Research & Reviews in Biotechnology & Biosciences **ISSN No: 2321-8681**
Website: www.biotechjournal.in **Mini-Review**
Volume: 9, Issue: 1, Year: 2022 **PP: 63.78**
DOI: https://doi.org/10.5281/zenodo.6620675 **Peer Reviewed Journal**

Plant based solutions for curing SARS-CoV and related strains

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Article History

Received: 12/03/2022
Accepted: 10/04/2022

Article ID: RRBB/124

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Abstract

COVID-19 is the most dangerous and destructive pandemic in human history. Since its outbreak, millions of people worldwide had lost their lives through its rapid spread in over 100 countries. It is officially designated as severe acute respiratory syndrome related corona virus (SARS-CoV-2). Although there were many other related strains in past that causes mild COVID symptoms but this pandemic requires the rapid development of vaccines and medicines for its cure. Out of all technology-based methods, plant-based technologies have proven to be more efficient and promising for combating SARS-CoV-2 and related strains. Plant expression systems can be used for the rapid production of effective medicine and chemicals. Plants can be used as stably transformed plants and transiently-transformed plants. Plants can also be used as oral vaccines for transferring antigens or antibodies (passive immunization) in the human body and in the diagnosis of viral antigen and antibodies as well. These approaches are more attractive due to their cost and immunity induction. There are many other potential chemicals in plants that have the capability to act as COVID-19 vaccine or an adjuvant for it. So, plant-based technologies can be regarded as an emerging approach for the treatment of COVID-19 and related strains.

Keywords – COVID, SARS-CoV-2, vaccines, antigen, antibodies

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1. Introduction

The pandemic COVID-19 caused by a novel corona virus (Severe acute respiratory syndrome corona virus 2; SARS-CoV-2) has led to massive efforts to develop vaccines and drugs in order to slow down the spread of the disease and has brought together researchers from all over the world to develop various diagnostics tools and therapeutics for combating the COVID-19 pandemic [1]. Keeping the economic condition of every country in mind, it is desirable to design a vaccine avoiding epitopes that can cause an unusual immune response. Since plants have enormous potential for the formation of biopharmaceuticals, it is high time to widen our knowledge about plant based technologies to exploit their potential in combating COVID-19 and related diseases [2].

The main features of corona viruses include the presence of envelop and positive single-stranded RNA [3, 4]. It has spherical body and with large spikes (16-21 nm long) extending from viral envelope [4].Slow mutation in SARS-CoV-2 is due to proofreading enzymes that prevent huge mistakes during replication. But researchers have seen 12,000 or more mutations in the SARS-CoV-2 genome. Mutations may either have no major effect on the virus and its pathogenicity or improve the infectivity of viruses [5].

2. Coronaviridae: A Family Of Coronavirus

Members of this family are larger from other RNA viruses. The length of genome is 25-32 kb with viral diameter of 118-136nm [4]. The main features of corona viruses include the presence of envelop and positive-single-stranded RNA [3, 4]. It has spherical body with 16-21 nm long spikes on viral envelope. RNA genomes of corona viruses have 7-10 ORFs and non-structural proteins. Non-structural proteins are needed for transcription and replication [4]. Non-structural proteins are long precursor proteins that are cleaved by proteases for its activation. For example, nsp1-nsp11 is formed by cleavage of REP1a protein and nsp12-nsp16 is formed by cleavage of REP1b. Spike (S) protein gene lies down stream of replicas-associated genes. Many small ORFs (accessory proteins) are present in between or overlap the structural protein genes. When these accessory proteins are damaged it may hamper the CoV's replication and pathogenesis. There are three membrane-bound proteins and one nucleocapsid (N) protein. Membrane bound proteins include spike (S), membrane (M) and envelope (E).Hem agglutinating and Esterase (HE) membrane proteins occur in few beta-corona viruses [4].

Coronaviridae (CoV) family causes moderate respiratory illness [6]. But SARS-CoV and Middle East respiratory corona virus syndrome (MERS-CoV) in humans can cause serious forms of respiratory diseases [6,7]. SARS-CoV-2 has 80% similarity with SARS-CoV-1 and 50% similarity with MERS-CoV [8,9]. SARS-CoV is far different from previously studied corona viruses in many ways, like peculiar tropism for Vero cells, growth at different temperatures and infection capabilities. These aspects are important for antiviral therapy and vaccine development [3].

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2.1 SARS-Associated Coronavirus Strains

A new strain of SARS-CoV was isolated from frozen sputum sample of patient who had alien respiratory disease and stored at -80˚C. This strain was named as SARS-CoV HSR1. Isolated strain was maintained in Dulbecco's modified Eagle medium with 10% fetal calf serum, penicillin/streptomycin and Fungi zone. The isolated strain was tested for cytopathic effects (CPE) in cell cultures. Vero cells were incubated with phosphate-buffered solution (PBS) with SARS-CoV HSR1 viral strain to check for infectivity of the strain. This method is called Plaque Infectivity Assay. Stereoscopic microscope was used for plaque examination and it's counting [3].

SARS-CoV HSR1 genome was fully sequenced by using 68 partially overlapping primers. SeqScape 2.0 software helps in editing and assembly of fragments for sequencing. PAUP software helps in estimating phylogenetic relationships. Multiple software, tools and techniques were used to identify or spot many sites in the genome, for example: 5′ UTR region was identified by 5′ rapid amplification of PCR ends (RACE) technique [3].

Studies on mutagenesis indicated that the infection mechanism of SARS-CoV-2 is controlled majorly by two factors, which are, (a) particular amino acid domain of nsp1 and (b) protein and host's small ribosomal unit interactivity. Deletion of 3 amino acids (KSF) and replacement of 2 amino acids (KS) from nsp1 can lead to development of new SARS-CoV-2 strains. Deletion of KSF amino acids from nsp1 protein can destroy the C-terminal region of nsp1 which can further lead to disrupted host's gene expression. Substitution of KS amino acids from nsp1 protein may lessen the loss of interferon alpha expression. This type of deletion can make the viruses less pathogenic and substitution of the above amino acids enhance the infectivity of virus by proper folding of nsp1 and inhibition of host's innate immunity [10].

The COVID-19 infection among UK population went to peak due to highly infectious SARS-CoV-2 variant VUI-202,012/01. This variant was formed due to N501Y mutation. A SARS-CoV-2 variant (501Y.V2) is known to be highly contagious. This variant is developed due to 3 spike protein mutations: K417N (substitution of lysine to asparagine at 417 amino acid position), E484K (substitution of glutamic acid to lysine at 484 amino acid position) and N501Y (substitution of asparagine to tyrosine at 501 amino acid position) [12].

D614G spike mutation in SARS-CoV-2 leads to the development of G614 variant. This variant is highly infective due to high viral loads. This variant is developed due to mutation at 23,403 nucleotide position in Wuhan reference strain. This change discards side-chain H_2 bond that enhances main-chain elasticity [12].

2.2 SARS-COV-2: Components and Mode of Action

SARS-CoV-2 is an RNA virus having four structural proteins – Membrane (M), Envelope (E), Nucleocapsid (N) and Spike (S) encoded by ORF1b.M and E proteins help in viral assembly and S protein helps in viral invasion [13]. It has a single-stranded positive-sense genomic RNA with an envelope around its genome [2,13]. It is classified into 4 groups – α-CoVs, β-CoVs, ɤ-

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CoVs and δ-CoVs [2,9,and 13]. First two forms affect mammals; the other two forms majorly infect birds [2,9]. It consists of the largest RNA among all RNA viruses (2600 bases to 3200 bases). RNA is packed into nucleocapsid helical in shape and then in the host-derived lipid bilayer. RNA has a 5′ end (cap structure) and 3′ end (multiple polys A tail) that can act as mRNA [13]. It has 74 spikes on its surface [7].

Glycosylated spike (S) activates an immune response in a host by cohering to angiotensin-converting enzyme 2 (ACE2) present on the cell surface of a host and start the infection process. S protein binding and invasion is facilitated by serine protease TMPRSS2 [9,14]. Other non-structural proteins like RNA-dependent RNA polymerase (RdRp), corona virus main protease (3CLpro), and papain-like protease (PLpro) are also produced by the viral genome [14] (in Table-I).Cellular serine protease helps in the breakdown of S protein and form S1 and S2 subunits. Receptor binding motif (RBM) in the receptor-binding domain provides space for binding of ACE-2 and similar to SARS-CoV-1. Viral capsid fuses into host cell membranes due to S2 subunit guidelines [6]. S1 subunit directs the binding of the virus to the ACE2 receptor on the host [13].The viral genome, on invasion into host cells, liberates a single-stranded positive RNA from which polyproteins are produced using the host's translation mechanism. PLpro helps in immune suppression by suppressing interferon factor 3 and NF- κB [14].

Table – I: Key proteins and their role in viral infection process [14].

Target Candidate	Full name	Role during viral infection
3CLpro	Corona virus main protease	A protease for the proteolysis of viral polyprotein into functional units
PLpro	Papain-like protease	A protease for the proteolysis of viral polyprotein into functional units
RdRp	RNA-dependent RNA polymerase	An RNA-dependent RNA polymerase for replicating viral genome
S protein	Viral spike glycoprotein	A viral surface protein for binding to host cell receptor ACE2
TMPRSS2	Transmembrane protease, serine 2	A host cell-produced protease that primes S protein to facilitate its binding to ACE2
ACE2	Angiotensin converting enzyme 2	A viral receptor protein on the host cells which binds to viral

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		S protein
AT2	Angiotensin receptor 2	An important effector involved in the regulation of blood pressure and volume of the cardiovascular system

2.3 Will Plants Really Be Helpful?

From the beginning of the human species, plants have been serving as the main source of medicines for treating various kinds of infectious diseases [15]. Plants have been used for over 30 years for large-scale production of diagnostic substrates and pharmaceutical products within a timeframe of few weeks. To fight against COVID-19, plant technologies may help to make (i) diagnostic substrates for finding infective agents, (ii) vaccines, and (iii) antivirals to cure viral symptoms [1]. Plants can be cultivated on large scale with economic stability and without bearing the growth of human pathogens [1,2]. This could help impoverished and developing countries with narrow means to afford health facilities during pandemics [2]. Also, Cellular machinery in plants allows post-transitional modifications that may be essential for functional protein formation [16]. Plant systems can efficiently synthesize multimeric or glycosylated complex proteins [2]. Plants have the potential for the treatment of COVID-19 and other diseases but scientific research is needed for the understanding of its prospects. Scientific research may include the study of biochemistry, genetics, and chemistry of medicinal plants for identifying relevant pharmacological properties (Table-II). According to JK Weng, plants have the potential to produce bioactive substances and their analogs via metabolic engineering which can be used further for other processes [15].

Traditional Chinese Medicine (TCM) is based on formulae which are derived out of which Lung cleansing and detoxifying decoction (LCDD). LCDD is a complex mixture of herbal remedies which is effective against SARS-CoV-2. Baicalein (one of its component) helps in repression of the main protease (3C-like protease) of SARS-CoV-2 that suppresses SARS-CoV-2 replication in Vero cells. Generalized functions of herbal components present in LCDD were to restrict viral replication, etc. [15].

The ayurvedic approach was also used to treat COVID-19 patients. Treatment was done in two stages – Jwara (1-13 days) and JwaraMukti (14-30 days). Medications like SudarsanaChurna, TalisadiChurna, and DhanwantaraGutika were given in the Jwara phase of treatment. In the JwaraMukti phase, VidaryadiGhritam medicine was given [17].

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Table – II: Medicinal plants and their relevant pharmacological properties [18].

Medicinal plant	Active compound	Relevant pharmacological property	Drug/ composition with active compound
Vitextrifolia (Chastetree)	Casticin (C19H18O8)	Immunomodulatory & Anti- inflammatory effect on lungs	US7604823
Punicagranatum (Pomegranate)	Punicalagin (C48H28O30)	Inhibited viral glycoprotein & Anti-HSV-1	US2008214656
Allium sativum (Garlic)	Allicin (C6H10OS2)	Proteolytic and hemagglutinating activity and viral replication	DB11780
Andrographispaniculata (Green Chiretta)	Andrographolide (C20H30O5)	Antiviral potential	US2017354639
Cynarascolymus (Globe artichoke)	Cynaratriol (C15H22O5)	ACE inhibitor	US6117844
Sphaeranthusindicus (East Indian Globe Thistle)	Tartaric acid (C4H6O6)	Inhibition of Mouse corona virus and Herpes virus Bronchodilation	Various compositions
Clitoriaternatea (Butterfly Pea)	Delphinidin-3-O-glucoside (C21H21O12)	Antiviral properties	US8609152
Embeliaribes (False black pepper)	1,4- Benzoquinone (C6H4O2)	Inhibition of ACE	Various compositions
Hyoscyamusniger (Black Henbane)	Hyoscyamine (C17H23NO3)	Viral Inhibition and Bronchodilator	DB00424
Eugenia jambolana (Black plum)	Ellagic acid (C14H6O8)	Protease Inhibitor	DB08846
Gymnemasylvestre (Australian Cowplant)	Tartaric acid (C4H6O6)	Inhibition of viral DNA synthesis	DB09459

3. POTENTIAL OF PLANT TECHNOLOGIES

3.1 Transient Expression and Molecular Farming

Molecular farming is a technology that uses the plant for the production of important products like antibodies, vaccines, hormones, etc [2]. Plants undergo transformation for transient expression of the target protein for scaling up of certain products [1,2].Extraction of biopharmaceuticals can be done from plant leaves or suspension of cell cultures in a bioreactor [1]. Recombinant proteins can be expressed in two ways – by stable transformation into the nucleus and by a transient transformation. Conventionally, the transgene is integrated into the nuclear genome [2]. Transient expression approaches in plants like Nicotianabenthamiana can be used for the production of required antigens, antibodies, etc. However, stably transformed plant

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species must be developed for generating vaccines that could be used at the post-pandemic stage [2]. Many viruses help in the development of transient and efficient expression systems in the plant, e.g. tobamoviruses, geminiviruses, etc. [2].

3.2 Approaches for recombinant protein expression in plants

Agrobacterium-mediated transformation – It is a widely used method for transferring major parts of DNA. DNA is inserted in such a way that it causes a fewer number of alterations. But the main disadvantage is that transgene is not added in proper manner which often cause position effects that make the expression of gene absurd. This type of insertion can also introduce certain silencing mechanisms.

Transplastomic technologies – It includes site-specific insertion of transgene via homologous recombination into the chloroplast. This leads to a high copy number of a transgene and higher production of protein. It avoids position effects and silencing mechanisms.

Viral-based vector methods – It express heterologous protein in plants and dependent on structured promoters, UTRs, and DNA/RNA replication mechanisms present in viruses.

Agrobacterium-mediated delivery of viral vectors – It is a high-yielding method for the fast generation of biopharmaceuticals [2].

The various approaches discussed in this review are:

3.2.1 Diagnostic approaches

Immediate and huge requirement for diagnostic kits is caused due to exponential growth of COVID-19. There are majorly two ways to develop the diagnostic kit, (i) to spot the virus itself and (ii) to find the antibodies made by the body in response to viral infection [1].

RNA-based assay – It was developed after GenBank was uploaded with the sequence of SARS-CoV-2. The reverse transcription-polymerase chain reaction (RT-PCR) method is used for the detection of viral RNA. This technique uses primers for initiation and universal positive control for standardization for different diagnostic laboratories. Primers can be synthesized easily, but universal positive control (thermostable, highly reproducible, and scalable) was not available. So, John Innes Centre (JIC), UK produced positive control using virus-like protein (VLPs) acquired from Cowpea mosaic virus (CPMV). VLPs are structurally similar to SARS-CoV-2 but didn't have genome [1]. So, Cowpea plants (Vignaunguiculata) [19] were used by the JIC group to produce and assemble artificial RNA having all of the SARS CoV-2 genome regions within CPMV-derived VLPs [1].

Antibody-based detection– S-protein helps in this type of detection as it provides exposure of the receptor binding domain (RBD) to the immune system of the body. S-protein/RBD is inoculated into mice for the production of hybridoma clones of antibodies. High-affinity antibodies for S-protein are scaled up using plants allowing the availability of antibodies for ELISA for virus identification. Plants allow rapid scaling up of recombinant virus protein and antibodies for high production in the short-term. Also, antibodies made from plants are stable and functional. Tobacco has been used by Diamante Company, Italy for

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producing antigens of SARS-CoV-2 for the spotting of serum antibodies in the ELISA test [1].

3.2.2 Vaccine development

Conventionally, the vaccine is developed by using attenuated strain but in the case of SARS-CoV-2, this technique has certain side effects like the chance of re-developed virulence. So, a vaccine based on protein responsible for antigenicity in SARS-CoV-2 with suitable adjuvants (prime-boost) or VLPs and certain antigens on its surface can be used as a safer and quicker alternative for vaccine development [1]. CureVac has successfully developed RNA-based vaccines [2].Tobacco, potato and turnip plants can be used for vaccine development [20].

Spike protein (Antigen-expression) – All four structural proteins can induce immune responses. But, N protein is not applicable for vaccine development as it has conserved sequences among corona viruses. Moreover, M and E protein trigger feeble immune responses. So, S-protein is used as primary targets for neutralizing antibodies in vaccine production, as it initiates the entry into cells. S2 subunit comprises highly preserved sequences (99%) among corona virus family, but the S1 subunit is 70% similar to other corona virus infecting humans, due to changes present in RBD (required for entry of virus). Vaccine candidate of SARS-CoV-1 target S-protein and control the infection of SARS-CoV-2 inducing processes like nullifying antibody responses, antibody-dependent cell-mediated cytotoxicity, or cross-reactivity for acquiring immunity. Kentucky BioProcessingCompany, USA is producing a COVID-19 vaccine via synthesis of SARS-CoV-2 protein subunits in tobacco plants using agrofilteration with Agrobacterium tumifaciens. S-protein vaccine creates a memory in the host and produces antibodies against the S1 protein sequence as a whole or for smaller RBD within [1].

S1 protein is made up of complex glycans and it is not known that S1 protein production in the plant will be successful for vaccine development for humans. But the old reports of expression of S-protein in transgenic Arabidopsis thaliana from S1 ectodomain of swine Transmissible gastroenteritis corona virus (TGEV) showed that when plant-based recombinant antigen was injected into mice TGEV- specific antibodies were formed. So, this indicates that the production of antigens in the plant can be immunogenic in the host (Capell T et al. 2020).RNA vaccines having new lipid nanoparticle (LNP) consists of mRNA that contains information for full-length and stabilized S protein. Shenzhen Geno-Immune Medical Institute established a vaccine that expresses immune-modulatory genes and viral antigens to trigger T-cells of the immune system. It is dependent on transduction processes to develop artificial antigen-presenting cells and this type of vaccine is called multiepitopic vaccine [2].

Virus-Like Particles (VLPs) are macromolecules that mimic virus structure but are devoid of genome and hence, non-infectious in nature [2]. Using VLPs for vaccine development has several advantages including its huge production by molecular farming in plants. It can act as a vaccine by expressing SARS-CoV-2 antigens on its surface [1]. It also cures certain disadvantages of vaccines with inactivated viruses [21]. Medicago Company developed

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VLP-based vaccines for SARS-CoV-2 with a production capacity of 10 million doses a month. Also, iBio Company of USA is developing VLP-based vaccines using tobacco plants [1,2]. IFN-ɤ and IL-4 production is increased due to the induction of cellular immunity through VLPs. SARS-CoV-2 epitopes can be expressed on VLP acting as a scaffold derived from unrelated viruses e.g. hepatitis B core protein [21].

Passive immunotherapy (Antibody-expression) – Inoculation of recombinant antibodies into the human body provides a primary response at the time of infection and gives the body a good amount of time for the production of its own antibodies against the pathogen. This can also be seen in the case of SARS-CoV-2 infection. Antibodies minimize the reaction of the virus by either counteracting the virus or by hindering cytokine that helps in viral infection. Recombinant antibodies can be produced by plant molecular farming as in the case of antibodies production for diagnosis of the virus [1]. Passive immunotherapy was first planned by Mapp Biopharmaceutical, USA and its partner LeafBio in 2014 for curing Ebola virus. As 2G12, produced in tobacco, was approved for a human clinical trial for treating HIV, a same strategy can be applied for treating SARS-CoV-2 by killing the virus. Rice can produce 2G12 and other two antiviral lectins that can be used as active cause for suppression of virus activity. The two potential antibodies – sarilumab/Kevzara and tocilizumab/Actemra interact with interleukin-6.-receptor (IL-6R) and can be effective for COVID-19 and both are undergoing clinical testing [1]. The protection of mucosal compartments can be achieved by the activation of secretory IgA production that can kill the virus. This process is called mucosal immunization [2].

Oral vaccines – Oral preparations comprising of freeze-dried biomass can be stored as gelatin pills or tablets. It proved to be advantageous over other methods with painful injections and risk of wrong injection passage [21]. Conventional vaccines are complex and costly. It requires higher efforts for purification, its low-temperature storage, and an expert person for administration. Oral vaccines were first permitted in 2006 by the United States Department of Agriculture (USDA). Oral vaccines are recombinant vaccines that allow the production of required antigens in huge amounts against particular pathogens introduced into plants. It is freeze-dried to control antigen dosage in fruits and vegetables. It is capable to secrete mucosal-specific antibodies and serum-specific antibodies (IgG). Sometimes it requires oral priming with adjuvants. In edible vaccines, the antigen is preserved by the cell wall of plant cells and when it enters an animal's body, it is digested by gut microbes for inducing an immune response. Digestive enzymes cannot digest these vaccines due to bioencapsulation of vaccines. Antigen uptake is done in two ways – Gut-associated Lymphoid Tissue (GALT) uptake and dendritic intestinal cells uptake. Generally expression level of antigens is limited leading to less immunogenicity. The expression level of an antigen in edible vaccines can be enhanced by the use of suitable promoters, e.g. CaMV 35S for dicot plants, Ubiquitin promoter for monocot plants, and actin promoter for rice [22].

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Multiepitopic vaccines – It helps in the production of practical vaccine design. The vaccine contains epitopes that help in the activation of immune system. Genetic vulnerability can be seen during the production of multiepitopic vaccines via plant systems. This concluded that SARS-CoV-2 has been progressed in two types – L and S. According to [11], S is less violent than the L type. This type of vaccine is formulated in such a way that it contains conserved epitopes amid different viral variants and has the ability to activate humoral responses. Adjuvants can also be used to enhance antigen complexity to trigger immune system with higher potential [21].

Immune complexes – These are produced in plants and are highly immunogenic agents. It activates humoral and cellular immunity as it contains antigen and antibody complex. For the novel SARS-CoV-2 virus, there are no antibodies available, so it could not be used for this virus. But due to similarities between SARS-CoV-1 and SARS-CoV-2 conserved protein, S-protein antibodies available for SARS-CoV-1 can cross react with SARS-CoV-2. Example includes ICs consisting of monoclonal antibodies and tetanus toxin fragment C was produced in tobacco plants [21].

Elastin-like polypeptide fusions – It helps in the conversion of the difficult and laborious process of extraction of antigen into simpler forms. It works on a unique property named reversible phase transition which enables change in temperature for extraction or precipitation of protein. This process is called the fusion of elastin-like polypeptides (ELP). Antigens are fused with ELP and expressed in transgenic plants, e.g. Mycobacterium tuberculosis antigens Ag 85B and ESAT-6 complexed with ELP is expressed in tobacco plants. These antigens after production were also inoculated in mice which caused activation of humoral responses that were long-lasting. It does not affect the quality of the antigen. ELP technology has the potency to generate antigens for SARS-CoV-2 [21].

Mucosal vaccines – These are majorly intranasal-administered vaccines. It protects the lungs and other mucosal membranes from respiratory diseases caused by pathogen invasion. There are many substitutes for the production of mucosal vaccines like promising antigen delivery, etc. [21].

Priming agents – Prime-boosting via different routes helps in the development of required immune profiles. Antigens generated in plants act as priming agents and are given by the oral route as boosting. Using these, secure and strong immune responses can be generated [21]. For example, Nicotiana benthamiana plants were used to produce cognate soluble HIV-1 subtype C gp140 antigens for immunological assays in rabbit [23].

3.2.3. Antiviral development

Hindrance of viral replication, slackening of infection, and providing ample amount of time to immune system to respond against the virus are the sole roles of antivirals. Carbohydrate-binding proteins (lectins) from plants can destroy the glycan structure of viruses which leads to viral inactivation, thus acting as antivirals. Griffithsia, red algae, generate griffiths in lectin that is less toxic to human cells and inhibit the entry of many viruses including corona viruses like

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SARS-CoV and MERS-CoV. It is not discovered that griffiths in can be effective against SARS-CoV-2, but due to the presence of highly conserved sequences of protruded S-protein in both SARS-CoV and SARS-CoV-2, cross-reaction can occur. Twenty plant lectins showed their effectiveness against SARS-CoV but the higher activity was showed by mannose-binding lectins because the most effective targets are high-mannose glycans. Antiviral like griffiths in and cyanovirin-N can be produced by transgenic rice lines. For antiviral production, plants are suitable for their large-scale production, scaling-up, and rapid access to antiviral proteins [1].

3.2.4. Ethanol for sanitizer production

Major component of sanitizer is alcohol. Due to the COVID-19 pandemic, the availability of sanitizers was limited. So, to cope with the needs of sanitization Madhucalongifolia or Mahua tree flowers are used. These are found in central and north Indian forests. This tree has several uses, e.g. feed, food, wood, alcoholic beverages, medicinal purposes, etc. Flowers have 72.9% sugars, 150mg calcium, 0.5% fat, 4.4% proteins, magnesium, vitamins, and iron. Fermentation is also called bioconversion of glucose/sugars into alcohol. The fermentation process involves four major steps- pretreatment, hydrolysis, fermentation, and product separation or distillation. It uses S. cerevisiae as a fermenter. M. latifolia is more appropriate for usage and produces ethanol in very less expenditure [24].

3.2.5. Inhibitor production

Angiotensin-converting enzyme 2 (ACE2) is a key receptor for SARS-CoV-2 for its entry into the host cells. It is highly expressed on the mucous membrane of the mouth. If ACE2 receptor binding is inhibited then the initial stage of viral infection, which is viral entry, will not occur [25]. According to Avicenna, ACE inhibitory activity is exhibited by various plant types, e.g. Allium sativum, Cinnamomumzeylanicum, Jasminumgrandiflorum, Tribulusterrestris, Vacciniummyrtillus, and Vitisvinifer [26].

3.2.6. Antibacterial and antihelmintic drugs production

Drugs combinations can be successful against the SARS-CoV-2 as indicated by energetics based modeling. Drug combinations adversely affect SARS-CoV-2 spike – ACE2 complex. For example, combination of hydroxychloroquine and azithromycin help in controlling SARS-CoV-2 associated pneumonia. Other antibacterial and anti-helmintic drugs are also used for the treatment of SARS-CoV-2 like Ciprofloxacin and Niclosamide. Different drugs have different mode of mechanism for treatment. The majority of drugs are undergoing clinical trials for their effectiveness on the COVID-19 (Pooladanda V et al. 2020).Plants like lettuce, barley and cucumber can absorb Ciprofloxacin from soil and the later can be isolated as drug [27]. Niclosamide is an aryl β-hydroxy-carbonyl pharmacophore motif which is present as the natural products from plants, fungus and bacteria [28].

3.2.7. Antiviral drug production

Similar processes can be applied for production of antivirals via plant machinery. Different kinds of drugs are used for treating SARS-CoV-2 [9,25]. Quercetin (produced in leafy vegetables, red onions

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Volume: 9, Issue: 1, Year: 2022 **PP: 63.78**
DOI: https://doi.org/10.5281/zenodo.6620675 **Peer Reviewed Journal**

and grains) possess antiviral activity against SARS-CoV and avoid its entry into host. Chloroquine (active component of the bark of Cinchona plant) is also effective. But Oseltamavir's effectiveness needed to be examined [25]. Also, a combination of remdesivir with Chloroquine can be effective against SARS-CoV-2 [9].

3.2.8. Interferon production

Recombinant DNA technology is used for the formation of interferon alfacon-1 (166 amino acid long sequence). Interferon alfacon-1 is successful against SARS-CoV-1. When it is combined with corticosteroids, it shows its effectiveness against SARS-CoV-2. When it binds to interferon receptor type 1 (IFNAR1 and IFNAR2c), it represents its antiviral functioning [25].Interferon alfacon-1 was produced by recombinant DNA technology in Nicotianabenthamiana plant [29].

3.2.9. Plant-derived chemicals in treatment

Essential oils possess antiviral activities. This is due to its introduction into the lipid bilayer of the envelope. This leads to changes in membrane fluidity. Essential oils can also breakdown phospholipid bilayer due to their lipophilicity. Hydroxyl groups of plant secondary metabolites form an association with an amino group of protein leading to inactivation of viral protein. Viral nucleocapsid and RNA expression system can be suppressed by terpenoids. This can lead to inhibition in viral entry. Viral entry can also be prevented by polyphenols that finely link to lipoproteins of viral envelopes and prevent the fusion of viruses into the host cell. Phenolic compounds from roots of Isatisindigotica have antiviral activities. Flavonoids inhibit SARS 3CLpro enzyme [7]. The summary of different approaches is given in Table – III.

Table – III: Plant system for combating SARS-CoV-2 and related antigens.

Plant Technologies	Target Product(s)	Plant(s) Used	Reference(s)
Diagnostic approaches	Universal positive control	Vignaunguiculata	[1,19]
	SARS-CoV-2 antigen	Tobacco plants	[1]
Vaccine Development	SARS-CoV-2 protein subunits	Tobacco plants	[1]
	VLP-based vaccines	Tobacco plants	
	2G12 and two antiviral lectins	Rice plants	
	Monoclonal antibodies	Tobacco plants	[2]
	Immune complexes	Tobacco plants	
	Antigens	Tobacco plants	[2,23]

Research & Reviews in Biotechnology & Biosciences **ISSN No: 2321-8681**
Website: www.biotechjournal.in **Mini-Review**
Volume: 9, Issue: 1, Year: 2022 **PP: 63.78**
DOI: https://doi.org/10.5281/zenodo.6620675 **Peer Reviewed Journal**

Antiviral development	Antiviral like griffiths in and cyanovirin-N	Rice lines	[2]
Ethanol for sanitizer production	Alcohol for sanitizer	Madhucalongifolia	[24]
Inhibitor production	ACE inhibitor	Allium sativum, Cinnamomumzeylanicum, Jasminumgrandiflorum, Tribulusterrestris, Vacciniummyrtillus,andVitisvinifer	[26]
Antibacterial and antihelmintic drugs production	Ciprofloxacin	Lettuce, barley and cucumber	[27]
Antiviral drug production	Chloroquine	Cinchona plants	[25]
	Quercetin	Leafy vegetables, red onions and grains	
Interferon production	Interferon alfacon-1	Nicotiana benthamiana	[29]
Plant-derived chemicals in treatment	Phenolic compounds	Isatisindigotica	[7]

4. CONCLUSION

Plants are being used to treat various infections for many centuries. Plants have different properties that are beneficial for human lives in various ways. These properties may be medicinal, strength, or edibility. But in today's time, plants have importance in various biological and biotechnological fields including treatment of SARS-CoV-2 and related strains, due to many other properties. These properties include transient expression system, molecular farming, easy creation of cell lines, etc. Transient expression system of plants is helpful for diagnosis of SARS-CoV-2. Molecular farming of SARS-CoV-2 antigens can act as a vaccine. Moreover, the expression of antigen in edible plants can act as an oral vaccine by directly consuming it. Plants naturally produce certain compounds, inhibitors, and chemicals that may inhibit the entry of the virus into the body. Many more potentials of plant-based compounds are into consideration. A vast range of clinical studies with strict protocols is required to estimate the accurate potential of antiviral phytochemicals against COVID-19 to ensure the fulfillment of internationally acceptable standards.

5. FUTURE PERSPECTIVES

In silico investigations and molecular docking of several plants have identified their potential to be used as medications for COVID-19 in the future. This analysis requires further experimentations for their approval [30,31]. 230 Indian medicinal

ISSN No: 2321-8681
Mini-Review
PP: 63.78
Peer Reviewed Journal

plants have inhibition properties against COVID-19 protease. Docking of compounds from Nigellasativa, e.g. Nigellicine, Thymol, etc, showed their possible inhibition action on main protease (M^{pro}) of COVID-19 [30]. Also, nine phytochemicals including Tenufolin (TEN) and Pavetannin C1 (PAV) are very effective against main protease enzyme of SARS-CoV-2 [6]. Stilbene-based natural compounds like Piceatannol and resveratrol also have the potential to combat COVID-19. Resveratrol hinder the entry of viruses into the cell by acting as an inhibitor for S-protein-ACE2 complex formation [32]. Plant lectins have the potential to adhere with envelope glycoprotein that avoids the entry of viral genome into the host. Mannose-binding lectins can be effective for treating corona virus. These lectins inhibit the early phase of viral replication by hindering viral attachment on the host. This leads to quelling of viral development [33]. Acaciades as a phytomedicine may be helpful in the development of the COVID-19 vaccine as it improves immune function, enhance the formation of T-cells, produces superoxide anions, aids lipid peroxidation, and break-down fatty coats of corona virus [34].

6. ACKNOWLEDGEMENT

We are thankful to the authors whose work has been cited in this review.

7. CONFLICTS OF INTEREST

There are no conflicts of interest.

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