

COVID-19 prevention and management: Potential applications of humic substances

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KEY WORDS

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ABSTRACT

Background: COVID-19 pandemic has forced the human population to rethink over the life-style and food habits being followed by them. During the current scenario, when cases of more virulent new strain are emerging and specific treatment are still underway, we must look back to the Darwin's theory of "Survival of the fittest" and in order to sustain the pandemic, we must follow the rule "Prevention is better than cure". Traditional Medicinal System can bring possible solution including prevention and control of COVID-19. Humic substances have been used for antiviral activities, signifying that it has potential applications in the management and prevention of infectious diseases.

Summary: Present article is focused on the assessment of the naturally occurring humic substances, which are major constituents of Shilajit, being used in traditional system of medicine and can be used against the prevention of COVID-19.

Key message: Humic substances not only improve the immune system, but also have anti-inflammatory, antioxidant and antiviral activities against human RNA viruses. Therefore, while the entire world is preparing to deal with alarming threat of community spread of COVID-19, Humic substances may play a vital role in improving the innate immunity for prevention and management of SARS-CoV-2 infection

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Introduction

Currently, the novel Coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 has posed a significant threat to global health (1, 2). Patients suffering from chronic medical conditions such as lung disease, heart disease, diabetes, chronic kidney disease, severe obesity or liver disease are at high risk of SARS-CoV-2 infection. Till now SARS-CoV-2 has caused millions of infections globally and the death toll has crossed the figure of billions (3). However, older people or those having compromised immune system are always at higher risk (4), although the young generation is not invulnerable and suffered during the second wave. Recent interventional trials suggest that populations at high risk may adopt the Indian traditional system of medicine. This system aims to focus on prevention through dietary management, lifestyle modification and preventive measures for improving immunity (5-7).

Despite the availability of vaccines, we have a long way to go to make them available globally. Thus, traditional medicine plays an important role, not only in mitigation of COVID-19 but also for other infections that might come in future. In this context, reconsidering Traditional Medicinal System can bring possible solutions including prevention and control of COVID-19 (8). COVID-19 has shown a direct association with adaptive and innate immune responses.

Traditional medicines play an important role in boosting immune responses against such pathogenic invasions and the fight against viral infections can be dealt with in a better way with good immunity (9).

The humic acid substances are various naturally occurring substances that are formed after decomposition of rocks and minerals into the upper layer of soil called humus. These are primarily humic acid, fulvic acid, minerals and some organic compounds. Shilajit is one such traditional formulation used in India for centuries, made up of humic substances and is used as a rejuvenator and anti-ageing medicine (10).

Humic substances and shilajit, are known to control antiviral activity, different components of humic substances are active against viral infections like human immunodeficiency virus, herpes simplex virus, influenza virus etc (11).

Here, we summarise the role of humic substances, which have been used since centuries for the treatment and prevention of diseases associated with inflammation.

Shilajit and Humic substances

Shilajit, also known as *Salajit*, *Silajatu*, *Mimie*, or *Mummiyo* in the north of India, is a brown to blackish powder from high mountain rocks. These rocks are especially located in the Himalayan Mountains between India and Nepal (10).

For centuries, it is known as an anti-ageing and rejuvenator compound in Ayurvedic medicine which is used to increase physical strength and boost human health in ancient Indian Ayurvedic medicine (12). Humic substances are the major components of Shilajit, which are formed by heteropoly condensation of carbohydrates, proteins, fatty acids, lignins, tannins and many other materials depending on their origin (13). The paramount sources of organic components of humic substances are water and soil (14).

The constituents of Shilajit include four primary chemical units i.e. (i) Selenium (selenoproteins or enzymes i.e. selenocysteine and seleno-methionine) (ii) Non-humic organic compounds comprising of conjugated and free derivatives (e.g. lipoidal, aminoacyl, fattyacyl), dibenzo-pyrones (iii) DCPs (dibenzo-pyrones-chromoproteins) and (iv) metallo-humates like fulvic acids (15). Various important components of Shilajit have been depicted in Fig. 1.

Sources of Humic substances

Humic substances are mostly present in marine, river water and soil etc. According to their source of origin, they differ in their characteristics. E.g. humic substances in streams are different to those from soils or the ocean. Aquatic humic substances account for almost 30 to 50% of the organic carbon in water (16). Marine humic substances have some specific aromatic character and are distinct from stream and soil humic substances. Various algal sources of fulvic acid have been derived from ponds and lakes (17). Algal derived fulvic acids are indeed yellow but same as terrestrially derived fulvic acids. Different sources known to produce humic substances are shown in Table 1.

Properties of Humic Substances

Fulvic acids are a family of natural organic acids and components of the humus. They are very similar to humic acids, differentiated by oxygen and carbon contents, degree of polymerization, acidity, inter-mediate molecular weight and color (26). Traditionally, fulvic acid is used for brain-related disorders such as Alzheimer's diseases, respiratory tract infections, fatigue, heavy metal toxicity, preventing hypoxia and is well studied for its effect on immune health and reducing inflammation (27, 28). It serves as an important vehicle that carries nutrients and vitamins to the exact place in our bodies (29). Traditionally, humic acid is consumed for boosting the immune system for treating the swine flu, avian flu, influenza flu and other viral infections (30).

Nutraceutical applications of Humic substances

There are many properties of fulvic acid and humic acid which are beneficial to humans, making it a good nutraceutical candidate. Fulvic acid has been described as an important component of nutraceutical beverages (31). The Fulvic acid acts as an electrolyte, balancing the ionic content of the body fluids and may act as an electron acceptor or donor as per the need of the cells. In addition to this, it also forms free complexes with various other ions leading to their increased bioavailability (32). The complexes formed with fulvic acid are low molecular weight complexes. As a result, they can easily pass through the plasma membrane. It is also known to increase the enzyme activity, particularly of respiratory enzymes (33).

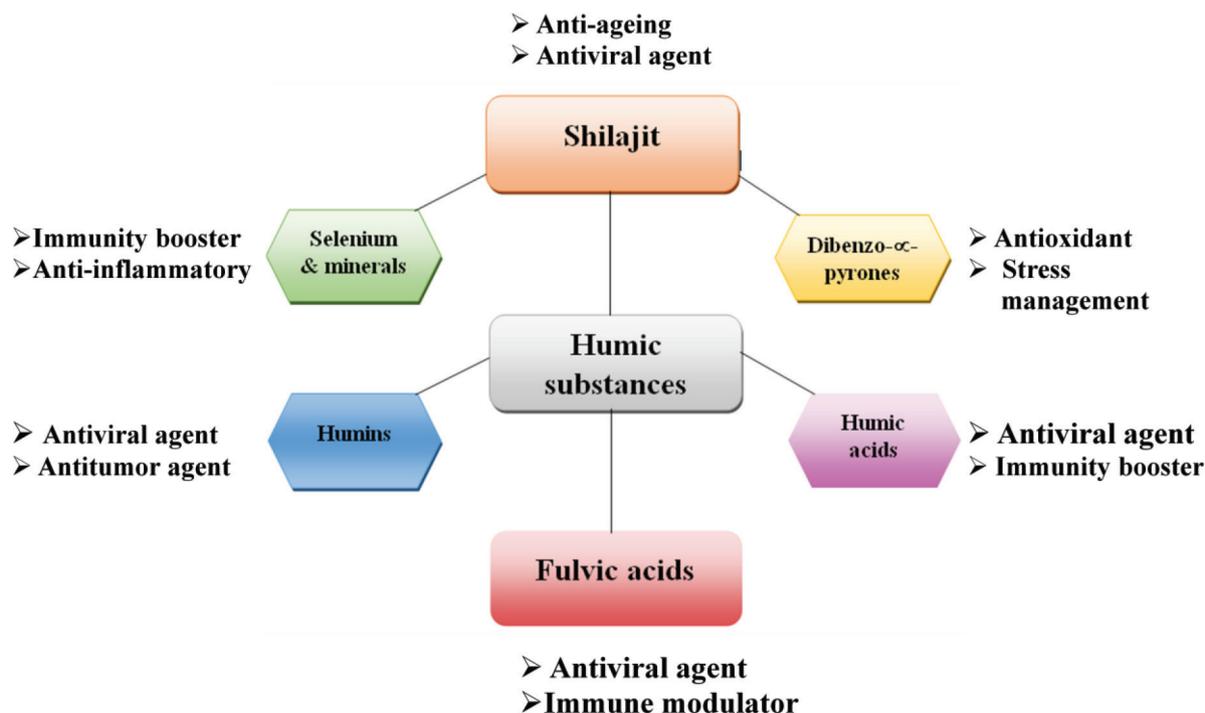


Fig. 1 Components of Shilajit (67)

Table 1: Common sources of Humic acid substances

Source	Example	Environment	Reference
Algal species	<i>Chlamydomonas intermedia chodat</i>	Pony lake, feather pond	(17)
	<i>Chlorococcum sp.</i>	Pony lake	
	<i>Cryptomonas sp</i>	Pony lake	
	<i>Navicula sp.</i>	Pony lake, feather pond	
	<i>Chroococcus sp.</i>	Pony lake, feather pond	
Plant source	cellulose, Hemicelluloses, Lignin, Tannins	Soil	(18, 19)
Fungal isolate	<i>Cercospora beticola</i>	leaf spot disease of the sugar beet (<i>Beta vulgaris L.</i>)	(20)
	<i>Pisolithus tinctorius</i>	ectomycorrhizal fungus	(21)
	<i>Myxotrichum sp.</i>	endolichenic fungus	(22)
Natural materials	Shilajit	Rocks, high mountain rocks	(23)
Water source	anion exchange, cation exchange, and molecule-adsorbing resins.	Jewell pond Oyster river	(24)
Soil	Isolation technique by using a modification of the method of Schnitzer and Skinner 1968	B2 horizon of a Podzol soil	(25)

The enzymes influenced by fulvic acid include alkaline phosphates, transaminase, and invertase. Due to its antioxidant nature, it acts as a free radical scavenger for the body. It increases the DNA content within the cell as well as increases the RNA synthesis. It also acts as a catalyst by forming a complex with vitamin and metal ions. Fulvic acid is an organic natural electrolyte and balances the ionic balance in body fluids (31).

In addition to various metabolic enhancing and protecting properties, fulvic acid has been found to be of immense importance in curing various diseases. It has been found to cure cases of dementia (34) and promote disassembly of Tau fibrils (35). Even in the case of diabetes, fulvic acid can increase superoxide dismutase activity in pancreatic beta cells leading to a reduction in hyperglycemia (36). It is also reported that a fulvic acid bath can cure ulcers with a 90 % success rate (37).

Activation of the immune system by fulvic acid to kill bacteria and promotes gut health is well established. It is effective in inhibiting gastroenterological bleeding with a 95.6% success rate (38). Similar to fulvic acid, humic acid have also been found to cure haemorrhagic fever and prevents cancers of oesophagus with a 100% success rate, 90% success rate in case of thyroid cancer, and with same success rate in case of curing ulcers (39). The cytotoxic effect of humic acid on adenocarcinoma has also been reported (40). Humic acid induces the release of plasminogen activators and converts plasminogen into plasmin, which degrades insoluble fibrin to soluble fibrinogen degradation product (41).

In nutshell, humic substances act as a booster of body functions and respiratory enzymes, working as an electrolyte and as a transporter of minerals. The humic substances may also provide a metabolic boost to a person by rejuvenating activity. Humic substances also act as prophylactic against various ailments including cancer, diabetes and other

diseases, making them excellent candidates for nutraceutical applications.

Immunomodulatory activity of humic substances

Many studies indicate that these acids help in reducing the release of proinflammatory mediators from cells (42). It is also shown that fulvic acid and humic acid at 200 µg/mL can lessen tumour necrosis factor alpha expression after an exposure to the endotoxin lipopolysaccharide in differentiated Human monocytes (43). Many studies have discovered a positive response to treatment on humic acid dosage in treating viral respiratory illness (44). Humic substances are biologically active immunomodulators which are affecting both the cellular and humoral branches of immune reactions. (45). A humic substance such as fulvic acid helps in the activation of the immune system so that it can eradicate bacteria, reduces pro-inflammatory markers. The anti-ageing property of humic acid is attributed to its ionic balance maintenance (46). Fulvic acid derived from carbohydrates can stop the succession of the wound infection. Many studies show the role of fulvic acid in macrophages, stimulating immune function and intracellular signalling (47, 48).

Shilajit

The lytic potential of Shilajit was found to produce T-cell mediated cytotoxicity and supplement activated lymphocytes. The shilajit used for treating lymphocytes has the ability to lyse ⁵¹Cr labelled tumour cells (49). The immuno-modulatory results in mice that were given by shilajit extract were evaluated. The white blood cell activity was observed before and at intervals after receiving the extract of shilajit or a placebo. The extract of shilajit increased the activity of the white

blood cells and it was observed that the experimental activity was dose dependant on the time of exposure (50). 25 and 50 mg/kg dose of shilajit for 5 days remarkably lowered the level of 5-hydroxy tryptamine, 5-hydroxy indole acetic acid, resulting in an increase in noradrenaline, dopamine level and its metabolites in the brain of rats. Such modifications in levels of neurotransmitters were found to be similar to those having increased humoural activity (10). Shilajit has been proven to provide immuno-potentiating properties, which improves the immunity that may complement conventional therapy in HIV. Compound formulation having pure shilajit as one of the most important constituents gained marked progress in the augmentation and symptoms in counts of CD4 and CD8 cells in clinical research studies of patients with HIV.

Selenium

Selenium is a key trace element involved in several crucial metabolic activities via enzymes or selenoproteins that are vital in providing protection against oxidative damage and thus helps to regulate immune function. It has also been established that an adequate quantity of selenium intake is necessary for the optimum function of both cellular and hormonal immune processes in humans (51). It also has several health benefits in managing high oxidative stress, human immunodeficiency virus, acquired immunodeficiency syndrome, rheumatoid arthritis, inflammatory or infectious diseases. It is also helpful for people at high risk of cancer especially prostate cancer (52). Selenoproteins such as seleno-methionine and selenocysteine are reported for managing inflammation and inducing immunity (53). The selenoproteins contain the 21st proteinogenic amino acid, e.g. selenocysteines, represent a group of redox-active proteins.

Selenium regulates both acquired and innate immunity. Selenium induces immunity by enhancing the growth and activity of T lymphocytes and other immune-competent cells (54). It has also been shown that deficiency of Selenium has a negative impact on immune cells during activation, cell differentiation, and cell proliferation (27). The evidence of immuno-stimulatory effect determined by NK cell activity, T cell proliferation, innate immune cell functions have been documented (55, 56). Consumption of selenium helps in regulating and activating the functions of B and T cells leading to adaptive immunity. Selenium levels *in-vivo* have a positive effect on the proliferation of clusters of differentiating CD4⁺ T helper cells (57). Lymphocytes with selenium deficiency do not easily proliferate in response to mitogen. Neutrophil chemotaxis also gets affected during selenium deficiency by affecting the macrophages, and leukotrienes B4 synthesis. Selenium deficiency is also known to affect the humoral system e.g. decrease in titers of IgM and IgG in humans. Deficiency of selenium in endothelial cells from asthmatics results in a raise in the expression of adhesion molecules and cause greater linkage of neutrophils (58). The most considerable relation between selenium and the immune system is the

effect of the micronutrient on neutrophil function. Neutrophils produce superoxide-derived radicals that help in killing microbes.

Dibenzo- α -pyrones

Oxidative stress causes different stress-induced diseases. Chronic stress is responsible for a significant increase in cortical Superoxide dismutase (SOD), with a concomitant decrease in glutathione peroxidase and catalase activities leading to an increase in lipid peroxidase activity. Both dibenzo- α -pyrones and dibenzo- α -pyrones chromoproteins significantly mitigate chronic stress-induced perturbations by normalizing activity of SOD and results in reducing the lipid peroxide (59). An immunomodulating antioxidant property of Shilajit extract has been attributed to the combination of dibenzo- α -pyrones and fulvic acid (60). Many products that originated from humic substances have been used and tested over decades for antiviral activities as well as antioxidant action, signifying that it has potential applications in the management and prevention of diseases (61). The anti-inflammatory action of potassium humate can be contributed to the suppression of the discharge of inflammatory associated cytokines (62).

Humic substances and Metallo-humates

Many products originated from humic substances have been used and tested over decades for antiviral activities as well as antioxidant action, signifying that it has latent applications in the management and prevention of diseases (63). The anti-inflammatory activities of potassium humate is able to be contributed to the suppression of the discharge of inflammation-associated cytokines (64).

The principal source of organic components of humic substances is water as well as soil. The humic substances have the capability to form complexes with inorganic and organic compounds and are stable as water-soluble and water-insoluble salts (65). Humic acids have high molecular weights ranging from a hundred to several thousand Daltons (66). Based on their solubility, these natural substances are divided into two important and major categories (i) Fulvic acid, which is soluble in both acid and alkali (ii) Humic acid, which is insoluble in acid

Therapeutic characteristics of Humic substances

Information on the medicinal use of humic substances has been studied by several researchers. Chinese Materia Medica pharmacological compendium established in the 15th century Ming Dynasty is one of the vital and earliest documentation in this field (62). In China 1978, humic acids and fulvic acids have been used for the treatment of a broad range of diseases and are referred to as "Wujinsan", meaning "Golden Medicine". Chinese drug organization agreed to the humic acid medicines (39). Due to the non-toxicity of humic acid and fulvic acid extract, it is recommended for internal and external use

(65). Transcript from Ayurvedic medicine strongly denotes to humic dating back over three thousand years (66). Humic and fulvic acids with enriched minerals can be assimilated in the body and helps to stimulate various precise and non-specific biological functions (67). In Natural Traditional Medication System Comprehensive Database, humic substances prevail as a therapeutic agent with a diversity of bioactive potential and then known as dietary supplements as well as cosmetic products (68).

Anti-inflammatory and pro-inflammatory properties of Humic substances

The anti-inflammatory activity of humic substances has been attributed to the various healing effect of peat therapy (69). Study reveals that sodium humate significantly suppresses the development of various edemas (70). Membrane protective activities of humic acid type substances are evidenced by inhibition in the lipoxygenase pathway of the arachidonic acid cascade, which is an integral part of the cell membrane (69). Fulvic acid can decrease cyclooxygenase 2 and prostaglandin E2 secretion (71). Antiallergic effects of fulvic acid extract from solubilized sludge are shown to decrease histamine and B-hexosaminidase in immunoglobulin-E-sensitized basophil cells and mast cells. Fulvic acid can lower the release of tumour necrosis factors and proinflammatory mediators (72).

Antiviral properties of Humic substances

The humic substances have been reported to have activity against human RNA viruses. The anti-viral therapies with humic acid as add-on therapy have been used against many human viruses (44). Some of the antiviral activities of humic substances have been discussed below

Herpes simplex virus

The effect of humic acids on an early stage of *Herpes simplex* virus replication inhibition has been confirmed by doing animal experiments (73). Shilajit, a well known humic substance has been studied for its antiviral activity against a group of viruses including *Herpes simplex* type 1 and 2 (74). Phenolic compounds and polyanionic substances of the humic acid have been studied as inhibitors of *Herpes simplex* virus type 1 replication. The functional group study showed the presence of carboxylic groups in the starting compounds improved the antiviral activity and lessen the cytotoxicity of polymers (75). The antiviral activity of synthetic low-molecular-weight humic acid polymers and naturally occurring humic acids have been studied in-vitro against HSV-1. Humic Acid polymers for example some oxidation products generally suppress virus multiplication with different strength and selectivity (76, 79).

Human Immunodeficiency virus

A polyphenolic compound with a molecular weight of about 1000 Dalton, designated HS-1500, was synthesized by

Synthetic Humate Analogues and derived by the oxidation of hydroquinone. The synthetic analogue HS-1500 has been shown to inhibit the infectivity of HIV particles by interference with virus entry mediated by the V3 loop (78). Further, more studies demonstrated the ability of humic acid to suppress *Human immunodeficiency* virus type 1 (79, 80). The mechanism of action analysis exposed virus penetration into host cells as the target of the anti-*Human Immunodeficiency* Virus type-1 activity.

Human respiratory syncytial virus

Shilajit has been investigated for its antiviral activity against *Human respiratory syncytial* virus. Partial virus inactivation and intervention with virus attachment mutually contributed to the antiviral activity of Shilajit (81).

Human Cytomegalovirus

Polycarboxylate (aurintricarboxylic acid) are effective against *Human cytomegalovirus* replication, without any cytotoxic effect towards humans. These compounds prevent the attachment of viral particles to the cell surface by inhibiting the ionic interactions between the two (82-83, 79).

Coxsackie virus

The humic substances are shown to be effective against *Coxsackie A9* (84, 44).

Influenza Virus

Humic acids have been reported to suppress *Influenza* virus (85, 86). Humic substances have proven to be more important as prophylactic candidates rather than therapeutic against viral infections (87).

Vaccinia virus

Humic acid has an inhibition effect selectively against some of the *Vaccinia viruses* (88). Reports revealed that humic substances inhibit the early stage of *Vaccinia* virus replication (79, 84).

Potential role of Humic Substance in Covid-19 treatment

Humic substances including Fulvic acid, Humic acid have been shown to have antiviral activity in vitro. The selenium and DCP have already been shown as Immunomodulatory substances, and boost our immune system which is natural means of protection already existing in our body. The mixtures of these components are being used in traditional medicine for centuries and have shown their value and trust already. The Humic acid substances to be effective against HIV (11) for which no vaccine is available and existing therapeutic agents have severe harmful side effects. These anti-viral activities are versatile and could be used against many other diseases including cancer (89). The use of these substances as a prophylactic measure could provide the needed solution

to tackle COVID-19 and many more emerging diseases, which may come in future.

In conclusion, humic substances have proven to improve the overall immune system, by forming a set of composite network of cells and proteins that protects humans from germs and infection. Having proven antiviral activities against several human viruses, again make them good candidates that can be used for preventing as well as managing COVID-19.

Abbreviations

COVID-19: Coronavirus disease 2019

SARS-CoV-2: Severe acute respiratory syndrome Coronavirus-2,

DP: Dibenzo-pyrones

DCPs: dibenzo-pyrones-chromoproteins

Se: Selenium

SOD: Superoxide dismutase

HS: Humic Substances

FA's: Fulvic acids

HA: Humic acids

HSV-1: *Herpes simplex* virus type 1

HIV: *Human immunodeficiency* virus

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Authors contributions

DK wrote the introduction of immunomodulatory properties and compiled the table. JKK wrote the antiviral properties. RSM wrote the components & properties of humic substances and prepared the graphical abstract. DC wrote the nutraceutical properties of humic substances. AC conceptualized, coordinated and corrected the manuscript. All authors read and approved the manuscript.

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Conflicts of interest

The authors declare that there are no competing interests.

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