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Ayurveda may play a preventive role against viral infections including SARS-CoV-2 by the inactivation of furin protease

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Ayurveda extends various concepts which direct us about the management of infectious diseases including deadly viral infections in either preventive or curative manner. In cases of emerging viral diseases where there is little scientific knowledge, a vigilant observation can help prepare plan and management in future outbreaks. This work summarizes few important viral pathogens that have gained particular attention in recent years and their possible remedies with ayurvedic medicine. To challenge against the deadly viruses without medicine, immunity is the only choice. Ayurvedic medicines guide us to strengthen our natural defense systems by increasing the body's immunity level. The recent viral pandemic of SARS-CoV-2 is a novel coronavirus, identified as the cause of COVID-19 and there is no approved drug to control the infection yet. The viral proteases including human proteases have significant role in disease progression. Among all the human proteases, furin is a promising target for therapeutic intervention in viral diseases as it cleaves and activates several types of viral proteins including the spike protein of SARS-CoV-2. Results obtained from *in silico* analysis of the common metabolites of tulsi (*Ocimum* sp.) revealed their effectiveness to inactive the furin enzyme. Interestingly, Ayurvedic medicines have been prepared as different formulations with several ingredients or metabolites might have potential to inactivate the furin protein by binding at various positions. Thus, it suggests that the combined or synergistic action of metabolites present in ayurvedic medicine may be a promising therapeutics to prevent viral diseases.

Keywords: Ayurveda, host immunity, COVID-19, furin protease.

Introduction

In an era of modernization and shifting demographic trends toward urban lifestyle, people has to face challenges like poverty, malnutrition, overuse, and misuse of antibiotics, or indiscriminate use of antimicrobials, have led to the emergence of microbial including, viral, bacterial and fungal diseases. Some are novel and some re-surfacing viral diseases. In recent past, the outbreaks of viral diseases e.g. chikungunya fever in the Americas and Indian sub-continent, Ebola virus disease in West Africa[1], and human infections with avian influenza viruses, Nipah virus, Corona viruses, etc. (together with the prevalent threat of another pandemic caused by a new influenza virus) prompts about our being vulnerable to upcoming or novel viral pathogens[2]. While infectious diseases account for ~20% of global mortality, viral diseases causing about one third of these deaths[3]. Emerging viruses e.g. Ebola, H5N1 and H7N9 (avian influenza), Middle East respiratory syndrome coronavirus (MERS-CoV) and the novel coronavirus causing COVID-19, represent a fraction of viral diseases, yet they attract public attention because of their potential to spread rapidly, as an epidemic/pandemic resulting in massive morbidity and mortality rates[4,5]. Emerging viruses are proclaimed to be "new" because of lack of knowledge of previous infection in humans. The RNA viruses showed higher rates of mutation as compared to DNA viruses, because of such wide scale losses. This happens because of viral RNA polymerases can't proofread newly replicated strand that is seen in DNA polymerases[6]. This extremely higher mutability of RNA viruses is one of the causes that make it difficult to design effective vaccines against these pathogens, which may quickly mutate to escape vaccine-induced population immunity.

Re-emerging viruses are those that were recognized previously but have adapted to become major health threats or have appeared in previously uninflected geographical locations. For instance, the incidence of dengue fever, caused by four closely related serotypes of dengue virus carried by *Aedes* mosquitoes, has been on a rise owing to the rapid urbanization which allows new breeding grounds for the insects[7]. This is the effect of over-crowding, improper sanitation, and stagnant water in discarded tyres, clogged drains, puddles etc. These mosquitoes dispersed geographically due to anthropogenic activity and have quickly adapted to new human-created ecological niches. Alongside, Hepatitis E virus largely affected several countries around the world, especially Eastern and Southern Asia. In cases of emerging viral diseases where there is little scientific knowledge, a vigilant observation can help prepare plan and management in future outbreaks. Further, early detection may help for rapid implementation of effective measures.

Research on virus-caused epidemics (Table 1) has not been the priority amongst the scientific community and therefore, treatment or vaccines for most emerging or re-emerging viral diseases have always taken a back seat. Many of these infections are either lethal or leave behind challenges such as permanent disability that affects livelihood of the survivors and their families. Due to high mortality or morbidity rates of viral diseases, exhaustive research has to be undertaken in this field. However, the recent outbreak of COVID-19 evolved into a pandemic within last three months in more than 200 countries. The situation demands an immediate need to explore all the therapeutic and prophylactic strategies that can be made available to stem the spread of the disease. Higher morbidity rate have been observed among the elderly one and immuno-suppressed patients. In this context, certain herbs used for treatment in Ayurvedic system may be used as prophylactic agents by increasing the immunity, or inhibit the viral entry and replication in multi-targeted actions is discussed and evidenced[15].

Ayurveda and viral remedy

Ayurveda contains various concepts which direct us about the management of infectious diseases in either preventive or curative manner. For example, the changes in weather or seasons can be predicted and thus, before starting of epidemics which are most likely to occur in that weather, adequate arrangements and planning can be done so as minimize the morbidity. In Sanskrit, the term Ayurveda refers to "The Science of Life". With its origin in India, Ayurvedic knowledge has survived over 5,000 years and therefore known as the "Mother of All Healing". (https://www.ayurveda.com/resources/articles/ayurveda-a-brief-introduction-and-guide).

Ayurveda emphasizes on prevention of diseases and focuses on right balance in a person life through positive thoughts, healthy diet and lifestyle and improving immunity by consuming various medicinal herbs. It proclaims that the entire universe is supported by the interaction of five elements – Space, Air, Fire, Water and Earth. The concept of "vata", "pitta" and "kapha" are the result of the above five elements, where, "vata" refers to the energy of doing work, "pitta" is the capability of digestion and metabolism, and "kapha" refers to the structure of a person. Ayurveda claims that diseases that vanquish populations together arise due to deranged Vayu (air), Jala (water), Desha (habitat) and Kala (seasons). Ayurveda also teaches about "Sansargaja" and "Upsragaja" which indicate that there are certain diseases which can be transmitted directly from infected persons to healthy persons (contagious diseases) and also some that are transmitted via respiration or are air-borne.

Ama is the toxic substance produced in the body, which promotes the incidence of diseases and sickness. Many diseases may have a common manifestation with Jwara (fever) and this concept of Ayurveda is dealing with diseases and their appearance is called as Vyadhi Sankara[16]. The same concept can be applied where the etiological factor causing the disease. Many etiological factors can cause one disease or may cause many diseases. This concept of Ayurveda is considered as Hetu Sankara[17]. Proximity or close-bodily contact via breath, secretions, clothing, utilization of sitting, sleeping place of diseased person all these are the factors for the Sankramika Rogalikekustha, Jwara, Sosha, Netrabhishyanda[18]. The Nidanarthakara Roga is a unique concept of Ayurveda. For example, Jwara will lead to the disease Raktapitta, while Raktapitta may lead to Jwara and it may lead to Sosha and finally death[19]. This line of infection

Table 1. Some viral pathogens that have gained particular attention in recent years are discussed below

Virus	Mode of transmission	Outbreak potential	Treatment availability (Drug/Vaccine)	Reference(s)
Enteroviruses (EVs)	Positive-stranded RNA viruses	Yes	Vaccine	8, 9
Reoviruse	Linear double-stranded RNA genomes, transmitted by vectors which are included in Culicoides midges, mosquitoes, black flies, sand flies and ticks		Vaccine	10
Avian influenza (AI)	Exposure to infected birds	Yes	Medication	World Health Organization. Influenza (Avian and other zoonotic). Available from: https://www.who.intlnews - room/fact sheets/detail/influenza- (avian-and-other-zoonotic)
SARS-CoV	Human to human transmission	Yes	Potential vaccine	21
MERS-CoV	Zoonotic viral illness, air-borne	Yes	Potential vaccine	World Health Organization. Middle East respiratory syndrome coronavirus (MERS-CoV). Available from: http://www.who.intlemergencies/mers -cov/en!, accessed on June 12, 2018
Rift valley fever (RVF) virus	Through contact of blood or fluids of infected animals as well as infected mosquitoes	Yes	Animal vaccination	World Health Organization. Rift Valley fever. Available from: https://www.who.intlnews-room/fact-sheets/ detail/rift-valleyfever, accessed on April 11, 2019
Yellow fever (YF) virus	Aedes mosquitoes	Endemic	Vaccine available	World Health Organization. Yellow Fever, Fact Sheet. Available from: https://www.who.intlnews-room/fact-sheets/detail/yellow-fever, accessed on January 15, 2019
Nipah virus (NiV)	Pteropus bats and pigs, also Human-to-human spread	Yes	No drugs or vaccines	11 World Health Organization. South-East Asia. Nipah virus outbreaks in the WHO South-East Asia Region. Available from: http://www.searo.who.intlentity/ emerging_diseases/links/nipah_virus_outbreaks_ sear/e

is associated with several viral diseases, wherein contact and use of infected person's belongings spreads the disease, leading to fever and associated symptoms to death.

Viral fevers may be managed or prevented during certain seasons by consuming medicinal herbs that empower immunity. For example during monsoon, use of Neem (*Azadirachta indica* A. Juss) leaves or its juice or using its bark powder, or powdered Sudrashan (*Crinum latifolium*) or Guduchi (*Tinospora cordifolia*), can be helpful. Appropriate fumigation of non living surfaces should be done with dry Neema leaves, Kapura, Vacha (*Acorus calamus* Linn.). Many of viral diseases such as chikungunya are rarely fatal and symptoms are self-limiting may last for 2–3 days[20]. However, in the long run it can cause post-infective complications such as post chikungunya arthritis resulting in long-term disabilities leading to medical and economic burden in affected areas.

The anti-inflammatory herbs Nirgundi (*Vitex negundo* Linn.), Shallaki (*Boswellia serrata* Roxb.), or Guggulu (*Commiphora wightii* Arn.) may be useful along with the medicines to control fever at the onset of chikungunya fever (https://www. planetayurveda.com/library/chikungunya-fever/). Simi-

larly, dengue fever causes rapid decrease in platelet count and lasts with prolonged debility. In dengue, along with antipyretic drugs, herbs that promote hematopoiesis and help in the platelet formation can be added. The same symptoms can be treated with various medications ayurvedic used in ayurveda[21]. A few popular herbs used in ayurvedic medicine (Table 2) may provide relief in various viral diseases.

Table 2. Applications of herbs against viral diseases

Viral disease	Curative plant	Part	Scientific name	Ref.
FLU	Tulsi	Leaves	*Ocimum sanctum*	22
Flu	Turmeric	rrhizome	*Curcuma longa*	23
Chikungunya	Giloy	Leaves	*Tinospora cordifolia*	24
Chikungunya	Sonth	Root	*Zingiber officinale*	24
Dengue	Papaya	Leaves	*Carica papaya*	25
Dengue	Green chiretta	Leave	*Andrographis paniculata*	26
Herpes simplex virus HSV1 and HSV2	Veld grape	Stem	*Cissusqua drangularis* L.	27
Rheumatism, spasm, cold/flu, etc.	Kewda	Leaves, flowers	*Pandanus odoratissimus* L.	28
New swine originating H1N1 strain of influenza A virus	Ashwa-gandha		*Withania somnifera*	29
Viral flu	Himala-yan onion	Leaves	*Allium wallichii*	30
Viral fever	Kalmegh	Leaves	*Andrographis paniculata*	12
Influenza A (H1N1)	Garlic	Bulb	*Allium sativum*	31
Influenza A (H1N1)	Indian leadwort	Roots and juice	*Plumbago indica*	31

Building immunity through ayrvedic medicine

Building good immunity is the key to combat viral diseases. Various ayurvedic medicines can strengths the body's natural defense systems. When any new virus interacts with human immune system, there's time to fortify our resistance by decreasing the toxins (ama) in the body, such that the virus doesn't find a weak body for spreading through it[32].

Medicinal plants having rasayana property (potential for mediating biochemical pathways), immune modulatory activity, and antiviral activity may prevent the adverse effect of the disease. Ayurvedic medicine against major diseases is a eco-friendly alternative. Unlike bacteria, viruses invade healthy living cells in order to reproduce. The body's own immune system offers the best prophylactic treatment by preventing the invasion, to suppress the symptoms by inhibiting viral reproduction. An Ayurvedic drug bolsters the body's defences at the cellular level, stimulating the immune system. Alkaloids are a common substance present in these drugs[33,34], besides several others (flavonoids, terpenoids, organosulfur compounds, sulfides, polyphenolics), which can stimulate white blood cell activity and increase the number of T-helper cells[35,36]. These cells coordinate the immune response, resulting in the production of antibodies and ridding the infectious agent. Quinolizidine alkaloids are a group having a quinolizidine or piperidine ring with alkaloid ring showed a diversified immunological function by stimulation of different signalling network[37]. A review on hepatoprotective and immunomodulatory activity of herbal agents has been described in details to increase the immunity[38].

Therefore, symptomatic treatment with known and established ayurvedic drugs not only help us to get relief in viral diseases but also strengthen the immunity for future attacks. There are reports on garlic (*Allium sativum*), green tea (*Camellia sinensis*), ginger (*Zingiber officinale*), purple coneflower (*Echinacea purpurea*), black cumin (*Nigella sativa*), licorice (*Glycyrrhiza glabra*), Astragalus, St. John's wort (*Hypericum perforatum*) etc. can be utilized as natural immune boosters[39]. Modes of their actions include boosting and functioning of immune system, activation and suppression of immune specialized cells, interfering in several pathways that eventually led to improvement in immune responses and defense system. In addition, some of these plants carry free radical scavenging and anti-inflammatory activities that are helpful sometimes against cancer insurgence[40].

The immune system (IS) is a complex integrated network of cells, tissues, organs and soluble mediators, evolved to defend the organism against any foreign insult that threatens the integrity of the organism. Plants/herbs contain various phytochemicals, namely, Fury compounds (furocoumarins and furano-chromone), alkaloids, polyacetylene/

polyenes, thiophenes or flavonoids, terpenoids etc., which follow various mechanisms to destroy or inhibit viral genomes/ functioning. Studies suggest that *Echinacea* stimulates immune functions in both healthy and immune suppressed animals[41]. In macrophages, phagocytosis and cytokine production (increased TNF-α, IL-1, IFN-β) have been enhanced following treatment with *Echinacea* extracts, increased leukocytes mobility as well as activation of natural killer cells has also been reasonably demonstrated in animals and humans. In addition to this, *Echinacea* products show anti-inflammatory, antiviral and antimicrobial effects[40]. Curcumin obtained from turmeric, or Indian saffron posse's immunomodulatory activity based on its interaction with different immunomodulators, which are present in dendritic cells, macrophages, B and T lymphocytes, and also as molecular components of the cells. The molecules like cytokines and several transcription factors participate in inflammatory processes through the definitive signalling pathways[42]. Due to the presence of a various other phyto-chemicals in turmeric, the use of whole *Curcuma longa* extract has been suggested to enhance the immune system in immunosuppressed patients. Similarly *Chlorophytum borivilianum* root extract exhibited a significant reduction in parasite count by the immunomodulation through enhanced Th1 immune responses and suppressed Th2 type of responses[43].

Various other plants have bioactive phytochemicals like flavonoids, terpenoids, organosulfur, sulfides, polyphenolics, alkaloids, lignans, thiophenes, and peptides with proven therapeutic applications against a diverse range of viruses[44]. The antiviral action of these agents is based on their antioxidant, free-radical scavenging ability, or inhibits nucleotide (RNA/DNA) synthesis, entry of viruses into the cells, or deterring viral reproduction and so on as presented in Table 3. It has been reported that extract of *Punica granatum* have antiviral activity against HSV-1 and HSV-2[45]. The punicalagin present in extract and juice of *P. granatum* has been suggested as the bioactive compound.

In cases of newly emerging viral diseases with little scientific knowledge and evidence, and vaccine or medication is still under research, ayurvedic drugs can help in combating the plight in cost-effective manner. Treating viral disease with plant derived compound, which is easily accessible and do not require laborious pharmaceutical synthesis, seems a

Table 3. Representative the action of bioactive compound of certain plants against viruses

Viral disease	Scientific name	Mechanism of action	Part	Clinical/preclinical evidence	Ref.
FLU	*Ocimum sanctum*	Masking/blocking of HA protein of the Virus[46]	Leaves	About 24 studies were reported on therapeutic effects of tulsi on metabolic disorders, cardiovascular disease, immunity, and neurocognition showing favourable clinical outcomes and no significant adverse events	47, 48
Flu	*Curcuma longa*	Curcumin showed the anti-influenza activity against influenza viruses PR8, H1N1, and H6N1; curcumin also modulates Apoptosis[49]	Rhizome	Curcumin showed good results in human clinical trials for a variety of conditions, including multiple myeloma, pancreatic cancer, myelo dysplastic syndromes, colon cancer, psoriasis and Alzheimer's disease. Curcumin interfered with the binding of the enveloped viruses to cells in a dose-dependent manner Mounce *et al.*, 2017	49, 50
Chikungunya	*Tinospora cordifolia*	Potent inhibitor of NS2B-NS3 receptor in Dengue virus[51]	Leaves	Potential medicinal properties reported by scientific research include anti-diabetic, antipyretic, antispasmodic, anti-inflammatory, anti-arthritic, antioxidant, antiallergic, anti-stress, anti-leprotic, antimalarial, hepato-protective, immuno-modulatory and anti neoplastic activities	25, 52, 53

Table-3 (contd.)

Chikungunya	*Zingiber officinale*	Undeciphered	Root	Fresh ginger dose-dependently inhibited viral attachment and internalization. Fresh ginger of high concentration could stimulate mucosal cells to secrete 1FN -? that possibly contributed to counteracting viral infection	54, 55
Dengue	*Carica papaya*	Maintain the normal level of hematocrit and accelerates the number of platelets in dengue-infected cases. The extracts are likely to possess membrane-stabilizing properties and protect	Leaves	The dengue infection in mouse model was established by inoculation of non -mouse adapted New Guinea C strain dengue virus (DEN -2) in AG129 mice	25, 57, 58

highly attractive alternative. Vigilant observation can help prepare planning and management in future outbreaks. Further, early detection may help in rapid implementation of effective measures.

In silico analysis for furin inactivation

More than 550 proteases are encoded by human genome and several viral pathogens utilize host proteases for their maturation59. Similarly, activation of bacterial toxins requires cleavage by proteases of the infected host. Therefore, host proteases are potential target for therapeutic intervention for a variety of viral diseases. Among all the human proteases, furin cleaves and activates more than 150 proteins60. Furin helps to recover from Herpes-, Corona-, Flavi-, Toga, Borna, Bunya, Filo, Orthomyxo, Paramyxo- and Retro-virus infections59. In Ayurveda, different plant metabolites are used to develop defense against many viruses. Here, we have undertaken a commonly used plant *Ocimum tenuiflorum* extract that help in the prevention of viral flu23. We have also listed the plant metabolites present61 in those extracts and exploited their role in furin inactivation.

Compound screening and energy minimization

Different metabolites present in Tulsi such as Oleanolic acid, Rosmarinic acid, Ursolic acid, β-Caryophyllene, β-Elemene, Germacren, Eugenol, Carvacrol, Linalool were chosen, based on their bioactivity and therapeutic efficacy. Each metabolites of tulsi was undergone energy minimization using ChemBio3DUltra 13.0 software, a high quality workstation where MM2 energy minimization of each metabolite was identified with stable molecular conformation (Fig. 1; Table 4). Minimum RMS gradient was taken as 0.010.

Molecular docking

Protein Data Bank file for Human Furin (PDB ID: 4RYD) was used as receptor molecule and Oleanolic acid (PubChem CID: 10494), Rosmarinic acid (PubChem CID: 5281792), Ursolic acid (PubChem CID: 64945), β-Caryophyllene (PubChem CID: 5281515), β-Elemene (PubChem CID:

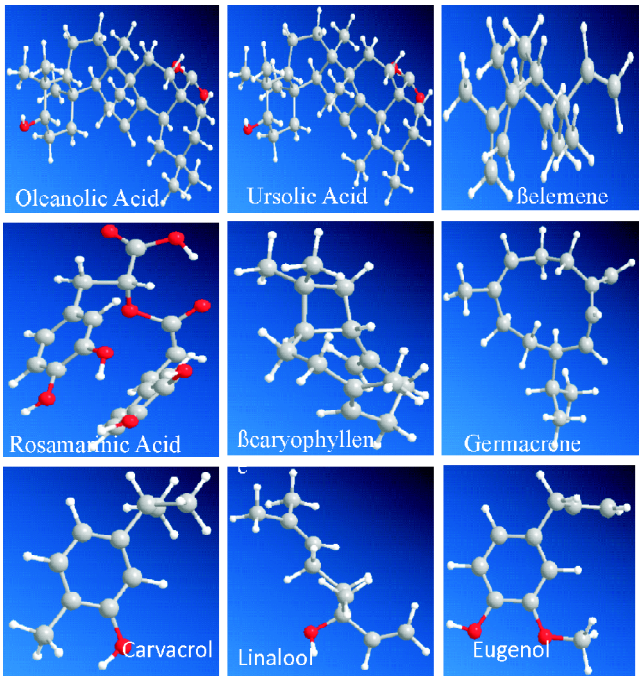

Fig. 1. Energy-minimized structure of different metabolites present in tulsi (*Ocimum* sp).

Table 4. Energy minimization calculation of different metabolites of Tulsi using ChemBio3DUltra 13.0 software

Ligand	Dipole/Dipole	Total energy after minimization (kcal/mol)
Oleanolic acid	–0.1976	88.2049
Rosmarinic acid	–6.6865	13.6561
Ursolic acid	–0.1994	91.0569
β-Caryophyllene	–	47.7206
β-Elemene	–	21.6279
Germacrene	–	25.1951
Eugenol	–1.3212	8.9420
Carvacrol	0.00	8.2352
Linalool	0.00	8.7798

6918391), Germacren (PubChem CID: 5373727), Eugenol (PubChem CID: 3314), Carvacrol (PubChem CID: 10364), Linalool (PubChem CID: 6549) was taken as ligand molecule for docking. Molecular docking was performed to monitor the interaction of different metabolites of Tulsi with Human Furin (PDB ID: 4RYD) using docking software PyRx version 0.8, together with Autodock Vina, was used for all docking calculations (http://pyrx.sourceforge.io/) to analyze the interaction between target protein and a small molecule[62]. Both selected protein and ligand files were loaded to PyRx as macromolecules and ligands respectively. Proteins were fixed while ligands were set to have rotatable torsions. Protein and ligand hydrogens were automatically added using the PyRx hydrogen (H) repair functionality. A box of size X: 23.6597; Y: 32.5622; Z: 3.1596 were defined around the active site of the protein with the exhaustiveness parameter set as 64 for all dockings. Autodock Vina automatically samples different conformation of the ligands to best fit to the active site of the protein[63]. The two dimensional ligand and receptor interaction can be viewed by PyMol, a molecular graphics visualizetion where ligand conformation and orientation of the molecule was notified relative to active site of the receptor.

Results and discussion

Furin is an endo proteinase of highly specific calcium dependent proprotein or prohormone convertases (PC) family[59] with a catalytic domain of homology to subtilisin and activates large number of secreted proteins by limited proteolysis. This type I transmembrane serine-protease is ubiquitously expressed and cycles from the trans-Golgi network to the cell membrane, as well as through the endosomal system[59]. Serine proteases make up at least one third of all known protease enzymes. Its large luminal/extracellular region have overall homology with the same region of other members of PC family. The greatest sequence similarity resides in the subtilisin-like catalytic domain; the aspartate (Asp), histidine (His) and serine (Ser) residues that form the CATALYTIC TRIAD and are rigorously conserved, and the catalytic domains of the other PCs showed 54–70% identical in sequence to furin. Serine proteases are known for their use

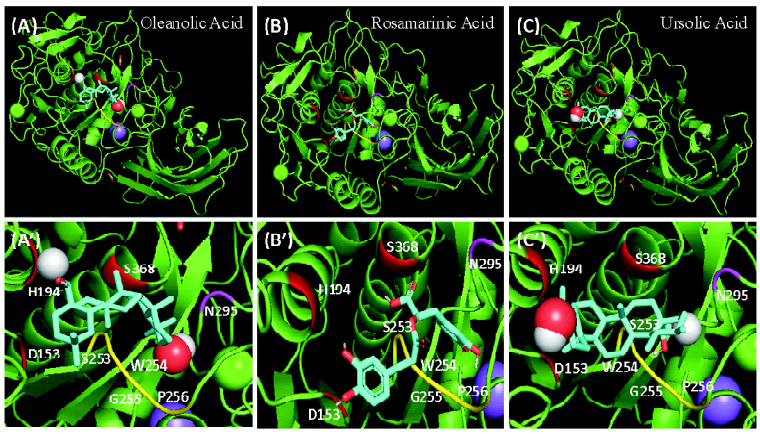

Fig. 2. Docked image of Human Furin (PDB ID: 4RYD) with Oleanolic acid viewed in Py MOL (A). Zoomed image of ligand binding site of Furin-Oleanolic acid complex (A′). Docked image of Human Furin (PDB ID: 4RYD) with Rosmarinic Acid viewed in Py MOL (B). Zoomed image of ligand binding site of Furin-Rosmarinic acid complex (B′). Docked image of Human Furin (PDB ID: 4RYD) with Ursolic acid viewed in Py MOL (C). Zoomed image of ligand binding site of Furin-Ursolic acid complex (C′). Red highlighted portions indicate catalytic triad, magenta colour represents oxyanion hole and yellow highlighted portion indicates site of binding.

of the catalytic triad (Asp153, His194, Ser368) in hydrolyzing peptide bonds. The -OH group at serine residue is responsible for the nucleophilic attack to the carbonyl carbon on the peptide bond of the substrate. The -OH group acts as the nucleophile, and the nitrogen on the histidine has the ability to accept the hydrogen from the serine -OH group. The aspartic acid residue's carboxyl group form hydrogen bonds with the histidine, making the nitrogen on the histidine more electronegative. A nearby pocket of positively charged residues also stabilizes the transition state of the deprotonated oxygen. This pocket is usually referred to as an oxyanion hole (Asn295). Oxyanion holes also aid with substrate insertion, preventing steric hindrance by substrates that otherwise would not fit.

From the results of molecular docking experiment of human furin (PDB ID: 4RYD) with different metabolites of tulsi, it shows a strong binding affinity of all the metabolites towards the catalytic site of furin thus revealed a preventive measure by blocking the catalytic triad and stops the viral entry within the body. In case of oleanolic acid, the binding

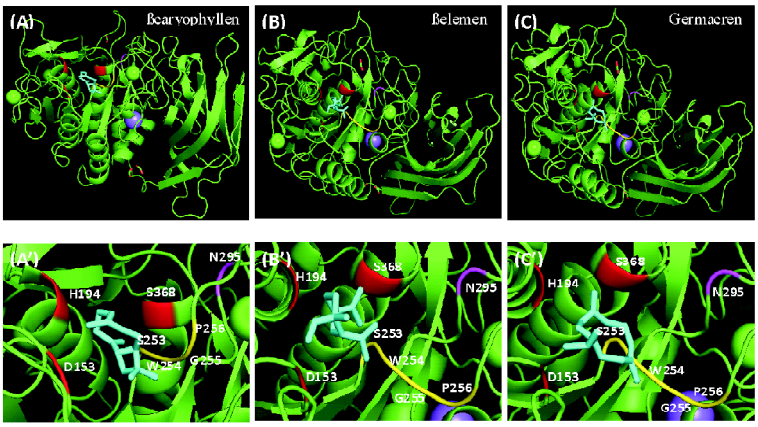

Fig. 3. Docked image of Human Furin (PDB ID: 4RYD) with β-cayophyllene viewed in Py MOL (A). Zoomed image of ligand binding site of Furin-β-caryophyllene complex (A′). Docked image of Human Furin (PDB ID: 4RYD) with β-elemene viewed in Py MOL (B). Zoomed image of ligand binding site of Furin-β-elemene complex (B′). Docked image of Human Furin (PDB ID: 4RYD) with Germacrene viewed in Py MOL (C). Zoomed image of ligand binding site of Furin-Germacrene complex (C′). Red highlighted portions indicate catalytic triad, magenta colour represents oxyanion hole and yellow highlighted portion indicates site of binding.

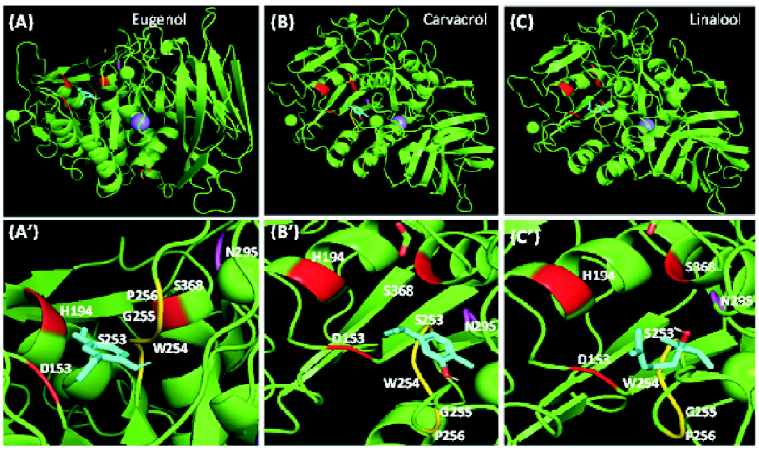

Fig. 4. Docked image of Human Furin (PDB ID: 4RYD) with Eugenol viewed in Py MOL (A). Zoomed image of ligand binding site of Furin-Eugenol complex (A′). Docked image of Human Furin (PDB ID: 4RYD) with Carvacrol viewed in Py MOL (B). Zoomed image of ligand binding site of Furin-Carvacrol complex (B′). Docked image of Human Furin (PDB ID: 4RYD) with Linalool viewed in Py MOL (C). Zoomed image of ligand binding site of Furin-Linalool complex (C′). Red highlighted portions indicate catalytic triad, magenta colour represents oxyanion hole and yellow highlighted portion indicates site of binding.

Table 5. Site-specific docking of different metabolites of tulsi with Human Furin (4RYD) and determination of binding free energy using PyRx

Receptor	Ligand	Catalytic Triad present in Furin	Binding free energy (kcal/mol)
4RYD:A(hFurin)	Oleanolic acid	His194, Ser368, Asp153	–8.1
4RYD:A(hFurin)	Rosmarinic acid	His194, Ser368, Asp153	–8.0
4RYD:A(hFurin)	Ursolic acid	His194, Ser368, Asp153	–7.6
4RYD:A(hFurin)	β-Caryophyllene	His194, Ser368, Asp153	–5.4
4RYD:A(hFurin)	β-Elemene	His194, Ser368, Asp153	–4.4
4RYD:A(hFurin)	Germacrene	His194, Ser368, Asp153	–5.5
4RYD:A(hFurin)	Eugenol	His194, Ser368, Asp153	–5.0
4RYD:A(hFurin)	Carvacrol	His194, Ser368, Asp153	–5.4
4RYD:A(hFurin)	Linalool	His194, Ser368, Asp153	–4.4

free energy is maximum (–8.1 kcal/mol) with respect to all the other metabolites when docked with human furin. The binding free energy was depicted in the Table 5 where the binding free energy was in the range (–8.1 to –4.4 kcal/mol). Apart from the catalytic triad, all metabolites form an interaction with the residues Ser253 to Pro256 which is also a binding inhibitor site for small molecule interaction.

Examination of the protein sequence of the S glycoprotein of SARS-CoV-2 reveals the presence of a furin cleavage sequence (PRRARS|V). By blocking the active site of furin, it can be suggested that it may further prevent the entry of virus. Thus in a nutshell it can be concluded that tulsi metabolites can be useful as a preventive compound against viral entry. Interestingly, all the metabolites have the binding affinity to furin with different binding energy at variable position. Therefore, it suggests the combined or synergistic action of plant metabolities may be energetic to prevent the viral diseases.

Conclusion

Ayurveda is one of the oldest traditional systems of medicine accepted worldwide but not explored as expected although this is the one main cord of the rich knowledge from different traditional systems. This review summarizes the application of few herbs for the prevention of viral infections. Ayurvedic drug revealed their immunomodulatory action which is helpful to fight against recent pandemic by SARS-CoV-2. Present *in silico* study revealed the efficacy of *Ocimum* metabolites to act as the inhibitor of furin protease, necessary for the entry of SARS-CoV-2 in human cell.

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