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Swati R Mahale
College of Forestry, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth Dapoli, Maharashtra, India

AD Rane
College of Forestry, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth Dapoli, Maharashtra, India

YB Patil
Punjab Agricultural University, Ludhiana, Punjab, India

SS Narkhede
College of Forestry, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth Dapoli, Maharashtra, India

Correspondence
Swati R Mahale
College of Forestry, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth Dapoli, Maharashtra, India

Seedlings of *Oroxylum indicum* Contain Traces of Chrysin in Their Roots

Swati R Mahale, AD Rane, YB Patil and SS Narkhede

Abstract
Oroxylum indicum (L.) Vent. belongs to family bignoniaceae and contain flavonoids of medicinal value, the chrysin is among them. Chrysin has many biological activities i.e. antibacterial, antioxidant, antiinflammatory, antiallergic, anticancer and antiestrogenic. A study was conducted to ascertain the variation of chrysin content in the roots of *Oroxylum indicum* among three different growth stages i.e. seedling, pole and tree. The chrysin content in roots extracted from three stages were analyzed by using HPLC. It was observed that, seedling stage had traces of chrysin (3.3148 µg ml^{-1}).

Keywords: Chrysin, *Oroxylum indicum*, Root, HPLC

Introduction
Oroxylum indicum (L.) Vent. (Bignoniaceae) is distributed throughout the country up to an altitude of 1200 m and found mainly in ravine and moist places in the forests and also distributed in Ceylon, Malaysia, Cochin, China, Philippines, Thailand, and Indonesia. Root bark is used in fever, bronchitis, intestinal worms, leucoderma, asthma, inflammation, anal troubles, etc. The fruits and seeds are also used as expectorant, purgative, and bitter tonic. The young shoots, flowers and unripe fruits are consumed as vegetable in northeastern. *Oroxylum indicum* is used as one of the important ingredients of well-known ayurvedic preparation ‘‘Dashmool’’. It is also used in other ayurvedic formulations such as amartarista, dantyadyarista, narayana taila, dhanawantara ghrita, brahma rasayan, and chyavanaprasa awalwha (Yadav *et al*. 2002).
Roots of their plants have always been an integral part of treatment of various ailments in Ayurveda as well as in diverse communities across India either singly or in combination. Several parts of this tree contain alkaloids and flavonoids of medicinal value (Grampurohit *et al*. 1992) [1]. The chemical constituents obtained from different parts of plant include baicalein-7-O-diglucoside (Oroxylin B), baicalein-7-O-glucoside, chrysin, apegenin, prunetin, sitosterol, oroxindin, biochanin-A, ellagic acid, baicalein and its 6- and 7-glucuronides, scutellarein, tetuin, antraquinone and aloe-emodin. Chrysin is a flavone which has many biological activities i.e. antibacterial, antioxidant, antiinflammatory, antiallergic, anticancer and antiestrogenic. Chrysin also have tyrosine inhibitory and moderate aromatase inhibitory activity. It can also inhibit the metabolism of the carcinogen benzo [α] pyrene by hamster embryo cells in tissue culture (Singh and Chaudhary, 2011) [4].

Material and Methods
The roots of *Oroxylum indicum* were extracted from randomly selected plant of three different growth stages namely seedling (6 months old), pole (less than 5 years) and tree (more than 5 years). The plant sample were collected from Dapoli, which is located on the west cost of India in the midst of the Western Ghats, at an elevation of 350 meter having a mean annual rainfall of 3300 mm and maximum temperature 24 °C and minimum of 21 °C with a lateritic soil type. The roots (less than 5 mm diameter) from the representative plants were extracted and thoroughly washed under running tap water to remove dust particles. The roots were air dried in shade at room temperature and fined powder was used for extraction by dissolving 50 mg root powder in 5 ml of methanol and sonicated in an ultrasonic bath for 15 min prior to injection in (HPLC High Performance Liquid Chromatography) system.
The standard stock solution of chrysin standard was prepared by dissolving 1 mg of standard Chrysin in 10 ml of methanol to get 10 µg ml^{-1} solution and sonicated for 10 minutes. The working standard solution was prepared into a series of 10 ml standard volumetric flask,

aliquots of 200µL, 400µL, 600µL, 800µL, 1000µL were drawn from 10 µg ml^{-1} stock solution and diluted up to the mark with the mobile phase used, to obtain a concentration range of 2 µg ml^{-1} to 10 µg ml^{-1} respectively. The mobile phase was prepared by mixing acetonitrile and water in volume ratio (36:66) containing 0.1 % TFA and filter through Ultipor Nylon 6, 6 membrane, 0.2 µm filter then sonicated for 15 minutes prior use in HPLC.

The Dionex Ultimate 3000 HPLC with UV detector and C18 column (150mm × 4.6mm, 5µm) was used to detect the presence of chrysin. Injection was carried by using 20 µl loop at 25°C with flow rate 1 ml /min and detection was carried out on 400 nm wavelength (Table 1).

Chrysin content among the three growth stages was analyzed using CPCS 1.0 analytical software using CRD (completely randomized design).

Table 1: Chromatographic conditions of HPLC to evaluate chrysin content

Sr. No.	Test conditions	Result
1.	Elution	Isocratic
2.	Wave length	400 nm
3.	Mobile phase	Acetonitrile: water ratio (36:66) containing 0.1 % TFA
4.	Column	C_{18} column (150mm × 4.6mm, 5µm)
5.	Retention time	2.197 min
6.	Flow rate	1 ml/min

Results and Discussion

It was observed that, the retention time for chrysin was 2.197 min. The calibration equation of chrysin was Y = 1.8162 x + 0.5854 with a correlation factor (r^2) of 0.9924. It was observed that the three growth stages i.e. seedling, pole and tree varied significantly for chrysin content in roots (Table 2). The roots from tree stage contained maximum chrysin (4.5352 µg ml^{-1}) followed by pole roots (4.2259 µg ml^{-1}), whereas minimum value for chrysin content was recorded in seedling roots (3.3148 µg ml^{-1}) (Figure 1-2).

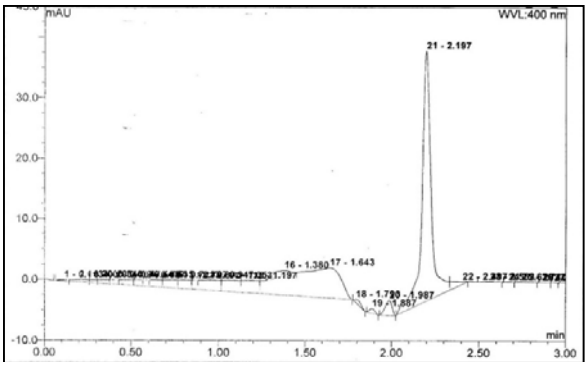

Fig 1: Chromatogram of standard chrysin

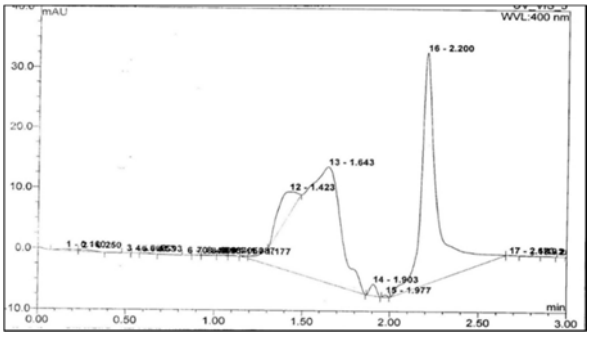

Fig 2: Chromatogram of sample extract from roots of *Oroxylum indicum*

Table 2: Variation in chrysin content at different growth stages

Growth stages	Chrysin Content (µg/ml)
Seedling	3.3148
Pole	4.2259
Tree	4.5352
CD at 5 %	0.5304

Quigley and Mulhall (2002) [2] suggest that phytochemicals concentration may vary according to the age of the root and/or the speed of growth. Sabio *et al*. (2007) [3] carried out experiment on *Radix puerariae* for determination of variation in isoflavonoids at different growth stage (1 to 5 year). They observed that the isofavonoids accumulation increased from second year to third year. At this stage, the plant produces numerous by-products by metabolism. They also observed that after the third year the concentration of isoflavonoids decreased as wood become woodier and produce fewer secondary products so five year plant has lower concentration as compared to third year.

Our study suggests that, there is no appreciable difference in the chrysin content at three different growth stages and this may be attributed to as advancement with the age of plant root become woodier and mature to produce more concentration of chrysin. So for commercial production of chrysin, seedlings can be used rather than mature plants. It will also reduce problems in root harvesting, cost of cultivation, cultural practices and time. It may be recommend that high density plantation of the species can be taken at short rotation to extract the chrysin from root.

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