IJPSR (2012), Vol. 3, Issue 05 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 January, 2012; received in revised form 23 February, 2012; accepted 23 April, 2012

ISOLATION AND CHARACTERIZATION OF POLYPHENOLIC COMPOUND QUERCITIN FROM *PHYLLANTHUS* EMBLICA

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Keywords:
Medicinal plants,
Quercetin,
Pharmacological activities

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ABSTRACT

The curative parts of medicinal plants are not simply its woody stem or its leaves but the number of chemical compounds (phytoconstituents) it produces and uses for its own growth like flavonoids, alkaloids, gum, mucilage, protein, carbohydrate etc. The genus Phyllanthus is widely used in medicine but still lot of work left to explore this genus. So the species, *Phyllanthus emblica* which is widely used in Indian traditional system are selected for various screening. The mentioned plant materials are rich source of polyphenolic compounds and some typical type of polyphenolic are responsible for various pharmacological activities. The isolation and characterization of Phytoconstituents was done from the alcoholic extract by the chromatographic method. The structure of the isolated compound was established on the basis of physical, chemical test and spectroscopic evidences (IR, UV, 1HNMR, MS). A flavonoidal structure was isolated from the alcoholic extract of the plant. The yellow colored compound which solubilizes in methanol, ethanol and water having m.p. 315^{o}C and Rf value 0.19 in mobile phase Isopropanol: formic acid: water (2:5:5) was identified by the various spectroscopical methods. So from this study it is concluded that isolated compound may be quercetin which is responsible for various pharmacological activities of the plants.

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 [1].

Among the popular species, *P. niruri, P. urinaria, P. emblica, P. flexuosus* and *P. amarus,* have received the most attention.

Emblica officinalis (EO) enjoys a hallowed position in Ayurveda- an Indian indigenous system of medicine. According to believe in ancient Indian mythology, it is the first tree to be created in the universe. It belongs to family Euphorbiaceae. It is also named as Amla, *Phyllanthus Emblica or* Indian gooseberry. The species is native to India and also grows in tropical and subtropical regions including Pakistan, Uzbekistan, Srilanka, South East Asia, China and Malaysia.

The fruits of *Emblica officinalis* are widely used in the Aryuveda and are believed to increase defense against diseases. It has its beneficial role in cancer, diabetis, liver treatment, heart trouble, ulcer, anemia and various other diseases.

Similarly, it has application as antioxidant, immunomodulatory, antipyretic, analgesic, cyto-protective, antitussive and gastro-protective. Additionally, it is useful in memory enhancing, ophthalmic disorders and lowering cholesterol level. It is also helpful in neutralizing snake venom and as an antimicrobial. It is often used in the form of Triphla which is an herbal formulation containing fruits of *Emblica officinalis*, *Terminalia chebula and Terminalia belerica* in equal proportions. It contains various alkaloids, benzenoid, furanolactone, Diterpene, triterpene, flavonoid, corilagin, sterol and carbohydrate. So in this regard an attempt has been made to isolate the polyphenolic compound quercetin from this plant.

MATERIAL AND METHOD:

Collection & Authentication: *Phyllanthus emblica* were collected from local market of Kanpur. The plant material was authenticated by Prof. J.P. Shukla, Department of Botany, D.B.S College, Kanpur, where a voucher specimen (PH/PE/22) is deposited for further reference.

Isolation of Phytoconstituent: Hundred grams of dry powder of the the fruits of *Phyllanthus emblica* were successively extracted with petroleum ether, ether, and chloroform for the removal of fatty material, steroids, terpenoids, etc. The remaining powder was exhaustively extracted in a Soxhlet apparatus with 95% ethanol. After the evaporation of ethanol under vacuum, the extract was dried. The dried residue dissolve in ethanol and performed the thin layer chromatography using the silica gel as stationary phase and mobile phase contain Isopropanol- formic acid-water (2:5:5).

On the chromatograms the yellow bands of quercetin with Rf, values of 0.19 were located. These bands were carefully eluted and extracted with 95% ethanol. The solvent was evaporated from the resulting extracts and residue was thus obtained which was subjected to various physical and spectral analysis [2].

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 alcohol and perform the following tests [3,4];

- **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.

RESULTS AND DISCUSSION:

Characterization of the compound: The yellow coloured compound, melting point 315^{o}C, have been isolated. The compound is soluble in methanol, ethanol and water and shows the spectra at particular wavelength.

UV Spectra: The UV spectra of the peaks were typical of flavonoid (UV maxima at 260–270nm and 340–370 nm). Peak showed UV absorption with maximum at 276 nm and 360nm.

IR Values: FT-IR data showed that the isolated compound presented the characteristic intensities of C=O absorption band at 1664.9 cm^{-1} and the OH stretch at 3408.1, 1611.9, 1450.3, 1383.2 and 1383.2 cm^{-1} Is the absorption band of ν C=C, ν C-OH, ν C-OH and ν C-O (Phenol)

Bands	**:**	**Frequencies**
ν (O–H)	:	(3408–3325) bd
ν(C=O)	:	(1664) s
ν (C=C)	:	(1611) v s
ν (C–OH)	:	(1319) s
ν (C–O–C)	:	(1261) v s

NMR values: The 1HNMR signals at δ: 12.45 (1H, s, OH-5); 10.80 (1H, d, 7-OH), 9.62 (1H, s, 3-OH), 9.39 (1H, s, 4'-OH); 9.33 (1H, s, 3'-OH); 7.78 (1H,d, 2'-H); 7.68 (1H, d, 6'-H); 7.03 (1H, s, 5'-H); 6.45 (1H, d, 8-H); 6.34 (1H, d, 6-H).

The spectrum of isolated compound showed product on aromatic groups ranging from 6 to 8 ppm, and a strong intramolecular hydrogen bonding at 12.45 ppm. The 1H NMR signals at 12.45 ppm should be attributed to hydroxyl protons of isolated constituent, which participated in a strong intramolecular hydrogen bond, between the hydrogen atom of the hydroxyl group OH(5) and the oxygen atom of the carbonyl group CO(4).

Mass Spectrum: Mass spectra of isolated constituent show Molecular ion *m*/*z* 301.1 was the most abundant ions. The greatest product ion from the parent ion *m*/*z* 301.1 was *m*/*z* 151.1. The above data represent that the isolated constituent may be quercetin.

CONCLUSION: From the above study, quercetin was isolated and characterized from ethanolic extract of *Phyllanthus emblica* Linn and this is a flavonoidal constituent.

Quercetin has anticancer, antiinflammatory, antiviral, fibromyalgia, metabolic syndrome etc [5]. Quercetin is frequently used therapeutically in allergic conditions, including asthma and hayfever, eczema, and hives [6]. Additional clinical uses include treatment of gout, pancreatitis and prostatitis, which are also, in part, inflammatory conditions. The common link is its ability to mediate production and manufacture of pro-inflammatory compounds.

However, its uses also may be important in cancer therapeutics. Quercetin is a recognized antioxidant and has been studied for its gastro-protective effects, inhibition of carcinogenicity either alone or in combination with chemotherapeutic agents, reducing risk of cataract. Again, the ability of quercetin to inhibit inflammatory leukotriene production may be a key to its beneficial impacts [7,8].

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