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Review Article

Plant Biotechnology; an Important Avenue for Medicine during Pandemics

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Abstract: The coronavirus pandemic that is still ongoing has let the world learn many lessons. One of the lessons is to search for a viable source of medicine against such viruses. Vaccines for those ailing with symptoms of such viruses are most importantly needed from a viable source. Plant biotechnology offers a platform called Biopharming, wherein vaccines are produced in a safe, biocompatible manner. These vaccines also promise to have the advantage of being produced cost-effectively once the complete map of the process is laid down and is upscaled. Vaccines are undoubtedly developed at an unprecedented rate and are the most effective strategy to fight such pandemics. However, as understood from a fundamental standpoint, the vaccine is a part of a preemptive strategy. Along with synthetic chemical compounds, phytochemicals cannot be overlooked as candidates for drugs against Severe respiratory coronavirus 2 (SARS-CoV-2). Compounds, for instance, *Glycyrrhizin* from the roots of *Glycyrrhiza glabra* have been shown as a very promising phytochemical against the SARS-CoV, which caused an outbreak in 2002-2003. Other chemical compounds, reserpine, emodin, betulonic acid, and apigenin isolated from different plants, were also effective against SARS-CoV. The production of these and many other compounds through plant biotechnology techniques such as transgenesis and gene editing followed by in vitro cultures is a vital avenue to be considered. Transgenesis offers the advantage of boosted production of existing phytochemicals or triggered production of novel compounds through in vitro cultures which serve as a reactor for the development of important phytomedicine. This article emphasizes the role of Biopharming in the production of vaccines against SARS-CoV-2 and any such future outbreak causing Virus or bacteria. The report also highlights the role of transgenesis and gene editing to produce medicine against the Virus.

Keywords: SARS-CoV-2, Pharming; plant biotechnology, in vitro cultures, transgenesis, Secondary metabolites

1. INTRODUCTION

The ongoing pandemic caused by the severe acute respiratory syndrome virus (SARS-CoV-2) has given a wake-up call to the world. Killing 2.6 million people and infecting 117 million across the globe so far, coronavirus disease (COVID-19) has become the deadliest disease in the past century [1]. When the SARS-CoV-2 was first identified in late 2019, scientists immediately geared up to start characterizing the Virus and looking for possible remedies against it. Owing to past scientific information and current robust scientific methods, the world was presented with five different vaccines against the coronavirus [2]. However, even though many countries are vaccinating their population against the Virus, the world is still lagging in tackling the pandemic [3].

Similarly, most currently approved vaccines are based on messenger RNA or nucleic acid isolated from the Virus [4]. These vaccines require a continued supply of many costly chemicals and reagents, along with the need for high maintenance requirements [5]. This calls for viable alternatives to vaccines. Plants offer one such platform for the production of medicine against the current and any potential pandemic virus [6]. Plants could produce secondary metabolites and other biomolecules with potent activity against viruses

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and bacteria [7]. However, plants too are subject to multiple constraints such as seasonal and geographical variations, non-uniform production of phytochemicals, and regulatory concerns.

Similarly, plants produce phytomedicine that is supposed to be used against symptoms caused by the Virus. While Vaccines, a preemptive strategy, are also needed in the fight against viruses. Plants offer pharming of vaccines as a strategy for producing safer vaccines [8]. Therefore, plant biotechnology is a very promising avenue to cope with the current and any future outbreaks. Tools of plant biotechnology that include transgenesis, gene editing, and pharming are the emphasis of this article which discusses their potential in detail. We aim to highlight the role of transgenesis and gene editing to produce medicine against the Virus.

2. PHARMING; AN AVENUE FOR VACCINES AGAINST COVID-19 AND OTHER PANDEMICS

Pharming or Biopharming entails the phenomenon of utilizing a living system to manufacture biological materials or drugs. Pharming employs living systems as bio-factories for the rapid and economically viable production of specific complex biomaterials on a high scale which in other cases may not be easily synthesized with already available manufacturing technologies. The first application of this approach was insulin production in 1978 by Genentech using a bacterial host Escherichia coli, which was subsequently commercialized in 1982 [8]. A technological leap was observed in Biopharming with the introduction of eukaryotic cells as production hosts for complex molecules, particularly those eukaryotic cells that have mammalian type post-translational modifications. Genentech, in 1987 commercialized the production of anticoagulant activase enzymes by remodeling *E.coli* fermenters for the production of Chinese Hamster Ovary (CHO) cells [8]. Following this development in technology, CHO cells were soon preferably utilized as hosts for extensive production of other complex biologics. In 2017, The estimated market worth of monoclonal antibodies was 123 billion USD, of which 87% mAb products were produced in CHO cells [9]. Biological materials production is currently dominated by fermentation-dependent technologies that generally take approximately 12 months to select clones, validating the required conditions and reaching the production capacity [10]. Non-Fermentation-based production of biological materials on an industrial scale is attributed to Transgenic animals, embryonated hen's eggs (EHE), and whole plants. Among these, whole plants need little input cost for biomass production and show the highest production capacity when a transient expression system is used [8]. Some of the strategies that could be used to employ pharming for the production of medicine against SARS-CoV-2 and any future outbreaks are shown in figure 1.

3. WHY PLANT BIOPHARMING FOR BIOLOGICS PRODUCTION?

Plant molecular pharming is a relatively novel idea for the production of proteins in plants. Molecular farming is a sub-discipline of plant biotechnology that uses certain plants as hosts to produce vital recombinant proteins, including vaccines, enzymes, and hormones [11]. Using plants for the production of diagnostic reagents and pharmaceutical proteins has been taking place for more than 30 years [12]. Molecular farming aims at the recovery and use of a particular recombinant protein rather than the plant itself. After playing its role as a host the plant is disposed of or used separately as a side stream, whereas the target protein is extracted, purified, and used for the purpose it was produced. Molecular pharming, in its inception, promised three advantages compared to other biologics, Low input cost required for growing plants, scalable production capacity, and insurance of product safety. This aroused the interest of researchers in the field and consequently resulted in an abundance of research publications for the expression of various proteins in plant-based expression systems. This development led to the establishment of several companies intending to commercialize this novel plant-based technology [13]. Plant molecular farming mainly encompasses using a whole plant such as cereals and tobacco, but the technology also invariably harness plant cells and tissue culture, aquatic plants, algae, moss, and performing in vitro plant-derived transcription and translation systems [8].

Low input cost and lesser biomass production requirements for plants compared to fermentation-

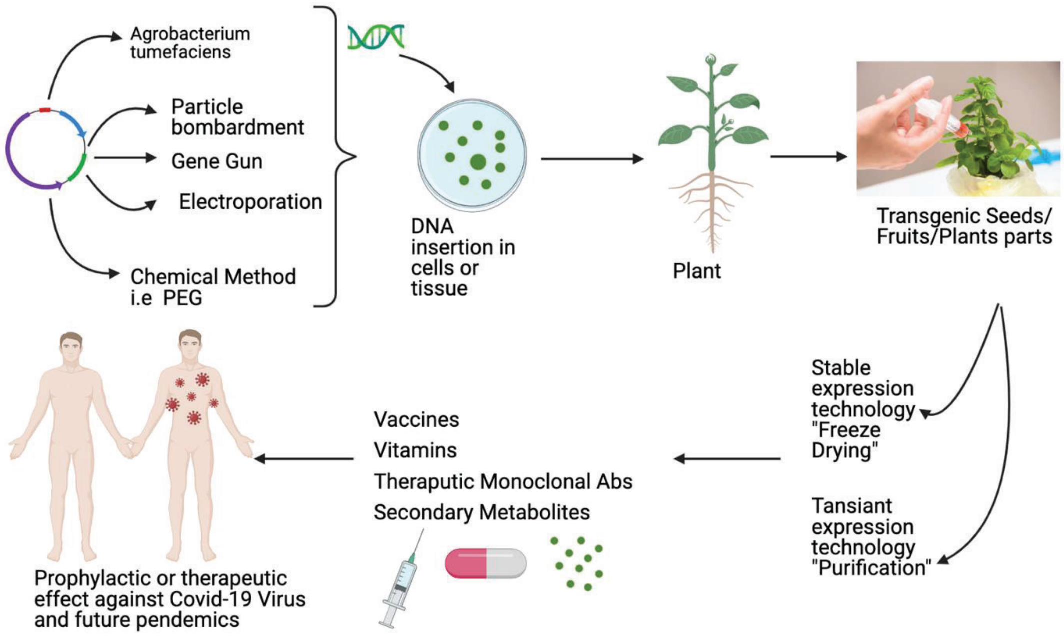

Fig. 1. Intervention points where transgenesis in plants will be of help to fight pandemics

based systems attracted the massive interest of researchers towards the field. Hence attempts were made to explore new prospects of using plants as low-price biofactories and using a carefully selected crop species will result in the production of edible vaccines. Efforts were made to scale plant-based biologics in crop plants such as rice, barley, maize, and safflower as production hosts [8]. Further investigations demonstrated production in plants based production systems in various other plants such as Sundews, moss, pitcher plants [14], corn, rice, barley, wheat, sunflower, tomato, soybean, carrot, lettuce, tobacco, tomato, Nicotiana benthamiana, and melon [15].

Molecular farming circumscribes several expression technologies that range from whole transgenic plant or more often a transient expression without the need for transgene integration [13]. In transient expression, an adult wild-type plant mostly tobacco—*Nicotiana benthamiana* or its relative *Nicotiana tabacum* is infiltrated with certain *Agrobacterium tumefaciens* strain or other viral vectors of the plant carrying that particular transgene [16]. The first generation of commercial biological products in the plant was centered on whole transgenic plants [17]. Currently, the arena of plant-molecular farming utilizes both transgenesis and transient expression strategies for entire plant production systems. Moreover, Systems based on cell cultures and plant-based systems are also in use [18]. The first genetically engineered plant-derived therapeutic approved by the FDA was manufactured by Protalix in 2012. Protalix biotherapeutics in Israel employed a transgenic carrot cell suspension system for the production of taliglucerase alfa, a drug for the treatment of an inherited metabolic disorder, Gaucher disease [19]. Although a broad range of plant-based systems has been tested under experiments, among them Nicotiana benthamiana is currently the host of choice for the production of biologics. The plant is a crucial production host of many Plant-based production companies such as PlantForm, Icon Genetics, Medicago, Capebio, iBio, Bioapp, and Leaf expression systems[20].

3.1 Plant Molecular Pharming during Times of Pandemics

In the past two decades, Viral outbreaks have strengthened the viewpoint that restraining an outbreak is best achieved with a faster detection system and spreading awareness about non-pharmaceutical interventions followed by immunization [21]. However, apart from the issues observed during the 2009 Pandemic of influenza A(H1N1), one of the main shortcomings observed was a global inadequacy to produce vaccines and inability to alleviate the spread due to the slow speed of production during the first wave. This was the result of dependence on an egg-based slow-yield vaccine manufacturing system [22].

Producing biological products in plant-based systems may serve as a practical arena for large-scale production within a couple of weeks, in contrast to longer periods required for production using cell-culture-based strategies [23]. A variety of plant species have been harnessed for antibodies, drugs, immunomodulatory proteins, vaccines, and biopharmaceuticals production, and they are regarded as living factories or bioreactors that inherently can produce biologics in a relatively short interval of time [24]. Vaccine against Newcastle disease virus (NDV) was first among plant-based vaccines to be approved for poultry by the United States Department of Agriculture (USDA), which demonstrated above 90% protection in poultry [25]. The only other product manufactured via a plant-based production approach to get licensed is the monoclonal antibody (scFv mAb). The antibody was harnessed to produce a recombinant Hepatitis B virus (HBV) vaccine in Cuba [26]. The fast tempo to respond to any viral outbreak was demonstrated by Mapp biopharmaceutical in the 2014 Ebola outbreak. The company established a quick production of an antibody cocktail against Ebola called ZMapp, which authorized an emergency approval for human use [27].

Molecular farming has resulted in several therapeutics and vaccines, to name a few vaccines production against the perilous Hepatitis B virus, cholera, and Dengue fever virus. The production of neutralizing monoclonal antibodies against HIV, Ebola virus, and therapeutic agent to provide treatment for those infected with Gaucher Disease [28].

VLP-based vaccines that have got approval are immunization against Norwalk virus, Bluetongue virus, Hepatitis B virus, and Papillomaviruses and up to 100 VLP-based vaccines are in the pipeline of clinical trials [29]. Lately, Medicago Inc has undergone phase III clinical trials of a quadrivalent nature plant-made-VLP vaccine. This development is considered a significant landmark in the production of plant-made biologics, and if it is approved for human use could promise explicit protection and a vast range of production options [30].

3.2 Services of Plant Molecular Pharming amid COVID-19 Pandemic

The outbreak of SARS-CoV-2 in December 2019 and its spread worldwide have created a myriad of challenges across the globe. These resultant challenges require efficient solutions in terms of public health and biomedical research [31]. This pandemic called for attention to specifically prepare and invest in those platforms that are more appropriate for flexible, quick, and environment-friendly production of medical remedies in terms of Diagnostics, Therapeutics, and vaccines against the emergence, re-emergence, and biological terrorism-related lethal diseases [29]. Plants have been consistently used for recombinant vaccine production for more than three decades and this phenomenon is termed “Molecular farming.” Therapeutics against COVID-19 in plants can be manufactured either by antibody expression against the Virus as passive protection or by the expression of SARS-Cov-2 antigenic components in plant-based expression systems [32, 33]. Vaccines produced in plant-based systems are generally termed third-generation vaccines. The procedure to manufacture a plant-made vaccine entails incorporating the candidate vaccine into a plant-based expression system. That plant expression promotes the candidate gene expression inside the plant machinery, which resultantly produces antigenic or protective protein.

The plant-based expression system serves as a bioreactor, producing the protein for many generations, thus ensuring an ongoing production and availability [23]. In addition, the SARS-CoV-2 Structural proteins, namely [Envelope (E), membrane (M), Nucleocapsid (N), and spike (S)] proteins elicit neutralizing antibodies(Nab) and activate cell-mediated immune responses [34].

Among structural proteins, the Virus uses the S protein to enter inside the cell via angiotensin-converting enzyme 2(ACE2) receptor binding. It thus makes S protein a tempting target to develop a vaccine against the Virus. The region of the S protein that interacts with the ACE2 receptor is RBD [23]. Bioinformatics-based epitope prediction through antigenic mapping of S protein has recognized essential immunogenic proteins that after expression in plants produces a vaccine against

SARS-Cov-2 [35, 36]. Two companies so far have proclaimed the development of antibodies and plant-based vaccines against COVID-19 causing viruses. A Canada-based company Medicago inc. had announced the production of Virus-like particles (VLPs) via a transient expression system soon after reaching out to the SARS-Cov-2 Spike (S) protein sequence [11]. The instant, continuous requirement for biologics during the COVID-19 and an observed inability of the current infrastructure to meet the demand has given rise to the perception of how a plant-based production system can help meet the immediate need for biologics [8]. Several plant-based biological drug manufacturing companies have started producing products related to SARS-Cov-2, using transient expression systems of N. benthamiana. Vaccines manufactured by Kentucky Bioprocessing and Medicago are in clinical trial stages, while two vaccines and a therapeutic product are yet in their developmental pre-clinical stages [8]. iBio is developing a VLP-Based COVID-19 in the tobacco plant. Likewise, a group of scientists at the Queensland University of technology has thoroughly assessed the genome sequence of N. benthamiana. They are putting their efforts to utilize the plant's genome for the production of vaccines against COVID-19 [23]. Also, researchers at The University of California San Diego are combining advanced manufacturing strategies with molecular plant farming in which the Virus can explicitly infect legumes but not humans and is engineered in such a way that mimics SARS-CoV-2 to provoke the immune system [23].

Plant-based vaccines may also face challenges in their developments the same way as other vaccines do. To fulfill the requirements of regulatory agencies for approval, this technology needs to be validated based on their safety by employing large-scale clinical trials. Certain plant-made biopharmaceuticals have got approval to be used in humans and the conduction of clinical trials for plant-made influenza vaccines is encouraging. Owing to its low cost, rapid availability, and safe nature, a plant-made vaccine may revolutionize the field of vaccinology in the years to come.

4. TRANSGENESIS

It is the process by which Transgenic plants express foreign genes with industrial or pharmaceutical value [37]. Scientists and physicians are working to understand the new viruses and pathophysiology of the diseases to discover potential treatment regimes, effective therapeutic agents, and vaccines [38]. One effective method is to insert valuable genes from an entirely different species into a target plant, yielding a transgenic plant that acts as a factory for therapeutic products and emergency manufacturing of antiviral drugs, vaccines, and diagnostic reagents

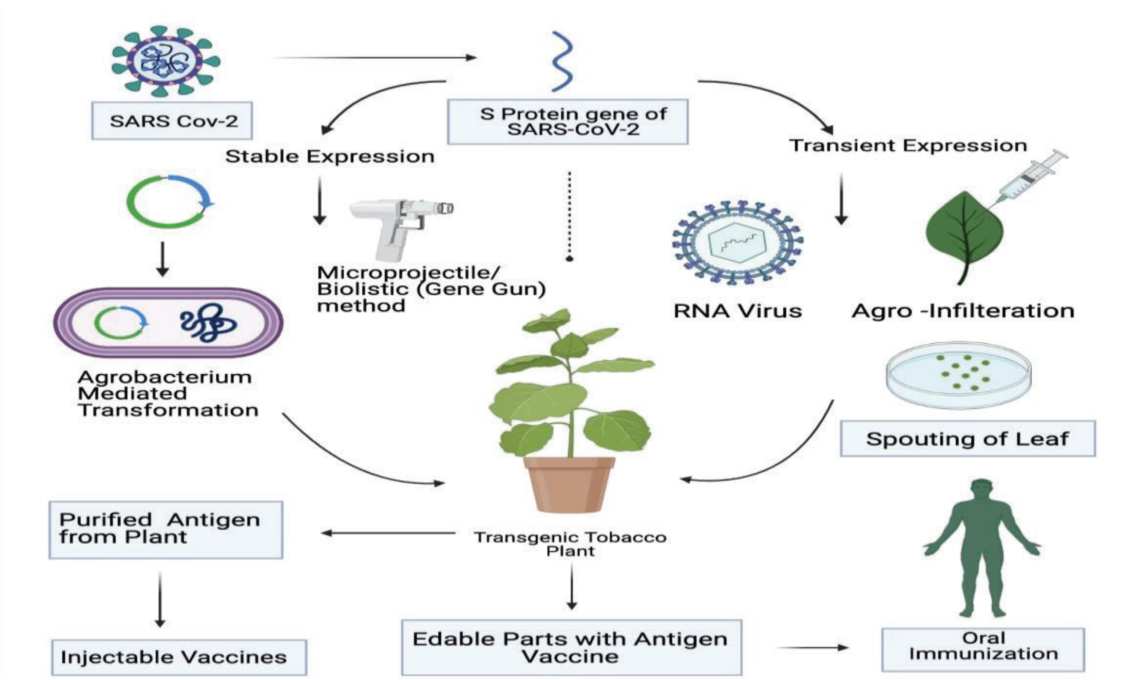

Fig. 2. Illustration of different strategies employed during pharming in plants potentially used to produce medicine against SARS-CoV-2 and other viruses

to reduce the spread of diseases and to save lives [12]. Different avenues where transgenesis in plants could help fight pandemics are given below (Figure 2).

4.1 Transgenic Secondary Metabolites against SARS-CoV-2

Plants are an essential source for numerous innovative bio-active compounds. A variety of different plant secondary metabolites (SM) are serving as vital drugs that exhibit extensive pharmaceutical and therapeutical properties with more minor side effects. To enhance the productivity and accumulation of target compounds transgenic plant cells can be manipulated in vitro [39]. To tackle future pandemics and COVID-19 caused by viruses, plant's secondary metabolites (PSMs) act as effective anti- SARS-CoV-2 molecules for further drug developmental processes and optimization [40].

Flavonols, a class of Flavonoids, can inhibit crucial proteins involved in an infective cycle of coronavirus. Flavonoids can inhibit SARS-CoV proteases, 3CLpro, PLpro, NTPase/helicase, and N protein of SARS-CoV. In fruits, a 72-fold increase in flavonol production was observed by chalcone isomerase gene overexpression from petunia in tomatoes. [41]. Glycyrrhizin obtained as an extract from licorice root is comprised of Glycyrrhetinic acid, Flavonoids, hydroxyl coumarins, and β-sitosterol that is spotted to have an essential anti-SARS-CoV activity [42].

Isoquinoline alkaloids like cepharanthine, tetrandrine, and fangchinoline can inhibit the expression of nucleocapsid and spike protein in SARS-CoV- OC43 in human lung cells. Such alkaloids can also intercalate DNA [43]. In berberine (isoquinoline alkaloid) biosynthesis, an enzyme (S)-scoreline 9-O-methyltransferase (SMT) is involved that controls the proportion of coptisine alkaloid: berberine and columbamine in cells of Coptis japonica. A 20% increase in enzyme activity was observed by this gene overexpression, which results in an increased level of columbamine and berberine from 79% in wild-type cells to 91% in transgenic cells. This study shows that an enzyme's overexpression in a pathway leads to increased flux of alkaloids which can be effective against COVID-19 and future pandemics [44].

4.2 Anti- SARS-CoV-2 MAbs Production in Plants through Transgenesis

Plant biotechnology gives a potential solution to the pandemic through the synthesis of affordable plant-made antibodies. In the case of passive immunization (antibody-mediated therapy), a great point is the cross-reactivity of the anti-SARS-CoV-1 Virus with SARS-CoV-2 Virus, proposing that the already produced biopharmaceuticals may well combat COVID-19. Hence the monoclonal antibodies (MAbs) are an effective therapeutic agent due to their potential for the COVID-19 treatment [11]. Researchers have discovered the capability of a plant expression system for manufacturing therapeutically appropriate human anti-SARS-CoV-2 MAbs like B38 and H4 that can be used as a diagnostic or therapeutic reagent. The MAbs can be expressed and assembled in Nicotiana benthamiana leaves using a geminiviral vector [45]. The B38 and H4 antibodies block the binding between the cellular receptor angiotensin-converting enzyme 2 (ACE2) and spike glycoprotein receptor-binding domain (RBD) of the Virus [46]. Researchers have further discovered the possibility of anti-SARS-CoV monoclonal antibody (MAbs) CR3022 and receptor-binding domain (RBD) of SARS-CoV-2 in N. benthamiana. The plant produced RBD showed specific binding to the SARS-CoV-2 receptor, angiotensin-converting enzyme 2 (ACE2) [47].

4.3 Vaccines Production through Transgenesis

Transient or stable expression of foreign genes results in the production of specific vaccines in plants. It has revealed that genes that encode antigens of viral and bacterial microbes can be expressed so that they hold their immunogenic properties [37]. The potential of plant expression systems for making vaccines against SARS-CoV-2 by expressing recombinant chimeric proteins, virus-like particles, sub-unit proteins, and other biologics are under study [48]. The expression of N protein in tobacco revealed that the injection and oral delivery of the N protein to the BALB-C mice increases the amount of IgG and IgA respectively in the experimental mice sera, representing the Anti-SARS-CoV activity of the vaccines [49].

By using transient expression systems, injectable vaccines can be produced that offer maximum protein yields and are now acquired at the industrial level to manufacture VLPs-vaccines and other biopharmaceuticals that help to provoke immunity against different antigens [11].

4.4 Production of Vitamins in Plants through Transgenesis against SARS-CoV-2

Several meta-analyses results have shown the significant benefits of a high dose of vitamin C injected intravenously (IV). Lactate secretion caused by the triggered immune cells can be inhibited by vitamin C treatment that possibly protects the innate immunity. This effect may help COVID-19 patients, as the SARS-CoV-2 usually affects the lower respiratory tract [50]. The vitamin C content can be enhanced two- to three folds in Arabidopsis thaliana by the overexpression of GalUR genes, this indicating the viability of engineering enhanced vitamin C levels in plants using this gene [51]. The level of vitamin E can also be increased by up to 4 fold in a transgenic Tobacco and A. Thaliana leaves by the plastidic expression

Table 1. Studies on the antiviral efficacy of several medicinal herbs against various coronavirus strains.

Coronavirus Strains	Plant species	References
SARS-CoV	*Lycoris radiate*	[75]
	Lindera aggregata	[76]
	Artemisia annua	
	Isatis indigotica	
	Pyrrosia lingua	
	Boenninghausenia sessilicarpa	[77]
	Lonicera japonica	[78]
	Eucalyptus spp.	
	Panax ginseng	
Bovine coronavirus (BCV)	*Amelanchier alnifolia*	[73]
	Cardamine angulata	
	Rosa nutkana	
	Verbascum Thapsus	
SARS-CoV (Hong Kong strain)	*Dioscorea batatas*	[79]
	Cassia tora	
	Taxillus Chinensis	
Ten different strains of SARS-CoV in fRhK4 cell line	Baicalin (*Scutellaria baicalensis*)	[80]
	Glycyrrhizin (*Glycyrrhiza uralensis*)	
HCoV-229E	Mulberry (*alba, Morus alba* var. *rosa*, *Morus alba* var. and *Morus rubra)*	[81]
	Calophyllum blancoi	[82]
	Pelargonium sidoides	[83]
SARS-CoV PLpro	*Psoralea corylifolia*	[84]
	Sambucus formosana	[85]
HCoV-NL63		
HCoV-OC43	*Stephania etrandra*	[86]
MERS-CoV EMC/2012	*Aglaia sp*	[87]

of the rat TATase gene [52].

5. GENE EDITING/GENOME EDITING IN PLANTS AGAINST PATHOGENS AND VIRUSES

Plants and pulses are the best sources of secondary metabolites, and they are known for the ability they show regarding human health [53]. Let's come to phytochemicals; those molecules which are synthesized in large amount by plants are known as phytochemicals [54]. Phytochemicals are non-nutritive substances present in massive amounts in plants [55]. Diet including fruits and vegetables can overcome the risk of various chronic diseases [56]. Also, phytochemicals can be used as a treatment for infections caused by bacteria and fungi [57]. It has been reported that there are multiple ways through which phytochemicals play a significant role in health; they can work as substrate, cofactors for an enzyme, and inhibitors. They can also work as a trap for toxic substances or fermentation substrate to multiple Bacteria and much more [58].

The success in the production of secondary metabolites by modifying or inserting new genes into in vitro culture has widened the chances of increasing or improving the production of phytochemicals [59]. This whole process took over three decades to demonstrate the feasibility of transformation in plants [60], which was the start of the genetic engineering era, and it compelled many countries for transgenic crops [59]. The availability of enough information regarding metabolic pathways made it easier for scientists to improve the quality and quantity of phytochemicals, leading to several successful experiments [61-64]. These plants were then used against many diseases, as scientists observed that various chronic diseases are inversely at risk to the food which contains antioxidant phytochemicals [65]. The success in improving and producing phytochemicals can be done by either qualitative or quantitative engineering approaches such as the engineering of β-carotene in rice grains [66, 67]. In rice, the elevation of iron content by two-fold increases [68] and enhances the ascorbic acid by seven-fold [69]. Moreover, the scientists also checked the activity of several plants against many pathogens. For example, mangrove plants were found very effective against many bacteria like Klebsiella pneumonia, Streptococcus pneumonia, Escherichia coli, and Enterococcus faecium [70].

Due to the unavailability of the vaccine and the treatment, Covid-19 caused high motility and morbidity. Scientists around the world tried different methods and techniques to overcome the pandemic. Such as Hyperbaric oxygen therapy (HBOT) [71], Packed red blood cell transfusions [72], Chloroquine/hydroxychloroquine treatments [73], and secondary metabolite; phytochemicals have a significant role in human health, a number of scientists were using different plants against the Virus. Such as the Indian Ayurvedic herb, Asparagus racemosus (Wiled). Through docking analysis, they used Asparoside-C, Asparoside-D, and Asparoside –F against SARS-CoV-2, and by their docking score and affinity, they confirmed that there is a receptor-binding domain on two proteins of SARS-CoV-2 viz. NSP15 Endoribonuclease and spike and were found that they are both effective against these proteins. [74].

Different experiments observed the antiviral potential of multiple medicinal compounds or phytochemicals against various strains of coronavirus (CoV), which are described in Table 1. Different Phytochemicals have different mechanisms against corona virus-like, Rosa nutkana, and Amelanchier alnifoliain habit or deduct the activity of enteric coronavirus [73], Torreya nucifera (Amentoflavone) inhibit the nsP13 helicase and 3CL protease, other plants like Black tea, also known as TheaFlavin is effective against SARS-CoV and inhibit its 3C-like protease [88].

6. CONCLUSION AND FUTURE PROSPECTS

Although treatment and vaccines for COVID-19 are available, we must continue the improvement of the treatment for a better antiviral effect. We should be cost-effective having more minor side effects while making an antiviral drug against particular viral proteins or gene the main problem we face is that while replicating the Virus continuously mutate itself, as studied in HIV and HSV and Hepatitis-B Virus. However, natural resources play an essential role in the development of new antivirals.

7. ACKNOWLEDGMENTS

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8. DECLARATION

We confirm that the manuscript contains original secondary data and is not published nor under consideration elsewhere. Moreover, the consent of all authors has been obtained.

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