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Role of mushrooms as a novel antiviral agents–lesson learnt from SARS-COV-2 infection

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Abstract
Coronavirus, SARS-CoV-2, producing the disease COVID-19 is a virus that aim at mostly the human respiratory system and other organs. SARS-CoV-2 is a novel strain that has not been earlier recognized in human beings, however, there have been earlier outbreaks of various versions of the coronavirus including severe acute respiratory syndrome (SARS-CoV1) and Middle East respiratory syndrome (MERS-CoV) which have been acclaimed as major pathogens that are an immense warning to public health and world-wide economies. Presently, no exact cure for SARS-CoV-2 infection has been recognized; however, certain medicines have shown noticeable effectiveness in viral inhibition of the disease. Natural substances such as herbs and mushrooms have earlier established immense antiviral and anti-inflammatory activity. Thus, the potential of natural substances as effectual treatments against COVID-19 may seem hopeful. One of the would-be candidates against the SARS-CoV-2 virus may be different types of mushrooms many of these are widely used as a raw material in various medical conditions. In this overview, we have evaluated mushrooms which are natural products with many biological activities in terms of the antiviral and anti-inflammatory effects.
Keywords: *Coronavirus; SARS-CoV-2, Herbs, Mushrooms, Antiviral agents, COVID-19 Infection antiviral sources, Pneumonia.*

Introduction

The new coronavirus, SARS-CoV-2, causes grave acute respiratory syndrome and has rapidly become a solemn hazard to public health (Shereen *et al*., 2021)[1]. After the origin of infection, cases were first identified in Wuhan, China, and then the virus has rapidly expanded in all the countries of the world. Over 20,046,642 cases have been recognized with more than 734,525 deaths being reported (Worldometer, 2020)[2]. Coronaviruses are enveloped positive-sense single-stranded RNA viruses. The viruses have been reported to cause illness in both animals and humans (Wang *et al.*, 2019)[3]. Coronaviruses have round or elliptical form with an estimated diameter of 60-140 nm (Cascella et al., 2020)[4]. Coronaviruses have four subgroups: alpha (α), beta (ß), gamma (γ) and delta (δ). Of these four identified subgroups, ß-coronaviruses tend to cause the most life-threatening disease in human populations (Velavan and Meyer, 2020)[5]. Two extremely pathogenic ß-coronaviruses have been recognized in humans, including severe acute respiratory syndrome (SARS-CoV-1) and Middle East Respiratory Syndrome (MERS-CoV) (Milne-Price *et al.*, 2014) [6]. Based on its genomic structure and phylogenetic relationships, the novel coronavirus SARS-CoV-2 has also been identified as a ß-coronavirus. A typical coronavirus contains around six open reading frames (ORFs) within its genome. Two-thirds of viral RNA, encode for 16 non-structure proteins. The rest of the virus genome encodes for structural and accessory proteins associated with the virus (Guo *et al.*, 2020)[7]. Four main structural proteins encoded by ORFs include a spike (S) protein, an envelope (E) protein, a membrane (M) protein, as well as the nucleocapsid (N) protein (Mousavizadeh and Ghasemi, 2020)[8]. The N protein is bound to the virus single-positive strand RNA and allows the virus to control host cells. The N protein also coats the viral RNA genome and has also been shown to play a vital role in viral replication and transcription. The M protein is found to be the most plentiful protein on the viral surface. The E protein is a membrane protein, and it plays an significant role in virus–host cell interaction and virus assembly (Boopathi *et al.*, 2020)[9]. SARS-CoV-2 possesses many variations in its genomic and phenotypic structure which largely impact the pathogenesis of SARS-CoV-2 (Mousavizadeh and Ghasemi, 2020; Wrobel *et al.*, 2020)[8,10].

Coronaviruses are zoonotic infections, with alpha and beta coronaviruses found mainly in mammals such as bats whereas gamma and delta are more frequent in pigs and birds. Previous coronavirus outbreaks found that SARS-CoV1 was transmitted from bats to humans and MERS-CoV from dromedary camels to humans (Velavan and Meyer, 2020)[5]. SARS-CoV-2 has also confirmed to be triumphant in making its transmission from an animal host to humans. Genomic studies of SARS-CoV-2 has shown 88% similarity between 2 earlier acute respiratory syndromes (SARS), highlighting that SARS-CoV-2 could have evolved from a coronavirus of bat origin ((Wang *et al.*, 2019; Wrobel *et al.*, 2020; Rothan and Byrareddy, 2020)[3,10,11]. The primary mode of human-to-human transmission of SARS-CoV-2 has been documented to be through respiratory droplets (Rothe *et al.*, 2020)[12]. Research have revealed that the primary target for this new virus seems to be the lung alveolar epithelial cells, which eventually results in the manifestation of respiratory symptoms (Rothan and Byrareddy, 2020)[11]. A study has revealed that SARS-CoV-2 shows higher affinity for the ACE2 receptor, thus explaining its high transmission rate compared to that of both SARS-CoV1 (Wrapp *et al.*, 2019)[13]. Patients with weaker immune system are more susceptible to this virus (Chen et al., 2019)[14]. One study showed that around 80% of patients reported being asymptomatic or had mild symptoms (Huang *et al.*, 2020)[15]. COVID-19 in comparison to SARS-CoV and MERS-CoV, COVID-19 seems to have a lower mortality rate[3] and that the patients often develop lymphopenia and an increase in C- reactive protein (Zhou et al., 2020) [16]. Lymphopenia and the presence of a cytokine storm leads to the pathogenesis of SARS-CoV-2 (Prompetchara et al., 2020)[17]. Studies are being done on understanding the nature of the disease in order to develop effectual treatments. There is a great need to develop an effective antiviral treatment for coronavirus infection.

Aims and Objectives

To review role of mushrooms as the therapeutic agent against SARS-COV-2 infection.

Material and Methods

Meta-analysis of related articles published in different journals had been done and important points are discussed in this article.

Discussions

Various medications have now been identified to control inflammatory crises such as steroids, nonsteroidal anti-inflammatory drugs, and immunosuppressant's (Bagad et. Al., 2013)[18]. The aim is to develop a drug with which has increased efficacy and has least adverse side effects (Bagad et. Al., 2013)[18]. Therefore, the use of natural medicinal products that are thought to be safe as a form of alternative therapy is now under consideration (Bagad et. al., 2013)[18]. (Lin *et al*. 2014)[19] highlighted in his studies about the antiviral capabilities of herbal medicines against several viruses such as coronavirus. Naturally occurring mushrooms have been thought to exhibit great antiviral activity against human coronaviruses. These naturally occurring compounds are supposed to effectively prevent coronavirus infection by affecting viral attachment and cell penetration and have been recognized as natural inhibitors against the number of enzymes present inside the virus (Lin *et al*. 2014; Lau et al., 2008)[19,20].

Using an artificial intelligence (AI) program, studies found that therapeutic natural herbs that could inhibit clathrin-mediated endocytosis could inhibit infection against COVID-19 (Stebbing *et al*., 2020)[21]. There is an urgent need to discover new antivirals that are cost-effective and have great efficacy for the management of viral infections (Lin *et al*. 2014)[19]. Mushrooms have earlier shown great antiviral and anti-inflammatory activity and thus serve as great resource of new antiviral treatments (Lindequist et al., 2005)[22]. Many people prefer the use of natural products in place of commercial pharmaceutically developed products for treating and preventing medical illnesses thus consider medicinal plants for health care (Jassim and Naji, 2003)[23]. Herbs are used as part of natural medicines in many Asian countries (Chen et al., 2020)[24]. Herbal plants are a rich source of new antiviral compounds[19]. *Prunella* is a genus of perennial herbaceous plants in the Lamiaceae family which have an antipyretic property and thus this antidotal herb is used in traditional Chinese medicine (Chen et al., 2020; Wang et al., 2019)[24,25]. Many studies investigating *Prunella* sp. have demonstrated an immense antiviral, antibacterial, anti-inflammatory, and anti-tumor properties (Bai *et al*. 2016; Fisher, 1932)[26,27]. Garlic (*Allium sativum* L.) is an aromatic herbaceous plant that has great medical importance, as it has shown to have antibacterial, antiviral, antifungal and antitumor effects (EL-Saber Batiha et al., 2020)[28]. Garlic has the ability to protect the human body from many illnesses due to the presence of more than two hundred chemical substances (Goncagul and Ayaz, 2010)[29]. It is also found to be effective against influenza B, herpes simplex viruses (HSV), cytomegalovirus (CMV), HSV type 1 and 2 and viral pneumonia (Weber et al., 1992; Bayan et al., 2014)[30,31].

Mushrooms are described as macrofungi which have unique fruiting and different mushrooms possess a variety of biological and pharmacologically active molecules and extracts derived from mushrooms have shown strong anticancer, antibacterial, antiviral, anti-inflammatory, antiatherogenic and hepatoprotective effects. Thus, mushrooms have a huge possibility for use as successful antiviral treatments with a less chance of adverse side effects (Lindequist et al., 2005)[22]. *Lentinula edodes* (Berk.) Pegler (1976) mycelia extract is a powder that is extracted from shiitake mushrooms which is found to have prominent antiviral activity and the main antiviral mechanisms was found to be inactivation as well as inhibition of viral replication. Also, it showed significant downregulation in the expression of pro-inflammatory cytokines such as TNF-α, IL-2 and

IL-11 thus causing anti-proliferative and immunomodulatory effects and regulation of the innate immune response (Vilček and Le, 1998)[32]. The innate immune response is a major factor for COVID-19 disease severity and disease outcome. COVID-19 patients inflammatory cytokines are markedly increased so the effects of this mushroom extract can be considered on SARS-COV-2 (Ren et al., 2018)[33]. Another species of mushroom with has shown antiviral effects is *Grifola frondosa* (Diks) Grey (1821) which is an edible mushroom also used in herbal medicine. The main active component in this mushroom is the *G. frondosa* polysaccharide (GFP) which has shown great anticancer power (He et al., 2018) [34]. Purified novel antiviral protein extract GFAHP from the same macrofungi is likely to be a novel antivirus protein which has shown great ability to inhibit in vitro replication of HSV type 1 (HSV-1). In murine models, higher concentrations of protein extract GFAHP has shown to strongly reduced the severity of neovascularisation and stromal keratitis caused by viral infections (Gu *et al*., 2007) and has shown to cause a significant decrease in virus production (Gu et al., 2007)[35]. D-fraction extracted from *G. frondosa* (GF-D) in combination with human IFN α-2b (IFN) has shown inhibitory effect on hepatitis B virus (HBV). Hence the combined use of GFD and IFN can synergistically inhibit HBV replication (Gu et al., 2006)[36]. In another study, the effects of GF-D on HIV-infected patients there was found to be increase in CD4+ cell count and a decrease in the viral load (Nanba *et al*., 2000)[37]. Abu-Serie *et al*., 2018[38] studies the antioxidant and the anti-inflammatory effects of *Ganoderma lucidum* Karst (1881)) aqueous extract (GLE) and *Chlorella vulgaris* Beijerinck (1890) ethanolic extract (CVE) which showed higher antioxidant and anti-free-radical effects and great ability in enhancing the cellular antioxidant indices. As oxidative stress and inflammation are two factors that are consistently linked to the pathogenesis of COVID-19, therefore there may be a great possibility of this combined extract as an alternative treatment (Abu-Serie *et al*., 2018) [38]. Chaga mushroom "*Inonotus obliquus* (Ach. ex Pers.) (Pilát, 1942)" can be a potential candidate against the SARS-COV-2 virus as it is also widely used as a raw material in various medical conditions (Hyun et al., 2006)[39]. This mushroom has shown to result reduction in nasopharyngeal inflammation (Pan *et al*., 2013)[40].

I. obliquus has shown to possess a powerful enzymatic system because of their parasitic mode of life (Shibnev *et al*., 2011)[41]. Extracts from this fungus have been used for its antitumor, antioxidant, hepatoprotective and anti-inflammatory properties (Lemieszek *et al*., 2011)[42]. It has been used as a source of bioactive compounds that exhibit cytostatic and cytotoxic effects and its antiviral effect shown to be promising [43]. A study done to show the effect of *I. obliquus* polysaccharides (IOP) in cats with feline viruses showed Inhibition of RNA viruses and DNA viruses in all the five viral subtypes (Glamoclija *et al*., 2015)[43]. Therefore, this mushroom is comparable to Tamiflu, which is an antiviral drug that prevent viral multiplication (Filippova *et al*., 2012)[44]. However, increasing resistance to these drugs has resulted in a need to develop alternative treatments to overcome the developing resistance. Chronic inflammation is the underlying pathogenesis of carcinomas, atherosclerosis, autoimmune diseases, and obesity, among others (Moro *et al*., 2012; Najafzadeh *et al*., 2007)[45,46]. It was reported that IOP can inhibit the induction of pro-inflammatory cytokines which has been associated with COVID-19 and is also has shown inhibiting the JAK-STAT signaling pathways which alleviate inflammatory responses (Van *et al*., 2009; Chen *et al*., 2019)[47-48].

Chaga mushroom was also found to have antitumor properties (Lee *et al*., 2008; Ma *et al*., 2013)[49,50] and is found to be effective against Hepatitis C and human immunodeficiency disease (Shibnev et al., 2015)[51]. It is studied that the bioactive molecules of chaga fungus also led to the activation of CD4+ T cells (Aras *et al.*, 2018)[52]. Cases of COVID-19 also showed similar inflammatory as these mushroom extracts have shown promising results in treating various viral diseases, the effect of this mushroom in COVID-19 infection could prove to be beneficial. Despite many advancements in science, no effective therapy has been approved for humans against these viruses, and so there is a great need to develop therapeutic treatments against it and effect of herbal agents should not be overlooked (Li *et al.*, 2019)[53]. This review may highlight the therapeutic potential of various mushrooms as a natural antiviral treatment against SARS-COV-2, therefore further research into characterizing the bioactive ingredients need to be done in order to develop an effective antiviral treatment against COVID-19.

Table 1 Antiviral effects of AbM, HE and GF (Human studies).

Viral agent	Experimental model	Mushroom product	Antiviral effect /Mechanism	Author/Year	References
WEE virus	*In vitro*, cell cultures	AbM extract	Anti-cytopathic effect induced by WEE virus in Vero cells	Sorimachi *et al.*, 2001	[54]
Polio virus	*In vitro*	AbM extract	Reduced virus replication Polio virus *In vitro*	Faccin *et al.*, 2007	[55]
HBV	HBV HEp G2 cells	GF extract	Induction of endogenous antioxidant enzyme	Gu *et al.*, 2006	[56]
HCV	Patients with chron. Infection (n = 4)	AbM extract	Normalized liver function	Hsu *et al.*, 2008	[57]
	Patients with chron. Infection (n = 5)	Mycelium extract incl. HE (Andosan) p.o.	Slight reduction (not significant)	Grinde *et al.*, 2006	[58]
HSV-1 or HSV-2	*In vitro* and in Mice	GF protein, topical admin.	Reduced virus production	Gu *et al.*, 2007	[59]
	HEp2 cultures	AbM polysaccharide and beta glucan	Inhibition	Minari *et al.*, 2011	[60]
	HEp2 cultures	AbM polysaccharide	Inhibition	Yamamoto *et al.*, 2013	[61]
	Mice ocular cutaneous and vaginal infections	AbM mycelial polysacc. p.o.	Reduced topical infections	Cardozo *et al.*, 2013	[62]
Influenza virus	*In vitro*	AbM metabolites	Direct antiviral action	Avtonomova *et al.*, 2014	[63]
	Plaque formation inhib. test	AbM extract	Inhibition	Eguchi *et al.*, 2017	[64]
Enterovirus 71	*In vitro*	GF polysaccharide p.o.	Blocked viral replication	Zhao *et al.*, 2016	[65]
Dengue virus	*In vitro*	HE Inhibition	Inhibition	Ellan *et al.*, 2019	[66]

Table 2 Anti- inflammatory l effects of AbM, HE and GF (Human studies)

Product, Applic.	Study in, of	Effects	Mechanism	Author/ Year	References
Mycelium extract incl. HE, GF, (Andosan) p.o.	Healthy Volunteers (n = 10)	Predominantly anti-inflammatory effect	Proinflammatory cytokines	Johnson *et al*., 2009	[67]
Mycelium extract incl. HE, GF, (AndoSan) p.o.	Healthy Volunteers (n = 8)	Antioxidant effect	iROS prod. & adhesion molec. expressio in MΦ& granulocytes	Johnson *et al*., 2012	[68]
Mycelium extract incl. HE, GF, (AndoSan) p.o.	IBD patients (50 UC &50 CD)	Improved symptoms & QoL, espec. in UC	Proinflammatory effect	Therkelsen *et al*., 2016a-c	[69-71]
AbM extract, p.o.	Rats, Pulmonary inflammation	↓ Lung damage induced by carcinogen	Attenuation of pulmonary inflammation & gross consolidation	Croccia *et al*., 2013	[72]
HE mycelium & erinacine A, p.o.	Rats, brain ischaemia	Protection against brain ischaemia injuryinduced neuronal cell death	Inhibition of iNOS/P3 MAPK Reduced IL-1β,IL-6, TNFα, nerve growth properties	Lee *et al*., 2014	[73]
AbM extract fractions	Mice, cerebral malaria	Improved consequence of cerebralmalaria	↓TNFα, IL-6, IL-1β Antimalarial activity	Val *et al*., 2015	[74]
HE extract & polysacc., po.	Rats, IBD	Improved damages in colonic mucosa of	↓MPO activ., $NF_{K}B$, TNFα, ↑T cell activ. Growth	Diling *et al*., 2017	[75]

Many studies have shown that different mushroom extracts or compounds isolated from fungi have anti-viral effects (Table 3).

Table 3 List of mushrooms with antiviral activities

Mushroom name	Virus	Compounds	References
Rhodotus palmatus (Bull.) Maire (1926)	Hepatitis C	Meroterpenoid and Sesquiterpenoids	[76] Sandargo et al., 2019
Boletus edulis Bull. (1782) *Fomes fomentarius* (L.) Fr. 1849 *Laetiporus sulphureus* (Bull.) Murrill (1920) *Lentinula edodes* Berk. *Morchella conica* Pers. *Morchella esculenta* Fr. *Phellinus igniarius* (L.) Quél. (1886) *Phellinus pini* (Brot.) Bondartsev & Singer, (1941) *Pleurotus ostreatus* (Jacq. ex. Fr.) Kummer (1871) *Porodaedalea pini* (Brot.) Murrill (1905) *Pyrofomes demidoffii* (Lév.) Kotl. & Pouzar (1964) *Cortinarius caperatus* (Pers.) Fr. (1838) *Terfezia boudieri* Chatin *Tricholoma anatolicum* H.H. Doğan & Intini 2015	Herpes simplex virus types 1 (HSV-1)	RC-183, Methanol and aqueous extracts, polysaccharide	[77-80] - Piraino and Brandt, 1999 - Doğan *et al*., 2018 - Santoyo *et al*., 2012 - Lee *et al*., 2010
Daedaleopsis confragosa (Bolton) J. Schröt. (1888) *Datronia mollis* (Sommerf.) Donk (1966) *Laricifomes officinalis* (Vill.) Kotl. & Pouzar 1957 *Trametes betulina* (L.) Pilát (1939) *Trametes gibbosa* (Pers.) Fr. (1836) *Trametes versicolor* (L.) Lloyd (1920)	Type A influenza virus of birds (H5N1) and humans A (H3N2)	Aqueous extracts	[81] Teplyakova *et al*., 2012
Ganoderma lucidum Karst. (1881) *Hericium erinaceus* (Bull.) Persoon (1797) *Lignosus rhinocerotis* (Cooke) Ryvarden (1972) *Pleurotus giganteus* (Berk.) Karun. & K.D. Hyde 2011 *Schizophyllum commune* Fr.	Dengue Virus 2	Hot aqueous, ethanol, hexane, ethyl acetate and aqueous extracts	[82] Ellan *et al*., 2019

Agaricus brasiliensis Fr. (1815) *Ophiocordyceps sinensis* Berk. *Ganoderma lucidum* Karst (1881) *Grifola frondosa* (Dicks.) Gray (1821) *Lentinula edodes* Berk. *Trametes versicolor* (L.) Lloyd (1920)	Human immunodeficiency virüs (HIV)	Polysaccharide	[83] Adotey *et al*., 2011
Cantharellus cibrius Fr. *Afrocantharellus platyphyllus* (Heinem.) Tibuhwa (2012) *Pleurotus citrinopileatus* Singer (1943) *Pleurotus djamor* (Rumph. ex Fr.) Boedijn (1959) *Lentinus sajor-caju* (Fr.) Fr. (1838)	Infectious bursal disease virüs, Poxviridae	Methanol extract	[84] Kidukuli *et al*., 2010
Hypoxylon fuscum (Pers.) Fr.	Echoviruses (E7, E13, and E19)	Methanol extract	[85] Ogbole *et.al*., 2018
Auriporia aurea (Peck) Ryvarden (1973) *Flammulina velutipes* (Curtis) Singer (1951) *Fomes fomentarius* (L.) Fr. 1849 *Ganoderma lucidum* Karst (1881) *Lentinula edodes* Berk. *Lyophyllum shimeji* (Kawam.) Hongo 1971 *Pleurotus eryngii* (DC.) Quél. 1872 *Pleurotus ostreatus* (Jacq. ex. Fr.) Kummer (1871) *Schizophyllum commune* Fr. (1815) *Trametes versicolor* (L.) Lloyd (1920)	H1N1 (influenza A virus subtype), HSV-2 (Herpes simplex virus types 2)	Mycelial extracts	[86] Krupodorova *et al*., 2014
Agaricus brasiliensis Fr. (1830)	Poliovirus type 1	Polysaccharide	[87] Faccin *et al*. 2007
Phellinus linteus (Berk. & M.A. Curtis) Teng.	Newcastle disease virus (NDV)	Crude extract	[88] Lee *et al*., 2011
Lentinus squarrosulus Mont. (1842) *Pleurotus ostreatus* (Jacq. ex. Fr.) Kummer (1871)	Human Cytomegalovirus (HCMV)	Methanol extract	[89] Roy *et al*., 2020

Many compounds are able to effectively prevent coronavirus infection by affecting viral attachment and cell penetration. Compounds such as myricetin, scutellarein and phenolic compounds from *Isatis indigotica* (Fortune) and *Torreya nucifera* (Zucc.) have been identified as inhibitors against the coronavirus enzymes, including nsP13 helicase and 3CL protease (Sevindik et al., 2018)[90]. The extract from *Houttuynia cordata* (Thunb.) has also been found to have an anti-coronavirus natural medicine, as it inhibits the viral 3CL protease and block the viral RNA-dependent RNA polymerase activity, thus showing antiviral mechanisms against SARS-CoV1 (Blagodatski et al., 2018)[91]. The antiviral and the anti-inflammatory effects of natural herbs and mushrooms against viral infections is depicted in (**Table 4)** showing an insight into the possibilities of using them as effective treatments against COVID-19.

Table 4. The list of medicinal herbs and mushrooms. (+ stands for the level activity, representing mild, moderate, severe, and very severe respectively).

Medicinal Herbs and Mushrooms Antiviral	Anti-Viral	Anti-Inflammatory	Anticancer
Prunella vulgaris	++	+++	++
Garlic (*Allium sativum* L.)	++	+	++
Zingiber officinalis	++	+++	+
Lentinula edodes mycelia (shiitake)	+++	+++	-
Grifola frondosa	++	+	++
Ganoderma lucidum aqueous extract (GLE)	+++	+++	-
Chlorella vulgaris ethanolic extract (CVE)	+++	+++	-
Inonotus obliquus	++	++++	++++

Conclusions

Mushrooms can be classified as poisonous, edible, and inedible and have been used by people as food, medicine, and poison. Edible mushrooms have high nutritional properties as they are low in fat content, rich in protein, vitamins, and minerals, and has low calories contents. In recent years, the interest in the research of the pharmaceutical potential of mushrooms is increasing. Many studies have shown that mushrooms have different biological effects such as antioxidant, antiallergic, immunomodulatory, antiviral, antibacterial, antiparasitic, antifungal, anticholesterolemic, hepatoprotective, DNA preservative, and anti-inflammatory. In addition to these biological effects, antiviral effects of fungi against COVID-19 can be used. In this context, mushrooms can be used to combat COVID-19 and many other viral diseases.

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