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Review article

Using metabolomics to discover the immunomodulator activity of food plants

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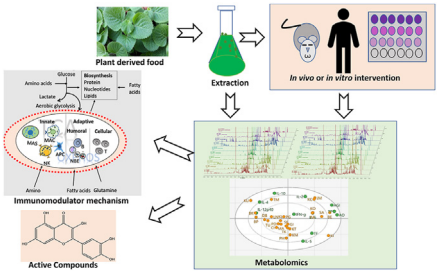
GRAPHICAL ABSTRACT



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ABSTRACT

Many edible plants exhibit immunomodulator activities that have beneficial effects on human health. These activities include the ability to activate, multiply, or suppress elements of the immune response. Some of these plants promote health by strengthening host defences against different diseases. In this article, we provide a comprehensive review of the constituents of several edible plants, their immunomodulatory activity, and mechanism of actions for *Carica papaya*, *Coffea* sp, *Asparagus cochinchinensis*, *Dioscorea alata*, beans, mushrooms, herbs, spices, and several vegetables. The studies reported here are pre-clinical (in vitro and in vivo) and clinical studies (limited in number). The bioactive compounds responsible for the immunomodulator activity of these plants were yet to be identified. This is because the plant is naturally a complex mixture, whilst the immune system is also an intricate system involving many cells and cytokines/chemokines. Metabolomics is a key tool for conducting global profiling of metabolites in a complex system. Therefore, it offers the ability to identify the presence of compounds in plant extracts associated with their immunomodulation effects. Likewise, metabolomics can also be used to detect any changes to metabolites in the cell as a response to treatment. Therefore, affected metabolic pathways that lead to the activation of certain immune responses can be determined from one single experiment. However, we found in this review that the use of a metabolomics approach is not yet fully developed for an immunomodulator study of food plants. This is important for the direction of future research in this field because unlike medicinal plants, food plants are consumed on a regular basis in small amounts with more obvious effects on the immune system. Information about possible bioactive compounds, their interactions (synergism, antagonism), and how the human body responds to them should be studied in a more holistic way.

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1. Introduction

Immune system dysfunction has been attributed to a number of illnesses, such as autoimmune diseases, allergies, and malignancies [1]. Discovering compounds that can act as immune response modulators, and thus be used to cure or prevent various diseases has been an interesting subject recently. There are many immunomodulator medications that have been developed that are both synthetic and natural products. However, the identification and development of new natural immunomodulators continues to be important because certain existing medicines can be less effective or have intolerable side effects [2].

Despite a large number of studies on the potential uses of plant derived immunomodulators, few have progressed to the clinical stage. Six of them, including curcumin, colchicine, epigallocatechin-3-gallate (EGCG), resveratrol, capsaicin, and quercetin have been reviewed elsewhere [2]. However, based on the available data, long research stages are still required to obtain an approved drug status. The authors of this previous review cited a lack of sound and meaningful clinical intervention studies as the key barrier to their research, particularly randomised double-blind placebo-controlled studies, which are referred to as the gold standard for determining a substance's curative or preventative potential. Another relevant issue involves the natural existence of plant extracts as complex mixtures of different phytochemicals. This characteristic can be both a benefit and a disadvantage for plant-derived bioactive chemical discovery efforts [3]. The rich molecular diversity of plants is connected to their special biological functionalities, which are incomparable to those of synthetic compounds [4]. Additionally, they require an elaborative purification step to isolate new pure compounds. Antagonism or synergy between components in this complex composition offers a significant intellectual challenge that can be time- and cost-consuming [5]. Recently, metabolomics has assumed an important role in addressing these challenges. Metabolomics-based studies on the identification of compounds from *Orthosiphon stamineus* Benth. that bind to the adenosine A1 receptor [5], cytotoxic compounds from *Plectranthus amboinicus* (Lour.) Spreng. [6], and cytotoxic compounds from rice brans [7] are some examples. Other successful metabolomics applications have also been reported on herb quality control to determine the purity of commercial oregano samples [8] and to differentiate ginger samples grown in two different locations [9].

There are few review papers published on the topic of plant-derived immunomodulatory compounds, but the subjects are mostly medicinal plants. A list of medicinal plants with immunomodulator effects has been reported in recent studies [1, 10]. For instance, oleanane-type saponins from *Cephalaria tchihatchewii* Boiss., tchihatchewoside A and tchihatchewoside B, significantly stimulated IL-1β cytokine secretion in vitro, indicating their potential to activate the innate immune response [11]. Terpenoid taraxerone, isolated from the Indian medicinal plant *Leucas lavandulifolia* Sm., displayed an in vitro immune-suppression effect by decreasing the peripheral blood mononuclear cells (PBMC) proliferation rate and interleukins IL-4 and IL-6 in phytohemagglutinin (PHA) - induced polymorphonuclear leukocytes (PMN) cells. The compound also exhibited a significant in vivo phagocytosis suppressive effect on experimental mice [12].

Many of the identified plants with immunomodulatory effects are edible and a part of a daily human diet. This is especially important because regular ingestion of these compounds, even in small amounts, may have noticeable impact on the body. This occurs by inducing the immune response to prevent or alleviate the effects of a disease or to temper the immune response in the case of an overreaction of the immune system. Therefore, the aims of this review were: (1) to analyse recent studies on the immunomodulation activity of various food plants (in vitro, in vivo, clinical stage) to determine if they included identification of active constituents and possible mechanisms of action, (2) to examine the use of the metabolomics approach to discover immunomodulators in food plants, and (3) to critically analyse the current plant-derived immunomodulator research and discuss how metabolomics could be applied to accelerate the identification of bioactive compounds of interest.

2. Methods

The literature cited in this review was retrieved from scientific databases, including Science Direct, Web of Science, PubMed, and Google Scholar, with relevant keywords, such as “plant”, “herbs”, “spices”, OR “food” AND “immunomodulator”, immunoregulation, OR “immune system”, AND “metabolomics” OR “active compound”. We selected papers written in English that discussed the immunomodulator activity of edible plants (vegetables, spices, fruits, and other plant-based foods, such as tea or coffee) using pre-clinical studies (in vitro or in vivo) or clinical studies. Only papers published between 2011–2021 were selected. Older papers explaining basic principles of the immune system were also included when they were relevant.

3. Immune responses and inflammation in brief

The term ‘immune system’ refers to the action of a number of cells, tissues, and organs in detecting, fighting, and eliminating foreign substances that enter the human body and may cause illness [13]. There are two type of immune responses, innate and acquired. The innate immune system is the first-line defence system that is genetically programmed to protect the body from pathogenic substances. It identifies pathogens by recognising the pathogenic molecular patterns released from injured cells using scavenger receptors and pathogen recognition receptors (PRRs) [14]. PRRs are mostly found on macrophages and dendritic cells and can later be stimulated to function as antigen-presenting cells (APC). PRRs can also be found on natural killer cells (NK), endothelial cells, and cells of the adaptive immune system [15]. Whether on the cell surface or intracellular, PRRs rapidly and efficiently alert the host to the presence of infection and activate pro-inflammatory and antimicrobial responses by modulating a range of intracellular signaling pathways, including adaptor molecules, kinases, and transcription factors. As a result, proinflammatory cytokines and type I interferons are secreted [16]. However, the innate immune system is not pathogen-specific. It relies on the ability of a group of proteins and phagocytic cells to recognise common characteristics of pathogens and then become rapidly activated to destroy intruders [17].

The adaptive immune system; however, is highly specific. It consists of T and B cells and involves antigen receptors that are created *de novo* in each individual [14]. In humoral immunity, one part of the adaptive immune system, helper T cells (THs) assist in the development of B cells into plasma B cells that are capable of producing antibodies against an antigen target. This immune response is designed to mitigate extracellular infections. Antigens are bound and neutralised by antibodies, leading to lysis or phagocytosis. In contrast, cell-mediated immunity, the other part of the adaptive immune system, is designed to fight intracellular infections and is mediated by T cells. T cells recognize pathogens only after antigens have been digested and presented on antigen-presenting cells (APC) with a major histocompatibility complex (MHC) molecule. THs secrete cytokines which assist activated T cells in adhering to the infected cell's MHC-antigen complex and differentiating into cytotoxic T cells. This leads to the lysis of infected cells [13]. T and B cells both play important functions in protecting against infectious diseases and have the ability to eliminate malignant cells [18]. Most PRRs in the innate immune system, especially the family of toll-like receptors (TLRs), have the capacity to elicit adaptive immune responses of several effectors, such as immunoglobulin M (IgM), immunoglobulin G (IgG), immunoglobulin A (IgA), T helper cell 1 (TH1), TH17 CD4+ T cells, and CD8+ T cell responses [19].

The entire immune system is an extremely complex interconnecting network that includes both pro- and anti-inflammatory agents. Interaction among cells induced by antigen presentation or soluble chemicals (cytokines, chemokines) facilitates communication between the adaptive

and innate immune systems, as well as the humoral and cellular immune systems (Figure 1). These interactions do not have to be mutually restrictive. The reaction to antigens is frequently a primary source of stimulation for adaptive immune cells to generate cytokines. These responses may activate or repress the target cells. Such communication networks are the consequence of the orchestration between the innate and adaptive immune systems, or of their elements [15].

Inflammation occurs when innate immune cells identify infection or tissue damage [20]. The body's defensive mechanism involves changes in circulation, such as increased blood flow and increased permeability of larger molecules and cells crossing blood capillaries from the circulating blood into the neighbouring tissues. The well-known symptoms of this process are redness, swelling, fever, and pain [13]. Excess inflammatory mediators are secreted by macrophages, monocytes, and other inflammatory cells during inflammation. Although the inflammatory response has an important role in protecting cellular physiological conditions, this process lasts only as long as needed to prevent any escalation of its undesirable conditions. Uncontrolled and excessive inflammation can lead to various health problems [21]. Overproduction of pro-inflammatory cytokines (such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), prostaglandin E2 (PGE2), nitric oxide (NO), and increased reactive oxygen species (ROS) production characterises the inflammatory response [13].

4. Recent reports on edible plants with immunomodulatory activity

4.1. *Carica papaya Linn.*

The fruit of *Carica papaya* Linn., or commonly known as papaya, is commonly consumed as food. The flowers and leaves of this plant are also eaten as vegetables. In terms of the immunomodulatory effects of papaya, there has been more research on the potential of the non-consumable component of the papaya (i.e. seeds) than on the papaya flesh itself [22]. Only reports on the edible part of papaya are discussed here. Papaya leaves are consumed as vegetables in many areas of Indonesia. The immunomodulatory activity of a water extract of papaya leaves (CP) on human PBMC has been reported [23]. The PBMC cytokine production with and without the presence of the CP fractions was measured. It was discovered that the production of IL-2 and IL-4 decreased dose-dependently. In contrast, TH1-related cytokines, IFN-γ, TNF-α, IL-12p40, and IL-12p70 increased at low CP concentration (0.124%) and then decreased at higher concentrations (0.25% and 0.50%), while the secretion of IL-5, IL-6, IL-10, and IL-15 was unaffected. CP did not affect PBMC proliferation. The authors concluded that CP modified not only innate but also adaptive immunity. In order to elucidate the active components, CP extract was also fractionated into portions containing

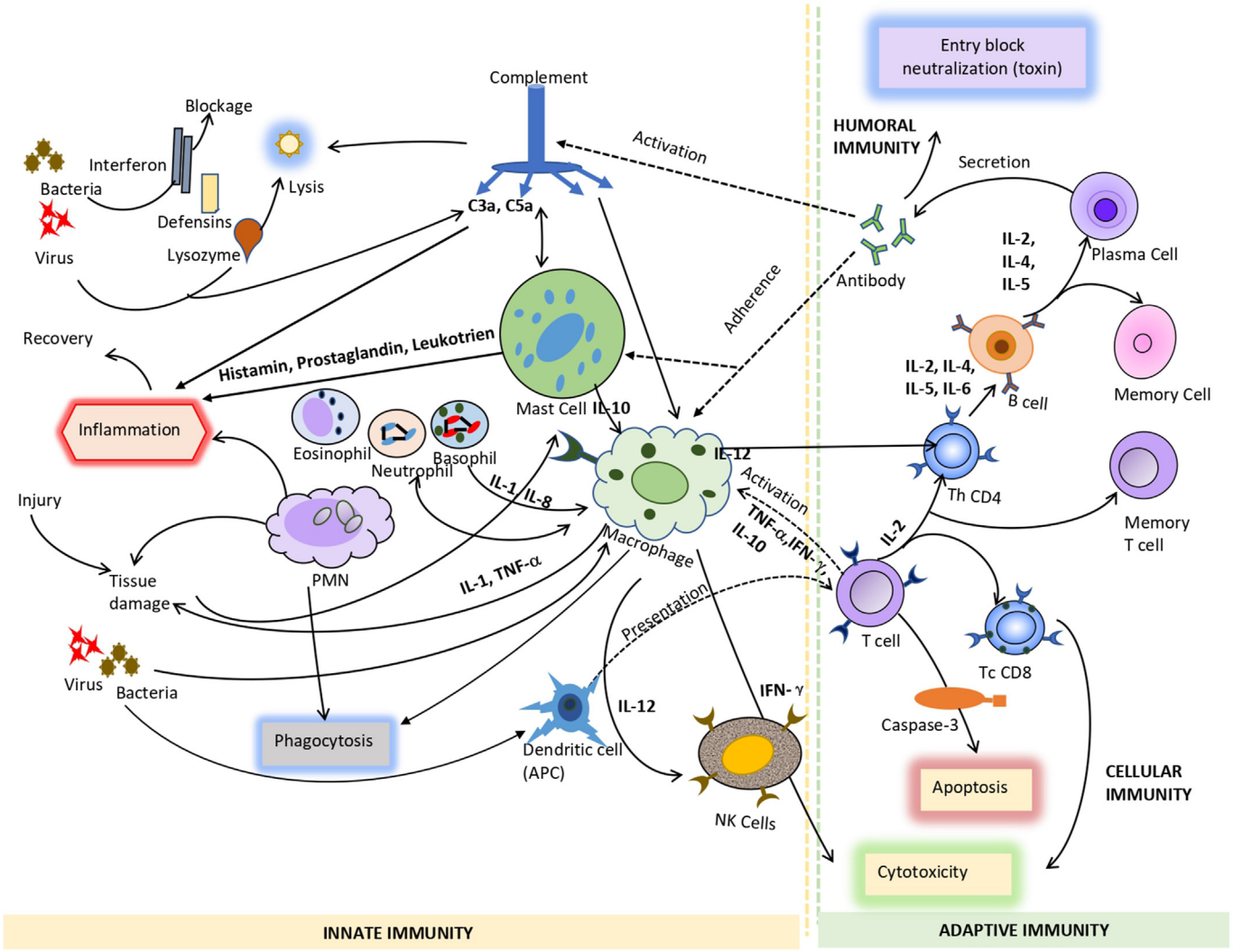

Figure 1. A simplified representation of the immune system, displaying the complex interaction network that involves both pro- and anti-inflammatory chemicals. Black arrows depict immune-cell interactions induced by antigen presentation or soluble molecules (cytokines, chemokines) that facilitate communication between the adaptive and innate immune systems, as well as the cellular and humoral immune systems.

components with different molecular weights using a cellulose membrane. The results showed that addition of a CP fraction less than 1000 MW upregulated TH1-type cytokine secretion similar to a treatment with CP crude extracts. However, no further analysis was conducted to elucidate the active compounds. CP was also found to induce apoptosis by activation of caspasse3/7 in Jurkat cells.

A study of the immunomodulatory effects of the most eaten part of the papaya, its fruits, in healthy humans was conducted a decade ago. Six male and six female participants (23–26 years old) were given controlled diets with 2 pre-exposure days. They were given 100 g of papaya fruit for each of the major three daily meals for two consecutive days [24]. Peripheral blood samples were taken in the morning before meals on the third and fifth days. A significant suppression of IFN-$\gamma^{+}CD4^{+}$, upregulation of IL-$4^{+}CD4^{+}$T cells, and upregulation of $CD3^{+}CD4^{+}CD25^{+}CD127^{+}$ were found in subjects consuming papaya compared to control, while the $CD8^{+}$T cells were not affected. The authors also conducted an in vitro experiment using the PMBC of six healthy male and six healthy female subjects (21–32 years old). The cells were treated with 125, 1000, 4000, 16000 μg/mL papaya extract. As a result, $CD4^{+}CD25^{+}CD127^{+}$ Tregs significantly increased at the dose of 4000 μg/mL among male subjects after 48 h incubation. In contrast, TNF-α, IL-6, IL-8, and IL-10 cytokines were significantly suppressed after 48 h in all subjects. Only the IL-1β of male subjects was upregulated and was positively correlated with Tregs, but there was no significant correlation found in female subjects. It was reported that IL-1β and Tregs expression are important for the TH2 role [25]. Based on these findings, the authors presumed that the immunomodulatory activity of papaya fruit was via enhancement of the TH2 role in mediating humoral immunity. TH2 was more prone to a decrease in $CD25^{+}$Tregs than TH1 [26].

In Japan, fresh papaya (FP) is often fermented by yeast and lactic acid bacteria and is commercially sold as a functional health food supplement. In one study, FP extract orally administered to mice sensitised by FITC (fluorescein isothiocyanate) or oxazolone (4-ethoxymethylene-2-phenyloxazol-5-one) suppressed allergic reactions. FTIC or oxazolone challenge was done on both the dorsal and ventral right ears. The results showed that FP treated mice had significantly lower ear swelling (in mm) compared to those of the control mice. Colon immunohistological observation showed that FP treated mice had significantly lower IgA and dendritic cell expression in the colon. FP treatment substantially reduced the level of IFN-γ, IL-10, and TNF-α in plasma. The authors concluded that antioxidant compounds found abundantly in papaya, such as vitamin C and carotenoids, might contribute to this FP immunomodulatory activity, although no experiment was conducted to prove it [27]. In a more recent study, a fermented papaya (9 g/d) preparation given to tube-fed elderly patients for 30 d restored PBMC cytolytic activity and natural cell cytotoxicity substantially increased. Other related cytokines measured in this study (IL-2, IL-6, IL-10, INF-γ, and TNF-α) were not affected. The phenolics profile of the fermented papaya was analysed using capillary electrophoresis (CE) - and liquid chromatography-time of flight- mass spectrometry (LC-TOF-MS). A number of compounds were detected (2-hydroxy-4-methylvaleric acid, m-hydroxybenzoic acid, 2, 5-dihydroxybenzoic acid, shikimic acid, hippuric acid, homovanillic acid, quinic acid, and m-aminophenol). However, the compounds that were responsible for the cytolytic increment were not explored [28].

4.2. Coffea sp.

A low molecular mass arabinogalactan-protein (AGP) consisting of galactose and arabinose was isolated from instant coffee powder of *Coffea arabica* L. beans. Its immunomodulating properties have been evaluated in mice splenocytes ex vivo [29]. The secretion of several TH1 cytokines (TNF-α, IFN-γ, and IL-2) was upregulated. It was concluded that AGP worked as a pro-T1 polarisation and bio immunological factor, with a focus on cellular immunity. In a newer study, coffee immunomodulatory activity in vivo was assessed using a T- cell receptor (TCR)-transgenic DO11.10 male and female mice allergic model [30]. The mice were administered daily with commercial sugar-free liquid coffee (50%, v/v) *ad libitum* in drinking water for two weeks. The secretion of IFN-γ, IL-2, IL-4, and IL-12p40 in the mice splenocytes was measured, but only IL-12p40 was significantly upregulated in the coffee-treated mice. The IgE in coffee-treated mice was lower than that in control mice, while the IgG1 and IgE levels were not affected.

Both of the above coffee studies did not explain the possible active compound responsible for the reported immunomodulatory effects. Caffeine is the most well-known active ingredient in coffee, but if it is solely responsible for the effects needs to be investigated further. A published review on coffee immunomodulator active components revealed that in numerous investigations, both caffeinated and non-caffeinated coffee had a similar effect on certain cytokines and other immunomodulator indicators, but different effects have been reported in other studies. Other components found in coffee, such as cafestol, kahweol, chlorogenic acid, and trigonelline were associated with antioxidants and the anti-inflammatory properties of coffee [31].

4.3. Herbs, spices, and vegetables

A great deal of research on vegetable immunomodulator activity has been published. Among the most recent studies is one that screened the in vitro proliferation activity of several methanolic extracts of vegetables and spices on the human lymphocytes. The tested concentrations for each sample were adjusted to the normal amount of daily consumption. It was found that the most potent samples were the flowers of torch ginger (*Etlingera elatior* (Jack) R. M. Sm.), lemon basil (*Ocimum albostellatum* (Verdc.) A. J. Paton), aromatic ginger (*Kaempferia galanga* L.), and celery (*Apium graveolens* L.). Although the active compounds were not identified, the authors hypothesised that the responsible active compounds were not necessarily phenolic compounds since the ^{1}H NMR spectra of the four most active samples varied. Only aromatic ginger showed clear signals in the aromatic regions of its ^{1}HNMR spectra [32]. More than a decade ago, a number of spices and vegetables commonly consumed in Japan were screened for inhibition activity against IgE-mediated β-hexominidase released from RBL-2H3 cells. Methanolic extracts of estragon and thyme were found to be the most potent. The active compound in estragon (IC_{50} 5×10^{-5} M) was identified as 7-methoxy coumarin, while luteolin, 5,4′-dihydroxy-6, 7, 3′-trimethoxyflavone, 5,4′-dihydroxy-6, 7, 8, 3′-tetramethoxyflavone, and 5-hydroxy-6, 7, 8, 3′, 4′-pentamethoxyflavone were identified as the active compounds in thyme with IC_{50} values of 6.4×10^{-6} M, 9.2×10^{-6} M, 1.1×10^{-5}, and 3.4×10^{-6} M, respectively. No cytotoxicity appeared at coumarin concentrations less than 1.0×10^{-4} M and flavone concentrations less than 5×10^{-4} M. Next, the authors further tested the inhibitory activity of each compound against phorbol 12-myristate 13-acetate (PMA) and A23187-induced β-hexominidase release from RBL-2H3 cells. Coumarin showed weak inhibitory activity ($>1.0 \times 10^{-4}$ M) while four flavones showed similar activities as in IgE-induced β-hexominidase RBH-2H3 cells [33]. Previously, ginger (*Zingiber officinale* Roscoe) was scientifically proven to be correlated with its traditional use to prevent the common cold. Zakaria-Rungkat et al. (2003) reported NK cell lysing activity was improved significantly after treatment with ginger water extract in both in vivo human (the dose was not mentioned) and mouse (10.4 mg/kg BW) experiments using lymphocyte cultures. Furthermore, the proliferative activity of B and T cells, as well as the CD3+ and CD4+ T cell subsets, were improved with the treatment of oleoresin or gingerol and shogaol fractions of ginger in almost the same manner [34].

The immunomodulatory activity of water extract of several common Umbelliferae family vegetables and spices, such as celery (*Apium graveolens* L., leaves and stem), coriander (*Coriandrum sativum* L., whole plant), carrot (*Daucus carota* L., root), fennel (*Foeniculum vulgare* Mil., root and aerial part), and parsley (*Petroselinum crispum* Nyman Ex A.W. Hill., whole plant) was studied. Analysis was done in vitro with human PMBC taken from healthy volunteers using vegetable and spice extract doses of

50, 100, and 200 μg/mL. At all concentrations tested, the extracts were able to significantly stimulate PMBC proliferation (except for fennel root) compared to the blank control. IFN-γ secretion was significantly stimulated by celery (both stem and leaves) and the areal part of fennel. The authors further tested several compounds commonly found in the studied vegetables and spices, such as bergaten, isopimpinellin, xanthotoxin, quercetin, and rutin. At a dose of 20 μg/mL, quercetin and isoipimpinellin significantly stimulated PMBC proliferation. IFN-γ secretion was strongly enhanced by bergetin, quercetin, and rutin at 2 and 20 μg/mL concentrations. Lower stimulation was shown by isopimpellin only at the 20 μg/mL dose, while xanthotoxin activity at the 2 μg/mL dose was stronger than at 20 μg/mL. At the dose of 20 μg/ml and 3 days of incubation time, quercetin was found to significantly increase total T cells, total B cells, active T cells, CD4+, CD8+, and NK cells compared to control (buffer only). After 6 days, only active T cells and CD4+ were increased significantly [35]. Previously, Mencherini et al. (2007) reported that aquaeous-ethanol (1:1) celery extract and its major compound, apiin, significantly inhibited NO2- release by LPS-stimulated J774.A1 macrophages with an IC_{50} of 0.073 mg/mL and 0.08 mg/mL, respectively. The expression of inducible nitric oxide synthase (iNOS) was also significantly inhibited by celery extract and apiin with IC_{50} of 0.095 mg/mL and 0.045 mg/mL respectively. The cells' viability was not affected by addition of the extract or apiin. In vivo, celery extract reduced inflammation in croton oil-induced mice when applied to the mice's ear at a dose of 900 μg/cm^2 [36].

The immunomodulatory effect of okra (*Abelmoschus esculentus* L.) polysaccharides was studied. It was concluded that crude okra polysaccharides could be useful ingredients for improving immune responses, such as phagocytic activity, spleen index, splenocyte proliferation, and modulating immunological responses via cytokine generation. This was based on the results obtained after two weeks of intervention with okra raw polysaccharide extract in mice with bacterial infections. At the 75 and 100 mg/kg doses, it increased phagocytic activity, spleen index, and splenocyte proliferation while the TNF-α levels increased in groups given crude polysaccharides at dosages of 25, 50, and 100 mg/kg. IL-17 levels decreased in all treatment groups. Crude okra polysaccharides also slightly increased NK cell activity and IFN-γ levels [37]. Further research by the same authors focused on the effect of okra raw polysaccharide extract (ORPE) on immune cells and cytokines in mice with diethyl nitrosamine induced-hepatocarcinogenic conditions. The study demonstrated that ORPE acted as an immune system suppressant or stimulant. ORPE treatment at the doses of 50, 100, and 200 mg/kg body weight directly inhibited regulatory T cell accumulation, suppressed macrophage activation, and balanced the level of effector T cells. However, at lower doses, it stimulated CD8 T cell activation and boosted interleukin-2 levels at all doses [38]. There are currently no reports on non-polysaccharides compounds from okra that show immunomodulator properties.

Red sea weed (*Nemalion helminthoides* [Velley] Batters) sulfated xylomannans can stimulate macrophage cells and significantly increase IL-6 and TNF-α cytokine production as well as generate nitric oxide (NO) in murine RAW 264.7. Similar effects were observed in vivo in BALB/c mice inoculated intravenously with xylomannans [39]. Sulfated and pyruvated polysaccharides (SPs) from green seaweeds *Caulerpa cupressoides* var. flabellata exhibited equal immunostimulatory effects in cultured murine macrophage cells. Generation of NO, reactive oxygen species, and proinflammatory cytokines (TNF-α and IL-6) were all elevated [40]. Previously, xylogalactomanans from *Caulerpa lentillifera* containing carboxyl and sulfated groups were found to stimulate macrophage proliferation in-vitro by increasing phagocytosis, NO generation, and acid phosphatase activity [41].

A food product called *geluring* created from a combination of *Gelidium* sp. and *Ulva lactuca* Linn. seaweed was reported to increase human lymphocyte proliferation and IL-2 production in vitro. Similar effects were reported with a treatment of unprocessed dried *Ulva lactuca* or *Gelidium* alone [42]. Geluring was reported to have high level of dietary fiber (29.19–29.83 % w/w). It had a total phenolic and flavonoid content of 1.38 mg GAE/g and 1.11 mg QE/g, respectively. However, whether the immunostimulation activity of this food product was associated with the fiber or phenolic/flavonoid content, was not clearly explained [43].

4.4. Beans

After cereals, legumes (Fabaceae/Leguminosae) play an essential role in the human diet as a source of proteins, minerals, vitamins, and phytochemicals important for the human health [44]. The research on immunomodulatory activity of beans that we covered in this study related to macrophage activation, which is a critical stage in modulating innate and adaptive immunological responses [45, 46].

The immunomodulatory activity of five common edible beans, including mungbean (*Phaseolus radi*atus L. var. typicus), rice bean (*Phaseolus calcaratus* Roxb), soybean (*Glycine max* (L.) Merr.), small bean (*Phaseolus radiatus* L. var. aurea), and wild soybean (*Glycine soja* Siebold & Zucc.), and their related ingredients was investigated in human PBMC [47]. Aqueous extracts of soybean considerably increased the human PBMC proliferation and IFN-γ release while aqueous extracts of mung bean, rice bean, small bean, and wild soybean only substantially increased the proliferation of human PBMC. Five known bioactive compounds present in soybeans (genistein, quercitrin, phytic acid, syringic acid and indole-3-acetic acid) were tested in the study. Genistein (20 μg/mL) exhibited a significant elevation of CD4+ T cells and a reduction of active T cells. Syringic acid caused immune cell alterations similar to genistein and a significant decrease in activated T cells. Phytic acid significantly reduced total B and active T cells, but did not significantly increase CD4+ T cells. Furthermore, at 20 g/mL, genistein, phytic acid, and syringic acid promoted a TH1-predominant immune response by enhancing the production of IFN- γ (TH1 cytokine) and considerably reducing the immunosuppressive IL-10 secretion.

A study of the in vitro immunomodulatory activity of soyabeans was reported in a previous study [48]. The immunomodulatory effects of ethanolic and methanolic soyabean extracts (SBE and SBM) at different doses (800 g/ml to 6.25 g/ml) were investigated for their effect on murine macrophage phagocytosis, NO, lysosomal enzyme activity, and myeloperoxidase activity. It was found that both SBE and SBM exerted immunomodulatory activities on the phagocytic response of peritoneal mouse macrophages and proliferation activity of mouse bone marrow cells and splenocytes. However, no dose-response relationship was observed. Release of cellular lysosomal enzyme was found at 200 μg/mL and 100 μg/mL of both SBE and SBM. The extracts induced significantly higher amounts of nitrite production and significant stimulation of myeloperoxidase activity at 50 μg/mL, 100 μg/mL and 200 μg/mL. In terms of proliferation response, SBE and SBM induced proliferation activity of both bone marrow cells and splenocytes while higher concentrations exhibited suppression of proliferation. SBE demonstrated the greatest proliferation increase, both with and without mitogen. Based on the results, the author found that soyabean ethanol and methanol extracts activated the non-specific immune system via phagocytosis and B cell proliferation through T cell independent pathways. Compounds in soybean that were associated with this activity were not identified.

Another study reported the immunomodulatory effect of drinking 240 mL of soymilk made from black soybeans (enriched with crude palm oil containing 295.24 μg carotenoid) on diabetic patients every day for one month [49]. Fasting blood glucose levels and HbA1c levels were significantly lower in the test group, while IL-6 and insulin levels increased significantly. The intervention also increased the number of CD4+ and CD8+ T cells. It was concluded that consumption of black soy bean milk caused potential antidiabetic and immunomodulatory activity in diabetes type 2 patients.

Compared to soy beans, there are more studies on the immunomodulatory effect of mung beans. The anti-inflammatory activity of mung bean ethanol extract on lipopolysaccharide (LPS)-induced mouse macrophage cells (J774) was studied. All pro-inflammatory cytokines,

including interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and inducible iNOS were significantly decreased in the cells treated with 3.7 mg/mL of mung bean fraction obtained from stepwise gradient of methanol in methylen chloride. Gallic acid, vitexin, and isoviteXin were found as major compounds in the active fraction [50].

In another study, the anti-inflammatory activities of mung bean (MB), germinated mung bean (GMB), and fermented mung bean (FMB) both in vitro and in vivo were reported. The viability of murine macrophage cells, RAW264.7, after treatment with extracts at 1.25, 2.5, 5, and 10 mg/mL was evaluated using MTT assay. All extracts at high dose (10 mg/mL) exerted a cytotoxic effect on cells after 72 h of incubation. It was found that only GMB and FMB dose-dependently inhibited NO release in RAW264.7 cells at 2.5 and 5 mg/mL concentrations. GMB and FMB aqueous extracts at 1000 mg/kg were shown in in vivo experiments to dramatically reduce ear oedema in mice. These activities were more efficacious than those of MB and FMB. The author suggested that the presence of flavonoids and phenolic chemicals, which are more abundant during germination and fermentation, could explain why GMB and FMB had better anti-inflammatory effects [51]. However, no experiment was conducted to confirm this.

The mung bean is also high in fiber and is composed predominantly of non-starch polysaccharides. Many reports denote the benefit of non-starch polysaccharides on immunomodulation activity. For example, polysaccharides isolated from mung bean water-alkaline extract was found to activate RAW 264.7 macrophages by increasing the release of NO, TNF-α and IL-6 from macrophages [44]. Verbascose, a penta-saccharide from the mung bean, could stimulate RAW264.7 cells proliferation in-vitro. Treatment with 200 g/mL verbascose had the greatest immunomodulatory effect, which was shown by an increase of IL-1β, IL-6, IFN-α, and IFN-γ secretion. Further in-vivo studies revealed that verbascose treatment at a dose of 90 mg/kg body weight for 8 d could significantly improve the spleen index, lysozyme activity in the spleen and serum, serum haemolysin level, and earlap swelling rate in mice [52].

4.5. Mushrooms

Edible mushrooms have been known as an excellent source of proteins, vitamins, minerals, and dietary fibers. It was also reported to have many health beneficial effects, including immunomodulation. Many papers were published on this topic and only some are discussed here. Methanolic extracts from edible mushroom *Agaricus bisporus* (J. E. Lange) Imbach., *Cantharellus cibarius* E. Sheld., *Cratelerrus cornucopioides* Lag., and *Lactareus deliciosus* (0.5 mg/ml) suppressed LPS-induced NO generation in LPS activated RAW 264.7 macrophages by inhibiting iNOS mRNA expression. Interestingly, mushroom extracts reduced LPS-stimulated IL-1 and IL-6 expression but were unable to block TNF-α production or TNF- mRNA expression in the activated macrophages. The author suggested that the observed anti-inflammatory effects, could have been mediated by the selective inhibition of different upstream factors in macrophage activation by LPS. Other species tested in this study with promising anti-inflammatory activities were *A. bisporus, C. cibarius* and *L. deliciosus*. They were able to suppress the production of NO and the expression of iNOS, IL-1β, and IL-6 in the macrophages [53]. Similar results were obtained in another study with wild Irish mushroom extracts. Ethanolic extracts of several wild Irish mushroom, including *Russula mairei, Lactarius blennius, Craterellus tubaeformis, Russula fellea,* and *Craterellus cornucopioides* exhibited selective anti-inflammatory activity by reducing the production of NO and IL-6 in LPS-activated RAW264.7 cells. However, the TNF-α cytokine was not affected [54].

The effects of five commercial edible mushrooms, white button and honey brown (both *Agaricus bisporus*), shiitake (*Lentinus edodes*), enoki (*Flammulina velutipes*) and oyster (*Pleurotus ostreatus*), on NO and TNF-α secretion in LPS and IFN-γ activated murine RAW 264.7 macrophages were reported. All five mushrooms induced the inhibition of NO production (IC_{50} values < 0.1 mg/mL). However, only oyster, shiitake, and enoki mushrooms (IC_{50} 0.035 mg/mL, 0.047 mg/mL, and 0.099 mg/mL, respectively) exhibited potent inhibition of TNF-α release. This study also tested the anti-inflammatory effects of mushroom extracts that had been processed twice, including ultrasonication and heating. When compared to the fresh samples, processed mushrooms displayed a considerable reduction of anti-inflammatory bioactivity, indicating that the responsible anti-inflammatory bioactive chemicals degraded during processing due to susceptibility to heating and other processing treatments [55].

Seven edible mushroom species, namely *A. subrufescens, G. frondosa, L. edodes, H. tessellatus, P. eryngii, P. nameko,* and *P. ostreatus*, were tested on their ability to stimulate dendritic cells (DCs), and the subsequent effects on T cells. DC stimulation was assessed through the measurement of IL-6, IL-10, IL-12, IL-17, TNF-α, and IFN-γ levels by DCs, as well as DCs expression. It was found that each mushroom extract promoted DCs in distinct ways as evidenced by the various cytokine secretion patterns of the DCs. Mushroom species having poor cytokine responses seemed to induce the reduction of several maturation markers, included MHC-II, CD40, CD86, and CD11c. Only *A. subrufescens* showed complete DC maturation among the seven mushroom species studied. Other species increased MHC-II and CD86 expression but had no effect on CD40 or CD11c expression. DCs endocytosed all the studied mushroom species through C-type lectin receptors (CLRs) activation. Additionally, stimulated DCs were found to enhance T cells but without the need for physical contact between DCs and T cells. This indicates the importance of CLRs in the mushroom immune system stimulation effect [56].

4.6. Dioscorea alata L.

The edible part of *Dioscorea alata* (DA), or the water yam, is its tuber. The DA tuber is consumed as a dietary supplement in Southeastern Asia and Africa [57]. The fresh tuber slices are widely used as functional foods in Taiwan, and the dried slices are used as traditional medicine in China (Liu et al., 1995). The in vitro immunomodulatory activity of DA aqueous methanolic extract in Swiss albino mice splenocytes at the dose of 5, 10, 20, 40, and 80 μg/mL was studied [58]. Splenocyte proliferation and levels of released cytokines were measured (IL-2, IFN-γ, IL-4, and IL-10). DA methanolic extract was shown to significantly increase splenocyte proliferation compared to untreated cells. The TH1-related cytokine secretion, IFN-γ, and IL-2 were significantly upregulated at a DA concentration of 20 μg/mL and higher compared to untreated cells. However, the TH2 related cytokine secretion (IL-4 and IL-10) were significantly downregulated at a DA concentration of 10 μg/mL and higher. This finding suggested that *D. alata* actively shifted the TH0 lymphocyte differentiation into the activation of TH1 immune response.

4.7. Asparagus cochinchinensis Merrill

Asparagus cochinchinensis Merrli tuber is a traditional herbal remedy with calming properties that is used to treat a variety of immune-related diseases. Lee et al. (2009) investigated the anti-inflammatory activity of 70% ethanolic extract from *Asparagus cochinchinensis* Merrill (ACE) in reducing mice ear oedema induced by 12-O-tetradecanoyl-phorbol-13-acetate (TPA). The results showed that ACE treatment for 10 d (200 mg/kg BW) significantly decreased both ear oedema thickness and weight. Since the production of pro-inflammatory cytokines IL-1β and TNF-α was also suppressed, the author hypothesised that the anti-inflammatory actions of ACE were related to the inhibition of IL-1β,TNF-α, and as a result of leukocyte proliferation [59].

Similar results were observed in a newer report. The effects of an ethyl acetate extract of *A. cochinchinensis* (EaEAC) in suppressing phthalic anhydride (PA)-induced skin inflammation in IL-4/Luc/CNS-1 transgenic (Tg) mice were studied [60]. The role of IL-4 cytokines during the anti-inflammatory action of EaEAC was investigated after a 2 week intervention. It was found that EaEAC treatment significantly decreased general phenotype biomarkers, including ear thickness, lymph node weight, immunoglobulin E (IgE) concentration, and the number of mast

cells infiltration. EaEAC treatment also reduced the level of IL-1β and TNF-α secretion. However, the associated active compounds were not discussed.

In Figure 2, we mapped all the plants mentioned in this review in a diagram of the overall immune system network. Each plant is placed in a location where it can have an effect. Table 1 also summarises all the plants, including the possible active compounds and the immunomodulator mechanism.

5. Metabolomics application in the research of immunomodulator compounds derived from edible plants

Searching several reputable scientific databases using the relevant keywords showed that there were few reports on the use of the metabolomics approach in elucidating the immunomodulatory effects of edible plants. Only four studies were found to be relevant and are discussed below.

5.1. Seed of date palm (Phoenix dactilyfera L.)

The date palm is an indigenous crop with high nutritional, medicinal, and commercial importance. Moreover, the pulp and seeds are also used in traditional medicine to cure various illness. NMR-based metabolomics was used to investigate the effects of Algerian Deglet date seed extract on the extracellular and intracellular metabolome of LPS-IFNγ-induced RAW 264.7 cells. The study was also designed to investigate the altered metabolic pathway and identify key metabolites associated with inflammation. The LPS-IFNγ-induced RAW 264.7 cells were treated with 100 μg/mL ethanolic date seed extract. The culture medium and the cell suspension were subjected to NMR analysis. Multivariate data analysis principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) were used to visualise the differentiation of normal (NML), induced (IND), and treated (DGS) cells and to identify potentially discriminating metabolites in both the extracellular and intracellular metabolomes. As a result, the intracellular metabolites of NML displayed a different pattern when compared to the IND cells. However, no obvious grouping of intracellular components was identified between the NML and DGS groups. Eleven of the DGS metabolites were considerably different from those in the IND groups, which included several amino acids (glycine, serine, leucine, and phosphocholine) and succinate. These findings suggest that the anti-inflammatory effect of DGS was associated with an improvement of energy metabolism and amino acid metabolism [61]. Several amino acids, such as glycine and leucine, were proposed to play role in the immune system by influencing effector cells, cytokine secretion, antigen presentation, and immune cell proliferation [62]. The succinate oxidation level was also reported to be important in switching the role of pro-inflammatory macrophages into anti-inflammatory macrophages [63].

5.2. Camellia nitidissima C. W. Chi

Camellia nitidissima C. W. Chi is a traditional medicinal and edible herb found in Southern China and Northern Vietnam. 3-cinnamoyltribuloside

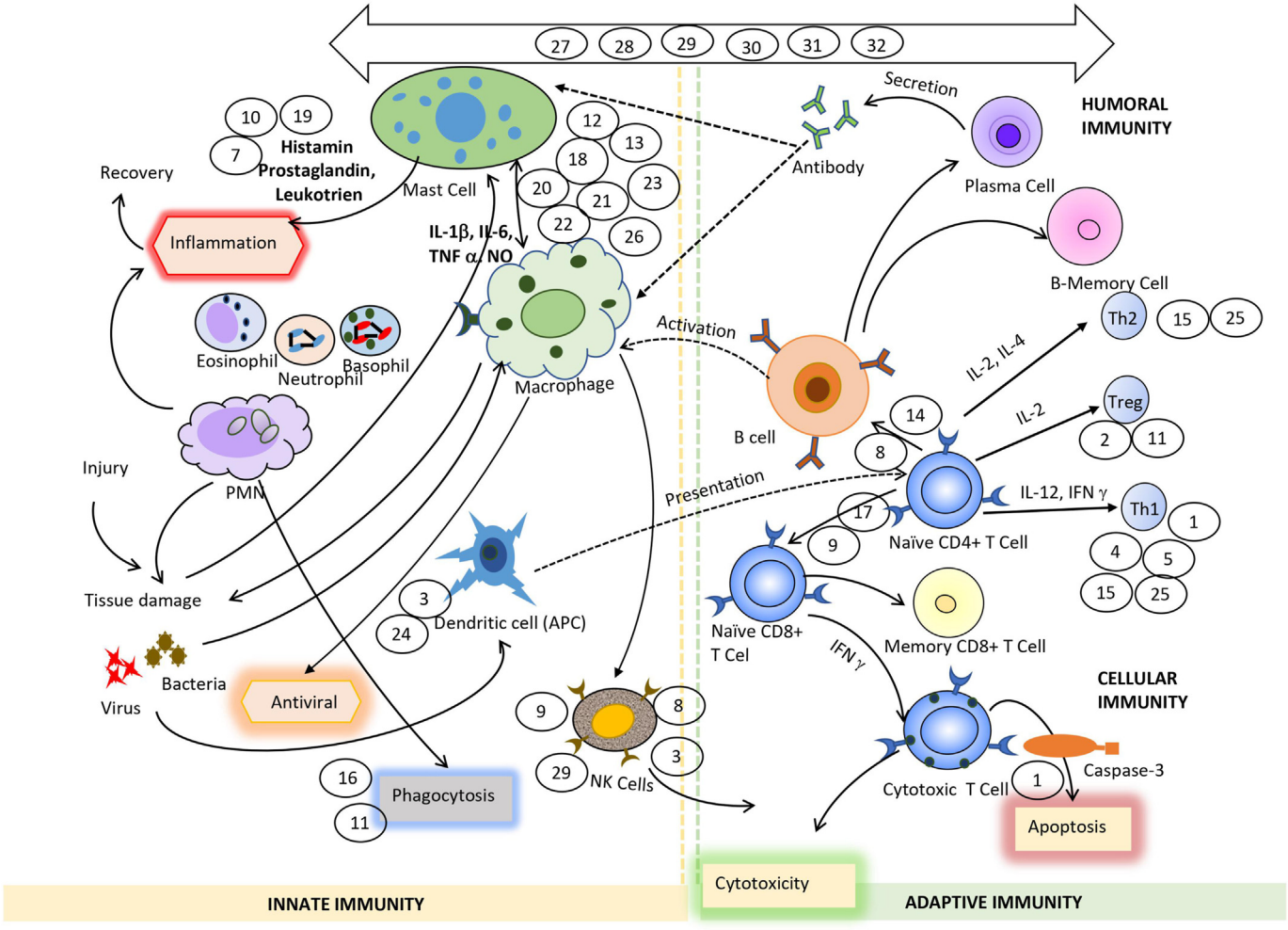

Figure 2. Overall immune system regulation in which the plants discussed in this review are placed in positions where they may have an impact.

Table 1. Summary of studies on the immunomodulator activity of edible plants as reported by targeted approach and metabolomics strategy.

No.	Plant	In vitro/in vivo/ clinical study	Active compounds	Metabolomics/ Targeted	Immunomodulator mechanism			
					Innate	Adaptive		Anaphylaxis
						Humoral	Cellular	
1.	*Carica papaya* Linn. (leaves) [23]	In vitro	ND	Targeted	√	-	√	-
2.	*Carica papaya* Linn. (fruit) [24]	In vitro and clinical study	ND	Targeted	-	-	√	-
3.	*Carica papaya* Linn (fermented fruit) [27, 28]	In vivo (mice) and clinical study	ND	Targeted	√	-	-	-
4	*Coffea* sp. (instant coffee powder) [29]	In vitro	ND	Targeted	-	-	√	-
5.	*Coffea* sp. (ready to drink beverage) [30]	In vitro	ND	Targeted	-	-	√	-
6.	Indonesian spices [32]	In vitro	ND	Targeted	-	-	-	-
7.	Spices (estragon and thyme) [33]	In vitro	In estragon: 7-methoxy coumarin In thime: 1. 5,4′-dihydroxy-6, 7, 3′-trimethoxyflavone 2. 5,4′-dihydroxy-6, 7, 8, 3′-tetramethoxyflavone 3. luteolin 4. 5-hydroxy-6, 7, 8, 3′, 4′-pentamethoxy flavone	Targeted	-	-	-	√
8.	Ginger (*Zingiber officina*le Rosc.) [34]	In vivo and clinical studies	A mixture of gingerol and shogaol	Targeted	√	-	√	-
9.	Umbelliferae vegetables (stem and leaves of celery, coriander, carrot, fennel, parsley) [35]	In vitro	Bergaten, isopimpinellin, xanthotoxin, quercetin, rutin	Targeted	√	-	√	-
10.	Celery (Umbelliferae) [36]	In vitro and in vivo	Apiin	Targeted	√	-	-	√
11.	Okra (*Abelmoschus esculentus* L) [37]	In vivo	Polysaccharides	Targeted	√	-	√	-
12.	Red sea weed (*Nemalion helminthoides*) [39]	In vitro and in vivo	Sulfated xylomannans	Targeted	√	-	-	-
13.	Green seaweeds *Caulerpa cupressoides* [40]	In vitro and in vivo	Sulfated and pyruvated polysaccharides	Targeted	√	-	-	-
14.	A mixture of *Gelidium* sp. and *Ulva lactuca* seaweed [42, 43]	In vitro	ND	Targeted	-	-	√	-
15.	Beans (*Phaseolus radi*atus L. var. typicus, *Phaseolus calcaratus, Glycine max, Phaseolus radiatus* L. var. aurea) [47]	In vitro	Genistein, quercitrin, phytic acid, syringic acid, indole-3-acetic acid	Targeted	-	-	√	-
16.	*Glycine max* [48]	In vitro	ND	Targeted	√	-	-	-
17.	Soymilk made from black soybean, enriched with crude palm oil [49]	Clinical study	ND	Targeted	-	-	√	-
18.	Mung bean [50]	In vitro	ND	Targeted	√	-	-	-
19.	Germinated and fermented mung bean [51]	In vitro	ND	Targeted	√	-	-	-
20.	Mung bean [52]	In vitro	Verbascose	Targeted	√	-	-	-
21.	Edible mushrooms (*A. bisporus, C. cibarius, C. cornucopioides, L. deliciosus*) [53]	In vitro	ND	Targeted	√	-	-	-
22.	Wild Irish mushrooms (*Russula mairei, Lactarius blennius, Craterellus tubaeformis, Russula fellea,* and *Craterellus cornucopioides)* [54]	In vitro	ND	Targeted	√	-	-	-
23.	Edible mushroom (*A. subrufescens, G. frondosa, L. edodes, H. tessellatus, P. eryngii, P. nameko,* and *P. ostreatus*) [55]	In vitro	ND	Targeted	√	-	-	-
24.	Seven edible mushroom species, (*A. subrufescens, G. frondosa, L. edodes, H. tessellatus, P. eryngii, P. nameko,* and *P. ostreatus* [56]	In vitro	ND	Targeted	√	-	-	-
25.	Water yam (*Dioscorea alata*) [58]	In vitro	ND	Targeted	-	-	√	-
26.	Asparagus (*Asparagus cochinchinensis* Merrli) [59]	In vitro	ND	Targeted	√	-	-	-
27.	Seed of date palm *(Phoenix dactilyfera L.)* [61]	In vitro	ND	Metabolomics	√	√	√	-
28.	*Camellia nitidissima* Chi [64]	In vitro	3-cinnamoyltribuloside	Metabolomics	√	√	√	-

(*continued on next page*)

Table 1 (*continued*)

No.	Plant	In vitro/in vivo/ clinical study	Active compounds	Metabolomics/ Targeted	Immunomodulator mechanism			
					Innate	Adaptive		Anaphylaxis
						Humoral	Cellular	
29.	*Lingzhi Ganoderma lucidum* (Leyss. ex Fr.) Karst [65]	In vitro	ND	Metabolomics	√	√	√	-
30.	*Clinacanthus nutans* [66, 67]	In vitro	Clinacoside B, lactic acid, alanine, clinacoside A, and valine	Metabolomics	√	√	√	√
31.	*Echinacea* species (*E. purpurea, E. angustifolia* and *E. pallida*) [69]	In vitro	ND	Metabolomics	√	√	√	-
32.	Yupingfeng granules [70]	In vitro	ND	Metabolomics	√	√	√	-

(3-CT), a cinnamoylglycoflavonoid extracted from the flowers of *C. nitidissima* Chi, was tested for its anti-inflammatory effect in LPS-activated RAW 264.7 cells. The results showed that in LPS-activated RAW 264.7 cells, 3-CT suppressed NO generation and mRNA expression of iNOS. Further, mRNA expression and ELISA assay results showed that 3-CT could decrease the release of inflammatory cytokines, such as TNF, IL-1, and IL-6 in LPS-activated RAW 264.7 cells. The anti-inflammatory metabolic pathway was investigated using ^{1}H- NMR-based metabolomics. Metabolites of LPS-activated RAW 264.7 cells (LPS, control, and high dose-, medium dose-, and low-dose treated groups) were extracted and subjected to NMR measurement. The data were analysed using multivariate data analysis PCA and supervised orthogonal signal correction partial least-squares discriminant analysis (OSC-PLS-DA) to evaluate variability among cell groups and to find distinct metabolites in each group. In the PCA, control cells were clearly separated from other groups. High-dose treated cells were also separated from the others, but the medium- and low-dose treated cells were in overlapping positions with the LPS group. Choline, taurine, glucose, lactate, alanine, and several other amino acids were found as distinct markers among cell groups. This correlated with the changes of the measured cytokine levels leading to a conclusion that in LPS-activated RAW 264.7 cells, 3-CT impacted the cholinergic anti-inflammatory system, oxidative stress, energy metabolism, and amino acid metabolism [64].

5.3. *Ganoderma lucidum (Leyss. ex Fr.) Karst*

Ganoderma lucidum (Leyss. ex Fr.) Karst. or Lingzhi, is a beneficial edible fungus used as dietary supplement worldwide to maintain health. It is also a medicinal fungus which is used to overcome fatigue, immunological diseases, and cancer. Recently, a study that looked into the immunostimulant effect of the oil of *G. lucidum* spores (GLSO), one of the most well-known *G. lucidum*-related products, was reported. The study first evaluated GLSO's immunostimulant activity in mice treated with 400 and 800 mg/kg GLSO by measuring macrophage phagocytosis activity in their splenocytes, and then used LC-MS based metabolomics to characterize the GLSO-induced mice faecal metabolome modification. The findings indicated that a high dose of GLSO (800 mg/kg) had immunostimulant activity in mice by enhancing macrophage phagocytosis and NK cell cytotoxicity. The metabolomics study revealed 143 substantially changed metabolites following GLSO treatment (29 downregulated and 114 upregulated). PLS-DA showed there were substantial metabolic changes between the control and GLSO-treated groups based on the relative abundance of these differentially expressed metabolites. Following GLSO treatment, KEGG metabolic pathways, including arginine and proline metabolism, tyrosine metabolism, porphyrin and chlorophyll metabolism, and serine and threonine metabolism were considerably enhanced. This study also supported the conclusion that amino acid metabolism had crucial role in immune system regulation [65].

5.4. *Clinacanthus nutans (Burm. f.) Lindau*

Clinacanthus nutans is shrub native to Southeast Asia that is widely utilized as traditional herbal medicine and is also consumed as vegetable. A study on the use of NMR metabolomics to link the chemical composition of *C. nutans* with its anti-inflammatory effects in LPS-IFNγ activated RAW 264.7 macrophages was reported. The *C. nutans* extracts were made with different solvents (deionized water and ethanol, 60 extracts in total) and the composition of the extracts was tested using an in vitro NO inhibition assay in LPS-IFNγ activated RAW 264.7 macrophages, with the water extract as the most potent one. Next, all the extracts were subjected to NMR analysis and the data was correlated to their NO inhibitory activity using partial least square analysis (PLS). PLS analysis revealed that clinacoside B, lactic acid, alanine, clinacoside A, and valine found in water extracts may have had a greater influence on the observed NO inhibitory activity than the others. Other compounds (clinamide A, B, and C, phytosterols, glutamine, orientin, and isoorientin) were also shown to have positive but weaker correlation with the activity [66]. Another study from the same researchers used NMR based metabolomics to reveal the essential biomarkers and underlying mechanisms of anti-anaphylaxis property of *C. nutans* leaf extract in an ovalbumin-induced anaphylactic rat model. The rats were treated with 125, 500, and 2000 mg/kg bw *C. nutans* water extracts. Their plasma and urine was subjected to NMR measurement and the data was analysed using multivariate data analysis. OPLS analysis of NMR data showed that *C. nutans* extract provided protection against ovalbumin-induced anaphylaxis by downregulating lipid and carbohydrate metabolism, and upregulating citrate cycle intermediates, propanoate, amino acid, and nucleotide metabolism [67]. Although not measured in this study, *C. nutans* leaf extract may interfere with adaptive immunity. Previous studies have reported that changes in cellular nutrient content have a complex effect on the signalling pathways and function of immune cells [68].

6. Metabolomics application in the research of immunomodulator compounds derived from medicinal plants

Similar to food plants, there were few results retrieved from the database about metabolomics applications for immunomodulator studies of medicinal plants which are not categorized as a part of daily food. Two recent examples are discussed below.

6.1. *Echinacea species*

Echinacea species are among the most widely used herbs in the treatment of flu and other upper respiratory diseases worldwide. A metabolomic technique based on ultra-high-performance liquid chromatography coupled with triple quadrupole mass spectrometry (UPLC-QqQ-MS) was used to conduct a comprehensive investigation of

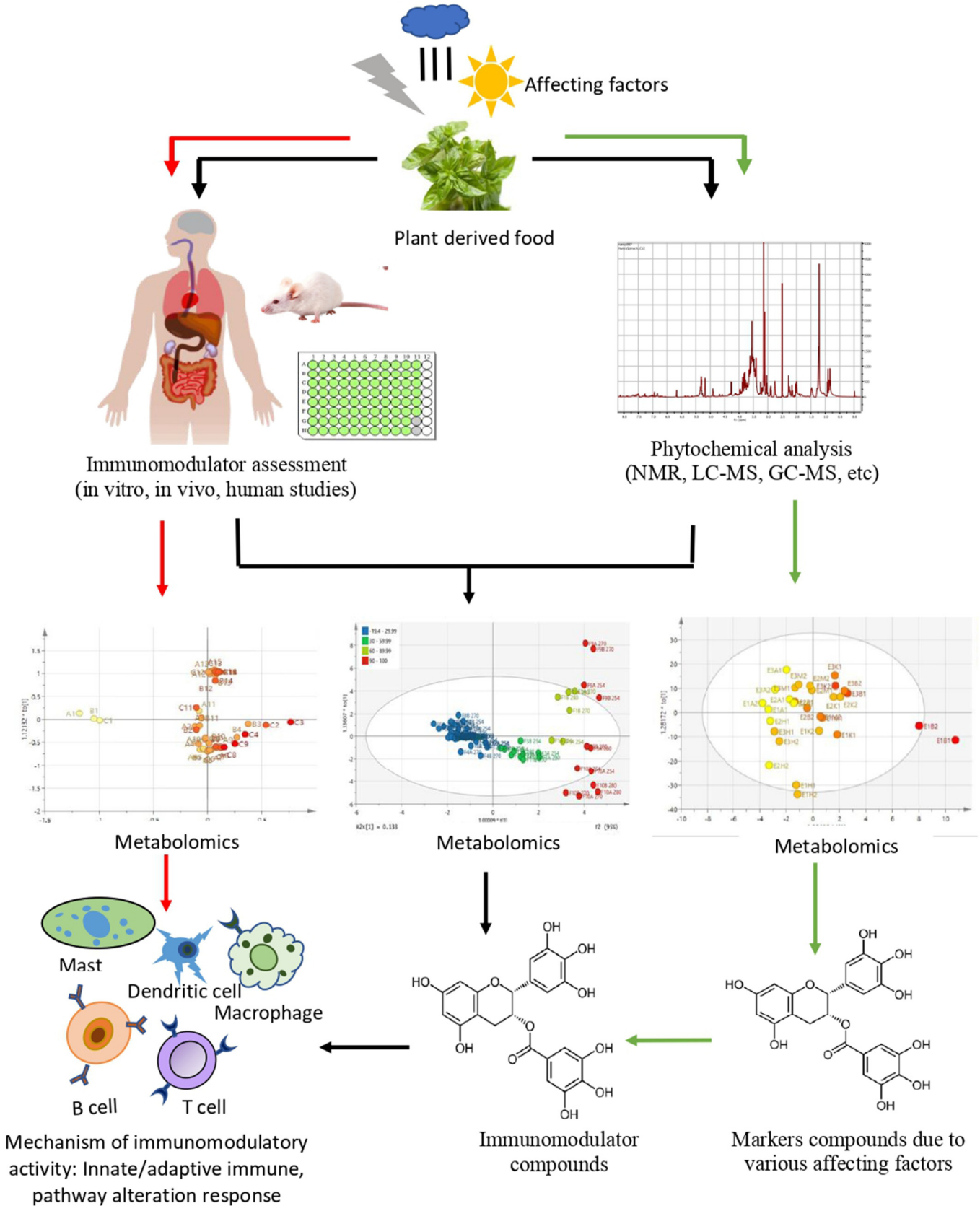

Figure 3. The proposed work flow for the use of metabolomics approach in the immunomodulator study of food plants.

immunomodulatory biomarkers among the various compounds found in the roots and plants of three *Echinacea* species *E. purpurea* (L.) Moench., *E. angustifolia* DC., and *E. pallida* (Nutt.) Nutt. OPLS analysis that correlated the UPLC-QqQ-MS and activity data demonstrated that 8,11-dihydroxy-2,4,9-dodecatrienoic acid isobutylamide, dicaffeoyl quinic acid, echinacoside, and 8-hydroxy-pentadeca-(9E,13Z)-dien-11-yn-2-one, were the compounds positively correlated to RELA (NF-κB downstream intermediate transcription factor p65) pathway activation in the human colorectal adenocarcinoma cell line Caco-2. NF-κB overexpression was also positively linked with 2-undecene-8,10-diynoic acid isobutylamide, dodeca-2,4,8,10-tetraenoic acid isobutylamide, and dicaffeoyl quinic acid. The findings also suggest that the polyenes' immunomodulatory action was linked to an increase in IL-6 production and a decrease in the RELA and NF-κB1 pathways. Additionally, alkylamides also contributed to an increase in IL6, NF-κB, and NO production [69].

6.2. Yupingfeng granules

Yupingfeng granules (YPFG) are a part of traditional Chinese medicine (TCM) made up of three medicinal herbs: Astragali Radix (Huangqi), Atractylodis Macrocephalae Rhizoma (Baizhu), and Saposhnikoviae Radix (Fangfeng). An integrated metabolomics and network pharmacology technique was used to determine the physiologically active constituents, possible biomarkers, and mechanisms of action in the immunoregulatory effects of YPFG in Sprague-Dawley rats. The rats were treated intraperitoneally with dosage of 1.6 g/kg bw which was equal to the adult daily dose of the TCM prescription. The metabolomics analysis revealed that YPFG modulated the levels of various bile acids and glycerophospholipids in order to restore the immunodeficient rats to their normal state. The compound target network was built with nine putative active components in YPFG as the target compounds. Some of the major mechanisms of action were the estrogen receptor, PPAR, MAPK, PI3K-

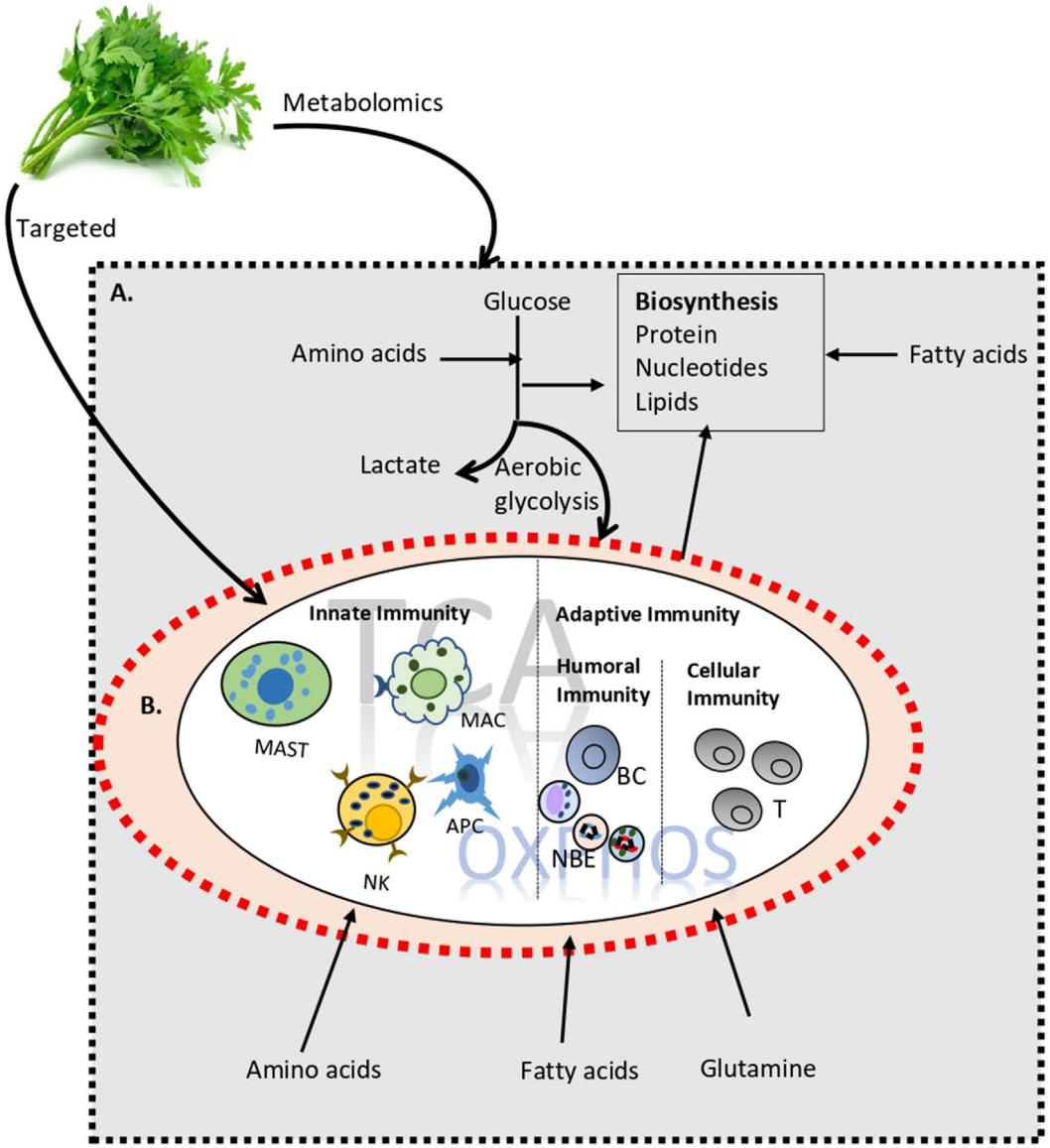

Figure 4. Comprehensive information is retrieved when a conventional targeted approach is used together with the metabolomics method for immunomodulator potential assessment of food plants. When metabolomics is used alone, or in combination with targeted research, a more comprehensive picture is obtained of how the intervention of bioactive components contained in plant-based foods affects the dynamics of the immune system. **A.** Comprehensive information obtained from metabolomics based- research to elucidate the effect of plant-based food to the immune-system. **B.** Information obtained from targeted research. MAST = mastocyte, MAC = macrophage, APC = antigen presenting cell, NK = Natural Killer, NBE = Neutrophy-Basophil-Eosinophil, BC = B cell, T = T cell, TCA = tri carboxylic acid cycle, and OXPHOS = oxidation phosphorylation that occurs in each immune cell.

Akt, JNK signaling pathways, and ubiquitin-mediated protein degradation. This suggests that the immunomodulatory mechanism was intimately tied to lipid metabolism and the inflammatory response. Based on the findings of metabolomics and network pharmacology, YPFG influenced lipid metabolism via the aforementioned pathways, and bile acid metabolism and inflammation [70]. Bile acids induce anti-inflammation of DCs, as well as regulatory T cells in the large intestine [71], while different types of glycerolipids were important nutrients for macrophage polarization [72].

7. Discussion

The summary of immunomodulatory profile of food plants discussed in this review is presented in Table 1. Most of these studies used a targeted approach. Apparently, the metabolomics approach is not widely applied for studying the health effects of food, despite the broader and deeper system information it yields compared to a targeted strategy. In metabolomics, the metabolome of the cells, which is the object of the in vitro experiments, in vivo experiments, or clinical studies, is measured by means of certain analytical tools. Various chromatographic and spectroscopic methods and combinations thereof can be chosen. The most often utilised analytical techniques in metabolomics are gas chromatography–mass spectrometry (GC–MS), liquid chromatography–mass spectrometry (LC–MS), and ^{1}H NMR. Each of these strategies has its advantages and limitations, which should be considered before choosing between them. NMR is an excellent tool for identifying and quantifying compounds, especially those present in a complex mixture. ^{1}H NMR measurements are based on the abundance of the isotope, which is present in nearly all metabolites. This allows the broad spectrum of metabolites to be detected. Calibration curves are not required to determine the relative quantities of compounds in the sample because the absolute number of hydrogen atoms responsible for the signal is represented by the integral of each signal. NMR also has good reproducibility and simple sample preparation. The limitations of NMR are related to the complex spectra and lower sensitivity compared to MS [73]. Moreover, MS is often combined with a chromatography technique (LC or GC). It offers high sensitivity but poses difficulties in the quantification of compounds because different compounds need specific calibration curves [73, 74]. The data resulting from this measurement needs to be pre-processed (e.g. baseline correction and noise removal) before it is subjected to multivariate data analysis. Different multivariate data analyses can be used, the most common ones are unsupervised and supervised partial least square – discriminant analysis (PLS-DA) or orthogonal projection to latent structure-discriminant analysis (OPLS-DA) [75]. Orthogonal partial least square (OPLS) or partial least square (PLS) analysis may also be used to correlate a plant's metabolome data with their immunomodulator activity. The final outcome data is the discovery of discriminating plant metabolites for each intervention group, as well as insight into the effects on the metabolome of the treated cells or organism. With comparison to the effects of known drugs, metabolomics and potentially other omics data, can help identify the possible mechanism of action, or may even help identify novel effects. Therefore, eventually the underlying immunomodulatory mechanisms of the extract and the individual active compounds that are present can be mapped in the human pharmacological network. Measuring the metabolome of the plant extracts itself, is important to learn about the variability of the metabolome considering factors, such as different geographical origins, different cultivars, different processing methods, or even harvesting time. These factors have been reported to influence the plant's metabolome profile, and thus its health-related bioactivities [76, 77, 78, 79, 80]. Taketa et al. (2008) used metabolomics to identify the sedative compounds in a plant by studying plant material from different sources and running the in vivo sedative activity of all ecotypes, followed by supervised multivariate data analysis in which the chemistry and the pharmacology were combined. This clearly showed great variability, but by separating the active and less-active extracts, the PLS-DA showed that modified triterpenes and flavonoids did correlate with activity [81]. Metabolomics is thus a multifunctional tool that can synthesise information about active compounds and their pharmacological effects. It is also the method of choice for quality control. Recent studies of Khoo and co-workers on *Clinacanthus nutans*, which we discussed in this review, are excellent examples of the application of metabolomics to this topic [66, 67]. The proposed work flow for the use of the metabolomics approach in the immunomodulator study of food plants is presented in Figure 3, while Figure 4 illustrates that a more comprehensive information can be retrieved when a conventional targeted approach is used together with the metabolomics method for immunomodulator potential assessment of food plants.

8. Conclusion

The aim of this review was to analyse the work published in the past decade on food plant immunomodulation. In fact, few studies have been published on this topic and the available data were too preliminary to define an evidence-based use of food as a medicine. In this review, we studied the immunomodulatory activity of several food plants, including *Carica papaya, Coffea* sp., *Asparagus cochinchinensis, Dioscorea alata*, beans, mushrooms, herbs, spices, vegetables, and several medicinal plants. Where relevant, the phytochemical substances thought to be responsible for the immunomodulatory activity were also discussed. This information, however, was very limited. Comprehensive research on the immunomodulatory effects of food plants is necessary for developing evidence-based functional food and drug discovery. The goal of the food plant research is to boost the human immune system with less side effects than synthetic drugs/supplements, because food plants are part of a daily diet. Moreover, downregulating the immune system is an interesting target for drug development (patients with transplants) or is useful for diseases in which the immune defense is overreacting, such as in COVID-19. Most of the relevant studies discussed in this review used a conventional targeted approach by testing the plant extracts or fractions in cell lines, mostly for targets of the immune system. The use of experimental animals also focused on measuring certain target proteins or genes. The same applies for studies with human participants. Studies using human participants were scarce and followed a more targeted approach. Because of the complexity of the immune system and the chemistry of the plant extracts, a systems biology approach should be chosen in which unknown constituents from the plant interact with different parts of the immune system. To date, the metabolomics approach has rarely been used in immunomodulator lead discovery studies. We found only several of such studies in our search. When compared to traditional targeted investigations, metabolomics studies covered more extensive features of the immunomodulatory effects of the tested plant that conventional studies do not capture. This should be emphasised in designing future studies on this topic. The following are examples of metabolomics-based studies that can be conducted to improve expected outcomes:

- Correlating the metabolome profile of the food plant with in vitro, in vivo, and clinical immunomodulator data. The result would be the active compounds responsible for the immunomodulator effect.
- Conducting metabolome profiling for the food plants with immunomodulator activity to observe the effects of various factors on the identified bioactive compounds.
- Conducting metabolome profiling to study the responses of cells from the research subjects (cell lines, animals, humans) after treatment with the food plant to determine metabolic pathway alterations and the immunomodulator activity of the plant.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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