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Research Article

# HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR QUANTIFICATION OF ELLAGIC ACID IN *IN VIVO* AND *IN VITRO* PLANT PARTS OF *OROXYLUM INDICUM (L.)* VENT

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**ABSTRACT**

**Objective:** This study was performed to investigate the comparative analysis of ellagic acid content in *in vivo* (stem, leaf, and root) and *in vitro* (callus, *in vitro* developed root and shoot) samples of *Oroxylum indicum* (L.) Vent. an important medicinal plant.

**Methods:** For *in vitro* culture, seedling explants were inoculated on MS (Murashige and Skoog's, 1962) medium, supplemented with N6-benzylaminopurine (BAP) and Kn alone and combination with indole acetic acid (IAA). Analytical method high performance liquid chromatography (HPLC) was developed for the quantification of ellagic acid in *in vivo* and *in vitro* samples.

**Result**: BAP combination with IAA was best for shoot multiplication, BAP with 2,4-D were used for callus proliferation. HPLC analysis of these extracts revealed that the quantity of ellagic acid present in both the samples, but maximum ellagic acid content was obtained in leaves among all plant parts.

**Conclusion:** Hence, this study showed the *in vivo* as well as *in vitro* samples contain ellagic acid. That can be used for its large scale production in future.

**Keywords:** *Oroxylum indicum,* Ellagic acid, High performance liquid chromatography.

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## INTRODUCTION

*Oroxylum indicum* (L.) Vent. commonly known as Shyonaka or Sonapatha, a member of Bignoniaceae. All parts of this plant are used in many Ayurvedic preparations. It has anti-inflammatory, diuretic, antiarthritic, antifungal, and antibacterial activity [1]. Previous reports have revealed that stem bark and leaves of this plant contain flavonoids, namely, chrysin, oroxylin-A, scutellarin, baicalein [2,3], and seeds of this plant contain ellagic acid [4]. It has been reported that baicalein possesses an anti-inflammatory [5], antiulcer [6], antioxidant [7], hepatoprotective [8], and immunomodulatory activity [9], while chrysin and baicalein both are reported to have antibacterial, antifungal, and antiviral activity [10,11].

Natural products which are obtained from the medicinal plant are important sources for biologically active drugs. Today the pharmacologically active ingredients of many Ayurvedic medicines are being identified and their usefulness in drug therapy being determined. Phytochemical studies have attracted the attention due to the development of new and sophisticated techniques. These techniques played a significant role in giving the solution to systematic problems and in the search for additional resources of raw materials for pharmaceutical industry [12].

Therefore, the present investigation was performed for quantification of ellagic acid from *in vivo* and *in vitro* samples by high performance liquid chromatography (HPLC) technique which will help in crude drug identification for various pharma industries.

## METHODS

### Plant materials and culture establishment

The plant parts of *O. indicum* (L.) vent were collected during the month of August-September 2012 from botanical garden of Hemchandracharya North Gujarat University (HNGU), Patan, Gujarat, India. The plant material was authenticated and identified from the Department of Botany, HNGU, Patan. *In vitro* plants were grown on MS media supplemented with 30 g/L sucrose and 0.8% agar from seedling explant, *viz*., apical bud, axillary bud, and hypocotyl. Different concentration of N6-benzylaminopurine (BAP) and indole acetic acid (IAA) were used for shoot multiplication and shoot elongation. NAA and IBA alone or in combination were used for rooting while the different concentration of 2, 4 -D, BAP and Kn were used for callus induction.

### Preparation of plant extract

The air-dried finely powdered plant samples (1.0 g each) of *in vivo* and *in vitro* samples, *viz*., *in vitro* root, *in vitro* shoot, and callus were soaked in 10 ml of methanol for 48 hrs at room temperature. The extracts were concentrated at 50°C and filtered through Whatman No.1 filter paper. The supernatants were collected, covered, labeled and used for the screening of quantitative analysis by HPLC method.

### HPLC analysis

HPLC technique was carried out to quantify the flavonoids of selected plant. For this purpose, extract was prepared from *in vivo* and *in vitro* samples using methanol extraction method. Ellagic acid was quantified in methanolic extracts using the HPLC method. The composition and the gradient elution conditions used were described previously [13] with some modification. The separation was achieved by 250×4.6 mm i.d. Symmetry- C18 5 µm column and water:Methanol:acetonitrile:Orthophosphoric acid (60:30:38:1, v/v/v/v) used as a mobile phase. The flow rate was 1.0 ml/minutes (gradient program) at room temperature and injection volume was 10 µL used. Detection wavelength was set at 262 nm. Quantification was made by comparison with standard solutions (from 2.0 to 12 µg/ml) retention times (tR) (minutes) of ellagic acid was 3.29. The quantification of ellagic acid was estimated using calibrated Shimadzu LC-2010 quaternary reversed phase (RP-HPLC) system.

## RESULTS AND DISCUSSION

Callus from hypocotyl and apical bud explants were significantly developed on MS media with 2, 4-D + BAP (2+2, 2+2.5). While highest

shoot length was obtained from apical bud with 3 mg/L BAP +0.3 mg/L IAA combination in MS media (data not shown).

The qualitative phytochemical analysis of *O. indicum* extract showed the presence of biologically active compound like phenol and flavonoids in both the samples *in vivo* and *in vitro* samples [14]. Further identification of ellagic acid has been carried out by HPLC. Ellagic acid is a phenolic compound [15]. It can act as an antioxidant and has been found to cause apoptosis in cancer cells [16]. Seeds, root bark and stem bark of this plant are reported to contain ellagic acid [4,17]. To evaluate the quantification of ellagic acid in *in vivo* and *in vitro* samples of *O. indicum*, a simple rapid and accurate RP-HPLC method was used. Standard ellagic acid (Sigma-Aldrich) was employed for the development of the method. The RP-HPLC system used a base deactivated C18 column with water, methanol, acetonitrile, and orthophosphoric acid as the mobile phase. Authentic standard ellagic acid is being resolved at the Rt 3.29 and almost same Rt was observed in methanolic extract of all the samples. Some unknown peaks were also observed in the extract. The amount of ellagic acid in plant samples was analyzed by comparing with the standard curve with known amount of it (2-12 µg/ml) (Fig. 1).

The chromatographic peaks of the samples of both *in vivo* and *in vitro* (Figs. 2 and 3) were confirmed by comparing their Rt those of the reference standards (Fig. 4). The higher amount of ellagic acid in the leaf sample was found to be 4.28 mg/g. All determinations were carried out at least three times (n=3) for each extract.

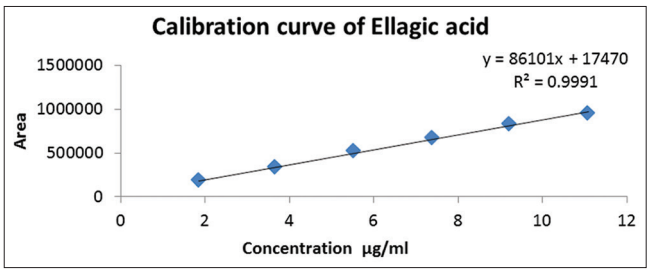


**Fig. 1: Standard curve of ellagic acid**

Phytochemical screenings in terms of quantification of phytoconstituent in the different parts of *in vivo* and *in vitro* were performed using RP-HPLC fingerprint in methanolic extract. Ellagic acid was detected in almost all the samples from plant parts as well as from *in vitro* samples (Fig. 5) by HPLC. Among all the samples, *in vivo* leaf contains highest ellagic acid content, whereas *in vivo* stem bark contain lower values of ellagic acid. There is no significant difference in callus and root bark. All the *in vitro* samples contain ellagic acid. The present finding is also significant as the ellagic acid accumulated in *in vitro* samples. Maitreyi *et al.* [13] reported quantification of ellagic acid in root bark of *O. indicum*, but the contents of ellagic acid in *in vivo* as well as *in vitro* samples have been reported for the first time in this study.

Mostafa *et al.* [18] found that different plant parts at reproductive stages (flowering and fruiting) contained flavonoids (using quercetin as standard) by HPLC. Arya *et al*. [19] found that 6 weeks old callus tissues derived from leaf explants of *Pluchea lanceolata* Oliverr and Hiern. produced high amounts of quercetin. Goswami and Reddi [20] reported quercetin production in cell culture of *Cassia angustifolia*. Sharma and Patni [21] quantified quercetin in *in vivo* and *in vitro* plant parts of *Grewia asiatica* Mast. They found the highest amount in leaf samples than callus.

The ellagic acid was found high in *in vivo* plant parts and it was also reported in all *in vitro* samples. Hence, this result will help to exploit their large scale production in a wide array of promoting benefits.

## CONCLUSION

Phytochemicals are very important in identifying new sources of therapeutically and pharmaceutical industry. This study revealed the presence of phytochemical in *in vivo* and *in vitro* samples. Hence, this

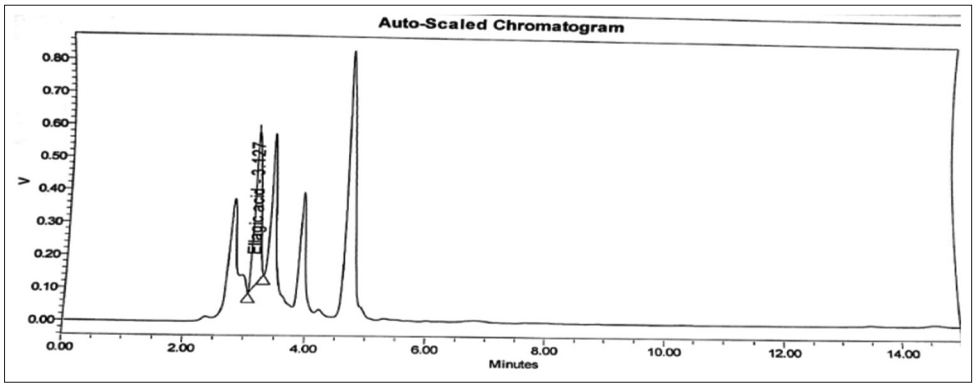


**Fig. 2: High performance liquid chromatography chromatogram of *in vivo* leaf sample**

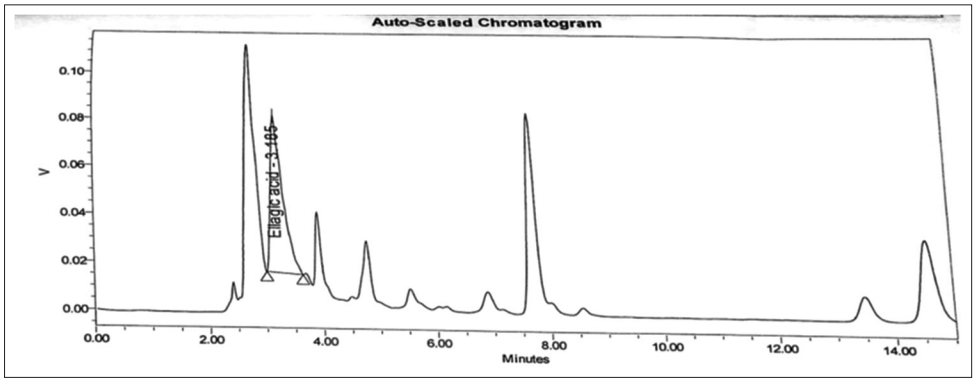


**Fig. 3: High performance liquid chromatography chromatogram of *in vitro* callus sample**

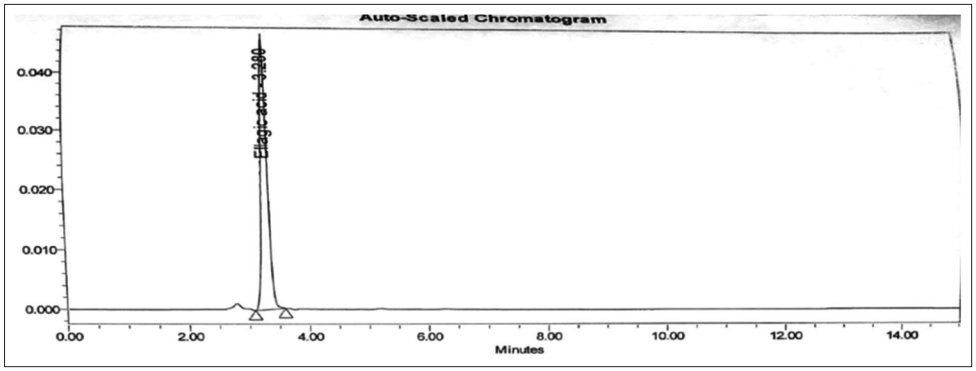


**Fig. 4: High performance liquid chromatography chromatogram of standard ellagic acid**

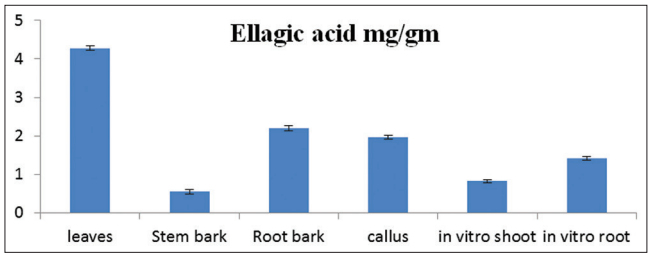


**Fig. 5: Quantification of ellagic acid in *Oroxylum indicum* L. vent**

study describes HPLC method to compare quantification of ellagic acid content in *in vivo* and *in vitro* plant parts. The investigation revealed that *in vivo* and *in vitro* samples contain ellagic acid. The results obtained are important for large scale production by tissue culture.

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