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A comprehensive review on Phyllanthus derived natural products as potential chemotherapeutic and immunomodulators for a wide range of human diseases

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ABSTRACT

Treatment options for most cancers are still insufficient, despite developments and technology advancements. It has been postulated that the immune response to progressive tumors is insufficient due to a deficiency in afferent mechanisms responsible for the development of tumor-reactive T cells. Many patients treated for cancer will have their cancer recurrence, often after a long remission period. This suggests that there are a small number of tumor cells that remain alive after standard treatment(s) – alone or in combination and have been less effective in combating metastasis that represents the most elaborate hurdle to overcome in the cure of the disease. Therefore, any new effective and safe therapeutic agents will be highly demanded. To circumvent many plant extracts have attributed for their chemoprotective potentials and their influence on the human immune system. It is now well recognized that immunomodulation of immune response could provide an alternative or addition to conventional chemotherapy for a variety of disease conditions. However, many hurdles still exist. In recent years, there has been a tremendous interest either in harnessing the immune system or towards plant-derived immunomodulators as anticancer agents for their efficacy, safety and their targeted drug action and drug delivery mechanisms. This review discusses *Phyllanthus Schum. & Thonn*. their chemopreventive and immunomodulatory properties over the past few years. Although, as many as 500 important bioactive phytochemical compounds have been isolated from *Phyllanthus* their chemotherapeutic, immunomodulatory properties, molecular targets and modes of action are yet to be enlightened in detail. Hence, the theme of this review is very useful for further research on *Phyllanthus species*. because many phytochemicals from these plants have demonstrated preclinical therapeutic efficacy for a wide range of human diseases.

1. Introduction

Conventional treatment of cancer includes surgery, ionizing radiation, chemotherapy, photodynamic therapy (PDT), or by combining these modalities (Ali-Seyed et al., 2016). It is been known that metastasis that represents the most elaborate hurdle to overcome in the cure of the disease (Jessie et al., 2016). Despite advances in technology which is aiding for various treatments, most cancers still not curable. Many traditional medicines in use are derived from plants, minerals and organic matter and many of them have been investigated for their pharmacological effects in accordance with modern medicine (Daniel et al., 2012; Arora, 2010). Paclitaxel from Pacific yew (*Taxus brevifolia*), capsaicin from chili peppers (*Capsicum species*), galantamine from the Caucasian snowdrop (*Galanthus caucasicus*), vinblastine, vincristine, their semi-synthetic derivatives from the Madagascar periwinkle (*Catharanthus roseus*), are examples of medicines based on plant bioactive principles. The phytochemicals that served as lead structures and/or were chemically altered are salicylic acid (acetylsalicylic acid), camptothecin (topotecan and irinotecan), artemisinin (artemether), dicoumarol (warfarin) and morphine (scores of derivatives) (Oberlies and Kroll, 2004).

The World Health Organization (WHO) has listed nearly 21,000 plants for medicinal purposes around the world. Needless to reiterate that various traditional medicinal systems practiced in India (Siddha and Ayurveda), China (TCM), Indonesia (Jamu) and in African countries (sangoma, n'anga, and inyanga) have proven success in treating various diseases including jaundice, diabetes, dysentery, tumors, vaginitis, kidney stones, diuretics dyspepsia (Singh et al., 2016) besides, they have used to treat hepatotoxicity, hepatitis B, and hyperglycemia, viral and bacterial diseases (Xia et al., 2011; Tewari et al., 2017). Most of the above countries are the resource of many potential medicinal plants with the record of as many as 2000 species (Tewari et al., 2017; Mustafa et al., 2011). In light of these observations, a large number of patients, especially in developing countries, prefer complementary and alternative medicines for treating and managing the symptoms of various diseases including cancer (Arora, 2010). A study by Sirisha et al. (2010) reported that nearly 17.1% population of developing countries uses medicinal plants for their health problems, whereas 29.6% of this

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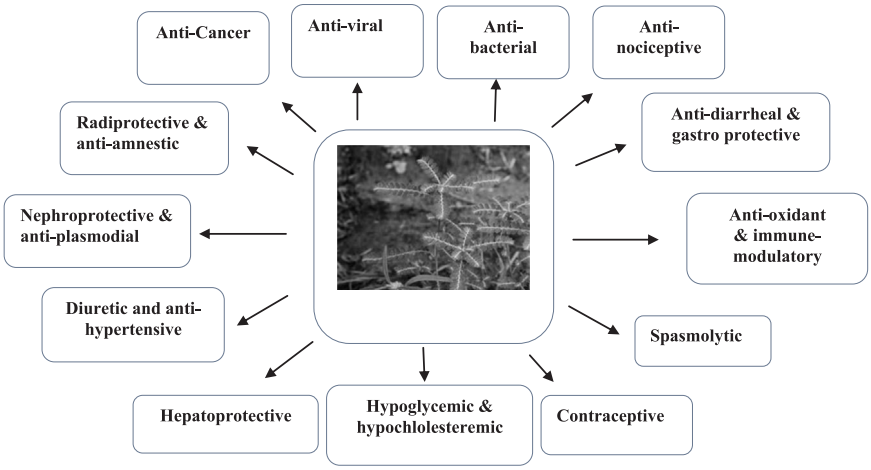

Fig. 1. Exhibits different pharmacological activities of *Phyllanthus species*.

population consumes herbal products for their health maintenance.

Drug formulations using herbal plants rich in bioactive compounds with anticancer and immunomodulatory activities attract natural product scientists with great interest for their efficacy, low risk of cytotoxicity on normal cells as well as minimal side effects. Many therapeutic effects of those compounds have been attributed for their chemoprotective effects (Ali-Seyed et al., 2016; Pang et al., 2018) and influence on the human immune system (Pang et al., 2018; van der Nat et al., 1987; Jantan et al., 2014; Yatoo et al., 2018). Despite many pitfalls, a majority of research and development still focuses on biochemicals, biologics, or single compounds as lead compounds that aim at particular targets linked with a disease. Although in reality it is difficult to attain single compound chemicals with high selectivity and potency, and low toxicity for targeted molecular/cellular targets and diseases, however, hope remains and gaining interest consistently (Abbott, 2011; Pan et al., 2013).

Phyllanthus species Schum. & Thonn. belongs to the family Euphorbiaceae, a small herb well known for its medicinal properties and widely used worldwide (Lee et al., 1996) and exhibits multiple pharmacological actions (Fig. 1) and used traditionally for the treatment of various ailments including hepatitis B and many types of cancer (Foo and Wong, 1992; Chevallier, 2000). This genus, consisting of more than 700 plus species, classified into 11 sub genera (Unander et al., 1995; Xia, 1997). The most studied 24 species are primarily belonging to subgenus Kirganelia, Cicca, and *Phyllanthus*. Most of these popular species have different vernacular names and are currently under practice as traditional medicine by different nationalities (Table 1). More than 500 bioactive principles have been isolated from *Phyllanthus*, the well-characterized ones are flavonoids, triterpenoids, tannins and lignins (Table 2) (Mao et al., 2016).

Phyllanthus elaborates different classes of organic compounds of medicinal importance including alkaloids, flavonoids, hydrolysable tannins (Ellagitannins), major lignans, polyphenols, triterpenes, sterols and volatile oil (Mao et al., 2016). Many lignans were isolated (from *P. amarus*, *P. fraternus*, *P. maderaspatensis*, *P. virgatus,* and *P. urinaria*) viz., phyllanthin (a bitter constituent) and hypophyllanthin (a non-bitter constituent) (Fig. 2) niranthin, nirtetralin, virgatusin, hinokinin and heliobupthalmin lactone (Jantan et al., 2014; Nahar et al., 2011; Shanker et al., 2011; Patel et al., 2011) Flavonoids such as rutin, quercitrin, quercetin, kaempferol, and astragalin (from *P. amarus* and *P. urinaria*) (Foo and Wong, 1992; Nara et al., 1977; Tripathi et al., 2006; Sharma et al., 2011) and numerous ellagitannins such as geraniin, corilagin, and phyllanthusiins (from *P. amarus* and *P. urinaria*) have proven to have therapeutic effects in many clinical studies (Etta, 2008). However, phytoconstituents are yet to be isolated from the following *Phyllanthus species*. such as *P. ajmerianus*, *P. kozhikodianus*, *P. rheedii*, *P. rotundifolius,* and *P. scabrifolius*.

The bioactive principles isolated from *Phyllanthus species* were used traditionally for the treatment of various ailments including, including anti-hepatotoxic, anti-lithic, anti-hypertensive, hypoglycemia properties, anti-HIV, anti-hepatitis B (Bagalkotkar et al., 2011), antitumor (Islam et al., 2008; Harikumar et al., 2009; Guha et al., 2010; Abhyankar et al., 2010; Lee et al., 2011) and immunomodulatory effects (Jantan et al., 2014). However, *Phyllanthus* derived important phytochemicals mostly present in the extracts and their anti-tumor, immunomodulatory properties, molecular targets and modes of action

Table 1
Describes common vernacular names used for *Phyllanthus species* in various countries.

S. No.	Language	Vernacular Names	Ref
1.	Tamil	Keelanelli (Keezhanelli)	(Divya et al., 2011)
2.	Hindi	Bhumi amla, Jangli amli	(Divya et al., 2011)
3.	Bengali	Bhui amla	(Divya et al., 2011)
4.	Rajasthani	Gugario	(Divya et al., 2011)
5.	Oriya	Bhuiaola	(Divya et al., 2011)
6.	Telegu	Nela urika	(Divya et al., 2011)
7.	Kannada	Neera-nelli; Kirunelli	(Divya et al., 2011)
8.	Malayalam	Kilanelli	(Divya et al., 2011)
9.	Sanskrit	Bhoomyaamlakee, Bhoodhatree, Tamalakee	(Divya et al., 2011)
10.	English	Black catnip, Carry me seed, Child pick-a-back, Gale of wind,	(Duque and Descoteaux, 2014), (Engleman et al., 2004)
11.	French	Poudre de plomb (ivory coast)	(Elisa et al., 2013), (Engleman et al., 2004)
12.	America	Yerba de la nina 10, Chanca piedra 13, Hurricane weed	(Duque and Descoteaux, 2014), (Engleman et al., 2004)
13.	Spanish	Yerba magica	(Duque and Descoteaux, 2014), (Elisa et al., 2013), (Engleman et al., 2004)
14.	Malay	pokok Dukung Anak	(Daniel et al., 2012), (Luo et al., 2016)
15.	Thai	Chanca Piedra, Quebra Pedra,	(Daniel et al., 2012), (Luo et al., 2016)

Table 2
Lists of *Phyllanthus species* derived phytochemical extracts and their bioactive principles.

S.No	Phytochemicals	Structural Description	Some important Bioactive principles	References
1.	Alkaloids	Alkaloids are a group of naturally occurring nitrogenous organic compounds of plant origin.	Securinine, nor-securinine, epibubbialine, isobubbialine, dihydrosecurinine.	(Engleman et al., 2004), (Etta, 2008)
2.	Flavonoids	Flavonoids are polyphenolic molecules containing 15 carbon atoms and are soluble in water.	Quercetin, kaempferol, astragalin, quercetin-3-O-glucoside quercitrin.	(Diwanay et al., 2004),(Geetha et al., 2005),
3.	Tannins	Tannin is a polyphenolic biomolecule that binds to precipitate proteins and various other organic compounds like amino acids and alkaloids.	Amarulone, geraniin, amariin,	(Gollnick et al., 2002),(Guha et al., 2010)
4.	Lignans	Plant lignans are also polyphenolic compounds derived from phenylalanine via dimerization of substituted cinnamic alcohols.	Phyllanthin, hypo-phyllanthin, 5-dimethoxy-niranthin, nirtetralin, phyltetralin, hinokinin, 4-(3,4-diethoxy-phenyl)-1-(7-methoxybenzo[	(Diwanay et al., 2004), (Harikumar et al., 2009)
5.	Sterols	Phytosterols, which encompass plant sterols and stanols, are phytosteroid similar to cholesterol, which occur in plants and vary only in carbon side chains and/or presence or absence of a double bond.	Amarosterol A, amarosterol B.	(Diwanay et al., 2004), (Engleman et al., 2004)
6.	Triterpenes	Triterpenes are a class of chemical compounds having three terpene units with the molecular formula C30H48 or consists of six isoprene units.	Phenazine and phenazine derivatives, 2Z, 6Z, 10Z, 14E, 18E,	(Diwanay et al., 2004), (Engleman et al., 2004)
7.	Volatile Oils	A volatile oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants.	Linalool,	(Diwanay et al., 2004),(Engleman et al., 2004), (Gautam et al., 2004), (Luo et al., 2016)

Fig. 2. Shows the structure and composition of some of the important bioactive principles of *Phyllanthus species*.

are yet to be enlightened in detail. Hence, this review discusses *Phyllanthus species* anti-cancerous, chemopreventive and immunomodulatory activities. Besides the above, this review also highlights some targets, which are involved in modifying immune responses in the tumor microenvironment. Therefore, the theme of this review is very useful for further research on this plant as many of the phytochemicals have already shown preclinical therapeutic efficacies for a wide range of human diseases, including HIV/AIDS and hepatitis B.

2. Chemopreventive potentials of *Phyllanthus species*

Previous studies (Bagalkotkar et al., 2011; Lee et al., 2011; Rajkapoor et al., 2007; Ramasamy et al., 2012; Ismail et al., 2012) have assessed different extracts of the *Phyllanthus* plants for various anticancer effects and the related mechanisms both in vitro and in vivo. Preclinical evidence using diverse cancer cell lines to establish that all cell lines considerably inhibited by *P. emblica, P. urinaria, P. polyphyllus, P. watsonii,* and *P. pulcher* by sparing normal cells. The extracts inhibited the growth of cancer cells by DNA fragmentation and dysfunction of mitochondria including up-regulated mitochondrial fission 1 protein and down-regulated optic atrophy type 1 and mitofusin 1 (Huang et al., 2014). Furthermore, the extracts also repressed the ability of cell invasion, migration, and adhesion. Additional investigations demonstrated that the fractions induced apoptosis, invasion, and migration by increasing the expression of various proteins such as caspase-3, 7,- 8, and p-JNK and decreasing the expression of ERK, p-ERK1/2, JNK, MMP-2, 9, Wnt, NF-κB, Myc/Max, and hypoxia ((Ngamkitidechakul et al., 2010; Zhao et al., 2015; Tang et al., 2013). To support the above, Ehrlich ascites tumor model was used for evaluating the antitumor activity of *P. polyphyllus* (Rajkapoor et al., 2007). This study has demonstrated that oral administration of methanol fraction of extract (up to 200 mg/kg) significantly reduced the solid tumor volume. Hematological parameters, protein, packed cellular volume (PCV), and antioxidant enzymes such as LPO, GPx, GST, SOD, and CAT were also greatly regulated.

It is well documented that *P. amarus* extract was found to be rich in alkaloids, flavonoids, hydrolysable tannins, triterpenoids, lignans, polyphenols, sterols and volatile oil (Ram et al., 2011) and reported that the aqueous extract of *P. amarus* increase the lifespan in rats with liver tumor as well as effectively inhibited the development of n-nitrosodiethylamine (NDEA) induced-hepato-carcinogenesis in animals (Joy and Kuttan, 1998). In these studies, it was found that the extract functioned through inhibition of the P-450 enzyme that suppressed the conversion of NDEA into hepato-carcinogenic active ethyl radical metabolite (Jeena et al., 1999). In addition, the aqueous extract induced the cell cycle arrest through the suppression of cdc-25 tyrosine phosphatase (Rajeshkumar and Kuttan, 2000). To support this, the 70% ethanol extracts of three *Phyllanthus species* including *P. amarus* were also reported to induce apoptosis in a hepatocellular carcinoma HepG2 cell line in vitro with an increase in caspases 3/7 activity. Besides the above, *Phyllanthus* extracts down-regulate the expression of COX2 and Bcl 2 (anti-apoptotic peptide) up to two-folds that lead to induction of apoptosis (Sureban et al., 2006).

In addition, Tang et al. (2010) detected the ability of the aqueous and methanol extracts of four *Phyllanthus species* including *P. amarus*, to induce apoptosis in skin melanoma MeWo and prostate PC3 human cancer cells. These extracts exhibited a cytotoxic effect through induction of cell cycle arrest as well as caspase-3 activity with a minimal necrotic effect. The hairy root methanol extract of *P. amarus* found to exhibit an anti-proliferative effect in the MCF-7 cells via induction of apoptosis by increasing reactive oxygen species (ROS) level as well as the reduction in mitochondrial membrane potential (ΔΨm) (Abhyankar et al., 2010). Additionally, Lee et al. (2011) determined the cytotoxicity and apoptotic activity of *P. amarus* in MCF-7 and lung cancer A549 cells alongside with anti-metastasis effect. Aqueous and methanol extracts of *P. amarus* induced apoptosis via triggering of caspases activity that was associated with cleavage of poly ADP ribose polymerase (PARP) and DNA fragmentation. Previous studies have also demonstrated that the aqueous extract from the leaves of *P. amarus* has acted against 20 methylcholanthrene (20 MC) induced sarcoma development through inhibition of DNA topoisomerase II and cell cycle regulatory enzyme tyrosine phosphatase cdc25 of Saccharomyces cerevisiae. The possible chemoprotective,as well as the mechanism of action of *P. amarus/niruri*, ***P. emblica, P. urinaria, P. polyphyllus, P. watsonii, and P. pulcher***is described in Fig. 3.

3. Immunomodulatory activity of *Phyllanthus species*

The immune system broadly categorized into two categories viz. (i) innate non-specific and (ii) adaptive specific or acquired the immune system. Immune system maintains normal homeostasis within the body. Numerous exogenous and endogenous factors influence the function and efficiency of the immune system, which results in either immunosuppressive or stimulatory function (Patil et al., 2012). Immune modulation well defines any influence that drug or phytochemical compound will have on immune system responsiveness. Immunomodulators are a variety of agents possessing an activity to modulate or regularize pathophysiological activities (Puri et al., 1994; Kumar and Kumar, 2017). For example, some bioactive molecule may stimulate T-suppressor cells (thus reduce immune resistance), and some may stimulate macrophages and natural killer (NK) cells. Others will enhance the production of antibodies within acting as antigens themselves. Besides the above, any bio-entity of synthetic or natural origin

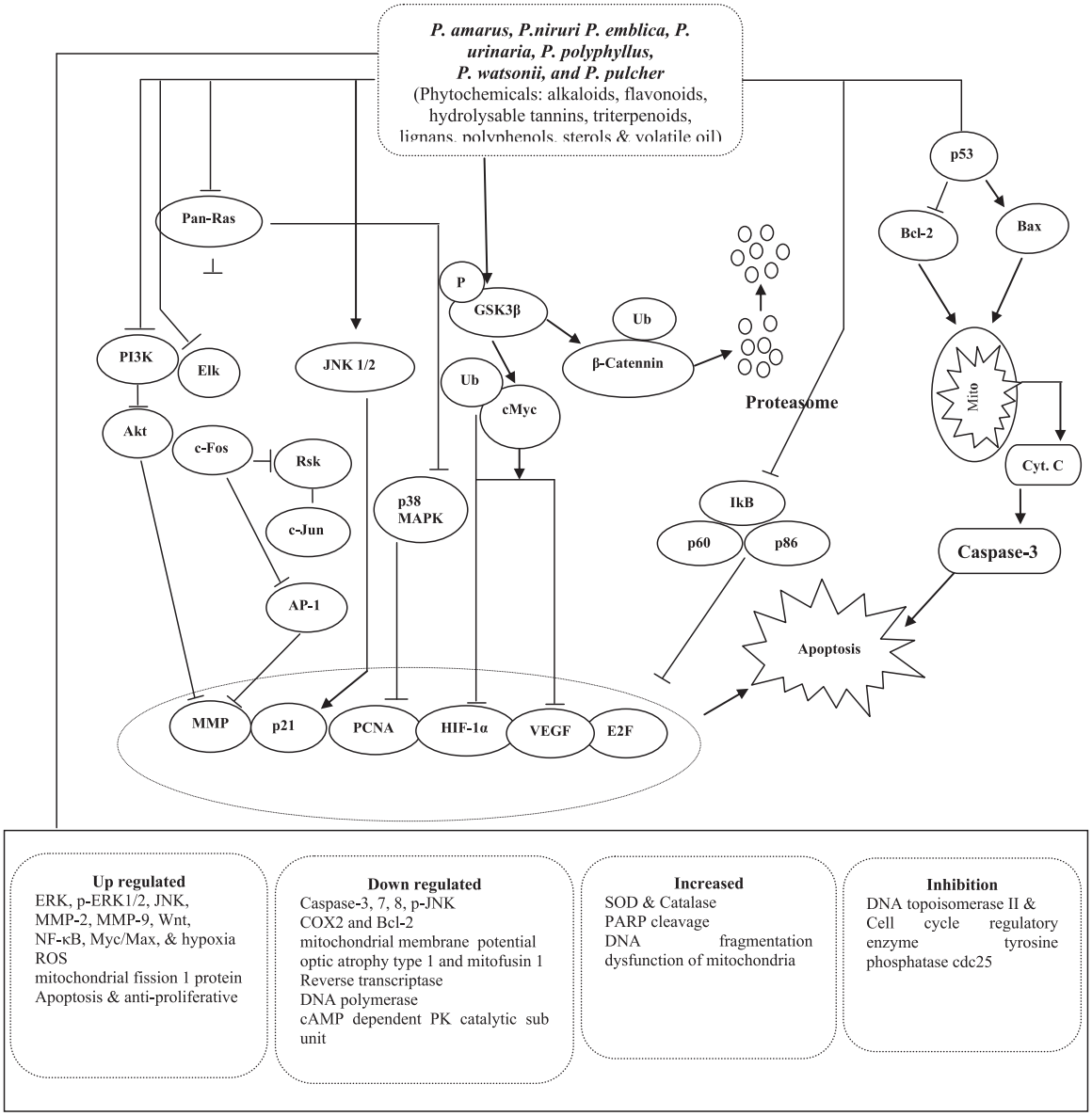

Fig. 3. Depicts the potential targets and possible mechanism of action of *Phyllanthus species* derived bioactive compounds on various apoptosis signaling pathways as well as multiple protein regulations in extract treated cancer cells.

with the capacity of modulating, suppressing or stimulating the components of either adaptive or innate immunity are also considered as immune modulators, restoratives, augmenters, or biological response modifiers. However, in clinical practice, they generally classified into immunoadjuvant, immunostimulant, and immunosuppressant (Ilangkovan et al., 2015; Khandelwal et al., 2016).

The development in clinical and experimental immunology strongly suggests that many infectious diseases and disorders arise because of stressful environmental conditions associated with suppression of the immune system (Stromberg and Carlson, 2010). It is now well recognized that immunomodulation of immune response could provide an alternative to conventional chemotherapy for a variety of disease conditions. For example, when the host's defense mechanism needs to be activated under conditions of impaired immune responsiveness (Luo et al., 2016) or when a selective immunosuppressant needs to be induced in a situation like autoimmune disorders and organ transplantation (Marta et al., 2014; Singh et al., 2016).

Immunomodulators have potential contributions to the treatment of various malignancies as they play a central role in supporting immune cells involved in our immunological defenses (Geetha et al., 2005; Vesely et al., 2011). It is been suggested that the immune response to progressive tumors is insufficient due to a deficiency in afferent mechanisms responsible for the development of tumor-reactive T cells (Baxter, 2007). As biological response modifiers, immunomodulators exert their antitumor effects by improving host defense mechanisms against the tumor. They have a direct anti-proliferative effect on tumor cells and enhance the ability of the host to tolerate damage caused by toxic chemicals (Oliver et al., 2015; Khalil et al., 2016).

Plant-based immunomodulatory therapeutics has gained attention in recent years. Plant extracts used in traditional therapy have reviewed for their immunomodulatory activities (Pang et al., 2018). To support this notion, many herbal preparations from the following flora such as *Tinospora cordifolia, Centella asiatica, Phyllanthus debelis, Trogonella foenum graecum, Pouteria cambodiana, Panax ginseng, Withania somnifera, Mangifera indica Picrorhiza scrophularii* have been shown to modify the immune function and have demonstrated a wide range of immunomodulatory effects (Davis and Kuttan, 2000; Makare et al., 2001; Diwanay et al., 2004; Gautam et al., 2004, Jayathirta and Misra, 2004).

Besides the above, recent innovative technologies and excessive investigations on immunomodulatory plant-derived natural products/extracts, and their active moieties with immunomodulatory potential may provide us with valuable natural entities to develop as novel agents to act as a counterpart for the current chemotherapeutic agents (Ilangkovan et al., 2015; Nfambi et al., 2015; Yoshizumi et al., 2017). Bioactive principles like polysaccharides, diterpenoids, glycosides, lactones, and alkaloids isolated from several plants have been reported as potential immunomodulatory agents (Winkler et al., 2004; Patwardhan and Gautam, 2005; Jantan et al., 2014). Several types of immunomodulators have also been identified from isolates and extracts of bacteria and fungi, mammalian proteins such as interferon, interleukins and cytokines and some synthetic chemicals (Schepetkin and Quinn, 2006), however, still a lot more need to be explored, so this field of research offers scope for further investigation.

The immunological effects of immunomodulators can be assessed based on their selective activities on the different components of the immune system, which comprise specific actions on immune cells, effector mechanisms, inhibition of nitric oxide (NO) and ROS production, secretion of inflammatory cytokines, signaling pathways in macrophage cells and phagocytosis activity (Pang et al., 2018). Several cytokines also play essential roles in the inflammatory process, especially interleukin-1 (*IL-1*) and tumor necrosis factor (*TNF*) (Dempsey et al., 2003). Both *IL-1* and *TNF* are commonly considered as major mediators of the biological responses to endotoxin/bacterial lipopolysaccharide (LPS). Other cytokines and growth factors [e.g., *IL-2, IL-6, IL-8*, and granulocyte/macrophage colony stimulating factor (GM-CSF)] also contribute to manifestations of the inflammatory response (Davoine and Lacy, 2014).

While many plant extracts have shown their therapeutic efficacy, which is attributed to influence the human immune system including *Phyllanthus species* as they have proven for their capacity to modulate, activate the immune system, evaluated in various clinical trials for the treatment of various human diseases (Tjandrawinata, 2011; Tjandrawinata et al., 2017). However, not much detailed information is available on the immunomodulatory effects of *Phyllanthus species*. However, a study by Yuandani et al. (2013) demonstrated that methanolic extracts derived bioactive principles of *P. amarus* and *P. niruri* have exerted their efficacy on chemotaxis, phagocytosis, and chemiluminescence of human phagocytes. In that study, they have shown that extracts have strongly inhibited the migration of polymorphonuclear leukocytes (PMNs). However, the *P. amarus* extract from Malaysian species exhibited only moderate inhibition in case of bacteria engulfment by the phagocytes but demonstrated strong ROS inhibitory activity.

In addition, bioactive principles such as phyllanthin and hypophyllanthin have shown relatively strong activity against PMNs chemotaxis, with lower IC50 values when comparedwith known standard drug ibuprofen. It is interesting to note that *P. niruri* has also been reported to improve humoral and cell-mediated immune responses to pathogens (Sarisetyaningtyas et al., 2006) and offered significant protection in different models of cancer in murine (Rajeshkumar and Kuttan, 2000; Yang et al., 2006; Rajeshkumar et al., 2002). On the other hand, ethanol extracts of *P. urinaria* and *P. amarus* established to have inhibitory effects on the chemotaxis of neutrophils and monocytes with IC50 values lower than 3 μg/mL. In addition, phagocytic activity and CD18 expression of neutrophils and monocytes were also down-regulated (Pang et al., 2018). A study using an animal model with oral administration of *P. reticulatus* extract demonstrated a significant increase in phagocytic activity, the percentage of neutrophil adhesion, and white blood cell (Kumar et al., 2004). Based on the above results, it is believed that most of the beneficial effects attributed to *P. amarus* and *P.niruri* are related to their immunomodulatory properties.

Professional phagocytes such as PMNs and macrophage cells play a significant role in our innate immune defense against infectious microbes and aid in antigen presentation (Jantan et al., 2011; Duque and Descoteaux, 2014). Several sequential steps are involved in phagocytosis, i.e. migration of phagocyte cells to the site of infection or death, adherence towards vascular endothelial cells, followed by degradation of the pathogen. Chemotaxis is the movement of phagocytes to the site of infection or death and is the earliest step of immune responses (Laarman et al., 2012). The ability of these cells to be chemotactically attracted to the site of initial microbial invasion or to an inflammatory focus is fundamental for the full activation of the immune response that follows. Accumulation of phagocytes at the infection site can be induced by either endogenous chemo attractant such as interleukin 8 (*IL-8*), leukotriene B4 (LTB4), platelet activating factor (PAF) or exogenous chemoattractant of bacterial cell product formyl methionyl-leucyl-phenylalanine (fMLP) (Jantan et al., 2011).

The leukocytes adhere stably to the endothelium cells because they possess cell surface expression of all three *CD11/CD18* leucocyte integrin. Binding of a receptor-specific ligand may lead, besides signal-transduction events, to internalization of the receptor-ligand complex, which leads to a subsequent down-regulation of surface receptor expression. Phagocytosis will occur at the site of infection by phagocyte-ligand interaction leading to a sequence of events known as oxidative burst (Jantan et al., 2011). The burst involves increased oxygen consumption and generation of highly reactive superoxide anion radical (O_2-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH-). In the phagosome, H_2O_2 and myeloperoxidase enzyme (MPO) activate a halogenating system, giving rise to hypochlorous acid (HClO) which is a potent bactericidal agent (Slauch, 2011). MPO is also involved in the production of highly toxic NO. Besides the defensive roles during the infections, the phagocyte-microbe interactions when excessively or

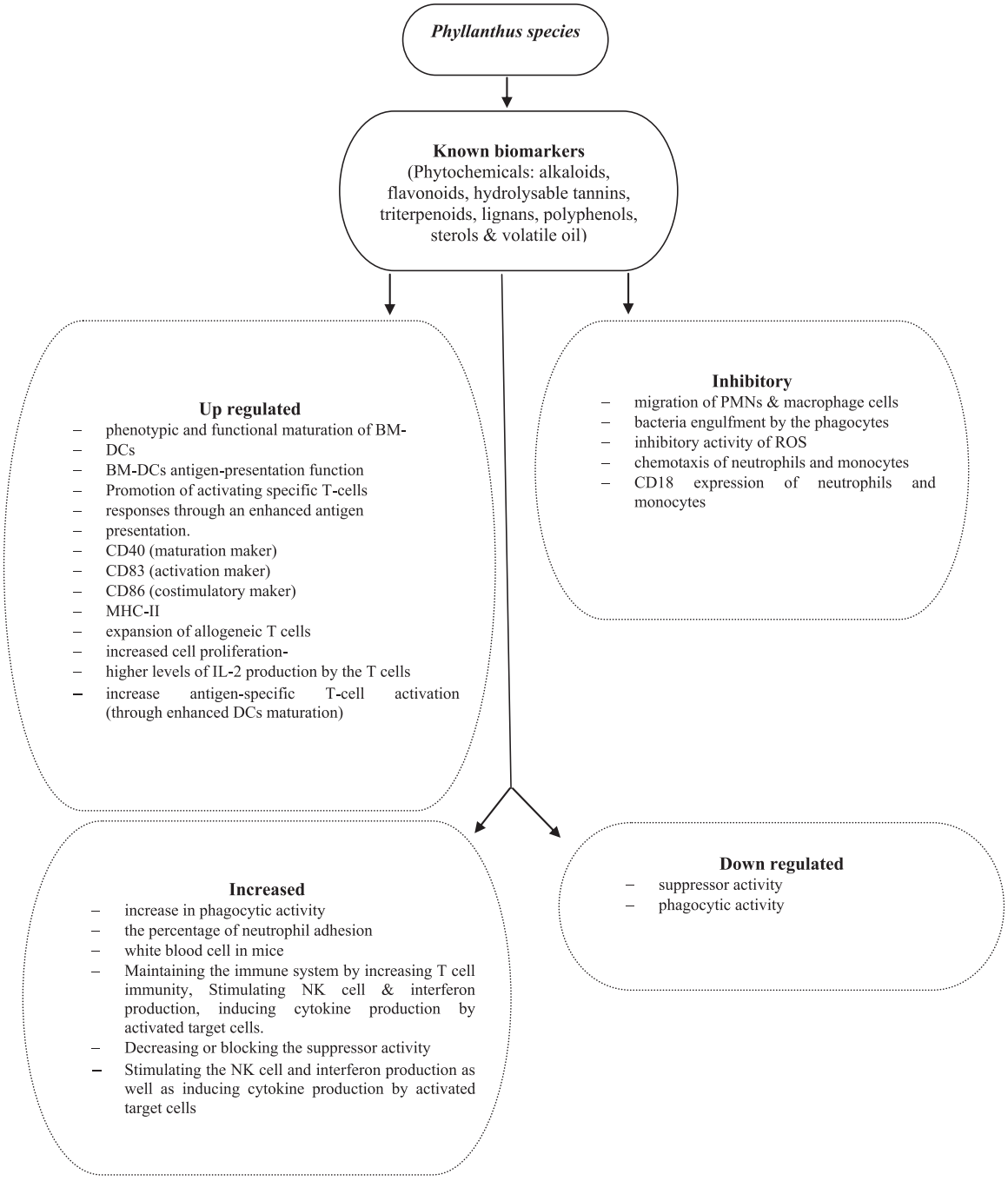

Fig. 4. Showing the immunomodulatory activity of *Phyllanthus species* and their major compounds on multiple protein regulations in immune cells treated with plant extracts.

inappropriately deployed can damage host tissues and contribute to the pathogenic conditions of various immune and non-immune chronic inflammatory diseases, including some rheumatoid disorders. Based on the above results, various disorders including the inflammatory one can be treated with inhibitors of phagocyte ROS.

Besides the above, dendritic cells (DCs) are professional antigen-presenting cells (APCs), which provide a link between the innate and adaptive immune responses and play important role in surveillance (Janeway and Medzhitov, 2002). Immature DCs (iDCs) distributed in peripheral tissues, where they continuously sample the environment for invading infectious agents by endocytosis (Banchereau and Steinman, 1988). Several studies have demonstrated that supplements and herbal products can promote or induce the maturation of DCs. This could explain some of their pharmacological and therapeutic effects (Wang et al., 2006; Lee et al., 2008; Liu et al., 2009), which stimulated the interest in understanding the mechanisms by which the whole extract of the plant could stimulate the immune system. However, nothing is known yet on the effect of the herb on the maturation and antigen-presenting function of DCs, which could also explain most of these therapeutic values of the herb on the immune system. Nworu et al. (2010) demonstrated that the aqueous extract of *P.niruri* enhances the phenotypic and functional maturation of murine bone marrow-derived DCs (BM-DCS) and their antigen-presentation function, in vitro. These activities could be relevant to the antitumor effects and to the enhanced antibody responses which have also been attributed to the herb, in their study they show that the aqueous extract of *P.niruri* promotes structural

and functional maturation of DCs, which are also more effective in activating specific T-cells responses through an enhanced antigen presentation (Yin-Quan et al., 2011).

P.niruri and related species has been the subject of many phytochemical studies and many active metabolites, responsible for the pharmacological activities, have been identified (Ilangkovan et al., 2015, 2016). Generally, biologically active lignans, glycosides, flavonoids, alkaloids, ellagitannins, and phenylpropanoids have been identified in the leaf, stem, and root of the plant (Divya et al., 2011). The phytoconstituent (s) responsible for the effects of *P. niruri* on DCs maturation has not yet been identified, however, the use of herbs in their original form with all the metabolites present in the natural balance are encouraged for reasons of safety and higher therapeutic potency. The whole aqueous extract of the aerial parts of *P. niruri*, the predominant form in which the herb is promoted and used (Nworu et al., 2010).

It is well established that DCs are particularly playing a crucial role in the initiation of primary T cell-mediated immune responses upon receiving signal. In this regard, previous reports (Divya et al., 2011; Watford et al., 2003) have demonstrated that *P. niruri* has upregulated the surface expression of structural maturation makers such as *CD40; CD83; CD86* and MHC-II (Tennenbaum et al., 200)). From the mechanistic point of view, this type of role by *Phylanthus species*. to induce the maturation markers of DCs is very important as it has been well associated with exhibiting changes in both innate and adaptive immunity. Besides the above, this study also highlights the critical role of *IL-12* by portraying as a linker cytokine which links both innate and adaptive immune systems by stimulating the production of various cytokines by effectors cells. For instance, *IL-12* induces *IFN-*γ production in NK and T cells which in turn increases these cells cytolytic activity against cancer cells (Watford et al., 2003).

In line with the above, previous studies (Yang et al., 2006; Rajeshkumar et al., 2002) have demonstrated that *P. niruri* extracts have exhibited protective activities against solid tumors as well as viral infections, where enhanced cell-mediated immunity is particularly important. These effects could be related to the enhanced DC maturation and functions induced by *P. niruri*extract (Rajeshkumar et al., 2002). As the most potent professional APC, matured DCs play a critical role in the presentation of engulfed primed antigens to T cells, which in turn elicits an immune response to that specific antigen (Rossi et al., 2005). The functional maturation of DCs always serves as a confirmative tool for antigen-presentation function (Sarisetyaningtyas et al., 2006). In this study, OT-1 transgenic mouse in which the *CD8* + T cells express a TCR specific for the Ova 257–264 (SIINFEKL peptide) as a source of the responder T cells was used. When Ova-pulsed and *P. niruri* -stimulated BM-DCs were cocultured with the T cells from OT-1 mice in 1:5 ratio, there was a significantly higher clonal expansion of allogeneic T cells as shown by increased cell proliferation and higher levels of IL-2 production by the T cells. These results indicate that *P. niruri* can potentially increase antigen-specific T-cell activation through enhanced DCs maturation. This could be a possible explanation for the reported increase in humoral responses with *P. niruri* herbal supplementation (Sarisetyaningtyas et al., 2006). The possible mechanism of action of *Phyllanthus species* is described in Fig. 4.

Furthermore, many clinical trials have shown that DCs, when appropriately armed with a tumor antigen (Ag), can promote antitumor immunity and clinically significant tumor regression. Previous studies have also shown the benefit of combining intradermal (i.t) injection of tumor-pulsed DCs (TP-DC) with chemotherapy or radiotherapy (Tong et al., 2001; Tennenbaum et al., 2003) but are associated with systemic toxicity and render less immunogenic when tumors treated with UV or ionizing irradiation, or frozen and thawed tumors (Gollnick et al., 2002). Moreover, cancer vaccines are the other potent options for cancer management (Kwak et al., 1992; Bendandi et al., 1999).

Many cancer treatment modalities such as chemotherapy (Palombo et al., 2014; Simon et al., 2015; Barbara et al., 2018), PDT (Thong et al., 2007; Ali-Seyed et al., 2011; Elisa et al., 2013) and extracts from various plants including *P. amarus* and *P. niruri* (Shimaa et al., 2018) have the capacity to induce many types of cell death including apoptosis or necrosis, or both and generate immunogenic peptides from tumor cells. Presentation of these peptides by APCs could lead to the activation and proliferation of peptide-specific cytotoxic *CD8* + T-cell clones. This process supported the shift from a *CD4* + to a *CD8* + T-cell infiltrate in the tumor in the patient. Some clinical findings (Ali-Seyed et al., 2011) are in agreement with previous animal studies using different photosensitizer for the observation of tumor-targeting *CD4* + and *CD8* + T-cell clones, not only at sites that have undergone cancer treatment but also distant metastatic tumors (Dougherty et al., 1998; Castano et al., 2006).

Besides the above, these treatments also alter the tumor microenvironment (Engleman et al., 2004) through the release of pro-inflammatory cytokines such as tumor necrosis factor a, interleukin (*IL-1*) and *IL-6*. Thus, this technique represents a useful tool to induce differential cell death. Tumor destruction after treatment results from direct cytotoxic effects as well as from the induction of a local inflammatory (Ali-Seyed et al., 2011). Based on its unique mechanism of tumor destruction, treatment modalities have the potential to create an environment at the tumor site that favors both tumor antigen loading and activation of various APCs including DCs, key requirements for induction of anti-tumor immunity by Phagocytosis. Phagocytes play important roles in recognition of the target structures, and subsequent engulfment of the TAAs, followed by intracellular destruction (Shimaa et al., 2018). DCs proven dual role in innate and adaptive immunity led us to explore their potential utility in tumor immunotherapy in combination with an alternative mode of therapy in the near future. Thus, the use of whole TP-DCs may represent a promising new approach to induce a potent immune response against weak antigens such as tumor proteins.

4. Conclusion

In summary, it is now well recognized that immunomodulation of immune response could provide an alternative to conventional chemotherapy for a variety of disease conditions. The present review suggests that the updated research data available for *Phylanthus species* to us warrant more attention by medical practitioners not only to the tumor-induced imbalance of the innate immune system but also to other diseases. In fact, the restoration of adequate antitumor immune response should be an important clinical goal. Immunomodulators containing extract derived bioactive principles with standardized doses can be considered as adjunctive therapy when regular treatments are given for various diseases. For example, this adjuvant therapy may improve tumor patients' quality of life, which is often, translates into better outcomes after standard treatment(s) – alone or in combination. In many cases, this type of combined treatments may be particularly effective to combat cancer. However, it warrants further validation with appropriate clinical trials, which are necessary to clarify the beneficial effect of combination treatments with currently available immunomodulatory effects of *Phyllanthus* derived bioactive substances

Conflicts of interest

The authors declare no conflict of interest.

Compliance with ethics requirements

This review article does not contain any studies with human or animal subjects rather quoted references only.

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