Physicochemical characterization of phyllanthin from Phyllanthus amarus Schum. et Thonn.

Hanh ND, Sinchaipanid N, Mitrevej A.

Source
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Abstract

Phyllanthin is a major bioactive lignan component of Phyllanthus amarus, with several known biological activities. This study dealt with the isolation and physicochemical characterization of phyllanthin. Phyllanthin was isolated from P. amarus leaves by column chromatography and purified by recrystallization to obtain phyllanthin crystals with a purity of more than 98%. UV, IR, MS, $^1$H NMR and $^{13}$C NMR spectra were employed to identify phyllanthin. The physicochemical properties of phyllanthin were characterized using differential scanning calorimetry, thermogravimetric analysis, X-ray diffraction, pH-solubility, ionization property and lipophilicity. The results indicated that phyllanthin crystals had the melting point and melting enthalpy range of 96.67-97.03 °C and 109.61-116.34 J/g, respectively. Three kinds of phyllanthin crystals, recrystallized by petroleum ether, absolute ethanol and 25% ethanol solution, showed only one polymorph and no polymorphic impurity. Phyllanthin in a solid state was found to undergo significant thermal decomposition above 200 °C. The compound demonstrated good stability in aqueous solution over a pH range of 1.07-10.02 for at least 4 h. The solubility of phyllanthin appeared to be pH-independent of pH range from 1.07 to 10.26. Ionization property studied by absorbance spectroscopy method was in agreement with the result of pH-solubility study, showing that phyllanthin has no $pK_a$ over a pH range of 1.12-10.02. The log $P_{ow}$ value of phyllanthin was found to be 3.30 ± 0.05 at pH 7.48, suggesting that phyllanthin may have good permeability through biological membranes. The findings could be useful tools for the development of stable and bioavailable oral dosage forms of phyllanthin.
MATERIALS AND METHODS:

The present review covers a literature across from 1980 to 2011. Some information collected from traditional Ayurvedic texts and published literature on ethanomedicinal uses of Phyllanthus amarus in different countries worldwide.

RESULTS:

Phytochemical studies have shown the presence of many valuable compounds such as lignans, flavonoids, hydrolysable tannins (ellagitanins), polyphenols, triterpenes, sterols and alkaloids. The extracts and the compounds isolated from P. amarus show a wide spectrum of pharmacological activities including antiviral, antibacterial, antiplasmodial, anti-inflammatory, antimalarial, antimicrobial, antitumour, antidiabetic, hypolipidemic, antioxidant, hepatoprotective nephroprotective and diuretic properties.

CONCLUSION:

The present review summarizes information concerning the morphology, ecology, ethnopharmacology, phytochemistry, biological activities, clinical applications and toxicological reports of P. amarus. This review aims at gathering the research work undertaken till date on this plant in order to provide sufficient baseline information for future works and commercial exploitation.


Evaluation of Antioxidant Activity and Characterization of Phenolic Constituents of Phyllanthus amarus Root.

Maity S, Chatterjee S, Variyar PS, Sharma A, Adhikari S, Mazumder S.

Source

Department of Biochemistry, University of Calcutta, 35 Ballygunge Circular Road, Kolkata-700019, India.

Abstract

The antioxidant property of the 70% aqueous ethanol extract of Phyllanthus amarus roots and its ether-soluble, ethyl acetate-soluble, and aqueous fractions were investigated by various in vitro assays. The root extracts showed higher DPPH, hydroxyl, superoxide, and nitric oxide radical scavenging and reducing power activity. Among all the samples, the ethyl acetate-soluble fraction demonstrated highest radical scavenging activity and total phenolics content. Twenty-eight different phenolic compounds were identified by LCMS/MS analysis of the ethyl acetate-soluble fraction. The majority of the compounds were found to exist as their glycosides, and many of these were gallic acid derivatives. Free epicatechin and gallic acid were also identified in the ethyl acetate-soluble fraction. The present investigation suggested that P. amarus root is a potent antioxidant and can be used for the prevention of diseases related to oxidative stress.


Nanoemulsified ethanolic extract of Pyllanthus amarus Schum & Thonn ameliorates CCl4 induced hepatotoxicity in Wistar rats.

Deepa V, Sridhar R, Goparaju A, Reddy PN, Murthy PB.

Source

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Abstract
Phyllanthus amarus (PA) is commonly used in traditional medicine for hepatoprotective activity. The major limitation is that, treatment requires a large quantity of herbal extract for a longer duration. Aim of the present study was to encapsulate ethanolic plant extract for sustained release of constituents in intestine and facilitate maximum absorption. The efficacy was compared for the hepatoprotective activity of nanoencapsulated ethanolic extract of P. amarus (NPA) and PA in carbon tetrachloride (CCl4) induced hepatotoxic male rats. Based on total phenol content (TPC), the loading efficiency of nanocapsules was 89% (pH 7.0) and optimum concentration was 2:18 (mg/mL) for plant extract: olive oil. Scanning electron microscopy (SEM) showed a spherical morphology, photon correlation spectroscopy (PCS) identified mean particle diameter as 213 nm and Fourier transform infrared spectroscopy (FT-IR) revealed that the phytoconstituents were stable. An oral dose of NPA (20 mg/kg body wt.) showed a better hepatoprotective activity than PA (100 mg/kg body wt.) and also repeated dose oral toxicity proved to be safe. These biochemical assessments were supported by rat biopsy examinations. In conclusion, the nanoemulsification method may be applied for poor water-soluble ethanolic herbal extracts to reduce the dosage and time.


Clonal propagation of Phyllanthus amarus: A hepatoprotector.
Xavier JR, Gnanam R, Murugan MP, Pappachan A.

Source
Scientist, Division of Biotechnology, Defence Institute of High Altitude Research, Defence Research and Development Organisation (DRDO), C/O 56 APO, Ladakh, Jammu and Kashmir, India.

Abstract
BACKGROUND:
The micropropagation protocol for Phyllanthus amarus, an important medicinal herb used widely for the treatment of hepatitis in ethnomedicinal systems, was standardized with shoot tip and single node explants.

MATERIALS AND METHODS:
The micropropagation was carried out for the hyperproducing ecotype (phyllanthin content 463.828 ppm; hypophyllanthin content: 75.469 ppm) collected from Aanaikatti, Coimbatore, and grown in mist chamber, CPMB, TNAU. For micropropagation studies, the leaves were trimmed off and the shoot tips (6 mm long) and nodal segments (single node) were used for initiation.

RESULTS:
Shoot tips and single node explants gave a maximum of 6.00 and 7.00 multiple shoots per explant with Benzyl Amino Purine (BAP) (1.0mg/L mg/L). Upon subculturing, a shoot length of around 7 cm with an average of eight internodes per shoot was observed after 20 days in the elongation medium supplemented with BAP (0.2 mg/Lmg/L) and Indole Acetic Acid (IAA) (2.0 mg/L). Seven to ten adventitious roots developed when the elongated microshoots were cultured in half strength MS medium with Indole Butyric Acid (IBA) (2.0 mg/Lmg/L) and NAA (1.0 mg/L mg/L) in 15-20 days after transfer. The rooted shoots acclimatized successfully to field conditions.
CONCLUSION:

A method for successful micropropagation of the valuable medicinal plant was established which will provide a better source for continuous supply of plants for manufacturing drugs.


Inhibition of hepatitis C virus replication by herbal extract: Phyllanthus amarus as potent natural source.

Ravikumar YS, Ray U, Nandhitha M, Perween A, Raja Naika H, Khanna N, Das S.

Source

Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560012, India.

Abstract

Hepatitis C virus infection is a major health problem worldwide. Developing effective antiviral therapy for HCV is the need of the hour. The viral enzymes NS3 protease and NS5B RNA dependent RNA polymerase are essential enzymes for polyprotein processing and viral RNA replication and thus can be potential targets for screening anti-HCV compounds. A large number of phytochemicals are present in plants, which are found to be promising antiviral agents. In this study, we have screened inhibitory effect of different plant extracts against the NS3 and NS5B enzymes of hepatitis C virus. Methanolic extracts were prepared from various plant materials and their inhibitory effects on the viral enzymes were determined by in vitro enzyme assays. Effect on viral RNA replication was investigated by using TaqMan Real time RT-PCR. Interestingly, Phyllanthus amarus root (PAR) extract showed significant inhibition of HCV-NS3 protease enzyme; whereas P. amarus leaf (PAL) extract showed considerable inhibition of NS5B in the in vitro assays. Further, the PAR and PAL extracts significantly inhibited replication of HCV monocistronic replicon RNA and HCV H77S viral RNA in HCV cell culture system. However, both PAR and PAL extracts did not show cytotoxicity in Huh7 cells in the MTT assay. Furthermore, addition of PAR together with IFN-α showed additive effect in the inhibition of HCV RNA replication. Results suggest the possible molecular basis of the inhibitory activity of PA extract against HCV which would help in optimization and subsequent development of specific antiviral agent using P. amarus as potent natural source.


Suppression of hepatitis C virus by the flavonoid quercetin is mediated by inhibition of NS3 protease activity.


Source

Molecular Hepatology Research Laboratory, Felsenstein Medical Research Center, Sackler School of Medicine, Tel-Aviv University, Petah Tikva, Israel.

Abstract

Phytochemicals exert antiviral activity and may play a potential therapeutic role in hepatitis C virus (HCV) infection. In this work, we aimed to isolate NS3 inhibitors from traditional Indian medicinal plants that were found, in our earlier study, to inhibit HCV NS3 protease activity and to evaluate their potential to inhibit HCV replication. A potent inhibitory effect of NS3 catalytic activity was obtained with Embelia ribes plant extracts. Quercetin, a ubiquitous plant flavonoid, was identified as the active substance in the fractioned extract. It was found to inhibit NS3 activity in a specific dose-dependent manner in an in vitro catalysis assay. Quercetin inhibited HCV RNA replication as analysed in the subgenomic HCV RNA replicon system. It also
inhibited HCV infectious virus production in the HCV infectious cell culture system (HCVcc), as analysed by
the focus-forming unit reduction assay and HCV RNA real-time PCR. The inhibitory effect of quercetin was
also obtained when using a model system in which NS3 engineered substrates were introduced in NS3-
expressing cells, providing evidence that inhibition in vivo could be directed to the NS3 and do not involve
other HCV proteins. Our work demonstrates that quercetin has a direct inhibitory effect on the HCV NS3
protease. These results point to the potential of quercetin as a natural nontoxic anti-HCV agent reducing viral
production by inhibiting both NS3 and heat shock proteins essential for HCV replication


Phyllanthus amarus has anti-inflammatory potential by inhibition of
iNOS, COX-2, and cytokines via the NF-kappaB pathway.

Kiemer AK, Hartung T, Huber C, Vollmar AM.

Source

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Abstract

BACKGROUND/AIMS:

Phyllanthus amarus is a herbal medicine traditionally applied in the treatment of viral hepatitis. Aim of this
study was to investigate potential anti-inflammatory properties of standardized P. amarus extracts
concerning a potential influence of P. amarus on endotoxin-induced nitric oxide synthase (iNOS),
cyclooxygenase (COX-2), and cytokine production in vivo and in vitro.

METHODS:

Investigations were performed in rat Kupffer cells (KC), in RAW264.7 macrophages, in human whole blood,
and in mice. Cells were stimulated with lipopolysaccharides (LPS) in the presence or absence of P. amarus
extracts (hexane, ETOH/H(2)O), mice were treated with galactosamine/LPS as a model for acute toxic
hepatitis. Nitrite was measured by Griess assay, prostaglandin E(2) (PGE(2)) by radioimmunoassay, and
cytokines by enzyme-linked immunosorbent assay. iNOS and COX-2 were determined by Western blot,
activation of NF-kappaB and AP-1 by EMSA.

RESULTS:

P. amarus EtOH/H(2)O and hexane extracts showed an inhibition of LPS-induced production of NO and
PGE(2) in KC and in RAW264.7. The extracts also attenuated the LPS-induced secretion of tumor necrosis
factor (TNF-alpha) in RAW264.7 as well as in human whole blood. Both extracts reduced expression of iNOS
and COX-2 and inhibited activation of NF-kappaB, but not of AP-1. P. amarus inhibited induction of
interleukin (IL)-1beta, IL-10, and interferon-gamma in human whole blood and reduced TNF-alpha
production in vivo.

CONCLUSIONS:

This work shows that standardized extracts of P. amarus inhibit the induction of iNOS, COX-2, and TNF-
alpha. Therefore, we report for the first time an anti-inflammatory potential of this traditionally employed
herbal medicine both in vitro and in vivo.

A comparative study of Phyllanthus amarus compound and interferon in the treatment of chronic viral hepatitis B.

Xin-Hua W, Chang-Qing L, Xing-Bo G, Lin-Chun F.

Source
Tropical Medicine Institute, Guangzhou University of Traditional Chinese Medicine, Guangdong, People’s Republic of China.

Abstract
Fifty-five patients with chronic viral hepatitis B were randomly divided into two groups. Thirty patients were treated with Phyllanthus amarus compound (PA Co) for three months in the treatment group, another 25 patients were treated with domestic recombinant human interferon alpha-1b (IFN-alpha 1b) for three months as controls. The total effective rate in the treatment group was 83.3%, showing no significant difference from the control (p>0.05). The normalization rates of ALT, A/G and SB in the treatment group were 73.3%, 80.0% and 78.2% respectively, which were significantly higher than that in the control (p<0.05). The negative conversion rates of HBeAg and HBV-DNA in the treatment group were 42.3% and 47.8%, showing no significant difference from the control (p>0.005). It is indicated that PA Co has remarkable effect for chronic viral hepatitis B in recovery of liver function and inhibition of the replication of HBV.


A trial of Phyllanthus amarus in acute viral hepatitis.

Narendranathan M, Remla A, Mini PC, Satheesh P.

Source
Department of Gastroenterology, Medical College, Trivandrum, India.

Abstract
The study was done to know whether the powders of Phyllanthus amarus plants favourably influence the duration of disease in patients with acute virus B hepatitis when compared to placebo. The powders of the plant were given in capsule form (300 mg capsules--3 capsules--3 capsules thrice daily) and an antacid powder in similar capsule was used as placebo. Persons with encephalopathy, preexisting medical conditions or serum bilirubin above 350 iu/l were excluded from the study. Fifty seven patients were randomized to receive either the placebo (28 cases) or the drug (28 cases). The two groups were comparable at the time of entry. Two cases from the placebo and one from the placebo and one from the drug group dropped out of the study. The duration of disease (time taken for bilirubin to come to below 2 mg%) was taken as the outcome measure. The duration of disease in the two groups was compared by Cox's proportional hazards analysis after adjusting for the variables that influence the duration of jaundice. Only initial serum bilirubin was an independent predictor of duration of jaundice. The an analysis showed that Phyllanthus amarus powders did not significantly reduce the duration of jaundice in persons with virus B hepatitis.


Phyllanthus amarus suppresses hepatitis B virus by interrupting interactions between HBV enhancer I and cellular transcription factors.

Ott M, Thyagarajan SP, Gupta S.

Source
Abstract

The Phyllanthus amarus plant suppresses HBV mRNA transcription in vitro and exhibits therapeutic potential in chronic HBV carriers, although further work is necessary to define its mechanism of action. Analysis in HuH-7 cells with transfected plasmids using a luciferase reporter showed that P. amarus specifically inhibited HBV enhancer I activity. To identify the mechanism of this HBV enhancer I inhibition, liver-enriched cellular transcription factors were co-expressed in HuH-7 cells. The C/EBP alpha and beta, as well as HNF-3 alpha and beta transcription factors, significantly up-regulated the HBV enhancer I activity. In contrast, co-transfection of HNF-I alpha or beta had no effect upon the HBV enhancer I activity. Exposure to P. amarus inhibited C/EBP alpha- and beta-mediated up-regulation of HBV enhancer I activity in a dose-dependent manner, whereas HNF-3 alpha- and beta-mediated up-regulation of HBV enhancer I was unaffected. In vitro gel shifts showed that P. amarus inhibited complexing of C/EBP transcription factors to a consensus oligonucleotide sequence, whereas DNA binding of AP-1 and SP-1 transcription factors was unaffected. As P. amarus down-regulates HBV mRNA transcription by a specific mechanism involving interactions between HBV enhancer I and C/EBP transcription factors, purification and further analysis of the active P. amarus component will advance insights into its antiviral activity.
Alexander cell line, an human hepatocellular carcinoma derived cell line which has the property of secreting HBsAg in the supernatant was used to study the antiviral property of phyllanthus amarus. Aquous extract of Phyllanthus amarus was evaluated for its in vitro ability to inhibit HBsAg secretion on a dose dependent manner. It was seen that P. amarus at 1mg/ml concentration on a single dose inhibited the secretion of HBsAg for a period of 48 hours. This experiment proved the anti hepatitis B virus property of P. amarus at cellular level and further confirmed its beneficial use in the treatment of acute and chronic hepatitis B and healthy carriers of HBV.


[Efficacy of Phyllanthus spp. in treating patients with chronic hepatitis B].
[Article in Chinese]
Wang MX, Cheng HW, Li YJ, Meng LM, Mai K.

Source
Henan Institute of Medical Sciences, Zhengzhou.

Abstract
The efficacy of Phyllanthus amarus produced in india, P. niruri gathered from hainan province and P. urinaria from henan province was assessed in a total of 88 cases of chronic hepatitis B with 11.42 and 35 each. It was shown that P. urinaria had the effect of seroconversion on HBeAg from positive to negative as well as on HBeAb from negative to positive, while the other two herbs had not. In addition none of these three herbs had similar effect on HBsAg.


A two-stage clinical trial of Phyllanthus amarus in hepatitis B carriers: failure to eradicate the surface antigen.
Doshi JC, Vaidya AB, Antarkar DS, Deolalikar R, Antani DH.

Source
Dr Balabhai Nanavati Hospital and Research Center, Juhu, Bombay.

Abstract
BACKGROUND:
There have been conflicting data in literature about the value of Phyllanthus amarus in treating hepatitis B virus-related disorders.

AIM:
To evaluate the role of Phyllanthus amarus in eradication of the virus in hepatitis B carriers.

METHODS:
Phyllanthus amarus was administered to 30 asymptomatic carriers of hepatitis B surface antigen (HBsAg) in a dosage of 250 to 500 mg thrice daily for 4 to 8 weeks.
RESULTS:
None of the 30 subjects cleared HBsAg. Phyllanthus amarus was well tolerated, with no clinical side effects or changes in the organ profiles for safety evaluation.

CONCLUSION:
Phyllanthus amarus is not effective in clearing HBsAg in asymptomatic carriers of the antigen.


Efficacy of Phyllanthus amarus for eradication of hepatitis B virus in chronic carriers.
Thamlikitkul V, Wasuwat S, Kanchanapee P.

Source
Department of Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Abstract
Sixty-five adult asymptomatic chronic carriers of hepatitis B virus were enrolled to the randomized controlled efficacy study of Phyllanthus amarus. Thirty-four received Phyllanthus amarus 600 mg per day for 30 days and 31 received placebo in identical capsules. The conversion rate of HBsAg was 6 per cent in the study group at day 30. When 20 subjects in the Phyllanthus amarus group were given a further 30-day treatment and 22 placebo recipients given Phyllanthus amarus 1,200 mg per day for 30 days, the conversion was observed in 1 (5%) in the higher dose group. Adverse effects were not observed in all patients receiving the plant. The results indicated that Phyllanthus amarus, whole plant except root, grown in the central part of Thailand, given at the studied dosage and duration, had a very minimal effect on eradication of HBsAg from Thai adult asymptomatic chronic carriers.


Effect of Phyllanthus amarus on chronic carriers of hepatitis B virus.
Thyagarajan SP, Subramanian S, Thirunalasundari T, Venkateswaran PS, Blumberg BS.

Source
Department of Microbiology, University of Madras, India.

Abstract
In a preliminary study, carriers of hepatitis B virus were treated with a preparation of the plant Phyllanthus amarus for 30 days. 22 of 37 (59%) treated patients had lost hepatitis B surface antigen when tested 15-20 days after the end of the treatment compared with only 1 of 23 (4%) placebo-treated controls. Some subjects have been followed for up to 9 months. In no case has the surface antigen returned. Clinical observation revealed few or no toxic effects. The encouraging results of this preliminary study recommend continued evaluation of this plant and the active principles isolated from it


Effects of Phyllanthus plant extracts on duck hepatitis B virus in vitro and in vivo.
Source

Department of Infectious Diseases, University of Sydney, Australia.

Abstract

The effects of extracts of five Australian Phyllanthus species (P. hirtellus, P. gunnii, P. gasstroemii, P. similis and P. tenellus), other plant extracts and the antiviral drug foscarnet on duck hepatitis B virus (DHBV) endogenous DNA polymerase (DNAp) activity were compared. All 5 Phyllanthus species caused 50% inhibition at concentrations of dry weight between 350-800 micrograms/ml, which is comparable with the effect described for P. amarus on the DNAp of human and woodchuck hepatitis B viruses. Incubation of P. hirtellus with 100 ID50 DHBV neutralized infection. However, neither P. gasstroemii extract, given by intraperitoneal injection (i.p.) at a dose of 20 mg/kg 3 times per week to ducklings early in the incubation period, or P. hirtellus extract, given to established DHBV carrier ducklings, prevented or eliminated infection.


In vitro effect of Phyllanthus amarus on hepatitis B virus.

Mehrotra R, Rawat S, Kulshreshtha DK, Goyal P, Patnaik GK, Dhawan BN.

Source

Postgraduate Department of Pathology, King George's Medical College, Lucknow.

Abstract

To evaluate the effects of P. amarus on hepatitis B virus (HBV) antigens and HBV-DNA, initial ethanolic extract and subsequent fractions of the plants were prepared. The whole plant material was dried, powdered and extracted with alcohol and subsequently fractionated in hexane, chloroform, butanol and finally in water. All the material were tested for in vitro effects on HBsAg, HBeAg and HBV-DNA in serum samples positive for HBV antigens followed by the screening of respective antigens by Elisa. HBV-DNA was determined by molecular hybridization. The extracts were effective against HBV antigens, the butanol extract being the most potent. Further chromatographic fractions showed an enhanced activity. The active fractions inhibited the interaction between HBsAg/HBeAg and their corresponding antibodies suggesting anti-HBs, anti-HBe-like activity and also an effect on HBV-DNA.


Hepatitis B virus and primary hepatocellular carcinoma: treatment of HBV carriers with Phyllanthus amarus.

Blumberg BS, Millman I, Venkateswaran PS, Thyagarajan SP.

Source

Fox Chase Cancer Center, Philadelphia, PA.

Abstract

A viricide capable of eliminating hepatitis B virus (HBV) from chronic carriers should, theoretically, decrease the risk of primary hepatocellular carcinoma. Extracts of Phyllanthus amarus have been shown to inhibit the DNA polymerase of HBV and woodchuck hepatitis virus (WHV) in vitro. Three of four recently infected WHV carriers treated i.p. with P. amarus extract lost WHV, animals infected for greater than or equal to 3 months showed a decrease in virus levels. Preliminary results in human carriers treated orally with P. amarus for 1 month indicated that approximately 60% of the carriers lost HBV during the observation period.