Review article

Natural products and their derivatives against the Ebola virus

Productos naturales y sus derivados contra el virus Ébola

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ABSTRACT

Background: Ebola is a virus that causes hemorrhagic fever, which has a high mortality rate and is therefore considered a public health problem and a bioterrorist agent. Although several therapeutic strategies have now been developed, the problem lies in the need to generate a long-lasting, cross-species immune response against multiple species of the virus. Natural compounds are a valuable and important source of chemical diversity including antiviral activity and may be useful as prophylactic and/or therapeutic agents against Ebola virus infections.

Objective: The objective of the review was to highlight the beneficial effects of plants as well as their bioactive compounds for the possible treatment of Ebola hemorrhagic fever.

Methods: The methodology consisted of a bibliometric search and analysis in four databases PubMed, Web of Science, Scopus and Cochrane Library using the descriptors: “traditional medicine”, “medicinal plants”, “herbs”, “phytochemicals”,

"herbal medicine", "hemorrhagic fever" and "Ebolavirus" and the search equation was adjusted in each of them.

Results: We obtained 293 research articles from which 20 articles were selected for critical analysis. The compounds acted through different mechanisms of action such as inhibition of viral proteins as well as interference in the different phases of the viral infection cycle.

Conclusions: Most of the compounds that showed promising effect for inhibition of infection by this virus include polar molecules such as: BanLec H84T, eugenol, ellagic acid, gallic acid, myricetin, curcumin, emodin, silvestrol resveratrol and 18β- glycyrrhetinic acid.

Keywords: antiviral agents; ebolavirus; hemorrhagic fever, ebola; medicine traditional; plants medicinal.

RESUMEN

Antecedentes: El Ébola es un virus causante de fiebre hemorrágica que presenta una elevada tasa de mortalidad, por lo que se considera un problema de salud pública y un agente bioterrorista. Aunque en la actualidad se han desarrollado varias estrategias terapéuticas, el problema radica en la necesidad de generar una respuesta inmunitaria duradera y transespecífica contra múltiples especies del virus. Los compuestos naturales constituyen una valiosa e importante fuente de diversidad química que incluye actividad antiviral y resultan útiles como agentes profilácticos o terapéuticos contra las infecciones por el virus del Ébola.

Objetivo: El objetivo de la revisión fue destacar los efectos beneficiosos de las plantas, así como sus compuestos bioactivos para el posible tratamiento de la fiebre hemorrágica del Ébola.

Métodos: La metodología consistió en una búsqueda y análisis bibliométrico en cuatro bases de datos PubMed, Web of Science, Scopus y Cochrane Library a partir de los descriptores: "traditional medicine", "medicinal plants", "herbs", "phytochemicals", "herbal medicine", "hemorrhagic fever" y "Ebolavirus", y se ajustó la ecuación de búsqueda en cada una de ellas.

Resultados: Se obtuvieron 293 artículos de investigación, de ellos se seleccionaron 20 artículos para su análisis crítico. Los compuestos actuaban a través de diferentes mecanismos como la inhibición de proteínas virales así como la interferencia en las diferentes fases del ciclo de infección viral.

Conclusiones: La mayoría de los compuestos que mostraron un efecto prometedor para la inhibición de la infección por este virus incluyen moléculas polares como: BanLec H84T, eugenol, ácido elágico, ácido gálico, miricetina, curcumina, emodina, silvestrol resveratrol y ácido 18β- glicirretínico.

Palabras clave: agentes antivirales; ebolavirus; fiebre hemorrágica; ebola; medicina tradicional; plantas medicinales.

Recibido: 06/12/2021

Aceptado: 27/07/2022

Introduction

Ebola is a filamentous single-stranded negative-sense RNA virus belonging to the Filoviridae family that causes hemorrhagic fever,[1] which is a severe disease with mortality rates between 50 % and 90 % in humans,[2] it caused the Ebola epidemic of 2014 and 2016 that resulted in 11355 deaths. Currently, WHO has reported two new outbreaks in Democratic Republic of Congo on June 1, 2020 and in Guinea on February 16, 2021.[3] This virus is not only considered a global public health problem, but also a category A pathogen and a terrorist agent.[4]

While several therapeutic strategies have been pursued including immunoglobulins against specific viral structures such as “ZMapp” monoclonal antibodies, small cell-penetrating antibody fragments targeting intracellular proteins, RNA interference, oligomer-mediated inhibitors, transfusion of convalescent blood/serum, gene expression inhibitors and vaccines such as rVSV-ZEBOV and AD26.ZEBOV + MVA- BN-FILO.[5] The problem lies in the need to

generate a long-lasting immune response since the virus remains in the seminal fluid of survivors up to 407 days after infection.[6] Another obstacle in the search for new antiviral agents is the need for a biosafety level 4 (BSL-4) laboratory to handle Ebola virus, as well as the need for inexpensive and effective vaccines and antiviral agents that are useful for any part of the world, including resource-poor countries.[7]

On the other hand, natural products and their bioactive chemical components have become a valuable source for the research of new antiviral drugs and currently phytochemical derivatives make up a high percentage of drugs approved by the Food and Drug Administration (FDA), in addition most of these have low toxicity, fewer side effects, are cheap and easily accessible.[8] Due to the approved therapeutic options for infections produced by this virus are very limited (vaccines or specific therapies), there is a very urgent need to find new prophylactic and therapeutic treatments, so the objective of this review was to address the role played by plant extracts and their derivatives against infections produced by this virus *in vitro* studies.

Methods

A bibliometric analysis was performed that included a search of all studies without date limit. In this way, we ensured the inclusion of all scientific production on the subject.[9] Therefore, all scientific articles until November 2021 were included.

The review of the scientific literature was carried out in four databases: Scopus, Web of Science, Medline (using the PubMed search engine) and Cochrane Library Plus, for which the following descriptors were used: “traditional medicine”, “medicinal plants”, “herbs”, “phytochemicals”, “herbal medicine”, “hemorrhagic fever” and “Ebolavirus”. Investigations in which plant mixtures were used and the compound or plant extract causing the inhibition of infection was not specified were eliminated. The extracted data are grouped in appendix, where each column includes the viral strain, cell line or computational models, type of plants as well as their purified components, the inhibitory concentration applied to biochemical

and enzymatic assays (IC50), the effective concentration applied to cell-based assays (EC50), the selective index (SI) and the mechanism of action, with the purpose of facilitating the understanding of the selected articles.

Results

After applying the search equation in the different databases, a total of 253 articles were obtained: 98 in Medline (via PubMed), 106 in Web of Science, 89 in Scopus and 0 studies in Cochrane Library. After eliminating repeated articles and applying the inclusion and exclusion criteria, 20 articles were selected from which (n = 10) were in silico studies, (n = 7) used extracts and purified their compounds and (n = 12) purchased the active principles.

In the 20 articles analysed, several plants extract and bioactive compounds were reported, which present various mechanisms of action against several therapeutic targets of Ebolaviruses such as:

- **Glycoprotein (GP)**

It is the main protein responsible for the binding and fusion between the viral and host membrane during virus entry, it has a trimeric structure formed by GP1 and GP2 subunits, it is also considered as a target for drug development since this way viral entry is blocked.[10] Some reported inhibitors correspond to compounds containing a diarylquinoline base such as those analysed by *Cui* et al.[11] of which SYL1712 stands out and was able to inhibit virus entry through its interaction with viral GP at an IC50 of 5μM, it also presented a CC50 equal to 241.9μM which indicated that it is not toxic to host cells. The same mechanism of action was reported for compounds ZINC32540717 and ZINC09410451, which is due to the presence of the pyrrolidine carboxamide group and the formation of halide bonds.[12] On the other hand, *Kuo* et al.[13] reported an inhibitory effect of this protein by the methanolic extract of *Perilla frutescens* through its binding with the viral particles which causes a neutralization and blocking of their entry into the cells.

- **Nucleoprotein (NP)**

It is composed of 739 amino acids and its interaction with VP35 is essential for the viral replication process.[14] Several molecules capable of inhibiting its activity have been reported, for example *Wang* et al.[15] through the use of virtual screening, affinity chromatography by MS and metabolomics identified three components of *Piper nigrum* (HJ-1, HJ-4 and HJ-6) which were capable of inhibiting the NP through the formation of oligomers and the reduction of its thermal stability, in addition the extract of this species has demonstrated larvicidal activity against the dengue vector *Aedes aegypti* Liston.[16] Similarly, another species that has been used due to its antibacterial and antiviral activity is *Glycyrrhiza uralensis*, for example two of its components have demonstrated antiviral activity, 18β-glycyrrhetinic acid against Ebola[17] and glycyrrhizin against SARS-CoV-2 strains,[18] hepatitis C virus[19] and H5N1 influenza virus.[20] Meanwhile, *Nasution* et al.[21] by molecular modelling reported two ligands with binding capacity to Ebola NP ZINC56874155 and ZINC85628951.

- **Viral proteins (VP)**

Structurally, EVOB is composed of four VP among which are VP24 and VP35 that participate in the assembly of viral structures such as the NP and suppress the host cell immune response through IFN inhibition, in turn the VP35 protein plays an important role by masking the viral double- stranded RNA (ds-RNA), which prevents its recognition by the RIG-1 receptors (retinoic acid inducible gene) of the innate immune system.[22] On the other hand, the VP30 protein initiates the viral transcription process and VP40 is involved in virus budding.[23] For this reason, several investigations have been carried out to inhibit these essential proteins, for example in the study performed by *Setlur* et al.[24] by means of virtual screening, molecular modelling and ADME studies, identified several ligands with binding capacity to VP, such as limonin which bound to VP24 and VP35, curcumin to VP30 and mahanine to VP40. Similarly, in silico analysis of curcumin, curcuminoids and their metabolites showed that they had the capacity to bind and inhibit several proteins simultaneously, such as bisdemethoxycurcumin to VP30, VP24, VP35, tetrahydrocurcumin to VP35 and VP30, curcumin to VP40 and

demethoxycurcumin to VP30.[25] These compounds have also demonstrated inhibitory activity against other viruses such as Zika and Chikungunya as they interfere in early stages of viral infection by inhibiting their entry into host cells.[26]

Likewise, through the screening of 7675 natural products from African plants, using molecular modelling studies, physicochemical profiles of pharmacokinetics and pharmacodynamics, as well as studies of binding mechanisms between these molecules and VP24, four compounds were identified, among which ZINC000095486070 stands out for its high binding affinity of -9.7 kcal/mol with EBOV VP24, in addition these compounds did not show toxicity and are considered suitable for *in vitro* and *in vivo* studies.[27]

By bioassay-guided fractionation of the ethanolic extract *Limonium morisianum*, *Daino* et al.[28] isolated the compound myricetin, which inhibited the VP35-dsRNA interaction with an IC50 value of 2.7μM. Additionally, this compound has demonstrated other biological effects, for example it inhibits HIV-1 integrase and reverse transcriptase and possesses antioxidant and prooxidant activity against pathogens due to the damage it induces at the carbohydrate and DNA level.[29] In another study, performed by *Ren* et al.[30] through virtual screening, molecular modelling and the use of the pharmacophore model, identified seven ligands (cpd1- cpd7) that share the same 4-acetyl-3-hydroxy-1-phenyl-1H-pyrrole-2 (5H)-one scaffold and were able to bind and inhibit the VP35 protein of the virus.

Against the VP40 protein, *Karthick* et al.[31] identified the molecules emodin-8-beta-D-glucoside and tonkinochromane_G, which were able to bind and inhibit this protein, in addition, through toxicity prediction studies and ADME (absorption, distribution, metabolism and elimination) it was demonstrated that emodin-8-beta-D-glucoside could be a possible candidate for the development of antiviral therapies against EBOV. Using the same methodology, *Mirza* et al.[32] identified thirteen compounds with inhibitory capacity to VP35 and VP40, which showed promising ADME properties and no toxicity was evidenced so that they can be tested *in vitro* and *in vivo* studies.

- **Phases of the viral cycle**

In addition to targeting specific therapeutic targets, there are compounds that present different mechanisms of antiviral action, for example lectins which are a

type of proteins that target the glucans present in their glycoproteins,[33] however clinical investigations on their use have been delayed due to their mitogenic effect on immune cells.[34] To avoid this problem *Covés-Datson* et al.[35] modified a banana lectin to obtain the compound H84T BanLec, which inhibited the entry of virus-like particles (VLP) as well as EBOV transcription and replication, and its administration in a single dose protected mice from murine-adapted EBOV infection, so it could be used in combination with some other agent in a prophylactic or therapeutic regimen. Another potential compound is eugenol, which has demonstrated antiviral activity against influenza A virus, herpes simplex type I and II[36] and in the study of *Lane* et al.[37] an antiviral activity against Ebola virus was evidenced at an EC50 of 1.3μM, while a cytopathic effect (CC50 > 50μM) was not observed. On the other hand, *Cui* et al.[38] reported the protective effect of *Rhodiola rosea* L. plant extract and its two isolated components ellagic acid and gallic acid against EBOV, which inhibited the early stage of the virus cycle before its internalization. The same mechanism of action was presented by seven Chinese medicinal plants against EBOV/HIV pseudo virus infection, of which the species *Prunella vulgaris* L. stood out due to its lower IC50 of 0.50 μg/mL;[39] in addition, this plant has demonstrated antiviral activity against HIV-1.[40] Similar results for this species were obtained in the study of *Zhang* et al.[41] where its aqueous extract inhibited infection up to 80 % by eGFP-ZEBOV at a concentration of 25μg/mL, the mechanism by which it acts is direct binding to viral particles which inhibits their early infection cycle.

Using high-throughput screening and the Ebola minigenome assay (MGA), *Luthra* et al.[42] identified nine benzoquinoline-type compounds that inhibited Ebola virus replication in a range of concentrations from 0.3 to 1μM, and no cellular cytotoxicity was evident (CC50 > 50μM). Finally, another molecule that showed antiviral capacity against Ebola is silvestrol, which inhibited infection in cells at a concentration of 10nM. The mechanism by which it acted was the inhibition of virus translation given that it decreases the activity of the host's eIF4A helicase, and in other studies this molecule has also shown antitumor and antiviral properties against coronavirus, Chikungunya and hepatitis E virus.[43,44]

It is important to take into account that medicinal plants and their derivatives not only possess antiviral activity but have also been used for the production of antibodies, such is the case of ZMapp, which consists of a combination of three

different monoclonal antibodies that act against the Ebola virus and have been produced transgenically in the tobacco plant of the *Nicotiana benthamiana* species by incorporating the genes of the antibodies thanks to *Agrobacterium tumefaciens*, which makes it a therapy of high specificity and low toxicity.[45]

Conclusions

Extracts of medicinal plants as well as their constituents are a valuable and powerful tool of chemical compounds with antiviral properties. The identification of compounds using high-throughput screening or in silico search increases their potency and selectivity. Some of the most promising compounds for Ebola prophylaxis and/or possible treatment include: BanLec H84T, eugenol, ellagic acid, gallic acid, myricetin, curcumin, emodin, silvestrol resveratrol and 18*β*-glycyrrhetinic acid, in addition it is worth mentioning that several of these compounds showed no toxic effects on cells so *in vivo* studies could be continued to determine the levels of safety and efficacy of each compound prior to use in the clinic.

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Appendix

Table - Characteristics of the 20 studies on plants and their derivatives against Ebolavirus

Viral strain	Cell lines	Test method	Plant species	Isolated compound	IC50 or EC50 (μM -μg/mL)	SI	Mechanism of action
EBOV glycoprotein (GP)-pseudotyped particles[13]	Huh-7	Time of drug addition assay Synchronized Infection Assay	*Perilla frutescens*	-	30	-	Inhibits EBOV glycoprotein (GP) and blocks its entry
Ebola virus/H.sapiens-tc/GIN/2014/ Makona-C05 (EBOV/Mak)[35]	HEK293 T/17, Vero E6, HeLa and Huh 7	Cell-based infection assay, infection assay (p4cis), replication/transcription (p1cis) by trVLP, entry, assembly and release assay by VLP	*Musa acuminata* Colla	lectin H84T BanLec dissolved in OMEM	Huh 7 and Vero E6: 20 trVLP: 5	-	Inhibits viral replication by 96 % and 67 %, inhibits trVLP viral cycle and inhibits VLP entry at a concentration of 100 to 250 μM
EBOV-GFP[37]	HeLa	Cell inhibition assay	-	eugenol p-anisaldehyde benzyl acetate phenethyl acetate	1.3 ± 0.5 2.8 ± 0.6 10 ± 5.0 10 ± 3.4	-	Compounds show antiviral activity
HIV-1/EBOV, HIV-1/H5N1, and HIV-1/LASV EBOV-GFP[11]	HeLa	TOA experiment and viral replication assay	-	SYL1640 SYL1642 SYL1654 SYL1655 SYL1657 SYL1658	2,96 5,21 4,98 2,65 3,56 8,65	64,3 29,2 44,7 49,9 60,3 12,7	Compounds inhibit viral entry and replication by binding to the GP of the virus

				SYL1660 SYL1683 SYL 1711 SYL1712	2,58 2,93 4,11 0,95	71,6 80,3 58,9 225,9	
EBOV-GFP[38]	HeLa	TOA experiment and infectivity assay	*Rhodiola rosea* L.	whole extract ellagic acid gallic acid	3,9 10,5 25,4	16,4 13,4 9,5	Act early in the infection cycle after initial cell attachment, but prior to viral/cell membrane fusion
EBOVpp[12]	TZM-bl	Molecular modelling and infectivity assay	-	ZINC32540717 ZINC09410451	0.05 ± 0.01 3.1 ± 0.02	-	Both compounds bind to GP to inhibit virus entry and infection
EBOV rVP35[28]	Expression of BL21AI viral protein in *E. coli*	Molecular modelling and fluorescence-based interaction assays	*Limonium morisianum* Arrigoni	whole extract myricetin epigallocatechin-3-gallate (EGCG)	19,2 ± 6,7 2.7 ± 0.9 43.5 ± 4.2	-	Inhibits the binding of VP35 to dsRNA
EBOV NP[15]	Expression of BL21 (DE3) viral protein in *E. coli*	Virtual screening, molecular modelling, binding affinity analysis by spectrometry of ligand NP with pure compounds	*Piper nigrum* L.	C21H27NO3 (HJ-1) C20H25NO3 (HJ-4) C20H27NO3 (HJ-6)	Kd = 24.4 ± 3.4 Kd = 20.1 ± 3.6 Kd = 33.8 ± 4.2	-	Compounds bind NP and promote the formation of NP oligomers
EBOV[31]	-	Molecular modelling, molecular interaction	*Polygonum cuspidatum* Sieb.	emodin-8-beta-D-glucoside tonkinochro	-	-	The compounds inhibit the activity of the

		analysis, ADME analysis and toxicity prediction.	etZucc	mane_G			VP40 protein
EBOV VP24[27]	-	Molecular modelling	African Medicinal Plant Library (AfroDB) and NANPDB	ZINC000095486070 ZINC000003594643 ZINC000095486008 sarcophine	-	-	Compounds bind and inhibit VP24 protein
EBOV[24]	-	Molecular modelling and ADME analysis	*Syzygium aromaticum* L. *Ferula assa-foetida* L. *Curcuma longa* L. *Murraya koenigii* L. *Vitis vinifera* L. *Syzygium aromaticum* L. *Ferula assa-foetida* L. *Murraya koenigii* L. *Ferula assa-foetida* L.	limonin samarcandin gummosin curcumin mahanine resveratrol limonin gummosin polyanthin mahanine mahanimbine gummosin	-	-	Inhibit VP 24 Inhibit VP 30 Inhibit VP 35 Inhibit VP 40

EBOV NP[17]	*E. coli* strain BL21 (DE3)	High-affinity mass spectrometry, SPR assay, FTS assay and molecular modelling	*Glycyrrhiza uralensis* Fisch	GC7 (18β-glycyrrhetinic acid) GC13 (licochalcone A)	Kd= 50 ± 7.3 Kd= >1000	-	Reduces the thermal stability of the NP protein, induces the formation of oligomers and disrupts the association between the viral ssRNA and the NP complexes
EBOV VP35[30]	-	PB-VS, QSARB-VS and coupling study	-	cpd1 cpd2 cpd3 cpd4 cpd5 cpd6 cpd7	3.70077 3.78533 3.81589 3.99457 3.97135 3.66696 3.57224	-	Inhibit the VP-35 protein
Pseudovirion EBOV/HIV[39]	A549	Inhibition test and TOA test	*Gardenia jasminoides* Ellis *Citrus aurantium* L. *Viola yedoensis* Makino *Prunella vulgaris* L. *Coix lacryma-jobi* L. *Pinellia ternata* (Thunb) Makino *Morus alba* L.	aqueous extracts	11.04 ± 1.66 38,35 ± 3,25 17.54 ± 5.93 0.50 ± 0.01 5.46 ± 1.37 5.47 ± 0.19 4.38 ± 1.10	> 27,2 > 21,1 > 38,2 > 124 > 21,2 > 21,4 > 27,6	Extracts could block virus entry

Pseudovirion EBOV-GP-V eGFP-ZEBOV[41]	HEK293 T Vero E6	Viral activity assay measured by fluorescence and addition time assay.	*Prunella vulgaris* L.	-	10 µg/mL 1.25 µg/mL	-	Inhibits EBOV entry and may increase 2G4 antibody activity
EBOV-GFP[24]	Vero E6	MGA and fluorescence viral inhibition assay	-	SW539 SW456	1 µM 0.3 µM	32 110	Compounds inhibit EBOV RNA synthesis
EBOV NP[21]	-	Molecular modelling and molecular dynamics simulation	-	ZINC56874155 ZINC85628951	-	-	Inhibit NP protein
Zaire VP Ebola[25]	-	Molecular modelling	*Curcuma longa* L.	Curcumin bisdemethoxycurcumin demethoxcurcumin tetrahydrocurcumin	-	-	The compounds inhibit several proteins simultaneously
EBOV Mayinga strain[44]	Vero E6 and Huh-7	Infectivity assay	*Aglaia foveolate* Pannell	silvestrol	10 nM	-	Inhibits translation initiation by targeting eIF4A factor

EBOV VP40 and VP35[23]	-	Molecular modelling, pharmacokinetic and toxicity studies	-	Timtec-ST45161107 Otava-7118230235 Timtec-ST50912611 Timtec-ST50616170 Analyticon-NP- 010155 Otava-0115540195 Analyticon-NP- 019744 Kihadarnin A Analyticon-NP- 005474 CID17597017 Analyticon-NP- 000375 Lactupicrin Analyticon_NP- 014205 Parfumine Analyticon-NP- 014522 Analyticon_NP- 003228 (Isorutarin)	-	-	Inhibit viral proteins VP35 and VP40.

Leyenda: SI: selective index; GP: glycoprotein; NP: nucleoprotein; VLP: virus-like particles; TOA: time-of-addition experiment; SPR: Surface Plasmon Resonance; FTS: fluorescence-based thermal shift assay; PB-VS: pharmacophore-based virtual screening; QSARB-VS: QSAR-based 3D virtual screening; MGA: minigenome assay; VP: viral proteins; eIF4A: eukaryotic initiation factor 4A; NANPDB: North African natural products database.

Conflict of interest

The authors declare that they have no conflicts of interest.