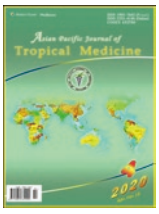

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Crimean-Congo hemorrhagic fever: etiology, diagnosis, management and potential alternative therapy

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ABSTRACT

Crimean-Congo hemorrhagic fever (CCHF) virus belongs to the genus *Nairovirus* and family Bunyaviridae. CCHF is a tickborne disease that has mostly been reported from Asia, Africa and Europe. Early diagnosis of CCHF is essential for patient care and preventing its spread to normal individuals. Treatment of CCHF is mostly limited to the use of ribavirin and palliative care. The practice of using interferon and vaccines has also been proved to be ineffective and unsafe. A search for an effective alternative treatment of the CCHF still continues. Therefore, the current review focusses on the cause, prevalence, mode of transmission, pathophysiology, signs, symptoms, diagnostic features and treatment options of CCHF. This review also highlights the possible alternative therapy in the form of antiviral medicinal plants which are effective against viral hemorrhagic fever. These medicinal plants have shown convincing evidence for their activities against different viral hemorrhagic fevers and may be used alone or in combination with existing therapies to achieve an optimum therapeutic response.

KEYWORDS: Congo fever; Dengue fever; Alternative therapy; Antiviral plants

1. Introduction

Crimean-Congo hemorrhagic fever (CCHF) commonly referred to as Congo fever is a tickborne zoonotic disease. It is caused by CCHF virus (CCHFV) that is transmitted vertically or horizontally to human hosts *via* tick bite[1]. Humans also get infected upon exposure to blood or other body fluids of already exposed animals such as goats, sheep and cattle that develop a state of transient viremia. The CCHFV was medically recognized 75 years ago, however, several instances of its outbreak have been reported in recent years.

The CCHFV is an RNA virus that belongs to the genus *Nairovirus*, family Bunyaviridae. Other genera of the family are *Orthobunyavirus*, *Tospovirus*, *Hantivirus* and *Phlebovirus*. The *Nairovirus* mainly spreads through tick bites. The CCHFV seven different sero-types and shares the sero-group with Hazara virus (HAZV) isolated from ticks parasitizing the wild rodents in the Hazara region of Pakistan[2]. Patients infected with CCHFV exhibit severe hemorrhagic afflictions[3]. The patients infected with CCHFV are difficult to diagnose and treat due to similarity with other hemorrhagic viral diseases and limited treatment options[4-8].

The CCHF is a rare disease with a global prevalence of less than one per million. The efficacy of antiviral drugs remains limited despite proven *in vivo* and *in vitro* effectiveness against the CCHF. Furthermore, the opinion about the time of administration and doses of medicines remains unclear[9]. Since there is no definitive treatment of the CCHF, naturally occurring herbal agents acting against CCHFV and associated complications should be explored. The process of new drug development for treating the CCHF is also complex due to limited number of patients. The current review summarizes the historical prospective, viral structure and strains, modes of transmission, signs and symptoms, diagnosis, allopathic and alternative treatment options.

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2. Brief historical depiction

The earliest mentions of the CCHF can be found in a thesaurus of a Persian physician who mentioned the area affected by the infection along with the sign and symptoms. The cause was revealed to be a hard tick or louse like organism parasitizing black birds. The first ever treatment against for CCHF was the essences and extracts of different plants orally administered to the patients or topically applied at the site of the tick bite. The CCHF was not recognized as a major disease for a long period until its occurrence in the Crimean region in 1944[10].

The earliest characterization of the virus was carried out in 1967 by Mikhail Chumakov in 1967. The newborn white mouse (NWM) inoculation method was effectively used in morphological, antigenic and physiochemical characterizations and isolation of CCHFV. In 1968, serological similarities were observed between the viral strains obtained from patients in various parts of the world that enabled to develop different antibodies and antigens which were helpful in the study of natural existence and behavior of the CCHFV[11].

The research on CCHFV remained sluggish for more than two decades because of the limitation to isolate the causative organism in the laboratory. However, it was found that some arboviruses could be isolated by introducing the inoculum into the cerebral region of the newborn mice. It was also observed that RNA in the CCHFV was particularly sensitive to sodium desoxycholate, chloroform and ether. It was able to pass through filters of 220 nm pore size. The virus showed significant resistance to freezing. It became inactive on exposure 60 ℃ for 15 min or 37 ℃ for 7 h. The size of virion is 100-130 nm and it is spherical in shape[12].

3. Prevalence and risk factors

Numerous outbreaks have been reported in South Asia, Middle East, Balkan region, Africa and Europe, especially after 2000[13-22]. Literature shows that the outbreak of CCHF usually occurs in early summer and spring. The variation in outbreak depends upon the changes in season that affects the tick population and viral load in the infected animals. Moreover, the geographical variations in the occurrence of the disease are due to the difference in the distribution of Ixodid ticks population[23]. Farm workers, shepherds, housewives, butchers, veterinarians, farmers, animal dealers and other individuals involved in handling of ticks exposed animals are mostly susceptible to CCHF. Moreover, health workers such as doctors and nurses are also at the risk of CCHFV infection due to its nosocomial nature. It was found that several individuals in direct contact with the CCHF patients' also got infected[23-26]. Afghanistan, Pakistan, India, Oman, Sudan, China, South Africa and Tajikistan are among the countries that experience 5-50 new cases of CCHF patients annually. More than 50 new cases of CCHF are reported from Iran, turkey, Uzbekistan or Russia annually[27].

4. Structure and replication of virus

There are 34 viruses in the genus *Nairovirus*, all of which are tickborne[28]. It develops lipid envelope around itself which is derived from the host it infects. The genome of the CCHFV is divided into 3 sections which are characterized according to their size as large (L), medium (M) and small (S)[16,29,30]. The lipid envelope consists of 2 types of glycoproteins namely GN and GC which play important role in the attachment of virion to the host cells. RNA-dependent RNA polymerase (RdRp) and the 3 segmented genomes of the virus are encapsulated in nucleoprotein, both of which are necessary to initiate viral replication in the host cells[31].

The receptor present on the surface of host cells has not been reported, however, it is indicated that glycoprotein GC present in viral envelope is involved in the attachment. Nucleolin, a host molecule, is important during the entry of virus into the host cell. The virus enters the cell *via* clathrin dependent endocytosis. As the virion gains access to the cytoplasm, low pH of endosome initiates conformational changes in glycoproteins of the virus that results in the fusion of the viral envelope and endosomal membranes releasing nucleocapsid into the cytosol followed by dissociation of nucleocapsids. Complementary RNA and mRNA are synthesized *via* RdRp[29]. The mRNA is used for synthesis of viral proteins while the complementary RNA is used for the synthesis of viral RNA. Once the replication process is completed and new viral RNA is formed, interaction occurs between viral RNA, RdRp and capsid proteins to develop new nucleocapsids. The translocation of glycoproteins occurs in the endoplasmic reticulum where GN and GC glycoproteins are formed by cleavage of precursor proteins. The final processing of the glycoproteins occurs in Golgi apparatus. The new viruses are transferred to host cytoplasm from where these are released[31].

5. CCHFV genotypes

The CCHFV strains are identified on the basis of partial or complete sequencing of the S-segment of negative stranded RNA to facilitate epidemiological studies as genotypes present in one geographical region of the world were different from the other such as Asia 1, Asia 2, Africa 1, Africa 2 and Africa 3, Europe 1 and Europe 2[32]. However, whole genome sequencing of the virus showed that the CCHFV exhibited enormous variety with greater exchange of M segments. Furthermore, co-infection of different CCHFV strains during blood meals of the ticks is implicated in such variations in strains[33].

6. Mode of transmission

Transmission of the CCHFV to human can occur *via* direct bite from infected ticks or through exposure to infected animals which act as host. Certain ticks only serve as carriers of the CCHFV without causing direct human infection. *Hyalomma* ticks are one of the main causes of human infections. The CCHFV sero-group can spread through different tick genera but mainly through Ixodid ticks[1].

The CCHFV infection can occur vertically and horizontally. Vertical transmission occurs in competent ticks capable of supporting viral replication. Adult females can transmit the virus to their eggs and the adult males can transmit the virus to adult females. Once the ticks suck infected blood, the virus reaches the midgut of tick where it replicates in the lining of the midgut and then spreads to other organs, eventually leading to high titers in the salivary glands and the reproductive organs. Horizontal transmission occurs between ticks and mammals during spring and summer upon consumption of blood meals[1]. It can also spread from infected to healthy humans by direct or indirect contact of skin, mucous membranes or other bodily fluids[34].

7. Pathogenesis and clinical features

Once the virus enters the host, it contacts the dendritic cells where it replicates and spreads to the nearby tissues, lymph nodes, blood monocytes and organs such as spleen and liver. The infection of the permissive parenchymal cells occurs due to the movement of tissue macrophages. The host lymphocytes remain uninfected, however, these are destroyed in large number due to the underlying illness. The intrinsic coagulation pathway is also initiated due to the production of cell surface tissue factors. The abnormalities in the endothelial cell function, platelet and coagulation factors disturb the homeostasis of body. The level of coagulation factors in the body is reduced due to hepatic dysfunction, disseminated intravascular coagulopathy, activation of different immunological and inflammatory pathways and direct injury to the endothelial cells and platelets. These pathological changes occur mainly due to the production of cytokines, pro-inflammatory mediators and chemokines in response to infected macrophages and monocytes. One research indicates that hepatocytes are principally affected by the infection[35].

The progression of infection occurs in 4 distinct phases. The first phase is the incubation phase lasting for 3 to 7 d. Incubation period is the time between the exposure to the CCHFV and manifestation of symptoms. The incubation period varies from patient to patient due to the difference in route of exposure, dose of infection and patient age. The second phase is the pre-hemorrhagic phase characterized by flu-like symptoms such as dizziness, fever, myalgia, headache, joint and orbital pain. An increase in the level of hepatic enzymes has also been observed during this phase. The third phase is the hemorrhagic phase that develops between 3 to 5 d after the onset of disease. It is characterized by oliguria due to the failure of renal system. The other characteristic is the development of disseminated intravascular coagulation[36]. Recent studies suggested that another condition known as virus-associated hemophagocytic syndrome had also contributed to the clinical features and severity of disease[37,38]. This condition is characterized by the cytopenia, fever, hepatomegaly and elevated levels of lactate dehydrogenase, triglycerides and ferritin. Hemophagocytosis is the most important characteristic of this syndrome that occurs in the liver, lymph nodes and bone marrow. The abnormally high activity and production of cytokines from helper T-cell macrophages and lymph nodes contribute to hemophagocytosis. Inflammatory cytokines are particularly high in fatal cases compared to non-fatal cases. The final stage is convalescent[39,40]. The fatality rate in CCHF lies between 40%-60%. In severe cases, death occurs mainly due to circulatory shock, disseminated intravascular coagulation and multi-organ failure. Recently, it was shown that one of the features of hemorrhagic syndrome was acute respiratory distress syndrome and alveolar hemorrhage[40,41]. The convalescent period occurs in the surviving patients between 10-20 d after the onset of disease. This stage is characterized by loss of hearing, weak pulse, tachycardia and alopecia[42].

8. Diagnosis of CCHF

Diagnosis of the CCHF is necessary as early as possible both for rapid recovery of patient as well as protection of healthy individuals. Differential diagnosis is necessary as the CCHF shares similarities with other diseases. Laboratory tests include detection of viral antigens and antibodies in patients by isolating the virus in a tissue culture or using the suckling mouse model followed by detection of the viral RNA by RT-PCR and the virus antigen by ELISA utilizing a recombinant virus N protein. Immunofluorescence assay is also used for the detection of virus specific IgM and IgG antibodies, viral antigens and recombinant protein N[16]. The CCHFV is biosafety level (BSL)-4 pathogen that hinders its handling and testing and necessitates the prevention of nosocomial infections[43].

During the early stages of infection with CCHFV, the sign and symptoms are non-specific to establish a firm diagnosis. It must be known that to where the patient has travelled in the recent past, if he/she has been exposed to a patient already suffering from CCHF infection or working in a setting where such patients and

specimens related to CCHF are being handled. Certain bacterial infections such as rickettsiosis (African tick bite and typhus fever), borreliosis and leptospirosis presented features like that of CCHF. Other hemorrhagic fevers such as Hanta virus fever, Yellow fever, meningococcal infection, Omsk hemorrhagic fever, dengue fever, Q fever and Kyasunar forest disease also result in symptoms similar to CCHF[44]. Malaria, hepatitis viral infection, leptospirosis, typhoid fever, salmonellosis, psittacosis, septicemic plague, measles, shigellosis, hemorrhagic smallpox, toxic shock syndrome and Ebola virus infection must also be considered while performing a differential diagnosis[45,46].

9. Clinical laboratory findings

Patients with CCHF develop leukopenia during the early stage followed by thrombocytopenia. Destruction of hepatocytes leads to a rise in the liver function tests such as alanine aminotransferase (ALT), creatine kinase, aspartate aminotransferase (AST) and lactate dehydrogenase. This elevation is more pronounced in chronic patients. Lysis of leukocytes occurs due to an abnormal elevation in myeloperoxidase expression. Similarly, level of fibrin degradation products, prothrombin and partial thromboplastin time also increase that indicate increased bleeding time[42]. However, plasma fibrinogen factor is decreased. Proteinuria, oliguria, hematuria and azotemia indicate the renal dysfunction[47]. These clinical laboratory findings are due to different underlying causes. Kupffer cells, hepatocytes and hepatic endothelial cells are the major targets of the virus. The CCHFV also inhibits the host immune response by interfering with cellular machinery. Platelet number decreases due to endothelial damage. Furthermore, the activation of coagulation cascade due to endothelial damage also results in the onset of disseminated intravascular coagulation and subsequent multi-organ failure[48,49]. Leakage in the vascular system is either due to the direct infection by virus or inflammatory cytokines[50]. The level of interleukin, IL-1 and IL-6, and tumor necrosis factor (TNF-α) are also elevated in chronic cases[42].

10. Treatment and management

Ribavirin, a synthetically produced purine nucleoside analogue, is the only antiviral drug that has shown favorable results against CCHFV. It is effective against a broad range of RNA and DNA viruses *in vitro*[43]. This drug was first used against CCHF during an outbreak in South Africa and Pakistan in 1985 and 1995 respectively. Similar results were reported from Iran and Turkey. However, both studies lacked control groups[51]. Different studies showed that ribavirin reduced viral load in the liver of suckling mice, however, it was ineffective in preventing viremia[52]. In clinical settings, ribavirin showed controversial efficacy in the early stages of disease[17,53,54]. The recommended dose of ribavirin for the treatment of CCHF infection is 30 mg/kg as a loading dose followed by 15 mg/kg for 4 d quarterly and then 7.5 mg/kg thrice for 6 d. Ribavirin being a teratogenic agent is contraindicated in pregnancy[55].

In addition to the generally used treatment with ribavirin and supportive care, other treatment options such as vaccines have been developed over the course of time. In 1970, CCHF vaccine was produced from brain tissue of mice and used in Russia. However, it lacked data on efficacy and safety in human[2]. Two different immunoglobulin preparations, namely CCHF bulin (IM) and CCHF venin (IV), developed from the plasma of surviving CCHF patients were boasted with one dose of CCHF vaccine. The results showed prompt recovery of seven severely ill patients[56]. However, a small number of patients and undefined protocols of the study were major limitations[17]. Different studies reported the efficacy of IFN-α in the treatment of CCHF due to its ability to inhibit virus in human hepatic and endothelial cells[57,58]. However, there is lack of sufficient data on the safety and efficacy of interferon. Furthermore, it was reported that the treatment with INF-α had been terminated due to severe adverse effects in CCHF patients[17].

The general treatments for CCHF are supportive therapy that requirs regular monitoring of patient's hematological status and coagulation situation. Aspirin should be avoided because of its effect on the coagulation system like other non-steroidal anti-inflammatory drugs. Electrolytes and fluid level must be monitored frequently. Proper administration of fluids, thrombocytes, erythrocyte preparations and fresh frozen plasma are necessary. Platelet transfusions are also carried out[59]. Recent studies indicated that the use of high dose methylprednisolone in CCHF patients had promising results. The reported dosage was 20-30 mg/kg/day intravenously for 5 d[17].

11. Alternative therapy

The treatment options for CCHF patients are limited to only a few antiviral drugs with variable efficacy. There are also limited supportive care options. Hence alternative medicine can also be explored for the treatment and supportive care of CCHF patients. As plants are the prehistoric sources of medicine and remain important regarding new drug development. The CCHF has a long history of incidents however, ethnobotanical use of medicinal plants against the disease has not been reported. The major reason behind the lack of any scientific data is the similarity of the disease with other hemorrhagic fevers, uneducated background of patients, late diagnosis and limited occurrence of the disease[42,52]. Therefore, we

reviewed several medicinal plants traditionally used and validated against different hemmorhagic fever causing viruses, which may be evaluated against CCHFV due to antiviral activity.

We searched various online databases such as ScienceDirect, Google Scholar, SCOPUS and PubMed were used for data collection regarding medicinal plants for CCHF during 2000-2019. The search terms included "alternate therapy", "herbal therapy", "Crimean-Congo hemorrhagic fever", "Congo fever", "hemorrhagic fever", "Dengue virus", "Lassa fever" and "Yellow fever virus". The process of selecting potential anti-CCHF medicinal plants is shown in Figure 1.

The review of literature revealed that at least 30 plants had shown antiviral activity against hemorrhagic fevers such as dengue, yellow fever, corona and SARS. These plants offer an opportunity to be explored for possible antiviral potential against CCHFV (Table 1). Several plants such as *Momordica charantia* and *Ocimum basilicum* have shown promising immunomodulatory activity in animals and *in vitro* models of disease. These plants may possibly alter or modulate the immune system in patients infected with CCHFV and may help ameliorate the morbidity *via* affecting cytokines and other inflammatory mediators involved in the infection.

12. Conclusions and prospective

In conclusion, the CCHF is a rare tick-borne zoonotic disease that is caused by CCHFV. The CCHFV is an RNA virus that has seven major genotypes mostly prevalent in Asia, Africa and Europe. Individuals handling the tick exposed animals are at risk of CCHFV infection. The CCHFV RNA and antigen are mainly detected by RT-PCR and ELISA method respectively. The CCHF causes hemorrhage, fever, thrombocytopenia, leukopenia and multi-organ failure. Like other hemorrhagic viruses, CCHFV targets different systems in the body, such as immune and digestive systems. Various antiviral drugs, such as ribavirin and INF- , are effective in CCHF patients to varying degree. This review also explored the realm of medicinal plants for the management of hemorrhagic fevers which should be investigated for their potential against CCHFV and its associated complications. The medicinal plants discussed in this review have not been tested against CCHF but possess diverse properties such as antiviral, immunomodulatory and hepatoprotective potentials. Several herbal drugs have shown significant activity against a wide array of viruses such as dengue, influenza A, Coxsackie virus B3, measles, corona and SARS viruses. These herbs should be explored for lead compounds that can be useful in the treatment of CCHF and other hemorrhagic viral infections. Moreover, these herbs can be used as nutraceutical against CCHFV infected patients to prevent immune over-activity and other CCHF related complications.

Conflict of interest statement

Authors declare that they have no conflict of interest.

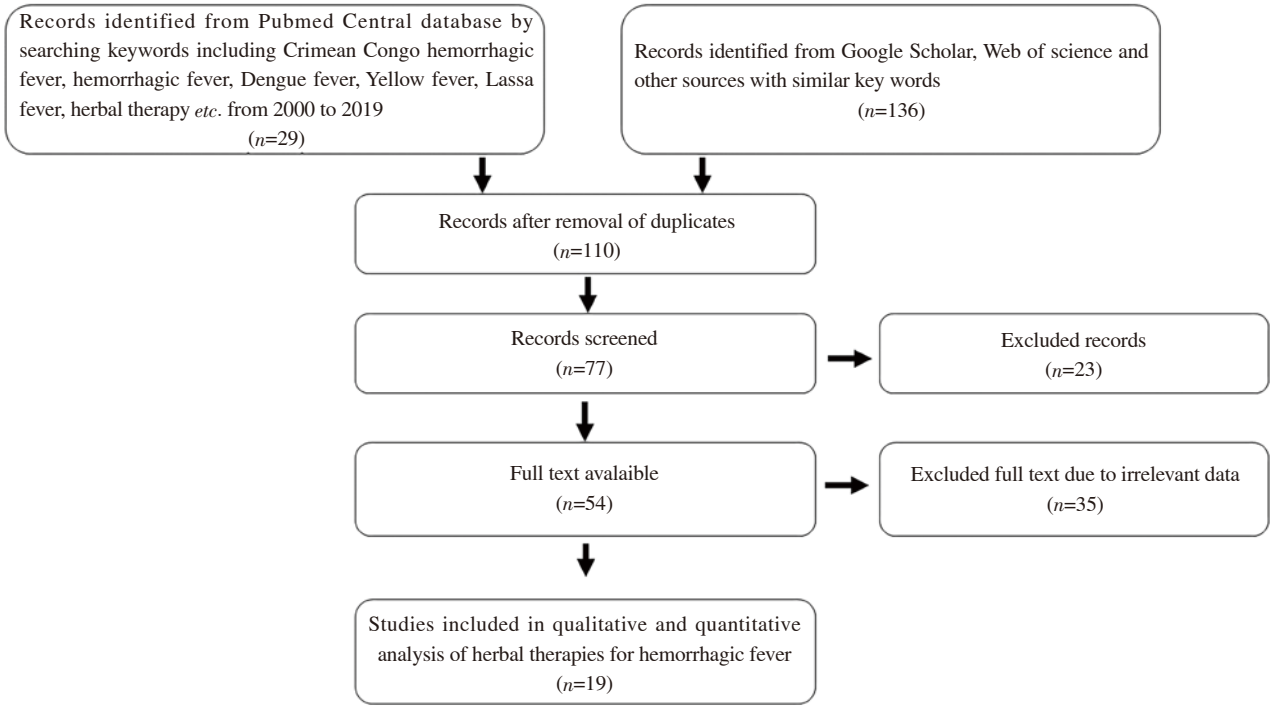

Figure 1. Selection criteria for potential herbal therapy of Crimean-Congo hemorrhagic fever.

Table 1. Medicinal plants with antiviral potential against different hemorrhagic viral diseases.

No.	Species name	Family	Name of assay/ Model used	Type of study	Degree of inhibition	Dose level	Possible mechanism	Antiviral activity against viruses	References
1	*Andrographis paniculata* (Burm.f.) Nees	Acanthaceae	DENV1-infected Vero E6 cells	*In vitro* Vero E6 cells	Maximum	None	Not shown	Dengue virus	[54]
2	*Momordica charantia* L	Cucurbitaceae	DENV1-infected Vero E6 cells	*In vitro* Vero E6 cells	Maximum	None	Not shown	Dengue virus	[54]
3	*Cymbopogon citratus* (DC.) Stapf	Poaceae	DENV1-infected Vero E6 cells	*In vitro* Vero E6 cells	Moderate	None	Not shown	Dengue virus	[54]
4	*Alternanthera philoxeroides* (Mart.) Griseb.	Amaranthaceae	Infected C6/36 cell lines	*In vitro* C6/36 cell lines	Maximum	47.43 mg/mL	Not shown	Dengue virus	[55]
5	*Azadirachta indica* A. Juss.	Meliaceae	Infected C6/36 cell lines and infected suckling mice	*In vitro* C6/36 cell lines and *in vivo* suckling mice	Maximum	120-30 μg/mL	Not shown	Dengue virus	[56]
6	*Boesenbergia rotunda* (L.) Mansf.	Zingiberaceae	Cell lines	Fluorogenic peptide substrate Boc-Gly-Arg-Arg-MCA	Maximum	None	Protease inhibition in DENV-2	Dengue virus	[57]
7	*Cladogynos orientalis* Zipp. ex Span.	Euphorbiaceae	Infected Vero cells	*In vitro* Vero cells infected with DENV-2	Maximum	12.5 μg/mL	Not shown	Dengue virus	[58]
8	*Cladosiphon okamuranus*	Chordariaceae (Sea weed)	Infected BHK-21 cells	*In vitro* BHK-21 cells infected with DENV-2	Maximum	10 μg/mL	Not shown	Dengue virus	[59]
9	*Cryptonemia crenulata* (J.Agardh) J.Agardh	Halymeniaceae (Algae)	Infected Vero cells	*In vitro* Vero cells infected with DENV-2	Maximum only against DENV-2	1.0 μg/mL	Not shown	Dengue virus	[60]
10	*Euphorbia hirta* L.	Euphorbiaceae	Not shown	Not shown	Not known	Not known	Increased blood platelets	Dengue virus	[61]
11	*Flagellaria indica* L.	Flagellariaceae	Infected Vero cells	*In vitro* Vero cells infected with DENV-2	Maximum	12.5 μg/mL	Not shown	Dengue virus	[58]
12	*Echinacea purpurea* (L.) Moench	Asteraceae	Infected cell lines	Various infected cell lines such as Vero, MDBK and HELA	Variable response in different viruses	Multiple	Various	Influenza virus, herpes simplex virus, respiratory syncytial virus	[62]
13	*Morus alba* L.	Moraceae	Vero cell lines	HSV infected vero cell lines	Maximum	1.6 mg/mL	Not shown	Herpes simplex virus	[63]
14	*Bupleurum chinense* DC. *Enteromorpha* spp. *Scrophularia scorodonia* L.	Apiaceae Ulvaceae Scrophulariaceae	Infected human fetal lung fibroblasts	*In vitro* human fetal lung fibroblasts (MRC-5; ATCCCCL-171) infected with HCoV-229E	Maximum	0.25–25 mmol/L (saikosaponins)	Effect on virus absorption and penetration	Corona virus	[64]
15	*Lycoris radiata* (L Hér.) Herb. *Artemisia annua* L. *Pyrrosia lingua* (Thunb.) Farw. *Lindera aggregata* (Sims) Kosterm.	Amaryllidaceae Asteraceae Polypodiaceae Lauraceae	Infected Vero E6 cells and hepG2 cells	*In vitro* SARS-COV infected Vero E6 cells and hepG2 cells	Variable	2.48 μg/mL	Not shown	SARS associated Corona virus	[65]
16	*Ocimum basilicum* L.	Lamiaceae	Infected cell lines	Cell lines infected by herpes viruses, adeno viruses, coxsackievirus B1 and enterovirus 71	Variable	Variable according to the virus	Variable	Herpes viruses, Adeno viruses, Coxsackievirus B1 and Enterovirus 71	[66]
17	*Sambucus nigra* L.	Adoxaceae	Madin Darbin canine kidney cells	Cell lines infected with the human HPAIV isolate A/Thailand/KAN-1/2004 (KAN-1, H5N1) and human strain B/Massachusetts/71	Moderate	Various	Altered cell surface proteins	Influenza A and B viruses	[67]

Table 1 continued.

No.	Species name	Family	Name of assay/ Model used	Type of study	Degree of inhibition	Dose level	Possible mechanism	Antiviral activity against viruses	References
18	*Pelargonium sidoides* DC.	Geraniaceae	Different cell lines and animal models used	*In vitro* different cell lines and in *vivo* animal models infected with the virus	Maximum	Various	impaired viral hemagglutination as well as neuraminidase activity	Influenza A virus	[68]
19	*Justicia adhatoda* L.	Acanthaceae	Madin-Darby Canine Kidney (MDCK) cell lines	Influenza virus infected MDCK cells	Maximum (methanolic extract)	10 mg/mL	Inhibited viral attachment and/or viral replication	Influenza virus	[69]
20	*Illicium parvifolium* subsp. *oligandrum* (Merr. & Chun) Qi Lin	Schisandraceae	Different cell lines	*In vitro* cell lines infected with coxsackie virus B3 and influenza virus A	Maximum	Spirooliganone B (3.70-5.05 μM)	Not shown	Coxsackie virus B3 and influenza virus A	[70]
21	*Houttuynia cordata* Thunb.	Saururaceae	Vero cell lines	*In vitro* Vero cell lines infected with DENV-2	Maximum	1.56 μg/mL	Inhibited viral entry and activity after absorption	Dengue virus	[71]
22	*Leucaena leucocephala* (Lam.) de Wit	Fabaceae	C6/36 cells and mice	*In vitro* DENV-1 infected C6/36 cell and YFV infected mice	Maximum	37 mg/L	Various	Dengue virus and yellow fever virus	[61]
23	*Piper retrofractum* Vahl	Piperaceae	Infected Vero cells	*In vitro* DENV-2 infected Vero cells	Maximum	100 μg/mL	Not shown	Dengue virus	[61]
24	*Cajanus cajan* (L.) Millsp.	Fabaceae	Infected embryonic chicken eggs and hep-2 cell lines	*In vitro* and *in ovo*, live attenuated measles virus strain infected cell lines and embryonated chicken eggs	Maximum	250 mg/mL	Not shown	Measles virus	[71]
25	*Quercus lusitanica* Lam.	Fagaceae	Infected C6/36 cells	DENV-2 infected C6/36 cells	Maximum	0.032 mg/mL	Down regulation of NS1 protein in infected cells	Dengue virus	[72]

Authors' contributions

The conceptualization and methodology were done by M.S, M.T and A.S. The formal analysis and investigation were carried out by M.T, M.F.A, and A.S. The resources and writing-original draft preparation were carried out by M.T, M.F.A, A.S and M.S. The writing-review and editing were performed by M.F.A, A.S and M.T. The supervision was done by M.S. The whole manuscript was read and approved by all the authors.

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