

RADIOPROTECTIVE AND FREE RADICALS SCAVENGING ACTIVITY OF PHYLLANTHIN: ISOLATED FROM *PHYLLANTHUS NIRURI*

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ABSTRACT

Background: The present study aimed at evaluation of in vitro antioxidant and radioprotective potential of lignan phyllanthin isolated from Phyllanthus niruri. Materials and Methods: Four preparative TLC isolates of P. niruri whole plant methanolic extract were assessed for hydroxyl radical scavenging ability. The hydroxyl radical scavenging potentials of S1 and S4 were found to be comparable [i.e. 33.4% (S1) and 26.5% (S4)], S1 exhibited significant ($p < 0.05$) antioxidant activity at 250 $\mu\text{g/ml}$ concentration. Compound S1 was identified as phyllanthin by TLC and HPLC analysis. Further authentication and characterized of phyllanthin was done by mp, UV spectrometry, elemental, IR, NMR and Mass analysis. Radioprotective effect of phyllanthin (S1) was studied against 4 Gy radiation induced chromosome damage in mouse bone marrow at a dose rate of 20, 40, 60 and 80 mg/kg, i.p. Bone marrow was scored for aberration in metaphase chromosomes after cytogenetic damage in the bone marrow cells was studied by chromosomal aberration analysis. Results: S1 fraction significantly reduced all types of aberrations at 40, 60 and 80 mg/kg dose. Phyllanthin significantly ($p < 0.001$) decreased percentage of severely damaged cell to only 0.91% at 80 mg/kg dose. Conclusion: Free radical scavenging appears to be the likely mechanism of radiation protection by the phyllanthin isolated from the P. niruri. Together these findings clearly prove that lignin phyllanthin content influences the radiation protective potential of P. niruri to a great extent.

KEY WORDS

Phyllanthus niruri, phyllanthin, lignan, antioxidant, radioprotection, chromosomal aberrations

INTRODUCTION

In recent years a lot of interest has developed worldwide in the area of radioprotectors. Radiation exposure as adjuvant of cancer therapy as well as nuclear and radiological emergencies resulting from a nuclear chain reaction or from decay of radiological wastes can cause hazards. In spite of intense global research in the field of radioprotection, no synthetic compound has been able to meet the criteria of a clinically acceptable radioprotector because of accompanying toxic effects. This has shifted the focus of researchers towards holistic approach for natural compounds as radioprotectors in view of their safety and multifaceted mode of action.

Ionizing radiations release high energy to break chemical bonds leading to induction of cellular oxidative stress owing to an imbalance of oxidants and endogenous antioxidants in the cellular milieu⁽¹⁾. The consequent oxidative stress is the outcome of imbalance between pro-oxidants (reactive oxygen species) and antioxidant defence system. As a result bio-molecules degrade, causing irreversible cellular dysfunctions and ultimately cell death. The extent of such damage is determined by the quantum of antioxidants in the system available at the time of exposure⁽²⁾. Endogenous antioxidants including non-enzymatic entities like glutathione, ascorbic acid and uric acid as well as enzymes like catalase, superoxide

dismutase and glutathione peroxidase play a crucial role to protect against radiation-induced damage. Exogenous agents may be useful in mitigating radiation damage that can retard generation of reactive oxygen radicals or scavenge them to restore normal redox state. Presence of large number of secondary metabolites having antioxidant activity helps in usage of herbs as an alternative due to their ability to combat adverse effect.

Phyllanthus niruri (syn *P. amarus*, *P. fraternus* Webster, family - Euphorbiaceae) commonly known as Jar amla or Jangli amla in Hindi and Bhumyamlaki in Sanskrit, This plant is a winter weed, growing in the tropical parts of India. The whole plant, fresh leaves as well as roots are used in traditional medicine as hepatoprotective drug ⁽³⁾. This plant is found to be effective in treating infective hepatitis and is used as an adjuvant with other herbal medicines. Sane *et al.*, reported that *P. niruri* protected rats against CCl₄ induced liver toxicity ⁽⁴⁾. Some drugs used in liver treatment like Liv 52 and Thiol (2-mercaptopropionylglycine, MPG) have shown good radioprotective effect against radiation induced chromosome damage in mouse ^(5 - 7). Methanolic extract (50%) of the aerial parts of *P. niruri* protect chromosomes against radiation damage by inhibiting induction of micronucleus in mouse bone marrow erythrocyte. The optimum dose for radioprotection of methanolic extract was 125 mg/kg which is much below the toxic dose as the extract was found to be non-toxic even at a dose of 2 g/kg ⁽⁸⁾. The alcoholic extract of *P. amarus* was demonstrated to possess radioprotective effect as shown by the increase in 30 days survival of mice exposed to lethal doses of whole body gamma radiation, chromosomal damage in bone marrow and intestine ^(9, 10). Ellagitannins and flavonoids isolated from *P. amarus* prevented radiation induced single strand breaks with effective prevention of lipid peroxidation and protein oxidation in mitochondria ⁽¹¹⁾.

Krithika *et al.*, reported *in vivo* antioxidative and hepatoprotective property of phyllanthin isolated from *P. amarus* ⁽¹²⁾. Isolation, characterization and quantification of phyllanthin and hypophyllanthin are reported by scientists like Tripathi *et al.*, Murali *et al.* and Nayak *et al.* ⁽¹³⁻¹⁵⁾. Lignans isolated from *Podophyllum hexandrum* (mayapple), *Linum*

usitatissimum (flex seed) and *Myristica fragrans* (nutmeg) are reported to have promising radioprotective potential ⁽¹⁶⁻¹⁸⁾. Though extract of *P. amarus* and *P. niruri* are reported to have radioprotective property, phytopharmacological correlation studies are still lacking to identify the active component responsible for the radioprotection. This study attempts to isolate the active lignan components in *P. niruri* to study *in vitro* antioxidant activity followed by screening for *in vivo* radioprotective effect of component showing the maximum antioxidant activity.

MATERIALS AND METHODS

Chemicals

Deoxyribose, colchicines, giemsa stain, trichloroacetic acid and thiobarbituric acid were obtained from Sigma Chemical Co., St. Louis, MO, USA. High performance liquid chromatography (HPLC) grade acetonitrile, water and methanol were purchased from Molychem (Mumbai, India). Standard phyllanthin was purchased from Natural Remedies (Bangalore, India). FeCl₃, EDTA, H₂O₂ and ascorbic acid were purchased from HiMedia, Mumbai, India. The rest of the chemicals used were of analytical grade.

Plant material and preparation of extract

Fresh aerial parts of *P. niruri* were collected from the fields of Bhopal, capital of Madhya Pradesh, India, during September-November of 2005-2006. The plant was identified by Sr Principal Scientist at CSIR-National Botanical Research Institute (NBRI), Lucknow, and a voucher specimen was deposited in the herbarium (voucher no. NBRI/D/2005/495). The aerial parts were air dried under shade and milled into coarse powder. Methanolic extract was prepared by refluxing with absolute methanol for 6 h in a Soxhlet apparatus at 40°C and residue concentrated under vacuum below 40°C. Qualitative phytochemical investigation was carried out to reveal the presence of carbohydrates, flavonoids, alkaloid, lignans, polyphenols, tannins, coumarins and saponins ⁽¹³⁾.

Isolation of components by chromatographic method

Thin layer chromatography: Freshly coated plates with silica gel G₂₅₄ was allowed to air dry in room temperature and transferred to oven for activation maintained at 105°C for 30 min. Alcoholic extract of *P.*

niruri was dissolved in chloroform and filter through Whatman filter paper. Different solvent system were tried to detect maximum possible phytoconstituents present in extract. Solvent system described in literature for lignins were tried and modification was attempted to achieve better resolution. The plates were placed into the developing chamber and allowed to run until it reaches a height of about 10 cm from the point of spotting. Developed plates were dried in a stream of hot air and sprayed with 10% sulphuric acid in methanol for detection of spots. After development the plates were heated in oven maintained at 110°C for optimal colour development of spots ⁽¹⁹⁾. Band scanning was done with CAMAG TLC scanner at 254 nm. The mobile phase hexane: acetone: ethyl acetate (7:2:1, v/v/v) and acetonitrile: water (80: 20, v/v) was optimized for final separation of components.

Preparative chromatography: Solvent system hexane: acetone: ethyl acetate (7:2:1) gave best resolution with separation of four compounds S1 to S4. Several round of preparative TLC was performed to recover the separated compounds. The spots were observed on a UV-detector at 254 nm and eluted with absolute methanol individually.

High performance liquid chromatography

Instrumentation and reagents: A Shimadzu HPLC system (SPD-M20A, Japan) equipped with a 680 quaternary pump, ASI-100 autosampler, 200 µl loop injector, a column oven, PDA-100 photodiode array detector and a degasser was used for setting the reverse phase liquid chromatographic conditions. Compound S1 was dissolved in methanol and then filtrated through a 45 µm membrane filter.

High performance liquid chromatography procedures: The chromatographic separation was performed on a reverse-phase Luna 5 µm C-18 100 Å, 250 × 4.6 mm, 5 µm particle size (Phenomenex, USA) column. The sample was eluted with an isocratic mobile phase of acetonitrile: water (80:20 for 30 min) at a flow rate of 0.5 ml/min. The detection wavelength and column temperature were set at 230 nm and 40°C respectively. A loading volume of 20 µl was injected under these conditions as well as an authentic sample of phyllanthin. Data analysis was performed using a

software named LC solution System provided by Shimadzu, Japan ⁽¹²⁾.

Characterization of compound

Melting point was determined using a digital melting point apparatus Toledo DSC 821 system in which the sample was press-sealed in an aluminium pan with a perforated lid and heated at a rate of 5°C/min in a nitrogen environment. The UV spectra of 5 µg/ml fraction in ethanol were obtained with Shimadzu (UV-1700 Pharmaspec) spectrophotometer while the IR spectra were recorded on a Jasco (FTIR- 5300) spectrometer using KBr pellet at a scanning speed of 2 mm/sec and with resolution set at 4 cm⁻¹. Elemental analysis was carried out on Heraeus Carlo Erba 1108 elemental analyser (Milan, Italy). ¹H NMR and ¹³C NMR spectra were recorded with a Bruker Deltonics (Avance 300) spectrometer in CDCl₃ at 300 MHz and the entire chemical shifts were relative to tetramethyl silane (TMS, d 0.00). The electron-impact mass spectra of the powdered sample was recorded on Shimadzu (QP 5000) spectrometer in CHCl₃, injected in HT-8 column, using helium as the carrier gas at a heating rate of 15°C/min and scan rate of 1 scan/sec in a scan range of 100-500 Delton.

In vitro antioxidant activity

Hydroxyl radical scavenging ability: The Fenton reaction was used to generate hydroxyl radicals in a test system, and the free radical scavenging activity was determined by the degradation of deoxyribose, as standardized by Elizabeth and Rao ⁽²⁰⁾. The hydroxyl radicals attack deoxyribose and initiate a series of reactions that eventually result in the formation of trichloroacetic acid reactive substance (TBARS). Radical scavenging by protectors results in inhibition of TBARS. The reaction mixture consisted of deoxyribose (3 mM, 100 µl), Ferric chloride (0.1 mM, 50 µl), EDTA (0.1 mM, 100 µl) and H₂O₂ (1 mM, 50 µl). Ascorbic acid (0.1 mM, 100 µl) in 550 µl of phosphate buffer saline (pH 7.4) was added. Each sample S1, S2, S3, S4 (50 µl each) was added to the reaction mixture at concentrations of 50, 100, 150, 200, 250 µg/ml to make a final volume of 1 ml and incubated for 1 h at room temperature. The mixture was then incubated for 20 min in a boiling water bath with 0.5 ml of 5% TCA and 0.5 ml of 0.5% of TBA, cooled and centrifuged. The absorbance in the supernatant was measured at 532 nm in a UV-Spectrophotometer.

Dimethylsulphoxide (DMSO) was used as the positive control. The results are expressed as percentage inhibition of formation of TBARS

In vivo radioprotective activity

Animals: Laboratory bred six to eight week old Swiss Albino mice of either sex, weighing 25-30 gm were used. The animals were housed and maintained under standard conditions of light (12:12 h L:D cycle), temperature (22±3°C) and humidity (50±5 %) in stainless steel cages. The animals had free access to standard pellets purchase from Golden Feed, Delhi and filtered acidified water *ad libitum*. Studies were conducted according to the guidelines of the CPCSEA, Chennai and approved by the Institutional Animal Ethical Committee (500/01/1/CPCSEA) of Jawaharlal Nehru Cancer Hospital and Research Centre, Idgah Hills, Bhopal, India.

Acute toxicity (LD_{50}) study in mice: Fraction S1 was freshly made in 5% tween 80 in water for Injection (WFI) and administered by intraperitoneal (i.p) route. Based on the OECD guidelines a Limit test was performed at 1000 mg/kg, (i.p) to categorize the toxicity class of the compound considering intraperitoneal route of administration and isolated nature of the compound. The animals (nulliparous and non-pregnant female mice) were fasted overnight with free access to water, weighed and a single dose of the test substance was administered. Animals were observed individually during first 30 min periodically during 48 h with special attention given during first 4 h and daily thereafter for total of 14 days (short-term toxicity).

Irradiation: The source of radiation was a ^{60}Co Theratron Teletherapy unit (Canada) in the Department of Radiotherapy, Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal. Unanesthetized animals were restrained in well ventilated perspex box and whole body was exposed at a dose rate of 1 Gy/min, in field size of $23 \times 23 \text{ cm}^2$ at a distance of 101 cm from the source⁽²¹⁾.

Study protocol

Animals were divided into 6 groups of 5 each and treated as follows: Gr I injected with 0.3 ml WFI and exposed to 4 Gy gamma radiation (Rt), Gr II sham Rt exposed control, Gr III and VI animals were injected with 20, 40, 60 and 80 mg/kg body weight of S1 fraction 1 h before whole body exposure to 4 Gy

radiation. Cytogenetic damage in the bone marrow cells was studied by chromosomal aberration analysis. After 22 h of irradiation all the animals were injected (i.p) with 0.025% colchicine and sacrificed 2 h later by cervical dislocation. Both the femurs were dissected out and used for chromosome preparation.

Chromosomal aberration study

Metaphase plates were prepared by the air drying method. Bone marrow from the femur was aspirated, washed in saline, treated hypotonically with potassium chloride (KCl) (0.0567%), and fixed in methanol: acetic acid (3:1), dried and stained with 1% Giemsa. Chromosomal aberrations were scored under light microscope (Olympus BX 60, Japan). A total of 500 metaphase plates were scored per animal. Different types of aberrations like chromatid breaks, chromosome breaks, fragments, rings and dicentrics, and cells showing polyploidy and SDC (severe damaged cells: with 10 or more aberrations of any type) were scored. When breaks involved both the chromatids, it was termed "chromosome type" aberration, while "chromatid type" aberration involved only one chromatid. When the deleted portion had no apparent relation to a specific chromosome, it was called a fragment⁽²²⁾.

Statistical analysis

Data were presented as Mean±SEM based up on variation between five mice. Statistical comparison of the data was done by one way ANOVA followed by Tukey's post-test using Graph PAD InStat Software. A p-value of 0.05 or less was considered to be statistically significant.

RESULTS

Extraction and phytochemical analysis: Colour of methanolic extract was dark brown having 8.97% yield. Qualitative phytochemical investigation revealed the presence of sugars, flavonoids, alkaloid, lignans, polyphenols, tannins, coumarins and saponins.

Identification of Compound: TLC of methanolic extract showed presence of two compounds with retention factor (R_f) of 0.63 (phyllanthin) and 0.84 in mobile phase acetonitrile: water (80:20), and four compounds with R_f 0.32 (phyllanthin), 0.61, 0.74 and 0.90 in mobile phase hexane: acetone: ethyl acetate

(7:2:1, v/v/v). Compounds isolated by preparative TLC as S1 (R_f value 0.32) yield 0.46%; S2 (R_f value 0.61) yield 0.44%; S3 (R_f value 0.74) yield 0.34%; and S4 (R_f value 0.90) yield 0.23%⁽¹²⁾. The retention time for phyllanthin on the HPLC column was 8.14 min for standard and 8.08 min for isolated fraction (Fig 2), in

a total run time of 30 min. Based on dead time of 2.41 min the capacity factor was calculated as 2.35 and the number of theoretical plates was 1632. The results were compared with standard phyllanthin sample (Fig 1).

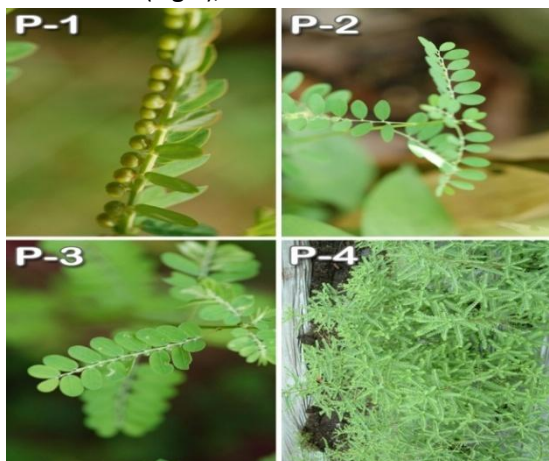


Fig: *Phyllanthus niruri* Linn. (amarus)

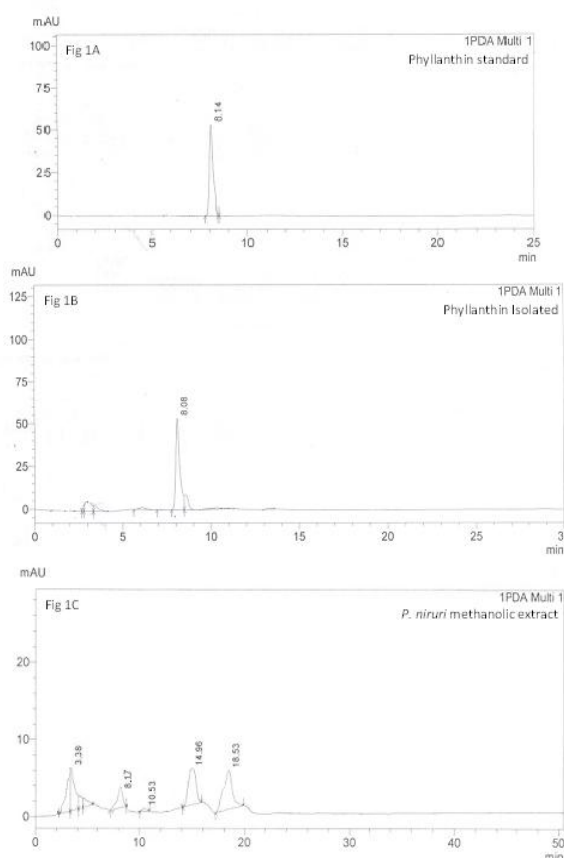


Figure 2: HPLC chromatogram

1A: Standard phyllanthin (retention time R_t 8.14); 1B: Isolated phyllanthin (R_t 8.08); 1C: Methanolic extract of *P. niruri* whole plant (R_t 3.38, 8.17 - phyllanthin; 10.53, 14.96 and 18.53) showed four prominent peaks.

To ascertain the identity of S1 component, characterization was done with UV, IR, NMR, MASS and elemental analysis. Fraction S1 gave bright yellow colour under UV light and positive colour reaction for lignin⁽¹⁵⁾. The compound had the following characteristics: m.p. 196-197°C; UV λ_{max} (EtOH) 230 (log E 4.29), 280 (2.35) nm; IR (KBr, cm^{-1}): 3201, 2917 (methylene $-\text{CH}_2$), 1515 (aromatic ring $-\text{C}=\text{C}-$), 1498 (methyl $-\text{CH}_3$), 1308 ($-\text{CH}$), 1159, 1103 and 1247 ($-\text{CO}$ stretch), 1085 (Cyclohexane ring vibrations C_6H_6), 946, 829 and 729 (aromatic and *trans* $-\text{CH}$); ^1H NMR (CDCl_3 300 MHz): δ 6.53 (2H, d), 6.60 (6H, dd), 2.52 (7H, dd), 2.03 (8H, m), 3.76 (oMe-3, s), 3.81 (oMe-4, s), 3.25 (oMe-9, s); ^{13}C NMR (CDCl_3 75 MHz): δ 133.2 (C & C'-2), 148.4 (C & C'-4), 112.5 (C & C'-5), 34.7 (C & C'-7), 40.8 (C & C'-8), 72.9 (C & C'-9); EIMS m/z (%): 418 (M^+ -9), 386 (M-OCH₃-5), 209 (M-C₁₁H₁₃O₂-20), 177 (M-C₁₂H₁₇O₃-15), 151 (C₉H₁₁O₂-100), 107 (15), 45 (34)⁽²³⁾.

In elemental analysis, the compound S1 corresponded to the molecular formula $\text{C}_{24}\text{H}_{34}\text{O}_6$. The molecular ion peak (M^+) of S1 was obtained at m/z 418. The base peak was obtained at m/z 151 (100%) was due to $\text{C}_9\text{H}_{11}\text{O}_2^+$ besides it gave peaks at m/z 346 (M - OCH₃), 209, 177 and 45 suggesting the nature of the side chain. The compound is suggestive to be phyllanthin which has the molecular formula $\text{C}_{24}\text{H}_{34}\text{O}_6$ [(+) 3, 4, 3', 4', 9, 9-hexamethoxy-8, 8'-butyrolignan] having absolute (8s, 8's) configuration comparing with IR and

NMR spectral data respective to the reported values (19, 24).

Hydroxyl radical scavenging ability: All the four compounds (S1, S2, S3, and S4) showed a dose-dependent inhibition of hydroxyl radical generation. S2 and S3 produced almost similar levels of inhibition, which was lower than that produced by DMSO. S1 showed significantly ($p < 0.05$) higher percentage of inhibition at 150, 200 and 250 $\mu\text{g/ml}$ concentration. S1 or in turn phyllanthin showed maximum inhibition of 33.4% at 250 $\mu\text{g/ml}$ (Table 1).

In vivo radioprotective activity: Acute toxicity study at 1000 mg/kg showed absence of any toxic signs. Depending on *in vitro* / *in vivo* correlation 20, 40, 60 and 80 mg/kg was selected for radioprotective activity assessment. Fraction S1 showed maximum antioxidant activity depending on which radioprotective was assessed on it. S1 showed extremely significant ($p < 0.001$) decrease in percentage chromosomal aberration in mice bone marrow exposed to radiation at 40, 60 and 80 mg/kg. Gama radiation exposure induced severely increased incidence of fragmentation, chromosome and chromatid breaks with 5.15% of severely damaged cell. S1 fraction significantly reduced all types of aberrations at 40, 60 and 80 mg/kg dose. Percentage of severely damaged cell was reduced to only 0.91% at 80 mg/kg dose of S1 (Table 2).

Table 1: *In vitro* hydroxyl radical scavenging ability of isolated compounds S1, S2, S3 and S4

Concentration ($\mu\text{g/ml}$)	Hydroxyl radical inhibition (%)				
	DMSO	S1	S2	S3	S4
50	5.3 $\pm$ 0.04	4.1 $\pm$ 0.58	4.5 $\pm$ 0.21	4.1 $\pm$ 0.63	3.7 $\pm$ 0.09
100	12.6 $\pm$ 0.43	12.3 $\pm$ 0.78	7.8 $\pm$ 0.48	6.5 $\pm$ 0.59	8.7 $\pm$ 0.92
150	16.9 $\pm$ 0.98	21.6 $\pm$ 1.33*	17.8 $\pm$ 0.56	14.4 $\pm$ 0.48	17.3 $\pm$ 0.55
200	20.5 $\pm$ 1.13	26.3 $\pm$ 1.10*	18.1 $\pm$ 0.78	16.7 $\pm$ 0.75	20.4 $\pm$ 1.26
250	24.4 $\pm$ 1.02	33.4 $\pm$ 1.15*	20.4 $\pm$ 1.92	20.1 $\pm$ 1.35	26.5 $\pm$ 1.06

* $p < 0.05$ compared to DMSO at the respective concentration.

Table 2: Effect of different doses of isolated compound S1 on whole body 4Gy irradiation induced chromosomal aberrations in the bone marrow of mice

Isolated Compound S1 (mg/kg, i.p)	% Aberrant cells	Types of aberrations (%)					Types of aberrant cells (%)		
		Chromatid breaks	Chromosome breaks	Fragments	Rings	Di-centrics	SDC	Poly-ploidy	Pul-verized
Control	0.881 ± 0.025	0.27 ± 0.01	0.06 ± 0.002	0.47 ± 0.06	0.00	0.00	0.00	0.00	0.00
4 Gy RT	29.10 ± 2.06 ^c	13.76 ± 0.73 ^c	5.18 ± 0.18 ^c	7.16 ± 0.45 ^c	1.60 ± 0.08	1.60 ± 0.02	5.15 ± 0.17	4.92 ± 0.61	1.64 ± 0.12
20 + RT	22.11 ± 1.36 ^{c,2}	10.42 ± 0.42 ^{c,3}	3.10 ± 0.16 ^{c,3}	6.11 ± 0.26 ^c	1.20 ± 0.06 ³	1.20 ± 0.01 ³	2.13 ± 0.13 ³	2.80 ± 0.32 ³	0.32 ± 0.06 ³
40 + RT	13.45 ± 0.99 ^{c,3}	8.06 ± 0.21 ^{c,3}	1.08 ± 0.02 ^{c,3}	4.09 ± 0.12 ^{c,3}	0.19 ± 0.002 ³	0.19 ± 0.02 ³	1.94 ± 0.11 ³	1.91 ± 0.24 ³	0.21 ± 0.01 ³
60 + RT	8.14 ± 0.95 ^{b,3}	6.02 ± 0.14 ^{c,3}	0.06 ± 0.002 ^{ns,3}	2.06 ± 0.03 ^{c,3}	0.00	0.09 ± 0.001 ³	1.46 ± 0.02 ³	0.14 ± 0.02 ³	0.00
80 + RT	3.06 ± 0.12 ^{ns,3}	2.01 ± 0.01 ^{a,3}	0.02 ± 0.00 ^{ns,3}	1.03 ± 0.02 ^{ns,3}	0.00	0.02 ± 0.00 ³	0.91 ± 0.03 ³	0.76 ± 0.04 ³	0.00

^ap<0.05, ^bp<0.01, ^cp<0.001 when compared to vehicle control. ²p<0.01, ³p<0.001 and ns = not significant when compared to negative control radiation control group.

DISCUSSION

Plants are valuable sources of novel biologically active molecules. *P. niruri* is a proved hepatoprotective drug and useful in severe jaundice. Free radical damage to biosystems is one of the major processes that contribute to degenerative diseases such as cancer and ageing. Free radical scavengers protect cellular DNA against indirect effects of ionizing radiation where hydroxyl radicals are believed to be the primary active species responsible for the damage. Alcoholic extract of *P. niruri* at a dose of 200 mg/kg, b.wt was effective in protecting the mouse bone marrow chromosomes against radiation induced chromosomal damage⁽⁸⁾. The plant contains several chemical compounds like flavonoids and lignans which have potential radioprotective effect⁽²⁴⁾. Research in the development of radioprotectors worldwide has focused on screening a variety of biological compounds from natural origin i.e., antioxidant cytoprotective agents, immuno modulators, vitamins and DNA binding molecules, have been evaluated extensively for their radioprotective potentials in both *in vitro* and *in vivo* models⁽⁵⁻⁷⁾. Most of the radioprotective drug available produces cumulative or irreversible toxicity and is unable to provide effective long-term

protection⁽²⁵⁾. In view of this, the search for less toxic and more potent radioprotector drugs are needed to be carried out.

The data obtained in this study demonstrate the antioxidant ability of the *P. niruri* methanolic extract. Four components (S1, S2, S3, and S4) were isolated from the crude extract, out of which S1 and S4 showed good *in vitro* antioxidant activity. S1 was the most effective and showed significantly higher antioxidant activity than that of DMSO. Therefore this compound was chosen for further radioprotective activity study. This compound was identified to be similar with reported structure of phyllanthin, which is a major lignin compound present in *P. niruri*. Lignin contains both hydrophilic and hydrophobic groups⁽²⁶⁾. Lignins are phenolic compounds and these compounds are well known antioxidants^(19, 27). Phyllanthin is a phenolic compound with two benzene rings and the presence of a phenolic group with a double bond in conjugation, which promotes radical scavenging. Phyllanthin has a basic structure, which is very favorable for its antioxidant function⁽²⁸⁾.

Curcumin contains a similar double bond in conjugation with the phenyl rings, having powerful free radical scavenging property⁽²⁰⁾. Plant derived ferulic acid readily forms a resonance stabilized

phenoxy radical which accounts for its potent antioxidant potential due to its phenolic nucleus and an extended side chain conjugation⁽²⁹⁾. Rajakumar and Rao have demonstrated that isoegenol having a double bond in conjugation with the phenyl ring plays an important role in the antioxidant activity of this compound⁽³⁰⁾. The presence of phenolic rings in *meta* and *para* position showed a good free radical scavenger activity. The polyphenolic flavonoids orientin and vicenin found in *Ocimum sanctum* have also been reported to have strong free radical scavenging activity *in vitro*⁽³¹⁻³⁴⁾.

Compound S1 (phyllanthin) showed good hydroxyl radical scavenging activity. The molecular structure of phyllanthin is highly suited for the free radical scavenging activity, which could explain the antioxidant activity of S1 compound observed *in vitro*. Selected compound S1 also showed very significant radioprotection in mouse bone marrow chromosomes and significantly reduced percent aberrant metaphases as well as complex aberrations like rings and dicentrics. Gamma radiation produces reactive oxygen radicals in water, which attack cellular DNA, and produced single and double strand breaks⁽³⁵⁾. Non-repair and mis-repair of DNA strand breaks lead to chromosomal aberrations. Radiation is one of the most severe causes of oxidative stress mediated by free radical flux. *P. niruri* extract administration increases the life span of rats with hepatocellular carcinoma⁽³⁶⁾. Extract of *P. amarus* is reported to have chemoprotective, antitumour and anticarcinogenic activity^(37, 38).

Phyllanthin and hypo phyllanthin are the major lignan compounds present in *P. amarus* and *P. niruri*^(13, 14). Phyllanthin has shown good *in vitro* antioxidant activity, must be protecting chromosomes by reducing DNA strand breaks through free radicals scavenging activity. Krithika *et al.*, has reported *in vivo* DPPH radical scavenging ability of phyllanthin⁽¹²⁾. Vitamin C and E have protective effect against γ -ray induced chromosomal aberrations improves survival and haematopoiesis in mice^(39, 40). Many flavonoids and lignans are already known for their antioxidant action and anti-apoptotic potential and thus contribute towards radioprotection via several mechanisms such as free radical scavenging, stabilization of mitochondrial cell potential,

regulation of cell cycle activities and time dependent apoptosis⁽⁴¹⁻⁴³⁾. Phyllanthin significantly inhibited the radiation-induced DNA damage in mouse bone marrow as indicated by decrease in DNA fragmentation in a dose dependent manner. Free radical scavenging appears to be the likely mechanism of phyllanthin radiation protection isolated from the *P. niruri*. The results of this study reinforce that presence of lignin phyllanthin influences the radiation protective potential of *P. niruri* to a great extent, which one of the bioactive radioprotective phytoconstituents in *P. niruri*.

CONCLUSION

This study showed lignin phyllanthin as one the active phytoconstituents responsible for radioprotector activity of *P. niruri* and free radical scavenging seems to play an important role in its protective mechanisms.

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