Asian Journal of Chemistry; Vol. 24, No. 5 (2012), 2351-2352

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

NOTE

Determination the content of Plumbagin in *Plumbago zeylanica* L. During Different Collected Periods by RP-HPLC

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(*Received*: 18 May 2011; *Accepted*: 26 December 2011) AJC-10900

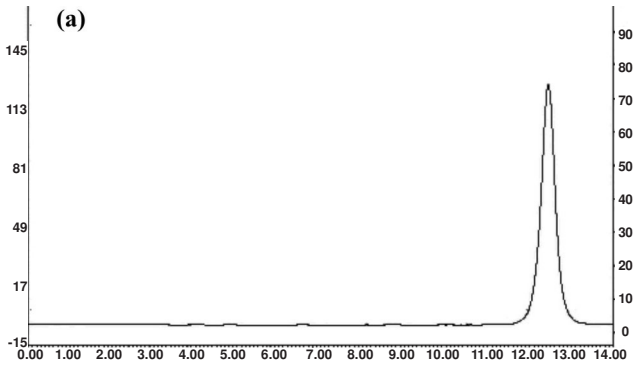
The content of plumbagin in leaves and branches of *Plumbago zeylanica* collected during different periods grown in Guangxi, China, were determined using RP-HPLC with methanol and H_2O (70:30) as mobile phase and UV detection at 254 nm. Linear range over 0.1220-1.2198 mg/mL of plumbagin was obtained. Recovery was within 98.6-102.8 %. The content in leaves and branches of *Plumbago zeylanica* is highest in October when the plant growth is most thriving and biggest in size. It is therefore considered that October is the best time for collecting leaves and branches.

Key Words: Plumbagin, Collected periods, HPLC, *Plumbago zeylanica*.

The plant of *Plumbago zeylanica* L., a semiclimbing shrub, is mainly distributed in the tropical and subtropical regions of Southeast Asia and China. All parts of the plant are considered to be a good source of a large number of bioactive substances. Previous phytochemical studies of *Plumbago zeylanica* have demonstrated that it contains many active constituents such as plumbagin, plumbagic acid, coumarins, steroids, naphthoquinones[1-3]. Pharmacological investigations have demonstrated that the antitumor, antibacterial, anti-oxidation, antiparasite, antiallergic effects as well as the antihigh blood sugar disorders of *Plumbago zeylanica* derived from plumbagin[4-6]. Therefore, plumbagin can be considered as the major active chemical constituent of *Plumbago zeylanica* and it can therefore be used as a chemical indicator for quality evaluation of the plant material to be used as medicine. The objectives of this research are to determine whether the different collected periods could result in different quality by determining the content of plumbagin in different samples. In this paper, a HPLC method using a diode array detector (DAD) has been developed for quantitative determination of plumbagin in leaves and branches of *Plumbago zeylanica*. Quantitative comparisons of the compound present during different collected periods are also reported to provide a scientific basis for rational exploitation of the plant resources.

Leaves and branches of *Plumbago zeylanica* were collected in 2009 and 2010 from Guangxi, China. The plant was authenticated by Associate Professor Ze-xiang DU of Guilin Medical University. Each sample was collected from the same plant and dried immediately in the shade. The samples were pulverized and passed through a sieve of 100 mesh before analysis. The standard of plumbagin was purchased from Sigma. Other chemicals and reagents used in these experiments were of the highest quality and only HPLC grade solvents were employed for HPLC analysis. The water was obtained in a Milli-Q water purification system.

The HPLC system was Agilent1100 series that equipped with a photodiode array detector (DAD). A Hypersil BDS-C_{18} column (250 mm × 4.6 mm, 5 μm) coupled with a C_{18} guard column (7.5 mm × 4.6 mm, 5 μm) was used at temperature of 30 ºC. The mobile phase was a mixture of methanol and H_2O (70:30) at a flow rate of 1.0 mL/min. The detection wavelength was set at 254 nm. The injection volume was 10 μL. A complete separation of plumbagin from other chemical compounds in the plant samples was successfully achieved (Fig. 1).



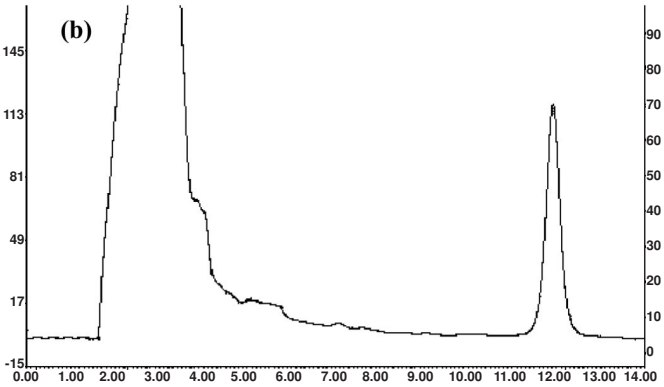


Fig. 1. HPLC Chromatograms of (a) plumbagin and (b) sample

Preparation of standard curve: 0.6099 g of plumbagin was weighed accurately into a 50 mL volumetric flask and methanol was added to volume to obtain a stock solution of the reference substance. The solution was filtered through a 0.45 μm membrane. The injection volume was 10 μL.

Sample pretreatment: Each of the fine powdered samples (10.2015 g) was weighed accurately and extracted with 80 mL methanol by a Soxhlet apparatus. Finally, the solution was filtered through a 0.45 μm membrane and the filtrate was subjected to chromatographic analysis.

Method validation: The proposed method for quantitative analysis of plumbagin was validated in terms of linearity, accuracy, repeatability and stability when compared with standard plumbagin.

Linearity was examined with a standard solution prepared in the range of 0.1220-1.2198 mg/mL of plumbagin. The linear relationship between the concentrations (mg/mL, x-axis) and peak area ratio (y-axis) was expressed by the following equation: y = 4750.4x - 9.0111. The correlation coefficient was 0.9998 and the calibration curve was a straight line.

The accuracy was confirmed by performing a recovery experiment, where one sample was spiked with known amounts of plumbagin. The recovery rates ranged from 98.6 to 102.8 % and the relative standard deviation (RSD) was 0.65 %.

Repeatability was investigated by analyzing five individual samples on the same day; the RSD of the results was 0.11 %. Stability was determined with the above same plant sample solutions as well as with the standard solution of plumbagin at time intervals of 1, 2, 3, 4, 5, 6, 7 h and the RSD of the results was 2.59 %. These data indicate that this protocol fulfills the requirements for a validated HPLC method.

Variation of plumbagin content in leaves and branches of *Plumbago zeylanica* with growth period: The content of plumbagin in leaves and branches of *Plumbago zeylanica* during different collected periods produced in Guangxi, China, was determined using the above developed HPLC method (Fig. 2). The content of plumbagin in leaves and branches of *Plumbago zeylanica* increased gradually from February and culminated in October, but decreased sharply later. The plant break dormancy and sprout in February. The sprouts then grow and develop into shoots. The content in leaves and branches of *Plumbago zeylanica* is highest in October when the plant growth is most thriving and biggest in size.

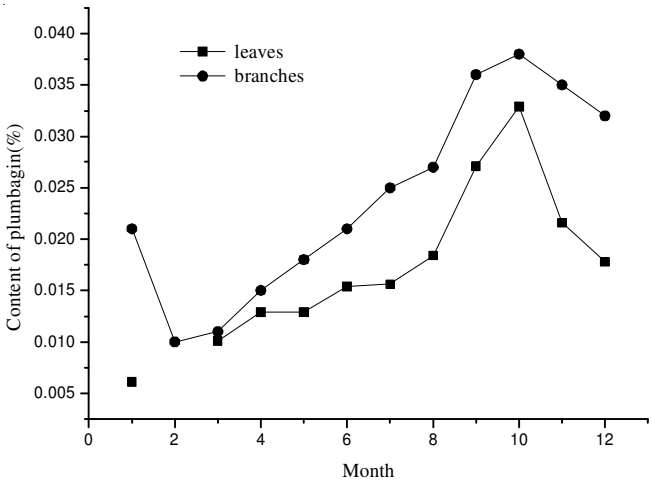


Fig. 2. Variation of plumbagin content in leaves and branches of *Plumbago zeylanica* with growth period

Conclusion

In conclusion, a convenient and reliable HPLC method using a diode array detector has been developed in our laboratory for quantitative analysis of plumbagin content in leaves and branches of *Plumbago zeylanica*. This analytical method was validated by its good linearity, accuracy, precision and stability. Utilizing this technology, we have successfully determined the content of plumbagin in 23 samples of leaves and branches of *Plumbago zeylanica*. The results showed there was a very large variation in the content of plumbagin in leaves and branches of *Plumbago zeylanica* from different collection time. This implies that the growing season has greater impact on the quality of *Plumbago zeylanica* in terms of the content of plumbagin. The content in leaves and branches of *Plumbago zeylanica* is highest in October when the plant growth is most thriving and biggest in size. It is therefore considered that October is the best time for collecting leaves and branches.

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