Asian Journal of Chemistry Vol. 22, No. 1 (2010), 593-596

Quantitative Estimation of Plumbagin in Various Parts of *Plumbago rosea* and *Plumbago zeylanica*

EDWIN SHEEJA*, S.B. JOSHI and D.C. JAIN
Department of Herbal Drug Research, B.R. Nahata College of Pharmacy and Research Centre, Mhow Neemuch Road, Mandsaur-458 001, India
E-mail: sheejapharm@rediffmail.com

In this work, the various parts of *Plumbago rosea* and *Plumbago zeylanica* have been standardized by estimating plumbagin. The dried plant materials were subjected to successive solvent extraction (petroleum ether (60-80 ºC), chloroform, acetone, ethanol and aqueous). The extracts that showed positive qualitative test were further taken for the estimation of plumbagin through HPTLC. The results indicated the concentration of plumbagin in the following manner, *P. rosea* root > *P. zeylanica* root > *P. rosea* stem > *P. zeylanica* stem> *P. rosea* leaf > *P. zeylanica* leaf.

Key Words: *P. rosea*, *P. zeylanica*, Plumbagin, HPTLC.

INTRODUCTION

Three species of Plumbaginaceae are recorded in India[1], of which two are medicinally important *viz.*, *Plumbago zeylanica* Linn. and *Plumbago rosea* Linn. These two plants are commonly used by the traditional medicinal practitioners of India in curing wide range of diseases[2]. Roots are commonly been used for both the species and are well known as abortifaciant[3]. The major constituents present in root are plumbagin[4], hydroxy-1,4-napthaquinone, sitosterol glycoside, fatty alcohol and tannins[5]. The aim of present study is to estimate one of the active constituent plumbagin in various solvents (petroleum ether, chloroform, acetone, ethanol and aqueous) from all parts of both the species. Different organic solvents have been used for complete extraction of any ingredients present in plumbagin.

EXPERIMENTAL

The plant materials (*P. zeylanica* Linn. and *P. rosea* Linn) were collected from Kanyakumari district, T.N and identified by Dr. H.S. Chatree, Botanist, Government Arts and Science College, Mandsaur, India. Voucher specimen (BRNCOP /P/006/ 2006, BRNCP/P/002/2006) was deposited in the herbarium of Department of Pharmacognosy, B.R. Nahata College of Pharmacy and Research Centre, Mandsaur for future reference.

Extraction: All the parts of both the species were separately shade dried and powdered. The powdered material was extracted using petroleum ether (60-80 ºC) for 72 h and successively extracted with chloroform, acetone, ethanol and water for

72 h each in soxhlet apparatus. The extracts were evaporated under reduced pressure to obtain solid masses and percentage yield of the extracts were determined (Table-1).

TABLE-1
PRELIMINARY PHYTOCHEMICAL STUDIES AND PERCENTAGE YIELD OF VARIOUS EXTRACTS OF BOTH THE PLANTS

Plant extracts	Yield (% w/w)	Constituents
Pz.L-P	2.83	Fats, steroids and napthaquinone
Pz.L-C	2.73	Steroids and napthaquinone
Pz.L-A	3.20	Tannins, flavonoids, triterpenoids and napthaquinone
Pz.L-E	14.26	Carbohydrates, glycosides, tannins, flavonoids and saponins
Pz.L-W	22.20	Carbohydrates, glycosides, tannins, alkaloids, flavonoids and saponins
Pz.S-P	1.23	Fats, steroids and napthaquinone
Pz.S-C	1.26	Steroids and napthaquinone
Pz.S-A	4.67	Tannins, flavonoids, triterpenoids and napthaquinone
Pz.S-E	3.65	Carbohydrates, glycosides, tannins, flavonoids and saponins
Pz.S-W	19.23	Carbohydrates, glycosides, alkaloids, tannins, flavonoids and saponins
Pz.R-P	1.32	Fats, steroids and napthaquinone
Pz.R-C	1.31	Steroids and napthaquinone
Pz.R-A	2.58	Tannins, flavonoids, triterpenoids and napthaquinone
Pz.R-E	13.10	Carbohydrates, glycosides, tannins, flavonoids and saponins
Pz.R-W	20.99	Carbohydrates, glycosides, alkaloids, tannins, flavonoids and saponins
Pr.L-P	2.32	Fats, steroids and napthaquinone
Pr.L-C	2.05	Steroids and napthaquinone
Pr.L-A	2.50	Tannins, flavonoids, triterpenoids and napthaquinone
Pr.L-E	4.57	Carbohydrates, glycosides, tannins, flavonoids and saponins
Pr.L-W	25.60	Carbohydrates, glycosides, tannins, alkaloids, flavonoids, proteins and saponins
Pr.S-P	1.65	Fats, steroids and napthaquinone
Pr.S-C	1.97	Steroids and napthaquinone
Pr.S-A	2.66	Tannins, flavonoids, triterpenoids and napthaquinone
Pr.S-E	3.71	Carbohydrates, glycosides, tannins, flavonoids and saponins
Pr.S-W	20.50	Carbohydrates, glycosides, tannins, alkaloids, flavonoids and saponins
Pr.R-P	2.65	Fats, steroids and napthaquinones
Pr.R-C	1.77	Steroids and napthaquinones
Pr.R-A	4.92	Tannins, flavonoids, triterpenoids and napthaquinones
Pr.R-E	15.88	Carbohydrates, glycosides, tannins, flavonoids and saponins
Pr.R-W	24.45	Carbohydrates, glycosides, alkaloids, tannins, flavonoids and saponins

Pz.L = *P. zeylanica* Leaves, Pz.S = *P. zeylanica* Stems, Pz.R = *P. zeylanica* Roots,
Pr.L = *P. rosea* Leaves, Pr.S = *P. rosea* Stems, Pr.R = *P. rosea* Roots
P = Petroleum ether (60-80 ºC), C = Chloroform, A = Acetone, E = Ethanol, W = Aqueous.

Phytochemical screening: In order to determine the presence of alkaloids, glycosides, flavones, tannins, terpenes, sterols, saponins, fats and sugars, a preliminary phytochemical study (colour reactions) with plant extracts was performed[6].

Estimation of Plumbagin in various extracts: The napthaquinone identified in the petroleum ether, chloroform and acetone extracts was further confirmed by HPTLC technique[7]. 1 mg/mL standard was prepared by dissolving 10 mg of plumbagin (National Chemicals) in 10 mL of petroleum ether and 10 mg/mL concentration of sample solutions were prepared by dissolving 100 mg of the extract in 10 mL of the respective solvents. Camag HPTLC system (Switzerland) equipped with a sample applicator Linomat IV, twin trough liner development chamber, Camag Scanner III combined with integration software CATS4.06 (Switzerland) and precoated aluminium silica gel F_{254} plate (Merck) were used for the study.

5 µL of (1 mg/mL) standard plumbagin and 5 µL of (10 mg/mL) sample solutions (extracts) were applied as 6 mm band width from about 1 cm of the edge of HPTLC plate using Camag Linomat IV applicator. The solvent system was chloroform:ethyl acetate:hexane:acetic acid (10:5:5:0.3). The chromatogram was developed and scanned at 366 nm using TLC scanner.

RESULTS AND DISCUSSION

The developed plates were observed in white R and UV (254 nm, 366 nm). The R_f value of the standard plumbagin was found to be 0.94. The quantity of plumbagin was estimated by comparing the peak area of the standard with that of the extracts. The following peaks were compared with that of the standard peak of plumbagin. The 4th peak (0.94) of petroleum ether extract, 4th peak (0.93) of chloroform extract and 5th peak (0.93) of acetone extract of *P. zeylanica* root; 5th peak (0.92) of petroleum ether extract, 5th peak (0.92) of chloroform extract and 6th peak (0.92) of acetone extract of *P. zeylanica* stem; 4th peak (0.93) of petroleum ether extract and 6th peak (0.92) of acetone extract of *P. zeylanica* leaf; 4th peak (0.93) of petroleum ether extract, 6th peak (0.93) of chloroform extract and 6th peak (0.92) of acetone extract of *P. rosea* root; 7th peak (0.92) of petroleum ether extract, 6th peak (0.92) of chloroform extract and 4th peak (0.93) of acetone extract of *P. rosea* stem; 7th peak (0.92) of petroleum ether extract and 4th peak (0.95) chloroform extract of *P. rosea* leaf but the chloroform extract of *P. zeylanica* leaf and the acetone extract of *P. rosea* leaf did not show any R_f which could be compared to that of the standard.

The amount of plumbagin present in petroleum ether and acetone extracts of leaves of *P. zeylanica* were found to be 2.65 % while the acetone extract had only 0.94 %. The chloroform extract, though showed positive chemical test for napthaquinone did not show any R_f value corresponding to that of standard plumbagin.

The amount of plumbagin present in petroleum ether, chloroform and acetone extracts of *P. zeylanica* stems were 1.94, 0.54 and 2.65 %, respectively and the amount in root were quite higher when compared to that of the other parts as 10.41, 1.30 and 7.26 %, respectively.

The amount of plumbagin present in petroleum ether and chloroform extract of leaves of *P. rosea* were found to be 0.71 % while the chloroform extract had only 3.49 %. The acetone extract, though showed positive chemical test for napthaquinone

did not show any R_f value corresponding to that of standard plumbagin. The quantity of plumbagin estimated in petroleum ether, chloroform and acetone extracts of *P. rosea* stems was found to be 2.27, 0.94 and 2.61 % and that in root was 21.89, 4.47 and 0.92 %, respectively. The concentration of plumbagin in various parts was found as follows: *P. rosea* root (27.28 %), *P. zeylanica* root (18.97 %), *P. rosea* stem (5.48 %), *P. zeylanica* stem (5.12 %), *P. rosea* leaf (4.20 %) and *P. zeylanica* leaf (3.59 %).

Herbal drugs are very complex in nature due to the presence of wide range of chemical constituents. The concentration of active marker in plant varies from time to time. This may be due to the climatic variations and, or failure to extract and estimate the actual content of active marker. Since plants are complex substances, solubility of one compound depends upon the other constituents present within the same matrix. Plumbagin is a type of constituent that is soluble in wide range of solvents[8]. Using a single solvent to estimate its content in various parts may result in an inaccurate result, which is observed from our studies. In Soxhlet extraction though the syphon got cleared and solvent remained in the siphon after extracting the plant materials with petroleum ether showed negative results for plumbagin, when the solvent was changed, the newer solvent (chloroform) again started to extract the remaining plumbagin. This may be due to a sudden change in polarity or the constituent that held plumbagin was not soluble in first solvent and was soluble in the second one. By this way the actual amount of any compound present in the plant materials could be determined. This method is very much useful for the estimation of plumbagin in various extracts from different parts of the plant.

ACKNOWLEDGEMENT

The authors are thankful to Department of Phytopharmacy and Phytomedicine, TIFAC-CORE in Herbal Drugs, J.S.S. College of Pharmacy for HPTLC studies.

REFERENCES

1. Anonymous, The Wealth of India, (National Institute of Science Communications and Information Resources, Council of Scientific and Industrial Research, New Delhi, Vol. 4, p. 148, 168 (2003).
2. A.I.C. Dorni, K.S. Vidyalakshmi, R.V. Hannah, G.V. Rajamanickam and G.P. Dubey, *Phcog. Mag.*, **2**, 239 (2006).
3. R.K. Kritikar and D.B. Basu, Indian Medicinal Plants, Delhi: Jayyed Press (1975).
4. P.U. Devi, B.S. Rao and F.E. Solomon, *Indian J Exp. Biol.*, **36**, 891 (1998).
5. Anonymous, The Wealth of India, New Delhi: Council for Scientific and Industrial Research, Vol. 8, p. 162 (1985).
6. K.R. Khandelwal, Practical Pharmacognosy, Nirali Prakashan, Pune, India, p. 152 (2005).
7. S. Manimaran, S.P. Dhanabal, S. Sudhakarraja and B. Suresh, *Indian J. Pharm. Educ. Res.*, **41**, 159 (2007).
8. Anonymous, ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/Plumbagin.pdf, accessed on 5/3/09.

(*Received*: 6 March 2009; *Accepted*: 12 September 2009) AJC-7875