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The use of *Phyllanthus niruri* L. as an immunomodulator for the treatment of infectious diseases in clinical settings

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ABSTRACT

Phyllanthus niruri L. (Euphorbiaceae) (*P. niruri*) has traditionally been used in many tropical countries to treat various ailments, such as kidney stones, chronic liver diseases, diabetes and viral infections. The versatile ethnomedicinal usage of the herb is tightly associated with its multiple pharmacological properties such as immunomodulator, anti-viral, antibacterial, diuretic, anti-hyperglycemia and hepatoprotector. The scope of this review is limited only to the clinical evidences demonstrating benefits of the plant *P. niruri* with its immunomodulatory properties, for the treatment of various infectious diseases. These evidences are expected to provide the plant a more significant place in the current clinical settings, particularly in the management of infectious diseases. *P. niruri* as an immunomodulator has scientifically been studied and evaluated in various clinical trials for the treatment of chronic hepatitis B, pulmonary tuberculosis, vaginitis, as well as varicella-zoster infection. In such diseases, the effective immune system is crucial to the treatment success and eradication of the pathogens. In those clinical studies, *P. niruri* has been proven for its capacity to modulate and activate the immune system. In fact, there are numerous *in vitro* and animal studies reporting potential benefits of the immunomodulatory properties of *P. niruri*, and numbers of randomized controlled clinical studies have been published to date. In the light of the scarcity of research to discover new, more effective and safe anti-infection chemical entities, that is also complicated with the growing threat from the new generations of drug resistant-pathogens, the utilization of nature-derived immunomodulatory agents, either alone or combined with the currently available antibiotics or antivirals, is undoubtedly promising and of clinical importance. Most of the studies on *P. niruri* warrant its potential benefits in various infectious diseases, and are expected to grant the herb an important place in the management of such diseases in the formal clinical practice.

1. Introduction

Plants stand as an infinite and important natural resource for drug development, novel chemotypes, pharmacophores, and other valuable bioactive agents, for a multitude of therapeutic indications. Many of the nature-derived compounds have been directly utilized as drug entities; while many others can also serve as chemical

models for the design, synthesis or semi-synthesis of novel drug molecules. A tremendous number of natural compounds are currently in various stages of clinical development, highlighting the significance and persisting viability of the nature-derived products as sources of new drug candidates[1-8]. This paper reviews *Phyllanthus niruri* L. (*P. niruri*), one herb species among the gigantic number of medicinal plants that have widely been studied worldwide.

P. niruri (Euphorbiaceae) is a worldwide distributed tropical plant acclaimed for its versatile ethnomedicinal uses[9,10]. It features multiple pharmacological properties such as an immunomodulator, anti-viral, antibacterial, diuretic, anti-hyperglycemia and hepatoprotector[10,11]. Therefore, it is not surprising that *P. niruri*

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has traditionally been used in many tropical countries to treat various ailments, such as kidney stones, chronic liver diseases, diabetes, and viral infections[12]. This plant is popularly known as stonebreaker, gale of the wind or seed-under-leaf. The other names of the herbal plant in assorted languages are *chanca piedra* (Spanish), *quebra-pedra* (Portuguese), *keezha nelli* (Tamil), *keezhar nelli* (Malayalam), *turi hutan* and *meniran hijau* (Indonesian)[11,13].

P. niruri is a 2-foot-height weed and has small leaves that grow in an alternate arrangement in 2 rows. The leaves are membranous and unusually thin and glaucous under its surface, elliptical in shape and have a narrow base with 2 stipules. The plant bears herbaceous branches and the light green bark is velvety. The plant's monoecious flowers are small and grow in pairs with the pale green color that is often flushed with red color tone. The fruits are tiny with the shape of depressed and globose capsule and contain endospermic and trigonous seeds[14]. Usually the entire plant of *P. niruri* is used in medicinal preparations by herbalists since the active phytochemicals, such as flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins and saponins, have been identified from various parts of the plant[15,16]. Extracts of this herb have been proven to have therapeutic effects in many clinical studies, which will also be presented in this review.

The scope of this review includes clinical evidences demonstrating the benefits of the plant *P. niruri* with its immunomodulatory properties, for the treatment of various infectious diseases. These evidences are expected to provide the plant a more significant place in the current clinical settings, particularly in the management of infectious diseases.

P. niruri has scientifically been studied and evaluated in various clinical trials on infectious diseases, such as chronic hepatitis B[17,18], pulmonary tuberculosis (TB)[19-22], vaginitis[23], as well as varicella-zoster infection[24]. In such diseases, the effective immune system is crucial to the treatment success and eradication of the pathogens. *P. niruri* has been studied for its capacity to modulate and activate the immune system, a pharmacological feature that is partly contributing to the ethnomedicinal applications of the plant extracts in the management of infectious diseases[25,26].

Ma'at demonstrated that *P. niruri* extract enhanced the activity and functions of immune system components, such as marked stimulation of natural killer (NK) cell cytotoxicity, tumor necrosis factor (TNF)- α secretion by T-helper 1 subset, and decreased interleukin (IL)-10 secretion by T-helper 2 subset. Therefore, *P. niruri* modulates both humoral and cellular immunity[25]. In line with such findings, Nworu *et al.* in their study reported that the aqueous extract of *P. niruri* was a potent murine lymphocytes mitogen, inducing marked increases in the expression of surface activation maker (CD69) and proliferation of B and T lymphocytes. The *P. niruri* extract-stimulated naïve splenocytes cultures also increased the production of interferon (IFN)- γ and IL-4 in a concentration-dependent manner. Phagocytosis, lysosomal enzymes activity, and TNF- α released by the murine bone marrow-derived macrophages were significantly augmented by pre-treatment with the extract. In addition, nitric oxide released by macrophages was also modulated by the extract[26]. The capacity of *P. niruri* extract as an immunomodulator has warranted numerous clinical studies of the extract for the treatment of infectious diseases.

Besides, a study by Liu *et al.*[27,28] and Mohan *et al.*[29] also quantitatively determined the antiviral activity of these herbs in well-defined *in vitro* systems.

2. Chronic hepatitis B

Chronic hepatitis B infection is confirmed by the presence of any of the viral antigens, including the hepatitis B virus surface antigen (HBsAg), hepatitis B e antigen (HBeAg), or hepatitis B virus (HBV) DNA at detectable levels in the blood, and the absence of antibodies to the virus core antigen (anti-HBc immunoglobulin M). The presence of the viral antigens should be found at two sequential tests performed at least 6 months apart. Such a chronic infection is attributable to the inadequate response of virus-specific T-cells, under which the exhausted T-cells show poor cytotoxic capacity, impaired cytokine production and prolonged expression of multiple inhibitory receptors[30-32]. In chronic hepatitis B, the level of virus replication, liver disease activity, and humoral responses can be markedly different between patients. Liver inflammation can be present or absent in chronic hepatitis B patients, regardless of the level of HBV replication. The profile of virus-specific CD8 (cytotoxic)-T cell response along the infection progress highly varies between patients and is associated with the level of HBV DNA replication rather than the liver disease activity[33].

The treatment of chronic hepatitis B is mainly aimed to suppress HBV DNA replication leading to the regression of hepatic necroinflammation and prevention of fibrosis progression, cirrhosis and its complications including hepatocellular carcinoma that at the end will improve survival[34]. Response to treatment is indicated by the reduction of serum HBV DNA load reflecting the suppression of HBV replication, then followed by diminished hepatic necroinflammation, fibrosis stabilization or even regression; the risk of reactivation still persists, however. If HBV DNA load can sufficiently be maintained under the subclinical threshold level and there is an effective immune response to clear the infected hepatocytes, the seroconversion of HBeAg may occur; thus the risk of reactivation is low. Further, if HBV replication is completely interrupted, reflected as the undetectable level of serum HBV DNA by sensitive assays, with stable HBeAg seroconversion, the HBsAg can no longer be detected (with or without HBsAg seroconversion), indicating the complete cessation of hepatic necroinflammation, thus no risk of reactivation[35].

To date, IFN- α or pegylated IFN- α , and long-term therapy with nucleos(t)ide analogues are the primary treatment strategies for chronic hepatitis B. IFNs exert antiviral, antiproliferative, and immunomodulatory activities. Nucleos(t)ide analogues, such as lamivudine, adefovir, entecavir and tenofovir directly inhibit HBV DNA polymerase, thus suppressing viral replication[36,37]. To date, pegylated IFN treatment given concomitantly with a nucleos(t)ide analogue antiviral is disappointing due to its short-term efficacy. However, long-term efficacy needs to be assessed using different schedules of combination (*e.g.* sequentially)[33,36]. Further, nucleos(t)ide analogue antivirals have to be indefinitely administered in the majority of patients as they cannot sustain their efficacy after discontinuation of therapy. Patients with chronic infections who then

withdraw their therapy before HBsAg loss or seroconversion are at high risk to relapse due to the prevailing HBV DNA (cccDNA) in the infected cells[35]. In general, the successful treatment rate of the standard agents at 1-year treatment is only around 20%, 48%–78%, 21%–93%, in terms of HBeAg seroconversion, normalization of serum ALT levels, and undetectable HBV DNA level, respectively[38]. Further, IFN use is limited due to its highly expensive cost and intolerable adverse effects. Long-term use of the current antivirals is also hindered due to the emergence of drug resistance[34,37]. The overall limitations of those currently available therapies for chronic HBV infection underline the need for more cost-effective and safer alternative therapies. Considering the immunological aspect, particularly the T cells, play a critical role in the pathogenesis of chronic HBV infection, combining the conventional antiviral therapies with safer and more affordable immunomodulatory agents able to stimulate and restore the antiviral T cell response directly and specifically may be an attractive alternative and rational strategy for treating chronic HBV infection[30,33,35].

As a folk medicine, the plants of *P. niruri* have long been used to treat chronic liver disease, including chronic hepatitis B infection[39,40]. Clinical studies of *P. niruri* for treatment of chronic hepatitis B carrier were started by Thyagarajan *et al.*[18]. In the 1990s, a major reorganization of the *Phyllanthus* genus was conducted, which classified *Phyllanthus amarus* as a variant of *P. niruri*[11,41]. Thyagarajan had also extracted three therapeutically active substances of the plant that showed activity against the hepatitis B surface antigen, improved the body's immune system, and protected the liver. The inability of the immune system to eliminate hepatitis B viruses from the liver cells keeps the body at the "carrier state" of hepatitis B infection. A study by Thyagarajan *et al.*[18], involved 37 patients chronically infected with HBV who were given a daily dose of *P. niruri* 600 mg extract for 30 days. Two weeks after the end of the treatment, 59% of the patients lost the HBsAg. Furthermore, after a 9-month follow-up, none of the cases showed any symptoms of HBsAg reappearance. The authors postulated that *P. niruri* might inhibit DNA replication of the virus, thus suppressing the proliferation. In another study involving 60 chronically HBV infected patients, Thyagarajan *et al.* reported that treatment of 200 mg of *P. niruri* herbs powder three times a day for 30 days resulted in seroconversion of HBsAg (59% vs. 4%, in *Phyllanthus* and placebo groups, respectively) within 15–20 days after the end of treatment and the HBsAg were still negative after 90 days of evaluation. In summary, this descriptive preliminary study concluded that *P. niruri* extract at a dose of 2 times 100 mg daily for 12 weeks has not yet demonstrated its benefits in terms of HBeAg clearance and ALT normalization[17].

Twenty-two randomized trials on *Phyllanthus* sp., with a total sample size of 1947 chronic hepatitis B patients were analyzed in a systematic review by Liu *et al.*[39]. The review included five and seventeen clinical trials with high- and low-quality methodology, respectively. The pooled analyses showed that the chance to reach serum HBsAg clearance was significantly higher with *Phyllanthus* species treatment compared to placebo or no intervention, with a relative risk (RR) of 5.64 [95% confidence interval (CI) 1.85–17.21; $P = 0.002$]. Further, *Phyllanthus* demonstrated no significant

difference with IFN treatment in the clearance of serum HBsAg, HBeAg and HBV DNA. Combination treatment of *Phyllanthus* and IFN, however, provided a significantly better chance for the clearance of serum HBeAg (RR 1.56; 90% CI 1.06–2.32; $P = 0.03$) and HBV DNA (RR 1.52; 90% CI 1.05–2.21; $P = 0.03$) than IFN alone. No serious adverse event was observed. The review concluded that *Phyllanthus* species may possess an antiviral property against the chronic HBV infection that protects liver function. The evidence is not robust, however, particularly due to the generally low methodological quality and non-standardized extracts of the herb used in those trials. Adequately large trials are still necessary.

A Cochrane systematic review by Xia *et al.*[40] reported that based on fifteen randomized trials with a total of 1284 chronic hepatitis B patients, the addition of *Phyllanthus* sp. treatment to an antiviral agent like IFN- α , lamivudine, adefovir dipivoxil, thymosin, vidarabine, or conventional treatment, significantly reduced serum HBV DNA (RR 0.69; 95% CI 0.52–0.91, $P = 0.008$; $I^2 = 71\%$), serum HBeAg (RR 0.70; 95% CI 0.60–0.81, $P < 0.001$; $I^2 = 68\%$), and induced HBeAg seroconversion (RR 0.77; 95% CI 0.63–0.92, $P = 0.005$; $I^2 = 78\%$) compared to the respective antiviral alone. However, the report also noted that the heterogeneity among those trials and high risk of bias were substantial. The trial sequential analysis did not support the result regarding serum HBV DNA. Mortality and hepatitis B-related morbidity, quality of life, or liver histology were not reported by any of the trials. No intolerable or serious adverse events due to *Phyllanthus* treatment were reported. Larger clinical trials with low risk of bias are needed to confirm such findings.

A randomized controlled study was also conducted by Lesmana *et al.*[42] involving forty patients with HBeAg-positive chronic hepatitis B, elevated serum glutamate-pyruvate transaminase levels, age of 18–75 years old, and naïve to treatments with either lamivudine or IFN. Trial medication was *P. niruri* extract 50 mg capsules or the placebo, given three times on top of vitamin B supplementation. Means of HBV DNA baseline level in *Phyllanthus* and placebo groups were (8.40 ± 0.64) and (7.69 ± 1.30) log₁₀ copies/mL, respectively. After 12 weeks of treatment, 65% (11 of 17) patients in the *Phyllanthus* group showed twice as much decrease in serum HBV DNA level [by (1.22 ± 1.86) log₁₀ copies/mL] as shown by similar percentage of patients (13 of 20) in the placebo group (0.69 ± 0.80 log₁₀ copies/mL). The remaining 35% (6 of 17) of patients in the *Phyllanthus* group showed their serum HBV DNA slightly increased [by only (0.08 ± 0.05) log₁₀ copies/mL], while 35% (7 of 20) of patients in the placebo group increased by (0.41 ± 0.50) log₁₀ copies/mL. *Phyllanthus* extract was found to be well tolerated, with an incidence of adverse events similar to that for placebo. The commonest adverse events were headache, fatigue, cold, hyperurinaria and loss of appetite. Of them, only hyperurinaria and loss of appetite were possibly related to *Phyllanthus* treatment. The proportion of patient in the *Phyllanthus* group experienced HBeAg clearance was slightly higher than that of the placebo (41% vs. 35%). However, this study did not measure the anti-HBe antibodies, thus the rate of HBe-seroconversion could not be confirmed in this study.

Those currently available clinical studies showed that *P. niruri* extract was capable to suppress the serum levels of HBV DNA. It

is likely that the herb exerts such favourable effect through both immunomodulatory and direct antiviral activity, which is yet to be proven in a further larger study. The direct antiviral activity of *P. niruri* might occur through the inhibition of polymerase DNA enzyme activity during the growth phase of hepatitis B virus[27-29]. On the other hand, *P. niruri* also exerts its immunomodulatory activity against the HBV infection through several ways. *P. niruri*-induced NK cell cytotoxicity increases the number of infected hepatocytes (host cells) that can be lysed. Enhanced TNF- α secretion by *P. niruri*-induced T-helper 1 subset also increases the major histocompatibility complex-class I molecule expression with virus peptide of hepatitis B, resulting in optimized specific-cytotoxic activity of T lymphocytes (CD8). In addition, decreased secretion of IL-10 by T-helper 2 subset diminishes suppression on monocyte/macrophage activity, either as phagocyte cells or antigen presenting cell. Therefore, hepatitis B viruses released to circulation or extra cellular milieu due to infected hepatocyte lysis by NK cells or cytotoxic T cells will then be eradicated whether by the complement system or by the phagocytic activity of monocytes/macrophages through the classical pathway[43]. Stimulation of T cell response by *Phyllanthus*, which is plausibly associated with either HBV DNA load or suppression, will induce HBV antigen seroconversion that ultimately leads to recovery from the disease. Patients' immune responses determine the successful immunomodulatory therapy with *P. niruri* extract. Recent evidence showed that the prone of being high or low responders is closely dependent on the human leucocyte antigen-type of the patients[43]. Thus, a combination of an antiviral agent and *P. niruri* extract as an immunomodulator may be a rational and synergistic therapy for an effective management of chronic hepatitis B infection. Further clinical studies with more robust methodology are needed to explore this possibility. Whether both agents should be given sequentially or simultaneously is another important thing which needs further investigation. An increased dose of *Phyllanthus* extract and extension of the treatment period up to at least 6 months is also interesting to be explored.

3. Pulmonary TB

The host cellular immune response plays a critical role to eradicate the intracellular pathogens, such as *Mycobacterium tuberculosis* (*M. tuberculosis*), the culprit of pulmonary TB in humans. T-lymphocytes together with their secreted cytokines that activate macrophage phagocytosis may contribute to the pathology of the disease and are fundamental for effective control of the disease progression[44,45]. IFN- γ , one among various cytokines secreted by the T-lymphocytes, seems to be responsible for augmenting the microbicidal activity of the phagocytes, thus controlling mycobacterial infection[46]. IFN- γ is biologically involved in the activation of macrophages and natural immunity, promotion of antigen-presenting cells, and development of T-helper phenotype and humoral immunity[47]. Defects in IFN- γ secretion due to depressed Th1 responses are prevalent in TB patients[48]. On the contrary, gradually improved immunity status of TB patients that appears as an elevated IFN- γ level is critically required to protect the body from and to combat *M. tuberculosis*. A TB therapy that is capable to stimulate IFN- γ secretion is a main

factor that determines the success of TB therapy.

The stimulation of IFN- γ secretion by treatment with *P. niruri* extract was demonstrated in two prospective, randomized, double-blind and placebo-controlled clinical studies on TB patients[19,21]. In those studies, *P. niruri* extract 50 mg three times daily or the matching placebo was given concomitantly with the WHO-standardized TB regimens[49]. In one study involving 40 active TB patients, a significant elevation of plasma IFN- γ level (+7.65 pg/mL from the mean baseline level of 5.24 pg/mL) was found after 2 months of concomitant treatment with *Phyllanthus*[21]. Aligned with that finding, another separate study involving 67 active pulmonary TB patients also reported a moderate IFN- γ elevation in those who received a 6-month concomitant *Phyllanthus* treatment[19]. In that study, the elevated cytokine level observed after 2 months of therapy with *Phyllanthus* extract was even maintained up to 6 months, albeit not significant due to the small statistical power. In subjects of the placebo group (*i.e.* those with the standard TB regimens alone), IFN- γ was slightly elevated after 2 months of treatment (+0.41 pg/mL from the mean baseline level of 7.73 pg/mL). However, in those subjects, IFN- γ level was then declining along the way from 2 to 6 months of the TB-therapeutic course[19].

Other than IFN- γ , *P. niruri* treatment on TB patients was also found to be associated with the elevated secretion of TNF- α [19]. A slight decrease of TNF- α level was observed earlier during the course, and then followed by its elevation for the last 4 months of therapy. Such an elevation indicated a favorable response of the body immune system against the growth of mycobacterial pathogens[19]. In active pulmonary TB patients, the levels of IFN- γ and TNF- α were found to be lower than those of healthy subjects[48]. In the process of mycobacterial infection control, the role of TNF- α seems to be more primordial. The cytokine is mainly secreted by monocytes, activated macrophages, T-lymphocytes, and dendritic cells, and acts in synergy with IFN- γ , upon various kinds of cells, to induce the production of reactive nitrogen intermediates and mediate the tuberculostatic function of macrophages[50,51]. TNF- α stimulates the immune cell migration to the infection site, contributing to the granuloma formation capable of controlling the disease progression. Induction of apoptosis is also associated with the control of *M. tuberculosis* by TNF- α . The IFN- γ and TNF- α locally produced by leucocytes critically contribute to the differentiation and activation of the recruited peripheral monocytes to devour the mycobacteria[44,52,53].

During *M. tuberculosis* infection, IL-10, an anti-inflammatory cytokine also known as human cytokine synthesis inhibitory factor, is produced and released primarily by macrophages and cytotoxic T-cells (CD8⁺), inhibiting the actions of NK cells against the pathogens[54]. In a randomized, double-blind, and placebo-controlled study, Amin[19] reported that *P. niruri* extract treatment in addition to the standard TB regimens suppressed human IL-10 secretion in 39 active pulmonary TB patients with moderate or severe radiological lesions and positive sputum acid-fast bacilli test. After 2 months of therapy, the IL-10 increased by 25% from the baseline level in group receiving standard TB regimens alone, compared to a suppression of 50% from the baseline in group receiving both *P. niruri* extract and the standard TB regimens. IL-10 suppression by *P. niruri* facilitated the inflammatory response required to eradicate the microbes. Such a

response also indicated a restored immunity of TB patients receiving *P. niruri* extract on top of the standard TB regimens.

Unlike IFN- γ and TNF- α , IL-10 primarily serves as an inhibitory cytokine; and therefore it plays an important role to regulate the balance between the inflammatory and immunopathological responses. In brief, it has an immunoregulatory function[55]. Active pulmonary TB patients particularly the anergic patients, demonstrated markedly elevated IL-10 level, both in serum and bronchoalveolar lavage fluid, suggesting that *M. tuberculosis* stimulates IL-10 production that in turn disrupts an effective immune response[48,56]. The higher the capacity of IL-10 production is, the greater the risk for developing the disease[57,58]. IL-10 was also found to downregulate *M. tuberculosis*-induced Th1 responses, thus inhibiting the synthesis of IFN- γ [56]. Therefore, the inhibition of IL-10 by *P. niruri* reverses the suppression on macrophages and T-lymphocytes observed in patients suffering from TB, indicating the restoration of the protective T-cell response.

IL-10 also possesses the capacity to directly inhibit the responses of CD4⁺ T lymphocyte[56]. Therefore, IL-10 suppression by *P. niruri* treatment also implies the optimization of CD4⁺ T lymphocyte activity as demonstrated in a randomized controlled clinical study by Raveinal[22]. Parallel with favorable effects of *P. niruri* on IFN- γ , TNF- α and IL-10, the study, which involved 40 TB patients with comparable baseline immunological status, reported that the addition of *P. niruri* 50 mg extract given three times daily to the standard TB therapy markedly elevated peripheral CD4⁺ count [from the baseline value of $(45.55 \pm 6.07) \text{ mm}^3$ to $(56.25 \pm 5.95) \text{ mm}^3$, $P < 0.01$] and CD4⁺/CD8⁺ ratio [from the baseline value of $(1.39 \pm 0.22) \text{ mm}^3$ to $(1.71 \pm 0.21) \text{ mm}^3$, $P < 0.01$]. Such increases were also significantly greater ($P = 0.010$) than those of group receiving the standard TB therapy alone[22]. Unlike the finding in *P. niruri* group, the increase of CD4⁺ count in that group [from the baseline of $(42.70 \pm 5.97) \text{ mm}^3$ to $(47.15 \pm 5.69) \text{ mm}^3$, $P < 0.05$] was not accompanied by a sizeable increase in CD4⁺/CD8⁺ ratio [from 1.36 ± 0.25 to 1.41 ± 0.20 , $P > 0.05$]. The level of CD8⁺ was not significantly affected by treatment in both groups[22].

Recent studies reported that a increased peripheral CD8⁺ lymphocytes and reduced CD4⁺/CD8⁺ ratio were observed in severe or advanced TB patients compared to those with less severe disease or healthy subjects[59-61]. In clinical practice, the ratio of CD4⁺/CD8⁺ has been used as a conventional indicator to evaluate the immunity of TB patients[60]. The increase in CD4⁺ count and CD4⁺/CD8⁺ ratio, which were reported by Raveinal after one month of treatment with *P. niruri*, indicated the presence of early immunity recovery of the TB patients[22].

The immunological findings found in clinical studies on *Phyllanthus* treatment were well correlated to those observed in preclinical studies. Further, those studies also reported that the enhancement in immunological parameters seen with *Phyllanthus*, as indicated by the improved balance of various cytokines, was also translated into positive clinical and radiological outcomes[19-22]. The addition of *P. niruri* extract to the TB standard therapy also speeded up the conversion of sputum acid-fast bacilli, which was found to occur within just 1 week after treatment initiation. Such an early sputum acid-fast bacilli conversion was observed in higher

proportion of patients receiving *Phyllanthus* treatment (52.9%) than that of placebo (39.4%)[19]. The difference between groups was not statistically significant due to the small sample size of the study. Yet, the finding provided a favorable clinical consequence, particularly for the community around which the patients lived. An earlier sputum conversion will reduce the risk of TB transmission among the community. The finding of this study suggests that TB treatment with *Phyllanthus* supplementation may potentially lower the risk of TB transmission.

The consistent immunological improvement reported in those clinical studies was aligned with the immunomodulatory activities of *P. niruri* demonstrated in many preclinical studies[62-64]. The herb promises a great potency to earn position in the management of TB therapy where it may act in synergy with the standard treatment of TB to achieve more optimized and successful outcomes.

The utilization of immunotherapy for TB infection is emergent, particularly because the current long-term use of antibiotic therapy for treating the disease has increased the emergence of antibiotic-resistant strains of the pathogens. In fact, the multiple and severe multiple drug resistant (MDR and XDR) forms of TB have become a serious global threat worldwide. A combination of antibiotics and immunomodulator agents could be the key of therapy, to minimize generation of drug resistant bacteria, shorten the duration of treatment, and lower the incidence of reinfection and reactivation[65-67].

4. Varicella-zoster infection

Varicella or chicken pox, a highly contagious infection, is known as a benign disease in childhood. However, in adults, varicella infections usually manifest more severely and adult patients are at risk of developing complications. Varicella-zoster, which is classified as herpes virus, is the causal pathogen of chicken pox as well as herpes zoster infection or shingles[68]. In immunocompetent patients, recovery from varicella infection critically depends on the effective body immune system. Therefore, it is advisable to use immunostimulating agents rather than antivirals to manage varicella infection in immunocompetent patients.

A randomized controlled study to examine clinical benefits of *P. niruri* extract in paediatric patients with varicella-zoster infection was conducted by Sarisetyaningtyas *et al.*[24], in a hundred children of 2–14 years old who were diagnosed to have uncomplicated varicella infection. In the study, the eligible subjects received either *P. niruri* extract at a dose of 25 mg/5 mL syrup three times daily or the matching placebo in a randomized, double-blind fashion. The study evaluated the course of recovery from varicella infection that is clinically characterized by disappearance of fever and new papules, followed by the crust formation in most parts of the body. The number of visible papules and crusts throughout the 4-day treatment was also measured quantitatively to indicate the clinical efficacy of the treatment.

The study reported that the clinical improvement of the *Phyllanthus*-treated patients was not significantly different with that of the placebo-control group. After two treatment days, fever had no longer persisted in all patients, suggesting that *P. niruri* had a neutral

effect on fever. After four treatment days, more than 50% of the crusty lesions had been aborted in a greater proportion of subject ($P = 0.053$) in *Phyllanthus*-treated group (43.1%) than in the placebo-control group (30.0%). The statistical number needed to treat was considerably low, *i.e.* 7.6, which means that we need to treat only 8 varicella-infected patients, to gain one patient with a complete recovery within 4 days after treatment initiation. Further, the study also reported that after 3 days of treatment, the crusts formation was found in more subjects of *Phyllanthus*-treated group than of control group. According to the clinical point of view, the result suggested that *P. niruri* extract was beneficial to shorten the time to recovery from varicella infection, particularly by accelerating crust formation, which was immediately followed by their disappearance indicating disease relief. The appearance of lots of crusts during the healing process is a clinically important indication that the infection is no longer contagious[24].

In the study, no subjects in both groups were inflicted with secondary skin infections or other central nervous system complications[24]. The finding was expected because such complications are rarely found in most immunocompetent patients with varicella infection[69].

The study provided a preliminary clinical evidence of *P. niruri*'s potential benefit to shorten the whole morphological course of skin lesions observed in varicella disease. Larger clinical studies are still necessary to further explore whether such a potential benefit was merely associated with the immunomodulatory activity of the plant or it possesses a direct antiviral activity. To date, there are no available data studying the herbs' direct antiviral effect on varicella-zoster virus.

5. Vaginal candidiasis

Vaginitis is the most predominant gynecological condition worldwide, with vaginal candidiasis (vulvovaginitis candidiasis), which is caused by *Candida albicans*, comprising up to a quarter of the diagnosis[70]. In fact, 75% of women experience vaginal candidiasis at least once during their lifetime[71]. Insufficiency of IFN- γ due to T-lymphocyte dysfunction was a predisposing factor to the recurrent vaginal candidiasis[72]. Promoting the host's specific immune response to candida is critical for the healing process of candidal infections[73]. Since the effectiveness and safety of currently available anti-fungal agents are limited, it is reasonable to apply a combination therapy of the anti-fungal and immunotherapy in dealing with candidiasis, particularly for the recurrent or refractory cases[74,75].

The efficacy of *P. niruri* in vaginal candidiasis based on its immunomodulatory property was clinically examined by Pramayanti *et al.*[23]. Under a double-blind fashion, thirty married female patients with vaginal candidiasis were randomly allocated to receive oral ketoconazole 200 mg given twice daily for 5 days, and either *P. niruri* 100 mg extract or its matching placebo given three times daily for 7 days. Follow-up and evaluation were continued up to 3 months after the treatment was ended. After 7 days of treatment, then a 1 and 3 month follow-up, IFN- γ levels in vaginal secretes of patients without *P. niruri* treatment were found lower [(100.76

± 28.54) pg/mL, (96.26 \pm 28.39) pg/mL and (91.35 \pm 30.37) pg/mL, respectively] than that of baseline [(105.11 \pm 28.67) pg/mL]. It is obvious that their IFN- γ levels dropped even lower than that of baseline at 1 and 3 months of follow-up. On the contrary, in patients receiving *P. niruri*, the IFN- γ levels after 7 days of treatment, and 1 to 3 months of follow-up [(138.00 \pm 34.67) pg/mL, (159.10 \pm 58.76) pg/mL and (128.48 \pm 24.92) pg/mL, respectively] were all significantly higher than that of baseline [(120.14 \pm 44.51) pg/mL], and significantly higher than those of patients without *P. niruri* treatment ($P = 0.004$, $P < 0.001$, $P < 0.001$, at 7 days of treatment, 1 and 3 months of follow-up, respectively)[23]. Elevation of IFN- γ level indicates an augmented activity of the T-helper 1 cellular immune response, with concurrent suppression of IL-4 and IL-10 secretion by Th2 subsets, and activation of the macrophages necessary for candida eradication from the vaginal tissues[72,76].

The study also reported that along with the elevation of IFN- γ in *P. niruri* group, a noticeably higher level of IL-12 in the vaginal secrets was observed after 7 days of treatment, and one to three months afterwards [(118.23 \pm 109.15) pg/mL, (128.31 \pm 112.76) pg/mL, (97.80 \pm 81.60) pg/mL, respectively] than those of baseline [(71.68 \pm 68.71) pg/mL]. There were no significant changes of IL-12 level observed in placebo group [(60.10 \pm 25.20) pg/mL, (60.13 \pm 28.04) pg/mL, (67.88 \pm 23.95) pg/mL, (55.47 \pm 20.44) pg/mL, at baseline, 7 days of treatment, one and 3 months of follow-up, respectively]. In specific cellular immunity, IL-12 is notably associated with the stimulation of IFN- γ production by NK and T cells as well as the augmentation of NK cell cytotoxicity[77].

Consistent with the immunological improvement, a high recovery rate (73.33%) was observed in patients receiving *P. niruri* at 7 days of treatment, also followed by a low recurrence rate after one and three months of follow-up period (18.2% and 45.5%, respectively). Those rates were all found much lower in patients without *P. niruri* treatment (26.67%, 50.00% and 100.00%, respectively)[23]. Finally, despite the necessity of more adequately powered clinical studies, the findings of the study have excitingly indicated the clinical benefits of chemo-immunotherapy in the management of vaginal candidiasis. Such an approach has been shown to accelerate the recovery as well as reduce the recurrence of the disease[78].

6. Tonsillopharyngitis

The immunomodulatory activity of *P. niruri* has also been studied in patients with acute tonsillopharyngitis. In a randomized, placebo-controlled study, *P. niruri* extract was given in a combined preparation with *Nigella sativa* (*N. sativa*) extract, which is known to possess an anti-inflammatory property[79,80]. One hundred and ninety-six enrolled patients were randomly allocated to receive either the active capsules containing a combination of *P. niruri* 50 mg extract and *N. sativa* 360 mg extract, or the matching placebo, under a double-blind fashion. The capsules were to be taken three times daily orally, for 7 days. The combined extracts greatly alleviated sore throat symptoms indicated by the visual analogue scale reduction in swallowing pain and swallowing difficulty within 6 h after administration. The reductions in *Phyllanthus-Nigella* treated group (29.29 \pm 19.46 or 58.71% from baseline; and 33.81

± 18.56 or 62.06% from baseline, respectively) were greater ($P = 0.008$ and $P = 0.001$, respectively) than that of the placebo (22.72 ± 19.08 or 46.44% from baseline, and 26.40 ± 20.36 , or 48.10% from baseline, respectively). Treatment with *Phyllanthus-Nigella* extract also provided a complete relief of the sore-throat in a significantly greater proportion of patients than the placebo did (60.0% versus 38.4% , $P = 0.022$)[79]. In the study, *Phyllanthus-Nigella* extract was efficacious in relieving the symptoms of acute tonsillopharyngitis with any baseline severity ranging from mild to severe. Yet, it showed a greater relief in those with moderate to severe baseline symptoms. This study alludes that *P. niruri* has a potential to be used in combination with other herbs to provide a synergistic effectiveness in the treatment of acute infections in clinical practice.

The anti-nociceptive property of *P. niruri*[81] altogether with the antibacterial activity of *P. niruri-N. sativa* combination might contribute to the effectiveness of the treatment with the combined extracts[82]. An *in vitro* study demonstrated that various pathogenic bacteria, including *Streptococcus pneumoniae*, one of the most common causes of bacterial tonsillopharyngitis, were sensitive to the combined *P. niruri-N. sativa* extract. The antibacterial potency of the *P. niruri-N. sativa* extract was much greater than that of either extract alone, suggesting that both extracts given in combination deliver a synergistic antibacterial effect[82-85]. The clinical benefit of the combination of *P. niruri-N. sativa* for the treatment of acute tonsillopharyngitis as confirmed through this study may contribute to minimizing the irrational and excessive use of antibiotics in such an infection, which is mostly viral in origin, thus in turn, the incidence of antibiotic-resistance will hopefully be reduced.

7. Conclusions

Altogether, all of the studies depicted *P. niruri*'s clinical efficacy as an immunomodulator through the activation and augmentation of the cellular immune system. Specifically, *P. niruri* activates neutrophils, macrophages or monocytes, and T and B lymphocytes. Activated phagocytic process by the neutrophils suggests an acceleration of the active eradication process, particularly of the extracellular pathogens, such as invading viruses, microbes, or fungi and their removal from our bodies. On the other side, the enhanced phagocytic profile of monocytes and macrophages by *P. niruri* induces the lysis of the intracellular pathogen-infected cells, rendering them being exposed to other immune components in the extracellular compartments. Further, the modulation of cytokine secretion by *P. niruri* treatment, as observed in various clinical studies, such as stimulation to IFN- γ , TNF- α , IL-4, IL-6, IL-12, and suppression to IL-10, strongly indicates that *P. niruri* influences our body's defense reaction involving cellular immune system against foreign pathogens.

It is also critical to note that in all of the studies published over the past two decades, no signs of toxicity or serious adverse reactions of *P. niruri* have been reported. The plant-derived extracts were proven to be practically safe in any of the human and animal studies, both for acute or chronic use[9,86,87]. Therefore, *P. niruri* is currently gaining popularity in many countries as an effective and safe herbal remedy for various infectious diseases. However, the use of the herb extract in formal practices is still low. In fact, there are

numerous *in vitro* and animal studies reporting potential benefits of the immunomodulatory properties of the species, and numbers of randomized controlled clinical studies have been published to date. In the light of the scarcity of research to discover new, more effective and safe anti-infection chemical entities, complicated with the growing threat from the new generations of drug resistant-pathogens, the utilization of nature-derived immunomodulatory agents, either alone or combined with the currently available antibiotics or antivirals, is undoubtedly promising and of clinical importance. Most of the studies on *P. niruri* warrant its potential benefits in various infectious diseases, and are expected to grant the herb an important place in the management of such diseases in the formal clinical practice.

Conflict of interest statement

We declare that we have no conflict of interest.

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