

POTENTIAL IMMUNOTHERAPY OF VARIOUS PLANT-DERIVED MONOCLONAL ANTIBODIES IN COLORECTAL CANCER: A REVIEW

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Abstrak

Pengobatan konvensional untuk kanker kolorektal dapat menyebabkan efek samping dan dalam waktu panjang dapat melemahkan sistem kekebalan tubuh. Penelitian terbaru mengenai pengobatan kanker menggunakan metode yang ditujukan pada sel target dan salah satu pengobatan terbaru tersebut adalah antibodi monoklonal (mAb). mAb memiliki banyak keunggulan dibandingkan dengan pengobatan konvensional. Tanaman sebagai sumber alternatif untuk produksi antibodi mAb dapat mengatasi kekurangan dari pengobatan konvensional tersebut. Tujuan dari tinjauan ini adalah untuk mengetahui potensi tiga tanaman sebagai sumber alternatif untuk produksi antibodi dalam mAb anti kanker kolorektal. Tiga tanaman yang telah diteliti sebagai mAb untuk kanker kolorektal adalah *Nicotiana benthamiana* sebagai rekombinan transien Nivolumab yang menargetkan pusat pemeriksaan kekebalan PD-1, *Arabidopsis thaliana* yang memiliki hasil protein terlarut total yang lebih tinggi, dan *Lemna minor* yang menunjukkan peningkatan sitotoksitas seluler yang bergantung pada antibodi yang lebih baik. Artikel ini akan mengulas *Nicotiana benthamiana*, *Arabidopsis thaliana*, dan *Lemna minor* sebagai sumber alternatif yang lebih baik sebagai mAb pada kanker kolorektal.

Kata Kunci: Antibodi monoklonal derivat tanaman, imunoterapi, kanker kolorektal.

Abstract

The conventional treatments for colorectal cancer can cause side effects and weakened the immune system. Recent studies on cancer treatments are aimed at targeted cells and one of them is monoclonal antibody (mAb). mAb has a lot of advantages compared to the conventional treatments. Plants as alternative sources for antibody production in mAb can overcome these advantages. The aim of this review is to know the potentials of three plants as alternative sources for antibody production in anti-colorectal cancer mAb. Three plants that have been studied as mAb for cancer colorectal are *Nicotiana benthamiana* as transient recombinant of Nivolumab that target the PD-1 immune checkpoint, *Arabidopsis thaliana* which has higher total soluble protein yields, and *Lemna minor* that showed better antibody-dependent cellular cytotoxicity enhancement. This paper will review *Nicotiana benthamiana*, *Arabidopsis thaliana*, and *Lemna minor* as more favorable alternative sources for mAb in colorectal cancer.

Keywords: Plant-derived monoclonal antibody, immunotherapy, colorectal cancer.

1. INTRODUCTION

Cancer can cause 10 million deaths in the worldwide every year with approximate percentage of mortality is 70% (Bulaon et al., 2023; Sung et al., 2021). Colorectal cancer is the most common gastrointestinal cancer with the third-most leading cause of death that related to cancer worldwide (Saraiva et al., 2023). A lot of conventional treatments are used to treat colorectal cancer such as radiotherapy,

surgery, and chemotherapy have some side effects in the immune system causing it to weakened and can cause failure in disease improvement. The current treatments for colorectal cancer are hormone therapy, hyperthermia, photodynamic therapy, stem cell transplant, immunotherapy, and targeted therapy that can target cells that undergo malignancy selectively with mild to moderate harm to normal surrounding tissues. Immunotherapy as anti-colorectal cancer therapy has increased in demand especially monoclonal antibodies (mAbs)

(Bulaon et al., 2023; Moussavou et al., 2015, 2018; Shin et al., 2019; Song et al., 2018, 2020). Immunotherapy for cancer has appeared with the method by increasing innate immune responses. Key mechanisms of action of immunotherapy for cancer are T-cell immune checkpoints and their inhibitors that can increase host's immune response to fight tumor cells (Bulaon et al., 2023; Esfahani et al., 2020; Rezaei et al., 2020; Stanculeanu et al., 2016).

Monoclonal antibodies are made from antibodies that contain two heavy chains and two light chains with disulfide bonds and consist of constant region and variable region. The structures of antibodies can prevent pathogen to enter or cause damage in cells and can recruit immunity response to destroy pathogens or tumor cells and interfere the function of antigen by binding with specific antigen (Moussavou et al., 2015; Nessa et al., 2020). From five classes of antibodies (IgA, IgD, IgE, IgG, and IgM), monoclonal antibodies are mostly from IgG1 (subunit of IgG). Monoclonal antibodies (mAbs) are important in biochemistry, biology, and medicine field because of their binding specificity and stability. mAbs can bind to different molecular targets (Donini & Marusic, 2019; Ecker et al., 2015; Moussavou et al., 2015; Shukla et al., 2017).

Chemotherapeutic mAbs act directly by binding cancer and immune cells, flagging cancer cells, attacking cancer cells, blocking cells growth, triggering cell-membrane destruction, blocking immune system inhibitors, preventing blood vessel growth, delivering chemotherapy, and delivering radiation treatment (Coulson et al., 2014; Nessa et al., 2020). Immune checkpoint inhibitor (ICI) is one of anti-cancer mAbs that target cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed cell death receptor-1 (PD-1), and programmed cell death receptor ligand-1 (PD-L1) (Calmeiro et al., 2021). mAbs contain glycans because IgG consists of glycoprotein that have impact in conformation of antibody and its effector functions (Dorokhov et al., 2016; Jennewein

& Alter, 2017; Komarova et al., 2017; Niwa & Satoh, 2015).

Recent studies showed the advanced technology of mAbs including modification and optimization of their glycosylation profiles that varies on the production system of mAbs (Dorokhov et al., 2016; Komarova et al., 2017; Liu, 2015). Plants have some advantages as biofactory systems in the production of mAb such as avoidance of ethical issues because of transgenic animals, easily to cultivate for containment conditions with minimal downstream processing demands, low large-scale cultivation process, and high production capacity (Besufekad & Malaiyarsa, 2017; Hefferon, 2017; Nessa et al., 2020). Plants can be developed rapidly, easily matured, available in large quantities, and widespread. The possibility animal or human pathogen contamination in plant biofactory systems are less likely to happen compared to transgenic animals or mammalian cell lines (Nessa et al., 2020; Oluwayelu & Adebisi, 2016). Many plants that can be engineered for plant-derived mAb production such as *Nicotiana benthamiana* (wild species of tobacco), *Arabidopsis thaliana*, and small aquatic plant *Lemna minor* (duckweed) (Donini & Marusic, 2019; Nessa et al., 2020).

2. REVIEWS

2.1 Colorectal Cancer

Cancer can cause 10 million deaths in the worldwide every year with approximate percentage of mortality is 70% (Bulaon et al., 2023; Sung et al., 2021). Colorectal cancer is the most common gastrointestinal cancer with the third-most leading cause of death that related to cancer worldwide. Risk factors for colorectal cancer are divided into modifiable and non-modifiable risk factors. The modifiable risk factors are excessive body weight, diabetes mellitus, alcohol and tobacco consumption, and western diet which poor in fiber and rich in processed and red-colored meat. The non-modifiable risk factors are family history with colorectal cancer, patients with Lynch syndrome, and patients with

inflammatory bowel disease. Clinical manifestations of colorectal cancer are sudden weight loss, rectal bleeding, abdominal pain, anemia, change in bowel habits, bowel obstruction, and loss of appetite. The most common location of colorectal cancer is in the left colon (rectum and sigmoid colon) (Saraiva et al., 2023).

A lot of treatments are used to treat colorectal cancer from the conventional therapies such as radiotherapy, surgery, and chemotherapy. These conventional therapies have some side effects in the immune system causing it to be weakened and can cause failure in disease improvement. The current treatments for colorectal cancer are hormone therapy, hyperthermia, photodynamic therapy, stem cell transplant, immunotherapy, and targeted therapy. These advanced treatments can target cells that undergo malignancy selectively with mild to moderate harm to normal surrounding tissues (Bulaon et al., 2023).

Immunotherapy as anti-colorectal cancer therapy has increased in demand. Immunotherapies using cytokines, interleukins, monoclonal antibodies (mAbs), humoral transfection agents, and vaccines have been produced by fermentation of mammalian cells, bacteria, and yeast (Bulaon et al., 2023; Moussavou et al., 2015, 2018; Shin et al., 2019; Song et al., 2018; Song et al., 2020). Immunotherapy for cancer has appeared with the method by increasing innate immune responses (Bulaon et al., 2023; Esfahani et al., 2020a; Stanculeanu et al., 2016). Key mechanisms of action of immunotherapy for cancer are T-cell immune checkpoints and their inhibitors that can increase host's immune response to fight tumor cells (Bulaon et al., 2023; Rezaei et al., 2020).

2.2 Monoclonal Antibodies

Antibody contains two heavy chains and two light chains with disulfide bonds.

Antibody consists of constant region and variable region. Constant region has a constant structure for different antigens. Variable region has different structures based on differences in antigens. V_H and V_L are amino acid terminal sequence domains of light and heavy variable chains. C_H and C_L are amino acid terminal sequence domains of light and heavy constant chains. These constants and variable regions can prevent pathogen to enter or cause damage in cells and can recruit immunity response to destroy pathogens or tumor cells and interfere the function of antigen by binding with specific antigen. Region that binds to Fc is called antigen-binding fragment (Fab) can engage with cell surface receptors (Fc receptors) and proteins of complement system (Moussavou et al., 2015; Nessa et al., 2020).

Antibodies or immunoglobulins are divided into five classes (IgA, IgD, IgE, IgG, and IgM) based on their immunological, physiochemical, and structural characteristics. Each class has different Fc regions and functions. IgA is a secretory immunoglobulin which has four light chains and four heavy chains that design as two tetramers like IgG with joining chain and secretory component. The expression of IgG in plants takes two genes while the expression of IgA takes four genes. Besides those five classes, there are smaller antibody derivatives namely minibodies, single-chain variable fragments (scFvs). Large single chains, Fab, F(ab)₂, diabodies, bispecific scFvs, nanobodies, antibody fusion proteins, and camelid antibodies (Edgue et al., 2017; Nessa et al., 2020).

Monoclonal antibodies (mAbs) are important in biochemistry, biology, and medicine field because of their binding specificity and stability. mAbs can bind to different molecular targets (Donini & Marusic, 2019; Ecker et al., 2015; Shukla et al., 2017). mAbs are mostly IgG1 (subunit of IgG) and produced in mammalian cell lines (Dorokhov et al., 2016; Komarova et al., 2017; Niwa & Satoh, 2015). Chemotherapeutic mAbs act directly by binding cancer and immune cells, flagging



cancer cells, attacking cancer cells, blocking cells growth, triggering cell-membrane destruction, blocking immune system inhibitors, preventing blood vessel growth, delivering chemotherapy, and delivering radiation treatment. Chemotherapeutic mAbs bind to cell surface antigens to target specific cancer cells. Cell surface antigens consists of antigens that associated with differentiation and growth. The examples of cell surface antigens are epidermal growth factor receptor (EGFR), cluster of differentiation (CD20, CD30, CD33, and CD52), carcinoembryonic antigen (CEA), receptor activator of nuclear factor kappa-B ligand (RANKL), human epidermal growth factor receptor 2 (HER2), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor (VEGF), extracellular matrix metalloproteinase inducers (EMMPRIN), fibroblast activation protein (FAP), and integrins ($\alpha 5\beta 1$ and $\alpha V\beta 3$) (Coulson et al., 2014; Nessa et al., 2020).

Immune checkpoint inhibitor (ICI) is one of anti-cancer mAbs that target cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed cell death receptor-1 (PD-1), and programmed cell death receptor ligand-1 (PD-L1). ICI acts by inhibiting T cells with PD-1 acting as inhibitory signal transducer in activated T cells and CTLA-4 as negative co-stimulator in T cell-DC interaction (Calmeiro et al., 2021). PD-1 as one of ICI belongs to CD28 or B7 T-cell receptors family that has function as immunotherapy by blocking its receptor that are mostly expressed on activated myeloid cells, B-cells, and T-cells. PD-1 expression levels are increased in tumor microenvironment that promoting evasion of host immune system. Nivolumab is classified as IgG4 mAb that act as PD-1 inhibitor. Nivolumab disturbs the connection between PD-1 receptor with PD-L1 and PD-L2 ligands. Nivolumab production was using transgenic mice immunized with PD-1 that expressed Chinese hamster ovary (CHO) cells. These cells showed high manufacturing expenditure that cause to high treatment costs. These limitation of Nivolumab has

led to advanced alternatives production beside CHO cells (Bulaon et al., 2023).

mAbs contain glycans because IgG consists of glycoprotein that have impact in conformation of antibody and its effector functions.^{19-21,24} Recent studies showed the advanced technology of mAbs including modification and optimization of their glycosylation profiles that varies on the production system of mAbs (Dorokhov et al., 2016; Komarova et al., 2017; Liu, 2015). These advanced studies are used as alternative production systems because of two causes. The first is because the production of mAbs in mammalian cell lines have some side effects caused by possible contamination of mAbs with viruses, foreign nucleic acids, or cell metabolism products. The second is because mAbs that contains new properties with increased efficiency are needed to overcome the limitations in conventional chemotherapeutic mAbs (Komarova et al., 2017).

2.3 Plant-Derived Monoclonal Antibodies

Chemotherapeutic mAbs have some limitations such as limited expandability of mammalian cell lines, potential human pathogen contamination, and high production expenditures. Such alternative organisms that can be used to overcome those limitations are plants, bacteria, yeasts, and insects (Moussavou et al., 2015; Nessa et al., 2020). Plants can be used as advanced biofactory systems because the advantage in large-scale antibody production as chemotherapeutic mAbs. Plants have some advantages as biofactory systems such as avoidance of ethical issues because of transgenic animals, easily to cultivate for containment conditions with minimal downstream processing demands, low large-scale cultivation process, and high production capacity (Besufekad & Malaiyarsa, 2017; Hefferon, 2017; Nessa et al., 2020).

The use of plants that those in mammalian cell lines have some benefits. Plants can be developed rapidly, easily matured, available in large quantities, and



widespread. The possibility animal or human pathogen contamination in plant biofactory systems are less likely to happen compared to transgenic animals or mammalian cell lines (Nessa et al., 2020; Oluwayelu & Adebisi, 2016). Anti-colorectal cancer mAbs using transgenic plants have a promising future for their large quantities with low possibility of animal and human contamination (Lee & Ko, 2017; Song et al., 2020).

Plants have similar secretory pathway and endomembrane system with human systems compared to other prokaryotic and bacteria systems. Plants can produce high yield of antibodies in shorter time but cannot activate immune responses like animal antibodies after introducing into non-self/foreign molecules (Nessa et al., 2020). Cells in plants have posttranslational modification mechanisms like in animals to produce therapeutic proteins such as antibodies that used in mAbs (Dorokhov et al., 2016; Nessa et al., 2020). Posttranslational modification of proteins in plant cells such as in antibodies are supported by chaperones that held the formation and folding of disulfide bonds and the addition of N-glycans is assisted by specific cellular glycosyltransferases. N-glycans hold responsibility for the differences of glycoproteins in plants and mammals. Complex N-glycans demonstrate substantial differences with sialic acid and β 1,4-linked galactose residues in animal cells and α 1,3-fucose and β 1,2-xylose in plant cells (Donini & Marusic, 2019).

mAbs from plants have shown better biological activity results compared from transgenic animals or mammalian cell lines that have advanced Fc- γ receptor binding and enhanced capability of antibody-dependent cellular cytotoxicity (ADCC) (Donini & Marusic, 2019; Marusic et al., 2018; Qiu et al., 2014). mAbs from plants are retrieved by cross-pollinating two transgenic lines that separately transformed with antibody light chain (L_c) or heavy chain (H_c) genes to obtain similar IgG molecules and IgA (Donini & Marusic, 2019). After that, many plant-derived

antibodies have successfully expressed as secretory IgA, single-chain antibody fragments (scFvs), Fab fragments, single variable domains, minibodies, camelid heavy-chain antibodies, scFv-Fc antibodies, and antibody fusion proteins or immunocytokines. These molecules can be used in the production of chemotherapeutic mAbs (Donini & Marusic, 2019; Lomonosoff & D'Aoust, 2016).

The first step in the process of making plant-derived mAbs is inserting selected DNA sequence of transgene (target antigen) into vector before it is moved into expression system. Then the transgene will be expressed in plant cells with transient or stable expression system. The transient expression can generate large quantities of protein in a shorter time (Rahimian et al., 2021). Many plants that can be engineered for plant-derived mAb production such as *Nicotiana benthamiana* (wild species of tobacco), *Arabidopsis thaliana*, and small aquatic plant *Lemna minor* (duckweed) (Donini & Marusic, 2019; Nessa et al., 2020; Yusibov et al., 2016).

2.3.1 *Nicotiana benthamiana*

Rapid scale-up and high biomass yield of high-volume seed production are the main benefits of tobacco rather than other plant species. Whole tobacco plants (leaves and stems) can be made into engineered therapeutic proteins that can increase the upstream production cost (Lim et al., 2015; Nessa et al., 2020). Tobacco plants contains toxic alkaloids and nicotine which need additional extraction procedure. *Nicotiana benthamiana* as a wild species of tobacco can produce N-glycosylated antibodies. *Nicotiana benthamiana* can produce higher amounts of leaf biomass compared to alfalfa plants (Besufekad & Malaiyarsa, 2017; Nessa et al., 2020).

Nicotiana benthamiana is the first plant-derived mAbs that produce by synthesizing catalytic IgG1 6D4 based on cDNA retrieved from murine hybridoma. The transformed plants will express genes encoding two light chains and two heavy chains in the same cell that can be resulted



in heterotetramers generation. The main benefit of transgenic plant into plant-derived mAb is the plants can be easily propagated and the seed can be stored with long duration of time (Komarova et al., 2017). *Nicotiana benthamiana* can be easily infiltrated by injecting into leaf intercellular space with a vacuum or a needleless syringe with a deliver system of plasmid DNA containing the target genes into plant cells using *Agrobacterium tumefaciens* (Komarova et al., 2017).

Nicotiana benthamiana can express mAbs to recognize cancer cells. mAb CO17-1A (IgG2a) binds cancer-associated antigen GA733 that is expressed on the surface of human colorectal carcinoma cells. Plant-derived mAb CO17-1A light and heavy chains were engaged to bind recombinant antigen GA733 and human SW948 colorectal carcinoma cells that express antigen GA733. Plant-derived mAb has similar effect of mammalian cell lines mAb CO17-1A by inhibiting tumor growth of human SW94 colorectal carcinoma cells (Moussavou et al., 2015).

Recent studies of Nivolumab show the production of anti-PD1 IgG4 into plant-derived mAb platform. This procedure including light chains and heavy chains into geminiviral vectors using *Agrobacterium tumefaciens* that were placed into *Nicotiana benthamiana* leaves using vacuum infiltration or needleless syringe injection. The procedures are resulting with a yield of 140 µg antibody/g of fresh leaves. The plant-derived mAb Nivolumab has expected monomer form and molecular weight that similar with mammalian cell lines N-linked glycosylation forms. The mechanism of action was also similar with conventional Nivolumab that have high affinity to human PD-1 thus can inhibit PD-1/PD-L1 binding and T cell response activation (Calmeiro et al., 2021; Rattanapisit et al., 2019).

Avaren-Fc (AvFc) is high-mannose glycans that targets cancer with the use of novel lectin-Fc fusion protein or lectibody in plants (Dent et al., 2022; Hamorsky et al., 2019; Oh et al., 2022). Large quantities of high-mannose glycans has found in many cancers such as colorectal cancer,

choleangiocarcinoma, hepatocellular carcinoma, pancreatic cancer, lung adenocarcinoma, prostate cancer, skin cancer, and ovarian cancer. Thus, AvFc can be a potential therapeutic for cancer. *Nicotiana benthamiana* can be made into plant-derived mAbs with modern techniques such as RNA interference (RNAi), transcription activator-like effector nucleases (TALENs), zinc-finger nucleases, and CRISPR/Cas9. From these techniques, *Nicotiana benthamiana* can be made into variants of biological glycoengineered that have higher levels of activity in vivo. Removal of α1,3-linked core fucose residues in plants using genetic engineering of the expression host can increase the affinity of mAbs for FcγRs (especially hFcγRIIIa) and other Fc-bearing molecules as therapeutic antibodies such as AvFc which has ADCC as major mechanism of action can be made as an advanced plant-derived mAbs (Dent et al., 2022).

2.3.2 *Arabidopsis thaliana*

Between other plant platforms, *Arabidopsis thaliana* has many advantages such as high total soluble protein yield, cost-effective transformation method, and short life span (Carneiro et al., 2015; Song et al., 2015; Song et al., 2020). Endoplasmic reticulum was fused into C-terminus of heavy chain in mAb, thus the accumulation in endoplasmic reticulum retention can signal peptide for high yields of anti-colorectal cancer mAb (Kang et al., 2017; Song et al., 2018; Song et al., 2020). Recent studies show the in vitro function and expression of anti-colorectal cancer mAbs produced in transgenic *Arabidopsis thaliana*. *Arabidopsis thaliana* have high total soluble protein levels and express light chains and heavy chains genes of recombinant therapeutic protein mAbs that can recognize EpCAM as target antigen in human colorectal cancer cells (Song et al., 2015; Song et al., 2020).

Arabidopsis thaliana that expressed anti-colorectal cancer mAb^PCOK and mAb^PCO were retrieved using *Agrobacterium*-mediated transformations.



The amount and expression of purified mAb^PCO were approximately two times lower than purified mAb^PCOK.^{5,8} *Arabidopsis thaliana* is used as a model for molecular and developmental plant biology because the availabilities and benefits in molecular mechanisms governing development, growth, and stress responses. *Arabidopsis thaliana* is bacteria-free and the purified proteins contain no endotoxins from bacterial contamination thus can reduce the need to test and purify (von Schaewen et al., 2018).

Arabidopsis thaliana has many advantages in protein expression. *Agrobacterium*-mediated transformation is widely and easily used in standard laboratories. Each transformed seeds are separated from each other physically and transformed seeds with high level expression will not suffer competitive growth disadvantages rather than in tissue-culture-base transformation protocol. After the identification, the transformed seeds with high level expression will be maintained in petri dish-based cell culture system at 25°C temperature with minimum light exposure and without using incubator. or large growth space. The generated cell lines will multiply in one week and can be harvested at 20-30 g for laboratory-scale experiments. Proteins produced in *Arabidopsis thaliana* will modify into post-translational formation to obtain active complexes, different with heterologous systems. Proteins that engaged in *Arabidopsis thaliana* programmed cell death pathway can trigger programmed cell death in the plant itself (Jeong et al., 2018; von Schaewen et al., 2018).

2.3.3 *Lemna minor*

Lemna minor or duckweed is a monocotyledon plant that belongs to Lemnaceae family. *Lemna minor* has smallest flower with the size in only a few millimeters. *Lemna minor* has simple biological structure with obovate, elliptic, or bilobed shape. *Lemna minor* undergoes asexual reproduction with short cycle which makes it genetically stable without any

genetic changes. *Lemna minor* can be produced from the budding pouch from mother or main plant. Other benefits are easy and simple cultures and can be produced in large-scale quantities. Because of these benefits, *Lemna minor* can be used as a potential recombinant protein in plant-derived mAb (Xu et al., 2015; Yang et al., 2021).

Transgenic *Lemna minor* can be regenerated in three months with the combination of *Agrobacterium*-mediated transformation of nodules and single strain of *Lemna minor* can be regenerated in six weeks. *Lemna minor* also has rapid clonal expansion of transgenic plants, high protein yield, full containment, fast growth rate at 36 hour doubling time, and high secretion of recombinant proteins.¹⁹ *Lemna minor* has been used in medicinal protein fields such as anti-interferon- α 2, human growth hormone, human granulocyte colony-stimulating factor, mAb, porcine epidemic diarrhea virus (PEDV) protective antigen and hemagglutinin. Protein's content of mAb in dry weight of *Lemna minor* is 5.6 g/kg that calculated from 2.8% of total soluble protein. Some studies before that use *Lemna minor* as the alternative in mAb showed the glycosylation was obtained by RNA interference in the process of co-expression of light and heavy strands of mAb (Yang et al., 2021).

Lemna minor has some advantages in biofactory system for plant-derived mAb compared to other plants. The first advantages are the size of *Lemna minor* is small and can easily absorb nutrients from water through roots or fronds. *Lemna minor* can also release proteins directly into culture medium. The second advantage is the growth rate of *Lemna minor* is fast with doubling time at one to two days in compatible environments. The third advantages are *Lemna minor* can adapt to wide temperature range and pH and can grow with rapid rate in compatible environments using inexpensive production costs. The optimum pH that suitable for *Lemna minor* growth is 4.5-7.2. *Lemna minor* can survive in the optimum temperature of 25°C that range from 2°C to

35°C. The fourth advantages are *Lemna minor* has high dry biomass or unit time and crude protein content containing high medicinal and nutritional value that can be used as new energy development, pharmaceutical manufacturing, and fodder processing. The last advantage is *Lemna minor* can easily access to sterile pure culture without chemical and microbial contaminations during the production of recombinant proteins in plant-derived mAbs compared to transgenic animals or mammalian cell lines (Lam et al., 2014; Peng et al., 2016; Xu et al., 2015; Yang et al., 2021; Ziegler et al., 2015).

3. CONCLUSION

Plants as advanced biofactory systems for the production of mAbs have some advantages as biofactory systems such as avoidance of ethical issues because of transgenic animals, easily to cultivate for containment conditions with minimal downstream processing demands, low large-scale cultivation process, and high production capacity. Many plants that can be engineered for plant-derived mAb production such as *Nicotiana benthamiana*, *Arabidopsis thaliana*, and *Lemna minor*. *Nicotiana benthamiana* can be engineered into Nivolumab that show the production of anti-PD1 IgG4 and recent studies of *Nicotiana benthamiana* show that the plant can be made into AvFc that can targets in colorectal cancer. *Arabidopsis thaliana* has many advantages such as high total soluble protein yield, cost-effective transformation method, short life span, and can express protein for mAbs that can recognize EpCAM as target antigen in human colorectal cancer cells. *Lemna minor* has some advantages in biofactory system such as rapid clonal expansion of transgenic plants, high protein yield, full containment, fast growth rate, and high secretion of recombinant proteins.

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