



Received on 11 December, 2011; received in revised form 15 March, 2012; accepted 30 March, 2012

## ISOLATION OF RUTIN FROM *PHYLLANTHUS AMARUS*

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### ABSTRACT

#### Keywords:

*Phyllanthus amarus*,  
Fractionation,  
Rutin,  
Spectroscopy

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General phytochemical screening of *Phyllanthus amarus* (Euphorbiaceae) revealed the presence of Saponin, tannins, glycoside and alkaloids etc. The aim of this study is to identify and characterize the bioactive principle from the plant. It has wide folk medicinal uses. The isolation and characterization of Phytoconstituents was done from the methanolic extract by the gradient fractionation method. The structure of the isolated compound was established on the basis of physical, chemical test and spectroscopic evidences (IR, UV, <sup>1</sup>HNMR, MS). A flavonol structure was isolated from the methanolic extract of the plant. The odorless, colorless compound which solubilizes in methanol having m.p. 186°C and R<sub>f</sub> value 0.46 in mobile phase Methanol: Glacial Acetic Acid: Water (90: 5: 5) was identified by the various spectroscopical methods. So from this study it is concluded that isolated compound may be rutin which is responsible for various pharmacological activities of the plant.

**INTRODUCTION:** The plants of the genus *Phyllanthus* (Euphorbiaceae) are widely distributed in most tropical and subtropical countries. It is a very large genus consisting of approximately 550 to 750 species and is subdivided into 11 subgenera. *Phyllanthus* genera contain many important phytoconstituent which are responsible for various type of pharmacological activity like very effective hepatoprotective agents in the Indian indigenous systems of medicine and are considered bitter, astringent, stomachic, diuretic, febrifuge, deobstruant and antiseptic<sup>1</sup>. Among the popular species, *P. niruri*, *P. urinaria*, *P. emblica*, *P. flexuosus* and *P. amarus*, have received the most attention.

*Phyllanthus amarus* known as “Bhui amla” (Family – euphorbiaceae) widely distributed throughout the rainforest region of Northern India. It is an annual herb grows up to 15-60 cm high have an erect stem. The

plant is most commonly used in the Indian Ayurvedic system of medicine in problems of stomach, genitourinary system, liver, kidney and spleen<sup>2</sup>. *P. amarus* have different classes of organic compounds of medicinal importance including alkaloids, flavonoids, hydrolysable tannins (Ellagitannins), major lignans, polyphenols, triterpenes, sterols and volatile oil<sup>3</sup>.

### MATERIAL AND METHOD:

**Collection & Authentication:** The *Phyllanthus amarus* was collected from Chandrashekhar azad agriculture university, Kanpur in the month of July-August. The plant material was authenticated by Prof J. P. Shukla, Department of Botany, D.B.S College, Kanpur where a voucher specimen (PH/PA/20) is deposited for further reference.

**Extraction and Isolation of Compound:** The dried plant material (100gm) is extracted with two successive quantities (2x 200 ml) of Methanol (80% v/v). The resulting filtered extract is carefully evaporated under vacuum in a rotary evaporator till it reaches up to 50-60 ml. The content of the flask is mixed with equal volume ether and the ethereal layer was separated. The hydro alcoholic layer is again extracted with the same volume of ether and the ethereal layer is separated. Both the ethereal layer is discarded. The hydro alcoholic layer is evaporated under reduced pressure to 10 ml. Same process was repeated with chloroform and discards the chloroform extract. Keep the concentrated residual liquid in refrigerator (0-5°C) over night when a solid crystalline substance appears.

The TLC of isolated crystal was performed using the mobile phase Methanol: Glacial Acetic Acid: Water (90: 5: 5) on silica gel G. The R<sub>f</sub> value was 0.46. The characterisation of the isolated compound was done on the basis of phytochemical analysis (shinoda test, zinc hydrochloride reduction test) and spectroscopic studies. (IR, UV, <sup>1</sup>HNMR).

**General and Physical Properties:** Appearance, color, taste, odor, solubility and melting point of the isolated constituents will be determined.

**Chemical identification of Constituents:** Little amount of the isolated constituent are dissolve in methanol and perform the following test-<sup>4,5</sup>

- **Shinoda Test (Magnesium Hydrochloride reduction test):** To the test Solution, add few fragments of Magnesium ribbon and add concentrated Hydrochloric acid drop wise and observe the color.
- **Zinc Hydrochloride Reduction Test:** To the test solution add a mixture of Zinc dust and conc. Hydrochloric acid. Heat the solution and observe the color.
- **Alkaline Reagent Test:** To the test solution add few drops of sodium hydroxide solution and observe the colour formation.

**Spectral Characterization of Constituents:**

- **Ultra Violet spectra:** The Ultra Violet spectra of the constituents (isolated from TLC and dissolved in methanol) are taken in double beam Shimadzu spectrophotometer (UV-1700) in between range 200 nm to 700 nm. Methanol was taken as reference solvent.
- **Infrared Spectra:** The IR spectrum of isolated constituents as KBr disc has been determined on a Perkin – Elmer Infrared Spectrophotometer. The structural assignments have been correlated for the characteristic bands as mentioned in results.
- **Proton NMR Spectra:** The proton NMR spectra was taken by dissolving the sample in DMSO – D<sub>6</sub> and run on a 60-MHz NMR Spectrometer. All chemical shifts reported are in reference to tetra methyl silane (TMS) at 0 ppm.
- **Mass Spectra:** The mass spectrum of rutin obtained by desorption chemical ionization (DCI) using ammonia as a reactant gas show a molecular ion M at m/e 611 amu. The prominent fragments and their relative intensities are mention in results.
- **Elemental Analysis:** Elementary analyses were obtained on the Isolated constituent.. These included percent carbon, per cent hydrogen and percent of oxygen.

## RESULTS:

**General and Physical Property:** It is pale yellow needles which gradually darken on exposure to light. It is tasteless and odorless. It is soluble in pyridine, formamide, methanol and alkaline solution. Melting point of the constituent was founded 189° C.

**Elemental Analysis:** The percentage of carbon 53.11 %, hydrogen 4.95 %and oxygen 41.93 % was found.

## Chemical Identification:

Test	Observation	Result
Shinoda Test	Green to blue color appears after few minutes.	Flavonol are present
Zinc Hydrochloride reduction test	It gives red color after few minutes.	Flavonol are present
Alkaline reagent test	Formation of an intense yellow color, which turns to Colorless on addition of few drops of dil. Acid.	Flavonol are present

**Spectral Characterization:**

- **UV Spectra:** The UV spectrum of rutin in metabolic solution shows two major absorption bands at 359 nm and 257 nm, which indicates the presence of flavonol structure.
- **IR Values:** 3330 OH (bonded) , 2920 CH stretch, 1660 C=O, 1620 C=C, 1600 Aromatic structure, 1510 C=C aromatic, 1360 C-O-C, 1295 C-O-C, 1200 C-O-C, 1060 C-O-C, 810 Substituted aromatics other fingerprint bands characteristics to rutin are seen following 970, 880, 730 and 700.

**NMR values:** Chemical shifts of Rutin in DMSO-D6 with respect to TMS.

Group	Position	Chemical Shifts (delta value)
CHMe	Rhamnosyl Me	103(d)
Rhamnoglucosyl		3.20; 3.40 (bS)
H-1 Rhamnosyl		4.40 (S)
H-1 Glucoosyl		5.30 (bS)
H- 6	Aromatic	6.17 (SL)
H-8	Aromatic	6.36 (SL)
H- 5	Aromatic	6.80 (d)
H- 6	Aromatic	7.50 (bS)

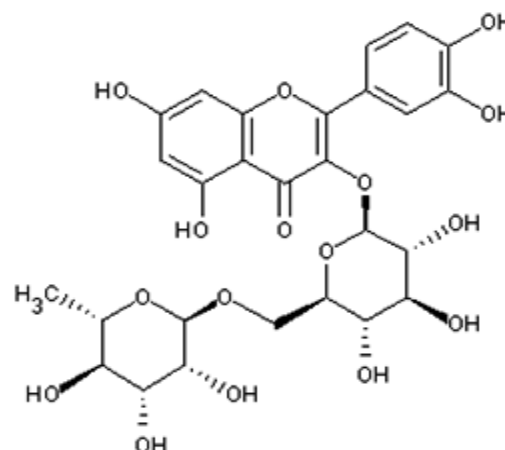
(S) = Singlet; (d) = doublet; (bS) = broad singlet; (SL) = Singlet showing long range coupling

**Mass Spectrum:** The mass spectrum of rutin obtained by desorption chemical ionization (DCI) using ammonia as a reactant gas show a molecular ion M at m/e 611 amu. The prominent fragments and their relative intensities are shown below

M/e	Relative intensity
303	100.00
611	28.88
628	2.22
164	57.77
180	66.66
304	44.44
308	22.22
320	24.44
326	42.22
449	11.11
465	33.33

General description and spectral analysis shows that the isolated constituent is rutin. Based on the melting point and other related data (UV, IR, <sup>1</sup>HNMR and

<sup>13</sup>CNMR) the structure of the isolated compound was proposed as;



RUTIN (C<sub>27</sub>H<sub>30</sub>O<sub>16</sub>, Mol wt. 610.53 g/mol)

**CONCLUSION:** From the above study, a flavonol structure (Rutin) was isolated and characterized from methanolic extract of *Phyllanthus amarus* Linn<sup>6, 7</sup>. Rutin inhibits platelet aggregation<sup>8</sup>, as well as decreasing capillary permeability, making the blood thinner and improving circulation.<sup>9</sup> It also shows anti-inflammatory activity, aldose reductase activity, antioxidant activity, can reduce the symptoms of haemophilia and reduce the cytotoxicity of oxidized LDL cholesterol which lowers the risk of heart disease<sup>10</sup>. There is also some evidence that rutin can be used to treat hemorrhoids, varicosis, and microangiopathy<sup>11</sup>.

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