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Bioactive Compounds from Lichens as Promising Biomaterial for the Treatment of Influenza Virus: A Review

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors NTT and TVC contributed equally to this study. Both authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Influenza viruses remain a significant threat that can cause severe morbidity and mortality responsible for epidemics and pandemics worldwide. Lichens produce distinct secondary metabolic products with a wide range of biological activities, which are promising as the diversity source of biomaterials for pharmaceutical application. Furthermore, biological activities of natural lichen metabolites such as anti-oxidants, anti-microbial, anti-insecticidal, antipyretic, and anti-cancer agents of lichen have been reported in several review papers. However, the antiviral activity against influenza virus has not been mentioned in any previously published papers. In the present study, we aim to highlight and discuss the antiviral effects against influenza viruses of natural lichens and its derivatives in the current literature with an update of recent findings. In conclusion, natural lichens and constituents promote the inhibitory effect on various infectious diseases especially for influenza virus infection, it, therefore, is promising as a biomaterial for anti-influenza virus drugs development.

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1. INTRODUCTION

Influenza viruses are a group of enveloped RNA viruses belonging to the *Orthomyxoviridae* family, and are some of the most common infectious respiratory diseases [1]. Three classes of influenza virus include types A, B, and C [1], but only types A and B can cause serious respiratory disease in humans, so they receive more attention. Seasonal and pandemic influenza viruses (IVs) remain a significant threat that can cause severe morbidity and mortality responsible for epidemics and pandemics worldwide [2]. At present, the two main methods for the control and treatment of influenza viruses include vaccination and antiviral drugs [3]. Three active groups of anti-influenza drugs are M2 protein inhibitors (amantadine and rimantadine), neuraminidase inhibitors (oseltamivir, zanamivir, peramivir and laninamivir), and a group that targets the inhibition of viral RNA polymerase (polymerase inhibitor). Favipiravir (T-705) is a polymerase inhibitor that was recently approved for use in Japan (in 2011) [2]. However, due to the slow response to vaccines in dealing with epidemic outbreaks, reduction in the effectiveness of vaccination has been reported recently. Moreover, the resistance of IVs to current anti-IVs drugs has been emerging, and seasonal influenza viruses continue to cause epidemics around the world each year. For example, according to the Centers for Disease Control and Prevention, many strains of influenza, including the 2009 pandemic H1N1 influenza, are now resistant to amantadine and rimantadine. So, there has since been extensive interest in developing a new antiviral treatment for this virus, and biomaterials from natural resources have been considered to be potential candidates for novel treatment against IVs infection. Therefore, it is urgently necessary to create/produce an effective, more potent and risk-free therapy for IVs infection.

Lichens are formed through the symbiosis between a fungus and a photosynthetic partner such as algae or cyanobacteria [4]. The worldwide lichen flora is estimated to include approximately 18,500 species and cover about 8% of the earth's land surface, and over 800 lichen secondary metabolites are known [5]. Lichens are well-recognized, self-supporting, and mutually symbiotic between a dominant fungal partner (mycobiont) and for which the fungus provides shelter for one or several photosynthetic green algae and cyanobacteria (photobionts), forming a unique symbiotic structure. Around 1050 lichen metabolites are currently known and have been reported to display diverse biological activities [6,7].

In addition, lichens have been well known for novel biological activities of lichens, that such anticancer, antibacterial, antioxidant, antiviral, and anti-inflammatory activities as well as healing properties, antiherbivoral, antiproliferative, antipyretic, allelopathic, and UV protecting effects, etc [8]. Lichens have been used as ingredients in traditional medicine for centuries, and many cultures have used lichens to treat a variety of ailments [9]. There is a plethora of related research on the biological functions of lichens, which have been reported in previously published papers. Among more than a thousand identified secondary metabolites from lichens, usnic acid has the most functional potential compound. Since its first isolation in 1844, usnic acid has become the most extensively studied lichen metabolite and one of the few that are commercially available [5]. Recently, several studies have reported that lichen acids showed potent antiviral activity against influenza viruses. Sokolov *et al*. (2012) reported that usnic acids have highly antiviral effects on the pandemic influenza virus A (H1N1) in MDCK cells [10]. Shtro et al. [11] and Shtro et al. [12] also reported the activity of some usnic acid (UA) derivatives against influenza virus *in vitro* and *in vivo*. Therefore, the exploitation and utilization of bioactive compounds from natural lichens have become crucial concerns, not only for pharmaceutical applications and the food industry, but also for other fields, especially for the development of new antiviral drugs. In this review, we summarize the antiviral effects of the natural extracts of lichens against influenza virus. As far as we know, there have been no reviews on the antiviral activity of natural lichens against influenza virus. Hence, this report is an endeavor to highlight the current literature with an update of recent findings, providing further insights to targets for anti-influenza drug development.

2. METHODS

A literature search was conducted using PubMed, Web of sciences, Scopus, BioMed Central, Science Direct and Google Scholar to search for

research studies published in the recent 20 years and only the articles in English have been selected. The keywords used included anti-influenza virus, biological activities of lichens, lichens metabolites, natural lichens compounds, lichen extracts, lichens against virus infection, lichen against influenza, influenza treatments, influenza drugs, or a combination of these terms. Finally, this current review which clearly described the biological activities of lichens, especially, highlighted the incidence and treatments of influenza virus of lichen acids and their derivatives *in vitro* and *in vivo* studies.

3. DIVERSITY OF LICHEN ACIDS AND BIOACTIVE COMPOUNDS FROM NATURAL LICHENS

To date, approximately 1050 secondary lichen compounds have been identified [7]. Two main classes of lichen secondary metabolites include depsides and depsidones and other substances from natural lichen extracts have been established in many previous studies (Table 1). According to Pavlovic et al. [13], there are four main lichen acids isolated from *Hypogymnia physodes,* which include physodalic acid, physodic acid; 3-hydroxy physodic acid, and isophysodic acid using HPLC combined with UV, MS, ^{1}H NMR and ^{13}C NMR [13]. Kosanić et al. [14] isolated and identified two main compounds, which are atranorin and fumarprotocetraric acid of acetone extracts of the lichens *Cladonia furcata, Cladonia pyxidata* and *Cladonia rangiferina*. Fernandez-Moriano et al. [15] reported that methanol extracts of two *Parmeliaceae* lichens: *Cetraria islandica* and *Vulpicida canadensis* consist of several lichens acids fumarprotocetraric acid, usnic acid, pinastric acid and vulpinic acid using HPLC. Sahin et al. [16] have isolated and quantified of evernic, fumarprotocetraric, lecanoric, stictic and usnic acids in three lichen species *Ramalina* (*R. farinacea, R. fastigiata,* and *R. fraxinea*). According to the study of Honda et al. [17], showing that acetone extract of *Parmotrema screminiae* is a remarkable source of norlobaridone, protolichesterinic acid, and atranorin acid. Newly, the study of Delebassée [18] reported the presence of cytochalasin E in lichens and more precisely in *Pleurosticta acetabulum* which is potent against human HT-29 colorectal cancer cells. The Fig. 1 shows the common lichen acids and constituents have been revealed in previous studies for their biological properties.

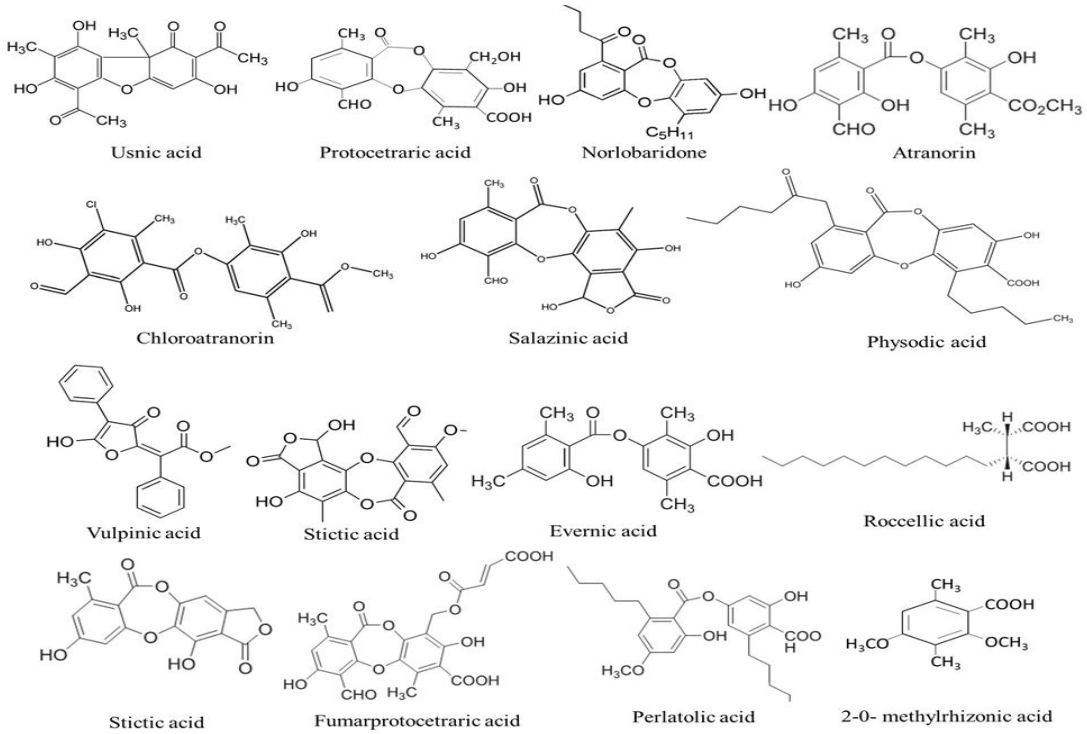

Fig. 1. Chemical structures of some common lichen acids

Table 1. Biological activities of lichen substances

Lichens compounds/Lichens	Biological activities	References
Usnic acid	Anticancer: Human ovarian carcinoma (A2780 cells); human lung carcinoma (A549 cells); Antibacterial: *S. aureus; B. subtilis*; Antioxidant; neurodegenerative disorders; Anti-inflammatory activity	[19,20,21,22,23]
Atranorin (*E. vexans*)	Anticancer: Lung cancer (H460, H1650, H1975, and LLC cells) Human prostate cancer androgen-responsive (LNCaP); antioxidant, antimicrobial and anticancer (FemX and LS174 cell)	[24,14]
Salazinic acid (*Parmelia sulcate*)	Antimicrobial activity	[25]
Protocetraric acid	Antimicrobial: *M. tuberculosis*	[26]
Stictic acid (*Ramalina farinacea*); (*Usnea articulate*); *Lobaria pulmonaria* (L.)	Antioxidant; Antimicrobial: *S. aureus; B. subtilis*; Anticancer: HT-29 and MCF-7 cells; and normal cell line: MRC-5 cells	[16,27,28]
Vulpinic acid (*Letharia vulpine*); (*Vulpicida canadensis*)	Photoprotective activity (HaCaT cells); Neuroprotective and anticancer activity (HepG2 and MCF-7 cell lines)	[29,15]
Evernic acid	Antioxidant; neurodegenerative disorders (U373-MG and SH-SY5Y cell lines)	[19,22]
Fumarprotocetraric acid (*Cetraria islandica*)	Antimicrobial: *B. cereus, B. subtilis, S. aureus, S. faecalis, P. vulgaris, L. monocytogenes, A. hydrophila, C. albicans,* and *C. glabrata;* neuroprotective and anticancer (HepG2 and MCF-7); (SH-SY5Y and U373-MG cell lines).	[30,15,31]
Norstictic acid	Anticancer: *in vitro:* Breast cancer cells (MDA-MB-231, MDA-MB-468, MCF-7, T-47D, BT-474, and SK-BR-3, MCF-10A); and *in vivo*: Female athymic nude mice	[32]
Barbatic acid	Antimicrobial: *S. aureus*	[33]
Divaricatic acid	Antimicrobial: *B. subtilis, S. aureus* and *P. aeruginosa*	[34]
Physodalic acid	Antioxidant, antimicrobial and anticancer	[19,13]
Physodic acid	Antioxidant, antimicrobial and anticancer (A375 melanoma cancer cells)	[19,35,36]
3-hydroxyphysodic acid	Antioxidant, antimicrobial and anticancer	[19]
Isophysodic acid	Antioxidant, antimicrobial and anticancer	[19]
Lobaric acid (*Stereocaulon alpium*)	Anticancer: Human colon adenocarcinoma HCT- 116	[37]
Variolaric acid (*Ochrolechia deceptionis*)	Anticancer: Human colon adenocarcinoma HCT- 116	[37]
Chloroatranorin (*Pseudevernia furfuracea*)	Antimicrobial activity: bacteria and yeasts	[38]
Diffractaic acid (*P. magellanica*)	Antimicrobial: *M. tuberculosis*; anticancer: human breast adenocarcinoma MCF-7	[26,37]
Protolichesterinic acid (*Parmotrema screminiae*)	Antioxidant: MCF-7, HeLa, HCT-116 and NIH-3T3 cells; Antimicrobial: *S. aureus* and *E. faecalis*	[17,37]
Norlobaridone (*Parmotrema screminiae*)	Antimicrobial: *Escherichia coli*, *S. aureus* and *E. faecalis*; Anticancer: Human cancer cell lines (HTC116; K562, J82, UM-UC-3, and BxPC-3)	[17,35]

Perlatolic acid (*Cladonia portentosa*); (*Cetrelia monachorum*)	Neurogenic activity (Neuro2A cells); Anti-inflammatory (*in vitro* using human A549, HEK-293/NF-κB-luc cells; *in vivo*: C57BL/6J male mice)	[39,40,41]
Gyrophoric acid (*Xanthoparmelia pokornyi*)	Anticancer (A375 melanoma cancer cells); Human cancer cell lines (A2780, HeLa, MCF-7, SK-BR-3, HT-29, HCT-116 $p53^{+/+}$, HCT-116 $p53^{-/-}$, HL-60 and Jurkat); Photoprotective activity (HaCaT cells)	[29,36,42]
Olivetoric acid (*Pseudevernia furfuracea* var. *ceratea*)	Antimicrobial activity: bacteria, yeasts, and fungi	[38]
Imbricaric acid (*Cetrelia monachorum*)	Anti-inflammatory *(in vitro* using human A549, HEK-293/NF-κB-luc cells; *in vivo*: C57BL/6J male mice)	[41]
Cytochalasin E (*Pleurosticta acetabulum*)	Anticancer: human HT-29 colorectal cancer cells	[18]
Parietin (*Xanthoria parietina* L.)	Anticancer: human cancer cell lines (A2780, HeLa, MCF-7, SK-BR-3, HT-29, HCT-116 $p53^{+/+}$, HCT-116 $p53^{-/-}$, HL-60 and Jurkat); Heavy metal tolerance	[42,43]

Table 2. Some review papers on the biological activities of lichen substances

Journal	Title	References
Mini-Reviews in Medicinal Chemistry	Atranorin – An Interesting Lichen Secondary Metabolite	[44]
Phytomedicine	Secondary metabolites from cetrarioid lichens: Chemotaxonomy, biological activities, and pharmaceutical potential	[8]
Planta Medica	Secondary metabolites isolated from *Xanthoria parietina* (L.) Th. Fr. lichen and their biological activity	[45]
Applied Microbiology and Biotechnology	Lichens as natural sources of biotechnologically relevant bacteria	[46]
Natural product research	Review of the biological properties and toxicity of usnic acid	[5]
Phytotherapy research	The Immunostimulating Role of Lichen Polysaccharides: A Review	[6]
ISBN 978-3-319-13373-7 DOI 10.1007/978-3-319-13374-4	Lichen Secondary Metabolites: Bioactive Properties and Pharmaceutical Potential (book chapter)	[47]
Phytochemistry Reviews	Lichens: a promising source of antibiotic and anticancer drugs	[48]
Pharmaceutical Biology	Biopharmaceutical potential of lichens	[49]
Zeitschrift fur Naturforschung - Section C Journal of Biosciences	Current Results on Biological Activities of Lichen Secondary Metabolites: A Review	[50]
Phytochemistry	Molecules of Interest Usnic acid	[51]
Naturwissenschaften	A review on usnic acid, an interesting natural compound	[52]
Applied Microbiology and Biotechnology	Pharmaceutically relevant metabolites from lichens	[53]

4. LITERATURE REVIEW OF THE BIOLOGICAL ACTIVITIES OF LICHEN SECONDARY METABOLITES

Numerous studies confirmed that lichen secondary metabolites had shown many biological properties including antioxidant, anticancer, antimicrobial, and antiviral (Table 2). On the other hand, huge reports have mentioned the toxicity and cytotoxicity of lichen constituents *in vitro* and *in vivo*. Herein, we are going to highlight and summary of toxicity and biological

activities of lichens its substances which focuses on the anti-influenza virus infections.

4.1 Toxicity and Cytotoxicity

For a long time, several studies have reported about the biological activities of lichen extracts and their derivatives both *in vitro* and *in vivo*. However, studies about its toxicological potential are limited. Manojlović et al. [54] examined the cytotoxic activity of salazinic acid, protocetraric acid and usnic acid in FemX and LS174 cells. The results showed that the IC50 values for tested lichen compounds relative to the cells ranged from 12.72 to 60.18 µg/mL. Brandao et al. [55] reported the cytotoxicity of lichen acids and phenolic compounds from various lichens. The authors isolated different metabolite compounds: atranorin, diffractaic acid, divaricatic acid, perlatolic acid, psoromic acid, protocetraric acid, norstictic acid, usnic acid, and lichexanthone from lichens such as *Parmotrema dilatatum, Usnea subcavata, Usnea sp., Parmotrema lichexanthonicum, Cladina confusa, Dirinaria aspera,* and *Ramalina sp.* against UACC-62, B16-F10, and 3T3 cells. This work showed that the cytotoxicity of lichen metabolites is varied with different GI_{50} (50% growth inhibition). In another study, Kumar et al. [56] have screened the cytotoxic effects of eight lichen extracts of various lichens: *Dermatocarpon vellereum, Xanthoparmelia stenophylla, Xanthoria elegans, Umbilicaria vellea, Melanelia disjuncta,* and *Lobothallia alphoplaca*. The results showed a wide range of cytotoxicity of different lichen extracts on HepG2 and RKO cell lines [56]. Up to date, most of the studies related to the toxicology of natural products are only focused in the predeployment phase, in vitro using cell lines models. Thus, further studies are urgently needed to evaluate the toxicity of lichen extracts and purified compounds, especially in a living organism using animal models or human trials.

4.2 Antioxidant Activity

Many biological effects of secondary metabolites have also been related to their antioxidant properties. However, only a few reports concerning the antioxidative nature of pure lichen metabolites are available in the literature; most of the publications describe the antioxidant activities of crude lichen extracts. Ranković et al. [57] investigated *in vitro* antioxidant activity of the lichens *Cladonia furcata, Lecanora atra* and *Lecanora muralis*. The result shows *Lecanora atra* had a potent free radical scavenging activity (94.7% inhibition) [57]. Manojlovic et al. [58] reported that extracts of lichen *Umbilicaria cylindrica* showed strong antioxidant activity. This study revealed that the predominant phenolic compound was salazinic acid. In another study published, Kumar et al. [56] evaluated the antioxidant capacities of fourteen saxicolous lichens from trans-Himalayan. This study revealed that these lichen species have a broad-spectrum free radical scavenging effect and high antioxidant capacity. In another study, Fernandez-Moriano et al. [15] demonstrated interesting antioxidant activities of *Cetraria islandica* and *Vulpicida canadensis* lichens extract which may be due to the predominant metabolites in the extracts which are fumarprotocetraric acid and usnic acid, respectively.

4.3 Anticancer Activities

Many types of cancers remain one of the most challenging diseases worldwide to treat and are the leading cause of human death, it, therefore, anticancer activities of lichen compounds have become the most importance and attractive topic for the research. Several studies have established the anticancer activities of crude extracts from various lichen using different cancer cell lines as a model. Earlier, Ranković et al. [57] reported anticancer activity of the acetone extracts of the lichens *Cladonia furcata*, *Lecanora atra* and *Lecanora muralis* against FemX (human melanoma) and LS174 (human colon carcinoma) cell lines with IC_{50} varied from 8.5 to 40.2 (µg/mL).

On the other hand, anticancer activities of various lichens components are known, such as usnic acid, lecanoric acid, gyrophoric acid, salazinic acid, lobaric acid, evernic acid, vulpinic acid, and protolichesterinic acid. Recently, various secondary metabolites from natural lichens have been reported with the potent anticancer activities. Backorova et al. [42] reported the anticancer activities of four typical secondary metabolites of lichens (parietin, atranorin, usnic acid and gyrophoric acid) using human cancer cell lines (A2780, HeLa, MCF-7, SK-BR-3, HT-29, HCT-116 p53 þ /þ, HCT-116 p532/2, HL-60 and Jurkat). In this study, the anticancer activity of usnic and atranorin were more effective compounds when compared with parietin and gyrophoric acid. In this work, the authors also investigated the mechanisms of the anticancer activity of usnic and atranorin on

A2780 and HT-29 cancer cell lines through the mitochondrial pathway (the expression of PARP, p53, Bcl- 2/Bcl-xL, Bax, p38, pp38) [42].

4.4 Antimicrobial Activities (antibacterial and antifungal)

Lichen acids and secondary metabolites from lichens have been established to have potency as antibacterial and antifungal agents. Schmeda-Hirschmann et al. [59] reported that extracts from the Andean lichens *Protousnea poeppigii* and *Usnea florida* showed antifungal activity against the pathogenic fungi such as *Microsporum gypseum*, *Trichophyton mentagrophytes* and *T. rubrum* with the minimum inhibitory concentration (MIC) values between 50 and 100 µg/mL. Later, Honda et al. [26] described the antimycobacterial activity against *Mycobacterium tuberculosis* of lichen phenolic substances from different sources such as *Parmotrema dilatatum, Parmotrema tinctorum, Pseudoparmelia sphaerospora* and *Usnea subcavata*. Among the studied compounds, diffractaic acid was the most active against *M. tuberculosis* with MIC value at 15.6 µg/mL, followed by norstictic acid and usnic acid (MIC value 62.5 µg/mL). While, hypostictic acid and protocetraric acid showed moderate inhibitory activity with MIC values of 94.0 and 125 µg/mL, respectively. In another study, Ranković et al. [57] reported that acetone extracts of lichen *Cladonia furcate* showed strongest antimicrobial activity among three tested lichens against ten different species of fungi: *Aspergillus flavus, Aspergillus fumigatus, Botrytis cinerea, Candida albicans, Fusarium oxysporum, Mucor mucedo, Paecilomyces variotii, Penicillium purpurescens, Penicillium verrucosum,* and *Trichoderma harsianum*. In this study, the antibacterial activities of lichen extracts were also examined using six species of bacteria which included *B. mycoides, B. subtilis, E. cloacae, E. coli, K. pneumoniae,* and *S. aureus.*

According to another study by Manojlović et al. [58], salazinic acid, protocetraric acid, and usnic acid showed the inhibitory effects on the growth of several bacteria *Bacillus mycoides, Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae*, together with several fungi such as *Aspergillus flavus*, *Aspergillus fumigatus, Candida albicans, Penicillium purpurescens* and *Penicillium verrucosum* with MIC values ranging from 0.015 to 1.0 mg/mL. Basile et al. [60] reported that acetone extract from lichen *Xanthoria parietina* displayed a robust antibacterial activity against both the Gram-positive and gram-negative bacteria, with MIC values ranging from 7.8 to 62.5 µg/mL. Recently, Honda et al. [17] reported that norlobaridone and protolichesterinic acid from *Parmotrema screminiae* lichens showed a potent antibiotic activity against *Staphylococcus aureus* and *Enterococcus faecalis*. According to another report [49], usnic acid, evernic acid and vulpinic acid showed the inhibitory effects on the growth of the Gram-positive bacteria *Staphylococcus aureus, Bacillus subtilis* and *Bacillus megaterium*, but had no inhibitory activity on the gram-negative bacteria *Escherichia coli* or *Pseudomonas aeruginosa*.

5. ANTIVIRAL ACTIVITIES OF THE SUBSTANCES FROM NATURAL LICHENS

5.1 Anti-influenza Virus

At present, several commercial drugs have been established for the treatment of influenza virus infection. Three classes of antiviral drugs, M2 inhibitors, NA inhibitors and polymerase inhibitors, have proven effective in preventing influenza viral infection (Fig. 2).

Despite the success of these drugs, concerns remain regarding drug efficacy, resistance, and cost. Therefore, the exploitation of the potential natural molecules for influenza therapy is remaining limited. Plant extracts and substances have been reported to have inhibitory activities against influenza viruses such as aronia, green tea, green tea by-products, cocoa, dandelion, black raspberry, phlorotannins, red algal lectin, various lectins, gallic acid, quercetin, chlorogenic acid, baicalin, etc. (Table 3). However, only a few studies reported on the antiviral activities against influenza virus of the extracts or pure compounds from natural lichens (Table 4). Among them, usnic acid and its derivatives are the most attractively studied molecules for the treatment of influenza virus infection. Sokolov et al. [10] observed that usnic acids and their derivatives against influenza virus A. Shtro et al. [11] also reported the novel derivatives of usnic acid are effective against influenza A virus. Later on, Shtro et al. [12] studied the inhibit effect of the derivatives of usnic acid against influenza viruses and protect mice from lethal influenza infection.

The results from those studies showed that usnic acid was highly effected against influenza virus

infection. For the understanding of the inhibiting effect, several studies focused on the role of lichen constituents in the influenza virus replication cycle. The studied mechanism of the inhibiting activity of lichen extracts and their derivatives have been suggested and discussed in Fig. 3.

5.2 Antiviral Activity Against Other Viruses

Usnic acid from lichens has been reported to have the potential effect against several types of viruses such as Epstein–Barr virus, human papillomavirus, respiratory syncytial virus and polio virus and arenavirus. According to the study of Perry et al. [61], usnic acid from several New-Zealand lichens showed high activity against Herpes simplex type 1 virus and Polio virus type 1. Earlier, in the study of Cohen et al. [62], which has been reported the evidence for the anti-herpes simplex virus type 1 (HSV-1) activities of anthraquinones, bianthrones and hypericin derivatives from several lichens. Esimone et al. [63] reported the antiviral activity of extracts from the lichen *Parmelia perlata* against three RNA viruses to include yellow fever virus, gumboro virus and polio virus. Later on, the study of Esimone et al. [64] revealed the antiviral effect of the fraction from the lichen *Ramalina farinacea* against both DNA viruses (adenovirus and HSV-1) and RNA viruses (HIV-1 and respiratory syncytial virus, RSV). Newly, Lai et al. [65] also reported the antiviral activities of phenolic compounds from the Nigerian lichen *Ramalina farinacea,* especially sekikaic acid showed a potent inhibitory effect against two strains of the respiratory syncytial virus (rg and A2 strain) with IC50 values of 5.69 and 7.73 µg/mL, respectively. Furthermore, recently, Vu et al. [66] reported that lichen metabolites (atranorin and its derivatives) showed the activity against Hepatitis C virus with IC50 values ranging from 12.8 to over 100 µM. Taken together, these studies demonstrate that lichen extracts and their constituents might represent a unique repertoire of novel antiviral agents.

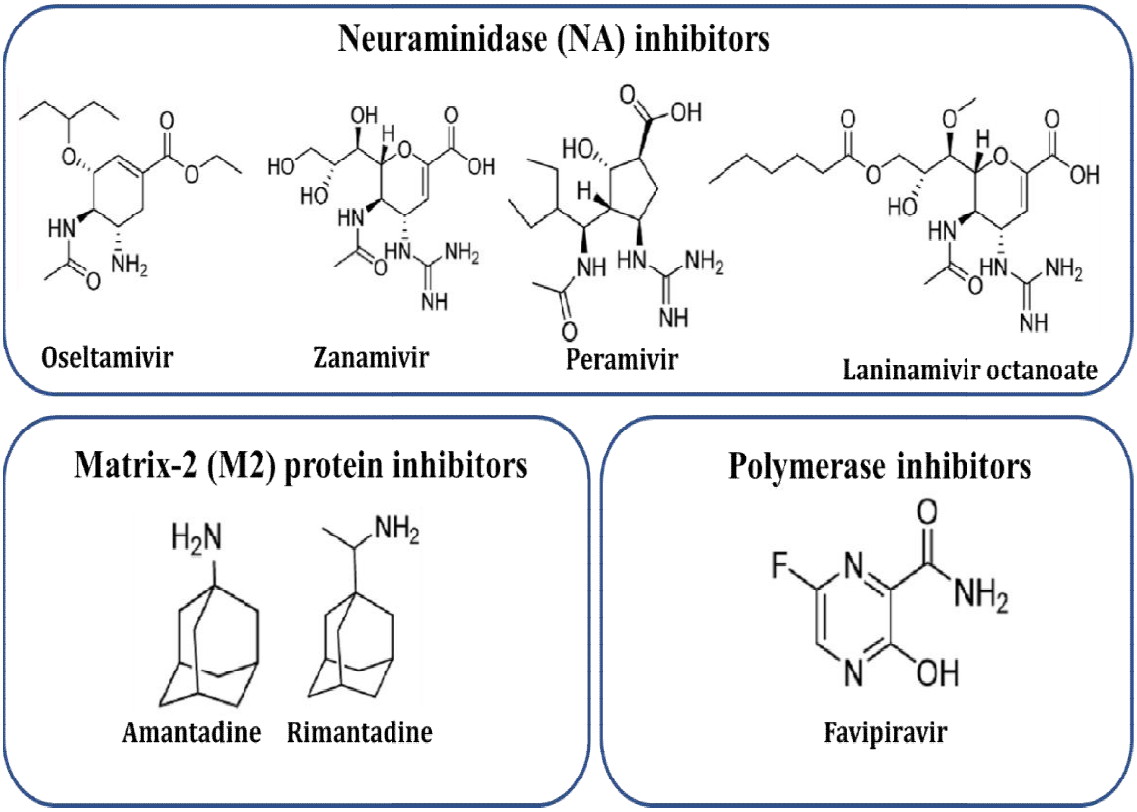

Fig. 2. Approved commercially anti-influenza virus drugs
(Adapted and modified from Ju et al., 2017) [2]

Table 3. Antiviral activity of natural constituents against influenza viruses

Natural compounds	Virus types	Studied models	References
Baicalin (7-D-glucuronic acid-5,6-dihydroxyflavone	Influenza virus H1N1-pdm09	*In vitro*: A549 and MDCK cells	[67]
Chlorogenic acid	Influenza A viruses (H1N1 and H3N2)	*in vitro:* A549 and MDCK cells and *in vivo*: BALB/c mice	[3]
Black raspberry (*Rubus coreanus*) Seed and Its Gallic Acid	The influenza A viruses: H1N1 and H3N2; influenza B strains	*in vitro:* MDCK cells and *in vivo*: BALB/c mice	[1]
Quercetin	Influenza A Virus (H1N1, H3N2)	*in vitro:* MDCK, A549 cells	[68]
Polygonum chinense Linn. extract	Viruses: H1N1 and H3N2; influenza B	293T and MDCK cells	[69]
Cocoa extract	Influenza virus A (H1N1, H3N2), human influenza virus B and avian influenza viruses (H5N1, H5N9)	*in vitro:* MDCK cells and *in vivo*: BALB/c female mice	[70]
High mannose-specific lectin (KAA-2) (red alga *Kappaphycus alvarezii*)	Influenza virus A (H1N1, H3N2), and influenza virus B	*in vitro:* MDCK cells	[71]
Various lectins and high mannose binding lectin ESA-2 (Red alga *Eucheuma serra*)	Influenza virus A (H1N1, H3N2), and influenza virus B	*in vitro:* human lung carcinoma NCI-H292 (H292), MDCK cells	[72]
Dandelion extracts	Human influenza virus A (H1N1, WSN)	*in vitro:* A549 and MDCK cells	[73]
Phlorotannins (Brown alga *Ecklonia cava*)	Influenza virus A (H1N1, H3N2, and H9N2)	*in vitro:* MDCK cells	[74]
Green tea by-products	Human influenza virus A(H1N1) and avian influenza virus (H9N2)	*in vitro:* MDCK cells and *in vivo*: BALB/c mice and Chickens	[75]

6. CHALLENGES: CULTURABLE CONCERNS AND SAFETY OF LICHENS

For the pharmacological application, huge amounts of lichens are required, so it is necessary to find a method to culture large quantities in a higher scale. However, up to now, only a few reports of the culturable ability of lichens as well as the understanding of their biosynthesis and production are available [76]. This is leading scientists to perform more research on the artificial culture of lichens. On the other hand, several studies have revealed that some lichen substances can cause allergies and are toxic to organs. For example, several natural lichen compounds were shown to be allergenic, such as atranorin, stictic, fumarprotocetraric and physodic acids [8]. In addition, several studies have demonstrated that usnic and other lichen acids can cause the hepatotoxicity [4,77,78]. Although usnic acid and its derivatives have been marketed in the United States, several reports of liver toxicity related to the ingestion of dietary supplements containing usnic acid have recently been documented [79]. Also, drug discovery from natural substances and molecules can be viewed as a challenging multidimensional problem, especially pertaining to safety. The exploitation and application of lichen metabolites in medicine is a promising field that requires interdisciplinary research by drug technologists, medicine chemists, nutritionists, and toxicologists. Thus, in the near future, there is an urgent need for specific analytical methods that need to be carefully assessed by *in vitro* and *in vivo* studies of risky lichen substances, as well as the assessment of potential negative impacts to the human organism.

7. FUTURE PERSPECTIVES

7.1 Multi-omics Approach to Identify and Explore the Mechanisms of Action of Specific Compounds from Natural Lichens

Lichens have produced an astoundingly vast pool of bioactive secondary metabolites with chemical diversity and varied in biological properties. However, the drug discovery screening of natural lichen substances and their derivatives is still mostly unexplored. Further investigation on the role played by a specific compound from natural lichens, its antiviral properties, and its drug development is highly required.

7.2 *In vivo* and Human Trial of Antiviral Properties of Lichen Secondary Metabolites

Since most of the studies were conducted using cell lines models, it is necessary to establish more *in vivo* and clinical studies to assess the mechanism of action for their therapeutic potential. The emergence of the fourth industrial revolution is creating an opportunity to establish new mechanisms of action based on next-generation sequencing and multi-omics approaches, such as metabolomics, proteomics, transcriptomics, etc. This advantage has, in turn, allowed for researchers to further explore the specific molecules from natural lichens for the prevention and treatment of influenza viruses.

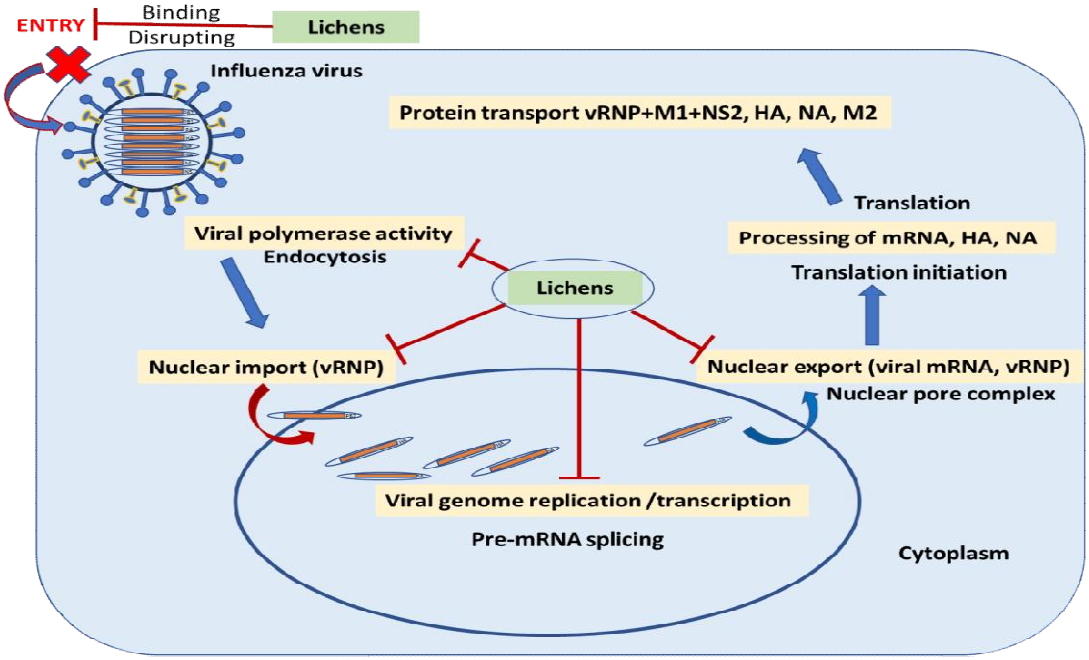

Fig. 3. Mechanism of virus-inhibiting action of lichens compounds studied in influenza virus life cycle

(Adapted and modified from Watanabe et al., 2010) [80]

Table 4. Antiviral activity of lichen substances against influenza viruses

Lichen compounds	Virus types	Studied models	References
26 compounds representing (+) and (-) isomers of usnic acid and their derivates	Influenza A virus (H1N1)2009	*In vitro*: MDCK cells	[10]
Usnic acid and derivatives	Influenza A viruses (H1N1 and H3N2)	*in vitro* and *in vivo*: MDCK cells and Inbred female mice	[11]; [12]

8. CONCLUSION

Regarding the unique properties of lichen acids and their derivatives, they are a potential beneficial biomedicine for many diseases, especially for the development of new drugs to treat influenza infection. However, since lichens produce a broad spectrum of unique secondary compounds, thus, the bioactive compounds from natural lichens for the development of drugs to treat IVs have remained elusive. Moreover, more specific *in vitro* as well as *in vivo* studies are necessary to understand the inhibitory mechanisms and establish the potential antiviral activity against IVs of specific molecules from natural lichens. The present review suggests that further investigation of pharmacological properties of the secondary lichen metabolites is highly urgent in order to support further understand their metabolism and defense properties in the human organism.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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