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Review Article

CHEMICAL AND PHARMACOLOGICAL PROPERTIES OF SELECTED MEDICINAL PLANT SPECIES FROM GENUS *PREMNA* AND THEIR IMMUNOMODULATORY POTENTIALS

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ABSTRACT

Epidemiological data today show an increase in immune diseases; hence increasing awareness to maintain and increase the body's immunity is essential. Immunomodulators are substances that improve the human immune system by stimulating, inhibiting, or regulating components in the immune system. Plants produce secondary metabolites which provide beneficial effects on human health, including immunomodulatory properties. Natural immunomodulators can be used to minimize side effects and toxicity compared to synthetic materials because using synthetic immunomodulators may result undesirable side effects, such as pulmonary toxicity, myelosuppression, alopecia, and nephrotoxicity. Besides that, using plants as medicine is safer to use, more effective against a wide spectrum of pathogens, and relatively cheaper than synthetic medicine. Some of the medicinal plants which have immunomodulatory properties belong to the genus *Premna* from Lamiaceae. Alkaloids, flavonoids, phenolics, saponins, terpenoids, polysaccharides, and fatty acids supported the pharmacological effect of *Premna* sp. as immunomodulatory agent. The present work reviews some species in genus *Premna,* which were the subject of literature search based on major scientific databases, including PubMed, Elsevier, SpringerLink, Science Direct, Scopus, Mendeley, and Google Scholar. This review presents a series of selected plants from genus *Premna* sp. including their chemical and pharmacological properties as well as immunomodulatory potential.

Keywords: *Premna* sp., Immune system, Immunomodulatory agent, Medicinal plants

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INTRODUCTION

Epidemiological data today show an increase in immune diseases [1]. Awareness to maintain and increase the body's immunity is essential because various disease-causing agents, such as bacteria, viruses, fungi, parasites, and other foreign microorganisms, can attack the body, causing infectious diseases [2–4]. Humans have an immune system that can recognize and react against pathogens [5]. Additionally, the immune system detects abnormal cells and infections and maintains the balance of body components and functions [6, 7]. Immune system, namely adaptive immunity, is highly effective and specific to attack an antigen [8]. Despite its high efficiency and specificity, imbalances in immune responses are unavoidable and may contribute to many diseases, such as allergies, autoimmune diseases, microbial infections, HIV/AIDS, and others [1, 7, 9].

There are several ways to increase the body's immunity, including using immunomodulators. Immunomodulators are substances that improve the human immune system by stimulating, inhibiting, or regulating components in the immune system [10, 11]. Immunomodulators can affect the work of cells, antibodies, cytokines, apoptosis, and components in the immune system. Immunomodulators are helpful for therapy in patients with impaired immunity, such as chronic infections and autoimmune disorders [12–14]. Immunomodulators are also known to have good potential, either as a single therapy or as additional therapy in treating viral diseases [12, 15]. Moreover, immunomodulators can be used in cancer patients to reduce the immunosuppression side effects of chemotherapy [7].

Immunomodulators affect the immune system's response by increasing (immunostimulants) or decreasing immune responsiveness (immunosuppressants) [10, 11]. Immunostimulants enhance immune responses to infections [11, 16]. They are also suitable for the treatment of immune-related diseases, such as rheumatoid arthritis, tumors, lupus, primary or secondary immune deficiencies, cancer, and changes in antibody transfer [2, 10, 14, 16]. On the other hand, immunosuppressants which reduce the immune response are ideal to be used by patients with transplanted organ and to treat autoimmune diseases such as pemphigus and allergies [10, 11, 13].

Immunomodulators can affect both innate and adaptive immunity. The mechanism of natural immunomodulators is that it can decrease pro-inflammatory cytokines (IL-1, IL-6, TNF, IL-8) in the body, increase the effect of IL-10, which is an antagonist to pro-inflammatory cytokines. Immunomodulators enhance phagocytic activity, phagocytic effect, and cell related to immunity [17]. Besides that, immunomodulators can also lessen excessive Th2 cells, lower IgE levels in allergic patients, stimulate antibody production, release hypersensitivity mediators and tissue responses to the mediators in the target organs [18]. Immunomodulators can also supress the immune system by decreasing antibody titer [17].

Immunomodulators can be derived from synthetic materials (such as levamisole and cyclophosphamide) or natural ingredients [13, 14, 19, 20]. Synthetic immunomodulators have several favorable impacts on the immune system. Despite the fact that these conventional immunomodulators have various useful functions, they also have a number of unwanted adverse effects that are developing with time and negatively impacting the body, such as pulmonary toxicity, myelosuppression, alopecia, and nephrotoxicity [21, 22]. Besides that, using synthetic immunomodulators can also cause diabetes, GI disorders, hypertension, hyperkalemia, hyperglycemia, hepatic fibrosis, lymphoma (related to the Epstein-Barr virus), and neurotoxicity (tremor, headache, motor difficulties, and seizures), hypertension, diabetogenic, elevated LDL cholesterol, tremor, hirsutism, hyperlipidemia, gum hyperplasia, hyperuricemia, and hyper cholesterolemia [7, 10, 16, 20, 22, 23].

The incidence of major adverse medication responses limits the long-term use of such medicines and requires the search for alternative techniques that are more effective and safer than these standard immunomodulatory agents [21]. Plants are known to produce secondary metabolites which provide beneficial effects on human health, including immunomodulatory properties [7, 11, 16]. Natural immunomodulators can be used to minimize side effects and toxicity [11]. Due to their numerous bioactivities, good tolerability, and high patient compliance, plant-based nutraceuticals provide an intriguing tool to be used for the treatment and prevention of immune system illnesses [24].

In addition, using herbal products has advantages, namely because of its safety, effective against a wide spectrum of pathogens, relatively cheap production costs, no significant environmental damage, and easy access for residents in rural places [16, 20, 25]. According to an Italian survey, herbal medicine is often used to improve quality of life [26]. Also, according to recent surveys, the most important reason for the preferred use of herbal therapy in Germany is dissatisfaction with conventional treatment [27].

An example of a medicinal plant with many benefits belongs to the genus *Premna* from Lamiaceae [28]. Genus *Premna* has more than 200 species and is distributed in tropical and subtropical Asia, Africa, Australia, and the Pacific Islands [29–31]. *Premna* is rich in phytochemical constituents such as essential oils, fatty acids, flavonoids, glycosides, diterpenoids and xanthones [28, 30, 32]. It is known that *Premna* has many health benefits [28]. Studies have assessed the immunomodulatory activity of extracts from *Premna* species [33–36].

Alkaloids, flavonoids, phenolics, and saponins in particular have been reported to have immune-modulating activities. Terpenoids, polysaccharides, and fatty acids have also drawn a lot of attention as potential immunomodulatory substances that could be used in the treatment of immunological diseases [24, 37]. Alkaloids are found in *P. latifolia*; flavonoids and terpenoids are found in most of *Premna* sp.; phenolics are found in *P. esculenta* and *P. herbacea*; saponins are found in *P. hispida* and *P. latifolia*; fatty acids are found in *P. integrifolia* and *P. odorata*.

Given the importance of the immunomodulatory potential of several species from genus *Premna*, the present work reviews some species in genus *Premna* which were the subject of literature search based on major scientific databases including PubMed, Elsevier, SpringerLink, Science Direct, Scopus, Mendeley, and Google Scholar. This review presents a series of selected plants from genus *Premna* sp., including their chemical and pharmacological properties as well as immunomodulatory potential.

Immunomodulators

Immunomodulators are substances that can be used to modulate the activity and function of the immune system so that it can avoid and treat the body from disease and maintain health [38]. Immunomodulators can be specific or nonspecific. Specific immunomodulators are limited to only one antigen, such as vaccination. In contrast, nonspecific immunomodulators can cause further changes in the immune response in innate immunity and adaptive immunity against various antigens [16]. There are two types of immunomodulators, namely immunostimulators and immunosuppressors [10, 11].

Immunostimulators induce the activity of immune system components and body's resistance against infection [10, 14, 16]. Immunostimulators can stimulate phagocytosis, complement system, T and B lymphocytes, synthesis of cytokines and specific antibodies, and the release of IFN-α and IFN-γ [16]. Secondary metabolites of plants that can be used to increase immunity are flavonoids, curcumin, phenolics, alkaloids, catechins, terpenoids, and carotenoids [7, 16]. Immunostimulators are indicated for diseases with low immune activity, such as AIDS, chronic infections, cancer, and tumors [14, 39]. Examples of immunostimulatory drugs are levamisole, thalidomide, and isoprinosine [10, 16, 38].

Immunosuppressors cause inhibition or suppression of immune system activity [13]. Immunosuppressors can inhibit lymphocyte proliferation and release pro-inflammatory mediators, such as prostaglandin E2 and TNF-α, and NF-B trans-activation activity [13, 20]. Immunosuppressors are indicated for diseases with high immune activity, such as autoimmune diseases (allergy, lupus, psoriasis), organ transplantation-related diseases [13, 39]. Secondary metabolites of plants that can be used to increase immunity are isoflavones, quinones, and stilbenes [38]. Examples of immunosuppressor activity drugs are mycophenolate, cyclophosphamide, and glucocorticoids [10, 38, 40].

Mechanism of phytochemicals in plant medicine as immunomodulators

Alkaloids are also reported to enhance immunological response, and several alkaloids are being studied for their immunostimulant characteristics [41]. Alkaloids inhibited tumor development by increasing WBC count, bone marrow cellularity, and total antibody formation, as well as the levels of Th1 lymphocytes mediated TNF-α, IL-12, and IL-1; decreasing expressions of Th2 mediated IL-4, IL-10, as well as cell viability; and preventing cardiac graft rejection [21, 38, 42]. Flavonoids may increase phagocytic capacity, macrophage activity, and interferon production [43, 44]. Flavonoids prevent oxidative cell damage, have a powerful anti-cancer effect, and protect against carcinogens at all stages. They suppress tumor formation, promotion, and progression in terms of anti-cancer action [41]. Flavonoids modulated IFN-γ expression; increased humoral and cell-mediated immune responses; reduced CD4+cell proliferation, INF-α and IL-2 production, p65/NF-B phosphorylation, chemokine, and cytokine expression [38].

Saponins have an anticancer impact on many cancer cells and suppress tumor cell growth via cell cycle arrest and apoptosis. Besides that, saponins augmenting cellular and humoral immune responses as well as Th1-mediated cytokines (IFN-γ, IL-2) while suppressing Th2-mediated cytokines (IL-4) [21, 41]. Several terpenoids have been shown to have antiarthritic or antiphlogistic activities, and their biological effects appear to be mediated via immunological mechanisms. These chemicals appear to have a dual effect on the immune system, initially increasing antibody formation and suppressing T-cell responsiveness. Terpenoids also increase lymphocyte proliferation [38].

Sterols in a certain ratio may be able to restore a balance between Th1 and Th2 cells, a sensitive equilibrium that affects the outcome of the immunological response [41]. Glycosides suppressed cancer growth by decreasing the expression of Th2-mediated TNF-α, IL-3, IL-4, IL-5, IL-9, IL-13, IL-17, and RANTES whilst increasing the expression of Th1mediated IL-2, IL-12, IL-10, and IL-γ in serum [21]. Phenolics reduced TGF-1, NF-B, and TNF-α levels, NO synthesis, IL-2, and T-cell proliferation; increased leukocyte count, thymus and spleen indices, IFN-γ production, NK cell cytotoxicity, and phagocytosis [21, 44].

***Premna* sp.: morphology, chemical constituents, ethnomedicinal uses**

Most *Premna* species are small trees or shrubs, rarely vines [29, 33, 45]. Many species have a series of small (usually<1.5 cm) intersecting triangular scales at the base of young branches. On older branches, these scales have usually fallen off, leaving scars of a series of dense bracts [31, 33]. The leaves of this genus are usually crossed. However, many species have examples of semi-paired or alternating leaf specimens. The crest is often present between the petioles and is sometimes confused with the stigma between the petioles. The leaves are hairy [29, 45].

In Southeast Asia, two basic calyx types can be recognized with certainty. The first seed always has four isomorphic leaves and largely preserves its shape during flower development and fruiting. The second type is more common and usually has 0 to 5 lobes of irregular shape. Two types of fruit can be identified. The first is a globular, stone-like fruit consisting of four fleshy mericarps, each with a seed. The second type is a tufted, almost single drupe-like fruit (four seeds present, but only one fully developed, the other three are only partially developed) [31, 33, 45].

Antioxidant activities

Many *Premna* sp. are known to have antioxidant activities, which in this review presents the antioxidant activity of *P. corymbosa*. The levels of antioxidants were dramatically enhanced in diabetic rats' various organs (liver, kidney, brain, heart, and pancreas) after treatment with *P. corymbosa* root extract (Rottl.). GSH levels were significantly elevated in diabetic rats treated with *P. corymbosa* (Rottl.) root extract. When compared to diabetic rats, treatment with *P. corymbosa* (Rottl.) root extract boosted the activities of SOD and CAT enzymes and may help to manage free radicals. The plant extract's impact was equivalent to the common medication, glibenclamide. When compared to diabetic control rats, root extract of *P. corymbosa* (Rottl.) significantly reduced levels of lipid peroxidation in STZ-induced diabetic rat tissues, which could be responsible for scavenging free radicals released by STZ and thus enhance both enzymic and non-enzymic antioxidants in diabetic rats treated with root extract of *P. corymbosa* (Rottl.) [47].

Table 1: Ethnomedicinal uses and pharmacological studies of some selected *Premna* sp

Premna species	Parts of plant	Chemical constituents	Ethnomedicinal uses	Pharmacological studies	Methods and findings
P. corymbosa	Leaves	Oxygenated sesquiterpenoids: spathulenol and caryophyllene oxide, sesquiterpene hydrocarbons: *allo*-aromadendrene, *(E)*-caryophyllene, *α*-copaene (Hung *et al.*, 2020)	Treat skin diseases [46]	Antioxidant [47]	**Methods:** Evaluate non-enzymatic antioxidants, enzymatic antioxidants, lipid peroxidation including basal, ascorbate-and peroxide-induced lipid peroxidation. **Findings:** Enzymatic antioxidants significantly increased, meanwhile, non-enzymatic antioxidants activities reduced in the diabetic rats treated for 30 d with 200 mg/kg of the root extract [47].
P. esculenta	Root	Phenols, tannins, terpenoids, flavonoids [48]	Treat gout, hook worm infection, hysteria, jaundice, leucorrhoea, lipoma (tumor), edema, snake bite, stomach disease, ureterolithiasis, and arthritis [49]	Analgesic [49]	**Methods:** Acetic acid-induced writhing test in mice and the radiant heat tail flick method in rats. **Findings:** The fractions showed a significant reduction in the number of writhings, inhibiting 85.96% and 61.98%. The extract produced an 88.49% prolongation of tail flick time at 90 min after oral administration at the same dose level [49].
				Anti-inflammatory [49]	**Methods:** Carrageenan-induced rat paw edema. **Findings:** The fractions showed a significant inhibition of paw edema by 22.68% and 17.24% [49]
P. herbacea	Root	Phenols, tannins, terpenoids, flavonoids [48], acetoxy group [50]	Treat scorpion and snake bite [48], convulsions [51], inflammation, and malaria [32]	Antitumor [52]	**Methods:** Ehrlich ascites carcinoma (EAC) model and the Dalton lymphoma ascites (DLA) model. **Findings:** Alcoholic extract at 500 mg/kg was the most effective in raising MST and reducing body weight in Ehrlich Ascites Carcinoma-induced tumors. The extracts and the fractions possessed potent antitumor activity by decreasing the solid tumor weight and volume [52].
P. hispida	Leaves	Saponins, flavonoids, tannins, and terpenoids [32]	Treat asthma, cough, rheumatism, fever, dropsy [32]	Antimicrobial [32]	**Methods:** Agar diffusion method against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus niger*. **Findings:** The methanol fraction, ethyl acetate fractions and methanol extract had considerable inhibitory zones diameter (IZD). The crude extract and solvent fractions' MICs ranged from 0.44 mg to 2.5 mg/ml [32].
P. integrifolia	Leaves	Linoleic acid, luteolin, phytol, *α*-humulene, spathulenol, eugenol [53]	Treat anorexia, hyperglycemia, dyspepsia, bronchitis, asthma, anemia [53]	Anti-inflammatory [54]	**Methods**: Carrageenan-induced inflammation model **Findings**: The extract showed 71.16% inhibition of carrageenan-induced anti-inflammatory activity [54].
P. latifolia	Leaves and bark	Carbohydrates, alkaloids, flavonoids, sterols, glycosides, triterpenes, tannins, and saponins [55]	Treat beri beri, vaginal discomfort, diarrhea, liver problems, and snake bites, cough, dyspepsia, and haemorrhoids [55]	Antidiabetic [56]	**Methods:** Measurements of a number of changes, including fluctuations in body weight and blood sugar, insulin, total cholesterol, triglycerides, high density lipoprotein, and low-density lipoprotein levels. **Findings:** *P. latifolia* normalizes the pancreatic and hepatic enzyme levels in diabetic rats, which in turn normalizes the blood glucose, insulin, and lipid profile. *P. latifolia* alcohol extract reduces blood glucose and cholesterol levels in diabetic rats [56].
				Antiviral [57]	**Methods:** The embryos in embryonated chik eggs infected with Newcastle Disease (NDV) vew and developmental defects. The NDV-induced cytopathic alterations were abolished by treatment with the plant extract at 500 µg/egg. **Findings:** The hemagglutination titre was found to be 512 in the group treated with the NDV virus alone, whereas it was reduced to 4 in the group treated with *P. latifolia* extract (500 µg/egg) [57].
P. microphylla Turczaninow	Whole plant	Blumenol C, β-cedrene, limonene, α-guaiene, cryptone, and α-cyperone [58]	Treat infections, appendicitis, and dysentery [58]	Antimicrobial [58]	**Methods:** By analyzing the presence or absence of inhibition zone diameters (IZD) and MIC values using the microdilution method. **Findings:** MIC of the essential oil of *P. microphylla* was 0.512 mg/ml with MIC values of 0.270 mg/ml (IZD: 15.1–15.4 mm) and 0.150-0.480 mg/ml (IZD: 9.0–21.8 mm). The essential oil demonstrated excellent antibacterial activity

					against the tested Gram–positive and negative bacteria. Following *E. coli*, *B. subtilis*, *S. aureus*, and *P. aeruginosa* in order of effectiveness was a MIC value of 0.150 mg/ml (IZD: 21.8 mm) [58].
				Anticancer [58]	**Methods:** MTT assay against HepG2 and MCF-7 cell line **Findings:** The essential oil significantly inhibited the development of HepG2 cells with an IC_{50} of 0.072 µg/ml and MCF-7 cells with an IC_{50} of 0.188 µg/ml [58].
P. odorata	Bark	Apigenin flavone glycosides, flavones, phenylethanoid glycosides, vitexin, acacetin, verbascoside, diosmetin, luteolin, apigenin [59]	Treat cold, flu, cough, headache, relieve abdominal pain [35]; phlegm, treat wounds [60]	Anticancer [61]	**Methods**: MTT assay against MCF-7 and HL-60 cell line. **Findings**: Cytotoxicity effect with IC_{50} value of 4.2, 2.7, µg/ml [61].
				Anticancer [62]	**Methods:** MTT assay against A549 cell line. **Findings:** Cytotoxicity effect of bark crude extract with IC_{50} value of 5.43 µg/ml and bark hexane fraction with IC_{50} value of 11.42 µg/ml) [62].
	Leaves	Iridoid glycosides, flavones [60]; sterols, triterpens, fatty acids; [63] acylated rhamnopyranosides, phenyl ethanoids [59]		Anticancer [62]	**Methods:** MTT against A549 cell line. **Findings:** Cytotoxicity effect of leaves hexane fraction with IC_{50} value of 16.78 µg/ml) [62].
P. serratifolia	Root	Sesquiterpene hydrocarbons: *α*-copaene and allo-aromadendrene. Oxygenated sesquiterpenoids: *(E)*-caryophyllene oxide, spathulenol, and humulene epoxide II, premnaodoriside A, p-anisaldehyde, methyl salicylate, p-vinyl anisole, acacetin, acacetin-7-O-glycoside, quercetin, kaempferol, and luteolin [46,64]	Treat genital infections, malignant wounds, unpleasant breath, white tongue, malaria, diarrhea, stomach problems, migraines, coughs, TB, and infectious diseases includes leuchorrea [29] beriberi, diabetes, dysentery, dysuria [48,64]	Anticancer [65]	**Methods:** MTT assay, Hoechst and AO/EtBr staining, ROS measurement, mitochondrial membrane potential, clonogenic and wound healing assays, this study assessed the cytotoxic capability of aqueous extract of root of *P. serratifolia* (AEPS) against Hep G2 cell line. **Findings:** The results showed that after 24 and 48 h of incubation, HepG2 cells treated with 1000 µg/ml AEPS died in 54% and 58% of cases. HepG2 cells treated with AEPS formed significantly fewer colonies during the clonogenic experiment than control-treated cells. AEPS was successful in preventing cancer cells from proliferating and colonizing. AEPS-treated HepG2 cells significantly reduced wound healing. Hoechst fluorescence significantly increased in HepG2 cells treated with AEPS (compared to control. After AO/EtBr staining in HepG2 cells treated with AEPS (1000 µg/ml) and untreated (control), there were notable alterations in the morphology of the cells. All of these findings demonstrated that AEPS has the ability to cause mitochondria-mediated apoptotic cell death [65]
				Anticancer [66]	**Methods:** Alamar Blue against SHSY-5Y cell lines. **Findings:** Cytotoxicity effect of compound 1 against SHSY-5Y with IC_{50} value of 1.5 µg/ml [66].
	Root Bark	Sabinene, Octen-3-ol, *α*-Phellandrene, Limonene, *α*-Copaene, *β*-Elemene, *β*-Caryophyllene, *γ*-Elemene, *α*-Humulene, *α*-Bulnesene, *δ*-Cadinene, Germacrene B, Phytol [67]		Anticancer [66]	**Methods:** Alamar Blue against B16 cell lines. **Findings:** Cytotoxicity effect of compound 1 against B16 with IC_{50} value of 4.7 µg/ml [66].

Analgesic activities

One of *Premna* sp., *P. esculenta*, has analgesic activity according to research by reducing the number of writhes with 85.96% and 61.98% inhibition, respectively, which was higher than the standard drug diclofenac sodium with 55.60% inhibition at a dose of 200 mg/kg body weight by the treatment of chloroform soluble fraction and ethyl acetate soluble fraction of ethanolic extract of *P. esculenta* roots. The ethanolic extract of *P. esculenta* roots exhibited 61.64% and 88.49% elongation of tail-flicking time following oral administration at the 200 mg/kg dosage level in the radiant heat tail-flick technique. The percentage elongation of tail-flicking duration rose with time and reached a peak at 90 min (88.49% elongation), which was more than that of the conventional analgesic morphine. During the observation period, the crude extract considerably improved the pain threshold, which was equivalent to morphine solution. At a dosage of 200 mg/kg, the CCl4 fraction of ethanolic extract of *P. esculenta* roots considerably increased stress tolerance capacity, with 34.24% and 19.41% elongation of tail-flicking duration at 60 and 90 min [49].

Anti-inflammatory activities

Anti-inflammatory is one of the activities of *P. esculenta* and *P. integrifolia*. According to research, the ethanolic extract of *P. esculenta* roots exhibited 61.64% and 88.49% elongation of tail-flicking time following oral administration at the 200 mg/kg dosage level in the radiant heat tail-flick technique. The percentage

elongation of tail-flicking duration rose with time and reached a peak at 90 min (88.49% elongation), which was more than that of the conventional analgesic morphine. During the observation period, the crude extract considerably improved the pain threshold, which was equivalent to morphine solution. At a dosage of 200 mg/kg, the CCl4 fraction of ethanolic extract of *P. esculenta* roots considerably increased stress tolerance capacity, with 34.24% and 19.41% elongation of tail-flicking duration at 60 and 90 min [68].

Another research showed that the anti-edematous effects of orally administered Methanolic Extract of *P. integrifolia* (MEPI) on carrageenan-induced paw edema in rats revealed dose-dependent and statistically significant anti-inflammatory activity. MEPI had strong anti-inflammatory effects at 200 mg/kg dosage (71.16% inhibition), whereas conventional indomethacin inhibited paw edema 75.72%. Because of their inhibitory effects on enzymes involved in the formation of the chemical mediator of inflammation, flavonoids and saponins are well recognized for their capacity to decrease pain perception as well as anti-inflammatory qualities [54].

Antitumor activities

One of *Premna* sp. activities is antitumor, supported by research conducted by Dhamija. The result showed that ethyl acetate at 250 mg/kg and 500 mg/kg significantly decreased the increase in body weight compared to the control. At 500 mg/kg, alcoholic extract greatly inhibited tumor growth. On days 12 and 15, the alcoholic extract and its ethyl acetate fraction were shown to be more active than Cisplatin and caused a decrease in body weight more than their zero-day weight. Alcoholic extract and its ethyl acetate fraction dramatically enhanced MST in EAC-challenged mice. The alcoholic extract was shown to be efficacious at 500 mg/kg, whereas the ethyl acetate fraction was found to be beneficial at 250 mg/kg. The alcoholic extract at 500 mg/kg was shown to be the most effective of the four treatments [52].

Both the alcoholic extract and its ethyl acetate component dramatically inhibited the formation of solid tumors. The ethyl acetate fraction at 250 mg/kg was shown to be the most efficient in suppressing tumor development (73.44%) of the four therapies tested. At 250 mg/kg and 500 mg/kg, parent extract (alcoholic extract) reduced tumor development by 50.12% and 56.90%. The ethyl acetate fraction was shown to be most effective at a dosage of 250 mg/kg, resulting in a 59.24% reduction in tumor weight. When compared to the control, alcoholic extract demonstrated a substantial tumor reduction in a dosage-dependent manner, with 37.22 and 42.91% reductions at 250 mg/kg and 500 mg/kg [52].

Antimicrobial activities

Research showed that the methanol extract of *P. hispida*, as well as the methanol fraction and ethyl acetate fraction, inhibited the development of all test microorganisms to varied degrees. The methanol extract was more effective against Bacillus subtilis (MIC: 0.57 mg/ml) than Tetracycline (MIC: 0.74 mg/ml). The MIC values of the ethyl acetate fraction against *Candida albicans* and *Aspergillus niger* were 0.44 mg/ml and 0.56 mg/ml, respectively, compared to 0.31 mg/ml and 0.51 mg/ml for fluconazole. Methanol extract had the most activity against all of the bacteria examined, whereas ethyl acetate has the highest activity against all of the fungus tested [32].

The growth inhibition zone diameters (IZD) and MIC values values showed that the *P. microphylla* essential oil had strong inhibitory effects against every Gram-negative bacteria and some Gram-positive bacteria, with MIC values of 0.270 mg/ml (IZD: 15.1-15.4 mm) and 0.150 mg/ml (IZD: 9.0-21.8 mm), respectively. With a MIC value of 0.150 mg/ml (IZD: 21.8 mm), *E. coli* was the most sensitive strain, followed by *B. subtilis* and *S. aureus* with MIC values of 0.270 mg/ml. (IZD: 15.4 mm). However, it lacked considerable action against *P. aeruginosa* and lacked antifungal activity. The absence of substantial effects on *P. aeruginosa* is related to the presence of a low concentration of terpinen-4-ol (0.7%).

Anticancer activities

One of *P. odorata* bark compounds, 4-hydroxyasarinin, showed cytotoxic effects on HL-60 and MCF-7 cell lines, with IC_{50} values of 2.7 and 4.2 µg/ml using a concentration of 10 l (5.0 µg/ml) using MTT assay [61]. *P. odorata* leaves crude extract [(12.37±2.30) µg/ml], *P. odorata* bark crude extract [(6.06±0.65) µg/ml], and *P. odorata* bark hexane fraction [(8.58±3.56) µg/ml] were all highly considered cytotoxic against HCT116 cell lines. The IC_{50} values of the extracts against MCF-7 for *P. odorata* leaves crude extract is 4.30±0.84 µg/ml, *P. odorata* leaves hexane fraction is 5.51±0.65 µg/ml, *P. odorata* leaves EA fraction is 13.71±1.39 µg/ml, *P. odorata* bark crude extract is 4.69±.063 mg/ml, and *P. odorata* bark hexane fraction is 8.42±2.11 µg/ml considered high cytotoxic activities against MCF-7 cell lines. *P. odorata* leaves hexane fraction [(16.78±7.19 µg/ml)], *P. odorata* bark crude extract [(5.43±1.72) µg/ml], *P. odorata* bark hexane fraction [(11.42±0.92) µg/ml] demonstrated potent cytotoxicity against A549 cell lines [62].

P. serratifolia also showed anticancer activities. Research showed that HepG2 cells treated with 1000 µg/ml aqueous extract of root of *P. serratifolia* (AEPS) induced 54% and 58% cell death after 24 and 48 h. AEPS treatment of Hep G2 cells resulted in considerable morphological alterations because it induced membrane breakdown and shrinking. Microscopic investigation revealed a substantial increase in DHE fluorescence in AEPS-treated cells. Fluorometric DCFDA was performed; enhanced fluorescence verified ROS generation in Hep G2 cells. These findings demonstrated that AEPS at 1000 µg/ml was effective in inducing ROS production and apoptotic cell death. There was a considerably rise in fluorescence intensity in AEPI-treated cells. The results showed that AEPS considerably changes mitochondrial membrane potential and causes mitochondrial depolarization [65].

During the clonogenic experiment in *P. serratifolia* as an anticancer, there was a substantial decrease in the number of colonies formed in AEPS-treated Hep G2 cells. The results indicate that AEPS was efficient in inhibiting cancer cell proliferation and colonization. AEPS (1000 µg/ml) treated Hep G2 cells showed a substantial reduction in wound healing. It is also established that AEPS efficiently limits wound healing by lowering cancer cell migratory capacity. There was a considerable increase in Hoechst fluorescence in AEPS (10 and 1000 µg/ml) treated HepG2 cells. It was discovered that AEPS had the ability to cause apoptotic cell death in treated Hep G2 cells. AO/EtBr verified AEPS's capacity to induce apoptosis. There was nuclear condensation and orange-stained apoptotic bodies were substantially greater in AEPS-treated cells [65].

Compound 1 was 21 and 23 times more effective than crude root bark extract against neuroblastoma and melanoma cells, respectively. In comparison to the traditional anticancer drug etoposide, the novel molecule was 5.2 and 9.6 times less effective on SHSY-5Y and B16 cells, respectively. The reported *in vitro* cytotoxicity findings against two typical cancer cell lines adds to *Premna* species' status as a rich source of new potential anticancer medicines [65].

Selected medicinal plants from genus *Premna* with immunomodulatory activities

Some medicinal plants from genus *Premna* with immunomodulatory activities will be discussed further on the following section.

P. odorata

Research conducted by Mohammad [35], reported that *P. odorata* might help patients infected with tuberculosis. It shows immunomodulatory and antioxidant effects of volatile oils extracted from *P. odorata* leaves, flowers, stems, and a combination of *P. odorata* essential volatile oils extracts (1:1:1) against TB-infected mice involving the TLR-4/NFκB signaling pathway. Positively infected TB mice showed a 78% decrease in Interleukin-10 (IL-10) but a 2,2-fold increase in the level of IL-10 by the treatment of leaves oil, 3,6-fold by flowers oil, 3-fold by young stems oil, and 5-fold by the combination of the three oils. Additionally, mice were treated with *P. odorata* VO extracted from leaves, flowers, and young stems, show significantly reduced serum IFN-γ levels by 57, 79, and 70%. In contrast, combining the three oils increased her IFN-γ serum levels to normal in infected mice or 100%. Oral treatment with leaves oil, flowers oil, and young stems oil significantly reduced serum IL-1β levels by 70%, 74%, and 65%. The 3-oil combination therapy had the best effect, as it restored IL-1β to normal mouse serum levels.

Moreover, mice were treated with *P. odorata* VO show globally reduced of serum TLR-4 levels by 58% (leaves oil), 73% (flowers oil), 67% (young stems oil), and 80% (combination of three oils) compared to infected mice. Similarly, NF-κB mRNA expression levels were increased approximately 19-fold in TB-infected mice. Oral administration of *P. odorata* VO extracted from oil of leaves, flowers, and young stems decreased NF-κB mRNA expression levels by 56, 78, and 45%. Again, the combination of the three oils showed a significant reduction in NF-κB expression levels to levels approaching normal levels.

P. pubescens

Restuati [34] stated that ethanol leaves extract of P. *pubescens* can be used as an immunostimulant. The most increased number of white blood cells is in the A2 treatment (ethanol extracts of P. *pubescens* 500 mg/kg bw and Sheep Red Blood Cells (SHRB) with a result of 9.88±2.31 x 103 mm3. The test count of lymphocytes shows that group A0 (Carboxy Methyl Cellulose) (76.47±3:10%) had the highest number of lymphocytes among the other three treatments. The least lymphocyte number was in group A3 (SRBC) because no immunostimulatory properties were added. The antibody titer test measured changes in antibody counts in the body's immune response and detected the highest antibody titers with A2 treatment, showed by IgM level of 1:05±3.96 ng/ml and IgG level of 9.48±5.90 ng/ml. These experiments also showed that A2 has the highest lysozyme level (0.04 µg/ml).

P. integrifolia

Azad [36] evaluated the immunomodulatory activity of petroleum ether, ethyl acetate, methanol, and water root extracts of *P. integrifolia* towards lymphocyte proliferation. The method used in lymphocyte proliferation assay was MTT assay. Lymphocytes treated with Con A significantly induced nearly two-fold proliferation. Out of the four extracts, the 50 µg/ml methanol extract was the only extract that showed significant lymphocyte proliferation. Meanwhile, PEE showed significant lymphocyte proliferation at 10 µg/ml, 25 µg/ml, and 50 µg/ml. These findings were further validated by cell counting. Methanol and PEE extracts of *P. integrifolia* showed potent immunomodulatory effects at nontoxic doses, suggesting potential therapeutic applications for various diseases, including cancer. PEE was further investigated for its effect on IL-2, an anti-inflammatory and immunomodulatory cytokine produced by LPS-challenged RAW 264.7 cells. PEE showed a dose-dependent increase in IL-2 production above 10 µg/ml. This significant induction of IL-2 by these extracts indicates the immunomodulatory and anti-inflammatory properties of PEE.

CONCLUSION

There has already been extensive research on the medicinal potential of more than 200+species of *Premna*. They have antioxidant, anti-inflammatory, analgesic, antidiabetic, antimicrobial, antitumor, antidiabetic, antiviral, and anticancer activities, among other possible benefits. Even though it has been extensively used by local people, no research has been done on all species. Therefore, more investigation is required, particularly into its immunomodulatory function because the immune system is crucial for maintaining body health. Several species of the genus *Premna* which are known to have immunomodulatory activity, are *P. odorata*, *P. pubescens*, and *P. integrifolia*. *Premna* sp. that has anticancer potential is still being studied *in vitro*; therefore, we need to broaden the research to learn more about the potentials and the mechanism.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

Declare none

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