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Anticancerous Potentiality of *in vivo* Grown Twigs and *in vitro* Generated Plantlets of Endangered *Oroxylum indicum* (L.) Vent.

Nishi Kumari, Rajeev Kumar, Kanak Sinha and Santosh Kumar*
Biotechnology Unit, University Department of Botany, B R A Bihar University, Muzaffarpur

Abstract: *The pharmacological and cytotoxicity evaluation of natural products, extracted from endangered medicinal plants have been reported in several cases. In the present study an attempt was made to evaluate the ethanol extract of both in vivo grown twigs and in vitro generated plantlets of Oroxylum indicum (L.)Vent. with respect to cytotoxicity against human cervical cancer SiHa cells and liver hepatocellular HepG2 cells. MTT assay measured the level of cytotoxicity of ethanol extract of both samples of in vivo and in vitro sources. In later case, plant tissue culture techniques were employed to raise in vitro generated plantlets, for which the nodal regions of in vitro seedling were taken as explants. MS medium, supplemented with BAP (2.5 µg ml^{-1}) proved better than 2,4-D for shoot initiation. Such differentiated shoots on transfer into half strength MS media with IBA (0.5 µg ml^{-1}) resulted into rooted plantlets and were exposed for ethanol extraction after 35 days incubation in acclimatizer room. The dose dependent cytotoxic effect of ethanol extract of in vivo sample in case of SiHa and HepG2 cells on extrapolation resulted into IC_{50} at 440 and 480 µg ml^{-1}, respectively whereas in case of in vitro samples, the IC_{50} for SiHa and HapG2 cells was deducted at 530 and 550 µg ml^{-1}, respectively. Thus, it was confirmed that the cytotoxic effect of natural products of O. indicum was more vigorous by 15% in field plants as compared to tissue culture generated plantlets and such difference could be possibly attributed to the changed habitat. The effect of extract on dying cells was further confirmed when apoptosis was measured by DNA fragmentation on agarose gel.*

Keywords: *Apoptosis, Cytotoxicity, HepG2 cells, Natural products, Oroxylum indicum, SiHa cells.*

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I. Introduction

Oroxylum indicum (L) Vent. has been reported to be an endangered forest tree of medicinal importance. The plant belongs to Bignoniaceae family and commonly known as Sonapatha or Midnight Horror and has been normally reported from moist places inside forests of India, Shri Lanka, Philippines and Indonesia [1]. The natural products from the extracts of medicinal plants, viz., alkaloids, flavonoids, etc., are used to cure bronchitis, jaundice, piles, small pox, leucoderma, cardiac disorder as well as against bacterial and fungal diseases. Recently Mao [2] has contributed the anticancer medicinal values of the plant in tribal. These properties of the extracts of the plant led the stakeholders in the Ayurvedic preparation especially Dashmularist [3]. All these reports have created utmost interest to pharmaceutical industries to explore the ongoing research of the impact of secondary metabolites to cure different ailments [4, 5]. Thus, standardization of natural products for their quality control is required [6]. The development of pharmaceuticals begins with identification of active principles, detailed biological assays and dosage formulations, followed by clinical studies to establish safety, efficacy and pharmacokinetic profile of the new drug [7].

O. indicum in natural population has been categorized as endangered and vulnerable group by the Government of India [8]. Destructive and non-sustainable collections have posed serious threats to the survival and availability of this highly useful tree [3]. The existence of natural, *in vivo* plants are far and few between even in Asian countries, so it is the potential urge to save the plants from its endangered status. Conventional methods for the propagation of plant depend on viable seeds but the low percentage of seed viability seeks alternative methods to preserve their elite genotypes. The endangered attributable character of the plant due to low regeneration, over exploitation and uprooting of whole plants with roots in many states requires mass multiplication and conservation through *in vitro* cultures [9]. It is, thus, imperative to develop *in vitro* plants through the technique of plant tissue culture. Under this impression, though the plant, *O. indicum* belongs to recalcitrant group, efforts have been undertaken to develop *in vitro* plants through the techniques of plant tissue culture.

Plant derived natural substances have recently become of great interest owing to their versatile applications [10]. Therefore, a comparative account of the extracts from the plant was prepared from both sources: *in vivo* growing natural plants and *in vitro* growing plantlets. These comparative accounts may lead to understand the comparative chemical complexity from both sources as well as new approach may be established

for the first time on the efficacy of the extracts of *in vitro* generated *O. indicum* plant. Large scale production of medicinal plant in shortest possible time is pre-requisite for pharmaceutical purpose for which tissue culture techniques can be employed for micropropagation especially for the vulnerable species which owe drug synthesis for chronic diseases. In case of trees which are recalcitrant, *in vitro* techniques, if successfully employed would be highly useful because generation through breeding is more difficult in trees. The long life cycle and the large size of trees such as of *O. indicum* prevents breeding [11].

Considering these principles and the data available through the literatures, the present study aims to establish efficient *in vitro* techniques for *O. indicum* based on the effective role of auxins and cytokinins, either separately or in combination for maximum shoot multiplication. Such attempts leads to formulate comparative evaluation of ethanol extracts on the cytotoxicity of both *in vivo* and *in vitro* samples of *O. indicum* against human cervical cancer SiHa cells and liver hepatocellular HepG2 cells. The study will certainly help in conservation and quality management of an endangered and vulnerable medicinal plant *O. indicum* with anti-cancer properties.

II. Materials and methods

The seeds of experimental plant, *Oroxylum indicum* (L) Vent. (Figure 1i) were collected in the month of January to March 2015 before dehiscence from bushes located behind Sanskrit college, Malighat, Muzaffarpur, India ($26^0$7'N and $85^0$27" E). Actually the capsule i. e. seed pods that hang and curve downward resembled the wings of a large bird. The capsules were furled and winged seeds, present in two layers were taken out. Wings looked papery in texture (Figure 1ii). After removing the wings, the seeds were washed with double distilled water followed by the treatment with 70% ethyl alcohol and 0.1% mercuric chloride for a brief period of 1-2 min. The seeds were again rinsed with sterile distilled water for complete elimination of even traces of these chemicals before they were placed on Murashige and Skoog [12] medium containing sucrose (3%) and agar (0.8%) in tissue culture jars for germination and seedling growth. The pH was maintained at 5.8 before addition of agar and autoclaving was done at 121^0 C for 15 min. The culture jars and tubes were dispensed with 40 and 15 ml medium, respectively.

Seed Germination:

The dewinged seeds were kept in Petri plates on moist filter paper for germination at $25+2^0$ C for 3 days. Since the result was not satisfactory, alternate approach, Tissue Culture MS medium with sucrose (3%) and agar (0.8%), which were sterilized at a pressure of 15 lbs cm^{-2} for 15-20 minute was considered for seed germination and seedling growth. Ten days old *in vitro* seedlings were used to take out nodal explants of about 2 cm with single axillary bud for further culture. The cultures were maintained in tubes and jars by placing in the culture room at 25 ± 2^0 C having 70% humidity. The light intensity of white fluorescent tubes was kept at 2000 lux under the photoperiod of 16 h light / 8 h dark.

Micropropagation:

MS culture medium supplemented with plant growth regulators (2,4-D: 1.0, 1.5; BAP: 1.5, 2.0, 2.5, 3.0; IBA: 1.0, 1.5, 2.0; NAA: 0.5 µg ml^{-1}) individually or in combination was used for micropropagation through nodal region of *in vitro* seedling. The effect of plant growth regulators in MS medium on callus differentiation and callus mediated plantlets was calculated up to 3 subcultures, each of 28 days. Thus differentiated plantlets were transferred to half strength and full strength MS media with IBA (1.0, 1.5, 2.0 µg ml^{-}1). Rooted plantlets were thoroughly washed with running water to remove the adhering agar and planted in jars having sand, soil and vermicompost in 1:1:1 ratio and then placed in acclimatizer room for 35 days before exposing in field.

Preparation of Ethanol Extracts:

The plant parts of *O. indicum* generated through *in vivo* as well as *in vitro* system were subjected for ethanol extraction separately. 1000 mg of plant materials from both sources were grinded into powder form in a pre-chilled mortar and pestle. The plant part powder of both sources was packed in Soxhlet apparatus separately, where 250 ml of ethanol were made to percolate for 24 hours. Later on, the extract was concentrated to dryness under reduced pressure in rotatory evaporator and dried in desiccators. Dark residues were obtained after concentrating the extract under reduced pressure. Thus obtained ethanol extracts of *in vivo* and *in vitro* sources were stored in desiccators for further cytotoxicity investigation.

MTT Assay

Procurement Cell lines and maintenance:

SiHa (Human cervical cancer) and HepG2 (Hepatic carcinoma) cell lines were procured from NCCS Pune, India. Cells were cultured and maintained in Dulbecco's Modified Eagle's Medium (DMEM) with 10% Fetal

Bovine Serum and antibiotics in a humidified atmosphere of 5% CO_2 at 37°C in culture dishes/flasks. Stock culture was maintained in the exponential growth phase by passaging as monolayer culture using in 0.02% EDTA. The dislodged cells were suspended in complete medium and reseeded routinely.

Cytotoxicity assay:

The cytotoxic effect was assessed against SiHa and HepG2 cells at the varying concentrations of plant extracts by the MTT assay. The 3-(4,5-dimethyl-2-yl)-2,5-diphynyl tetrazolium bromide (MTT) is metabolic substrate which is reduced by the mitochondrial succinate dehydrogenase enzyme and forms formazan crystal. In brief, cells were seeded over night at the number of 1×10^4 per well and then incubated with various concentration of extracts for 48 hrs. At the end of the treatment, medium was removed and cells were incubated with 20µl of MTT (5mg ml^{-1} in PBS) in fresh medium (50µl) for 4 hrs in CO_2 incubator. After the treatment formazan crystal, formed by mitochondrial reduction of MTT were solubilised in DMSO (150 µl $well^{-1}$) and the absorbance was read at 570 nm after 10 min incubation on the iMark Microplate Reader (Bio-Rad, USA). Percent cytotoxicity was expressed as IC_{50}.

Detection of DNA fragments into smears

The cancer cell lines used in the present study, SiHa and HepG2, provided another evidence of the anti cancer property in the extracts prepared from *O. indicum*. Both SiHa and HepG2 cells ($1X10^5$) were treated with ethanol extracts at IC_{50} in the well of culture plate. The cells were incubated for 48 hour in the treatment and after 48 hours cells were harvested and pelleted by centrifugation at 10000 rpm in high speed centrifuge. As per the procedure of Sambrook and Russel [13], DNA from ethanol extract treated cancer cells of SiHa and HepG2 were isolated. The procedure required SDS/proteanase K treatment, phenol chloroform extraction and ethanol precipitation, finally it was dissolved and stored in TE buffer. The DNA samples were made to migrate on 1.2% agarose gel electrophoresis. After electrophoresis, the gels were stained with ethidium bromide and visualized as DNA smear under UV light of Gel documentation. ƛ –*Hin*d III DNA was used as marker.

III. Results

Plantlet Regeneration

Initial problem of seed dormancy in germination was overcome by obtaining *in vitro* germinating seedlings in phytohormone free MS medium (Fig. 1iii). Seeds were marked with swollen bases (Fig.1iv) and phenol secretion that caused browning of the seeds (Fig.1v). The explants exuded high amount of phenol during micropropagation, activated charcoal (25µg ml^{-1}) when added on MS medium either alone or in all combination of auxin and cytokinin, secretion of phenol was completely inhibited (Figure 1vi). Such seedling nodal explants with single axillary bud were inoculated on MS medium fortified with 2,4-D (1.0-1.5 µg ml^{-1}) or BAP (1.0-3.0 µg ml^{-1}) to induce multiple shoots. After 12-15 days, basal periphery of seedling nodal explants got swollen but MS medium with 2,4-D in another fortnight could induce only callus proliferations (Fig. 1vii) in 60% explants. Shoot formation was found from these nodal explants on MS medium supplemented with BAP (1.0-3.0 µg ml^{-1}) in 90% cases. However maximum number and height of shoot were achieved in combination of BAP (2.0 µg ml^{-1}) and NAA (0.5µg ml^{-1}). Routine sub-culturing of shoots in the similar medium of BAP (2.0 µg ml^{-1}) and NAA (0.5 µg ml^{-1}) after 4 weeks resulted into elongated shoots (Fig. 1viii). The length of shoot proliferation with an average length of 3.2 cm within 45 days was marked with multiple shoots on sub culturing (Table-1).

Rooting and Hardening

These micro shoots were transferred into half strength and full strength MS medium supplemented with IBA at different concentration (1.0, 1.5 and 2.0 µg ml^{-1}). It was found that within 35 days, the half strength MS medium with IBA (1.5 µg ml^{-1}) was more effective in generating roots (Fig. 1ix) in 70% cases as compared to full strength MS medium (Table 2). Such rooted plantlets having 2-3 small leaves (Fig. 1x) were successfully placed in polythene bags having sand, soil and vermicompost in 1:1:1 ratio. These rooted plantlets were acclimatized in presence of 70% humidity for another 35 days with 16 hour light before finally transferred to the soil. The mortality rate was nearly 60% and the grown up regenerants were morphologically very similar to the plants raised in the field.

Cytotoxicity assay against SiHa and HepG2 Cell lines:

Sufficient amount of extracts of both *in vivo* grown field plant and *in vitro* generated plantlets of *O. indicum*, obtained separately in ethanol solvent with the help of soxhlet apparatus and rotatory evaporator were screened in terms of cytotoxicity for bioactive compounds. The cytotoxicity effect was assayed against SiHa and HepG2 cells at the varying concentration of plant extracts of both *in vivo* and *in vitro* samples of the experimental plants *Oroxylum indicum* by MTT assay (Figure 2). The data of MTT assay were used to extrapolate IC_{50} of the extracts of *in vivo* grown and *in vitro* generated plantlets of *O. indicum* against SiHa and

HepG2 cells. It was 440 and 530 µg ml^{-1} in *in vivo* and *in vitro* samples, respectively against SiHa cells and 480 and 550 µg ml-1 against HepG2 in case of *O. indicum*. It was noticed that the *in vivo* samples were more cytotoxic against SiHa and HepG2 cells as compared to *in vitro* extract samples of the experimental plants. The IC_{50} of *in vitro* samples was less than 17% as compared to *in vivo* samples against SiHa cell line and 12.8% against HepG2 cell line. On comparing these data, it could be summarised that the *in vitro* samples had, on an average 15% less cytotoxic effect on the cancer cell lines as compared to that of *in vivo* samples. This could be attributed to change in their growing habitat.

Validation of apoptosis measurement by DNA laddering

During the investigation, SiHa cells were treated with ethanol extracts and the DNA was directly extracted and was placed on agarose gel electrophoresis. DNA fragmentation was seen as a stepwise ladder of DNA fragments on the gel under Gel Documentation system. The data, represented in Figure 3 proved that DNA laddering was pronounced for ethanol extract at IC_{50} against HepG2 and SiHa cells. These results confirmed that the bio-ingredients present in ethanol extracts could induce apoptosis of HepG2 and SiHa cells. The ethanol extract was found effective against SiHa and HepG2 cancer cell lines since the phenomenon of apoptosis was proved by DNA fragmentation when DNA was isolated from extract treated cancer cells. This further confirmed the effective role of natural products of *O. indicum* towards cancer cell lines.

IV. Discussion

The present investigation deals with anticancer potentiality of the natural chemical constituents present in the ethanol extracts of a very important but endangered plant, *Oroxylum indicum* (L). Vent. The cytotoxicity assay of *in vivo* plant extract confirmed dose dependent growth inhibition of cancer cell lines. Thus, an elaborate MTT assay was carried out against human cervical cancer SiHa cells and liver hepatocellular HepG2 cells. A number of such studies were carried out and the anticancer potentiality of *in vivo* plant extract was compared with that of *in vitro* generated plantlet extracts in *Coleus forskohlii* [14] *Vernonia divergens* [15], *Mucuna pruriens* and *Tinospora cordifolia* [16]. The basis of such studies was on the finding of the exhibition of three peaks in *in vitro* samples as against single peak in *in vivo* sample of the extracts in *Coleus forskohlii* during spectrophotometric scanning within the visible range [14]. Thus the difference in maxima *in vivo* and *in vitro* samples even in *O indicum* (Unpublished data) required *in vitro* organogenesis of the experimental plant to establish comparative potentiality of *in vivo* grown field plant and *in vitro* generated plantlets of *O. indicum* for which the technique of plant tissue culture was adopted.

Seeds are the natural choice in the propagation of angiosperm plants. However, many tree-plants exhibit seed dormancy and do not respond even in favourable conditions. Experiencing the same problem in the present investigation, an alternate technique of Tissue Culture was undertaken. The MS basal medium successfully broke the seed dormancy of *Oroxylum indicum*, first by separating outer seed coat structure followed by swelling [17]. An adverse situation of phenol secretion was noticed during seed germination and micropropagation that caused browning of the seeds or explants. But supplementation of activated charcoal (25µg ml^{-1}) in MS basal medium completely inhibited phenol secretion. The inhibitory action of activated charcoal to prevent exudation of phenols was earlier reported in *O. indicum* [18,19]. Shoot proliferation on MS medium fortified with BAP was also successfully achieved in other members of family Bignoniaceae [20]. The regenerants of shoot developed rooting system on IBA (1.5 µg ml^{-1}) supplemented MS medium but the success was achieved when half strength of MS medium was introduced. Earlier Parmar and Jasraj [21] too have reported the effectiveness of half strength MS as against the full strength with IBA (2.5 µg ml^{-1}) for root induction in *O. indicum*. The difference in concentration could be attributed to the changed habitat as well as of soil profile.

The routine ethanol extraction of both *in vivo* and *in vitro* sources on examination for anti cancer activities added a new candidature for the human welfare along with other drugs in order to inhibit cancer cell proliferation [7]. *In vitro* cytotoxicity assays can be used to predict human toxicity and for the general screening of the chemicals [22, 23] In the present case, the results obtained from the cytotoxicity assay indicate that inhibition of SiHa and HepG2 cells was gradually increased by the addition of ethanol extract in MTT assay. It was a remarkable findings that the cytotoxicity potentiality of *in vivo* and *in vitro* samples of the experimental plant was only 15% whereas it was nearly more than 20% in plants like *Vernonia* [15], *Mucuna* and *Tinospora* [16] which speaks volume of the importance of *Oroxylum indicum.* Morever, the DNA fragmentation in the form of DNA smear on agarose gel of SiHa and HepG2 cells due to apoptosis, which is a physiological process of cell elimination, was an oblique support for the anticancer properties of the chemical constituents present in ethanol extract of *O. indicum.* Thus, the present findings may be in confirmation with possible applications of drug formulaion from *Oroxylum indicum* for cancer prevention as conceived by Chiu and Wu [24].

V. Conclusion

The present study demonstrated that the natural bio constituents of ethanol extract of *Oroxylum indicum* was effective against cancer cells with a difference in *in vivo* and *in vitro* samples. Further research of clinical proof through *in vitro* system on animal models may resolve the *in vivo* efficacy of natural products of *O. indicum.*

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References

[1]. Bennet SSR, Gupta PC and Rao RV. Renerated plants. ICFRE Dehradun.1992; 147-149.
[2]. Mao AA. *Oroxylum indicum* vent –A potential anticancer medicinal plant. India Journal of traditional knowledge . 2002; 1(1): 17-21
[3]. Yasodha R, Ghose M, Santan B, and Gurumurthi K. Importance of biotechnological research in tree species of Dashmula. Indian Forest. 2004; 130: 79-88.
[4]. Latha C, Shriram VD, Jahagirdar SS, Dhakephalkar PK and Rojatkar SR. Antiplasmid activity of 1- acetoxychavicol acetate from *Alpinia galanga* against multi resistant bacteria. Journal of Ethnopharmacol. 2009;123: 522-525
[5]. Pavitra PS, Sreevidya N and Verma RS. Antibacterial activity and chemical composition of essential oils of *Pamburus missionis*. Journal of Ethnopharmacol. 2009; 124: 151-153.
[6]. Jadhav RB, Patil CR, Bhpoe S, Murumkar CV. Herbal drug standardization and quality assurance of raw materials: a rational approach. Natural Product Rad. 2003; 2:134-140.
[7]. Mehdi SJ, Ahmad A, Irshad M, Manzoor N and Rizvi MMA. Cytotoxic effect of carvacrol on human cervical cancer cells. Biology and Medicine. 2011; 3(2): 307-312.
[8]. Ravikumar K and Ved DK. One hundred red listed medicinal plants of conservation concern in southern India. Foundation for revitalization of local health traditions, Bangalore India. 2000.
[9]. Jayram K and Prasad MNV. Genetic diversity in *Oroxylum indicum* (Linn.) Vent. (Bignoniaceae), a vulnerable medicinal plant by random amplified polymorphic DNA marker. African Journal of Biotechnology. 2008; 7(3): 254-262.
[10]. Khan A, Ahmad A, Manzoor N and Khan LA. Antifungal activities of *Oscimum sanctum* essential oils and its lead molecules. Natural Product communications. 2000; 5(2): 345-349
[11]. Dalal MV and Rai VR. *In vitro* propagation of *Oroxylum indicum* Vent. A medicinally important forest tree. Journal of Forest Resources. 2004; 9: 61-65.
[12]. Murashige T, and Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant. 1962; 15:473-497.
[13]. Sambrook J and Russell DW. Molecular cloning : A laboratory manual. Third edition. Cold Spring Harbor Laboratory Press, Plainview, NY. 2001.
[14]. Kumar S, Prakash A, Vohra R and Verma AK. Evaluation of anticancer activities of ethanolic extracts of *in vivo* and *in vitro* grown *Coleus forskohlii* briq. Perspective in Cytology and Genetics. 2011; 15: 19-30.
[15]. Kumar R, Prakash A, Kumari S, Utkarshini, Kumari N, Kumar R, Sinha K and Kumar S. Cytotoxicity of Ethanol extract of *in vivo, in vitro* and biotized grown plants of *Vernonia divergens* on EAC cell lines. International Journal Pharmacognosy and Phytochemical Research. 2014; 6(4): 678-684
[16]. Kumar R. Antibacterial, antidiabetic and anticancer activities of natural products of some medicinal plants of Muzaffarpur distt. Ph D thesis; 2018: B R A Bihar University, Muzaffarpur.
[17]. Amusa TO. Effect of three pre treatment techniques on dormancy and germination of seeds of *Afzelia Africana.* Journal of Horticulture and Forestry.2011; 3(4): 96-103
[18]. Dennis TT.. The role of activated charcoal in plant tissue culture. Biotechnology advances. 2008; 26(6): 618-631.
[19]. Bansal YK and Gokhale M. Effect of additives on micropropagation of an endangered medicinal tree *Oroxylum indicum* (L) vent. Intechopen. Com .2012 ; 183-196
[20]. Shukla S, Shukla SK and Misra SK.In vitro plant regeneration from seedling explants of *Sterospermum personatum* D C. A medicinal tree. Trees. 2009; 23: 409-413
[21]. Parmar VR and Jasrai YT. Effect of cytokinin and auxin on micropropagation of *Oroxylum indicum* (L) Vent: A mountain tree of India. International Journal of Life Science Research. 2014; 2: 58-64.
[22]. Clemedson C and Ekwall. Over view of the final MEIC results. The in vitro- in vitro evaluation. Toxicology in vitro. 1999; 13: 657-663
[23]. Scheer ME, Ekwall Ba and Dierickx JP. In vitro long term cytotoxicity testing of 27 MEIC chemicals on hepG 2 cells and comparision with acute human toxicity data.Toxicology *in vitro*. 2001; 15: 153-161.
[24]. Chiu HF and Wu YC. Cytotoxic constituents of the stem bark of *Neolitsea acuminatissima*. Journal of Natural Products. 2002:65: 255-258.

Table No. 1: Effect of cytokinin (2,4-D; BAP) and auxin (NAA) on shoot multiplication of *Oroxylum indicum* through nodal explant, grown on MS medium

Growth regulators (µg ml-1)			No. of shoot per explant	Shoot length (cm)	Response (%)
2,4,D	BAP	NAA			
1.0	-	-	Callus	NR	50
1.5	-	-	Callus	NR	70
-	1.5	-	1.0±0.72	0.9±0.3	38
-	2.0	-	1.0±0.64	1.2 ±0.3	56

-	2.5	-	2.5±0.92	1.5 ±0.4	72
-	3.0	-	1.2±0.88	0.5 ±0.17	65
-	2.0	0.5	2.2±0.80	1.5 ±0.45	74
-	2.5	0.5	2.8±0.58	2.8±0.80	90
-	3.0	0.5	2.1 ±0.7	2.2 ±0.5	58

Table No. 2: Root induction on *in vitro* generated shoot of *Oroxylum indicum*

Medium strength	Growth regulator IBA (µg ml-1)	No. of roots per shoot	Root length (cm)	Response %
½ MS	1.0	1.0±0.32	1.2±0.1	50
	1.5	1.4±0.72	1.8±0.8	70
	2.0	1.0±0.28	1.0±0.2	40
MS	1.0	0.6±0.22	0.8±0.4	28
	1.5	1.0±0.44	1.0±0.6	50
	2.0	0.8±0.34	0.8±0.4	30

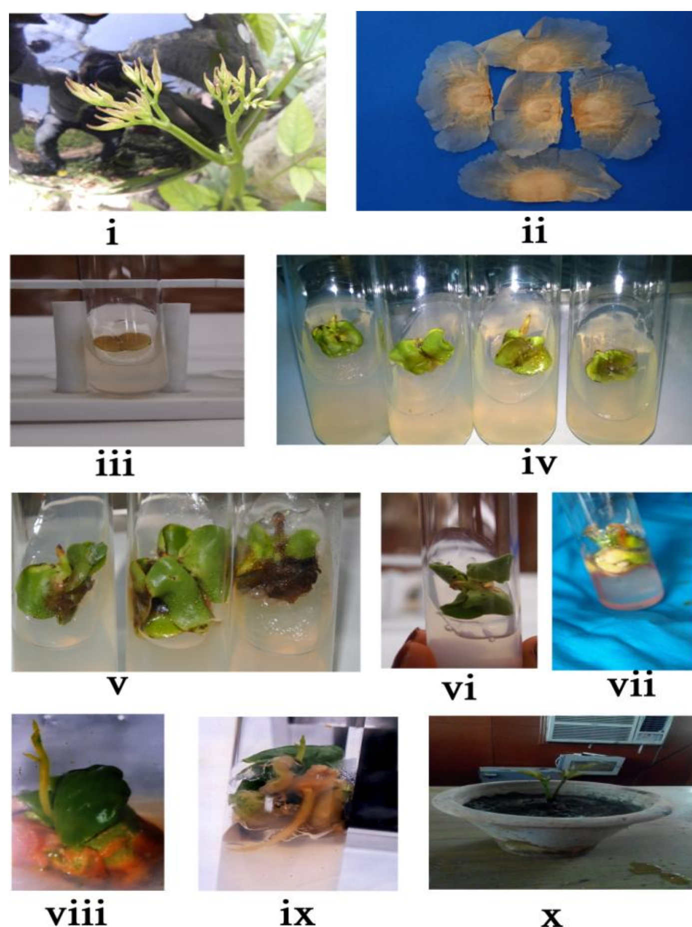

Figure 1: (i) A twig of *Oroxylum indicum*, grown in field (ii). Winged seeds of Oroxylum indicum
(iii) Seed in MS basal medium (iv) Seeds in MS medium with swollen base
(v) Phenol secretion leading to browning of seeds and medium
(vi) Complete inhibition of phenol by activated charcoal (25µg ml^{-1})
(vii) Callus proliferation from nodal explant (MS + 2,4-D)
(viii) Differentiation of elongated shoot (MS + BAP+ NAA)
(ix) Differentiation of root in on shoot (1/2 MS + IBA)
(x) Rooted plantlets of *Oroxylum indicum* with a pair of leaf in pot after acclimatization.

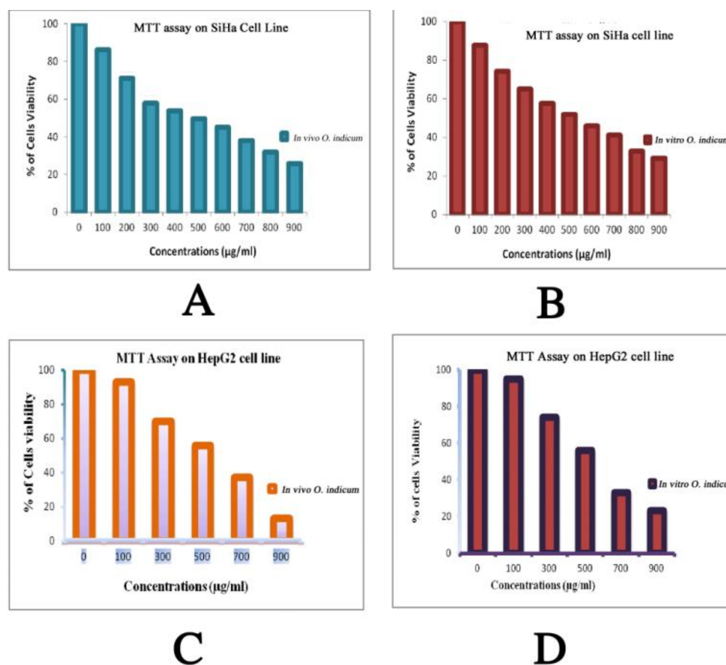

Figure 2: Dose dependent effect of ethanol extract on % viability of SiHa and HepG2 cells.
(A) SiHa cells treated with 100 to 900 µg ml^{-1} of *in vivo* samples of *O. indicum*
(B) SiHa cells treated with 100 to 900 µg ml^{-1} of in vitro samples of *O. indicum*
(C) HepG2 cells treated with 100 to 900 µg ml^{-1} of *in vivo* samples of *O. indicum*
(D) HepG2 cells treated with 100 to 900 µg ml^{-1} of *in vitro* samples of *O. indicum*

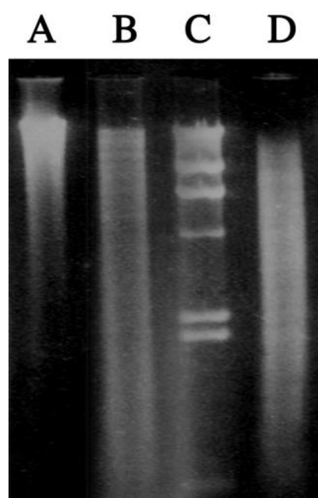

Figure 3: Agarose gel electrophoresis of DNA isolated from cancer cells after treatment with ethanol extract of *O. indicum*
(A) Total DNA from untreated SiHa cells
(B) DNA smear of treated SiHA cells treated
(C) ƛ –*Hin*d III DNA was used as marker
(D) DNA smear of treated HepG2 cells

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