Quantitative and Qualitative Analysis of Plumbagin in the Leaf and Root of *Plumbago europaea* Growing Naturally in Kurdistan by HPLC

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Abstract

Plumbago (Plumbaginaceae) is a genus of 10-20 species of flowering plants used in traditional Indian medicine, native to warm temperature to tropical regions of the world. The roots of *Plumbago europaea*, the Iraqi species of *Plumbago*, have been used for the treatment of cancer, rheumatoid arthritis, and dysmenorrhea. The main active constituents from dried powdered leaves and roots of *Plumbago europaea* were extracted by Soxhlet apparatus using ethyl acetate, the main active constituent was characterized by spectroscopic analysis (IR, ^{1}H NMR, and ^{13}C NMR) as plumbagin. Quantitative and qualitative study of plumbagin in the roots and leaves extracts was carried out by HPLC technique using analytical column (Eurospher 100, C18, 5 µm, 250 x 4.6 mm) with 10% solvent (B) isocratic elution of methanol (solvent A) and water (solvent B) at flow rate 0.75 ml/min and detection wave length of 270 nm. The percentage of plumbagin in the root and leaf extracts was recorded to be (1.9 %) and (1.5 %) respectively.

Keywords: *Plumbago europaea*, plumbagin, HPLC

الخلاصة

يضم جنس *Plumbago* ١٠-٢٠ من انواع النباتات الزهرية المستخدمة في الطب الشعبى فى الهند, ينمو بصورة طبيعية في المناطق الحارة الى المناطق الاستيوائية من العالم. يستعمل جذور النوع العراقي *Plumbago europaea* في علاج السرطان ،الروماتيزم و طمث الرفث. تم استخلاص المكونات الكيميائية الرئيسية للأوراق والجذور المجففة ل *Plumbago europaea* وذلك باستخدام جهاز Soxhlet والمذيب أثيل اسيتيت. تم تشخيص المادة الكيميائية الرئيسية المعزولة باستخدام تحليل الأطياف (^{13}C NMR , ^{1}H NMR ,IR) بمادة plumbagin . تم اجراء دراسة نوعية و كمية لمادة plumbagin في المستخلصات الاوراق و الجذور باستخدام تقنية كروماتوغرافيا تحت الضغط العالي (HPLC) باستخـدام العمـود المعاكـس (Eurospher 100 C18 column , 4.6 mm i.d. x 250 mm, 5 µm) و تحت الظروف التالية: ميتانول (المذيب A) و الماء المقطر (المذيب B) كمحلول ناقل وذلك بالغسل الثابت مع ١٠ % من المذيب B و سرعة جريان ٠.٧٥ ml/min . تم الكشف بالأشعة الفوق البنفسجية بالطول الموجي ٢٧٠ nm. كانت النسب المؤية لمادة plumbagin في كل من المستخلصين الجذر والورق ١.٩ % و ١.٥ % على التوالي .

Introduction

Plumbago (Plumbaginaceae) is a genus of 10-20 species of flowering plants, native to warm temperature to tropical regions of the world [(1)]. Roots of *Plumbago* species are used in traditional Indian medicine, immunosuppressive and antitumour activities have been demonstrated [(2)]. *Plumbago europaea* have been used extensively in China and other Asian countries for treatment of cancer, rheumatoid arthritis, dysmenorrheal [(3)]. The chemical profile of the *Plumbago* genus is marked by the presence of naphthoquinones, flavonoids and terpenoids [(4)]. *Plumbago europaea* is naturally occurring plant in Kurdistan region (Kurdish name: rashky kalak) traditionally used for wart skin infection, hence the aim of this research works directed towards qualitative and quantitative analysis of the main active constituents in the leaf and root of the *Plumbago europaea* by HPLC.

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Materials and methods

Plant materials

Plumbago europaea leaves and Roots were collected from Susae village Kurdistan region in Iraq during June 2007, authenticated by the department of biology, college of Education, university of Salahaddin.

Extraction of the active constituents

Plumbago europaea leaves and roots were collected, dried in air for seven days and powdered separately with mechanical grinder. 25 gm of dried powdered leaves and roots were extracted separately in the Soxhlet with 400 ml ethylacetate for 5 hr. The extracts were evaporated in vacu by rotary evaporator to yield 2.134 gm of dark green color residue of total leaf extract (P1) and 1.1gm of orange yellow color residue of total root extract (P2).

Quantitative separation of the major active constituents by TLC

The major constituent in P1 and P2 extracts was isolated quantitatively using preparative TLC. Fifteen gm of silica gel GF254 was mixed with 30 ml distilled water to prepare the slurry which spread on one glass plate of (20 x 20) cm by DESAGA spreader to obtain 0.75 mm thickness layer of silica gel sorbent [5]. The plates were activated for 1 hr in oven at 110 ºC before use. The mobile phase used was (toluene: ethyl acetate (93:7)).

Development of TLC plates

Four hundred mg of P1 and P2 extracts were dissolved separately in 10 ml of ethyl acetate and applied as a line by a capillary tube on silica gel plate (25 plates) fore each extract. One major band which was detected visually and under UV 366 in the plates of P1 and P2 extracts, scraped off from the plates and silica gel containing the isolated major band was dissolved separately in chloroform: ethanol (3:1). The mixtures were filtered through Buckner funnel and the filtrates obtained from P1 and P2 extracts were evaporated to dryness in vacue to get constituent (A). The main active constituent (A) was characterized by spectroscopic analysis (IR, ^{1}H NMR, and ^{13}C NMR).

HPLC analysis

HPLC technique was used for qualitative and quantitative study of plumbagin in P1 and P2 extracts obtained from *Plumbago europaea* using analytical column (Eurospher 100, C18, 5 µm, 250 x 4.6 mm), mobile phase used was methanol: water (90:10), flow rate of 0.75 ml/min, injection Volume of 20 µL, detection wave length was set to 270 nm, and temperature adjusted to 33 ºC.

Sample preparation used in HPLC analysis

The sample extracts from *Plumbago europaea* were prepared by extraction of 1 g of dried powdered leaf and root separately with 35 ml ethylacetate for two hours by refluxing two times, the extracts were filtered, and evaporated to dryness by rotary evaporator, and dissolved separately in 5 ml methanol, filtered through 0.45 µm membranes before injection to the HPLC. The Knauer HPLC instrument equipped with ChromGate software provided by Knauer was used for this analysis. The calibration curve was plotted using single level calibration, made by preparation of solution (1mg/ml) of standard plumbagin (Sigma Aldrich, USA) in methanol. The calibration graph was obtained from Chromo Gate software, figure (1).

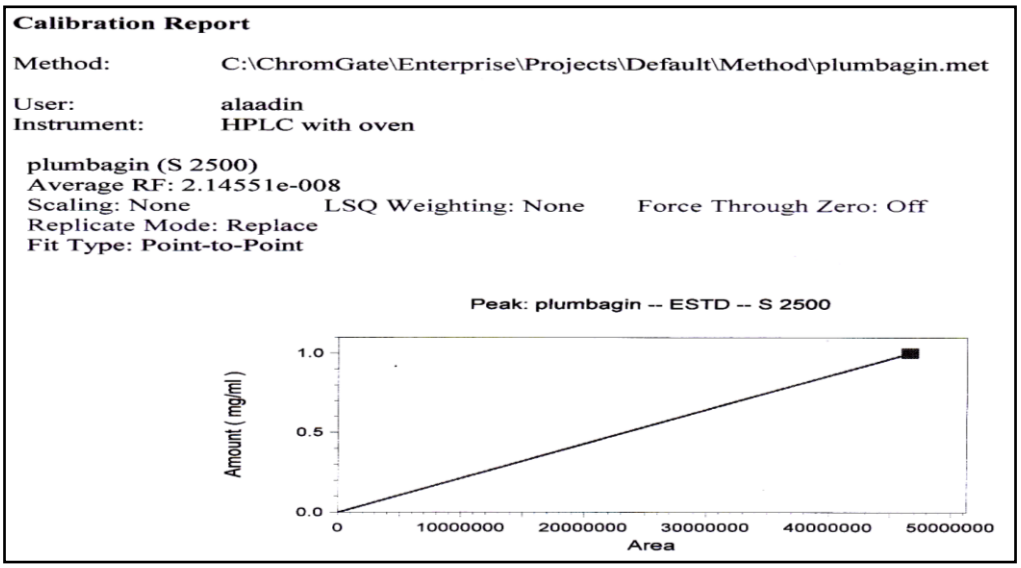
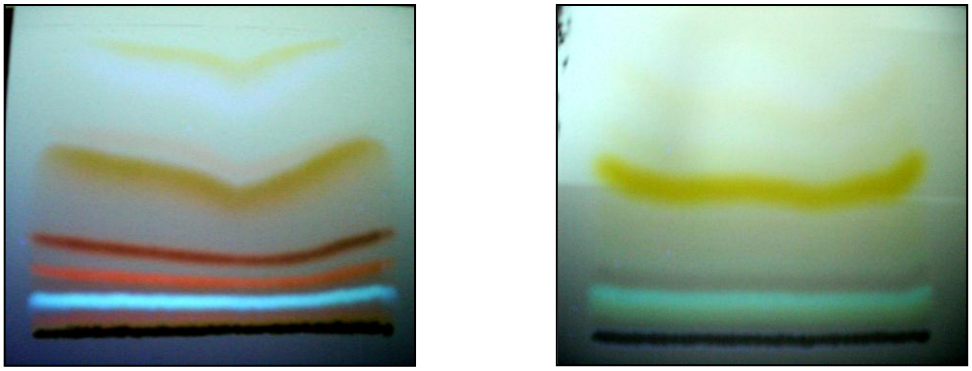


Figure (1): Calibration curve of authentic plumbagin.

Results

The major band on the chromatogram corresponding to authentic standard compound was isolated in both chromatograms of P1 and P2 extracts, figure (2). The purity of the isolated constituents was confirmed by TLC.The IR spectrum for the isolated constituent (A) figure (3), showed stretching vibration bands at (3419, 3000, 1725-1710, 1648 and 1611) cm^{-1}. ^{1}H-NMR spectrum figure(4), as shown in $CDCL_3$ showed a singlet peak at (3.323-3.332) δ, two doublet strong bands at (6.68 and 7.054) δ, multiplate peaks at (7.314) δ , and a singlet peak at (7.613). While ^{13}C NMR figure (5), the data were 46.744, 47.028, 47.312, 47.596, 47.880, 48.163, 48.447, 116.104, 118.157, 120.151, 121.891, 125.726, 125.799, 126.332, 128.365, 129.370, 132.088, 146.423, and 150.581. HPLC chromatogram of P1 and P2 extracts indicated the presence of plumbagin by comparing the retention time with that of the standard plumbagin (Rt 6.567), figure (6), and the concentration of plumbagin was calculated by using ChromGate software depending on the calibration curve of the standard plumbagin. The results showed higher concentration of plumbagin in the root part of the plant than in the leaf, table (1).



(A) **(B)**

Figure (2) TLC Chromatogram of preparative separation of the major constituent from A-P1 extract, B- P2 extract.

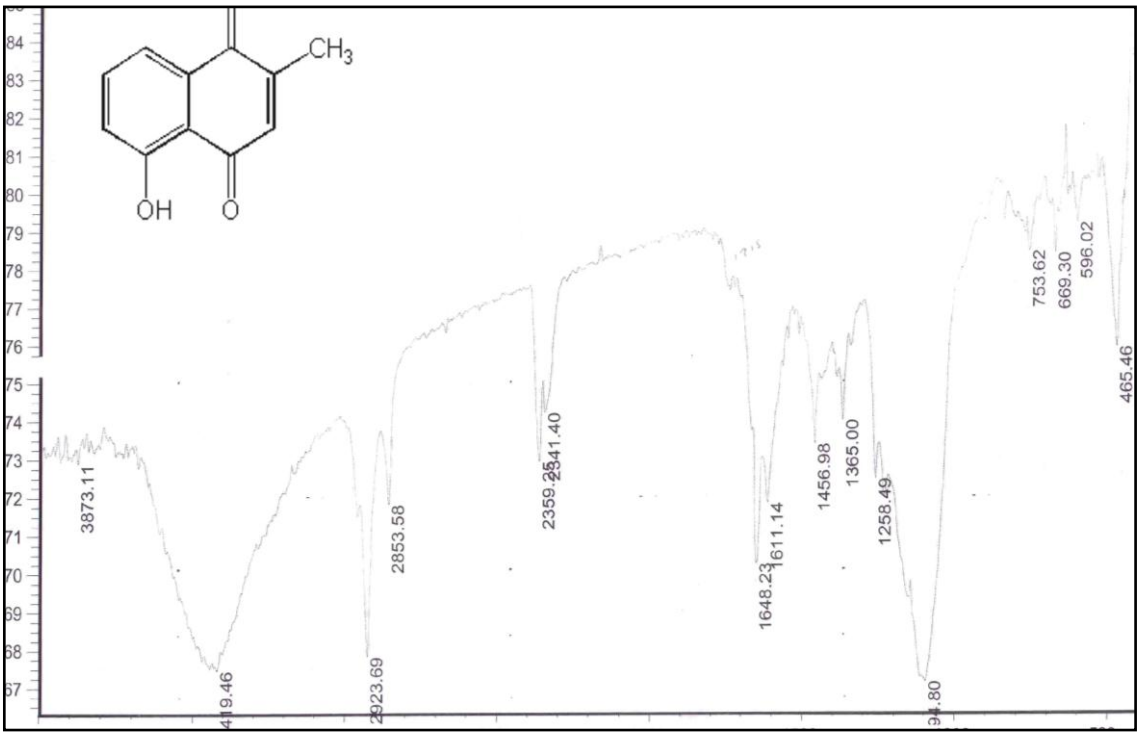


Figure (3): IR spectrum of isolated constituent (A).

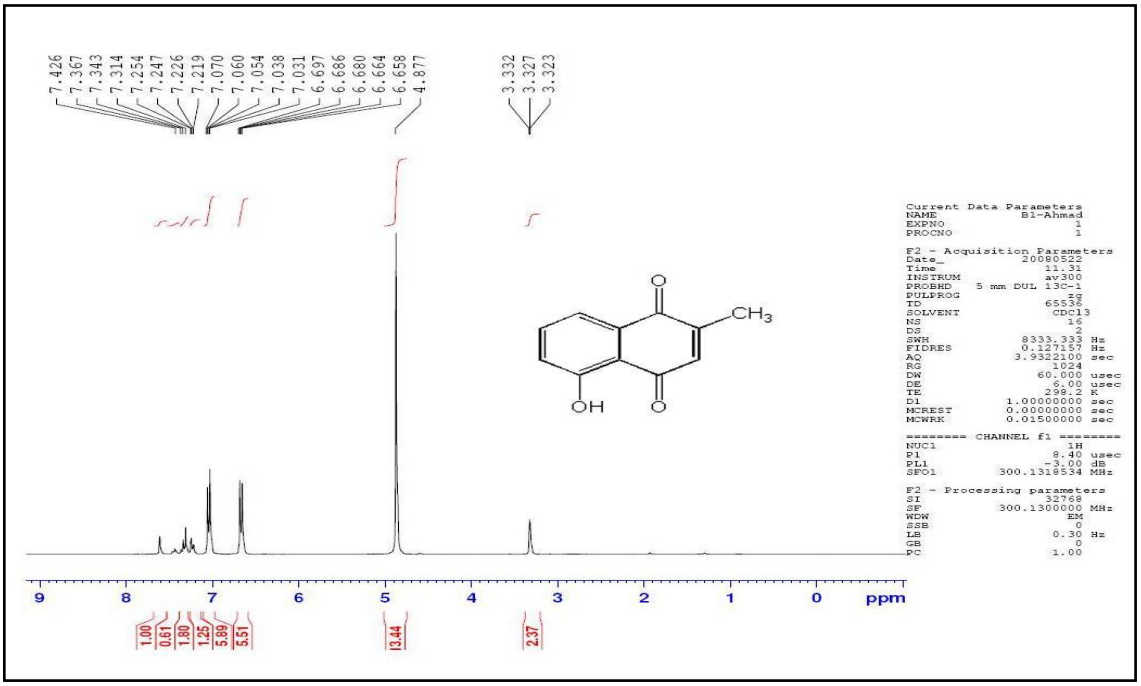


Figure (4):[1]H NMR spectrum of isolated constituent (A).

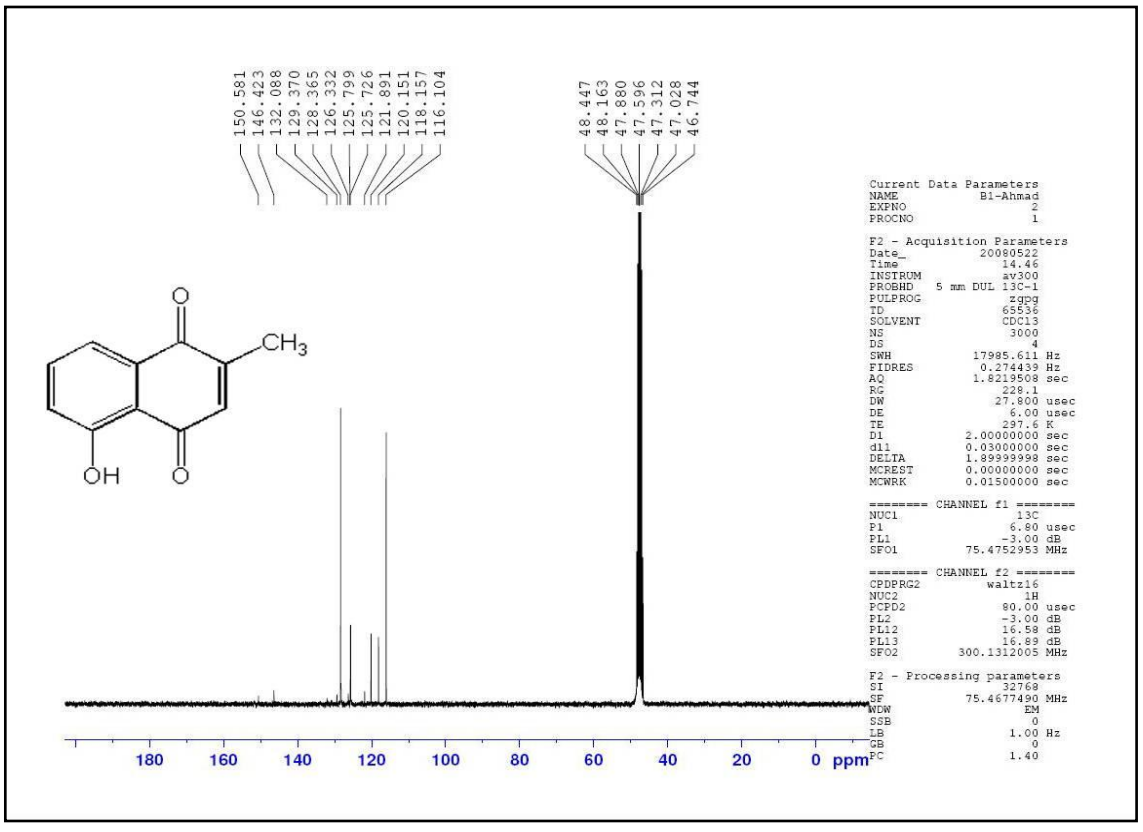
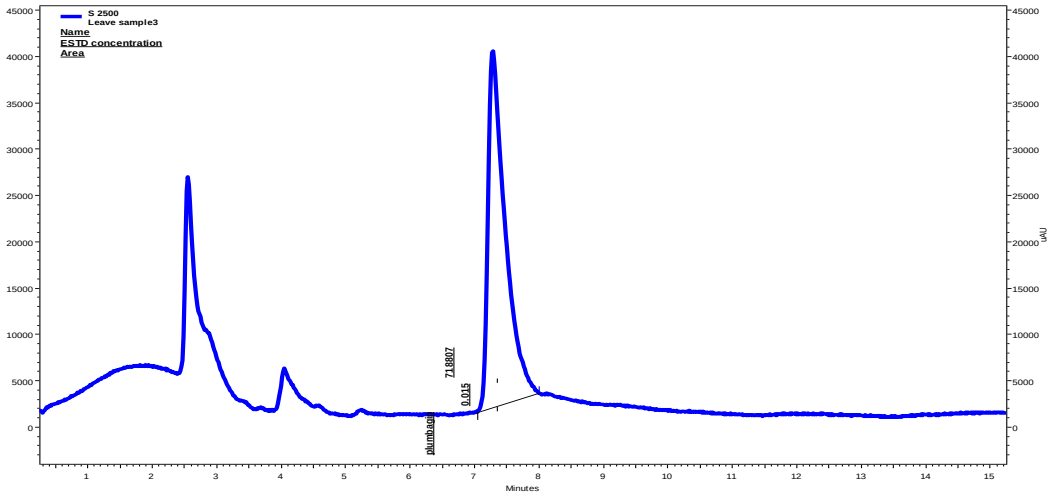
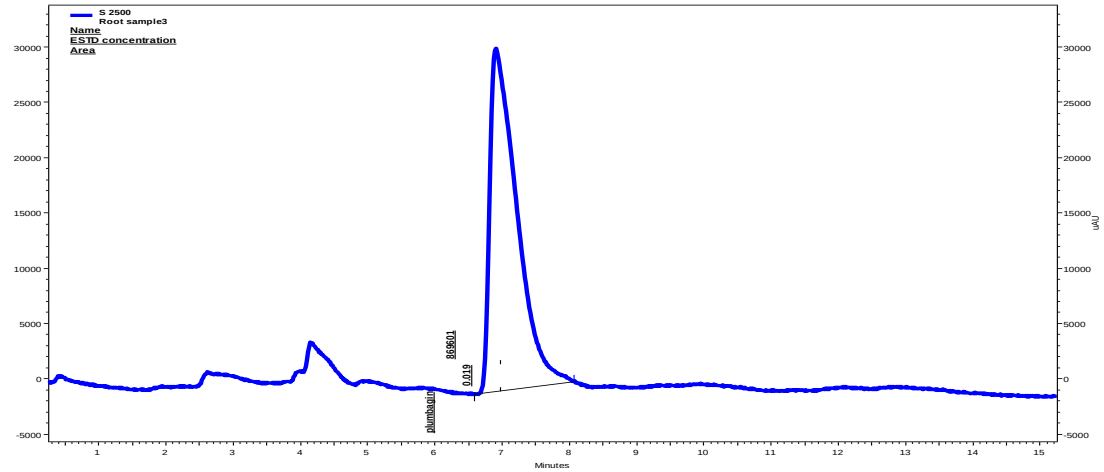


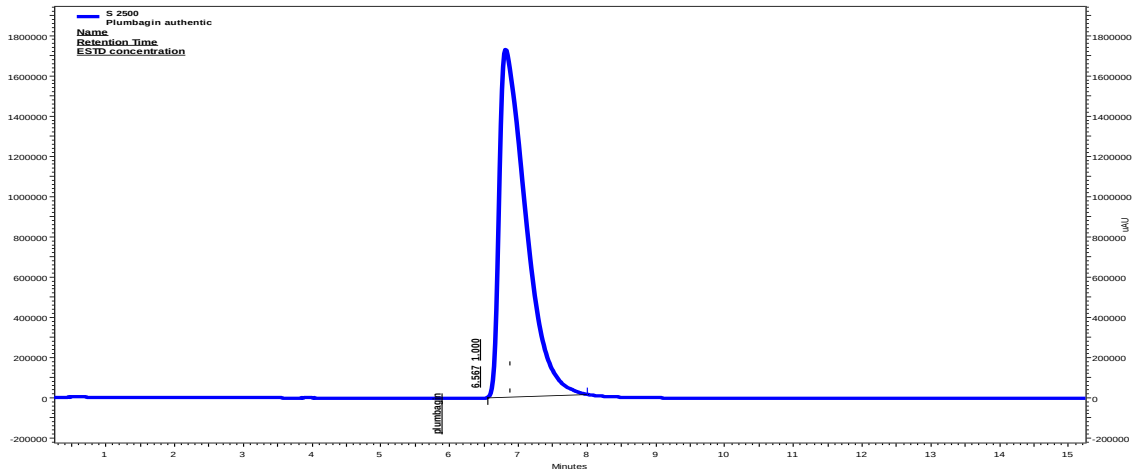
Figure (5): [13]C NMR spectrum of isolated constituent (A).



(A)



(B)



(C)

Figure (6): HPLC chromatogram, A- leaves extracts (P1), B- roots extracts (P2), C- Standard plumbagin

Table (1): Quantitative study of plumbagin in P1 and P2 extracts by HPLC

No	Extract	Area under the curve of samples	Area under the curve of standard plumbagin	Concentration (%)
1	P1	718807	46608959	1.5
2	P2	896601		1.9

Discussion

Preparative TLC technique was used for separation and isolation of the major constituent in P1 and P2 extracts. The spectroscopic data (IR, ^{1}H NMR, and ^{13}C NMR) obtained confirmed the chemical structure of the isolated constituent (A) to contain the important functional groups of plumbagin which agrees with the data obtained for the same compound in other research works [6, 7, 8]. HPLC technique was used successfully for this study; different conditions of HPLC were used for qualitative and quantitative analysis of plumbagin in other species of the genus Plumbago [9, 10]. Plumbagin was identified qualitatively and quantitatively in P1 and P2 extracts by comparing the retention time with that of the standard sample. The result indicates that the HPLC method was efficient for qualitative identification and quantitative determination of plumbagin.

References

1. Huxley A., ed. (1992), New Horticultural Society dictionary of Gardening, Vols.4, Macmillan.
2. Evans WC (2005): Treace and Evans Pharmacognosy, 15th ed. London, UK, W.B. Saunders Company.
3. Binita B. Chaplot., Ashok M., Dave and Yogesh T., Jasrai (2006), A Valued Medicinal Plant-Chitrak (*Plumbago zeylanica* L.): successful Plant Regeneration through Various Explants and Field Performance, *Plant Tissue Culture and Biotechnology*, 16(2), 77-84.
4. Selma R. De Paiva., Lucilene A. Lima., Maria Raquel Figueiredo and Maria axiliadora C. Kaplan (2004),Plumbagin quantifications in roots of Plumbago scandens L.obtained by different extraction techniques, Annals of the Brazilian Academy of Sciences, 76(3) 499-504.
5. Joseph C. Touchstone and Murrell F.Dobbins (1977), Practical of Thin Layer Chromatography, *Awilly-Interscience Publication*, Pages 25, 42,311.
6. Nisha Mathew., Paily K.P., Abidha P., Kalyanasundaram M., Balaraman K. (2002). Macrofilaricidal activity of the plant *Plumbago indica/rosea*, *Drug Development Research*, 56(1), 33-39.
7. Nayana S. Kapadia., Shalini A. Isarani., Mamta B. Shah (2005),A simple method for isolation of plumbagin from roots of *Plumbago rosea*, *Pharmaceutical Biology*,43 (6), 551-553.
8. Kwang-Soo S., Samkeun L. and Byeongjin C. (2007), Antifungal Activity of plumbagin from Leaves of *Nepenthes ventricosa x maxima* against phytopathogenic Fungi, *Plant Pathology Journal*, 23(2), 113-115.
9. Yuan L., Fanq D., Chao L., Qing-Yan M., Ze-Wen G. (2006), Determination of plumbagin in different parts of *Plumbago Zeylanica* by RP-HPLC, *Zhongguo Zhong Yaoza Zhi*. 31(20), 1684-1686.
10. Mei Z.-n., Zhao Y.-h., Li X.-k. (2007), HPLC determination of plumbagin in different sections of *Plumbago zeylanica* Linn. From previous sources of Yunnan, *Chinese Journal f Pharmaceutical analysis*, 27(6), 901-903.