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Antiproliferative effect of aqueous bark extract of *Oroxylum indicum* L. on *Vigna radiata* L. (Green gram) seedlings

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ABSTRACT

The present investigation was carried out to evaluate the antiproliferative activity of aqueous bark extract of *Oroxylum indicum* L. on *Vigna radiata* L. (Green gram) seedlings. For different concentrations of plant extract (250, 500, 1000 and 2500 µg/mL) were treated in a 24 well microplate containing the *Vigna radiata* L. seeds (n=30) for 24, 48 and 72 hours. Colchicine (50 µg/mL) was used as standard drug. Seed germination and seedlings growth were measured. Mitotic index was calculated for the proliferation of cells. The treatment with plant extracts significantly inhibits the germination of seeds, roots and shoots growth, and reduced the mitotic index in meristem ells of *Vigna radiata* L. at dose and time dependent manner. Qualitative phytochemical screenings of the aqueous stem bark extract of *Oroxylum indicum* L. revealed the presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenolic compounds and flavonoids. Saponins were found to be in high concentration in the extract.

Keywords: *Oroxylum indicum* L., Colchicine, Mitotic index, Phytochemicals, *Vigna radiata* L.

INTRODUCTION

The disease cancer is a major global health issue and the antiproliferative pharmacological efficacy of plant derived secondary metabolites appear to explain the chemo preventive or anticancer effects [1]. Some of the effective anticancer and anti-neoplastic drugs target on the cell cycle progression machinery [2]. Various plants derived active compound act as antitumor and an apoptosis inducer in cancer cells [3]. The finding of effective anticancer agents like vinblastin and vincristine isolated from *Catharanthus roseous* and paclitaxel from the *Taxus brevifolia* provide convincing evidence that plants are a major source of novel anticancer chemotherapeutic agents [4]. Therefore, the interest exists to explore the competent antiproliferative agents from the natural products of plant origin.

The plant *Oroxylum indicum* L. (Family: Bignoniaceae) is a medicinally important forest tree species and is frequently reported to be used in traditional health practices [5-7]. It is native to the Indian sub-continent, in the Himalayan foothills and Eastern and Western Ghats [8], and extends into the Southern China, Indo-China and the Malaysia ecozone [9]. The plant has been used in Indian traditional Ayurvedic formulations [10-11]. *Oroxylum indicum* L. also possesses strong hepatoprotective [12], antiprostatic hyperplasia [13], antioxidant [14], anti-inflammatory [15], antiproliferative [16-17], antitumor [18-20], and gastroprotective [21] activities. Taking this information into account, the present work was carried out to evaluate the antiproliferative effect of aqueous stem bark extract of *Oroxylum indicum* L. on *Vigna radiata* L. (Green gram) seedlings.

MATERIALS AND METHODS

Drugs and chemicals

Colchicine, ethanol, methanol, glacial acetic acid and acetocarmine were obtained from obtained from MERCK, Mumbai, India. Other chemicals used in the present study were of analytical grade and purchased from the reputed manufacturers.

Collection and preparation of plant extract

The plant *Oroxylum indicum* L. was collected on 25th March, 2013 from the Rajiv Gandhi University campus, and identified with the help of taxonomist from the Department of Botany, Rajiv Gandhi

University. The voucher specimen (LBC/RGU/2013/01) was deposited in the unit of Plant Systematic and Pharmacognosy Research Laboratory, Centre with Potential for Excellence in Biodiversity (CPEB), Rajiv Gandhi University for future reference. The fresh stem bark of *Oroxylum indicum* L. was washed, shade dried, powdered. 10 g of powder was dissolved in 100 ml of double distilled water for overnight, filtered and kept at -20 °C for further use. Working dilutions of all drugs were made in distilled water.

Experimental plant model

Vigna radiata L. (Green-gram; Family: Fabaceae) was used as plant model. Good quality and free from any external injury *Vigna radiata* L. (Green-gram) were purchased from the local market. Green-gram seedling root apical meristem cells were used to determining the cell cycle delay and metaphase arresting activity for the validation of antiproliferative activities. Colchicine (50µg/mL) is used as standard drug for the growth retardation of green-gram seedlings.

Treatment of *Vigna radiata* L. (Green-gram) seedlings

Germination test of *Vigna radiata* L. was performed according to ISTA (International Rules for Seed Testing) rules 2018 [22]. For the germination of seeds, four different concentrations of aqueous stem bark extract of *Oroxylum indicum* L. (250, 500, 1000 and 2500 µg/mL) were placed in a 24 well microplate and *Vigna radiata* L. seed (n=30) was dropped into each well. The microplate was then covered with a lid and allowed to germinate at room temperature. Colchicine (50µg/mL) treated group served as positive control and tap water alone in a microplate was considered as negative control. The dosage of colchicine was based on previous study [23]. Seed germination and root length were measured at 24, 48 and 72 hour intervals both in control and test groups. The shoot growth microphotography was taken by using Nikon D3200 camera. The seeds that did not germinate were simply discarded and no other parameters could be measured on these seeds.

Mitotic index (MI) analysis

The analysis of total cell counted, total cell in mitotic stage and mitotic index (MI) were determined by our previous report [24]. Two doses of aqueous stem bark extract of *Oroxylum indicum* L. (250 and 2500 µg/mL) were selected for the mitotic index analysis of *Vigna radiata* L. root apical meristem cells at 0, 24, 48 and 72 hour intervals. The roots were cut and length were measured with standard scale and then fixed in Carnoy's fixative (aceto: methanol, 3 part methanol: 1 part acetic acid) for 24 hours. The root tips were then passed through 100%, 90% and 70% alcohol and preserved in 70% absolute alcohol for mitotic index analysis. The root tips were then passed through 50% and 30% alcohol up to distilled water. The apical root tips were separated and stained in 2% aceto-carmine and squashed under a cover slip. Slides were studied under the bright field light microscope at 400x, using Leica DM5000B ((Leica Microsystems, Germany) microscope for cell cycle phase (interphase, prophase, metaphase, anaphase, and telophase) observation. Each experimental group of slides, 500-600 cells were analyzed, calculating the total number of cells in whole cell cycle and total number of cells in mitotic stage of *Vigna radiata* L., and thereby determining the mitotic index (MI).

The Mitotic index (MI) was calculated by the following equation:

$$\text{Mitotic Index} = \frac{\text{Number of cell in mitotic stages}}{\text{Total number of cells counted}} \text{ X } 100$$

Phytochemical analysis

The phytochemical analysis of the aqueous stem bark extract of *Oroxylum indicum* L. was carried out to determine the presence of secondary metabolites like alkaloids, carbohydrates, glycosides, proteins and amino acids, saponins, phytosterols, phenolic compounds and flavonoids, and oils and fats, using the standard qualitative procedures [25-27].

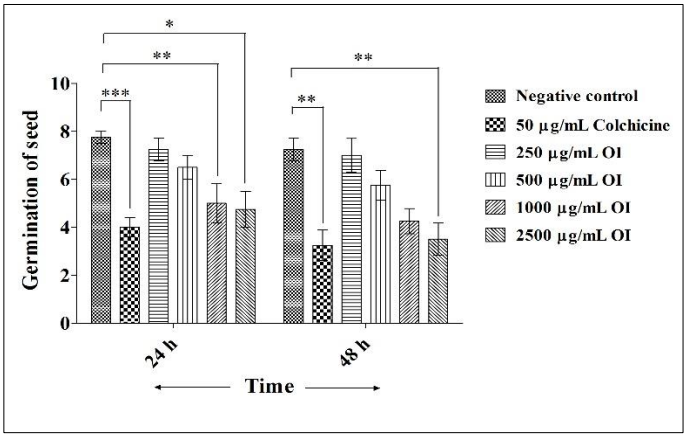
Statistical analysis

All data were expressed as mean ± SEM (n=30). Statistical analysis is done by two-way ANOVA followed by Bonferroni's post test using Graph pad Prism 5.0 (Graph Pad software, San Diego California, USA) software. The level of significance was set at P <0.05.

RESULTS

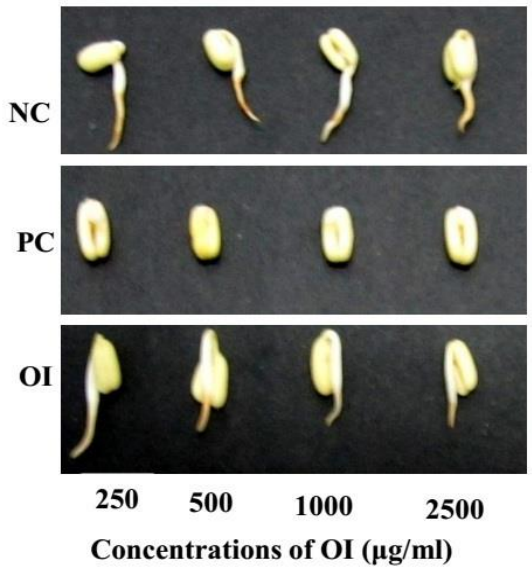
Effect on seed germination, root and shoot growth retardation

The aqueous stem bark extract of *Oroxylum indicum* L. investigated in this study revealed a dose and time dependent inhibitory effects on seed germination, shoot growth and root length in *Vigna radiata* L. At higher concentration of *Oroxylum indicum* L. bark extract (2500 µg/mL) significantly (P< 0.05; P< 0.01) inhibited seed germination at time dependant manner compared to the negative control of respective groups (Figure 1). 250 µg/mL of *Oroxylum indicum* L. bark extract treated group also significantly (P< 0.01) inhibits the germination of green gram seeds at 24 hours of treatment when compared with the negative control (Figure 1). Colchicine (50 µg/mL) induced group significantly (P< 0.001; P< 0.01) decreased the germination of seeds, both in 24 and 48 hours' time interval when compared to the respective negative controls (Figure 1). Treatment with aqueous bark extract of *Oroxylum indicum* L. reduced the shoot growth of green gram at dose and time dependently. While colchicine treated group showed the complete retardation of shoot growth (Figure 2).



NC: Negative control, PC: Positive control (Colchicine 50 µg/mL), OI: *Oroxylum indicum* L. aqueous stem bark extract (250, 500, 1000 and 2500 µg/mL) treated group. Values were expressed as mean ± SEM (n=30). Statistical analysis is done by two-way ANOVA followed by Bonferroni's post test. Level for significance was set at P<0.05. Significant at $^{***}P$<0.001, $^{**}P$<0.01 and $^{*}P$<0.05 compared to negative control.

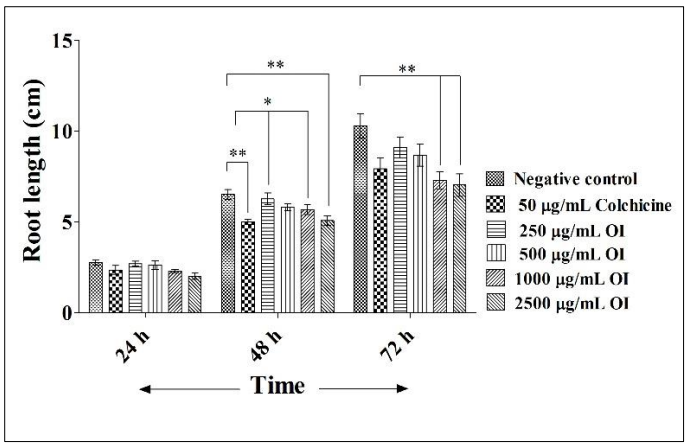
Figure 1: Effect of aqueous stem bark extract of *Oroxylum indicum* L. on the seed germination of *Vigna radiata* L.



NC: Negative control, PC: Positive control (Colchicine 50 µg/mL), OI: *Oroxylum indicum* L. aqueous stem bark extract (250, 500, 1000 and 2500 µg/mL) treated group. Photographed by using Nikon D3200 camera.

Figure 2: Representative photomicrographs of shoot growth of *Vigna radiata* L.

High concentration of *Oroxylum indicum* L. bark extract (2500 µg/mL) treatment significantly ($P< 0.01$) reduced the root length of green gram seedlings time dependently when compared to the negative control of the respective groups (Figure 3). Treatment with 1000 µg/mL of *Oroxylum indicum* L. bark extract showed a significant effect ($P< 0.05$; $P< 0.01$) on the root apical meristem growth of green gram seedlings at 48 and 72 hours time interval (Figure 3). Similarly, colchicine treated group also reduced the root length in a time dependant manner.



NC: Negative control, PC: Positive control (Colchicine 50 µg/mL), OI: *Oroxylum indicum* L. aqueous stem bark extract (250, 500, 1000 and 2500 µg/mL) treated group. Values were expressed as mean ± SEM (n=30). Statistical analysis is done by two-way ANOVA followed by Bonferroni's post test. Level for significance was set at $P<0.05$. Significant at $^{**}P<0.01$ and $^{*}P<0.05$ compared to negative control.

Figure 3: Effect of aqueous stem bark extract of *Oroxylum indicum* L. on the root length of *Vigna radiata* L.

Effect on mitotic index

The findings indicate that the treatment with *Oroxylum indicum* L. aqueous stem bark extract (250 and 2500 µg/mL) significantly decreased the rate of mitotic index of *Vigna radiata* L. root apical meristem cells in a dose and time dependent manner when compared to the respective negative controls (Table 1). The standard drug colchicine (50 µg/mL) also significantly reduced the mitotic index in all the experimental groups.

Table 1: Effect of aqueous stem bark extracts of *Oroxylum indicum* L. (250 and 2500 µg/mL) on the mitotic cell division of *Vigna radiata* L. root apical meristem cells.

Exposure time (h)	Group	Total cell counted	Total cell in mitotic stage	Mitotic Index (MI) (%)
0 h	NC	7999	97	1.21 ± 0.276
	PC	5483	81	1.47 ± 0.246
	OI 250 µg/mL	4080	50	1.22 ± 0.042
	OI 2500 µg/mL	4969	67	1.34 ± 0.281
24 h	NC	1863	138	7.40 ± 0.909
	PC	2017	71	3.52 ± 0.390^{b}
	OI 250 µg/mL	2000	50	2.5 ± 0.158^{a}
	OI 2500 µg/mL	2613	93	3.55 ± 0.370^{b}
48 h	NC	2147	130	6.05 ± 0.821
	PC	2265	71	3.13 ± 0.411^{b}
	OI 250 µg/mL	3456	60	1.7 ± 0.085^{b}
	OI 2500 µg/mL	4633	84	1.81 ± 0.560^{b}
72 h	NC	2883	148	5.13 ± 0.808
	PC	4414	112	2.53 ± 0.375^{a}
	OI 250 µg/mL	4700	80	1.7 ± 0.125^{a}
	OI 2500 µg/mL	4143	87	2.09 ± 0.355^{b}

NC: Negative control, PC: Positive control (Colchicine 50 µg/mL), OI: *Oroxylum indicum* L. aqueous bark extract (250 and 2500 µg/mL) treated group. Values were expressed as mean ± SEM (n=30). Statistical analysis is done by two-way ANOVA followed by Bonferroni's post test. Level for significance was set at $P<0.05$. Significant at $^{a}P<0.01$ and $^{b}P<0.001$ compared to respective negative control.

Qualitative phytochemical analysis

Qualitative phytochemical analysis revealed that the aqueous stem bark extract of *Oroxylum indicum* L. possess alkaloids, carbohydrates (present in moderate concentration), glycosides, saponins (present in high concentration), phytosterols, phenolic compounds and flavonoids. Proteins and amino acids, and oils and fats were found to be absent (Table 2).

Table 2: Qualitative phytochemical analysis of aqueous stem bark extract of *Oroxylum indicum* L.

Chemical constituent	Phytochemical test	Aqueous extract
Alkaloids	Dragendorff's test	+
	Mayer's test	-
	Wagner's test	-
	Hager's test	-
Carbohydrates	Molish's test	++
	Fehling's test	-
	Barfoed's test	-
	Benedict's test	-
Glycosides	Borntrager's test	+
	Conc. H_2SO_4 test	-
Proteins and amino acids	Millon's test	-
	Biuret test	-
	Ninhydrin test	-
Saponins	Foam test	+++
Phytosterols	Salkowski's test	+
Phenolic compounds and flavonoids	Ferric chloride test	-
	Lead acetate test	-
	Alkaline test	+
Oils and fats	Spot test	-

+ =Present in low concentration; ++ = Present in moderate concentration; +++ = Present in high concentration; - = absent

DISCUSSION

Despite the current availability of a large quantity of anticancer agents, there is a continuous search for new compounds that may be more effective and safe. Different experimental models have been used for the assessment of anticancer compounds, which are based either on *in vivo* studies that include xenografts in nude mice, carcinogen induced tumors in rodents, transgenic and knockout mice or on *in vitro* studies on tumor cell lines [28]. However, there is a greater emphasis on *in vitro* studies for the initial screening that is followed by validation in an animal model. A simple plant model that has been widely used for such studies is the root tip meristem of *Allium cepa*, where cells are actively dividing [29]. However, due to chemical treatment of the bulbs for long term storage, this model posses problems in growing root tip meristem cells. Therefore, in this study the *Vigna radiata* L. was chosen as a plant model to evaluate the antiproliferative effect of the aqueous bark extract of *Oroxylum indicum* L.

Many earlier studies has reported the antiproliferative activity of medicinal plant in *Vigna radiata* L. model system such as *Nigella sativa* [30], *Azadirachta indica* [31], *Synedrella nodiflora* [32], *Clerodendrum viscosum* [23], *Sorghum bicolor* [33], *Parthenium hysterophorus* [34], and *Moringa oleifera* [35].

In this study, the evaluation of antiproliferative effects of aqueous stem bark extracts of *Oroxylum indicum* L. in *Vigna radiata* L. test system demonstrated that the species significantly inhibited the rate of seed germination, root and shoot growth at dose and time dependent manner. Increasing concentration of plant extracts significantly exerted the growth inhibitory effects on all the parameters when compared to negative control groups. This indicates the antiproliferative capacity of the aqueous stem bark extracts of *Oroxylum indicum* L. Moreover, these results are in agreement with the previous study reports representing the seed germination, root and shoot growth retardation is a result of the suppression of cell division [36]. A number of earlier studies have suggested that the level of root growth inhibition increases with the increasing plant extract concentrations [23, 37].

The mitotic index is used as an indicator of adequate cell proliferation, which can be measured using the *Allium cepa* L., *Vigna radiata* L. and other model systems [38]. In this study the treatment of aqueous stem bark extracts of *Oroxylum indicum* L. (250 and 2500 µg/mL) was found to be associated with a significant reduction in mitotic index in the root tip meristematic cells of *Vigna radiata* L. when compared to negative controls. Reduction in the mitotic index could be due to inhibition of DNA synthesis or blocking in the G_2 phase of the cell cycle, preventing the cell from entering mitosis [39]. This results indicates that the plant extract posses antiproliferative property.

In the present investigation, the qualitative phytochemical screening of aqueous stem bark extract of *Oroxylum indicum* L. witnessed the presence various secondary metabolites of alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenolic compounds and flavonoids. Saponins are found to be in high concentration in the extract. Saponins have been reported to possess antiproliferative [40-41] and anticancer activities [42-43]. Presence of alkaloids, flavonoids, phenols and saponins were in agreement with the earlier reports [44-45]. It is indicating that the inhibitory action against germination and seedling growth and significant reduction in mitotic index of *Vigna radiata* L. may be due to the presence of secondary metabolites in the plant extract which have adverse effects on the cell cycle progression machinery. The present study is further supported by previous reports where different secondary metabolites affect the seedling growth and cell division [46, 32].

CONCLUSSION

The present study we concluded that the secondary metabolites present in the aqueous stem bark extracts of *Oroxylum indicum* L. significantly inhibited the rate of seed germination and seedling growth, and reduced the mitotic index in *Vigna radiata* L. It can be signify that the use of *Oroxylum indicum* L. bark extract in high concentrations may potentially therapeutic for inhibiting the cell cycle in eukaryotic organisms. Thus, the plant *Oroxylum indicum* L. can be a strong antiproliferative agent for the management of proliferative diseases and cancer progression. Further research will be needed to isolate the active compounds and to determine their influence on the cell cycle regulatory gene expression.

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