RESEARCH PAPER | Pharma | Volume : 3 | Issue : 11 | Nov 2013 | ISSN - 2249-555X

Simultaneous Determination of Phenolic Compounds in *Melia Azedarach*. Linn Leaves by High-Performance Liquid Chromatography

KEYWORDS: *Melia azedarach*. Linn, HPLC, Phenolic compounds, Quercetin,Rutin and Kaempferol.

Mohammed Fazil Ahmed
Nizam Institute of Pharmacy & Research Center, Deshmukhi, Pochampally (M), Near, Ramoji Film City, Nalgonda,(AP), INDIA-508284.

A. Srinivasa Rao
Bhaskar Pharmacy College,Yeknapally, Moinabad(Mandal), R.R(Dist), Hyderabad-500075.

ABSTRACT *Rutin, Quercetin and Kaempferol are the most important phenolic compounds of Melia azedarach. A simple and rapid HPLC method was developed for simultaneous determination of Rutin, Quercetin and Kaempferol in ethanolic leaves extract of Melia azedarach. Acetonitrile and Phosphate buffer (pH=5.8) in ratio of 55: 45 used as mobile phase. The phenolic compounds were determined by Athena C18 column type at 254 nm with flow rate of 1 ml/min. Retention time of standards, Rutin, Quercetin and Kaempferol were found to be 2.357, 6.093 and 9.373 respectively. While the Retention times of Rutin, Quercetin and Kaempferol in Melia azedarach are 2.403, 6.143 and 8.903 which are found to be matching with standards retention time values respectively. Thus this HPLC method was found to be convenient and simple for quantitative analysis of phenolic compounds in Melia azedarach.*

INTRODUCTION

Plants are potential sources of natural antioxidants. It produces various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive.[1] ROS, which include free radicals such as superoxide anion radicals ($O_2^{\cdot}$), hydroxyl radicals ($OH^{\cdot}$) and non free-radical species such as H_2O_2 and singled oxygen ($^1O_2$), are various forms of activated oxygen. These free radicals responsible for cellular injury and aging process.[2] Plant phenolics are commonly found in both edible and non-edible plants, and have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential.[3] It is an established fact that polyphenolic compounds possess remarkable antioxidant activities which are present quite commonly in the plant family Meliaceae. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants.[4] Therefore; the need exists for safe, economic, powerful and natural antioxidants to replace these synthetic ones.

Rutin is the rhamnoglucoside of the flavonoid quercetin, and found in many plants[5] and used for treatment of various diseases related to the vascular.[6]Quercetin is a flavonol, it is plant derived flavonoid used as a nutritional supplement found in fruits and vegetables. Quercetin is thought to have potent antioxidant, Antidiabetic and anti tumour, and antiviral, anti inflammatory benefits.[7] Quercetin is mainly found in many often consumed foods include green apple, onion, green tea, lemon as well as many seeds, flowers, barks, and leaves.[8] Kaempferol occurs naturally in a variety of fruits, vegetables, wine and tea. Kaempferol can be isolated from tea, broccoli, witch-hazel, propslis, grapefruit, and other plant source.[9]kaempferol is one of the most important flavonoids that inhibit heart, spinal cord, and brain disease. Itinhibits both oxidative susceptibility of low density lipoprotein (LDL) in vitro,and platelet aggregation.[10]

Melia azedarach Linn. (Family: Meliaceae) is a shrub or small evergreen and medium-sized deciduous tree.It has been useful in fever, thirst, nausea, vomiting, and skin diseases.[11,12] Leaves and fruits showed antifeedant activity.[13,14] The stem extracts showed larval mortality[15] and insecticidal activity. The plant has also showed antifungal[16], antibacterial [17],cytotoxic [18], antimalarial [19], anthelmintic [20], antilithic [21] and antifertility activity.[22] The present study was designed to for simultaneous determination of Rutin, Quercetin and Kaempferol in ethanolic leaves extract of *Melia azedarach* by high-performance liquid chromatography.

MATERIALS AND METHOD

Reagents and Materials:

All chemicals and solvents used were of analytical grade. The standard Rutin and Quercetin were purchased from Yucca Enterprises,Mumbl (purity >97%).The standard Kaempferol MP Biomedicals, Mumb(purity >97%). Phosphate buffer (pH=5.8) and solvent Acetonitrile used as mobile phase were obtained from S.D.Fine Chem Limited, Mumbai. The column type was, Athena C18 250X 4.6 (CNW Technology).

Plant material:

The basic plant material of *Melia azedarach* leaves was obtain from Zaheerabab,Medak Dist. The plant was identified and authenticated by Department of Botany and Research office (Botanist) Anwar-ul-loom college of Pharmacy, Hyderabad.

Extraction of plant material for HPTLC analysis:

The leaves of *Melia azedarach* were dried under shade and powdered in a mechanical grinder. The leaves powders of *Melia azedarach*, weight about (250 g) were individually packed in the thimble of soxhlet apparatus and extracted with ethanol at 55°C for 18 h. The extract was concentrated to get dry residue and stored in the dessicator and it was used for subsequent experiments. Preliminary photochemical screening revealed the presence of Polyphenols, flavanoids and glycosides.

Preparation of standard and sample solutions

Phosphate buffer (pH=5.8) and solvent Acetonitrile used as mobile phase.10 mg of Standard Rutin and Quercetin were dissolved in 25ml of mobile phase, while 15mg of kaempferol were dissolved in 25ml of mobile phase and 10 mg of Sample solution of extract *Melia azedarach* were dissolved in 25ml of mobile phase as above as standard preparation.

Chromatographic conditions:

Flow rate	: 1 ml/min
Detection	: 254 nm
Injection quantity	: 0.02ml
Column used	: Athena C18 250X 4.6
Column temperature	: 35°C

Volume : 3 | Issue : 11 | Nov 2013 | ISSN - 2249-555X

Mobile phase ration : 55: 45 % v/v
Mobile phase : Phosphate buffer (pH=5.8) and Acetonitrile

The operating temperature was maintained at room temperature. Identification of the compounds was achieved by comparison with retention times of standards with the samples.

Assay formula

$$\frac{\text{Sample Area}}{\text{Standard Area}} \times \frac{\text{Standard weight}}{\text{Standard dilution}} \times \frac{\text{Sample dilution}}{\text{Sample weight}} \times \text{Standard Purity}$$

RESULTS AND DISCUSSION

Quantification of Rutin, Quercetin and Kaempferol in Melia azedarach:

The retention time (Rt) of standards Rutin, Quercetin and Kaempferol were found to be 2.357, 6.093 and 9.373 with 100% area(Fig:). While the retention time (Rt) of Rutin, Quercetin and Kaempferol in *Melia azedarach* extract, was found to be 2.403, 6.143 and 8.903 respectively (Fig: 5.10) which are matching with standards Rt values respectively. The amount of rutin, quercetin and kaempferol in ethanolic leaves extract of *Melia azedarach* was found to be 10.4%, 0.07% and 0.11 % w/v respectively.The mobile phase include acetonitrile and Phosphate buffer (pH=5.8) were tested and the results showed the good resolution and good peaks shape.

Fig: 1 HPLC Chromatogram of standard Rutin:

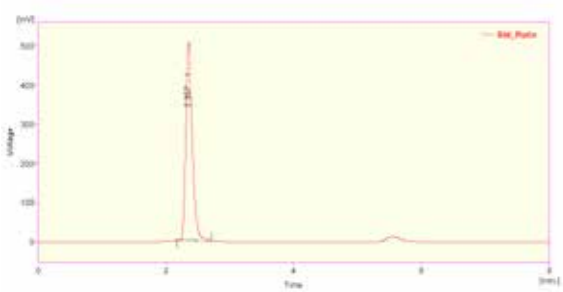

Fig: 2 HPLC Chromatogram of standard Quercetin:

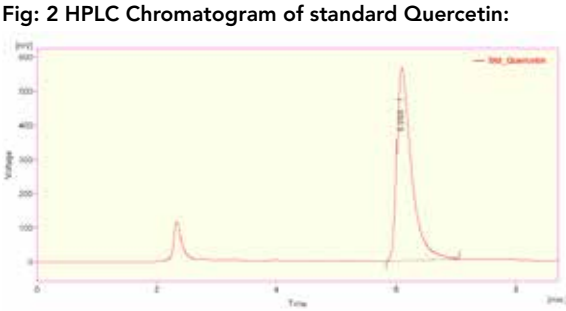

Fig: 3 HPLC Chromatogram of standard Kaempferol:

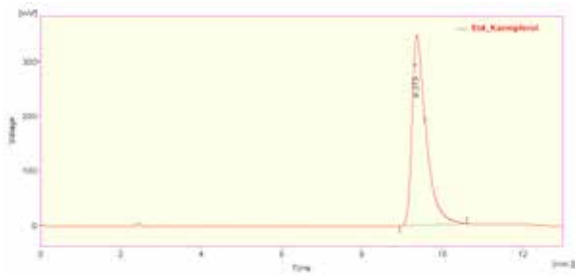

Fig: 4 HPLC Chromatogram of extract Melia azedarach(M1):

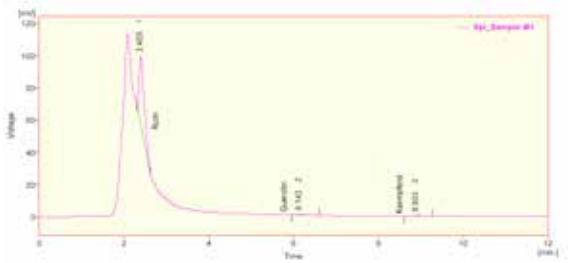

Table: 1 Retention time, Height and % Area of Standards Rutin, Quercetin and Kaempferol:

Standards	Retention time(min)	Area(mV.s)	Height(mV)	Area (%)
Rutin,	2.357	3700.301	505.494	100
Quercetin	6.093	9594.659	564.435	100
Kaempferol	9.373	8468.410	348.791	100

Table: 2 Retention time, Height and % Area of Rutin, Quercetin and Kaempferol in extract Melia azedarach:

Standards	Retention time(min)	Area(mV.s)	Height(mV)	Area (%)
Rutin,	2.403	358.182	42.504	96.64
Quercetin	6.143	6.335	0.386	1.71
Kaempferol	8.903	6.101	0.336	1.65
Total		370.619	43.227	100

CONCLUSION

The HPLC in-house analytical methods were developed, which was found to be excellent technique for simultaneous determination of rutin,Quercetin and Kaempferol in ethanolic leaves extract of *Melia azedarach* . The cost and Running time per analysis are found to be relatively low in comparison with other methods. Hence this method can be apply for the Quantitative analysis of rutin, Quercetin and Kaempferol. Furthermore, the method can be used as quality control for phenoloic compounds and was found to be efficient, simple and rapid.

Volume : 3 | Issue : 11 | Nov 2013 | ISSN - 2249-555X

REFERENCE

1. Lu F, Foo LY (1995). Toxicological aspects of food antioxidants. In:Madhavi DL, Deshpande SS, Salunkhe DK, editors. Food antioxidants. New York: Marcel Dekker. | | 2. Gülçin I, Oktay M, Kıreçci E, Küfrevio lu I (2003). Screening of antioxidant and antimicrobial activities of anise (Pimpella anisum L.) seed extracts. Food. Chem. 83: 371-382. | | 3. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB (1995).The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free. Radical. Res. 23: 375-383. | | 4. Ito N, Fukushima S, Hasegawa A, Shibata M, Ogiso T. Carcinogenicity of butalated hydroxyanisole in F344 rats. Journal of National Cancer Institute 1983;70:343-47. | | 5. The European Agency for the evaluation of Medicinal products, Committee for veterinary medicinal products "Ruta graveolens", EMEA-542, March, 1999. | | 6. Toker G, Turkoz S, Erdemogly N; High performance liquid chromatographic analysis of rutin in some Turkish plants II. J Chem Soc Pak. 1998; 20(4):240-243 | | 7. Spencer et al., Flavonoids: Modulators of brain function. British Journal of Nutrition. 2008; 99: 60–77. | | 8. http://www.jci.org/cgi/content/full/107/2/135?ijkey=a1e09ce2dbca283cec170598f240b15d5f4304f&keytype2=tf_ipsecsha | 9. Yoshida, T.; Konishi, M.; Horinaka, M.; Yasuda, T.; Goda, A. E.; Taniguchi, H.; Yano, K.; Wakada, M.; Sakai, T. Kaempferol sensitizes colon cancer cells to TRAIL-induced apoptosis. Biochem. Biophys. Res. Commun. 2008, 375, 129-133. | 10. Kowalski, J.; Samojedny, A.; Paul, M.; Pietsz, G.; Wilczok, T. Effect of kaempferol on the production and gene expression of monocyte chemoattractant protein-1 inJ774.2 macrophages. Pharmacol. Rep. 2005, 57, 107-112. | 11. Koul O, Isman MB, Ketkar CM. Properties and uses of neem, Azadirachta indica. Can J Bot 1990; 68: 1-11. | | 12. Chatterjee A, Pakrashi S. Treatise on Indian medicinal plants. New Delhi: Publications and Information Directorate; 1994, p. 80-82. | | 13. El-Lakwah FA, Mohamed R, Darwish AA. Evaluation of toxic effect of chinaberry. Annals Agri Sci 1995; 33: 389-398. | | 14. Gebre amkak A, Azerefegne F. Insecticidal activity of chinaberry, endod and pepper tree agains the maize stalk borer (Lepidoptera:Noctuidae) in southern Ethiopia. International J Pest Management 1999; 45: 9-13. | | 15. Valladares GR, Ferreyra D, Defago MT, Carpinella MC, Palacios S. Effects of Melia azedarach on Triatoma infestans. Fitoterapia 1999; 70: 421-424. | | 16. Carpinella MC, Herrero GG, Alonso RA, Palacios SM. Antifungal activity of Melia azedarach fruit extract. Fitoterapia 1999; 70: 296-298. | | 17. Sallem R, Ahmed SI, Shamim SM, Faizi S, Siddiqui BS.Antibacterial effect of Melia azedarach flowers on rabbits.Phytotherapy Res 2002; 16: 762-764. | | 18. Zhou H, Hamazaki A, Fontana JD, Takahashi H, Esumi T,Andcheer CB, et al. New ring C-Seco limonoids from Brazilian Melia azedarach and their cytotoxicity. J Nat Prod 2004; 67:1544-1547. | | 19. Ofulla AVO, Chege GMM, Rukunga GM, Kiarie FK, Githure J, Kofi TMW. In vitro antimalarial activity of extracts of Albizia gummifera, Aspilia mossambicensis, Melia azedarach and azadirachta indica against Plasmodium falciparum. Afr J Health Sci 1995; 2: 309-311. | | 20. Pervez K, Ashraf M, Hanjra AH. Anthelmintic efficacy of Melia azedarach against gastrointestinal nematodes in sheep. Applied Parasitol 1994; 35:135-137. | | 21. Christina AJM, Haja Najumadeen NA, Vimal kumar S, Manikandan N, Tobin GC, Venkataraman S, et al. Antilithiatic effect of Melia azedarach on ethylene glycol induced nephrolithiasis in rats. Pharm Biol 2006; 44: 480-485. | | 22. Keshri G, Lakshmi V, Singh MM. Pregnancy interceptive activity of Melia azedarach Linn. In adult female Sprague-Dawley rats.Contaception 2003; 68: 303-306. |