Received: 24 January 2022 | Revised: 18 March 2022 | Accepted: 6 April 2022

DOI: 10.1002/ptr.7475

REVIEW

WILEY

Emerging trends of edible vaccine therapy for combating human diseases especially COVID-19: Pros, cons, and future challenges

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Abstract

The researchers are still doing efforts to develop an effective, reliable, and easily accessible vaccine candidate to protect against COVID-19. As of the August 2020, nearly 30 conventional vaccines have been emerged in clinical trials, and more than 200 vaccines are in various development stages. Nowadays, plants are also considered as a potential source for the production of monoclonal antibodies, vaccines, drugs, immunomodulatory proteins, as well as used as bioreactors or factories for their bulk production. The scientific evidences enlighten that plants are the rich source of oral vaccines, which can be given either by eating the edible parts of plants and/or by oral administration of highly refined proteins. The use of plant-based edible vaccines is an emerging trend as it possesses minimum or no side effects compared with synthetic vaccines. This review article gives insights into different types of vaccines, the use of edible vaccines, advantages of edible vaccines over conventional vaccines, and mechanism of action of edible vaccines. This review article also focuses on the applications of edible vaccines in wide-range of human diseases especially against COVID-19 with emphasis on future perspectives of the use of edible vaccines.

KEYWORDS
conventional vaccine, edible vaccine, plant-extracts, SARS-CoV-2, transgenic plant

1 | INTRODUCTION

Vaccination was first introduced in nearly 200 years ago by Edward Jenner (1796) for smallpox disease (Concha et al., 2017). Vaccination causes the body to mount an adaptive immune response to the antigenic material that has been delivered to the body. It prepares the body to fight against new infections in contrary to the classical ways, in which treatment is usually done after the onset of a disease (Gunasekaran & Gothandam, 2020). Various diseases such as typhoid fever, cholera, poliomyelitis, and tuberculosis have been controlled all around the world by mass vaccination (Saxena & Rawat, 2014). The traditional childhood vaccines are administered against six diseases, which include diphtheria, tetanus, whooping cough, measles, polio, and tuberculosis (TB). In addition, now vaccination against hepatitis B, pneumococcal illness, rubella, and rotavirus are routinely being administered all over the world.

From 1996 to 2000, about 1.7 to 44.2 million hectares of land used for growing transgenic crops and the future of edible vaccines is revealed by this massive increase. The number of countries farming them increased from 6 to 13, indicating that transgenic crops are gaining widespread approval in both developed and developing countries (Jan et al., 2016). The majority of the edible vaccines were against viruses and bacteria that cause a deadly infection in humans, animals, as well as in poultry. So far, no edible vaccine has been

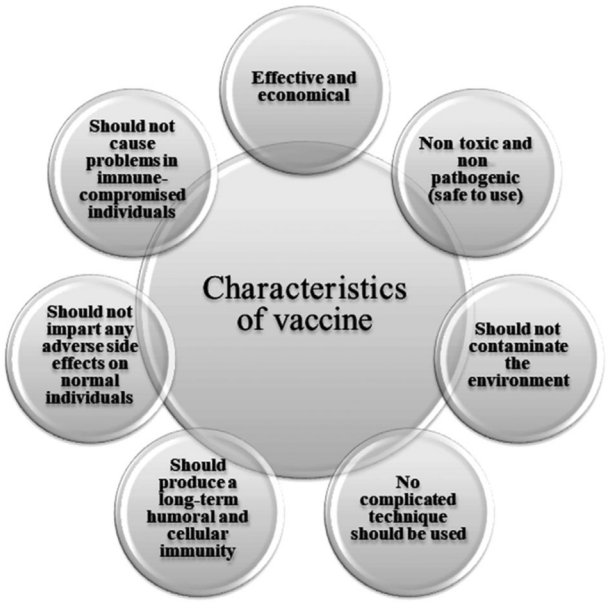


FIGURE 1 Feature characteristics of reliable vaccines effective for multiple diseases

approved by the United States Food and Drug Administration, since such vaccines are regarded as genetically engineered crops (Lal, Ramachandran, Goyal, & Sharma, 2007). In the light of this spectacular research, this review focuses on the uses, challenges, and promises of the edible vaccines.

Despite the children's global vaccination program against six life-threatening diseases, almost 20% of the children remain unvaccinated, particularly in the remote areas of the world. Nonvaccination results in the death of approximately 2 million individuals every year. This is due to the difficulties in the production, distribution, and delivery of vaccine. We need to deal with this problem of the unvaccinated population to prevent the spread of diseases and epidemics (Jan et al., 2016). According to the literature (Ramsay et al., 1999), 100 % vaccination is required. Because of the unimmunized population of remote areas, the infection could spread in immunized "safe" areas, having a lower rate of herd immunity (Haq, Mason, Clements, & Arntzen, 1995).

The key issues to be resolved are the limitations on vaccine manufacturing, availability, and distribution. There are no vaccines available for certain infectious diseases. Vaccination by DNA, for example, is exceedingly expensive and unreliable. Vaccines can be used as an alternative, but the process is costly, and some people may not want to use it. Immune reactions that are not ideal arise (Jan et al., 2016). Aside from being a costly methodology, the storage and transportation of vaccines is another issue. Many of them require the use of a refrigerator; as a result, they are considered to be eliminated. There is a hunt for universally acceptable, storable solutions to these difficulties. Their distribution systems, in particular, are simple to administer and dependable in emerging nations (Khan et al., 2019). To combat this, we need an alternative and easy method of vaccine delivery, which is more immunogenic than the previously used methods of vaccine production. On contrary, the edible vaccine provides a reliable alternative.

In this review, we discussed about the different aspects of vaccine and edible vaccine including characteristics and types of vaccines, limitation of conventional vaccine, production, and mechanism of action, advantages, disadvantages, application, and future prospects of edible vaccines. It also highlights the importance of use of this delivery method for vaccination against COVID-19 to get rid of current pandemic. According to various studies (A. U. Kumar, Kadiresen, Gan, & Ling, 2021; R. S. Kumar & Kiran, 2019; Mishra, Gupta, Khatri, Goyal, & Vyas, 2008), a reliable vaccine has various feature characteristics as presented in Figure 1.

2 | METHODOLOGY FOR LITERATURE SEARCH AND STUDY SELECTION

2.1 | Data source and search strategy

We searched different electronic databases from the beginning of online indexing years till 2022 including Pub-Med, Google Scholar, and Scopus. The key words we used in our search strategy were "Vaccines," "Edible vaccine," "Plant based vaccines," "SARS-CoV-2," "Vaccine against Covid-19," "Transgenic plant," and "conventional vaccine." All the terms were looked up in the title, abstract, and keywords.

2.2 | Study selection

The articles were screened twice. All references were gathered, and duplicate and triplicate articles were deleted. The authors worked independently in the first phase for choosing titles and abstracts from an electronic database articles in order to find possibly acceptable articles. In the second phase, the authors independently reviewed and read all of the articles chosen in the first phase, removing those that did not match the eligibility requirements. The reviewers were contacted at all stages in the event of any concerns or differences, and all issues were settled by consensus.

2.3 | Data extraction

All the selected articles were analyzed carefully for the extraction of data for giving explanation regarding vaccines, types of vaccine, vaccine production, mechanism of action, vaccines against different diseases, production of transgenic plants and of edible vaccine, SARS-CoV-2, edible vaccine against COVID-19, and advantages of edible vaccine over conventional vaccine.

TABLE 1 Schematic representation of different types of vaccine against pathogens; the text indicates against which pathogens certain vaccines are licensed and when each type of vaccine was first introduced

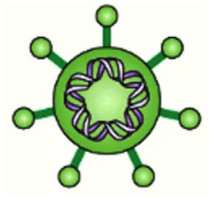
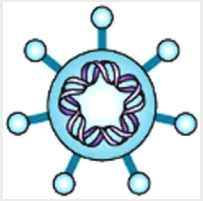
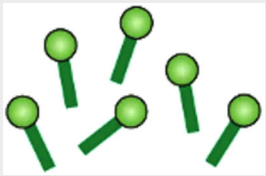
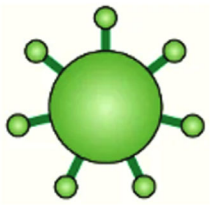
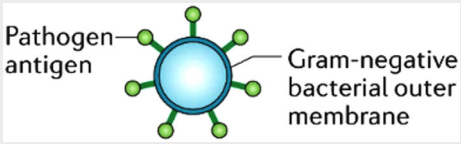
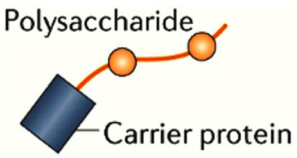
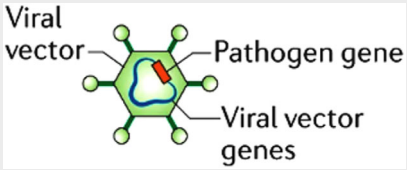
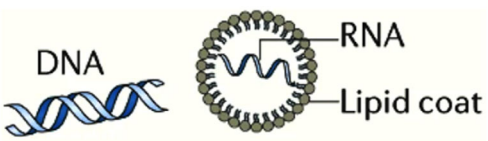
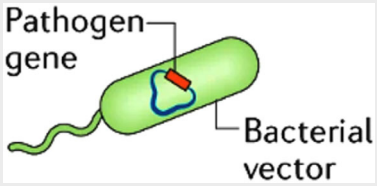
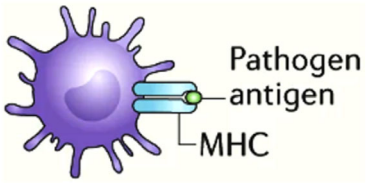
Types of vaccine	Shapes	Licensed vaccines using this technology	First introduced
Live attenuated (weakened or inactivated)		Measles, typhoid, mumps, Japanese encephalitis, rubella, yellow fever, influenza, oral polio, rotavirus, BCG, varicella zoster	1798 (smallpox)
Killed whole organism		Whole-cell pertussis, hepatitis A, polio, Japanese encephalitis, influenza, rabies	1896 (typhoid)
Toxoid		Tetanus, diphtheria	1923 (diphtheria)
Subunit (purified protein, recombinant protein, polysaccharide, peptide)		Pertussis, hepatitis A, typhoid, influenza, pneumococcal, hepatitis B, meningococcal	1970 (anthrax)
Virus-like particle		Human papillomavirus	1986 (hepatitis B)
Outer membrane vesicle		Group B meningococcal	1987 (Group B meningococcal)
Protein-polysaccharide conjugate		Haemophilus influenza type B, typhoid meningococcal, pneumococcal	1987 (H. influenzae type B)
Viral vectored		Ebola	2019 (Ebola)
Nucleic acid vaccine		SARS-CoV-2	2020 (SARS-CoV-2)

TABLE 1 (Continued)

Types of vaccine	Shapes	Licensed vaccines using this technology	First introduced
Bacterial vectored		Experimental	N/A[a]
Antigen-presenting cell		Experimental	N/A[a]

Note: BCG, *Mycobacterium bovis* Bacillus Calmette–Guérin (Pollard & Bijker, 2021).
[a]Data not available.

2.4 | Inclusion criteria

The book chapters, research, and review articles published until 2022 about edible vaccine, vaccine, vaccine against different diseases, corona virus, and COVID-19 vaccines were included in this review.

2.5 | Exclusion criteria

We excluded the studies on the basis of following criteria:

1. Studies reporting incomplete data.
2. Studies in non-English language.
3. Studies published in unauthorized journals.
4. Studies involving detailed explanation on immunological aspects of vaccine.
5. Duplicates and conference proceedings.

3 | RESULTS AND DISCUSSION

3.1 | Types of vaccines

Vaccines against microbes can be designed in a variety of ways. These decisions are usually made based on the microbe's basic characteristics, such as the infection mechanism in host cells and immune system's reaction as summarized in Table 1. Recent advances in molecular biology, on the other hand, have opened up new avenues for vaccine creation (Yadav, Yadav, & Khurana, 2014). Some of the major types of vaccine are as follows:

Live-attenuated vaccines: These are the earliest type of vaccines where the weakened live infectious organism is used as a vaccine. Most common live attenuated vaccines are against rubella, mumps, measles, and varicella.

Inactivated vaccines: These are the type of vaccines in which remains of dead microorganisms are used as a vaccine. Vaccine against hepatitis A, rabies, tick-borne encephalitis, Japanese encephalitis, and cholera are some classical examples of inactivated vaccines (Vetter, Denizer, Friedland, Krishnan, & Shapiro, 2018).

Toxoid vaccines: These are the vaccine in which toxins produced by the organism are used as the vaccine. Toxoid vaccines aim to prevent the root cause of the disease rather than the disease itself. Vaccine against tetanus, diphtheria, and a cellular Pertussis are most commonly used Toxoid vaccines (Anderson & May, 1985).

Biosynthetic vaccines: As the name indicates, these are the vaccines that are synthetically made by researchers and have an almost similar structure and characters to the disease-causing organism. Vaccine against hepatitis B is an example of biosynthetic vaccine.

DNA vaccines: In this type, plasmid DNA with antigen's DNA sequence is used as a vaccine. This recombinant plasmid DNA is injected directly into a specific tissue or muscle where it is expressed (Kim & Yang, 2010). There is no FDA-approved DNA vaccine, yet but DNA vaccines against HIV, TB, and malaria are in clinical trials (Nascimento & Leite, 2012).

Recombinant vaccines: These are the vaccines in which a recombinant plasmid with the gene coding for antigen is inserted into the bacteria and the protein is expressed inside the bacteria. Then purified antigen protein is used as a vaccine. The most recently developed recombinant vaccine is against human papillomaviruses (Fernández-San Millán et al., 2008).

Edible vaccines: In order to prepare the edible vaccine, the plant is genetically modified to express antigens in its edible part; when the edible part is consumed, it induces an immune response (Van Buren & Schaffner, 1991). Edible vaccines for hepatitis, diarrhea, rabies, cholera, and cystic fibrosis are under clinical trials (Qian et al., 2008; Tacket et al., 1998; Tacket et al., 2000).

Vaccines can also be classified based on methods of their application, which include the injection method, one of the most commonly

used methods, while the other methods are spray application and oral intake. The mode of activation of the immune system is affected by the route through which antigen is administered, as the mode of activation of immune response is determined by the class of antigen-presenting cells (APC) in administration of antigen (Hiatt, Caffferkey, & Bowdish, 1989).

According to WHO report (1992) the vaccines for children should be economical, easy to apply, and easy to store under normal environmental conditions (Qian et al., 2008). As it is said, "Let thy food be thy medicine," scientists suggest that vaccines against diseases can be produced by plants as potential source of natural recombinant vaccine. A plant-based vaccine can be produced against several diseases including dental caries, diarrhea, acquired immunodeficiency syndrome (AIDS), etc. (Daniell, Khan, & Allison, 2002). Their production is very fast and can be increased easily. According to an estimate, antigen required for vaccinating the whole Chinese population against hepatitis B could be grown on only 40 ha of land, while all children in the world can be vaccinated by just 200 ha of land (Ruf, Hermann, Berger, Carrer, & Bock, 2001).

3.2 | Pros and cons of conventional vaccines

Although conventional vaccines were the biggest breakthrough for the prevention of infectious diseases, they also have many limitations. One of the main concerns is biosafety. Although the bacteria or viruses are very carefully attenuated through controlled processes, the chances of reverting these bacteria's or viruses should not be ignored. There are also chances of failure of quality tests, which can lead to undetected vaccine contamination with the bacteria or virus (Kurup & Thomas, 2020). Since the vaccines have highly specific expiration dates and refrigeration requirements (not heat stable). Specialized conditions are required for the storage and transportation of conventional vaccines. According to the literature (Aboul-Ata et al., 2014; H. T. Chan & Daniell, 2015; Kurup & Thomas, 2020; Rybicki, 2017; Webster, Thomas, Strugnell, Dry, & Wesselingh, 2002), the key limitations of conventional vaccines are as follows:

1. Conventional vaccines are costly to prepare and are administered in multiple doses and there is a need of incorporating adjuvant
2. The parenteral route is the most common route of conventional vaccines administration for which trained personnel are required.
3. There is a possibility of secondary effects of parenteral vaccine injection which include localized inflammation at the site of inoculation fever, and in rare cases, hypersensitivity.
4. The injectable vaccines have a low mucosal response because they can only promote systemic humoral responses, but the T cell effector activity and mucosal immunity are critical for the infectious disease prevention.

Not all the pathogenic agents can be cultured in external media as some of the agents require biosecurity and biosafety infrastructures that all the countries cannot finance due to their highly pathogenic properties. As a result, in many countries, the manufacturing of certain vaccines is still limitations of traditional methods of vaccine production; the need for alternative techniques arises. The development of plant-based vaccines known as "edible vaccines" is one of the leading trends in vaccines development due to its several advantages over conventional methods of vaccine development (Stern & Markel, 2005).

3.3 | Edible vaccine

In 1990s, Arntzen first introduced the concept of edible vaccine. In the early 1990s, the scientific evidence that plants are edible led to their usage in oral vaccines (Shah, Trivedi, Vachhani, & Joshi, 1990). The desired gene(s) can be introduced into the plant genome and expressed in various plant tissues, including edible sections. These genes code for antigens that protect animals and humans from viral, bacterial, or parasitic infections. The vaccine can be given either by eating the edible part of the genetically engineered plant or by oral administration after producing a high yield of refined protein (Mor, Gómez-Lim, & Palmer, 1998). Edible vaccines have advantage of long-lasting immunity without the possibility of a relapse reaction. In recent years, research investigations have attempted to resolve the drawbacks of traditional vaccine by stimulating the efforts of edible vaccine development (Huang, Liao, Chang, & Liu, 2006).

The production of surface antigen from Streptococcus mutants in tobacco was the first evidence of an edible vaccine. Because this bacterium causes tooth decay, it was thought that stimulating a mucosal immune response might not allow the bacteria to colonize the teeth surface that would lead to protection against tooth decay. Edible vaccines are similar to unit preparations in that they include antigens, but they do not contain any genes that may cause complete infections to mutate or create negative impact on human health. As a result, there is no way of causing infection, which is especially important in immune-compromised individuals (Daniell, Streatfield, & Wycoff, 2001).

Various forms of highly efficacious plant-based expression systems have been developed over the last few decades. More than 100 different types of recombinant proteins have been successfully expressed in various plant tissues, including plant-derived vaccine antigens (B. V. Kumar et al., 2013). In 2006, the United States Department of Agriculture approved first plant-based vaccination against Newcastle disease virus after testing, that revealed 90% protection against a large amount of viral antigen (M. Sharma & Sood, 2011).

3.4 | Advantages of edible vaccines over conventional vaccines

The plant-based edible vaccines have a number of advantages proving them as future of vaccination. Since plants have less stringent requirements of sunlight, water, and minerals, the production, purification, sterilization, packaging, and distribution of edible vaccines do not

TABLE 2 Comparison between the conventional and plant derived edible vaccines

Conventional/ traditional vaccines	Plant based edible vaccines	References
Comprised of weakened	Live attenuated or killed pathogen, comprises of plasmid/vector carrier system or metal particles containing small segment of target DNA sequence	Mercenier, Wiedermann, and Breiteneder (2001); Taylor and Fauquet (2002)
Injected intramuscularly or subcutaneously thus painful immunization procedures	Given orally that is, needle-less vaccination thus easier administration for children	Mishra et al. (2008); Streatfield (2005)
Ineffective to induce a protective response at mucosal surfaces	Effective in inducing protective response at mucosal surface	Streatfield (2006); Yuki and Kiyono (2003)
Possess residual virulence	No residual virulence	Lal et al. (2007); Mishra et al. (2008); Streatfield (2006)
Need extensive safety precaution	Have a wide of safety	Altindis et al. (2014); Daniell et al. (2001)
Production difficulty and expensive	Relatively easy to produce and relatively cheap	Giddings, Allison, Brooks, and Carter (2000); Govea-Alonso, Cardineau, and Rosales-Mendoza (2014); Nochi et al. (2007)

necessitate a sophisticated framework, saving vaccine research expenses in the long run. Therefore, it is relatively simple to bulk produce on-site, transportation, and then store without the need of refrigeration. The use of plant tissues to express vaccines provides a heat-stable environment allowing oral delivery of the vaccine (Hudu, Shinkafi, & Umar, 2016). An edible vaccine can be consumed in the form of fruits or vegetables. There is no need of adjuvants to boost immune responses as plant compounds such as lectins added in the edible vaccines act as adjuvants (Hafiz & Eyob, 2015). The use of syringes and needles is also eliminated that lowers the risk of infection. Subunit vaccines (vaccines that have not been attenuated) have a higher level of safety. Unlike traditional immunization, orally administered vaccinations activate mucosal immunity as well.

Another significant benefit of edible vaccine is the potential to produce several components, which is enabled by the crossing of two plant lines. Second-generation vaccines are multicomponent vaccination proteins that allow numerous antigens to approach M (microfold) cells at the same time (Kessans, 2011; Khadwal, Singh, Singh, Sharma, & Sharma, 2020). Further, increasing the knowledge of extraordinary benefits of edible vaccine are giving edible vaccinations to women to immunize the baby in utero via transplacental transfer of maternal antibodies or through breast milk to immunize the infant. Edible vaccinations may protect newborns against infections such as group-B Streptococcus, respiratory syncytial virus, and others, according to research. When compared to regular vaccines, edible vaccines have a high level of compliance, particularly among children, and oral delivery reduces the need for skilled medical professionals (Hirlekar & Bhairy, 2017).

Another advantage of edible vaccines is that the expression of antigen in the seed allows for a longer maintenance and stability (Richter, Thanavala, Arntzen, & Mason, 2000). After an individual intakes an edible vaccine, the antigens are protected from damage by gastric secretion by the outer wall of plant cells, allowing them to be delivered to the intestinal mucosal surfaces, where they are absorbed through various mechanisms to stimulate a strong and specific immune response (Saxena & Rawat, 2014). Also, the manufacturing of edible vaccines can be ramped up quickly through breeding. Table 2 gives a brief comparison between conventional/ traditional and edible vaccines.

3.5 | Production of edible vaccine

3.5.1 | Insertion of antigenic gene into the plant

The antigenic gene must be inserted into the plant of interest using genetic engineering techniques to create an edible vaccination. According to the literature reported previously (Aswathi et al., 2014), insertion of a transgene can be done by using the following two methods:

1. Direct gene delivery method (without combining with vector)
2. Indirect gene delivery method (by combining with the vector)

Direct gene delivery method

Biolistic method. In the biolistic method, a gene is introduced to plant using a gene gun, that bombards metal particles (1–3 μm in diameter) coated with DNA to plant cells. Metal could be gold or tungsten (Rice, Ainley, & Shewen, 2005). The pros of this method are that it allows the delivery of multiple heterogeneous genes. The cons of this method are the use of expensive instruments, and the genes which are introduced can also be unstable. Vaccines developed by the biolistic method include canine parvovirus, tetanus, rotavirus, cholera, plague, anthrax, and Lyme disease (Streatfield, 2006).

Indirect gene delivery method

Agrobacterium-mediated gene transfer. Agrobacterium is a plant infecting bacterium, which causes tumors by inserting its transfer

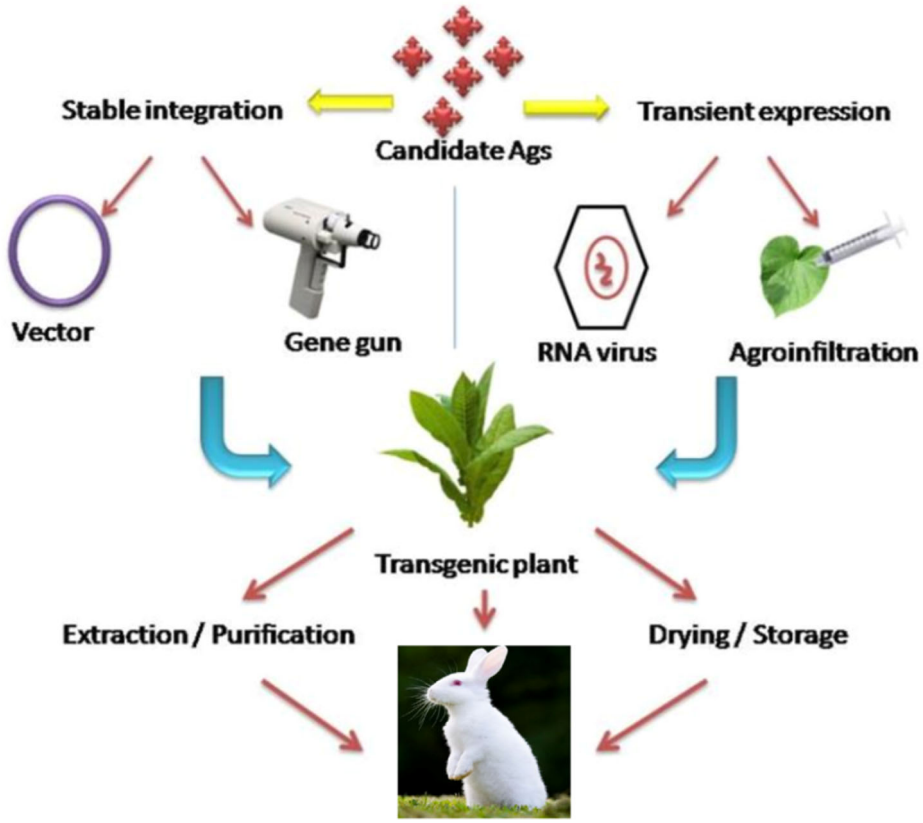


FIGURE 2 Different delivery methods for the formation of a transgenic plant for edible vaccine production (Aswathi et al., 2014)

DNA (tDNA) into the host genome. A molecular biologist has made use of this process to introduce the gene of interest into the host plant genome. The tDNA of agrobacterium is integrated into the chromosomal DNA of the plant by a process similar to conjugation (Komari, Ishida, & Hiei, 2004; Li, Sun, Lu, & Liu, 2011). The transformed cells are selected during tissue culture and transfer into a transgenic plant. Time duration depends on the type of species, which ranges from 8 weeks to 18 months to regenerate a transgenic plant (Hirlekar & Bhairy, 2017; Walmsley & Arntzen, 2000). TB, Ebola, avian flu virus, diarrhea, and dengue are examples of this method.

Electroporation. DNA is introduced into a plant cell by subjecting it to a long-duration high-voltage electric pulse that causes pores in the plasma membrane. The cell wall is weakened enzymatically because it is an obstacle to the entry of DNA into the cell. Hence, these pores allow DNA to enter the cell (Hirlekar & Bhairy, 2017).

Transgenic plant screening. The transformed cells and full plate are screened using herbicide and antibiotic resistance genes as a marker, which contains the foreign genes and expresses the desired product as shown in Figure 2. The gene of interest incorporates in plant chromosome randomly cause different antigen-expressing level for every independent line (Gunn, Singh, Giambrone, & Wu, 2012).

3.6 | What makes plant a beneficiary candidate?

Those plants that are suitable for production of edible vaccines are called candidate plants. According to the literature (Khadwal et al., 2020; Kim & Yang, 2010; Qian et al., 2008; Thanavala et al., 2005), the factors which make a plant a good candidate for an edible vaccine are as follows:

- It should have prolonged shelf-life
- Must have fast growth rate such as tomato and tobacco
- The edible part of the plant or plant itself can be stored for a very long time without any degradation. One of the great example is cereals, that is, wheat, rice, and maize

Those plant which takes a long time to grow and mature are considered bad candidates such as vegetables and fruits that produce on trees. The plants should be easily transformable. Most suitable plants are those on which research has already been done and has the most effective transformation. Recombinant proteins are highly expressed in green fruits that have been reviled by expression analysis of antigen. So, green fruits are suitable candidates for the edible vaccine.

3.7 | Model plants for edible vaccines

For the production of edible vaccines, plants that have been used previously are Barley, tomato, tobacco, banana, lettuce, pea, rice, wheat, maize, potato, spinach, alfalfa, carrot, soybean, papaya, and cucumber (Moss, Cutts, & Griffin, 1999). The data presented in Table 3 show the list of suitable antigens involved in edible vaccines and also cover the advantages and disadvantages of the plants used for edible vaccines.

TABLE 3 Showing antigens used, advantages and disadvantages of the plants used for edible vaccine

Plant host	Antigens	Against disease	Advantages	Disadvantages	References
Banana	HBsAg (surface protein of hepatitis B)	Hepatitis B	Can be eaten raw, economical, grow rapidly, have high vitamin A which increases immune response	Spoils rapidly, take 2–3 years for complete growth, high cultivation space requirement	Mason, Warzecha, Mor, and Arntzen (2002)
Tomato	Surface protein	Norwalk virus, diphtheria, pertussis, tetanus	Grows rapidly, planted broadly, heat-stable, high vitamin A content to boost immune response	Degrade easily, have less shelf life	Soria-Guerra et al. (2007)
Rice	CTB	Cholera, foot and mouth disease, psittacosis, allergy	Used as pediatric food, can be stored for a long time	Grows slowly, need specific glasshouse condition	Tacket (2009)
Tobacco	HPAIV H5N1, Virus VP1 protein	The avian flu virus, chicken infectious anemia, epidemic acute gastroenteritis, swine edema disease	Good in evaluating recombinant proteins, is a multi-harvest crop.	Toxic alkaloids incompatible with oral delivery	Mason et al. (1996)
Potato	HBsAg, CP	Hepatitis B, diarrheal diseases	Safely stimulating antibodies, inexpensive, and kept for a long time without preservation	Cooking is required, as this denatures antigen and reduces immunogenicity	Thanavala et al. (2005)
Lettuce	HBsAg	Hepatitis B	Direct consumption, high yield	Spoils rapidly	Hefferon, (2014); Sobrino et al. (2001)
Maize	LT-B	Diarrhea, porcine, reproductive and respiratory syndrome	Cheaper and does not need to be refrigerated	Due to cooking protein can be degraded	R. S. Kumar and Kiran (2019); Youm et al. (2008)
Pea	Hemagglutinin protein (H), surface protein	Rinderpest virus, Norwalk virus	Short life cycle, high in protein content	Needs cooking so it can reduce immunogenicity	Lal et al. (2007)
Alfalfa	Antigen eBRV4	Rota viral diarrhea (BVR)	Comparatively efficient transformation system; high protein level in leaves; leaves are taken uncooked	Potential for out crossing in the field; deep root challenging for cleaning field	Mason et al. (2002)
Spinach	GP/NP (fusion), Tat protein	Rabies, anthrax, HIV	High in vitamin A content, grown in a short time	It contains a high quantity of oxalic acid which blocks the absorption of iron	De Muynck, Navarre, Nizet, Stadlmann, and Boutry (2009)
Carrot	SubunidadUreB	*Helicobacter pylori*, HIV	Can be eaten raw, grown in a short time	Less shelf life and spoil readily	Concha et al. (2017)
Papaya	Synthetic peptides	Cysticercosis	Direct consumption, high antigenic expression	Limited shelf life, it took a long time of 6–9 months to grow	Concha et al. (2017)
Quinoa	VP2 protein	Infectious bursitis virus.	High in protein for a seed, a whole amino acid, can be cultivated easily	Can cause some allergic reactions and inflammation in the stomach	Concha et al. (2017)

3.7.1 | Tobacco (*Nicotiana tabacum*)

In 1990s, the first edible vaccine was developed in tobacco (Saxena & Rawat, 2014). The tobacco plant is adequate for the production of recombinant protein and is a perennial plant. The core disadvantage is that it causes toxicity due to its high composition of toxic alkaloids (Tregoning et al., 2003). Tobacco is used to develop a vaccine against the avian flu virus, chicken infectious anemia, epidemic acute gastroenteritis, and swine edema disease (R. S. Kumar & Kiran, 2019).

3.7.2 | Banana (Musa)

Banana was considered a perfect expression system. For example, it can grow in both tropic and sub-tropical regions of the world where 3rd world countries are located. In 2005, the first report of antigen expression in bananas was given by Kumar and colleagues. The surface antigen (HBsAg) gene of hepatitis B was transformed into a banana cultivar embryo cell. In the leaves, a high expression level of 19.92 ng/g was observed. To confirm the expression of HBsAg in leaves, reverse transcription polymerase chain reaction was also used (Aryamvally, Gunasekaran, Narenthiran, & Pasupathi, 2017). Because of the time-consuming during growth and development, the banana is no more considered an ideal candidate for the construction of an edible vaccine. As the banana tree takes 2 to 3 years for maturation. It is used to construct a vaccine against hepatitis B (Aryamvally et al., 2017; van Eerde et al., 2019).

3.7.3 | Tomato (*Solanum lycopersicum*)

Tomato can be used for the production of the vaccine, as it is taken as a salad so that helps in easy oral delivery (Dalsgaard et al., 1997). Tomato is an ideal candidate for vaccine development as it is easily transformable, heat-stable, grows in a short period, broadly cultivated, has high vitamins, and has excellent biomass. This composition enhances immune response. Thus, the tomato is a green vaccine factory (Sohrab, 2020). However, the drawback is that it spoils quickly. Tomato is used to develop a vaccine against AIDS/HIV, anthrax, and rabies (Davod, Fatemeh, Honari, & Hosseini, 2018; van Eerde et al., 2019).

3.7.4 | Lettuce (*Lactuca sativa*)

Escherichia coli B-subunit of the thermolabile protein is expressed in lettuce, which is the cause of enteric disease in both animals and humans and illustrates the possibility of lettuce as the edible vaccine. Glycoprotein E2, a swine fear hog pest virus, was expressed by lettuce in 2005. In developing stages, recombinant *Lactuca sativa* showed immunogenicity against hepatitis B in Poland (Hefferon, 2014).

3.7.5 | Pea (*Pisum sativum*)

Pea is another important model plant as it has a short life cycle and is high in protein content. The drawback is that it needs cooking so it can reduce immunogenicity. Pea plants are used in the expression of a protective antigen (PA) against rinderpest virus (RPV) and hemagglutinin protein (H) (Sahoo, Mandal, Dwivedi, & Kumar, 2020).

3.7.6 | Rice (*Oryza sativa*)

Rice is extensively used in the production of an edible vaccine. This is because of the availability and abundance of rice in third world countries and is harmless for animals and humans (Tacket, 2009). It has also been shown by an experiment that rice-based edible vaccines cause allergy (Aryamvally et al., 2017; Mason et al., 2002). Rice is used to a made vaccine against cholera, foot and mouth disease, psittacosis, and allergy (Mason et al., 2002).

3.7.7 | Maize (*Zea mays*)

A protein is expressed by the maize plant that is used in the development of a vaccine against the hepatitis B virus (HBV). This is cost-effective and does not require a refrigerator for its storage. The main drawback is it causes degradation of the protein.

3.7.8 | Potato (*Solanum tuberosum*)

Potato is an ideal plant for edible vaccine development, these are drought resistant, decrease the risk of degradation of proteins, and can be consumed raw (Aryamvally et al., 2017) nutritional value, abundant biomass, and high stability of recombinant proteins, long shelf-life, and short growth cycle. The disadvantage is, cooking is required, as this denatures antigen and reduces immunogenicity. Potato is used for the development of a vaccine against the HBV (Davoodi-Semiromi, Samson, & Daniell, 2009; Jani et al., 2002).

3.7.9 | Spinach (*Spinacia oleracea*)

Spinach is also used for the construction of an edible vaccine. It is used to develop a vaccine against the HIV-1 Tat protein and anthrax. Experiments showed that parts of PA were produced as a translational fusion with a capsid protein of tobacco mosaic virus (TMV) and spinach was injected with the transgenic virus (Mason, Lam, & Arntzen, 1992).

3.7.10 | Alfalfa (*Medicago sativa*)

Alfalfa is a plant that is related to the pea family. It is utilized to provide immunity to animals because it is frequently used as cow feed. Humans, on the other hand, consume alfalfa, which has been utilized in herbal therapy for over 1,500 years (Aryamvally et al., 2017). When administered orally to mice, the transformation and production of the bovine retrovirus (BRV) peptide, eBRV4, in alfalfa aids in generating immunity in the mother as well as the suckling infants (Kim & Yang, 2010).

3.7.11 | Carrot (*Daucus carota subsp. sativus*)

The carrot was combined with *A. thaliana* to create an edible vaccine for surface HIV antigen expression, and it was found that rats treated

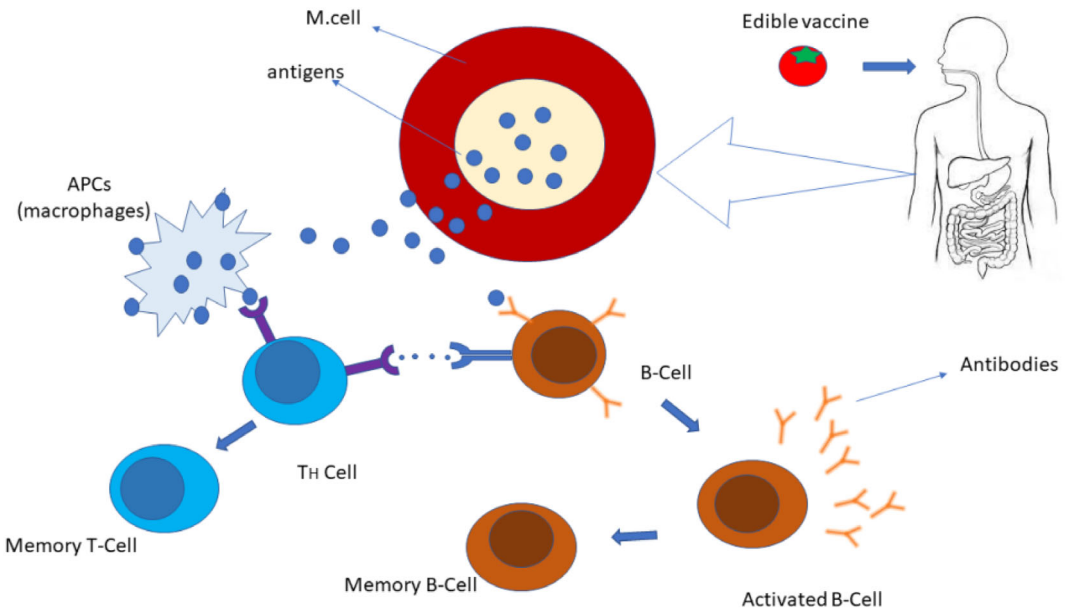


FIGURE 3 Mechanism of action of edible vaccine: edible vaccine stimulate the immune system. APCS presents the antigen to the B-cells to activate them to release antibodies which will fight against the virus

with carrot had a greater beneficial effect than nontreated animals. Carrots have a good impact on HIV therapy, not only because they are nutritious, but also because for the edible vaccine they are employed as a proof-of-concept model species (Sahoo et al., 2020).

3.7.12 | Soybean (*Glycine max*)

E. coli thermolabile toxin's B-subunit was expressed in soybean seeds endoplasmic reticulum (ER), yielding a total antigen level of up to 2.4% of entire *Glycine max* seed protein without any problems after drying for further processing. Furthermore, when this protein is given to rats orally, it causes a rise in systemic IgG and IgA levels (Sahoo et al., 2020).

3.7.13 | Papaya (*Carica papaya*)

Synthetic peptides were expressed in 19 transgenic papaya clones in 2007 to produce a papaya vaccine to combat cysticercosis caused by Taenia sodium. The vaccine was evaluated in rats, with a 90% immunogenic response in animals that have been vaccinated. These edible vaccines may provide significant alleviation in the disease's two main carriers, both people and pigs (Hefferon, 2014; Sahoo et al., 2020).

3.8 | Mechanism of action of an edible vaccine

Mucosal immunity is primarily stimulated by the edible vaccine. Both the innate and adaptive (T and B cells) arms of the immune system are present in this form. The mucosal-associated lymphoid tissues are well-structured in composition (MALT). Secretory immunoglobulin A (SIgA) also plays an important role in preventing microbe and toxin adherence on mucosal surfaces. To boost vaccine efficacy, novel platforms for pathogen or toxin-specific SIgA and systemic IgG administration must be developed.

Initiation of the mucosal immune system (MIS) is required for an edible vaccination to work. MIS is the main line of defense for the mucosal surfaces that line the digestive system, where most human and animal infections start infecting people. The detection of an antigen by specialized cells known as M-cells is the first step in inducing a mucosal immune response (Fragoso et al., 2017). These cells can be found in the mucosal membranes of lymphoid tissues like Peyer's patches in the small intestine. The antigen is channeled by M-cells to underlying tissues, where it is internalized and processed by APC. Antigenic epitopes are then displayed on the APC surface, where they activate B cells with the support of helper T cells (Hernandez et al., 2014). Activated B cells travel to the mesenteric lymph nodes, mature into plasma cells, and then migrate to mucosal membranes to release immunoglobulin IgA. When IgA molecules pass through the mucosal epithelial layer on their way to the lumen, they combine with membrane-bound secretary components to create SIgA. When SIgA is transported into the lumen, it interacts with antigenic epitopes to neutralize the pathogen (Walmsley & Arntzen, 2000) as graphically represented in Figure 3.

To boost vaccination effectiveness, innovative vaccine delivery platforms based on the elicitation of pathogen- or toxin-specific SIgA, as well as systemic IgG, are needed. So far, edible or intradermal vaccine formulations are the most well-known vaccine delivery methods which induce both mucosal and systemic immunity. Immunity is stimulated in gut-associated lymphoid tissue by oral vaccinations (Criscuolo et al., 2019).

3.9 | Applications of an edible vaccine against diseases

3.9.1 | Edible vaccine for rabies

Rabies is a fatal virus that transmits from animals to human beings (Yusibov & Koprowski, 1998). According to the WHO, more than 55,000 individuals die each year as a result of this condition (Loza-Rubio et al., 2012). Antibodies against rabies could be induced in mice by tomato plants expressing rabies antigens. TMV can also be used as an alternative. Cauliflower mosaic virus (CaMV) transformed tomato plants carrying the rabies virus's gene (ERA strain) glycoprotein (G-protein) and animals were found immunogenic to them (Tacket, 2009). The glycoprotein of the rabies virus has been expressed in a variety of systems including plants, yeast, adenovirus, baculovirus, and vaccinia, and it has been recognized as the primary antigen. Several plants, including spinach, tomato, tobacco, and more recently, the carrot plant, have expressed the G protein. In mice, these vaccinations were able to protect the challenge (E. Rybicki, 2009).

3.9.2 | Cancer therapy

Monoclonal antibodies have been successfully designed through several plants, which have been proven to be effective against cancer therapeutics. Monoclonal body in soya bean (BR-95) is an effective drug that targets doxorubicin, which is linked to lung tumors, breast cancer, ovarian tumor, and colon cancer (Massa et al., 2007).

3.9.3 | Edible vaccine for Ebola virus

An important example in mid-2014 is the great number of fatalities caused by the outbreak of the Ebola virus in Africa. There is no vaccine or globally tested treatment is available against the Ebola virus. Three monoclonal antibodies were transiently expressed by *Nicotiana benthamiana* plants that recognize Ebola virus surface G protein isolated from persons who fight against Ebola infections, demonstrating that plants can be effectively used as big pharmacies (Lou et al., 2007).

3.9.4 | Edible vaccines for diarrhea

One of the leading causes of death among children younger than 5 years old is enteric infections. *E. coli* is often regarded as the most common cause of bacterial diarrheal illness. The most effective way for prevention, spread, and control of these infections. So it is essential to overcome the limitations of the current vaccines by utilizing modern vaccine gears or vaccination approaches (Van der Laan, et al., 2006). The administration of the edible vaccine can trigger mucosal immunity, release antibodies, cells-mediated immune response, so colonization of infective agents on mucosal lines could be prevented this way (Jeshvaghani et al., 2019). Scientists at Cornell University introduced transgenic tomatoes against severe diarrhea caused by the Norwalk virus. The tomato produced surface protein specific to the disease causative agent. A researcher has informed when transgenic tomatoes are fed to the mice; an immune response is generated against the virus in mice (Miller & Ross, 2005). Also, for the expression of a transgenic protein, a banana was studied as it eradicates the cooking procedure as well as grows locally. The identification of a specific promoter is necessary for this expression. Furthermore, the expression of hepatitis B surface antigen in potato and lettuce and rabies G protein in spinach and has been reported (Mason et al., 1992).

3.9.5 | Edible vaccine against HIV

Transgenic tomatoes were generated when two genes of HIV protein along with CaMV promoters are injected with a needle. The expression of the protein in tomatoes was confirmed by running a polymerase chain reaction in various portions of the plant, including the second-generation plant as well as ripe fruit (Van Buren & Schaffner, 1991). Initial success has been achieved in splicing HIV protein into CPMV to produce an edible vaccine. Recently, for Tat protein expression cloned into TMV spinach has been successfully inoculated (Bhatia & Dahiya, 2015).

3.9.6 | Edible vaccines against measles

In terms of effectiveness and safety, the currently available measles vaccination bears promise. However, the live attenuated vaccination for measles has little oral effectiveness and may be destroyed if a "cold-chain" of refrigeration is maintained, posing distribution and storage issues (Khadwal et al., 2020). Millions of people live in areas where measles is endemic and resources are scarce Crude *Quillaja saponin* extracts stimulate measles' virus–specific immune responses in mice, following oral immunization with plant-based measles virus haemal-glutenin protein (Webster, Thomas, et al., 2002). Measles is spread by respiratory droplets from one individual to another. It is severe fevered infection; the onset is flu-like with high fever, cough, and conjunctivitis, red spots with a bluish-white center on the buccal mucosa called Koplik's spots. Measles antigens expressed in plants are antigenic and immunogenic both after invasive and oral vaccination (B. V. Kumar et al., 2013; Tacket et al., 1998).

3.9.7 | Edible vaccines against human papilloma viral disease

Human papillomavirus (HPV) is a common disease that is transmitted sexually worldwide. HPV is known to be a major cause of cervical cancer in women as well. Urgent attention is required to develop an edible vaccine that could confer protection against HPV (Autran, Carcelain, Combadiere, & Debre, 2004). A study revealed the isolation of a genetic sequence for the synthesis of an HPV protein envelope and virus-like particles (VLPs) were generated using this sequence.

Moreover, these VLPs were reported to be noninfectious and were speculated to be efficient oral immunogens successfully inoculated used for the treatment of HPV disease (Arakawa, Chong, & Langridge, 1998).

3.9.8 | Edible vaccine against diabetes

Diabetes affects more than 100 million people throughout the world. Type I diabetes, also known as insulin-dependent diabetes mellitus or juvenile-onset diabetes, is a form of diabetes that mostly affects youngsters, accounting for about 5–10% of diabetes diagnoses in America (Mason et al., 1992). Diabetes in mice can be averted by feeding them the plants modified to generate a diabetes-related protein, according to Ma and Hein 1965 of the University of Western Ontario (Ashraf et al., 2005). Transgenic plants tobacco and potatoes containing the gene for 1GAD67 were created by a Canadian team and obese diabetic mice were fed upon them, which developed diabetes that is insulin-dependent on their own. The interesting results were observed; only 20% of the prediabetic mice developed diabetes fed with transgenic plants, while the rest 70% of non-treated mice were diagnosed with the disease. Increased levels of IG1, an antibody associated with cytokines were also shown in treated mice, which support oncogenicity and prevent diabetes disease in animals (Yusibov, Streatfield, & Kushnir, 2011).

3.9.9 | Edible vaccines against dental caries

Antibodies against Streptococcus mutants, a common tooth decay bacterium, were produced in transgenic plants using a similar technique. The plant developed antibodies that could easily neutralize the infection, protecting the patient from dental illness (Ma et al., 1998). Individual transgenic plants expressing single antibodies, on the other hand, are required to provide effective protection against tooth disease. Initially, chains must be generated, which can then be hybridized to create a plant that produces full antibodies with both heavy and light chains (Khadwal et al., 2020).

3.9.10 | Edible vaccine against cholera

The cholera toxin antigen gene was put into the cells of an organism that causes crown gall, a plant disease. By infecting the alfalfa plant with the modified crown gall disease, new genes were introduced, and the cells of the newly infected plant were grown with the cholera antigen, and the alfalfa plant was regenerated from the infected cells (Kanagarajan, Tolf, Lundgren, Waldenström, & Brodelius, 2012).

3.9.11 | Edible vaccine against hepatitis-B

Globally, 1 million instances of this disease are estimated to be fatal each year. In the poorer countries, where infectious illnesses are still the leading cause of mortality, there is a pressing need to make subunit vaccinations, which are technically complex, accessible (which are almost exclusively biotechnology products) (Lakshmi & Kumar, 1992). Another study successfully analyzed when plant-produced HBsAg is administered orally, it can trigger an immune response. It was realized that the identification of an immunogenic HBV protein that may activate the human immune system to create defensive antibodies is a major determinant in the development of a HBV vaccine (Horn et al., 2003). Furthermore, one must optimize and construct a quantifiable measure of vaccination success, which necessitates the optimization of dosage levels and timings (Khadwal et al., 2020). The safety and immunogenicity of orally given HBsAg produced in transgenic potatoes were investigated in this clinical experiment. Health care workers in a randomized, placebo-controlled, double-blind trial volunteered to eat several dosages of genetically modified or control potatoes that had a history of past parenteral vaccination with the approved conventional hepatitis B vaccine. The participants' safety, responses to the vaccination vehicle, and immunological response to HBsAg were all assessed (Kapusta et al., 1999).

3.9.12 | Role in autoimmune diseases

Rheumatoid arthritis, diabetes, multiple sclerosis, lupus, and transplant rejection are among the most common autoimmune disorders being researched (Weiner, 1997). In one clinical investigation, diabetic mice were fed potatoes that could produce glutamic acid decarboxylase (GAD) protein and insulin. The protein was discovered to be effective in reducing immunological attacks and delaying the onset of high blood sugar levels. In addition to the above diseases, an edible vaccine against malaria, tetanus, Alzheimer's disease, foot and mouth disease, anthrax, dengue fever, *Helicobacter pylori*, influenza, and Japanese encephalitis, has also been produced (Khadwal et al., 2020).

3.9.13 | Edible vaccine for coronavirus disease 2019

Coronaviruses, a family of viruses, were found in human beings for the very first time in the 1960s (Kahn & McIntosh, 2005). The 229E and OC43 strains of human coronaviruses, which cause the common fever or cold, were the first viruses to be examined (Geller, Varbanov, & Duval, 2012). Emerging viruses like the severe acute respiratory syndrome–related CoV (SARS-CoV) of Southern China (2003), the Middle East respiratory syndrome–related CoV (MERS-CoV) of Saudi Arabia (2012) (Drosten et al., 2003), and SARS-CoV-2, the recently recognized coronavirus in the City of China, Wuhan, are all examples of coronaviruses 2019 (Tan et al., 2020). MERS-CoV, SARS-CoV, and SARS-CoV-2 are the coronaviruses known to infect people, causing acute sickness; however, OC43, 229E, NL63, and HKU1 cause minor indications (Corman, Muth, Niemeyer, & Drosten, 2018). SARS-CoV-2 is the seventh most common coronavirus infecting humans (Mahmood, Nasir, & Hefferon, 2021). The

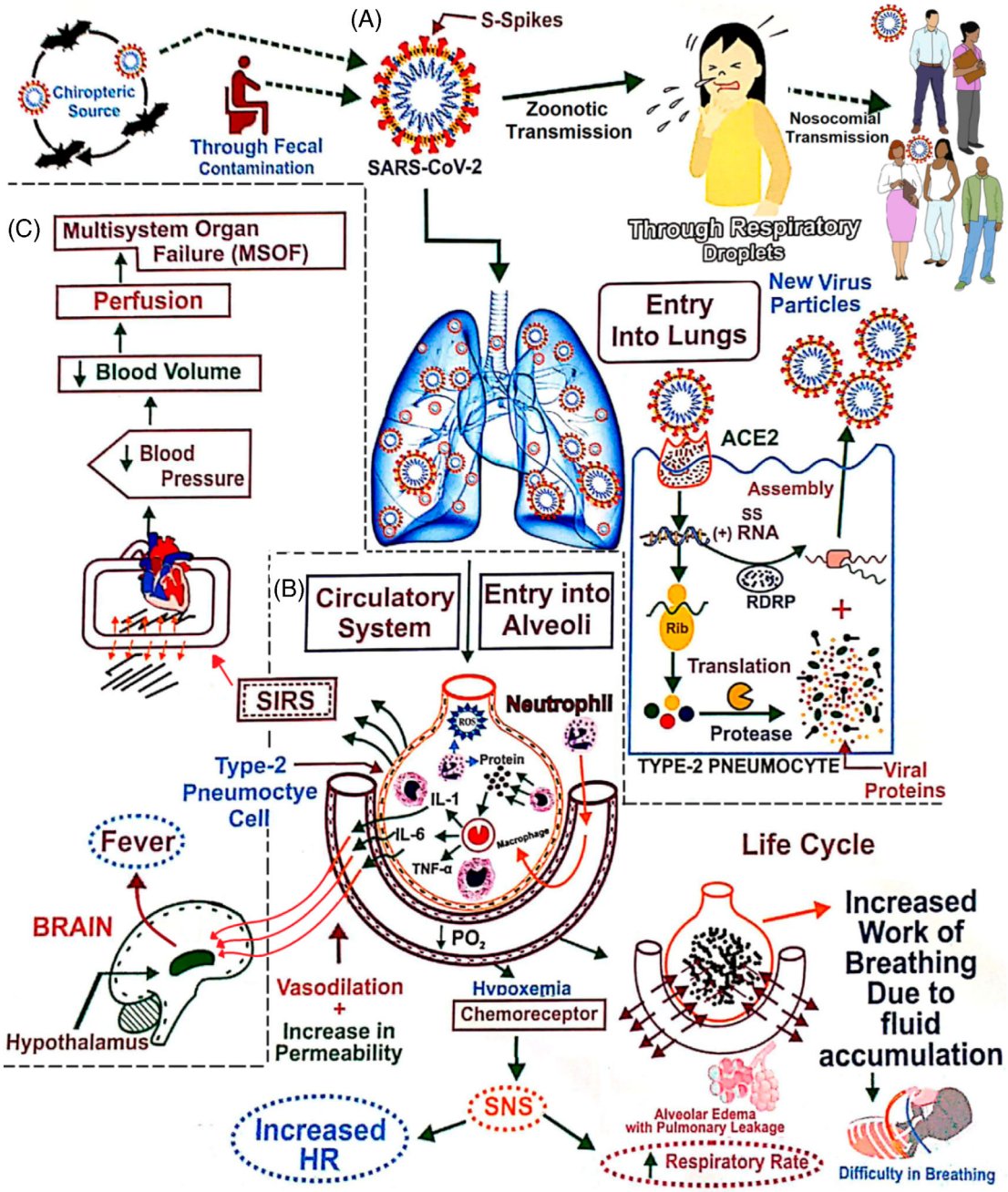


FIGURE 4 Diagrammatic illustration of the spread of COVID-19 infection and its effect on human immune, circulatory, and respiratory systems. (a) The virus that causes COVID-19 spreads via different routes, which may include transfer from a nonhuman animal to humans (chiropteran source, zoonotic transmission), hospital-acquired infections (nosocomial), or through fecal contamination. As the viral RNA enters human lung cells, it initiates the synthesis of viral machinery in the host cells (RNA-dependent RNA polymerase [RDRP] synthesizes a complementary strand of RNA, viral proteins are also synthesized) that results in the synthesis of new virus particles (b) COVID-19 may lead to hypoxemia as a result of inflammatory responses to the viral infection affecting the sympathetic nervous system (SNS) that leads to increased heart rate (HR) and alveolar edema which is difficulty in breathing due to fluid accumulation. (c) During COVID-19, the higher levels of cytokines (IL-1, IL-6, and tumor necrosis factor-a (TNF-a) lead to a hyper-inflammatory response by recruiting macrophages and diffused intravascular coagulation. This cascade of events may result in severe respiratory pain, failure of different organs, or pneumonia

mechanistic spread of SARS-CoV-2 from different species of animals to humans, as well as human-to-human transmission and how it affects the lungs and other body organs to cause severe symptoms, has been shown in Figure 4, which leads to death finally.

Beta coronavirus was immediately identified as the causal culprit (Zhu et al., 2020). The genome of this virus has 29,903 nucleotides and shows 89.1% nucleotide resemblance with the SARS-like family of coronaviruses earlier discovered in bats (Mahmood et al., 2021).

TABLE 4 Briefly shows the data of candidates for vaccine formation against coronavirus disease 2019 (COVID-19) along with their shots, speed, immune response, benefits, and drawbacks (O. Sharma, Sultan, Ding, & Triggle, 2020)

Platform	Candidates in clinical trials phases	Kind of candidate vaccine	Targeted antigen	Shots of vaccine	Speed of action	Immune response	Benefits	Drawbacks
DNA	Inovio Pharmaceuticals-Phase½	DNA plasmid vaccine with electroporation	Spike protein	Multiple	Fast	Show cellular and humoral response	Vigorous immune response generated by the electroporation method Made using genetic sequence and cultivation is not required	Though believed to be harmless, electroporation is complex and challenging DNA based vaccine has not formed previously
RNA	Moderna/NIAID-Phase 3	Lipid nanoparticle [LNP]-encapsulated mRNA	Spike protein	Multiple	Fast	Show cellular and humoral response	Made using genetic sequence and cultivation is not required	LNP is sensitive to heat Ability to make huge scale unknown RNA based vaccine has not formed previously
	BioNTech/Fosun Pharma/Pfizer-Phase 3	LNP-mRNAs	Spike protein					
Nonreplicating viral vector	AstraZeneca/University of Oxford-Phase 3	AZD1222	Spike protein	Single	Medium	Show cellular and humoral response	Can be produced on huge scale -Harmless and efficient immunologically as presented by Ebola	Pre-existing immunity can hinder medical use and decrease immune reaction
	CanSino Biological Inc./Beijing Institute of Biotechnology-Phase 2	Adenovirus type 5 vector	Spike protein					
Inactivated	Wuhan Institute of Biological Products/Sinopharm-Phase 3	Inactivated	Complete virus	Multiple	Medium	Generally humoral	A pathogen is killed and so, no threat of decline	Threat of vaccine-increase sickness Generally trigger a weak immune reaction
	Beijing Institute of Biological Products/Sinopharm-Phase 3	—	Whole virus			—		
	Sinovac-Phase 3	Inactivated + aluminum adjuvant	Whole virus			Generally humoral—aluminum adjuvant boosts response more vigorous		

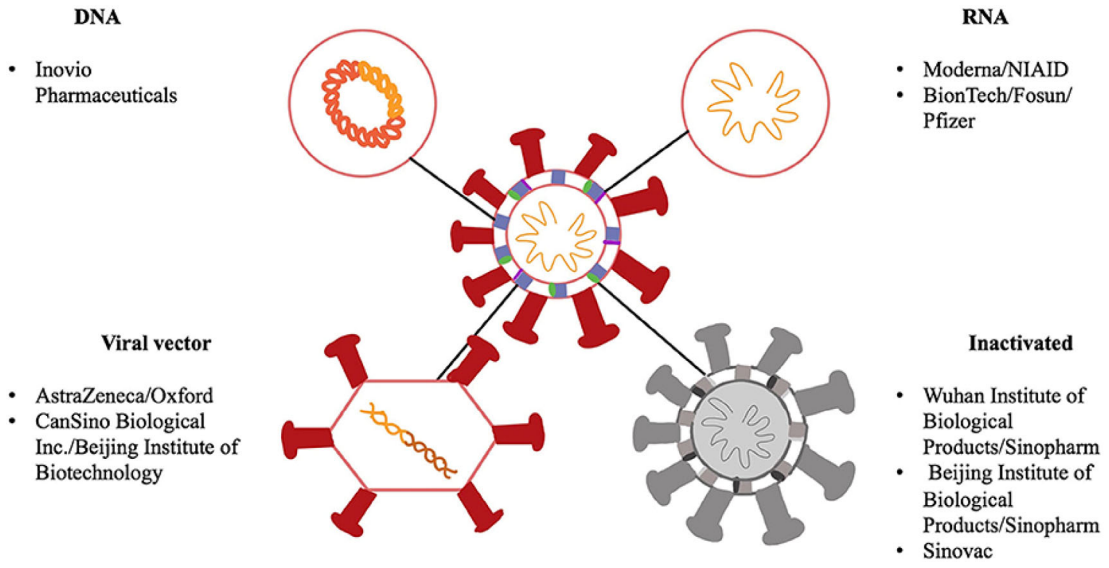


FIGURE 5 Different types of vaccines available against coronavirus disease 2019 (COVID-19) approved for clinical trials (O. Sharma et al., 2020)

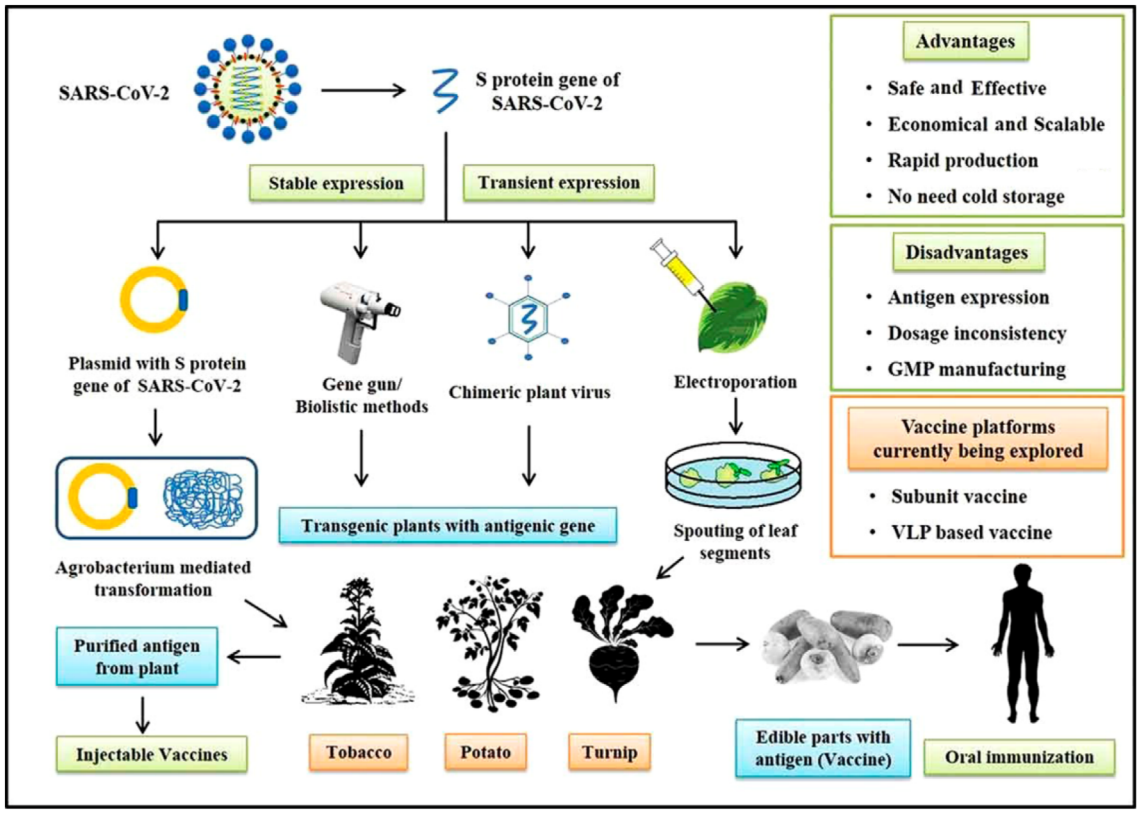


FIGURE 6 Process of the production of edible vaccine against coronavirus disease 2019 (COVID-19) (Dhama et al., 2020)

Despite similarities to MERS-CoV and SARS-CoV, the virus is entirely different (Tan et al., 2020). Initially, it was assumed that the new virus would be less severe than MERS-CoV and SARS-CoV. A rapid increase in cases and interpersonal spread provides further evidence and however, suggested this is an extremely deadly virus (J. F.-W. Chan et al., 2020). The World Health Organization on March 11, 2020, proclaimed that epidemic caused by 2019—nCoV a pandemic and disease named COVID-19 (Mahmood et al., 2021).

The major outbreak of severe acute respiratory syndrome coronavirus-2, causing cold or pneumonia-like symptoms in people

and had converted into a global pandemic named COVID-19. In terms of amount, COVID-19 is causing about 1 million deaths that have exceeded the rate of the severe acute respiratory syndrome and respiratory syndrome in the Middle East. COVID-19 deaths have overtaken, coronary cardiac diseases, cancer, and even cold death after the few months of novel coronavirus arrival.

The creation of a COVID-19 vaccine that is successful in combating the disease's spread is the major aim of various countries around the world. Table 4 briefly shows the data of plant candidates for vaccine formation against COVID-19 along with their shots, speed, immune response, benefits, and drawbacks Figure 5, depicting the types of vaccine candidates against COVID-19 with approved consent of WHO for clinical trials. Moreover, developments of oral vaccines are also considered as one of the available approaches. The edible vaccine has gained its status among scientists because of its known cost-effectiveness and manufacturing process (A. U. Kumar et al., 2021). Furthermore, Dhama et al. (2020) reported a brief description of the production of edible vaccines for COVID-19 as pictorial illustration presented in Figure 6. The lack of specialized antiviral medications or therapies for human coronaviruses emphasizes the need of taking preemptive steps to control the virus's transmission (Cutts, Henao-Restrepo, & Olive, 1999).

As shown in previous work on coronavirus vaccines, the glycoprotein (S) of the spike induces defensive antibodies in the body. The Spike (S) protein can be used to produce a vaccine against the COVID-19. When the gene of Spike (S) protein or subunit of Spike-like is inserted into a plant expression vector the desired plant such as lettuce, tomato, or cucumber, can be transformed [99]. The resulting transgenic plants can be eaten raw as salad and immunized the human being to combat the novel virus. Many groups of scientists are working together on a vaccine to defend humans against the new CoV. The therapeutic vaccine choices for COVID-19 have been recommended and discussed in a published article. Although, to avoid any future pandemics and control the spread of the virus the formation of a defensive vaccine is of great importance (Sohrab, 2020). Medicago, iBio, Zyus, Centre for Research in Agricultural Genomics, Beijing CC-Pharming, Newcotiana, Kentucky Bioprocessing, and Daniel Garza are biotech giants which are working on an edible vaccine against COVID-19 by using a transgenic plant approach (A. U. Kumar et al., 2021).

Medicago, a Canadian biopharmaceutical company, has created a coronavirus VLP after getting the genetic sequence of SARS-CoV-2 in just 20 days. They employed a technique that involved introducing a genetic sequence encoding the COVID-19 spike protein into Agrobacterium, a common soil bacterium that plants eat (Mani et al., 2020). The created plants form a VLP that is made up of a plant lipid membrane and the COVID-19 spike protein. *Nicotiana benthamiana*, a closely related to tobacco plant, is being used by Medicago to create SARS-CoV-2 virus VLPs (COVID-19: The VLPs are identical in size and form to coronaviruses), but they lack RNA/DNA and so are not infectious (Peyret et al., 2021). Medicago completed Phase 1 clinical trials satisfactorily and is now progressing on Phase 2 clinical trials (Capell et al., 2020). Medicago has already developed VLPs containing influenza virus hemagglutinin, demonstrating their safety and efficacy in animal models and human clinical studies. The cost of making a VLP-based vaccination from plants is a fraction of the cost of making a conventional vaccine (Mohammadinejad et al., 2019).

Kentucky Bioprocessing, on the other hand, is developing its own fast-growing GM tobacco and has openly declared that it is previously undertaking preclinical tests and can produce up to 3 million dosages per week (Rosales-Mendoza, Márquez-Escobar, González-Ortega, Nieto-Gómez, & Arévalo-Villalobos, 2020).

The third private-sector research group is a collaboration between iBio in the United States and Beijing CC-Pharming in China, which is coupling COVID-19 VLP culture with a lichenase carrier immunostimulatory adjuvant in GM tobacco (Das, Samantarai, & Panda, 2021).

In Mexico, as a first step toward developing a COVID-19 vaccine, Garza is making efforts. In a conference with the Cornell Alliance for Science, Garza remarked, "The creation of an edible vaccination to combat SARS-CoV-2 has so far been a little-explored substitute, even if the profits are clear." "Under this premise, the problem would be solved by producing a fusion protein having vaccine-like properties that could be expressed in tomato plants" (Rosales-Mendoza et al., 2020).

To implement a reverse vaccination technique, Garza and an interdisciplinary team of scientists use tools of bioinformatics and computational genetic engineering techniques. Through "in silico" examination of the pathogen genome, they determine the antigens most probable the candidate of vaccine to stimulate an immune reaction using bioinformatics methods. They proceed to optimize the tomato plant nucleotide sequence and the *A. tumefaciens* genetic transformation, once the candidate sequence has been determined. "Expression in tomato plants using the newly found epitopes allows us to achieve significant amounts of recombinant protein expression," Garza explained. To put it another way, prior bioinformatics modeling saves time and effort by focusing on antigens that elicit a strong defensive reaction to combat the pathogen, making them a good candidate for developing a viable and scalable vaccine (Capell et al., 2020).

3.10 | Pros and cons of edible vaccines

There are several limitations and challenges regarding the safe supply and production of edible vaccines. Plants producing edible vaccines may face difficulties in commercialization in states that do not allow the sale of transgenic food or do not ready to permit the entrance or consumption of transgenic plants (or portion of plants) that produce edible vaccines (Mercenier et al., 2001). In certain regions, some people believed that transgenic plants and foods are injurious to the health, so there is a serious need to be aware of the people from this kind of myth.

Because many mRNAs from the transgene promote gene silence in the plant genome, trials to increase the number of antigens

TABLE 5 The data comprise the currently developed edible vaccine involved in clinical trials

Plant host	Against disease	Antigen	Clinical trial	References
Potato	Diarrhea	LT-B	Phase 1	Tacket et al. (1998, 2000); Thanavala et al. (2005)
	Diarrhea	CP	Phase 1	
	Hepatitis B	HBsAg	Phase 1	
Maize	Diarrhea	LT-B	Phase 1	Tacket, Pasetti, Edelman, Howard, and Streatfield (2004)
	Cystic fibrosis, pancreatitis		Phase 2	
Spinach	Rabies	GP/NP (fusion)	Phase 1	Yusibov et al. (2002)
Lettuce	Hepatitis B	HBsAg	Phase 1	Kapusta et al. (1999)
Rice	Cholera	CTB	Phase 1	Nochi et al. (2009); Kurokawa et al. (2013)

produced result in immature plant growth and lower fruit formation (Laere et al., 2016). The transgenic plant can induce allergies in certain people. Edible vaccines might trigger hypersensitive responses during posttranslational modifications, and oral acceptance, when combined with an oral adjuvant, to normally trigger the mucosal immune response, can exacerbate allergic reactions to several other proteins present in the daily food stuff (Maxwell, 2014). Because of significant variation in the glycosylation patterns of plants and human beings, the role of edible vaccines may be impeded (Pascual, 2007).

Another drawback of edible vaccines is the complexity around the determination of an appropriate oral administration quantity, which may need multiple administration rounds, raising the overall cost of their use (Shakoor et al., 2019; Yoshida et al., 2011). Required dosage varies from generation to generation, protein content, weight, patient age, ripeness of the fruit, plant to plant, and amount of the food consumed (Moffat, 1995). Overeating of these plants containing antigens that trigger the immune response may lead to immune system overstimulation. The location where oral vaccine-producing plants are produced is another essential element to consider. To minimize seed or pollen damage during plant cutting, the absolute governor should be trained to protect the atmosphere where such plants are cultivated (Webster, Cooney, et al., 2002).

It is also worth noting that, while developing an edible vaccination has been promoted as a way to stimulate the immune response by ingesting a portion of a plant, the procedure is challenging to standardize antigen concentrations in various plant tissues. Even with plants produced via in vitro asexual circumstances, the main challenge is the plants' inherent genetic diversity, for example, somaclonal variation (Wigdorovitz et al., 1999). The person may develop immunological tolerance to a specific vaccination protein or peptide. Some foods in raw form cannot be eaten (e.g., soya bean, potato) because they need cooking that causes denaturation of the protein present in them, as edible vaccines are reliant on plant stability (Salazar-González, Bañuelos-Hernández, & Rosales-Mendoza, 2015). One of the limitations of edible vaccines is that they are not site-specific and fail to reach their site of action (Aryamvally et al., 2017). Ecological problems or biodiversity concerns are elevated for the genetically modified plants or seeds that outflow into the wild. Furthermore, plant-based vaccines and non-plant-based vaccines of identical plants cannot be differentiated. Traditional tomato and edible vaccine tomato plants look similar so there is always a chance of misunderstanding (Pelosi, Shepherd, & Walmsley, 2012).

The formation of vaccines into a balanced seed form or leaf formation is preferred; however, spoiling must be avoided to minimize antigen loss or seeping into the environment. The gastric tolerance to oral vaccines/therapeutics is the most common concern with vaccination. Immune suppression with triamcinolone can be used to solve this issue. Although, this has to be done in minor quantities to avoid any major health concerns or even death. Selin and Lyubomska suggested numerous dosages of vaccines over a definite period (Miller & Ross, 2005). Developing countries should be aware of the use of an edible vaccine. These concerns must be considered to meet the excellence standards for vaccines set by World Health Organization (Witkamp & van Norren, 2018).

3.11 | Future perspectives

Yield improvement has a crucial influence on economic feasibility, as this is one of the most significant holdups in oral vaccine technology (Levine, 2006). It is difficult to predict how quickly new items will become available and well-known by purchasers in this field of study. In theory, it is now feasible to transfer an organism's gene into any plant and have that gene express a novel product will form in any portion of the plant, rather it is root, shoot, seed, or leaf. Food is being increasingly regarded not only a fundamental basis of sustenance; nevertheless, a commodity as well along with distinct medical capabilities (Khan et al., 2019). Shortly, edible vaccinations could be made from the coffee plant and natural grass, which are both regularly consumed by humans and animals and considered as a promising solution against potential hazards associated with conventional parenteral vaccines (Sahoo et al., 2020).

Mainly, we predict proteins such as insulin, human growth hormone (HGH), antibodies, and antihemopoietic proteins (e.g., factor VIII) will be used for pharmacological purposes toward fighting against human and veterinary diseases but as the model proteins antigens for edible vaccine manufacture (Richter et al., 2000). In designing new pharmaceutical proteins plant production system offers broader flexibility. Time is not far away when we eat appealing fruits and vegetables to prevent ourselves from infective agents (Tacket, 2007).

Monoclonal antibodies are usually utilized for the cure of arthritis and tumor. The price is reduced in the formation of 1 kg of monoclonal antibody from about 3 million to more than 100 dollars via a plant expression system. Moreover, the use of a plant expression system to produce medically valuable proteins has the added benefit of removing any undesirable impurities that might be present when utilizing an animal cell culture method to make the protein (Yoshimatsu et al., 2012).

Currently, in the market, commercially plant-based oral vaccines are not present. However, based on research claiming that different phases of clinical trials are now going well as presented in Table 5, as well as plant-based edible vaccination products could be accessible in the not-too-distant future in the market. Furthermore, since this technology develops innovative vaccination technologies and manufacturing, the social and efficient influence of the technology might prove to be massive (Cui, Li, & Shi, 2019). In the upcoming edible vaccine combat, anthrax, plague, and smallpox etc. can be formed in a huge number (up to millions of doses) within a short time.

4 | CONCLUSION

Edible vaccines, the much safer and inexpensive substitutes of traditional vaccines, can be produced without the need for sophisticated equipment and tools. These edible vaccines are economical, needle-free, do not require refrigeration, appeal to kids, stored nearby at the place of consumption, and delivered in the form of salad, which can trigger both systemic and mucosal responses. The edible vaccines can reduce the tremendous use of antibiotics and cope with the major challenge of antibiotic resistance. Developing and mostly underdeveloped countries will have profited more from this economical method of edible vaccine production and the vaccine products would be accessible to the population.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Fatima Khalid, Reema Tahir, Manahil Ellahi, and Nilofer Amir: Performed equally in the bibliographic search and writing of the manuscript. **Fatima Khalid and Reema Tahir**: Contributed in the construction of the tables and figures and reference editing. **Ammarah Hasnain and Syed Faheem Askari Rizvi**: Contributed to the conceptualization of the study design as well as reviewing and proof reading of the manuscript.

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How to cite this article: Khalid, F., Tahir, R., Ellahi, M., Amir, N., Rizvi, S. F. A., & Hasnain, A. (2022). Emerging trends of edible vaccine therapy for combating human diseases especially COVID-19: Pros, cons, and future challenges. *Phytotherapy Research*, *36*(7), 2746–2766. https://doi.org/10.1002/ptr.7475