http://www.e-journals.net

ISSN: 0973-4945; CODEN ECJHAO
E-Journal of Chemistry
2012, **9(1),** 313-317

High Performance Thin Layer Chromatographic Determination of Chrysin in *Oroxylum Indicum* Vent. from Different Geographical Regions of India

K SRILATHA SRINIVAS and A. SARAF APARNA*

Department of Botany, The Institute of Science, Mumbai-32, India
draparnasaraf@yahoo.co.in

Received 24 May 2011; Accepted 16 July 2011

Abstract: HPTLC fingerprints can be used for documentation and quantification of chemical markers to identify morphological and geographical variation in herbal raw materials. The current work was aimed at developing phytochemical fingerprints of *Oroxylum indicum* vent. from different geographical regions of India *viz.* Western Ghats (Maharashtra) and Northern Uttar Pradesh. Fingerprint of flavonoid, chrysin was developed for roots, leaves and bark of the plant from different regions using cold extraction and suitable solvent system. HPTLC technique was successfully used for evaluating regional variations. The fingerprints were also utilized for quantification of the flavonoid, chrysin from different parts of the plants from two distinct geographical regions. Maximum content of chrysin was found in plant collected from Western Ghats than in U.P.

Keywords: *Oroxylum indicum* vent. HPTLC, Chrysin, Quality control

Introduction

Standardisation is an essential measurement for ensuring the quality control of herbal drugs. The HPTLC fingerprint of botanically authenticated raw material serves as a primary reference against which unknown material can be characterized[1]. *Oroxylum indicum* Vent. (Family- Bignoniaceae) syn: Shyonaka is an important herb in Ayurvedic medicine and indigenous medical system for over thousands of years[2]. *Oroxylum indicum* have been used as a single drug or as a component of well known Ayurvedic formulations like chyavanprash, dashmularista *etc*[3]. The root bark and stem bark possess antiallergic properties and are used in treating allergic disorders, urticaria, jaundice, asthma, sore throat, laryngitis, hoarseness, gastralgia, diarrhoea, dysentery, erythema and measles[4,5] . The plant contains flavonoids like chrysin, oroxylin and baicalein as active principles[6]. Chrysin was reported to have broad spectrum biological activities[7-9] and hence was selected as marker for standardization so as to assure a consistent and acceptable quality herbal product.

Since different parts of *Oroxylum indicum* find use for different clinical indications, it is important to establish quality of plant raw material for its constituent plant part composition. The present study aims at developing chromatographic fingerprint of *Oroxylum indicum.* The method developed is found to be rapid, sensitive and precise for simultaneous quantification of chrysin from different plant parts of *Oroxylum indicum* collected from two different geographical regions, village, pophli, kumbharli Ghats, near chiplun raigarh, Western ghats and Kehri village, foothills of Himalayas, Northern UP. The source of plant powder raw material and also its constituent plant part can be easily identified from the HPTLC fingerprints which have been developed.

Experimental

Whole plant parts of *Oroxylum indicum* were collected during the flowering season from two different geographical regions, Western Ghats (Village, Pophli, kumbharli Ghats, near Chiplun, district Raigarh, Maharashtra) and Kehri village, foothills of Himalayas, Northern UP. The plants were identified and authenticated at Blatter's Herbarium, St. Xavier's College, Mumbai. The plants collected from different regions were sorted out and individual plant parts were separated. The plant parts were then washed and dried in an oven at 45 ^{0}C for four days, powdered and sieved thro BSS Mesh No. 85. This powder was used for analysis.

Ethanolic extracts of air dried powder of various plant parts such as root, stem and leaf were prepared from the plant of *Oroxylum indicum* vent. These extracts were used for developing HPTLC finger prints.

Chromatography

Procedure

The HPTLC analysis was performed on aluminum plates pre-coated with silica gel $60F_{254}$ (Merk, Germany). Extract was applied on the plate of 10x10 cm as bands of 10 mm width of each with the help of CAMAG linomat IV sample applicator. The plates were developed in a CAMAG twin-trough chamber previously equilibrated with a mobile phase for 20 min. Solvent system for flavonoids chloroform: methanol: formic acid (8.8:0.7:0.5). Each plate was developed up to 8 cm, air dried and scanned at wavelength of 254 & 366 nm using CAMAG TLC Scanner 3. The chromatograms were recorded. Then the plates were derivatized with alcoholic $Fecl_3$ and heated at 105 ^{0}C on hot plate till the development of colour of bands and observed under white light. The colour of recorded bands and R_f values were recorded. Standard chrysin (Figure 1) (99% purity) was procured from Sigma Aldrich. The solvents used for analysis were of analytical grade (Qualigens Fine Chemicals)

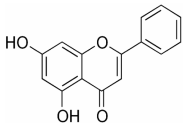

Figure 1. Structure of chrysin ($C_{15}H_{10}O_4$) 5,7-dihydroxyflavone; 5,7-dihydroxy-2-phenyl-4*H*-benzo[b]pyran-4-one

Assay procedure

The standard solution of 1 µg/µL of chrysin was prepared in ethyl alcohol. The plant extract utilized was of concentration 800 µg/µL. 20 µL of plant extracts and 2 µL and 5 µL standard chrysin solutions were spotted on the HPTLC plate. The amount of chrysin present in the plant extract was calculated by comparison of area measured for the sample to that for the standard.

Results and Discussion

The plant *Oroxylum indicum vent* is of immense medicinal value. Traditionally, the stem, bark, seeds and leaves used for the treatment of the diarrhea, typhoid and stomachache. It has also been used as antitussive, analgesic and anti-inflammatory agents for the treatment of cough, bronchitis and other lungs diseases. In India, roots are used in ayurvedic preparation called "Dasamoola" *i.e.,* used as an astringent, anti-inflammatory, anti-helminthic, antibronchitic, antileucodermatic, anti-rheumatic, anti-anorexic and for treatment of leprosy and tuberculosis[10]. It is also used in other ayurvedic formulation such as amartarista, dantyadyarista, narayana taila, dhanawantara ghrita, brahma rasayana, chyavanaprasa awalwha, *etc.* Since different parts of the plant have different therapeutic applications it is important to distinctively identify the plant part constituent of the plant powder. The work carried out here has led to the development of HPTLC fingerprint patterns for different plant parts of *Oroxylum indicum vent* from different geographical regions. This distinctively helps us to identify the plant part and also gives an idea about the region of collection. The marker chrysin was detected and quantified accurately using silica gel F_{254} HPTLC pre coated plates with mobile phase chloroform:methanol:formic acid (8.8:0.7:0.5) (Figure 2 and 3). Spectra of standard chrysin and overlay of the samples was also obtained (Figure 4 and 5). The developed fingerprints showed distinct variation in leaf, stem and root of plant collected from U.P. In case of plants collected from Western Ghats, chrysin content was maximum in roots with little difference in the chrysin content in leaf and stem (Table 1).

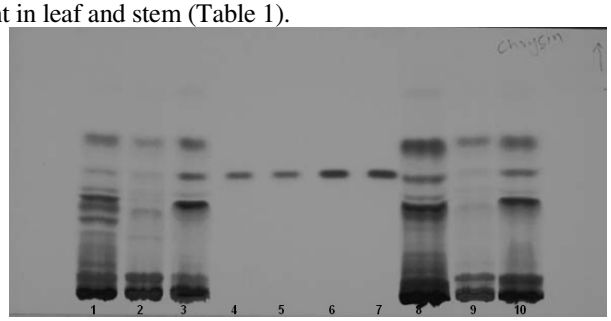

Figure 2. Chromatographic plate of standard chrysin with *Oroxylum indicum* vent at 254 nm BD*

**Ethanolic extract of Oroxylum indicum vent (1=WG leaf; 2= WG stem; 3= WG root; 4,5= Ch 2 µL; 6,7=C h 5 µL; 8= UP leaf; 9= UP stem; 10= UP root) WG: Western Ghats; UP: Uttar Pradesh; Ch: standard chrysin*

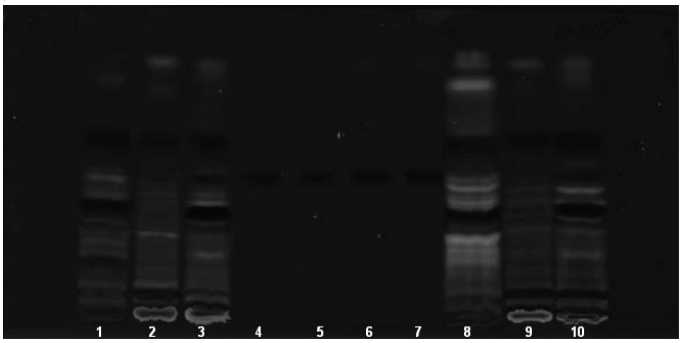

Figure 3. Chromatographic plate of standard chrysin with *Oroxylum indicum* vent at 366 nm BD

**Ethanolic extract of Oroxylum indicum vent (1=WG leaf; 2= WG stem; 3= WG root; 4,5= Ch 2 µL ;6,7=Ch 5 µL; 8= UP leaf; 9= UP stem; 10= UP root)WG: Western Ghats; UP: Uttar Pradesh; Ch: Standard Chrysin*

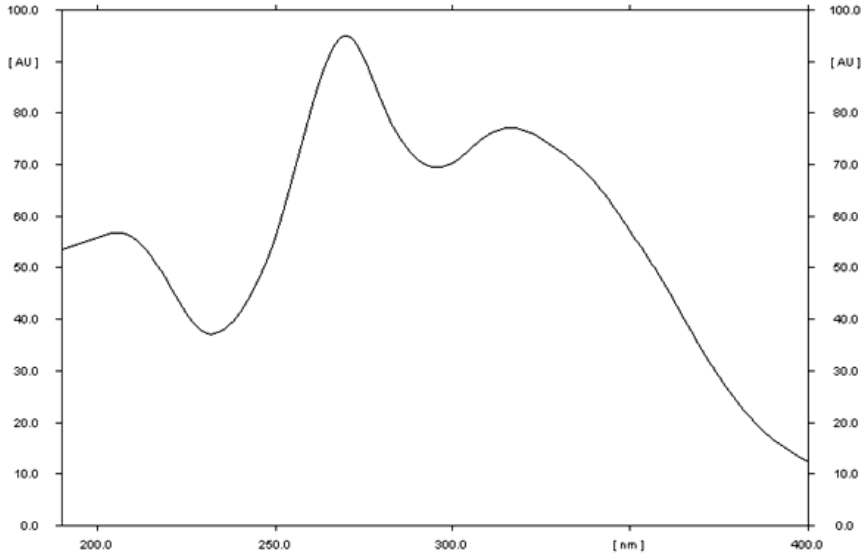

Figure 4. Spectra of standard chrysin

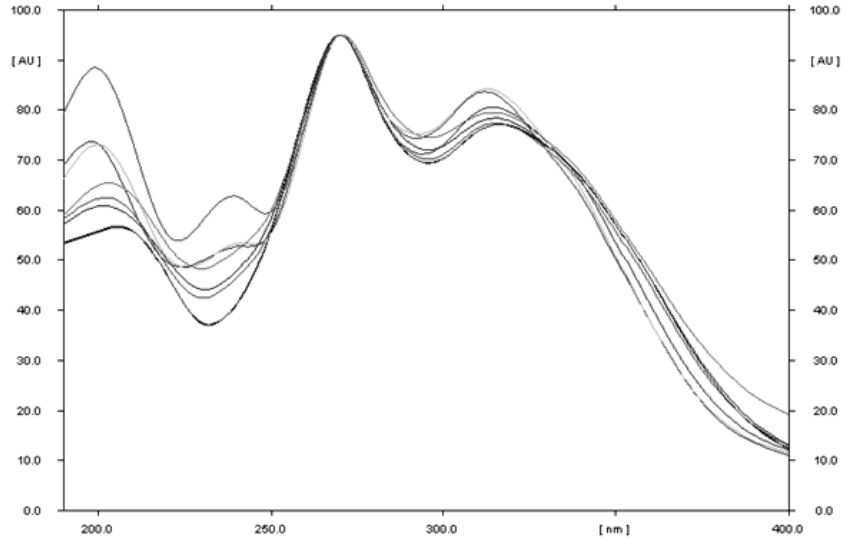

Figure 5. Overlay of spectra of standard chrysin and chrysin from samples in *Oroxylum indicum* vent. collected from different geographical regions of India

Table 1. Result of chrysin content in *Oroxylum indicum* vent. from different plant parts collected from different geographical regions of India.

Geographical Region	Chrysin content in different parts of *Oroxylum indicum* vent.		
	Root	Stem	Leaf
Uttar Pradesh	0.011%	0.002%	0.006%
Western Ghats	0.014%	0.004%	0.007%

The presence of chrysin in all plant parts justified the use of every plant part in ayurvedic preparations. The chromatogram developed by this techniques show variations in the phytochemical marker chrysin which helps us to identify the difference in phytochemical constituents due to the geographical region of collection and due to the constituent part of the plant in medicinal plant raw material.

Conclusion

Standardisation of plant materials is the need of the day. An HPTLC fingerprint is suitable for rapid and simple authentication and comparison of subtle differences among samples of identical plant resource from different geographic locations. The work carried out for the development of chromatographic fingerprint of *Oroxylum indicum* vent demonstrate that HPTLC technique was successfully used for evaluating regional variations and quantitate chemical markers in the herbal raw material. The results for quantification of chrysin indicated that there is distinct variation in the chrysin content in the plants obtained from the two geographical regions *viz*. Western Ghats and U.P. The chrysin content is found to be more in plants collected from Western Ghats than in U.P. The fingerprints so developed are useful in confirming the identity and purity of the medicinal plant raw material.

References

1. Eike Reich and Anne Schibli, High-Performance Thin- Layer Chromatography for the Analysis of Medicinal Plants, Thieme, New York. Stuttgart. 2007, 224- 240.
2. Mall S B, Medicinal Herbs in Bagmati zones, ICIMOD, Kathmandu, Nepal, 1994, 68.
3. Mao A A, *Indian J Traditional Knowledge*, 2002, **1(1),** 17-21.
4. Anonymous. The Ayurvedic Pharmacopoeia of India. Government of India, Ministry of health and family welfare department of Indian system of medicine and Homeopathy, New Delhi, India, 1998 pp. 209-210
5. Warrier P K, Nambiar V P K and Ramankutty C, In: Oroxylum indicum. Indian medicinal Plants. Vol 4, Orient Longman Ltd., Madras, India, 1995, 186-190.
6. Chen L J, Games D E and Jones J, *J Chromatogr A,* 2003, **988(1),** 95-105.
7. Villar I C, Jimenez R, Galisteo M, Garcia-Saura M F, Zarzuelo A and Duarte J, *Planta Med.,* 2002, **68(9),** 847-850.
8. Joon-Su Shin, Kyoung-Soon Kim, Myoung-Bohn Kim, Jae-Hoon Jeong and Bak-Kwang Kim, *Bioorg Med Chem Lett.,* 1999, **9**, 869-874.
9. Sathiavelu J, Senapahy G J, Devaraj R, Namasivayam N, *J Pharm Pharmacol*, 2009, **61(6),** 809-817.
10. Kalaivani T and Lazar Mathew, *We Int J Sci Tech.,* 2009, **4**, 45-52.