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Inhibition of experimental gastric lesion and inflammation by *Phyllanthus amarus* extract

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Abstract

Methanolic extract of *Phyllanthus amarus* Shum & Thonn (Euphorbiaceae) 50, 200, and 1000 mg/kg body weight significantly inhibited gastric lesions, induced by intragastric administration of absolute ethanol (8 ml/kg). Mortality, increased stomach weight, ulcer index, and intraluminal bleeding were reduced significantly by *Phyllanthus amarus*. Biochemical analysis indicated that reduced glutathione (GSH) of gastric mucosa produced by ethanol administration was significantly elevated by treatment with *Phyllanthus amarus* extract. Aqueous and methanol extracts of *Phyllanthus amarus* produced an inhibition of rat paw edema up to 42% compared to control in 3 h and continued up to 8 h. Anti-oxidant activity of the extract as well as presence of tannins in the extract may be responsible for these observed activities.

Keywords: *Phyllanthus amarus*; Ethanol toxicity; Gastric lesion; Anti-inflammatory

1. Introduction

Phyllanthus amarus is traditionally used to treat flu, dropsy, diabetes, and jaundice (Foo, 1993). It is also being used to treat hepatic and urolitic diseases and have diuretic activity. *Phyllanthus amarus* inhibited hepatitis B virus polymerase activity and decreased episomal hepatitis B virus DNA content and suppressed viral release into the culture medium (Lee et al., 1996). Simultaneous administration of *Phyllanthus amarus* extract along with carcinogen has been reported to inhibit the hepatocellular carcinoma development induced by NDEA and increased the life span of hepatocellular carcinoma harbouring animals (Joy and Kuttan, 1998; Rajesh and Kuttan, 2000). In chemically induced liver toxicity models *Phyllanthus amarus* significantly protected the liver tissue (Prakas et al., 1995). *Phyllanthus amarus* has potent free radical scavenging activity and could scavenge superoxides and hydroxyl radicals and can inhibit lipid peroxides (Joy and Kuttan, 1995). As the inflammation is mainly produced by the oxidative burst of the macrophages, many anti-oxidants may be effective to reduce the inflammation. Infusion of the young shoots of *Phyllanthus amarus* has been recommended to lessen the edematous swelling and ulcers (Mhaskar et al., 2000). In the present study, we have checked anti-inflammatory activity of *Phyllanthus amarus* using experimental paw edema produced by carrageenan administration. We have also looked the protection of gastric lesions by *Phyllanthus amarus* extract.

2. Materials and methods

2.1. Extraction of *Phyllanthus amarus*

Leaves and stems of *Phyllanthus amarus* were collected from Thrissur district of Kerala and were dried at 50 °C. A voucher specimen of the plant was identified by Regi Raphael K, Botanist, Amala Cancer Research Centre, Thrissur, Kerala (Voucher No: EUP, 9) and has been kept at Amala Ayurvedic Hospital and Research Centre.

2.1.1. Preparation of alcoholic extract

Dried parts of *Phyllanthus amarus* were powdered and this powder was extracted twice in five volumes of 75% methanol by stirring overnight and centrifuged at room temperature. This supernatant was evaporated to dryness at 50 °C under reduced pressure using a rotary evaporator. The yield of the extract was 8%.

2.1.2. Preparation of aqueous extract

Powdered *Phyllanthus amarus* (50 g) was extracted twice overnight with 250 ml of distilled water at room temperature.

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The supernatant was collected and evaporated to dryness at 50 °C under reduced pressure. The yield of the extract was 10%.

Both methanolic and aqueous extracts had almost similar TLC pattern. Major extracted products in both cases were tannins as seen by ferric chloride test. However, yield of the aqueous extract was higher than methanol extract. Extracts were prepared freshly before each experiment.

2.2. *Induction of ethanol-induced gastric lesion*

Adult male Wistar rats weighing 120 g were used for the experiment. They were grouped into four groups of five animals each. All the animals were fasted for 16 h and deprived of water for 12 h prior to the experiments. Group I acted as animals treated with alcohol alone. Group II—IV were treated with 1000, 200, and 50 mg of *Phyllanthus amarus* extract/kg body weight as a single dose 30 min prior to the experiment. Acute gastric lesion was induced by absolute ethanol (Robert et al., 1979). Briefly, absolute ethanol (8 ml/kg body weight) was administered intragastrically to control and drug-treated animals. Each animal was sacrificed by ether overdose 1 h after administration of ethanol and stomach was excised, opened along the greater curvature and washed gently with ice cold solution. The stomach weight and intraluminal bleeding were recorded.

The extent of erosion of stomach mucosa was assessed from a scoring system designed by Merazzi–Uberti Turba as follows: 0, no erosions; 1, one to three small erosions (4 mm or smaller); 2, more than three small erosions or one large erosion; 3, two large erosions; 4, three to four large erosions; and 5: more than four large erosions or lesion proliferation (Giordano et al., 1990). The results were expressed in terms of an ulcer index, which is the average severity of erosions per rat each group on the scale from 0 to 5.

2.3. *Biochemical analysis*

The mucosa of glandular stomach was removed by scraping with a blunt knife and 10% homogenate was prepared. Reduced glutathione (GSH) in the gastric mucosa was determined by Ellman's reaction using 5′5′-dithio-bis-2-nitro benzoic acid (DTNB) was described by Moron et al. (1979). Briefly 125 μl of 25% trichloro acetic acid (TCA) was added to 0.5 ml of homogenate to precipitate proteins. The tubes were cooled in ice for 5 min and the mixture was further diluted with 0.6 ml of 5% TCA and centrifuged at 9000 x *g* for 10 min. 0.3 ml of the supernatant was taken for estimation. For this purpose the volume of the aliquot was made up to 1 ml with 0.2 M sodium phosphate buffer (pH 8.0) and 2 ml of freshly prepared DTNB solution (0.6 mM in 0.2 M phosphate buffer pH 8.0) was added to the tubes. After 10 min the intensity of the yellow color formed was read at 412 nm in a spectrophotometer. Reduced glutathione (Sisco Research Laboratories, Mumbai, India) was used as a standard. The protein content of the gastric mucosa was quantified by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

2.4. *Histopathology*

A portion of the stomach from each group was fixed in 10% formalin. The formalin fixed specimens were embedded in paraffin and sectioned (3-5 μm) and stained with hematoxylin and eosin and histochemical sections were evaluated by light microscopy.

2.5. *Statistical evaluation*

The values are expressed as mean ± standard deviations. The results were analyzed statistically by analysis of variance. Values of P less than 1% ($P < 0.01$) were considered to be statistically significant.

2.6. *Determination of anti-inflammatory activity*

Anti-inflammatory activity was determined by carrageenan-induced mice paw edema method of Langrange et al. (1974). Female inbred strains of Balb/c mice weighing 25-28 g (6-7 weeks old) were used for the experiment. They were divided into four groups of three animals each. Group 1 acted as control. Groups 2-4 received 500, 250, and 100 mg/kg body weight of *Phyllanthus amarus* extract orally as a single dose 1 h prior to the experiment. Paw edema was induced by injecting carageenan (200 μg/20μ1) into the sub-plantar region of the left paw. The thickness of paw edema was measured by venire calipers before treatment and after injection with carageenan. Measurement was continued at 60 min intervals up to 8 h and further at the 24th hour. The inhibition of paw edema was calculated by comparing the difference in paw thickness of the control and treated group. Experiment was repeated twice and average values were taken.

3. Results

3.1. *Effect of Phyllanthus amarus extract in gastric lesion*

The present investigation indicate that rat mucosal gastric injury induced by ethanol was significantly and dose dependently reduced by methanolic extract of *Phyllanthus amarus* (Table 1). Administration of absolute ethanol to fasted rats resulted in severe gastric damage visible from the outside of the stomach as thick reddish-black lines. After opening, the gastric lesions were found in the mucosa and consisted of elongated bands, 1-10 mm long, usually parallel to the long axis of the stomach. They were located mostly in the corpus (the portion of the stomach secreting acid and pepsin). No gross lesions developed in the fore stomach (the nonsecretory part of the stomach). Intragastric administration of the absolute ethanol to rats resulted in 50% mortality due to

Table 1
Effect of *Phyllanthus amarus* administration on mortality, stomach weight, and intraluminal bleeding of rats treated with absolute ethanol

Treatment	Dose (mg/kg)	Mortality		Stomach weight (g/100 g body weight ± S.D.)	Intraluminal bleeding	
		Number			Number	
Normal rats	0	0/5	0	0.68a ± 0.05	0/5	0
Ethanol	0	5/10	50	1.02[d] ± 0.13	5/5	100
Ethanol + *Phyllanthus amarus*	50	1/5	20	0.85[c] ± 0.10	2/4	50
Ethanol + *Phyllanthus amarus*	200	0/5	0	0.79[6.0]. ± 0.05	0/5	0
Ethanol + *Phyllanthus amarus*	1000	0/5	0	0.70[a,] ± 0.08	0/5	0

[a,b,c,d] Result of the significance test done by ANOVA method.

acute reaction of the alcohol and its metabolites. Increased mortality in the controls were found to be aggrevated due to the fasting (16 h) and deprivation of water (12 h). The rats, which died, had perforated lesions and severe intraluminal bleeding. Stomach weight in alcohol-treated rats was increased to 1.02 ± 0.013 g/100 g body weight as compared to normal rat stomach weight 0.68 ± 0.05 g/100 g body weight possibly due to inflammation. In the treated animals because of scavenging of the oxygen radicals generated by ethanol, the mortality rate and increase in stomach weight induced by ethanol was found to be significantly less (Table 1). All animals treated with absolute ethanol caused intraluminal bleeding in the glandular portion of the stomach, while all animals in the *Phyllanthus amarus* (200 and 1000 mg/kg) pretreated group were found to be significantly protected from intraluminal bleeding.

Ethanol administration to rats produced gastric damage with an ulcer index of 4.75 ± 0.5 and 48.8% reduction in gastric mucosal GSH. *Phyllanthus amarus* pretreatment (50, 200, and 1000 mg/kg) significantly reduced the ulcer index to 3.5±0.6, 2.0±0.5, and 0.6±0.5, respectively and reduced the depletion of GSH to 36, 16.5, and 5.5%, respectively (Table 2).

Histological analysis of ethanol-treated rat stomach revealed the presence of necrotic debrii in the lamina propria of the mucosa which are infiltered with polymorphonuclear leucocytes. The depth of the lesion extended up to the muscularis mucosae with red blood corpuscles extravasation. The submucosa of the corpus was markedly thickened by edema but devoid of polymorphonuclear leucocytes. Histologically, the stomach of the *Phyllanthus amarus* pretreated groups (250 and 1000 mg/kg) showed superficial erosion in the mucosa and moderate degree of sub-mucosal edema with neutrophilic infiltration.

3.2. Anti-inflammatory activity of *Phyllanthus amarus*

Development of paw edema was observed in both control and treated group after carrageenan injection. Thickness of the paw was found to be increased initially upon injection of carrageenan due to volume effect. Difference in the thickness of mice paw edema was further increased during the time interval of 60-180 min in control group. Water extract of *Phyllanthus amarus* (100, 250, and 500 mg/kg) produced an inhibition of 26, 33, and 39%, respectively at 3 h (Fig. 1). While the methanol extract of *Phyllanthus amarus* (100, 250, and 500 mg/kg) produced an inhibition of 29, 37, and 42%, respectively at 3 h (Fig. 2) and significant inhibition of paw oedema was observed throughout the course of the experiment up to 8 h.

Table 2
Effect of *Phyllanthus amarus* administration on the ulcer index and glutathion (GSH) content of the mucosa of rats treated with absolute alcohol

Treatment	Dose (mg/kg)	Ulcer index f S.D.	Inhibition (%)	GSH (nmol/mg (%) reduction protein) in GSH	
Normal rats	0	0	100	12.7a ± 0.8	
Ethanol	0	4.75[a] ± 0.5		6.5[b] ± 0.6	48.8
Ethanol + *Phyllanthus amarus*	50	3.5[b] ± 0.6	26.3	8.1c f 1.1	36
Ethanol + *Phyllanthus amarus*	200	2.0c ± 0.5	57.9	10.6[d] ± 1.1	16.5
Ethanol + *Phyllanthus amarus*	1000	0.6[d] ± 0.5	87.4	12.0a f 0.5	5.5

[a,b,c,d] Result of the significance test done by ANOVA method.

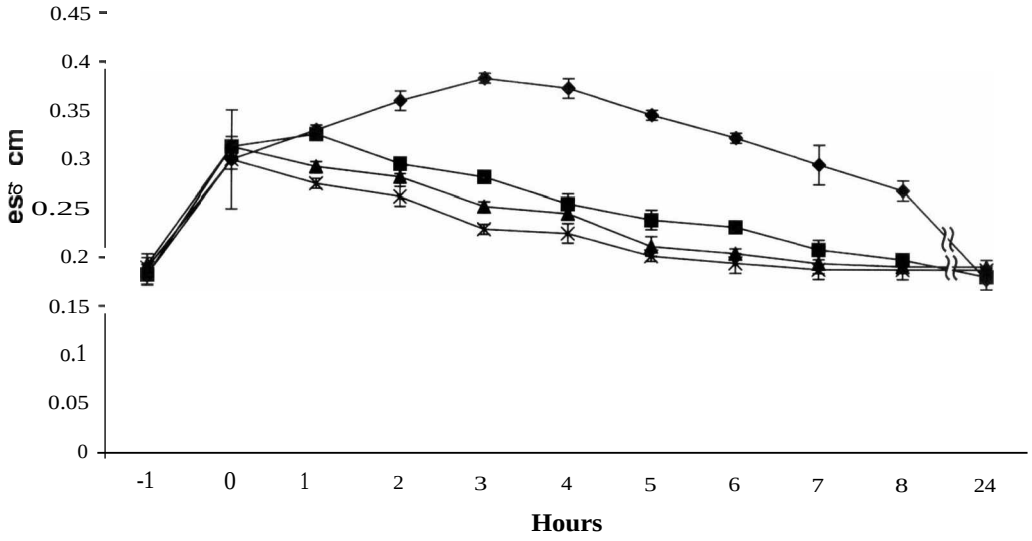

Fig. 1. Anti-inflammatory activity of water extract of *Phyllanthus amarus*. (O) Control treated with the extract; (■) 100 mg/kg; (A) 250 mg/kg; (x) 500 mg/kg.

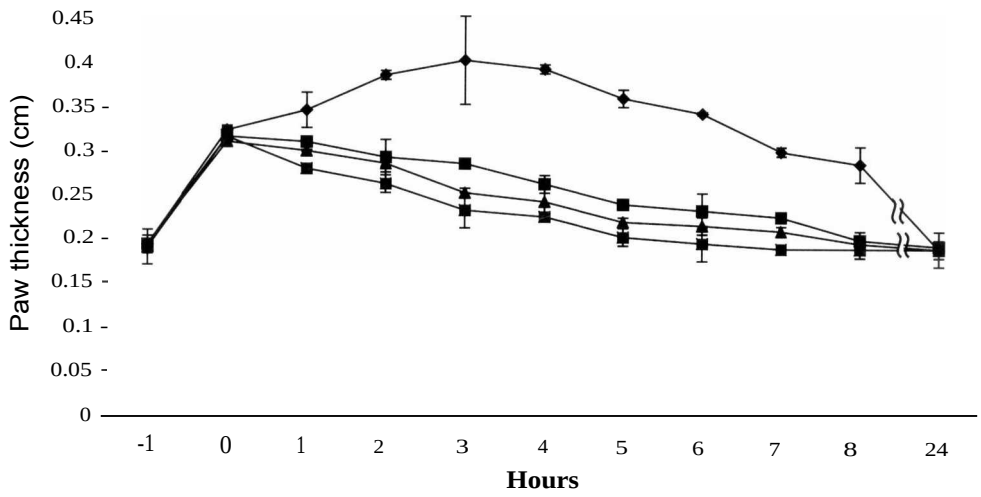

Fig. 2. Anti-inflammatory activity of methanolic extract of *Phyllanthus amarus*. (●) Control treated with the extract; (■) 100 mg/kg; (A) 250 mg/kg; (x) 500 mg/kg.

4. Discussion

In the present study we have checked the anti-lesion and anti-inflammatory activity of *Phyllanthus amarus* extract which is a very important plant in the herbal medicine practice. *Phyllanthus amarus* has been shown to be an effective medicine against viral hepatitis as it has been shown to suppress the mRNA transcription of hepatitis B virus (Lee et al., 1996). It has been shown to be useful to reduce the Hbs/Ag antigen found in human hepatitis carriers (Unander and Blumberg, 1992). Our recent findings indicate that the extract had a significant activity to suppress the chemicarcinogenesis induced by chemicals such as 3-methyl cholanthrene (Rajesh and Kuttan, 2001) and *N*-nitroso diethylamine (Joy and Kuttan, 1998). The mechanism of action of the extract seems to be (a) suppression of proliferation, (b) suppression of activation of carcinogen, and (c) anti-oxidant activity of the extract.

The present study indicating that the extract has significant effect in reducing inflammation and lesion is again reflective of its activity as scavenging oxygen radicals. Reactive oxygen species has been shown to have significant effect on the cellular system, damages its structure, and induces alteration especially in its high molecular weight components. Large doses of ethanol has been shown to specifically effect the inner lining of the stomach producing erosion. In liver ethanol converted to ethanal (aldehyde) which is more toxic. Ethanal has been shown to reduce GSH levels in liver tissues. Fall in GSH increases lipid peroxidation. Pretreatment with the *Phyllanthus amarus* extract may either produce a protective lining on the stomach and reduces oxygen radical production and thereby reduces

its effect on the liver. Similar mechanism could also be postulated for its anti-inflammatory activity of *Phyllanthus amarus*. Inflammation produced by carrageenan is mainly due to macrophage activation and there by the producing of oxygen radicals. *Phyllanthus amarus* extract could inhibit the oxygen radicals and there by reduces the inhibition.

Another possible mechanism for the activity of the extract to inhibit gastric lesion produced by alcohol may be due to the formation of a protective layer of the polyphenolic compounds present in the extract with the protein of the stomach lining by hydrophobic interaction. Moreover the extract may inhibit the prostaglandin synthesis like nonsteroidal anti-inflammatory drugs. It has been shown that *Phyllanthus amarus* extract could inhibit the onset of diarrhea induced by castor oil and reduced frequency of defecation and also reduced gut meal travel distance significantly (Odetola and Akojenu, 2000). This effect has been attributed to the inhibition of prostaglandin synthesis. Extract may also inhibit the macrophage migration which can reduce the inflammatory response produced by carrageenan.

Several active compounds have been identified in *Phyllanthus amarus* extracts. Most common among them are lignans like phyllanthin and hypophyllanthin (Somanabandhu et al., 1993), flavonoids like quercetin, astragalin (Nara et al., 1977), ellagitannins like amarinic acid (Foo, 1995) as well as amarin (Foo, 1993) and phyllanthisiin D (Foo and Wong, 1992). Hydrolysable tannins purified from *Phyllanthus amarus* were found to be potent inhibitors of rat liver cyclic AMP-dependent protein kinases. These hydrolyzable tannin inhibitors are the most specific and potent plant-derived inhibitors of cyclic AMP-dependent protein kinase catalytic sub unit yet found (IC50 0.2-17 fM) (Polya et al., 1995). It also showed anti-genotoxic properties (Gowrishankar and Vivekanandan, 1994). Phyllanthin, a diaryl butane lignan, isolated from *Phyllanthus amarus* showed a significant protection against CCl_4-induced elevation in transferase levels and significantly increased protein level (Syamasundar et al., 1985). Anti-viral agents: repandusinic acid (Ogata et al., 1992) and niruriside (Qian-Cutrone et al., 1996) isolated from *Phyllanthus amarus* were shown to inhibit HIV transcription in tissue culture. Active compounds responsible for the anti-ulcerogenic and anti-inflammatory activity produced by the extract have not been clearly understood.

References

Foo, L.Y., 1993. Amariin, a di-dehydro hexahydroxy diphenoyl hydrolysable tannin from *Phyllanthus amarus*. Phytochemistry 33, 487-491.

Foo, L.Y., 1995. Amarinic acid and related ellagitannins from *Phyllanthus amarus*. Phytochemistry 39, 217-224.

Foo, L.Y., Wong, H., 1992. Phyllanthisiin, an unusual hydrolysable tannins from *Phyllanthus amarus*. Phytochemistry 31, 711-713.

Giordano, 0.S., Guerreiro, E., Pestchanker, M.J., 1990. The gastric cytoprotective effect of several sesquiterpene lactones. Journal of Natural Products 53, 803-809.

Gowrishankar, B., Vivekanandan, 0.H., 1994. In vivo studies of a crude extract of *Phyllanthus amarus* L by tannery effluents. Mutation Research 322, 185-192.

Joy, K.L., Kuttan, R., 1995. Antioxidant activity of selected plant extracts. Amala Research Bulletin 15, 68-71.

Joy, K.L., Kuttan, R., 1998. Inhibition by *Phyllanthus amarus* of hepatocarcinogenesis induced by *N*-nitrosodiethylamine. Journal of Clinical Biochemistry and Nutrition 24, 133-139.

Langrange, P.H., Mackaness, G.B., Miller, T.E., 1974. Influence of dose and route of antigen injection on the immunological induction of T-cells. Journal of Experimental Medicine 139, 528-530.

Lee, C.D., Ott, M., Thyagarajan, S.P., Shfritz, D.A., Burk, R.D., Gupta, S., 1996. *Phyllanthus amarus* down regulates hepatitis B virus m RNA transcription and translation. European Journal of Clinical Investigations 26, 1069-1076.

Lowry, H.D., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with folin phenol reagent. Journal of Biological Chemistry 193, 265-275.

Mhaskar, KS., Blatter, E., Caius, J.F., 2000. Kirtikar and Basu's Illustrated Indian Medicinal Plants, vol. 9. Sri Satguru Publications, Delhi, India, p. 3074.

Moron, M.A., Depierre, J.W., Mannervick, B., 1979. Levels of glutathion, glutathion *S*-transferase activities in rat liver. Acta Biochemistry and Biophysics 582, 67-68.

Nara, T.K., Glyeye, J., Cerval, E.L., Stanislan, E., 1977. Flavonoids of *Phyllanthus niruri, Phyllanthus urinaria, Phyllanthus orbiculatus*. Plantes Medicinales et Phytotherapie 11, 82-86.

Odetola, A.A., Akojenu, S.M., 2000. Anti-diarrhoeal and gastrointestinal potentials of the aqueous extract of *Phyllanthus amarus* (Euphorbiaceae). African Journal of Medicine and Medical Sciences 29, 119-122.

Ogata, T., Higuchi, H., Mochida, S., Matsumoto, H., Kato, A., Endo, T., Kaji, A., Kaji, H., 1992. HIV-1 reverse transcriptase inhibitor from *Phyllanthus niruri*. AIDS Research in Human retroviruses 11, 1937-1944.

Polya, G.M., Wang, B.H., Fooly, L.Y., 1995. Inhibition of signal regulated protein kinases by plant derived hydrolysable tannins. Phytochemistry 38, 307-314.

Prakas, A., Satyan, K.S., Washi, S.P., Singh, R.P., 1995. P. *urinaria, P. niruri* and *P. simplex,* on carbon tetrachloride induced liver injury in the rat. Phytotherapy Research 9, 594-596.

Qian-Cutrone, J., Huang, S., Trimble, J., Li, H., Lin, P.F., Alam, M., Klohr, S.E., Kadow, K.F., 1996. Niruriside, a new HIV REV/RRE binding inhibitor from *Phyllanthus niruri*. Journal of Natuaral Products 59, 196-199.

Rajesh Kumar, N.V., Kuttan, R., 2000. *Phyllanthus amarus* extract administration increases the life span of rats with hepatoprotective carcinoma. Journal of Ethnopharmacology 73, 215-219.

Rajesh Kumar, N.V., Kuttan, R., 2001. Cancare—a herbal formulation inhibits chemically induced tumors in experimental animals. Indian Journal of Experimental Biology 39, 654-659.

Robert, A., Nezamis, J.E., Lancster, C., Hanchar, A.J., 1979. Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. Gastroenterology 77, 433-443.

Somanabandhu, A., Nityangkuru, S., Mahidol, C., 1993. ^{1}H and ^{13}C NMR assignments of phyllanthin and hypophyllanthin lignans that enhance cytotoxic responses with cultured multidrug-resistant cells. Journal of Natural Products 56, 233-239.

Syamasundar, K.V., Singh, B., Thakur, R.S., Husain, A.. Kiso, Y., Hino, H., 1985. Antihepatotoxic principles of *Phyllanthus niruri* herbs. Journal of Ethnopharmacology 14, 41-44.

Unander, D.W., Blumberg, B.S., 1992. In-vitro activity of *Phyllanthus* (Euphorbiaceae) species against the DNA polymerase of hepatitis viruses: effects of growing environment and intra- and inter-specific differences. Economic Botany 45, 225-242.