Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 2, 2012

Research Article

HPLC ANALYSIS OF VITEXIN AND FINGERPRINT OF *VITEX NEGUNDO* L.

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Received: 30 Aug 2011, Revised and Accepted: 29 Sep 2011

ABSTRACT

High Performance Liquid chromatography was done for quantification of vitexin and for examining the fingerprint of *Vitex negundo* methanol leaf extract. The analysis was carried out using Lichrosorb C-18 column at 25°C, with methanol: water as mobile phase. The flow rate was 1.0 ml/min and the detection was at 210 nm. The amount of vitexin present in *V. negundo* collected from five different locations of Western Ghats ranged from 22.91 to 83.69 µg/ml. Although the amount of the marker flavonoid (vitexin) was different, HPLC fingerprints of all samples were well conserved. The highest amount of vitexin was found in Karaiyar accession and was considered as superior genotype. The compiled data will be useful for taxonomy, to improve the quality control and authenticity testing of phytopharmaceuticals containing *V. negundo* extracts.

Keywords: Fingerprint, High Performance Liquid Chromatography, *Vitex negundo,* Vitexin.

INTRODUCTION

Plants have limitless ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10 % of the total and their contents vary depending on climate, regions of cultivation and seasons of harvest which make it difficult to ensure batch-to batch uniformity. The quality control of standardized herb extracts is essential for the therapeutic reproducibility, efficacy and safe application of extract[1]. Conventional quality control mainly focuses on the analysis of the active constituents of herbal medicines[2].

Recently the chromatographic fingerprint technique was introduced as a tool to evaluate the quality of herbal samples or their derived products[3,4,5]. A quick, sensitive and accurate analytical method is required for the analysis of a large number of plants samples. In the past decade, the chromatographic fingerprints established by TLC, HPLC, HPTLC, GC and CE have been recognized as rapid and reliable means for the identification and qualification of herbal medicines[6,7].

HPLC method is gaining importance for qualitative and quantitative analysis of plant extracts, being useful for quality control of phytochemical compounds[8]. This method is able to quantify the marker compounds in plants. The identity, consistency and authenticity of samples can be determined by comparison of their chromatographic fingerprints (Chromatogram) using similarity analysis and chemometric methods[9]. This method enables the simultaneous identification of the major bioactive constituents present in medicinal herb[10].

Vitex negundo L. (Verbenaceae), a large, aromatic shrub or sometimes a small slender tree, upto 4.5 m in height found throughout the greater part of India. The whole plant is used in anticancer, inflammations, antiseptic, antipyretic, diuretic, antihistamine, antioxidant, antibacterial, CNS depressant, antifungal, snake venom neutralization, mosquito repellant activity, insecticidal, larvicidal efficacy, antinociceptive, antiandrogenic, hepatoprotective, antifertility, skin aging inhibitor and anti dopaminergic effects[11]. Constituents previously isolated from the plant include eight lignans, flavonones, non diterpene, pentacyclic triterpenoids and flavonoid glycoside[12]. Vitexin (Flavonoid) has potent and broad antitumor efficacy in preclinical models of ectopic growth of breast, prostate, liver and cervical cancer cells[13].

The objective of this study was to develop a HPLC procedure for the analysis of vitexin and for the fingerprint of leaf extracts of *Vitex negundo* plant collected from five different locations of Western Ghats of India.

MATERIALS AND METHODS

Herbal Materials

The wild, fully grown *Vitex negundo* leaves were randomly collected from five different locations of Western Ghats of India such as Kodhayar, Karaiyar, Maruthamalai hills, Billigirirangan hills and Peringalkunthu. The plants were identified and checked with the Herbarium of Botanical Survey of India (BSI), Coimbatore. Then the collected specimen was dried and mounted on Herbarium sheet and deposited in St. Xavier's College Herbarium (XCH), Palayamkottai, Tamilnadu, India. The voucher number of the deposited specimen is XCH: 28075 - *V. negundo*.

Preparation of standard

Standard stock solutions (500 µg/ml) were prepared by dissolving 50 µg vitexin in 5 ml of warm methanol and was made up to 100 ml with distilled water and sonicated for 20 minutes. Standard solution was prepared by diluting the stock solution with 50 % methanol to obtain the desired concentration.

Preparation of sample solutions

The leaves were shade dried for a week and powdered using mixer grinder. 25 gms of the leaf powder from each location was continuously extracted with 100 ml of methanol for 18 hrs at 60°C using Soxhlet apparatus. The methanol extract of each sample was used for HPLC analysis. All extracts were filtered through a Whatman no.1 filter paper. 30 ml filtered extract was evaporated by a rotatory vaccum evaporator. The evaporated residues with constant weight were stored prior to analysis in dark at 4°C. 200 mg of extract was dissolved with 4 ml methanol, sonicated at 35 °C for 15 minutes and filtered through 0.45 µm filter and applied (50 µl) on to HPLC column.

HPLC conditions

The HPLC analysis was carried out on a Shimadzu, LC-10 AT VP, consisting of SCL-10Avp system controller, degassing unit DGU-14A, low-pressure gradient flow control valve FCV-10ADvp, auto injector SIL-10ADvp with 500 µl loop, column oven CTO-10AC, a UV detector SPD-10Avp using a 254 (5µm). The temperature was maintained at 25ºC with injection volume of 200 µl and flow rate of 1ml/min. Active compound was separated using reverse-phase LiChrosorb C-18 column with the methanol:water as mobile phase and detected at 210 nm. The chromatography system was equilibrated by the mobile phase.

Validation parameters

The calibration curve for the vitexin was determined by the external standard methods. Three determinations were carried out for each solution. The calibration curves were obtained by plotting the peak of the vitexin versus the concentrations of the standard solutions. The intermediate precision was determined and it was expressed as relative standard deviation (R.S.D. %) of the concentration of Vitexin. In all samples the peaks of interest were found to be pure and free of eluting compounds. Calibration data was calculated from peak area and height with linear equation and correlation coefficient

(r^2). The peak area was used for quantification of vitexin in *Vitex negundo* leaf extracts.

RESULTS AND DISCUSSION

The method developed was applied for the identification of appropriate markers for *V. negundo* leaves collected from five locations of Western Ghats. Vitexin was selected as a potential marker (Fig 1), since it was present in all the samples and was well resolved from the other peaks. Vitexin peaks were identified in the extracts by comparing the retention time with that obtained from the standard solution, by spiking the standard solution into the sample solution and by a comparison of its UV spectra with that of the standard (Fig 2). The retention time of vitexin peak in the extract of five populations varied from 5.10 to 5.39 (% RSD = 0.18 %), compared to 5. 20 min in case of standard vitexin (Fig 3). The retention times were due to the complex matrices in different extracts.

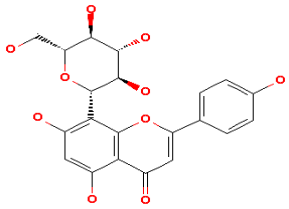

Fig. 1: Structure of Vitexin compound

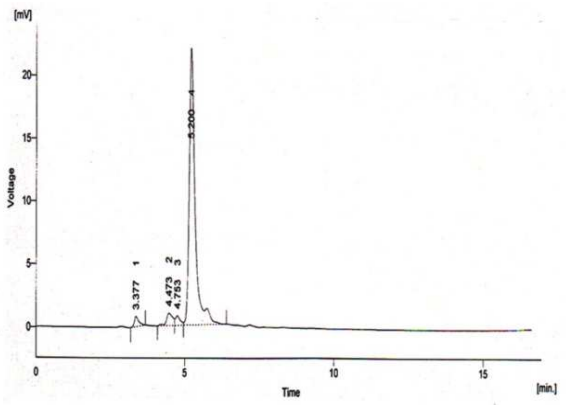

Fig. 2: Chromatogram of vitexin standard

Results showed that the *V. negundo* leaf extracts contained various amount of vitexin (22.91 to 83.69 µg/ml) as one of the constituents (Table 2). The highest amount of vitexin was found in the leaf extract from N_2, while the least amount was from N5. Different vitexin contents from each source may be due to the varied climate conditions. Excellent linearity was observed for vitexin between peak area as confirmed by the correlation coefficient of 0.036 (Table 1). Upon examining the relative intensity with the base peak, which was always the most peak in methanol extracts of *V. negundo* from five accessions (Fig 4).

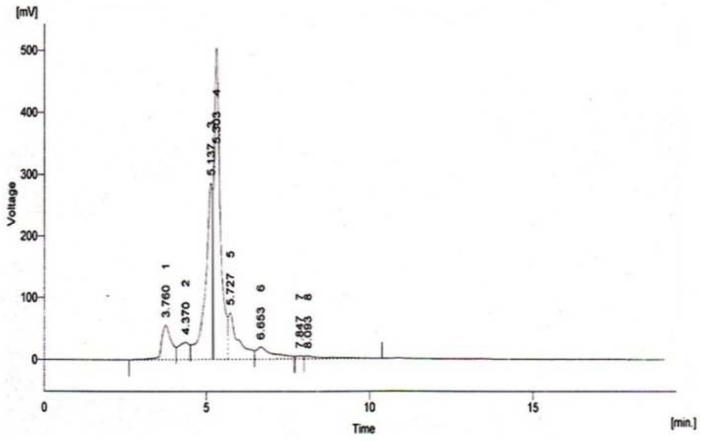

Fig. 3: A chromatogram of showing separation of vitexin in *V. negundo* from karaiyar accession

Table 1: Calibration data of vitexin in *V. negundo* (*n*=3)

Sample	Peak area Mean±SD	% of area	% RSD	Peak height Mean±SD	% of area	% RSD
tandard	298.728±1.0	90.5	0.33	21.988±1.0	89.6	4.54
N_1	21911.836±6.1	81.5	0.27	999.082±6.1	86.7	0.61
N_2	6846.711±1.0	44.4	0.14	504.062±1.0	51.6	0.19
N_3	10973.534±6.1	78.6	0.55	378.184±1.5	76.8	0.39
N_4	10235.723±1.0	75.6	0.97	340.875±1.0	75.3	0.29
N_5	25000.744±1.0	77.1	0.39	998.962±1.0	78.6	0.10
Linear equation	Y= 956.6x+12124			Y= -16.34x+693.2		
Correlation coefficient (r^2)	0.036			0.006		

N_1-Kodhayar, N_2-Karaiyar, N_3-Maruthamalai hills, N_4-Billigirirangan hills, N_5-Peringalkunthu, RSD- Relative Standard Deviation

Table 2: Assay of vitexin in V. negundo leaf extracts (*n*=3)

Sample	Vitexin (µg/ml)	% RSD
N_1	73.35045	1.36
N_2	83.690662	1.19
N_3	36.7342	2.72
N_4	34.264358	2.91
N_5	22.91959	4.36

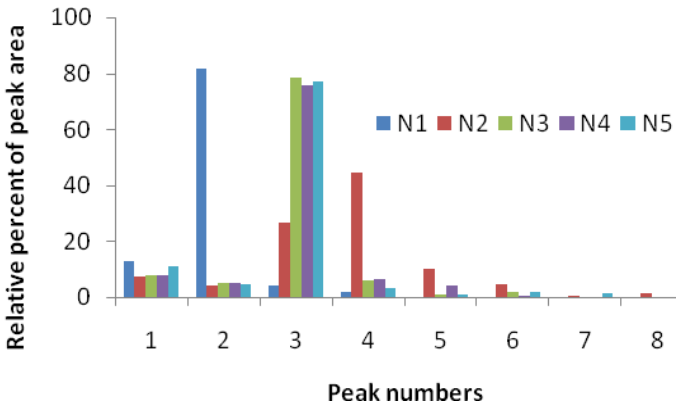

Fig. 4: Prevalent of characteristic components in the analyzed *V. negundo*

The optimized HPLC condition was utilized to reveal the fingerprint of *V. negundo*. The analyzed samples were from wild plants and were grouped according to the geographical locations of the collected samples. The chromatogram profiles of the extracts were complex and contained at least four components. All populations showed distinction in number of compounds and difference in the concentration of the marker compound, vitexin, which was present in all profiles. In addition to the marker compound, many other peaks could be detected using the optimized HPLC condition. The fingerprint pattern of *V. negundo* extracts and the appropriate marker, a relative percentage of peak area of each component was calculated based on the base peak.

Upon examining the relative intensity with the base peak, which was always the most abundant peak in every sample. The data suggested that this pattern evaluation is suitable for natural product extract, whose components may vary with environmental factors. Although the characteristic components were present at different population levels, the fingerprint characteristics were highly conserved. Importantly vitexin can be isolated and identified from the *V. negundo* with low % RSDs, suggesting that vitexin is an appropriate marker for the leaf extract of the plant. Therefore, established HPLC method can be used to validate the quality and authenticity of phytopharmaceuticals containing methanol leaf extract of *V. negundo.*

The broad range of vitexin that could be determined with this method is particularly noteworthy. The developed HPLC technique for the analysis of vitexin has several advantages over existing methods. HPLC was done in methanol extract of *Passiflora foetida* collected from ten different locations in Thailand, the vitexin compound was quantified from ten samples[14].

Several factors such as worldwide changes in seasonal patterns, weather events, and temperature changes, biotic and abiotic stresses may affect the production of secondary metabolites in plants[15,16]. Campothecin compound was quantified from methanol extract of *Nothapodytes nimmoniana* collected from different locations of Western Ghats of India using HPLC, which showed that the concentration of camptothecin was affected by geographical origin and climatic conditions[17].

CONCLUSION

This study describes a rapid and efficient HPLC procedure for the analysis of vitexin in *V. negundo.* The accessions which showed high amount of vitexin are identified. N_2 accession of *V. negundo* collected from Karaiyar was considered as the superior genotypes. The flavonoid was an appropriate marker for the leaf extracts of the plant. Additional chromatographic analysis can be performed on different samples to generate unique fingerprint that provide a general overview on the patterns and trends of the extracts and this method has the advantages of simplicity, precision, rapidity and reliability, allowing its quality control of manufacturing process. The compiled data will be useful for taxonomy, to improve the quality control and authenticity testing of phytopharmaceuticals containing *V. negundo* extracts.

ACKNOWLEDGEMENT

The authors are grateful to the Indian Council of Medical Research, Government of India, New Delhi (Ref: 59/12/2006/BMS/TRM dt. 26.03.2009) for financial support.

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