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Plant Cell and Callus Cultures as an Alternative Source of Bioactive Compounds with Therapeutic Potential against Coronavirus Disease (COVID-19)

A Ferid¹, Arifullah Mohammed^{1,2*}, Shaik Ibrahim Khalivulla³, Mallikarjuna Korivi⁴ and Mohammad Khairul Azhar Abdul Razab⁵

¹Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli 17600, Malaysia.

²Institute of Food Security and Sustainable Agriculture (IFSSA), Universiti Malaysia Kelantan, Jeli 17600, Kelantan, Malaysia.

³Faculty of Pharmaceutical Sciences, UCSI University, Cheras 56000, Kuala Lumpur, Malaysia.

⁴Exercise and Metabolism Research Center, College of Physical Education and Health Sciences, Zhejiang Normal University, Jinhua City 321004, Zhejiang, China.

⁵School of Health Sciences, Health Campus Universiti Sains Malaysia, 18150 Kubang Kerian, Malaysia.

E-mail: aurifullah@umk.edu.my

Abstract. Plant cell and callus cultures are important tools for the mass production of bioactive compounds (secondary metabolites) from plants cell or tissue under a controlled environment. From past few decades the bioactive compounds assumed to play a key role in the development of novel drugs against several viral diseases, causing serious threat and even death to thousands of human lives. The recent, pandemic coronavirus disease (COVID-19) outbreak upraised the importance of developing an effective therapeutic drug or vaccine as quick as possible to treat or prevent further spread. The research studies are in progress to find coronavirus therapeutics among existing antiviral drugs. Of these drugs, hydroxychloroquine and azithromycin have emerged as frontrunners and shown early promising results in treating COVID-19 in both *in vitro* and *in vivo* studies. However, these drugs have adverse side-effects and they became ineffectual due to eventual drug-resistance. Research is continuing by several means in search of potential therapeutics with minimal side-effects. The natural bioactive compounds from a plant sources generally have minimal toxicity can exert inhibitory capacity against coronavirus is of great interest. Up to now, various phytochemical compounds, namely arctiin, scutellarin, forsythoside, kaempferol, secoxyloganin, nicotianamine, saikosaponin, reported to have anti-SARS-CoV-2 activity. The mechanism of action appears to be inhibiting virus replication and blocking viral infection. Due to time taking cultivation, expensive extraction and isolation of bioactive constituents, it is essential to develop alternative techniques for the mass production of bioactive compounds in a less timeframe using *in vitro* methods of plant cell and callus culture methods. In the present work, we highlighted the importance of modern biotechnological approaches including cell or tissue or callus culture methods and plant-based antiviral compounds currently being tested to treat novel coronavirus.



1. Introduction

Natural products derived from plants have been used traditionally as an alternative or complementary medicine to treat vast number of diseases like common cold, headache, nausea, muscle pain, cough, diabetes, wounds, and other viral infectious diseases with their diverse range of chemical constituents. The complex chemical structure and their pharmacological relevance with respect to their structure activity relationship are important in the drug discovery. Many life-saving drugs have been derived from natural products (medicinal plants) in modern medicine [1]. Many plants contain molecules (secondary metabolites) that are clinically important, such as phenolics, flavonoids, terpenoids, tannins and saponins, which have been using for the treatment of different diseases including antiviral drugs [2]. Secondary metabolites can be obtained by extracting directly from various organs of the plant species. However, the extraction and isolation procedures are expensive, takes longer time and also obtain in low yield. Therefore, it is necessary to develop alternative approaches for the mass production of bioactive compounds using plant tissue (plant cell and callus) culture method. This method has several advantages such as continuous and reliable production of natural compounds without environmental challenges [1, 2].

Herbal and modern medicine have been using for the treatment of various diseases throughout the world. Emerging viral infections have been posing a serious threat in the last few decades causing thousands of deaths globally. Recent, deadliest novel viral coronavirus pandemic outbreak upraised the importance of developing an effective therapeutic drug or vaccine in short timeframe to prevent further human loss [1-3]. Virus causes a multitude of diseases and are responsible for various human pathogenesis ranging from the common cold to cancer. Viruses are obligate intracellular parasites, composed of a protein coat and a nucleic acid. Virus contains an outer capsid of protein coat that covers an inner core of genome, either DNA or RNA, but not both [1, 2]. These submicroscopic infectious particles (≤ 200 nm \emptyset) may also contain additional proteins, especially enzymes (polymerase) need to produce new viral DNA or RNA. Unlike bacterial cells, which are free-living organisms (non-symbiotic), viruses must carry out their propagation by utilizing a host cell and can only reproduce inside of a particular host [2]. Viruses are mainly categorized by the type of nucleic acid they contained (RNA or DNA), and the shape of their protein capsule. They also encode proteins resembling cellular proteins and force their host cells to produce more virions. Thus, inhibiting viral proteins without distressing the host cell is very challenging. Several incurable diseases and disorders including cancer have been linked with viral infections. Moreover, due to accelerated urbanization, increased international travel, infected food supplies, and contaminated drinking water, disease outbreaks caused by newly emerging and re-emerging viruses represent a serious threat to public health system, particularly when the vaccines and antiviral medicines are unavailable [3, 4].

The coronaviruses are a large group of viruses that are common in people and few animals including bats and camels. These viruses usually cause mild sicknesses, such as cough and common cold. But, certain types of coronavirus can infect the lower airway, causing severe infections like pneumonia or bronchitis. In the past few decades, coronavirus outbreaks have arisen in human populations around the globe, each distinctive but also with some resemblances. The recent novel coronavirus outbreak has spread to over 213 countries and affected millions of people worldwide. Due to this WHO declare it as a pandemic disease and yet, the studies are underway to find a therapeutics or vaccine to treat this disease [5-7].

Hence, in the present paper highlighted the therapeutic options currently being tested to treat novel COVID-19 and an alternative/complementary plant-derived antiviral compounds with related pharmacological mechanisms to fight against this disease. The paper also covers modern biotechnological approaches such as plant cell culture, hairy root culture, plant metabolic engineering, that has been used to improve and/ or produce novel antiviral bioactive compounds.

2. Antiviral and herbal medicine against coronavirus disease 2019 (COVID-19)

2.1. Antiviral drugs

The global health crisis caused by novel coronavirus (COVID-19) has persuaded researchers to develop antiviral drugs and vaccine. However, the development of these therapeutic agents will take long time. According to WHO, vaccine development could take minimum of >18 months to possibly many years

[12]. Although various existing drugs have been used to combat infectious diseases, currently there are no clinically approved antiviral drugs or vaccine for COVID-19 [5, 6, 7, 13]. However, repositioning of old drugs for COVID-19 virus is an interesting strategy because of available information on safety profile, side effects and drug interactions of the studied drugs which could help the researchers scramble to develop an effective antiviral drugs/vaccines [14]. Studies on existing antiviral drugs, including chloroquine (antimalarial drugs), favipiravir (Japanese anti-flue drugs), azithromycin (antimicrobial) and the Ebola drug (remdesivir) were studied in this way and still several drugs are in progress. Of these drugs, chloroquine, hydroxychloroquine and azithromycin were emerged as frontrunners and shown early promising results in treating novel coronavirus [5-8].

2.2. Herbal medicine as source for drug-discovery against COVID-19

Medicinal plants are a source of active constituents used in the treatments of various diseases caused by a number of pathogens. Nearly 25% of drugs prescribed throughout the world today are originated from plants and according to WHO, 80% of the world population rely on herbal medicines (plant-derived products) for primary health care. In recent years, the herbal medicines have been used for disease prevention and as alternative medicines [15]. Traditional herbal products and plant-derived drugs have been proven to be potent sources of antiviral agents with their broad spectrum of therapeutic efficiency [16]. A range of various natural products are shown to inhibit the virus replication without affecting the host cell or with limited side effects [15] and also regulate the host immune response against viral infection [17]. In 2003, patient with SARS-CoV was treated with traditional herbal medicine and reported that the mode of action of herbal medicine was by decreasing the steroid related side-effects, and improvement of recovery symptoms [12, 18].

Recently couple of reports on plant secondary metabolites for the inhibition of COVID-19 explains the importance of plants for the treatment of coronavirus [19]. Still, there has been much pharmacological research investigation on different plant species is ongoing at different parts of the World for the potential of anti-COVID-19 therapeutics. Despite all the efforts being made to fight the recent novel coronavirus disease outbreak, very little attention was given on herbal medicines. Yet, extensive research on herbal medicine are still urgently needed to find an alternative and complementary medicine for the treatment of patients with novel corona virus infection. Natural compounds with antiviral activities against SARS-associated coronavirus is summarized in Table 1.

Table 1. Natural compounds with antiviral activities against SARS-associated coronavirus

S. No	Plant extract/ compound	Plant species/ family	Virus/target site	EC ₅₀	Mode of Action	References
1	Lycorine from <i>Lycoris radiata</i>	<i>Lycoris radiata</i>	SARS-CoV	15.7±1.2 nM	Block viral infection and replication	[20]
2	Glycyrrhizin, licorice roots	<i>Helichrysum petiolare</i>	SARS-CoV	300 mg/L	Inhibiting virus replication (SARS-CoV)	[21]
3	Saikosaponin A	<i>Bupleurum falcatum</i>	CoV 229E	8.6±0.3 µmol/L	Inhibit the early stage of HCoV-229E infection,	[21, 22]
4	Saikosaponin B2	<i>B. falcatum</i>	CoV 229E	1.7±0.1 µmol/L	Inhibit the primary stage of HCoV-229E	[21]
5	Saikosaponin C	<i>B. falcatum</i>	CoV 229E	19.9±0.1 µmol/L	Inhibit primary stage of infection, viral attachment	[21]
6	Saikosaponin D	<i>B. falcatum</i>	CoV 229E	13.2±0.3 µmol/L	Inhibit the early stage of HCoV-229E	[21]
7	Oroxylin A	<i>Scutellaria baicalensis</i>	RSV	14.5 µg/mL	Inhibition of respiratory syncytial virus <i>in vitro</i>	[23]
8	Wogonin	<i>Scutellaria baicalensis</i>	RSV	7.4 µg/mL	Inhibition of respiratory syncytial virus <i>in vitro</i>	[23]
9	Baicalin	<i>Scutellaria baicalensis</i>	RSV	20.8 µg/mL	Inhibition of respiratory syncytial virus <i>in vitro</i>	[23]
10	Phenolic compounds from <i>Isatis indigotica</i>	<i>Isatis indigotica</i>	SARS-CoV	217 µM	3CL protease inhibitor	[24]
11	Myricetin and scutellarein	<i>Aglaia perviridis</i>	SARS-CoV	-	SARS-CoV helicase inhibitor	[24]
12	Chlorogenic acid	Mongolia medicine	SARS – CoV	-	Inhibition of SARS-associated virus	[25]
13	Rimantadine		SARS – CoV	-	Inhibition of SARS-associated virus	[26]
14	Lopinavir	<i>Ginkgo biloba</i>	SARS – CoV	-	Inhibition of SARS-associated virus	[26]
15	Ribavirin	<i>G. biloba</i>	SARS - CoV	-	Inhibition of SARS-associated virus	[26]
16	<i>Isatis indigotica</i> root	<i>Isatis indigotica</i>	SARS – CoV	Ranging from 10 to 1000 µM	Inhibitory effects on SARS-CoV cell replication	[27]
17	Scutellarin (<i>Erigeron breviscapus</i>)	<i>Erigeron breviscapus</i>	SARS-CoV	48.13±4.98 µM	Block the infection of 2019-nCoV	[28]
18	Hesperetin	<i>Isatis indigotica</i>	SARS – CoV	8.3 µM	Block the infection	[28]
19	Nicotianamine	<i>Veronicaalina</i>	SARS -nCoV	84 nM	Block the infection	[28]
20	Daidzin	<i>Genista tinctoria</i>	SARS – CoV	-	Block viral infection	[29]

3. Plant Cell and callus culture Technology for the Production of novel bioactive compounds

In the past few decades, the outbreak of several diseases has demonstrated the power of biotechnology. The World leaders are leaning on biotechnology and pharmaceutical industries, with the hope of possible solution for the treatment of COVID-19. In the context of plant cell culture, biotechnology offers new techniques applicable to pharmacognosy, such as developing a mechanism to produce various therapeutic compounds or antiviral agents from plants and, also provide mechanism for mass production of existing and novel substance [1, 2, 30, 31].

Modern biotechnological approaches such as plant cell culture appear as a sustainable alternative approaches for the production of secondary metabolites [32], and have opened up the possibility of production of high-value secondary metabolites including therapeutic agents which are clinically useful to treat various ailments [33, 34]. Production through plant cell culture perhaps more expensive than by traditional field-grown plants. However, plant cell culture has a number of advantages over field-derived plant sources, including better quality, production of huge quantities of secondary metabolites at a high efficiency within short time, and year-round production [32]. Various types of plant-derived compounds have been reported to have therapeutic activities such as antiviral, anti-cancer, anti-inflammatory, and antioxidant properties [25]. It is appropriate to bring this information together with recent advances in phytochemistry, and plant cell culture to provide a comprehensive guide to produce high value secondary metabolites, which are used to fight viral diseases.

3.1. Conventional strategies for production of novel antiviral compounds

Secondary metabolites (PSMs) produced by plants is served as a defence chemical compounds against invading pathogens and herbivores. In this regard, the technique available to improve the production of secondary metabolite, elicitor, aims to juggle the cells for a possible biotic-abiotic attack by using elicitors that can trigger the defence response. This results in the release of various chemicals by the cells upon elicitors attack. Elicitors can be divided into two types biotic (biological origin, derived from the pathogen/plant) and abiotic (physical and chemical factors). Elicitors contain signalling molecule such as methyl jasmonate (MeJA), microbial cell wall extracts (e.g., yeast extracts), inorganic salt, heavy metal, physical agents (e.g. radiations) among others. Therefore, the application of indigenous and exogenous elicitors can activate multitude of elicitor induced proteins, some of which are perhaps linked with the accumulation of secondary metabolites in the plant cell culture. Therefore, this technique could have a significant contribution for the production of high value natural products and ultimately essential for pharmaceutical and food industry [35].

Several factors should be optimized in order to boost secondary metabolite production of the *in vitro* cultures. Among them, selection of a mother plant with high contents of the desired substances, growth medium compositions, acidity, inoculum density and the culture room condition (i.e. temperature, light and humidity). The culture medium is one the main factor that significantly affects the biomass and metabolite production, and thus the choice of the appropriate culture medium composition is vital step and should also be based on the physiological requirement of a particular species [35, 36]. Culture medium comprises, salt strength, nitrate and phosphate levels, plant growth-hormone type and concentration, sucrose (carbon source), and so forth. For example, sucrose plays an important role in the signal transduction system through altering gene expression and developmental process [32].

3.2. Plant metabolic engineering approaches for production of novel antiviral compounds

Plant metabolic engineering is an emerging branch of biotechnology that permits altering of specific pathways to improve the production of existing or novel compounds (secondary metabolites) [34]. The technique needs in-depth understanding of the gene expression involves in the biosynthesis of many plant secondary metabolites through metabolomics study [37]. Engineering the biosynthetic pathways allows alteration of secondary metabolite structure to produce novel bioactive compounds having enhanced therapeutic biological activities. This may occur, by overexpressing genes encoding regulatory enzymes involved in biosynthetic pathways, therefore it is possible to increase the productivity of the *in vitro* plant cell culture. Another approach is to suppress the expression of competitive metabolic pathway [38]. This whole procedure needs a collective knowledge of molecular biology, genomics, proteomics, and metabolomics. The main purpose of metabolic engineering is to produce high quality secondary

metabolite with better-quality and quantity. Metabolic engineering also creates a good understanding of biosynthetic pathways and their corresponding final product [36].

The following endogenous plant species contains active phytochemicals possessing anti-SARS-CoV activity and the plant tissue culture was performed for other pharmacological activities. The ethanol extract of leaves of *Torreya nucifera* exhibited good SARS-CoV 3CL(pro) inhibitory activity (62% at 100µg/mL) and on the bioactivity-guided fractionation, a biflavone, amentoflavone (IC₅₀ 8.3µM) showed most potent 3CL(pro) inhibitory effect [39] and abietane diterpenoids were derived from the suspension cultured cells of the same species [40]. The other endangered tree fern, *Cibotium barometz* was used as both ornamental and traditional Chinese medicinal plant [41]. This species showed significant inhibition of SARS-CoV 3CL protease activity with IC₅₀ values of 44 µg/ml [42]. From the *Paulownia tomentosa*, a series of 12 geranylated flavonoid compounds with high inhibition against SARS-CoV were derived as pain like protease [43]. The roots of the same species were micropropagated to improve the shoots and to enhance the quality to transfer to the green house [44]. These studies suggesting the *in vitro* cell culture and micropropagation of the endangered plant species could be beneficial to get enhanced amounts of the active phytochemicals and improve the potential to survive in the natural atmosphere.

4. Future prospects and conclusion

Even today infectious diseases are deadly, though some of them can prevent with drugs or vaccines. Due to complex structure of virus, the development of anti-viral drugs is not an easy process. In addition, the development of inexpensive, non-toxic and effective antiviral drugs including phytochemicals needs high efficiency or needs advanced technologies. Hence, it is important to continue the search for potent natural antiviral agents, which can be expected to prolong the efficacy of drug therapy for infected diseases including COVID-19. Many natural products are found to possess strong antiviral activity and their discovery can further help to develop therapeutic agents. Modern biotechnological approaches such as plant cell culture and metabolic engineering offers an opportunity to improve the secondary metabolites production in terms of quality and quantity. Therefore, these technologies should be integrated in the search of antiviral bioactive compounds from medicinal plants. We strongly believe that plant cell and callus culture plays remarkable role and contribute to antiviral-drug development.

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