Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2012, 4 (5):1530-1533
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Structural comparison of Jatropha Gossypifolia with 1-phenylnaphthalene using HPLC Technique

Sujata Deo, Farhin Inam, Anupama N. Jadhav*

Department of Chemistry, Institute of Science, Nagpur

ABSTRACT

Plant lignans are natural products resulting from the phenylpropanoid metabolic pathway. Lignans are of considerable interest in the treatment of cancer and other diseases. After a survey on plants containing lignan, Jatropha Gossypifolia (J.G) with lignan Arylnaphthalene was selected, which showed structural similarity with synthesized lignan (1-phenylnaphthalene). Plant Jatropha Gossypifolia has potent anticancer activity, have already been proved by various researchers. In a follow-up study, the lignans synthesized in our laboratory were also tested for its invitro cytotoxic activity using MTT assay. The results were found to be significant. A simple, rapid and specific method for the analysis of structurally similar lignan in the plant Jatropha Gossypifolia with synthetic organic compound (1-phenylnaphthalene) by a sensitive High Performance Liquid Chromatography technique is developed. The lignan peak was separated from endogenous peaks on phenomenex ODS 5µ C_{18} column (250 x 4.6mm) with mobile phase comprising of phosphate buffer: acetonitrile (70:30), pH-2 at a flow rate of 1ml/min and Ultra Violet detector at 230nm. Lignan 1-phenylnaphthalene was eluted at 4.047 minute and lignan from plant J.G was eluted at 4.077 minute.

Keywords: Lignan, 1-phenylnaphthalene, Jatropha Gossypifolia, HPLC

INTRODUCTION

The term “lignan” was introduced in 1936 by R. D. Haworth to denote structures that are comprised of two phenylpropanoid units linked by the central carbons of their side chain [1]. Lignans have long been of interest to organic chemists due to their diverse biological properties. Although their molecular backbone consists only of two phenylpropane (C_6-C_3) units, lignans show an enormous structural diversity. Lignans also possess significant pharmacological activities, including anticancer, anti inflammatory, immunosuppressive, cardiovascular, antioxidant and antiviral actions [2-8]. Lignans are found in roots, rhizomes, stems, leaves, seeds and fruits. J.G Linn finds frequent use in the Indian traditional medicine, its most important application have been reported to be in the treatment of cancer [9, 10]. Arylnaphthalene is a naturally occurring lignan, which is extracted from the plant J.G. The present work reports the use of HPLC for the identification and comparison of lignans from synthetic and natural origin.

MATERIALS AND METHODS

Synthesis of 1- Phenylnaphthalene Lignan:
Perkin condensation of aromatic aldehydes with β-benzoyl propionic acid gives α-arylidene-γ-phenyl-Δ,β-butenolides [11]. The butenolides were cleaved with alcoholic sodium carbonate to afford α-arylidene-β-benzoylpropionic acid [12].This keto acid was then treated with different reagents like CH_2N_2, formaline to get various derivatives. Cyclization of α-arylidene-β-benzoyl propionic acid and its derivatives gave 1-Phenylnaphthalene and Pericarbonyl lactone lignans [13].

Chromatographic Conditions:
The mobile phase A was made by preparing 70% phosphate buffer of pH-2 & mobile phase B was made up of 30% Acetonitrile. Separation were performed on a phenomenex ODS C18 column (250 x 4.5nm), 5µm. The mobile phase flow rate was 1.0ml/min. First the column was saturated with a mobile phase of phosphate buffer: Acetonitrile (70:30). The reference and sample solutions prepared in HPLC grade methanol were run into this system separately one after another. Lignans with 1-phenylnaphthalene structures were resolved properly with sharp peak in the above selected mobile phase for both reference and sample solutions and their chromatograms were recorded.

Instrumentation:
Comparative study of plant and synthetic lignan was carried on a Shimadzu 1100 HPLC system, which consisted of a binary gradient pump LC 10 ADvP, UV-VIS detector at 230 nm and manual injector 7725I(Rheodyne) with 20 µl loop and a reversed phase 5µ Phenomenex ODS C18 column (250× 4.6mm). Mobile phase used for the studies consisted of phosphate buffer pH-2: Acetonitrile (70:30) with a flow rate of 1 ml/min.

Reagents:
1)Acetonitrile (E-Merck Limited)
2)Methanol (HPLC Grade)
3)HPLC grade water was used for the preparation of buffer.
4)All other reagents were of analytical reagent grade.

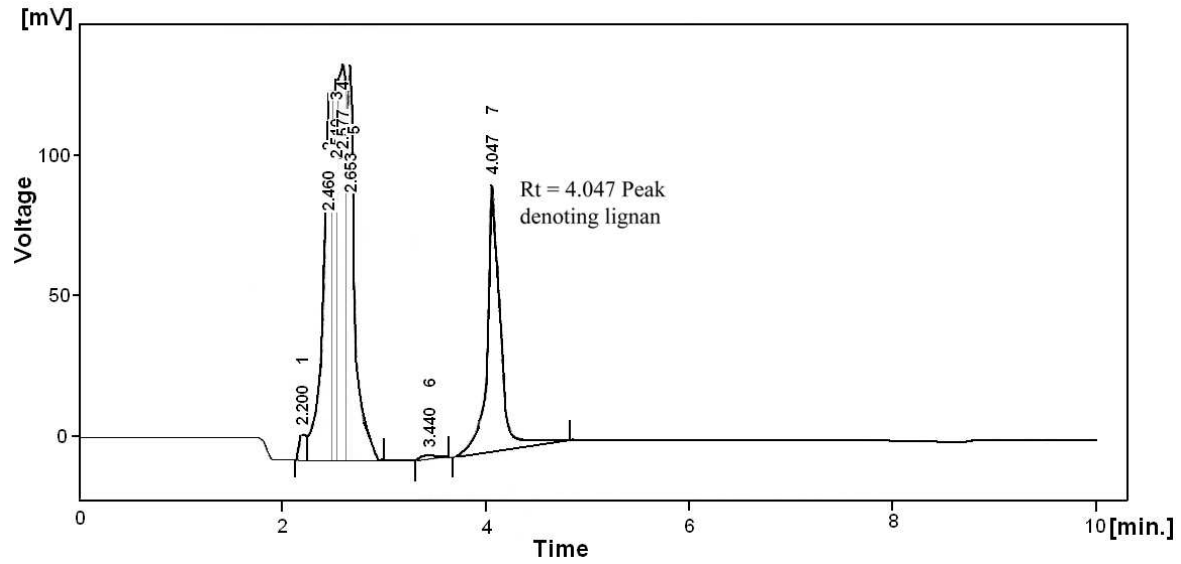

Figure 1: HPLC chromatogram of 1-Phenyl-6,7,8 trimethoxy naphthalene lignan.

METHOD
Preparation of standard solution of 1-Phenylnaphthalene:
The reference compound 1-phenyl-6,7,8 trimethoxy naphthalene lignan was synthesized in the laboratory of Chemistry, Institute of Science Nagpur. Identity and purity was confirmed by HPLC chromatographic and spectral methods and the structures were confirmed by their UV, ^{1}H NMR & IR data. The standard solution for HPLC

analysis was prepared in HPLC grade methanol having concentration of 20µg/ml. The standard chromatogram of 1-phenylnaphthalene is shown in Figure 1.

Preparation of sample solution for Assay:
The plant Jatropha Gossypifolia Linn was collected from Shree Shail Medifarm, Nagpur plant nursery and was botanically identified and confirmed by Department of Botany, RTM, Nagpur University. The plant specimen voucher number is 9461. In our present work, the whole plant of Jatropha gossypifolia was subjected to extraction using petroleum ether (60-80^{o}C). The extract was then concentrated for further analysis. The sample solution for HPLC analysis was prepared in HPLC grade methanol having concentration of 20µg/ml. Chromatogram of the sample is shown in Figure 2.

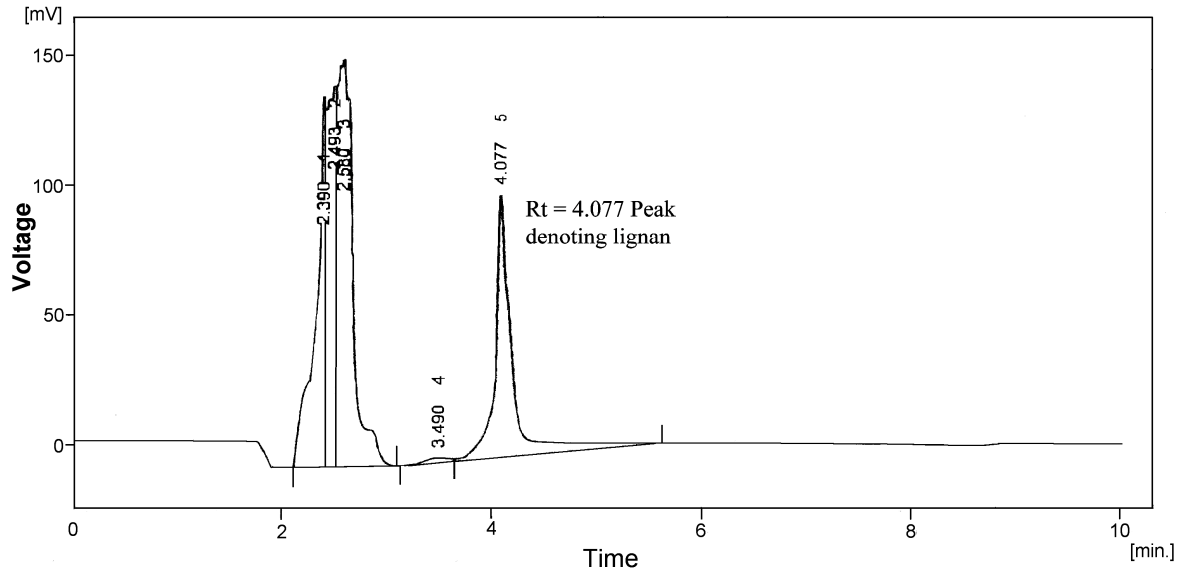

Figure 2: HPLC chromatogram of *Jatropha gossypifolia*

RESULTS

A single prominent peak denoting lignans at Retention time (Rt) 4.047 minutes for the synthesized compound was observed which was then compared with the Retention time of the plant extract of Jatropha gossypifolia and was found that the patterns of their chromatogram was more or less similar except for some variation in the relative intensity of peaks. The Retention time for plant Jatropha gossypifolia was found to be 4.077 minutes.

Increased peaks with better resolution were observed for plant lignan and synthesized lignan. Arylnaphthalene lignan in plant J. G. was identified by HPLC based on the comparison of retention time with the reference compound.

DISCUSSION

The present study was carried out to develop a simple and precise HPLC method for the comparison of 1-phenylnaphthalene type lignan with arylnaphthalene lignan in plant Jatropha gossypifolia.

CONCLUSION

The reverse phase HPLC method was developed for the comparison of structurally similar lignan in plant Jatropha gossypifolia with 1-phenylnaphthalene lignan.

This study has shown that HPLC can be used as an effective and rapid method for the characterization and identification of lignans from synthetic and natural origin and further for faster development of new lignan drugs for anticancer study.

Acknowledgement
We are thankful to Dr.M.M.Gadegone, Director, Institute of Science, Nagpur for making available the facilities at the Department and Kotagle sir of S.K.B College of Pharmacy, Kamptee for the HPLC analysis.

REFERENCES

[1] Haworth, R.D. *Ann. Rep. Prog. Chem.* **1936**, 33, 266.
[2] T. Hirano, M. Gotoh and K. Oka, *Life Sci*., **1994**, 55, 1061; L. U. Thompson, M. M. Seidl, S. E. Rickard, L. J. Orcheson and H. H. S. Fong, *Nutr. Cancer*, **1996**, 26, 159.
[3] L.Kangas,N. Saarinen, M. Mutanen, M. Ahotupa, R.Hirsinummi, M. Unkila, M. Perala, P. Soininen, R. Laatikainen, H. Korte and R. Santti, Eur. *J. Cancer Prev*., **2002**, 11, S48.
[4] N.Kuku Kboyaci and Bilge Sener, *Journal of Medicinal Plant Research*, **18 June 2010**, Vol 4 (12), pp 1136-1140.
[5] Shin-Yong Park, Sung Haklee, woo Hyruk Choi et. al., *Planta Med*. **2007**, 73(7):674 – 678.
[6] D. D. Kitts, Y. V. Yuan, A. N.Wijewickreme and L. U. Thompson, *Mol. Cell. Biochem.*, **1999**, 202, 91.
[7] S. Yamauchi, T. Ina, T. Kirikihira and T. Masuda, *Biosci., Biotechnol., Biochem.*, **2004**, 68, 183.
[8] (a) Yuan-Bin, Cheng Meng-Ping Chang, et. al., *Journal of Natural Products*, **2009**, 72(9), pp 1663-1668. (b) Ren_Wnag, Jainy, Jin-Rong Zhou et. al., *Journal of Natural Products* **2007**, 70(2), pp 283-286.
[9] Hartwell, J.L. (**1969**) *Lloydia* 32, 153.
[10] Hartwell, J.L., **1982**. *Plants used against cancer.* A survey Quarterman Publications Lawrence, M.A.
[11] S. Deo; F. Inam; R.P. Mahashabde; A. N. Jadhav. *Asian J Chem*, **2010**, 22, 3362.
[12] A. A. Avetisyan ; G. G. Tokmadzhyan. *Chem Heterocycl Compd*, **1987**, 23, 595.
[13] SPB Oveden; J Yu; S. S. Wan; G. Sberna; R. M. Tait. *Phytochemistry*, **2004**, 65, 3255.