Antiviral activity of Andrographolide against Ebola virus, Dengue fever and SARS coronavirus

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Abstract

Andrographolide (**1**) a bitter, diterpenes lactone is the most biologically active constituent from *Andrographis paniculata*. Andrographolide showed *in vitro* inhibition of the growth of Ebola virus (Zaire) with EC50 activity of 10 µM which is twenty-five-fold the activity of the control Favipiravir (EC50 = 250µM) in the Crystal violet (Plaque reduction/ Neutral red (Toxicity) assay. It also showed significant activity against Dengue fever virus with EC50 value of 0.56 µg/ml in the Visual (Cytopathic effect/ Toxicity) Assay and EC50 of 0.58 µg/ml in the Neutral Red (Cytopathic effect/ Toxicity) Assay (comparable to the values obtained with 6-Azauridine as the positive control EC50 = 0.32 µg/ml and 0.38 µg/ml in the respective bioassays; and against SARS Coronavirus. Andrographolide had an EC50 of 1.2 µg/ml in the Visual (cytopathic effect/toxicity) assay and 1.1 µg/ml in the Neutral Red (cytopathic effect/toxicity) assay. The compound exerts several immunomodulatory properties. In experimental models, it effectively reduced the levels of proinflammatory cytokines such as IL-1/3, IL-6, GMCSF, and TNF-a. The compound has a good pharmacokinetics profile and relatively non-toxic even at high doses making it an experimental drug for the treatment of viral infections, with possible application in the control of the Novel Coronavirus, Covid – 19.

Introduction

Andrographis paniculata (Burm. F) Nees, - Family: Acanthaceae) commonly known as the “king of bitters,” is an herbaceous plant cultivated in many tropical and subtropical countries for its medicinal values. *A. paniculata* is a traditional remedy used for the treatment of an array of diseases including fevers, cancer, diabetes, hypertension and microbial infections. It has been used as a general bitter tonic, antidote for snake- bite and poisonous stings of some insects, and to treat dyspepsia, influenza, dysentery, malaria and respiratory infections1,2. Extracts from the plant and their constituents exhibit a wide spectrum of biological activities including antitumour, anti-inflammatory, antidiabetic and antiviral properties 3,4. The leaves are used in Nigeria as a treatment for febrile conditions, upper respiratory tract infections and in the management of hypertension5.

A. paniculata has been found useful in the treatment of human immunodeficiency virus (HIV-1) infections in phytotherapy. Reports have shown that when tested under laboratory conditions, andrographolide affects several facets of HIV-1 infection6. These include (i) inhibition of HIV-1 cell-to-cell transmission, (ii) inhibition of viral replication in peripheral blood mononuclear cells (PBMCs), and (iii) inhibition of CD4+ T-cell death. In an open-label, phase I clinical trial in 13 HIV-1-positive patients indicated that the patients displayed increased CD4+ T-cell counts after 6 weeks of andrographolide administration. At the molecular level, andrographolide acts as a kinase inhibitor and down-regulates the expression of the cell cycle-related enzymes cyclin B and cyclin-dependent kinase 1 (CDK1) in either cancer cells or HIV-1 infected cells. It has also been suggested that by combining andrographolide with other natural compounds that target other molecular sites necessary for viral replication, the prospect of formulating a safe and cost effective "natural cocktail" to control HIV-1 infections is possible6.

The plant has been shown to be effective in the treatment of upper respiratory infections7 as evidenced by the outcome of several clinical trials. A meta-analysis of thirty-three clinical studies, which involved 7175 patients in cohorts on the use of *A. paniculata* for symptomatic relief of acute respiratory tract infections in both children and adults found the preparations containing the herb to be effective when compared against a placebo. *A. paniculata* therapy consistently shortened the duration of cough, sore throat and sick leave/time to resolution when compared with usual care. The ethanol extract of *A. paniculata* has also been found to possess *in vitro* antiviral activity against Dengue fever virus8.

Andrographolide (**1**), a major bioactive constituent of *A. paniculata*, has shown biological activity that suggests that it may be largely responsible for the observed pharmacological activity of the plant. The reported activities by various investigators include anti-inflammatory effects in experimental models for asthma, stroke, and arthritis, anticancer, antibacterial, antitumor, antidiabetic, anti-HIV, and antiviral4. Andrographolide exhibits antioxidant property as evident in several test systems9. The observed antioxidant and anti-inflammatory properties of andrographolide may be partly responsible for its use in the prevention and treatment of metabolic syndrome10. The compound has been reported to possess activity against hepatitis C virus[11] and Chikungunya virus (CHIKV), with a 50% effective concentration (EC50) of 77 μM without cytotoxicity and the observed activity being independent of the cell type used in the assay.12.

Andrographolide exhibited a strong and consistent anti-inflammatory effect in several inflammatory disease models13. There is also increasing evidence supporting endogenous antioxidant defense enhancement by andrographolide through Nrf2 activation, although the exact pathway leading to NF-jB and Nrf2 activation by andrographolide has yet to be elucidated. However, studies have showed that andrographolide downregulates inflammatory iNOS and COX-2 gene expression by inhibiting the activation of NF–κB and STAT3 by modulating the expression of SOCS1 and SOCS3 signaling14. Other studies have also confirmed that the compound attenuates innate immunity through NF-κB Signaling Pathway15.

A bifunctional fluorescent andrographolide probe (called ANDRO-NBD) with comparable bioactivity to andrographolide has been synthesized to investigate the uptake kinetics, cellular distribution and molecular target of andrographolide16. The use of this probe showed that andrographolide entered cells rapidly as indicated by the fluorescent signal of the molecular probe which could be detected in nucleus, cytoplasm, mitochondria, and lysosome. The study also showed that several putative target proteins of andrographolide, including NF-κB and hnRNPK were covalently bound to the biomimetic compound.

Ebola Virus Disease

Ebola virus infection in humans is a lethal and accidental dead-end event. There is no approved drug or vaccine currently available for the prevention or treatment of Ebola virus disease. Experimental therapies for the Ebola virus disease can be classified into two main categories: those designed to directly block the virus replication and those that act indirectly by boosting the host immunity and reduce the deleterious

host responses. The pathogenesis of Ebola haemorrhagic fever disease still remains not properly understood, however, intense inflammatory response resembling septic shock is the major clinical features17. It is the system-wide release of pro-inflammatory cytokines that heralds the characteristic vascular instability, hypotension and shock, and ultimately multi-organ system failure18. It has been suggested that the consequence of these events rather than direct viral infection that results in much of the reported morbidity associated with Ebola virus disease19. Available data also suggest that a better clinical outcome is linked to strong well regulated innate inflammatory response of the patient20.

Dengue Fever

Dengue fever is caused by viruses transmitted by mosquitoes. It is the most prevalent arthropod-borne viral diseases globally in terms of morbidity and mortality. Dengue fever is often confused with malaria fever. It is endemic in more than 110 countries in Asia, Latin America and Africa with about two fifths of the world populations are at risk, with an estimation of around 100 million of infections, 2.1 million cases of dengue hemorrhagic fever and 200 thousand deaths worldwide every year. There is currently no vaccine to prevent infection with dengue virus and drug treatment are often not available on time to prevent usual medical complications and death. The development of a dengue vaccine has met with limited success because of complication posed by the antibody-dependent enhancement effect. Therefore the development of a plant- based antiviral compounds seems a feasible and viable alternative in combating dengue disease.

SARS – Corona Virus

The Covid – 19 pandemic caused by Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV – 2) has indeed become the greatest threat to global health in recent history with over 1,289,380 confirmed cases, more than 70,590 deaths, and 270,372 recovered cases as at April 06th 2020. Hitherto, Coronaviruses (CoVs) were considered as relatively harmless respiratory pathogens to humans until the two outbreaks of severe respiratory tract infections caused by SARS Coronavirus 21 and the Middle East respiratory syndrome coronavirus (MERS-CoV)22. It is believed that the outbreaks were as a result of zoonotic CoVs crossing the species barrier and caused high pathogenicity and mortality rates in human populations. This latest and perhaps most deadly outbreak of Coronavirus has brought the danger of the coronaviruses CoVs to global attention and has also highlighted the importance of global collaborative response and the need for controlling infectious pathogens at international borders23. The current CoVid -19 pandemic has underscored the need and urgency to investigate naturally occurring compounds as possible anti-infective agents for the prevention and/or treatment of these emergent life-threatening infections in humans.

Results And Discussion

Andrographolide is represented by the following chemical structure (see Figure 1).

Table 1: Anti-Ebola Virus Activity

	Favipiravir	Andrographolide
EC_{50} – compound conc. that reduces viral replication by 50%	250 µM	10 µM
CC_{50} – compound conc. that reduces cell viability by 50%	>1000	>100 µM
SI_{50} – CC_{50}/ EC_{50}	> 4	>4

Table 2: Anti-Dengue Virus Activity

Visual (Cytopathic effect/ Toxicity) Assay	6-Azauridine	Andrographolide
EC50 – compound conc. that reduces viral replication by 50% CC50 – compound conc. that reduces cell viability by 50%	0.32 µg/ml >100	0.56 µg/ml CC50 4.2 µg/ml
SI50 – CC50/ EC50	> 310	7.5

Neutral Red (Cytopathic effect/ Toxicity) Assay	6-Azauridine	Andrographolide
EC50 – compound conc. that reduces viral replication by 50% CC50 – compound conc. that reduces cell viability by 50%	0.38 µg/ml 12 µg/ ml	0.58 µg/ml 3.5 µg/ml
SI50 – CC50/ EC50	32	6

Table 3: Anti – SARS Coronavirus Activity

Visual (Cytopathic effect/ Toxicity) Assay	M128533	Andrographolide
EC50 – compound conc. that reduces viral replication by 50%	0.042 µg/ml	1.2 µg/ml
CC50 – compound conc. that reduces cell viability by 50%	>10	32 µg/ml
SI50 – CC50/ EC50	>240	27

Neutral Red (Cytopathic effect/ Toxicity) Assay	M128533	Andrographolide
EC50 – compound conc. that reduces viral replication by 50%	0.64 µg/ml	1.1 µg/ml
CC50 – compound conc. that reduces cell viability by 50%	>10 µg/ ml	33 µg/ ml
SI50 – CC50/ EC50	>160	30

The results show that andrographolide (**1**), a naturally occurring molecule that contains an exocyclic ene-γ-lactone moiety, exhibits antiviral activity against Ebola virus, Dengue virus and SARS Coronavirus. Andrographolides showed *in vitro* inhibition of the growth of Ebola virus (Zaire) with EC50 activity of 10 µM which is twenty-five-fold the activity of the control Favipiravir (EC50 = 250µM) in the Crystal violet (Plaque reduction/ Neutral red (Toxicity) assay (Table 1). It also showed significant activity against Dengue fever virus with EC50 value of 0.56 µg/ml in the Visual (Cytopathic effect/ Toxicity) Assay and EC50 of 0.58 µg/ml in the Neutral Red (Cytopathic effect/ Toxicity) Assay (comparable to the values obtained with 6-Azauridine as the positive control EC50 = 0.32 µg/ml and 0.38 µg/ml in the respective bioassays (Table 2); and in the test against SARS Coronavirus, andrographolide had an EC50 of 1.2

µg/ml in the Visual (cytopathic effect/toxicity) assay and 1.1 µg/ml in the Neutral Red (cytopathic effect/toxicity) assay (Table 3). The compound belongs to a class of electrophilic natural products that can form a covalent bond with their targets through Michael addition reaction. It is readily absorbable by the gastrointestinal tract, or by parenteral route, to provide the highest degree of bioavailability of the compound in a dosage formulation for the potential treatment of patients with Ebola virus disease and other virus infections.

Previous studies have reported the antiviral activity of andrographolide against the Epstein–Barr virus24, human immunodeficiency virus25, hepatitis C virus , herpes simplex virus 126, Chikungunya virus11, influenza virus27 and via different mechanisms. Andrographolide also ameliorates the virus induced suppression of cellular immunity during infection because of its inhibitory effects which involves modulation of multiple signaling of several pro-inflammation pathways/ targets. Several other mechanisms have been suggested for the mode of the antiviral activity of andrographolide, including inhibition of the DNA-binding activity of NF-κB through forming a covalent bond with its p50 subunit28; The possible synergistic activity with other antiviral agents like acyclovir in the treatment of Herpes simplex virus infection has been suggested29. Andrographolide has been shown to be a putative broad spectrum antiviral agent with potential benefit in the treatment of Ebola virus disease, Dengue fever and Coronaviruses30.

Method

Isolation of Andrographolide

Leaves of *A. paniculata* were procured from the AgroBION farms and authenticated by Mr. Alfred Ozioko of the International Centre for Ethnomedicine and Drug Discovery, Nsukka, Nigeria. The plant material was processed following a method described earlier31, with some modifications. About 1 kg of powdered dried leaves of *A. paniculata* were extracted with methanol using a Soxhlet extractor. The methanol extract was concentrated under reduced to yield a brown viscous residue (250 g) which was suspended in H2O and separated into hexane, ethyl acetate and water-soluble fractions. The ethyl acetate fraction was concentrated *in vacuo* and chromatographed on silica gel column chromatography with a chloroform–methanol of increasing polarity to obtain three main fractions. The first pooled fraction was further subjected to dry-flash chromatography using hexane–methanol of increasing polarity to yield andrographolide (130 mg). The identity of the compound was determined from the proton NMR and co-TLC with an authentic sample that was kindly provided by *Natural Remedies* Pvt, India.

Biological Assays

Anti-Ebola in vitro Assay

The antiviral *in vitro* Assay against Ebola virus (Zaire) was conducted under agreement with the United States National Institute of Health (US-NIH) Antiviral Screening Program at Utah State University. Institute for Antiviral Research. Vet Science/Bacteriology Bldg Room 204. 5600 Old Main Hill Logan, UT 84322-

5600. U.S.A. Using a Vero cell line, with the compound (ARB 14-000931) was dissolved in DMSO and tested at concentrations of 0.1 – 100 µM; the control Favipiravir was tested at concentrations of 1 – 1000 µM. The method used was essentially as described by Smee and others with some modifications32.

Dengue Fever *in vitro* Assay

The antiviral *in vitro* Assay against Dengue virus 2 (New Guinea C) was conducted under agreement with the United States National Institute of Health (US-NIH) Antiviral Screening Program at Utah State University.

Institute for Antiviral Research. Vet Science/Bacteriology Bldg Room 204. 5600 Old Main Hill Logan, UT 84322-5600. U.S.A. Using a Vero cell line, the compound (ARB 14-000931) was dissolved in DMSO and tested at concentrations of 0.1 – 100 µM; the control Azauridine was also tested at concentrations of 1 – 1000 µM. The method used for the assay followed the protocol described by Smee and others with some modifications32.

SARS coronavirus *in vitro* Assay

The antiviral *in vitro* Assay against SARS coronavirus (Urbani) was conducted under agreement with the United States National Institute of Health (US-NIH) Antiviral Screening Program performed under contract at Utah State University. Institute for Antiviral Research. Vet Science/Bacteriology Bldg Room 204. 5600 Old Main Hill Logan, UT 84322-5600. U.S.A. Using a Vero 76 cell line, with the compound (ARB 14- 000919) dissolved in DMSO and tested at concentrations of 0.1 – 100 µg/ml; the control M128533 was tested at concentrations of 0.01 – 10 µg/ml. The assay was conducted following a previously described method with some modifications33.

Declarations

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Author contributions:

M.M.I and E.S. planned the project; C.O.O. and M.T. conducted the extraction and isolation of the compound. All the authors contributed in the writing of the manuscripts and reviewed the final draft before submission.

Competing interests:

The author(s) declare no competing interests.

Data availability:

All data generated or analysed during this study are included in this published article.

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Figures

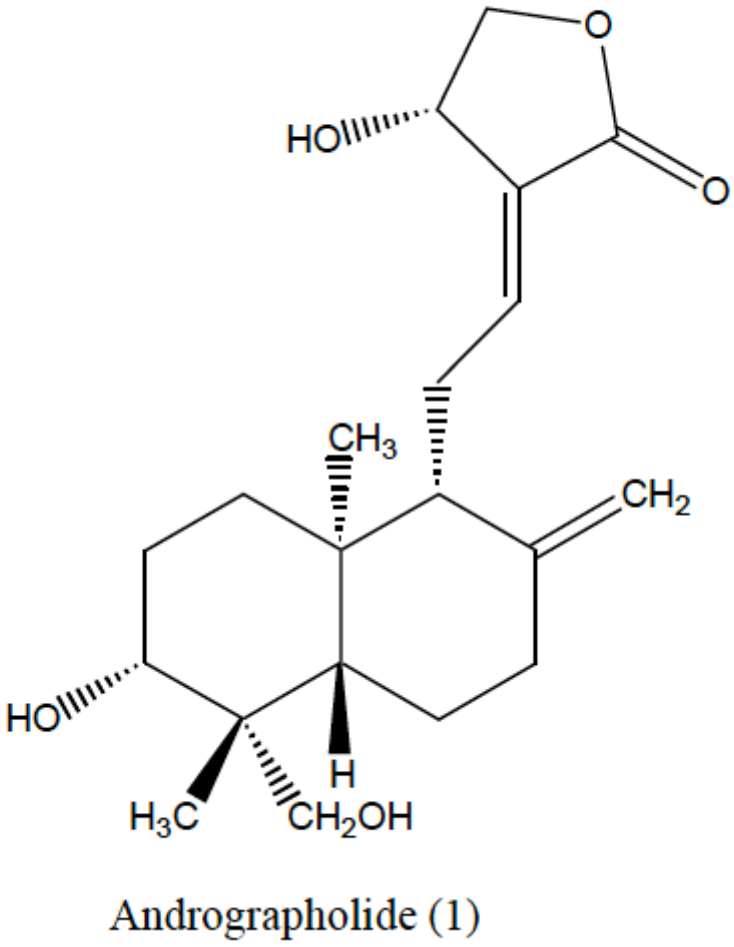

Figure 1

Chemical Structure of Andrographolide