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Spectrophotometric Determination of Hepatoprotective Principle of *Phyllanthus niruri* Linn

S. VENKATESH¹, B. MADHAVA REDDY¹, C. KASI VISWANATH² AND M. RAMESH*

University College of Pharmaceutical Sciences, Kakatiya University, Warangal-506009.

¹G. Pulla Reddy College of Pharmacy, Mehidipatnam, Hyderabad-500028.

²Stanmax Pharma Pvt. Ltd., Prashanth Nagar, Uppal, Hyderabad-500039.

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A simple and sensitive spectrophotometric method in ultraviolet region has been developed for the determination of phyllanthin in different parts of *Phyllanthus niruri*. Phyllanthin shows maximum absorbance at 280 nm. Beer's law is obeyed in the concentration range of 5-50 µg/ml. The proposed method is precise, accurate and reproducible.

Phyllanthus niruri Linn. belongs to the natural order Euphorbiaceae. It is an annual herb and chiefly distributed in the tropical and sub-tropical regions of the world¹. It is incorporated in large number of reputed hepatoprotective herbal formulations. A number of compounds have been isolated from the plant, which include lignans, alkaloids, flavonoids and sterols. The presence of phyllanthin, which belongs to the class of lignans is reported to be responsible for the hepatoprotective activity²⁻⁵. No standard method has been reported for the estimation of this active principle. An attempt is made here to determine the percentage of phyllanthin by spectrophotometer, using methanol as a blank.

The percentage of phyllanthin was evaluated separately for leaf-midrib (including fruits) and stem-root by this method.

A Shimadzu model 250 UV/Vis spectrophotometer with 1 cm matched quartz cells was used. Silica gel G and methanol (analytical grade) were obtained from S. D. Fine Chemicals, Mumbai and all other organic solvents used were of laboratory grade. Fresh herbs of *P. niruri* were collected locally in the month of September, 2002 from two different locations viz., Osmania University Campus and Ghatkesar. Botanical identification was performed and a voucher specimen (PN/WP/02) is being maintained in the phytochemistry department of G. Pulla Reddy College of Pharmacy (GPRCP), Hyderabad, A.P. The stem-root was separated from the herb. Both the samples were air-dried at room temperature and ground into powder.

*For correspondence

E-mail: Mullangir@hotmail.com

TABLE 1: ESTIMATION OF PHYLLANTHIN IN DIFFERENT PARTS OF *P. NIRURI*

Preparation	Percentage of phyllanthin	
	Leaf-mid rib Including fruits	Stem-root
Sample-I	1.49	0.42
Sample-II	1.40	0.39
Sample-III	1.51	0.36
Average	1.47	0.39
S.D	0.06	0.03
C.V	3.99	7.69

Estimation of phyllanthin percentage in the samples collected from different parts of *P. niruri* viz., leaf-mid rib and stem-root.

About 1 g of (40 #) dry powder was extracted with petroleum ether (3x25 ml) by gently warming on water bath. The combined petroleum ether extract was concentrated to about 2 ml volume and residue was completely applied on a chromatographic plate as a band with a reference spot of standard phyllanthin (isolated at GPRCP and characterized using chromatographic and spectral techniques) on both sides. The preparative TLC was run using silica gel G as a stationary phase and toluene:ethyl acetate (5:1) as a mobile phase.

The visualization of the phyllanthin was done in iodine chamber, having R_f value 0.45. The distinct band was scraped off, extracted with methanol (3x5 ml) and filtered through Whatmann filter paper. The volume of filtrate is made up to 50 ml with methanol and considered as sample stock solution. The 5 ml of stock solution was adjusted to 10 ml and the absorption was measured at 280 nm on a Beckman spectrophotometer using methanol as blank.

Recovery studies were carried out by adding known amount of pure phyllanthin to pre-analyzed crude drug and again analyzed to estimate drug content. The concentrations of the phyllanthin in all the samples under investigation were calculated with the help of the standard curve. Standard

TABLE 2: RECOVERY STUDY DATA

Amount of phyllanthin added (mg)	Recovery in mg	% Recovery*
10	9.89±0.82	98.9
15	14.84±0.24	98.9
20	19.82±1.24	99.1

*Average of three determinations. Absolute recovery of phyllanthin from crude drug (pre-analyzed) at three different concentrations.

solution (100 µg/ml) of phyllanthin was prepared by dissolving 10 mg of phyllanthin in 100 ml methanol. Appropriate dilutions of phyllanthin were made in methanol to produce working stock solutions of 50, 40, 30, 20, 10 and 5 µg/ml. The standard curve of phyllanthin was plotted by taking absorbance of solutions containing 5, 10, 20, 30, 40 and 50 mg/ml concentration and across this concentration range, phyllanthin obeys Beer Lambert's law. The results of analysis and recovery studies are presented in Table 1 and 2, respectively.

The results indicate that the yield of phyllanthin is more in leaf-midrib in comparison to the stem-root. The spectrophotometric method described here is simple, sensitive and easy for the routine estimation of the active principle of *P. niruri*.

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