COMPARATIVE PHYTOCHEMICAL ANALYSIS OF IN VITRO AND IN VIVO PLANT PARTS OF OROXYLUM INDICUM (L.). VENT. – AN ENDANGERED MEDICINAL PLANT

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ABSTRACT
Oroxylum indicum (L.) Vent. is used extensively in the preparation of both traditional and modern medicines. In present study, comparative account of phytochemical screening of methanolic extract was performed in in vivo plant parts like leaf, stem bark and root and in vitro developed Callus. Callus was cultured on Murashige and Skoog (MS) medium supplemented with BAP (2mg/L) and IAA (0.2mg/L). Result obtained from in vivo and in vitro phytochemical screening of methanolic extract shows the presence of flavonoids, alkaloids, saponin, tannins, glycosides, phenol, that show wide medicinal properties. Root bark shows better accumulation of secondary metabolites in any other plant parts and callus.

Key Words: Oroxylum indicum (L.) Vent., phytochemicals screening, TLC analysis.

INTRODUCTION
Medicine plant has been proved to be the most useful in treatment of many diseases since from ancient time as they provide an important source of most of the drugs in the world. Medicinal herbs are moving from fringe to mainstream use with a great number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals[1].

Plant produces a variety of secondary metabolites that have been a source of medicine. These metabolites are being used, as precursors or as lead compound in the pharmaceutical industry for making various drugs against many diseases.

Oroxylum indicum (L.) Vent. Family Bignoniaceae is commonly called as Sonapatha, Syonaka and Midnight horror which is widely found in India, shri lanka, china, Philippines and Malaysia [3]. This plant is a very important herbal medicine in many Asian countries and is used in traditional medicine as a cure of various diseases [3]. This plant possesses a flavonoid viz. Baicalein used to check proliferation of human breast cancer cell line MDA - MB - 435 [4]. This plant possesses antioxidant, antifungal, antimicrobial, anti-inflammatory, antibacterial, anti-arthritis, anti-cancer property [5]. Root extract of this tree has been used for long in Ayurvedic preparations like Dashmularisht and Chyawanprash [6]. It is also one of the important ingredients in ayurvedic formulation such as Amartarista, Dantyadyarista, Narayana taila, Dhanawantara ghrita, Brahmas rasayana, Awalwha, Chyavanprasha [7]. Because of its indiscriminate collection and over exploitation for medicinal purpose has pushed this plant to the list of endangered plant species of India [8]. The present investigation is aimed at screening both in vivo and In-Vitro developed samples for their biological compound.
MATERIALS AND METHODS

Collection of plant samples
The leaves, stem bark and root bark of Oroxylum indicum (L.) Vent. were collected from botanical garden of Hemchandracharya north Gujarat University (HNGU), Patan, in the month of August, 2012. The plant material was authenticated and identified in the Department of Botany, HNG University, Patan, Gujarat, India. Callus was developed from plant parts viz. apical bud, axillary bud by culturing it on MS media.

Method for callus development
Apical bud and axillary bud of Oroxylum indicum (L.) Vent. were rinsed with distilled water and sterilized with 0.1% mercuric chloride for 40 to 50 seconds. Following rinsing with sterile distilled water about 3 times. Explants were cut aseptically in small piece (1.0-1.5 cm) with the help of sterilized scalpels. Explants were cultured in sterile culture tubes containing 10 ml of MS media supplemented with BAP (1, 2, 3 mg/L) and IAA, 0.2 mg/L. Cultures tubes were incubated at 25±2°C with photoperiod of 16 h in light and 8 h in darkness inside the culture room. The initiated callus was subsequently sub-cultured into a fresh MS media.

RESULTS AND DISCUSSION

Best callus initiation and multiplication of callus was found in MS medium supplemented with BAP (2 mg/l) and IAA (0.2 mg/l) (Table 1). After ignition, color of callus light cream which turned in to brown after shifting it in to the multiplication media. The intensity of the brown color was dark as with respect to the culturing period. After extraction, the extracts were subjected to qualitative phytochemical tests. The preliminary phytochemical screening of in vivo and in vitro samples of this plant revealed the presence of many bioactive compounds in it different parts as shown in Table-2.

Leaf extract
Phytochemical analysis shows the presence of alkaloids, flavonoids, tannins and phenols, saponins, sterols and complete absence of quinines and glycosides in leaf part.

Stem Bark extracts
Stem Bark is found to contain ellagic acid also contain different flavonoids. Phytochemical screening shows the presence of of alkaloids, flavonoids, tannins, sterols, saponins and phenols, similar result were reported and complete absence of quinones and glycosides in stem bark.

Root bark extracts
Root extract gives positive result for the presence of flavonoids, alkaloids, glycosides, tannins, phenols, sterols, saponins while quinines was completely with same hormone concentration and combination up to 6 weeks and then used for screening.

Preparation of plant materials
The freshly collected samples as well as callus were washed thoroughly with distilled water and air-dried under shade at room temperature for 7-10 days. After drying, the samples were reduced to small piece; material was ground in to fine powder using mortar-pestle then sieved using a muslin cloth. Powdered samples were then stored in air tight containers for further use.

Preparation of extract
The air-dried finely powdered plant samples (2.0 g each) were soaked in 20 ml of methanol for 24 hr. at room temperature. The extracts were filtered through Whatman No.1 filter paper. The supernatants were collected, covered, labelled and used for the screening of various phytochemicals.

Phytochemical analysis
The phytochemical analysis of methanolic extract of the dry leaves ,stem bark and root bark as well as callus were carried out to determine the presence of following bioactive compounds using the standard qualitative procedures. The presence of bioactive compounds indicates the medicinal value of the plants. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against various diseases. The phytochemical screening of leaf, stems bark, root bark and callus contain most of the secondary metabolites analyzed. Phytochemical study on Oroxylum indicum has been earlier reported.

Callus
In vitro developed Callus from apical bud explants cultured after 6 weeks shows the result like in vivo samples. Callus gives positive result for alkaloids, flavonoids, tannins, phenols, sterols and glycosides and completely absence of quinone. Several compounds has been identified from different parts of the plant, however in current screening step, certain important metabolites are shown to be a present in not only reported plant parts but also in vitro developed callus.

TLC analysis
TLC analysis showed one similar spot with Rf value 0.68 in both in vivo and in vitro samples. Leaf, root bark and callus give same Rf value 0.09. one additional Rf value 0.54 is present in shoot bark, root bark. Stem bark give Rf value 0.08 which is near
with 0.09. Leaf and root bark gives R₄ value 0.09. TLC profile of methanol extract in optimized solvent systems of Oroxylum indicum bark is summarized in Table-3.

Table 1: Effect of growth regulators on in vitro regeneration from explants of Oroxylum indicum (L.) Vent.

<table>
<thead>
<tr>
<th>Explant</th>
<th>Growth regulators(mg/L)</th>
<th>BAP</th>
<th>IAA (mg/L)</th>
<th>Callus initiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical bud</td>
<td></td>
<td>1</td>
<td>0.2</td>
<td>- + +</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>+ ++ +++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td>+ + ++</td>
</tr>
<tr>
<td>Axillary bud</td>
<td></td>
<td>1</td>
<td>0.2</td>
<td>+ + +</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>+ + ++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td>+ + +</td>
</tr>
</tbody>
</table>

+; low concentration, ++; moderate concentration, +++; high concentration, -; absent

Table-2: Phytochemical analysis of in vivo and in vitro sample of Oroxylum indicum.

<table>
<thead>
<tr>
<th>Test for phytocompound</th>
<th>Name of test</th>
<th>Name of sample</th>
<th>Leaf</th>
<th>Stem bark</th>
<th>Root bark</th>
<th>Callus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Dragendorff</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannin &amp; Phenol</td>
<td>Gelatin</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FeCl₃</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Lead acetate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Alkaline reagent test</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mg ribbon test</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Lead acetate</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Legal’s test</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kellar kiliani test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sterol</td>
<td>Libermann Burchard test</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salkowski test</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>
Table-3 $R_f$ value of *in vivo* and *in vitro* samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>$R_f$ Values</th>
<th>$R_f$ Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At 254 nm</td>
<td>At 366 nm</td>
</tr>
<tr>
<td>Leaf</td>
<td>0.09, 0.17, 0.43, 0.68, 0.77</td>
<td>0.09, 0.17, 0.35, 0.43, 0.54, 0.68</td>
</tr>
<tr>
<td>Stem bark</td>
<td>0.08, 0.14, 0.54, 0.68</td>
<td>0.08, 0.12, 0.54, 0.68</td>
</tr>
<tr>
<td>Root bark</td>
<td>0.09, 0.17, 0.54, 0.68</td>
<td>0.09, 0.12, 0.32, 0.54, 0.68</td>
</tr>
<tr>
<td>Callus</td>
<td>0.07, 0.09, 0.12, 0.68</td>
<td>0.04, 0.07, 0.09, 0.68</td>
</tr>
</tbody>
</table>

Figure 1: 6 weeks callus initiated from apical bud respectively

Figure 2: 8 week callus

Fig. 3  TLC plate showing spots under 366 nm

Fig. 4  TLC plate showing spots under 254 nm
CONCLUSION
The result from present study revealed that the methanolic extract of both in vivo and in vitro samples of *Oroxylum indicum* (L.) Vent. have significant amount of phytochemicals. So, it can be used for the treatment of various diseases. Thus, we can conclude that production of secondary metabolites can be obtained by using tissue culture technique without harm to the species.

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