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Research Article

QUANTITATIVE ANALYSIS OF TOTAL PHENOLS AND FLAVONOIDS IN *IN VIVO* AND *IN VITRO* SAMPLES OF *OROXYLUM INDICUM* (L.) VENT

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ABSTRACT

Objective: This paper presents flavonoids and phenol content of methanolic extract of *in vivo* and *in vitro* sample of *Oroxylum indicum* (L.) Vent. an important medicinal plant. Selected plant contains number of phytochemical in its various parts such as stem bark, root bark, and leaves. This study involves quantitative determine of phenolics and flavonoids content using Folin–Ciocalteau and quercetin as standard.

Methods: For investigation of *in vitro* culture, callus was prepared using apical bud and axillary bud as an explants. Explants were cultured on MS medium supplemented with different concentration of 6-benzylaminopurine (BAP), kinetin (KN) and indole-3-acetic acid (IAA) for shoot multiplication and BAP, KN and 2,4- dichlorophenoxyacetic acid 2,4-D for callus induction.

Results: BAP combination with IAA was best for shoot multiplication, BAP with 2,4-D were used for callus proliferation. The flavonoid and phenolics contents in the *in vivo* and *in vitro* plant parts were found to be present in methanolic extract. The results show high phenolic and flavonoids content from *in vitro* root of the plant parts.

Conclusion: There is great variation in secondary metabolites accumulation in different plant parts in both *in vitro* and *in vivo* developed condition.

Keywords: Flavonoid, Phenol, *Oroxylum indicum.*

INTRODUCTION

Medicinal plants are the most important source for plant based drugs. Plants synthesize many compound called primary metabolites, which include protein, fats, and carbohydrates. Plants also synthesize additional compounds called secondary metabolites. The secondary metabolites are known to play a major role in the protection of plant in their environment and also represent an important source of pharmaceuticals [1]. Plant cell cultures are an attractive alternative source to the whole plant for the production of high-value secondary metabolites [2-9]. Hence, the question is, whether the concentration of secondary metabolites is same in the fresh sample as well as *in vitro* samples? Therefore, to find out the answer, the present study was planned.

Oroxylum indicum (L.) Vent. is commonly known as shyonaka or sonpatha is small to medium sized deciduous tree of family Bignoniaceae. It is distributed throughout the India, up to an altitude of 1200 m and found mainly in ravine and moist places in the forests [10] and also in Himalayan foothills, Eastern and Western Ghats and North East India [11]. Different plant parts are used in Ayurveda and traditional medicine. Its different plant parts are used for the different ailments such as fevers, cough, cancer, diarrheas, ulcer, dropsy, jaundice and preventing other respiratory disorders [12]. Root extract of this tree has been used for long in ayurvedic preparations like Dashmularisht and Chyawanprash [13]. It is also one of the important ingredients in ayurvedic formulation such as Amartarista, Dantyadyarista, Narayana taila, Dhanawantara ghrita, Brahma rasayana, Awalwha, Chyavanprasha [14]. This plant possesses antioxidant, antifungal, antimicrobial, anti-inflammatory, antibacterial, anti-arthritic, anti-cancer property [15].

The leaves and stem bark are reported to contain flavonoids namely chrysin, oroxylin-A, scutellarin, baicalein [16,17]. Root bark is reported to contain chrysin, baicalein, biochanin-A and ellagic acid [18]. Seeds are reported to use in perfume industry [19]. Baicalein is reported to possess an anti-inflammatory [20], anti-ulcer [21], antioxidant [22], hepatoprotective [23] and immunomodulatory activity [24]. Baicalein is also reported to check proliferation of human breast cancer cell line MDA-MB-435 [25].

The basic aim of the research was to determine the total phenolic content (TPC) and total flavonoids content (TFC) from the *in vivo* and *in vitro* samples.

METHODS

Plant materials

The leaves, stem bark and root bark of *O. indicum* (L.) Vent. were collected from botanical garden of Hemchandracharya North Gujarat University (HNGU), Patan, in the month of August-September 2012. The plant material was authenticated and identified from the Department of Botany, HNGU, Patan, Gujarat, India.

Methods for callus and *in vitro* plant development

Seeds of *O. indicum* (L.) Vent. were soaked in different plant growth regulators like gibberellic acid (GA3), kinetin (Kn), 6-benzylaminopurine (BAP) and zeatin for overnight. The seeds were surface sterilized in 0.01% (v/v) tween-20 for 2 minutes. Followed by 0.1% (w/v) $HgCl_2$ for 2 minutes. Each treatment was followed by repeated washing minimum of 3 times in autoclaved distilled water. Surface sterilized seeds were inoculated in glass tubes containing MS media supplemented with 30 g/L sucrose and 0.8% agar. After 5-6 weeks, from 8 to 10 cm long seedling, the apical bud, axillary bud, and hypocotyl were used as an explants source. Different concentration of BAP and indole-3-acetic acid (IAA) were used for shoot multiplication and shoot elongation. NAA and IBA alone or in combination were used for rooting while different concentration of 2,4- dichlorophenoxyacetic acid (2,4-D), BAP and Kn were used for callus induction. Each treatment had eight replicates of culture and experiments were repeated thrice. The seed germinated root, *in vitro* developed root, Young (6 weeks) and dark callus (15 weeks) as well as *in vivo* samples like leaves, stem bark and root bark were used for quantitative analysis of phenol and flavonoid.

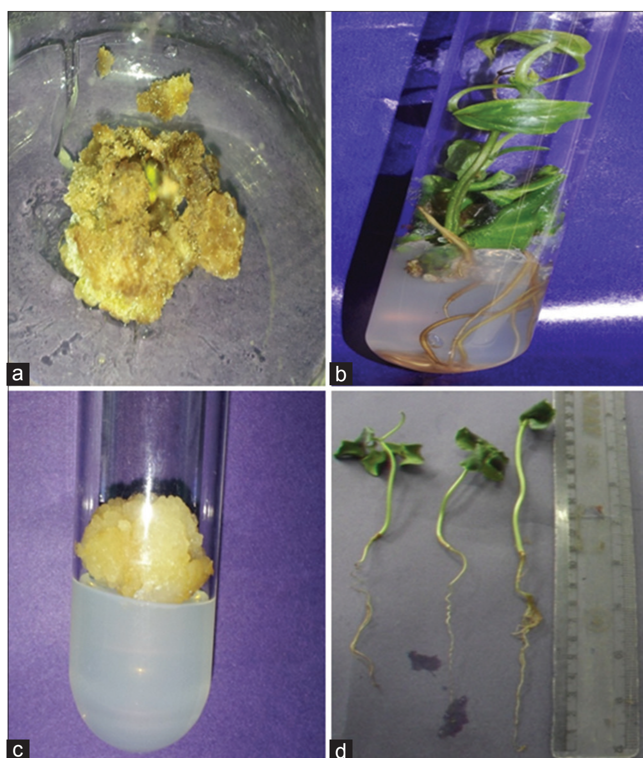

Plate 1: *In vitro* plant parts of *Oroxylum indicum* (a) Dark callus, (b) *in vitro* developed plant, (c) young callus, (d) seed germinated plant

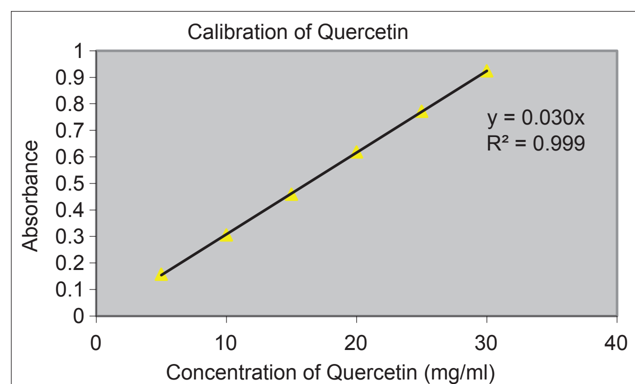

Fig. 1: Standard curve of quercetin for flavonoids determination

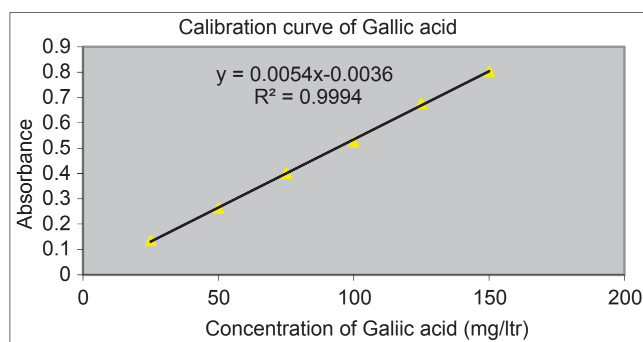

Fig. 2: Standard curve of gallic acid for phenolics determination

Extraction of samples

The freshly collected samples such as leaves stem bark, root bark well as *in vitro* samples viz. Seed germinated root, *in vitro* developed root, dark callus and Young callus Vent. were washed thoroughly with distilled water and air-dried under shade at room temperature for 7-10 days. After drying 5 g each plant powder was extracted in 50 ml of methanol by maceration (48 h). The extract was filtered through Whatman (No 1) filter paper. Each extract was prepared freshly for the analysis.

Total flavonoids determination

The TFC of *in vivo* and *in vitro* plant parts was determined using the aluminum chloride assay through colorimetry [26]. Each plant extracts (0.5 ml of 1:10 g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 minutes; the absorbance of the reaction mixture was measured at 415 nm with an ultraviolet (UV) - visible (UV-1700 pharmaspec, shimadzu, Japan). The calibration curve was prepared by preparing quercetin solutions at concentrations 5-30 g/ml in methanol (Fig. 1).

Total phenols determination

Total phenols were determined by Folin–Ciocalteu reagent [27]. A dilute extract of each plant extract (0.5 ml of 1:10 g/ml) or gallic acid (standard phenolic compound) was mixed with Folin–Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml, 1 M). The mixtures were allowed to stand for 15 minutes and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 25, 50, 75, 100, 125, 150, mg/L solutions of gallic acid (Fig. 2) in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass).

RESULTS AND DISCUSSION

Out of four growth regulators (GA3, Zeatin, BAP and Kn) GA3 had a significant effect on seed germination with all control condition. Hence, various explants *viz.* hypocotyl, apical buds and axillary bud were collected from about 6 to 7 weeks old seedling developed under GA3 treatments and inoculated for callus formation. The results of *in vitro* development show that 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 the apical bud with 3 mg/L BAP + 0.3 mg/L IAA combination in MS media (Plate 1).

TFC

During the present investigation, TFC was found to be maximum in *in vitro* developed root when compared to others *in vivo* and *in vitro* plant parts. TFC of methanolic extract of *O. indicum* (L.) were between 0.192±0.002 mg/g and 1.025±0.002 mg/g. The flavonoid contents of the extracts in terms of quercetin equivalent (the standard curve equation: y=0.030x, R^2=0.999). There is no significant difference between *in vitro* developed root (1.02 mg/g) and *in vivo* root (0.98 mg/g). Leaf showed 0.882 mg/g, stem bark contain 0.576 mg/g, dark callus 0.361 mg/g while young callus contain 0.302 mg/g. Whereas, seed germinated root contain the least amount (0.192 mg/g).

TPC

For the TPC quantification, gallic acid was used as a standard compound, and it was expressed as mg/g gallic acid equivalent using the standard curve equation: y=0.005x−0.003, R^2=0.999. Where y is absorbance at 765 nm and x is total phenolics compound expressed in mg/g. TPC was found highest in also *in vitro* root (12.42 mg/g). Followed by *in vivo* leaf (11.1 mg/g), dark callus (10.59 mg/g), root bark (10.32 mg/g), seed germinated root (3.03 mg/g), stem bark (0.729 mg/g) and young callus (0.675 mg/g). TPC and TFC present in methanolic extract of *in vivo* and *in vitro* plant parts are shown in (Figs. 3 and 4).

Agarwal and Kamal [28], reported the presence of three flavonoids content from *in vitro* parts. Maximum amount of flavonoids was observed in multiple shoots of *Momordica charantia* L. Gawri and Upadhyay [29] were found higher values in callus compare to *in vivo* samples. Arya and Patni [30] were also quantified maximum flavonoids in *in vivo* leaf of *Inpluchea lanceolata* Oliver & Hiern. Whereas quercetin

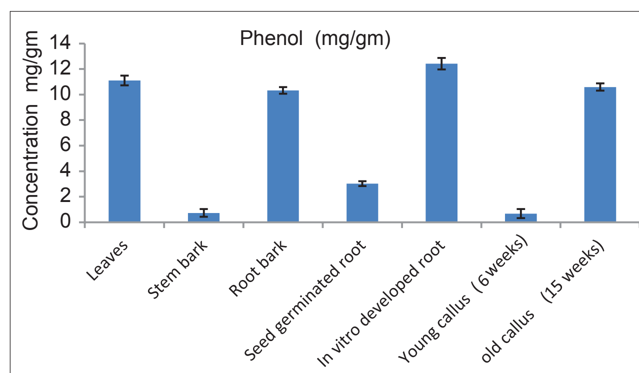

Fig. 3: Quantitative analysis of phenol

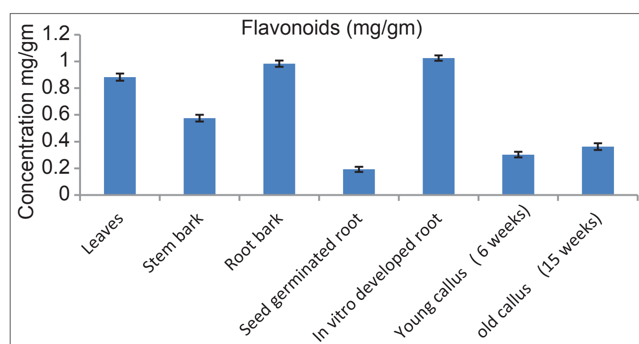

Fig. 4: Quantitative analysis of flavonoids

presence highest in callus. Sharma [31] was reported qualitative and quantitative estimation of flavonoid, found highest in *in vivo* parts compared to *in vitro* samples of *Grewia asiatica* Mast. Hence, all these results show increased production of phytochemicals like phenols and flavonoids but their variation in its accumulation in its different parts.

CONCLUSION

The present investigation revealed that *in vitro* developed root and leaf contain a significant amount of phenol, *in vitro* developed root and *in vivo* root bark contain flavonoids then other plant parts. Least amount of secondary metabolites were found in young callus, although it was also observed in old callus (15 weeks). By this study we can conclude that with the use of tissue culture technique increased secondary metabolites production, it also helps to correlate the relationship of secondary metabolites to biological activities and evaluated *O. indicum* (L.) Vent. as a potential source of bioactive compounds.

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