Research Article

# Determination of Phenolic Compounds in flowers of *Michelia Champaca* L. by HPLC Analysis

**T. Ananthi*[1], R. Anuradha[2]**

[*1]Assistant Professor, PG and Research Department of Biochemistry, S.T.E.T Women's College, Mannargudi, Tamil Nadu, India.
[2]Head, PG and Research Department of Biochemistry, S.T.E.T Women's College, Mannargudi, Tamil Nadu, India.
***Corresponding author's E-mail:** elangani576@gmail.com

**Accepted on:** 19-06-2015; **Finalized on:** 31-07-2015.

**ABSTRACT**

In the present study, High Performance Liquid Chromatography has been used for detection and quantification of flavonoids and phenolic compounds in methanolic flower extract of *Michelia champaca*. The quantitative determination was conducted by HPLC equipped with ultraviolet detector. Optimal separation was achieved by isocratic conditions elution with solvent A (water –acetic acid) and solvent B (methanol). The standard markers (Gallic acid, caffeic acid, ferulic acid, rutin and quercetin) were identified by retention time and co-injected with reference standard and quantified by external standard method at 280 nm. Retention time and peaks were used as parameters to determine the presence of specific compounds. Distinct peaks and retention time were recorded, and on that basis Caffeic acid (Rt=9.300), Ferulic acid (Rt=24.283), Gallic acid (Rt=5.58), Quercetin (Rt=12.017) and Rutin (Rt=10.800). The data provided the basis for its wide uses of the therapeutic effects of this plant.

**Keywords:** *Michelia champaca*, retention time, flavonoids

## INTRODUCTION

High-performance liquid chromatography (HPLC) has been the most widely employed chromatographic technique in flavonoid analysis during the past 20 years[1-5]. It has added a new dimension to the investigation of flavonoids in food and plant extracts. The separations are far more rapid than classical methods and provide high resolution and sensitivity[6]. HPLC of flavonoids is a widely used methodology and easily adapted to the quantitation of individual compounds. It has the advantage of generating a chemical fingerprint, which can be used in defining the identity and quality of a given sample.

*Michelia champaca* L. (Magnoliaceae) commonly known as Svarna champa, a tall handsome tree with yellow fragrant blossoms, is commonly used by many traditional herbal preparations. The plant is also reported to have significant wound healing,[7] antimicrobial,[8] antidiabetic,[9] antitumor[10], anti-inflammatory,[11] antioxidant,[12] and antiinfective[13] properties. Therefore, the aim of the present study was to develop a simple, routine, reproducible, and accurate HPLC method for the determination of flavonoids in methanolic flower extract.

## MATERIALS AND METHODS

### Collection of Plant material

The *Michelia champaca* flowers were procured from the local areas of Udumalaipettai, Coimbatore District, Tamilnadu. The collected plant material was botanically identified and confirmed by Dr. S. John Britto, The Director, Rapinat Herbarium, St. Joseph's College, Tiruchirappalli. The herbarium specimens were preserved for further reference (Voucher No. 001). The flowers were chopped into small pieces, shade-dried and coarsely powdered by using a pulverizor.

### Preparation of Extract

2 g flowers of *Michelia champaca* suspended in 50 ml of 80% methanol was extracted at 80 KHz using an ultrasonic device for 30 min (twice) at 45 °C. The resulting extract was collected, filtered and dried at 50 °C under reduced pressure. The dried crude extract was dissolved in the 100 ml mobile phase, filtered through 0.45 mm membrane filter (Millipore) and the extract was injected into HPLC.

### Preparation of Standards for HPLC

Standard stock solutions of gallic acid, ferulic acid, caffeic acid, rutin and quercetin were prepared in methanol at concentrations of 2, 4, 6, 8 and 10 µg/ml and filtered through HPLC filter 0.45 mm membrane filter (Millipore).

### Analysis of flavonoids by HPLC

The flower extract was analyzed for flavonoids using a HPLC method, Shimadzu Corp., Kyoto, consisting of a LC-10ATVp pump, SCL 10A system controller and a variable Shimadzu SPD- 10ATVp UV VIS detector and a loop injector with a loop size of 20 µl was used. The peak area was calculated with CLASSVP software. Reverse phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250×4.6 mm i.d., particle size 5 µm, Luna 5 µ C-18; phenomenex, Torrance, CA, USA) at 25 °C. The gradient elution of solvent A (water-acetic acid; 25:1 v/v) and solvent B (methanol) had a significant effect on the resolution of compounds. Detection wavelength was 280 nm. Gallic acid, caffeic acid, ferulic acid, rutin and quercetin were used as internal and external standards. Phenolic acids

present in each sample were identified by comparing chromatographic peaks with the retention time (Rt) of individual standards. The amount of each phenolic acid is expressed as µg/g.

## RESULTS AND DISCUSSION

HPLC method is one of the most fast and reliable method for identification of plant phenolics. The chromatographic separations of Gallic acid (Rt - 5.750), Caffeic acid (Rt - 9.450), Rutin (Rt - 10.517), Quercetin (Rt - 12.400) and Ferulic acid (Rt - 24.175) standard shown in Figure 1. The content of each flavonoid was calculated from the corresponding calibration curve and presented as the mean of five determinations as shown in Table 1.

The HPLC Result showed based on the Retention time (Rt), Gallic acid (Rt - 5.558), Caffeic acid (Rt - 9.300), Rutin (Rt - 10.800), Quercetin (Rt - 12.017) and Ferulic acid (Rt - 24.283) content in *Michelia champaca* flowers was found to be 0.001µg/gm, 0.1 µg/gm, 1.1 µg/gm, 0.2 µg/gm, and 0.2 µg/gm. The obtained value was compared with standard (Figure and Table.2).

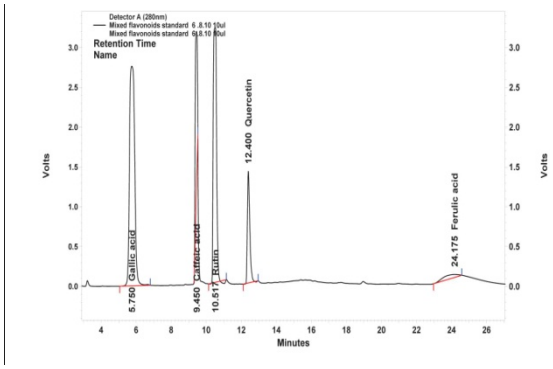


**Figure 1:** HPLC chromatogram of flavonoids standard

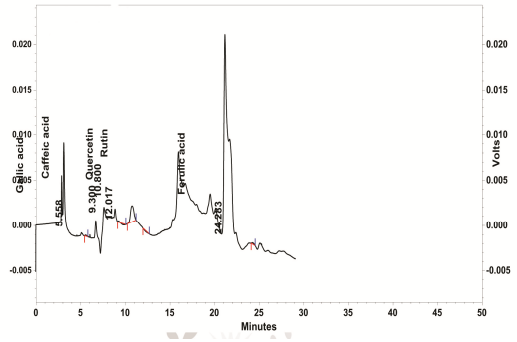


**Figure 2:** HPLC Chromatogram of *Michelia Champaca* L Flowers

**Table 1:** Retention time and area of five kinds of standard flavonoids

| Detector A (280nm) | | | | | |
|---|---|---|---|---|---|
| **Retention Time** | **Area** | **Height** | **Concentration** | **Units** | **Name** |
| 5.750 | 56744802 | 2757981 | 10.000 | µg/ml | Gallic acid |
| 9.450 | 17443471 | 1882880 | 10.000 | µg/ml | Caffeic Acid |
| 10.517 | 42056735 | 3198304 | 10.000 | µg/ml | Rutin |
| 12.400 | 13396467 | 1402866 | 10.000 | µg/ml | Quercetin |
| 24.175 | 2810655 | 36358 | 10.000 | µg/ml | Ferulic acid |

**Table 2:** HPLC Validation data for *Michelia champaca* flowers

| Retention Time | Area | Height | Concentration (ug/ml) | Name* |
|---|---|---|---|---|
| 5.558 | 1411 | 103 | Below Detection Limit | Gallic acid |
| 9.300 | 1435 | 150 | 0.1 | Caffeic acid |
| 10.800 | 46653 | 1768 | 1.1 | Rutin |
| 12.017 | 2876 | 0 | 0.2 | Quercetin |
| 24.283 | 2588 | 131 | 0.2 | Ferulic acid |

Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these compounds were found to be present in the extracts, it might be responsible for the potent antioxidant capacity of pomegranate. These secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value. For example saponins have hypotensive and cardiodepressant[14].

The work supported by the HPLC analysis of methanolic extract of *Zanthoxylum armatum* fruits. The HPLC chromatograph will help as standard chromatogram in future studies, comparing the retention time of isolated compounds with given literatures. The good separation of the peaks which could be identified in the chromatogram, as flavonoid (Rt=2.8) for compound I at λ max 277 nm and anthraquinone (Rt=2.2) for compound II at λmax 273 nm[15].

## CONCLUSION

Analysis of flowers of *Michelia champaca* for determination of flavonoids was carried out with the help of HPLC. From the results it is evident that the extract

contained an especially high concentration of Rutin (1.1µg/ml) followed by Quercetin (0.2 µg/ml), ferulic acid (0.2µg/ml) and Caffeic acid (0.1 µg/ml) were also detected. The presence of polyphenols provide the basis for its wide uses of the therapeutic potential of *Michelia champaca*.

## REFERENCES


1. Wulf LW and Nagel CW. Analysis of phenolic acids and flavonoids by high-pressure liquid chromatography. J. Chromatogr. 116(2), 1976, 271–79 .
2. Hertog MGL and Hollman PCH. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. J. Agric. Food Chem. 40, 1992, 1591–98.
3. Das. DK. Naturally occurring flavonoids: structure, chemistry and high performance liquid chromatographic methods for separation and characterization. Meth. Enzymol. 234, 1994, 410–20.
4. Robards K and Antolovich M. Analytical chemistry of fruit bioflavonoids: a review. Analysis, 122, 1997, 11–34.
5. Merken HM and Beecher GH. Measurement of food flavonoids by high performance liquid chromatography. A review. J. Agric. Food Chem. 48, 2000, 577–99.
6. Lamuela-Raventos RM and Waterhouse AL. A direct HPLC separation of wine phenolics. Am. J. Enol. Vitic. 45, 1994, 1–5.
7. Dwajani S and Shanbhag TV. *Michelia champaca*: Wound Healing Activity in Immuno suppressed Rats. The Internet Journal of Alternative Medicine. 7(2), 2009, 1540-1545.
8. Khan MR, Kihara M, Omoloso AD. Antimicrobial activity of *Michelia champaca.* Fitoterapia. 73, 2002, 744-48.
9. Jarald EE, Joshi SB, Jain DC. Antidiabetic activity of flower buds of *Michelia champaca* Linn. Indian Journal of Pharmacology. 40(6), 2008, 256-60.
10. Hoffmann JJ, Torrance SJ, Wiedhopf RM, Cole JR. Cytotoxic Agents from Michelia champaca and Talauma ovata: Parthenolide and Costunolide. J Pharm Sci. 66, 1977, 883-84.
11. Vimala R, Nagarajan S, Alam M, Susan T, Joy S. Antiinflammatory and antipyretic activity of Michelia champaca Linn. (White variety), Ixora brachiata Roxb. and Rhynchosia cana (Wild.) D.C. flower extract. Indian Journal of Experimental Biology. 35(12), 1997, 1310-14.
12. Hasan SMR, Hossain MM, Akter R, Jamila M. DPPH free radical scavenging activity of some Bangladeshi medicinal plants. Journal of Medicinal Plants Research. 3(11), 2009, 875-79.
13. Oumadevi R, Guy R, Francisco ER, Kiban C. Screening for anti-infective properties of several medicinal plants of the Mauritians flora. J Ethnopharmacol, 109(2), 2007, 331-37.
14. Olaleye MT, Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa.* Journal of Medicinal Plants Research. 1, 2007, 9–13.
15. Dinesh K Metha, Rina Das and Anil Bhandari. Phytochemical Screening and Hplc Analysis of Flavonoid And Anthraquinone Glycoside In Zanthoxylum Armatum Fruit. International Journal of Pharmacy and Pharmaceutical Sciences. 5(3), 2013, 190-193.


**Source of Support:** Nil, **Conflict of Interest:** None.