuable and authoritative resource of chemical compounds displaying antiviral properties. Structure modification of these compounds may help in improving and increasing their potency. The development of antiviral drugs is a challenge, and some antiviral medications can only prevent virus replication or inhibit further infection. In this review, different methods and its possible mechanism showing antiviral property have been highlighted. In the present situation, there is no proper development of antiviral drugs due to which the world is searching for its remedies in nature. All the listed plants and their compounds in this review are highly potent with promising results against coronavirus. These can be further screened for invasive tests and used for making different formulations or may be polyherbal formulations considering its safety profile and toxicity.

5. Declarations

Author contribution: conceptualization, writing, referencing, Sindhu KC, Conceptualization, referencing, writing, supervision, Draft preparation, Manoj Pandit, writing, reviewing, editing, Amit Kumar Shrivastava

Conflict of interest: the author declares no conflict of interest

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