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Review Article

Phyllanthin: A Potential Lead Molecule for the Future Needs

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ABSTRACT
Phyllanthin is an active lignan present in various *Phyllanthus* species and number of studies revealed that it exhibits various biological activities that include antioxidant, hepatoprotective, anticancer, antidiabetic, immunosuppressant and anti-inflammatory activities. After thorough examination of existing literature it was discovered that there is currently no comprehensive review available on this significant phytocompound. Hence, an attempt was made to present the physicochemical properties, enhancement techniques of yield and bioavailability, synthesis, pharmacological applications and toxicity studies of phyllanthin. This report also highlights semisynthetic derivatives and mechanisms of action of phyllanthin for various biological activities.

Keywords: *Phyllanthus*, *Phyllanthus amarus*, lignan, hepatoprotective, anticancer, semisynthetic derivatives, bioavailability.

INTRODUCTION

Natural products, mainly the plant-derived constituents, have long been sources of drugs. There are number of advantages using plants and phytoconstituents compared to pharmaceuticals available in modern medicine. Several classes of secondary metabolites are synthesized by plants and, among those, lignans are recognized as a class of natural products with a wide spectrum of important biological activities. The term "Lignan" was first introduced by Haworth (1948) to describe a group of dimeric phenyl propanoids (Fig-1) where two C6-C3 are attached by its central carbon (C8), as shown in Figure-2.

Aromatic amino acids L-phenylalanine and L-tyrosine are produced from shikimic acid pathway, and then converted into a series of cinnamic acid derivatives further reduction of these acids via coenzyme A forms three alcohols p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol, which act as main precursors for all lignans[1]. Lignans exist abundantly in the *Phyllanthus* genus, which consist of more than 600 species of shrubs, trees and rare herbs belong to the Euphorbiaceae family.

Phyllanthin is the major therapeutically active lignan present in various *Phyllanthus* species such as *Phyllanthus amarus* (*Phyllanthus niruri*), *Phyllanthus urinaria*, and *Phyllanthus maderaspatensis* etc. A number of studies proved that phyllanthin possess hepatoprotective activity against carbon tetrachloride, galactosamine and ethanol treatment[2,3,4]. It was revealed that phyllanthin effectively acts against diabetes and various chemical and virus induced liver abnormalities including hepatitis[5], antifibrotic[6] and anti-inflammatory[7]. Phyllanthin is also known to possess immunomodulatoty[8], nephroprotective[9], and anticancer[10] properties. After thorough examination of existing literature it was discovered that there is currently no comprehensive review available on this significant phytocompound. The purpose of this review is to deliver a comprehensive update on the status of the research carried out on the Phyllanthin in the fields of pharmacognostical, physicochemical and pharmacological.

Phyllanthin

Phyllanthin (Fig-3) chemically known as (4-[(2S, 3S)-3-[(3, 4-dimethoxyphenyl) methyl]-4-methoxy-2-(methoxymethyl) butyl]-1, 2-dimethoxybenzene). Molecular formula and molecular weight of phyllanthin was found to be $C_{24}H_{34}O_6$ and 418.51 respectively. It is a diaryl butane type lignan linked through C8–C8^0 of phenyl propanoid units. The stereochemistry in phyllanthin was identified as 8(S), 8^0(R). A single crystal X-ray analysis revealed the full 3-dimensional structural characterization of phyllanthin[11].

Phyllanthin obtained from plants belongs to *Phyllanthus* species. It was also synthesized in laboratory using (+) 2, 3-diveratryl succinic acid and resolution was secured through its cinchonine salt[12].

Physicochemical properties

Phyllanthin showed maximum UV absorbance at two wavelengths, 230nm and 280 nm, displayed IR spectrum vibrations at 2999, 2917 and 2868 cm^{-1} (C–H aliphatic stretch); 1516 and 1464 cm^{-1} (C=C ring stretch) and 1141 cm^{-1} (C – O – C stretch) and shown molecular ion peak in MS spectrum at 418 m/z and a base peak at 151 m/z. Phyllanthin crystals showed the melting point and melting enthalpy range of 96.67–97.03 ^{0}C and 109.61–116.34 J/g, respectively. Crystals, recrystallized form petroleum ether, absolute ethanol and 25% ethanol solution, revealed only one polymorph and no polymorphic impurity and 25% ethanol solution gave high crystallinity. It thermal decomposition was found to be above 200 ^{0}C. It has stability in aqueous solution over a pH range of 1.07–10.02 for at least 4 h and expected to be unaffected by the

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variation in pH throughout the GI tract. Solubility was found to be in the range of 7.32–8.57 mg/ml and appeared to be pH-independent and it has no pKa over a pH range of 1.12–10.02. The log P_{ow} value was found to be 3.30±0.05 at pH 7.48 by shake flask method, it indicates good permeability through biological membranes[13].

Enhancement of yield

Considering the importance of Phyllanthin various researchers tried to increase its yield by using various techniques.

By using different harvesting techniques

Phyllanthin content was observed more in plants growing at higher altitudes as compared to lower altitude[14]. When three areas having different soil, profile and color taken it is revealed that the content of the phyllanthin does not fluctuate significantly and are not very much dependent on any biotic or abiotic factor in particular to enhance or decrease them[15].A study was carried out to know the effect of Nitrogen, magnesium and shading revealed that 2.39 g of urea and 0.48 g of MgSO4 per plant and 3 months of harvest produced the highest phyllanthin yield[16].It was revealed that heavy metals such as chromium and cadmium at certain level enhance concentration of secondary metabolite phyllanthin in *Phyllanthus amarus* due to abiotic stress[17,18].

By genetically modifying

When transgenic lines and wild plants was compared using HPLC it was discovered that transgenic lines contained higher phyllanthin content (0.3–0.81% w/w) than wild plants (0.09% w/w)[19].CIM-Jeevan a cultivar of *Phyllanthus amarus* plant producing high amount phyllanthin and hypophyllanthin was developed by using y-irradiation of superior germ plasm[20].

By using biotechnology

A study depicted enhancement of phyllanthin yield by immobilization technique, HPTLC was used to compare the phyllanthin contents in calcium alginate immobilized cells obtained from fresh grown plants and MS medium was supplemented with different abiotic elicitors. The study revealed that an increase in the content of phyllanthin and hypophyllanthin were elicitor concentration dependent and silver nitrate treatment gave a maximum yield of hepatoprotective bioactives as compared to the other abiotic elicitors used[21]. Another immobilization technique developed to enhance phyllanthin and hypophyllanthin by using calcium alginate immobilized cells obtained from fresh grown plants and M. S. medium which was supplemented with different precursors and phytohormones. It was revealed that cinnamic acid treatment gave maximum yield of hepatoprotective bioactives as compared to other precursor and phytohormones[22].

An experiment performed on callus by manipulating the different concentrations of phytohormones revealed that 12 week old callus culture induced in MS media containing phytohormones 2, 4-D (1mg/L) and Kinetin (0.5mg/L) gave the maximum yield of phyllanthin (0.805%) [23]. When thidiazuron is used in Murashige and Skoog (MS) medium caused the regenerated shoots to produce about two time's higher phyllanthin and hypophyllanthin than the leaves of naturally grown plant[24]. When MS medium containing callus was treated with gibberellin acid, coconut water, sugarcane juice and water-melon extract and estimated by HPTLC it was revealed that watermelon extract, enhances maximum phyllanthin yield followed by sugarcane juice, coconut water and gibberellin acid[25].

A research work found an endophytic fungi from different parts of *Phyllanthus amours* producing bioactive compounds phyllanthin and hypophyllanthin[26]. A team developed micropropagation method to overcome the uncertainty in stable supply by the hyperproducing ecotype which was able to produce phyllanthin in good yield[27]. In a study cDNA library from fresh young leaves of *Phyllanthus amarus* was created and used it for generation of EST database to explore the presence of metabolite synthesizing enzymes in the library. This EST's can be used as a first-hand reference at the molecular level to identify the genes of phyllanthin biosynthetic pathway[28].

Some researchers tried to increase the yield by enhancing phyllanthin extraction from plants by using various methods such as supercritical fluid extraction[29, 30], pressurized liquid extraction[31], soxhelation[32], and other conventional methods.

Enhancement of bioavailability

To address the phyllanthin low bioavailability some research had been carried out. a research group developed phyllanthin-loaded self-microemulsifying drug delivery system which contain phyllanthin/capryol 90/cremophor RH 40/transcutol P in ratio of (1.38:39.45:44.38:14.79) w/w resulted very good enhancement of solubility, in-vitro release and in-vivo oral bioavailability in rats compared with pure phyllanthin[33]. Conventional and PEGylated liposomes of phyllanthin were developed through film hydration technique. Extended drug release for over 24 h was observed in in-vitro drug release studies[34]. By using coacervation technique phyllanthin containing chitosan based microcapsules were developed and more than 60% of phyllanthin released after 120h. This microcapsules showed enhanced growth inhibitory activity towards *Staphylococcus aureus* and stronger anti-oxidation potential on both human fibroblasts and keratinocytes[35]. A mixed micellar lipid formulation (MMLF) of Phyllanthin along with piperine by using phosphatidylcholine was formulated to increase the bioavailability and hepatoprotective activity against CCl4 damaged hepatotoxicity. The complex MMLF normalized adverse conditions of rat livers more efficiently than the non-formulated phyllanthin. The present findings indicate that the MMLF is helpful in solving the problem of low bioavailability of phyllanthin[36].

Semisynthetic derivatives

To further enhance the potential of phyllanthin, derivatives were prepared by some groups. Bromo derivative of phyllanthin was synthesized and along with phyllanthin, it was tested for curative impact on cythion induced activities in albino rats with special reference to blood serum (VLDL, LDL, and HDL) hepatotoxicity. The results reveals that there is a declining trend in the values of these constituents in cythion treated albino rats. Administration of phyllanthin and Bromo derivative phyllanthin showed

remarkable curative effect[37]. Dibromophyllanthin was synthesized and characterized by NMR[38]. Iodo derivative of phyllanthin was synthesized and characterized by x-ray crystallogaphy[39] Around 28 derivatives of phyllanthin were synthesized and evaluated for anti-HIV activities. Four compounds showed good anti-HIV activity[40].

Nano particles synthesis: It was found that phyllanthin is able to help in the synthesis of nanoparticles. A simple and efficient way for the production of anisotropic gold and silver nano particles using the phyllanthin extract was established. $HAuCl_4$ greatly reduced by phyllanthin than $AgNO_3$ at constant amount. Phyllanthin –OCH3 group plays an important role in formation and stabilization of nano particles by donating the electrons and interacting with metal ions[41]. A green, high-yield, fast, and low-cost approach was made to reduce silver ions to nano sized silver particles by using plant broth of *Phyllanthus amarus* containing phyllanthin. Plasmon absorbance of silver nanoparticles was proven by UV-visible spectrum[42].

Pharmacokinetics

A study was evaluated the pharmacokinetic parameters of phyllanthin in rat plasma after intravenous and oral administration. Results revealed that after intravenous administration, phyllanthin showed a gradual decline in its plasma concentrations and estimated volume of distribution (V_d) of the phyllanthin was relatively small with a mean value of 0.20 l/kg shows that it was not widely distributed into the tissue compartment. Phyllanthin appeared to be cleared slowly from the body as evidenced by its small mean clearance values of 0.04 l/kg h. Following an oral administration, phyllanthin showed a rapid rise with a T_{max} of 1 h followed by a gradual decline to 0 after 24 h. Mean C_{max} values was 0.18 µg/ml. The calculated absolute bioavailability found to be 0.62%. The low oral bioavailability may be due to its poor aqueous solubility causing only a small fraction available in dissolved form for absorption after oral administration[43].

Pharmacological activities

The scientific literature revealed that phyllanthin has been reported to possess the antioxidant, hepatoprotective and various other activities such as anticancer, anti-inflammatory, antifibrotic, vasodilator, anti-microbial, immunomodulatory effects and anti-diabetic activity etc.,

Antioxidant

Aqueous extract of *Phyllanthus amarus* whole plant possesses an effective in vitro antioxidant activity, which may be due to radical-scavenging & reducing power. This effect attributed to the total phenolics, among which phyllanthin and hypophyllanthin is regarded as important bioactive compounds[44]. A study revealed that lignan phyllanthin is responsible for radioprotector activity of *Phyllanthus niruri* by scavenging free radicals[45].

Hepatoprotective

Phyllanthin shown hepatoprotective activity against CCL4, galactosamine and alcohol induced hepatitis.

Against CCL4

A combination therapy containing silymarin and standardized extract of *Phyllanthus amarus* tested against CCl4-induced hepatotoxicity in rats and it showed synergistic effect for hepatoprotection. This improved activity with ethanolic extract of *Phyllanthus amarus* due to the higher concentration of phyllanthin in ethanolic extract than aqueous extract of *Phyllanthus amarus* as estimated by HPLC[2].

A research group evaluated the hepatoprotective and antioxidative property *Phyllanthus amarus* extract by invitro techniques[46] (DPPH assay and human hepatoma HepG2 cell line) and invivo mice model[47].The results indicated that phyllanthin effectively alleviated the changes induced by CCl4 in a concentration-dependent manner, with much smaller strengths as compared to *Phyllanthus amarus* extract and lowered 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) metabolism and increased the release of transaminases which were adjusted with co-incubation with *Phyllanthus amarus*. Even Histopathology and ultramicroscopic studies correlated well with the biochemical findings, as phyllanthin treatment reversed the alterations induced by the toxin and the subcellular features of phyllanthin treated mice were similar to those present in the normal mouse liver. Study suggested that in vivo anti-hepatotoxic potential of phyllanthin may be responsible for the liver protecting property of *Phyllanthus amarus*[48].

Protective effects and possible mechanism of phyllanthin was investigated against carbon tetrachloride (CCl4)-induced hepatic damage in common carp. The results showed that administration of phyllanthin inhibited the adverse changes caused by CCl4 in serum and liver tissue and data from histopathology also suggested that phyllanthin may be used as a hepatoprotective agent to prevent liver diseases in fish[49]. Oral pre-treatment of phyllanthin at a dose of 120 mg/kg prior to CCl4-treatment effectively prevented the hepatic damage by reducing alanine aminotransferase (ALT) and aspartate aminotransferase (AST)[50].

Against Galactosamine-induced

Phyllanthin and hypophyllanthin isolated from a hexane extract of *Phyllanthus niruri* along with two other constituents protected against carbon tetrachloride- and galactosamine-induced cytotoxicity in primary cultured rat hepatocytes[3]. Protective mechanism of lignans from *Phyllanthus amarus* against galactosamine/ lipopolysaccharide-induced hepatitis in mice was evaluated and correlated it from in-silico molecular docking studies. In-vivo and in-silico results suggest that pretreatment of standardize mixture of lignans exhibit potent hepatoprotection against galactosamine/ lipopolysaccharide -induced hepatitis in mice and the liver protective effects may be due to the inhibition of inflammatory mediators[51].

Against Alcohol induced

Alcohol induces oxidative stress when taken at higher concentrations and along with polyunsaturated fatty acid (PUFA) elevated lipid peroxidative products and antioxidant defense potential lost greatly. An experiment was carried on alcohol and heated PUFA induced hepatotoxicity shown that *Phyllanthus niruri* effectively decreased the oxidative stress and associated damage and it was hypothesized that phyllanthin along with other

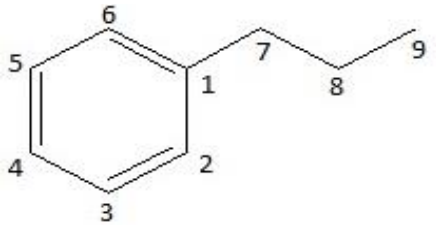

Figure 1: Phenylpropanoid.

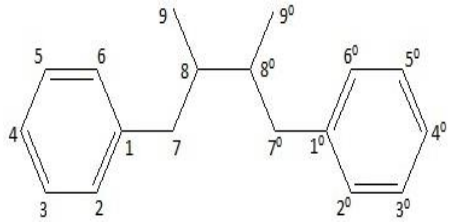

Figure 2: Lignan structure.

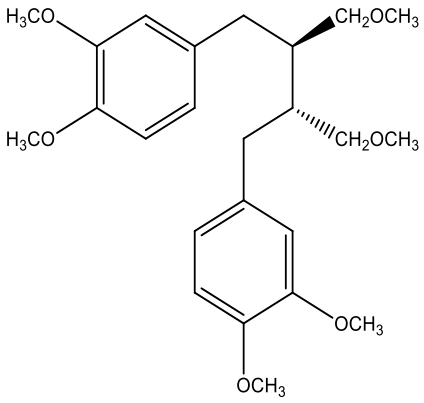

Figure 3: Phyllanthin.

Figure 4: *Phyllanthus* species.

compounds was responsible for this effect[4]. Protective effect of phyllanthin was investigated against ethanol-induced rat liver cell injury. Phyllanthin antagonized the ethanol-induced oxidative stress and restored the antioxidant capability of rat hepatocytes along with level of total glutathione, and activities of superoxide dismutase (SOD) and glutathione reductase (GR) which were reduced by ethanol. Results suggested that hepatoprotective effect of phyllanthin against ethanol was due to its antioxidant activity[52].

Anti-HBV

Different extracts from *Phyllanthus rheedei* were evaluated for Anti-HBV activity by using PLC/PRF, Hep3B, FLCII10 and HepG2215 cell lines and analysis was done by using ELISA, SQRT-PCR and immuno blotting techniques. From the screening experiments it was shown that the ethanol extract of this plant has the maximum activity in lowering the viral markers like hepatitis-B surface antigen (HBsAg), HBV Core and HBV X protein and whole virions with comparatively lesser cytotoxicity. The dose responses of this particular extract were further established. The ethanol extract was then evaluated by using HPTLC revealed the presence of phyllanthin, hypophyllanthin, ellagic acid and Quercetin and it was proved through standards[53].

Anticancer

Preventive and curative role of *Phyllanthus amarus* lignans was examined in tumor bearing mice by taking a mixture (1:1) of phyllanthin and hypophyllanthin. Antitumor activities against Ehrlich ascites carcinoma in the Swiss albino mice was proved by the decrement of tumor volume, and packed cell volume and viable cell count were observed in lignans treated mice. Treatment with test compounds increased the survival time and normal peritoneal cell count[54]. Antigenotoxic property of a crude extract of *Phyllanthus amarus L was* proved by using two types of tannery effluents. The root meristems was pre-treated with effluents caused the maximum incidence of mitotic anomalies, were then exposed to the crude extract of *Phyllanthus amarus* showed a significant reduction in the frequency of chromosomal alterations[55]. Multidrug resistance constitutes the major obstacle to the successful treatment of cancer and it is thought to be mediated by the super-expression of P-glycoprotein (Pgp). Pgp extrudes drugs from the cells, therefore reducing their cytotoxicity, and its activity inhibition may reverse the MDR phenotype. Lignans from *Phyllanthus amarus* reversed the cytotoxic effect and the multidrug resistance (MDR). Results revealed that along with other compounds phyllanthin also showing a potent action as MDR reversing agents, mainly due to their ability to synergize with the action of conventional chemotherapeutics[56].

Anti-inflammatory

Chondroprotective potential of *Phyllanthus amarus* extract and its lignans, phyllanthin and hypophyllanthin was evaluated in experimental cartilage explant degradation model induced by IL-1β. They show decrease in sulfate glycosaminoglycans level and matrix metalloproteinase-2 activity in culture medium consistent with an increase in uronic acid and proteoglycan contents in the explants when compared to the IL-1β treatment. Improved activity was observed in *Phyllanthus amarus* crude extracts than those of the purified components[7]. Hexanic extract of *Phyllanthus amarus* containing phyllanthin, niranthin, and 5-demethoxyniranthin did not show antihypernociceptive activity in experimental autoimmune encephalomyelitis[57]. Protective effect of phyllanthin and hypophyllanthin was examined against colitis model of inflammatory bowel disease in Wistar rats showed that *Phyllanthus amarus* exert a preventive anti-inflammatory, antioxidant and anti-apoptotic effect by the neutrophil infiltration inhibition, inhibition of pro-inflammatory mediator production and

reducing DNA damage due to the presence of phyllanthin and hypophyllanthin phytoconstituents[58].

Study designed to evaluate anti-arthritic activity of *Phyllanthus amarus* extract containing 2.5% phyllanthin and hypophyllanthin against Freund's complete adjuvant induced arthritic rats and it was revealed that extract mitigated the changes brought by arthritis and suggested that it has prominent anti-arthritic activity[59]. An in vitro study suggested that the anti-inflammatory activity of *Phyllanthus simplex* petroleum ether extract (PSPE) and *Phyllanthus simplex* ethanol extract (PSEE) was mediated by their inhibitory action on NO production in a dose dependent manner ($p < 0.05$) and based on previous studies they concluded that phyllanthin and gallic acid were present in extract responsible for its anti-inflammatory activity by controlling NO production[60].

Antifibrotic

Chronic injury to liver causes synthesis of extracellular matrix components resulting in progressive fibrosis and eventually cirrhosis. Transforming growth factor-β1 (TGF-β1) transduces its signal by binding to TGF-β type 1 receptor kinase or activin like kinase (ALK5) receptor and mediates hepatic fibrosis by increasing the transcription of downstream entities such as collagen via $Smad_2$ and $Smad_3$. Antifibrotic potential of phyllanthin was evaluated against important profibrotic mediator transforming growth factor β1 and predominant extracellular matrix (ECM) components collagen and fibronectin. The outcomes revealed the molecular mechanism of phyllanthin which acts by suppressing the expression of inflammatory cytokine tumor necrosis factor-α and prevents activation of nuclear factor-kβ in hepatic tissue[6] and it was correlated by molecular docking experiments, which shown inhibitory role of phyllanthin on TGF- β type 1 receptor kinase[61] . Research conducted to examine the antifibrotic activity of *Phyllanthus maderaspatensis* hexane extract (PmHE) in Wistar rats based on preventive and curative treatment strategies revealed that post treatment with PmHE (200 mg/ kg) reversed CCl4 induced hepatic fibrosis in rats. Analytical methods HPLC and HPTLC were used to confirm the presence of phyllanthin and quercetin. It was hypothesized that mechanism of action may be scavenging the reactive oxygen species, produced from inflammatory cells and liver cells, reducing collagen deposition and down regulating the over expression of αSMA and collagen III at proteins and mRNA levels[62].

Vasodilator

Modulating effects of phyllanthin and hypophyllanthin on vascular tension was investigated using the in vitro model of isolated rat aorta showed that sustained contraction induced by phenylephrine was significantly relaxed by phyllanthin and hypophyllanthin. It was revealed phyllanthin more active than hypophyllanthin against contractile responses upon cumulative addition of $CaCl_2$ and mechanism of action may be via the endothelium independent [63].

Immunosuppressive effects

A study revealed that the standardized ethanol extract of *Phyllanthus amarus* was able to suppress the immune response of both specific and nonspecific immunity in Wistar-Kyoto rats through various pathways. Immunosuppressive activity of 80% ethanol extract of *Phyllanthus amarus* in Wistar-Kyoto rats exhibited significant inhibition of the phagocytic activity of neutrophils by down regulation of the percentage of CD11b and CD18 expression in neutrophils, of chemotaxis, and of phagocytosis of E. coli by neutrophils. It was shown that phyllanthin and hypophyllanthin were main constituents in extracts, which may be responsible for this activity [64]. Immunosuppressive effects of phyllanthin on cellular and humoral immune responses evaluated by using Balb/C mice. Phyllanthin dose-dependently inhibited CD11b/CD18 adhesion, the engulfment of *E.coli* by peritoneal macrophages molecules, NO and MPO release in treated mice. It was revealed that phyllanthin significantly inhibited T and B lymphocytes proliferation, down-regulated the Th1 (IL-2 and IFN-γ) and Th2 (IL-4) cytokines ,reduced the expression of CD4+ and CD8+ in splenocytes and inhibited the sheep red blood cell (sRBC)-induced swelling rate of mice paw in delayed type hypersensitivity[8].

Immunomodulatory effect of the standardized methanol extracts of *Phyllanthus amarus* and *Phyllanthus urinaria* and their biomarkers phyllanthin and hypophyllanthin was examined. It was found that they were able to modulate the innate immune response of phagocytes especially on the chemotactic migration of phagocytes, phagocytic ability, and on the release of ROS. Phyllanthin exhibited higher inhibitory effects on the phagocytic activity of neutrophils particularly in inhibiting ROS production and bacteria engulfment as compared to hypophyllanthin[65]. Effects of constituents of the extract of *Phyllanthus amarus* on nitric oxide (NO) production as well as lymphocyte proliferation and cytokine release from phagocytes were evaluated. Among the compounds tested, the lignans, especially phyltetralin and phyllanthin, showed strong inhibition on lymphocyte proliferation with half maximal inhibitory concentration (IC50) values of 1.07 μM and 1.82 μM, respectively. The compounds constituting the extract of *Phyllanthus amarus* were able to inhibit the innate immune response of phagocytes at different steps[66].

Antimicrobial

Antimicrobial activity of the methanolic extract of *Phyllanthus amarus* was explored against some drug resistant pathogenic bacterial strains by disc diffusion and agar dilution method. The extract showed significant concentration-dependent antibacterial activity particularly against gram-negative microbes. They showed antibacterial action was mainly due to the isolated phyllanthin[67].

Antidiabetic

An investigation was done by a group to endorse the ethnopharmological information of *Phyllanthus niruri* against Type-2 diabetes mellitus (T2DM) through molecular docking and pharmacophore modeling .They found that phyllanthin had affinity for aldose reductase and can be used against T2DM[68].

Antihyperuricemic

Oral antihyperuricemic activity of lignans was evaluated in potassium oxonate and uric acid-induced hyperuricemic rats. Study revealed that among isolated lignans phyllanthin significantly reversed the plasma uric acid level of hyperuricemic animals to its normal level in a dose-dependent manner, comparable to that of allopurinol, benzbromarone, and probenecid which are used for the treatment of hyperuricemia and gout[69]. Mechanism for antihyperuricemic effect was investigated by using xanthine oxidase assay and uricosuric studies in potassium oxonate and uric acid induced hyperuricemic rats. Results showed that methanol extract exhibited in vitro xanthine oxidase inhibition and a moderate in vivo xanthine oxidase inhibitory activity. However, the lignans display poor xanthine oxidase inhibition in vitro and a relatively weak in vivo inhibitory activity whereas intraperitoneal treatment with methanol extract and lignans, phyllanthin, hypophyllanthin and phyltetralin exhibited higher urinary uric acid excretion and clearance. It was revealed that methanol extract acting via both uricosuric action and xanthine oxidase inhibition, whereas lignans were acting through uricosuric action only[9].

Miscellaneous

Effect of phyllanthin and hypophyllanthin on the functioning of P-glycoprotein (P-gp) and multidrug resistance protein 2 (MRP2) was explored using the in-vitro model of Caco-2 cells. Fluorescence spectroscopy used to determine the activity and it was revealed that hypophyllanthin and phyllanthin inhibited P-gp function, but neither compound affected MRP2 activity. It was proved that both phyllanthin and hypophyllanthin could reversibly inhibit P-gp function[70]. Acute toxicity of standardized methanolic extract of *Phyllanthus amarus* (MEPA) was evaluated by using female albino rats. Outcome revealed that phyllanthin and hypophyllanthin were safe[71]. Estrogenic properties of phyllanthin and hypophyllanthin against carbofuran induced toxicity in female rats was studied by inducing toxicity with carbofuran on estrous cycle in virgin Wistar rats and recovery from the damaged estrous cycle with treatment of *Phyllanthus amarus* lignans viz. phyllanthin and hypophyllanthin. It was known that phyllanthin and hypophyllanthin transformed into enterolignan(s) systemically, which is expected to be responsible for augmenting estrus cycle in rats[72]. A study evaluated the role of phyllanthin, extracts and some other active constituents against β-glucuronidase inhibitory action. It was revealed that phyllanthin and hypophyllanthin show their hepatoprotective effect through a mechanism other than of β -glucuronidase inhibition[73]. Mechanism of inhibitory effect of *Phyllanthus amarus* and its major lignans on human microsomal cytochrome P450 was evaluated by using human liver microsomes and selective substrates. It was revealed that phyllanthin and hypophyllanthin were potent mechanism based inhibitors of CYP3A4. The study suggested that co-administration of *Phyllanthus amarus* with drugs that are metabolized by CYP3A4 may potentially result in herb-drug interactions[74].

CONCLUSION

Phyllanthin is a major active lignin which exists in various *Phyllanthus* species and it exhibits various biological activities. It shows significant hepatoprotective, anticancer, antidiabetic, immunosuppressant, anti-inflammatory and various other activities. Physicochemical properties, various yield and bioavailability enhancement techniques, biosynthesis, synthetic route, pharmacological applications, mechanism of action, herb-drug interactions, toxicity studies and semisynthetic derivatives of phyllanthin has been discussed. This review is presented in view of immense importance of phyllanthin in the health care system. Further research by taking phyllanthin as lead molecule will have great scope. Further development of safe, simple and economic extraction and isolation techniques, laboratory synthesis and derivatives by exploring the structure activity relationship of phyllanthin for the prevalent and rare health problems will be useful for the future needs.

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