Research Article

In vitro Cytotoxicity Studies of the Anti-Cancer Potential of Fractions of Root Bark of *Oroxylum Indicum in* Human Breast Carcinoma Cells

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ABSTRACT

The use of medicinal plants for the treatment of various diseases is as old as human civilization and has obtained a worldwide significance in the primary healthcare system. Inspite of their structural complexicity and many unknown chemical constituents, they have been frequently prescribed because of their use and efficacy, contributing to the disclosure of their therapeutic properties. *Oroxylum indicum*, commonly known as Syonakh (tetu), belongs to the family *Bignoniaceae*. It is used as an astringent, carminative, diuretic, stomachic, aphrodisiac and has high potential for stimulating digestion, curing fevers, coughs and preventing other respiratory disorders. The present study has been conducted to evaluate the anticancer potential of different fractions of root bark of *Oroxylum indicum*. The different fractions were tested for their cytotoxicity using the brine shrimp lethality assay, and MTT assay using MCF7 breast cancer cell line. The chloroform, ethylacetate and n-butanol fraction showed lethality in the brine shrimps. The n-butanol fraction of *Oroxylum indicum* showed the highest toxicity on MCF7 cell line, with 70.41% inhibition in the MTT assay. In conclusion, amongst all the tested fractions, the *n*-butanol fraction of the root bark of *Oroxylum indicum*, might be considered as potential source of anticancer compounds. Further studies are necessary for chemical characterization of the active principles and more extensive biological evaluations.

Keywords: *Oroxylum indicum,* MCF7 breast cancer cell, MTT assay.

INTRODUCTION

Cancer is the excess cell proliferation, which cannot be completely abolished by chemotherapy. One of the most common cancers amongst women is breast cancer and its metastatic malignancy is being a major cause of mortality since years. The chemotherapeutic agents are not only toxic for tumour cells but also for normal cells and thus rating cancer as a fatal disease due to lack of availability of effective drugs. Moreover, the chemotherapeutic agents are highly expensive, mutagenic, carcinogenic and teratogenic in nature. Resistance to most of the available anticancer agents such as anthracyclines and taxanes, and its increasing incidence are the foremost obstacle in current breast cancer therapy. Therefore, researchers are giving efforts to find out the suitable anti-cancer drug of plant origin which ultimately might be useful in the treatment of cancer[1].

In the recent years medicinal plants have received a considerable attention in the recent years as potential chemotherapeutic agents[2]. More than 150 000 plant species have been studied; many of them containing therapeutic substances are being used since ages[3]. Majority of the population in third world countries relies almost exclusively on plant products for their primary health care (Mans)[4]. The metabolites possessing anticancer properties, such as flavonoids, terpenoids, alkaloids and phenylpropanoids were isolated from natural sources[5, 6].

The cytotoxic phytochemicals such as vinca alkaloids or paclitaxel (Taxol) from the natural sources are often used in cancer. They serve as model for synthetic compounds[7,8]. Such drugs as these have been customarily isolated as single plant extracts or fractions, thereof or have been mixtures of fractions/extracts from different plants and used subsequent to their evaluation of safety and efficacy in model systems and humans[9]. Various *in vitro* and *in vivo* experiments have demonstrated that most of the phytochemicals act by interfering with several cell signaling pathways and lead to cell cycle arrest and/or differentiation induction (Chathoth) apart from their apoptosis-inducing potential and cyto-toxic potential[10].

Oroxylum indicum (L.) Vent. (also known as Shivnak, Sonapatha, Shyonaka or Midnight horror) is a deciduous tree belonging to *Bignoniaceae* family characterized with few branches. It has been used in Ayurveda and other traditional health systems since centuries back[11]. It is a member of the well-known 'Dasamula' group, an ingredient of several important Ayurvedic formulations used to treat various ailments. Every part of this plant has medicinal property. The root of Shyonaka is an astringent, bitter tonic, stomachic, anodyne, anti-inflammatory and expectorant. It stimulates digestion, cures fever, cough and other respiratory disorders and is useful in diarrhoea, dysentery, abdominal pain, thirst, vomiting, anorexia, rheumatism, worms, leprosy and other skin diseases, oedema and urinogenital disorders[12]. The leaves are useful in stomachalgia, flatulence, ulcers and also splenomegaly. The tender fruit is also reported for

improving digestion, promoting taste and destroying piles and worms. The mature fruits are useful in pharyngodynia, cardiac disorders, helminthiasis, gastropathy, bronchitis and haemorrhoids.

This species is reported to have a variety of medicinal properties like anticancer[13,14] antiulcer[15], antidysenteric[16], antimicrobial and anti-inflammatory activity[17].

Apart from its ability to treat several other ailments, the bark decoction of *O. indicum* has also been reported for its use in treating cancer, despite the lack of mechanistic information about this therapeutic modality[18].

O. indicum bark extracts were furthermore reported to possess anti-proliferative property on human breast cancer cells[19] and also anti-inflammatory activity[20].

However, such studies were performed using polar extracts unlike our study using non-polar extracts from the same plant. Previous phytochemical studies of *O. indicum* led to the identification of ellagic acid[21], 5,7-Dihydroxy flavone (chrysin)[22], 5-hydroxy-8-methoxy-7-0-β-Dglucopyranuronosyl flavones[23], Stigmast-5-en-3-, 5,6,7-trihydroxy flavone (baicalein)[24,25] 4',5- Dihydroxy-7-methoxy isoflavone (pratensol)[26], 3-(4- hydroxy phenyl)-2-propenoic acid and 3, 4', 5, 7-tetrahydroxy-flavonol[27]. However, the antineoplastic effects of the crude extract of *O. indicum* have hitherto not been extensively studied and can have significance since there could be a synergy between the different phytochemical constituents, which may have been undetected in other studies. This study is aimed to access the *in vitro* cytotoxicity of the fractions of root bark of *Oroxylum indicum* using brine shrimp lethality assay and MTT assay in human breast carcinoma cells[28].

MATERIALS AND METHODS

Procurement of plant material and extraction procedure

The fresh root bark of *Oroxylum indicum* was collected from Van-Aaushadhi Ektrikaran Udyan, Ahwa, Dang forest, Gujarat, India. The voucher specimen (#404) was deposited in the department of Pharmacognosy and Phytochemistry at the KBIPER, Gandhinagar. The root bark was dried and powdered to a 60 mesh size (≈250 m). The powder of the root bark after defatting with petroleum ether (0.32 % w/w) was dried, then moistened with ammonia (NH3) solution, and extracted with chloroform (0.78% w/w), ethyl acetate (1.52% w/w) and *n*-butanol (1.68 % w/w), successively. The dried fractions were then stored in airtight containers until usage.

Screening of the extracts by cytotoxic activity

Brine shrimp lethality bioassay

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of the fractions of root bark of *Oroxylum indicum*[29,30]. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a conical shaped vessel (1 L), filled with sterile artificial seawater (prepared using sea salt 38 gm/L and adjusted to pH 8.5 using 1 N NaOH) under constant aeration for 48 h.

After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each well containing 2.0 ml of brine solution. In each experiment, 0.5ml of the plant extract was added to 2.0 ml of brine solution and maintained at room temperature for 24 h under the light and surviving larvae were counted. Experiments were conducted along with control (vehicle treated), different concentrations of plant extracts (10, 100, 250, 500 and 1000 µg/ml) of the test substances in a set of three well per dose. Standard drug solution of appropriate concentrations was added to the wells to get final concentration of 0.1, 1.0 and 10.0 µg/ml respectively. Each concentration of test/std was added in triplicates.

Cell lines and maintenance

MCF7 (human breast carcinoma) cell line was procured from National Centre for Cell Science (Pune, India). MCF7 was maintained in Minimum essential medium (MEM) (Eagle) with Non-essential amino acids, with 10% fetal bovine serum in a humidified atmosphere at 37 °C with 5% CO2. The cell line was maintained in their growing phase at 70% confluency with regular passaging.

Cytotoxicity assessment: MTT assay

Extracts were tested for its cytotoxicity by MTT-assay[31,32]. MCF7 cells were seeded in their respective culture medium (200 µl, 1 x 10^4 cells/well) in a 96-well plate and incubated at 37 °C for 24 h with 5% CO_2 supply. After incubation, the control wells were replenished with fresh medium and the test wells were treated with 10, 100, 250, 500 and 1000 µg/ml of extracts. The cells were further incubated for 48 h maintaining the same conditions. After the treatment incubation period, medium in each well was replenished with 200µl of fresh medium plus 20µl of MTT (0.5 mg/ml). The plate was then incubated for 4 h in the same conditions after which the absorbance was measured at 570 nm using ELISA reader.

Percentage cytotoxicity was calculated by the following formula

% Cytotoxicity = [(Ac-At)/Ac)] X 100

Ac is the mean absorbance of the control wells and at is the mean absorbance of test wells with a particular extract dosage.

Statistical analysis

The IC_{50} values were obtained by non-linear regression using the Graph-pad prism program.

RESULTS

Brine shrimp lethality assay

The cytotoxicity on the tumor cell lines was evaluated using the brine shrimp lethality assay and the results are presented in Table 1. The *n*-butanol fraction obtained from root bark of *Oroxylum indicum* was the most active in this assay, presenting an IC_{50} of 636.43µg/ml.

Table 1: Brine shrimp toxicity of different fractions of root bark of *Oroxylum indicum*

Conc. (µg/ml)	Pet ether		Chloroform		Ethyl acetate		n-butanol	
	Total no. Of survivors	% Mortality	Total no. Of survivors	% Mortality	Total no. Of survivors	% Mortality	Total no. Of survivors	% Mortality
10	28	6.67	29	3.33	27	10.00	28	6.67
100	27	10.00	28	6.67	25	16.67	21	30.00
250	26	13.33	27	10.00	23	23.33	18	40.00
500	26	13.33	21	30.00	20	33.33	14	53.33
1000	25	16.67	19	36.67	17	43.33	11	63.33

MTT assay

Percentage Cytotoxicity of the n-butanol fraction of *Oroxylum indicum* on MCF7 cell line is as shown in Figure 1, having IC_{50} of 516.4 µg/ml.

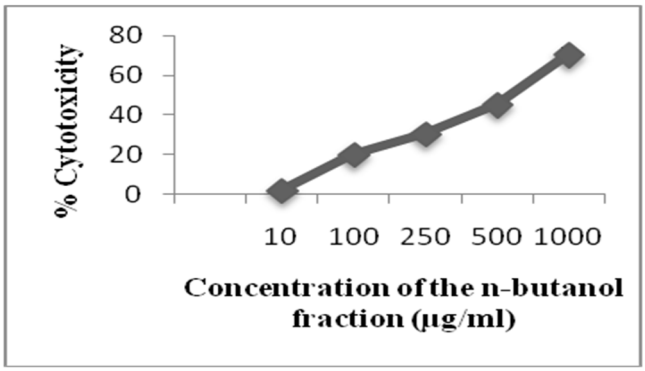

Figure 1: Percentage cytotoxicity (MTT assay) of the n-butanol fraction of *Oroxylum indicum* on MCF7 cell line

DISCUSSION

Over the past twenty years, the interest in the pharmacological effects of bioactive compounds on cancer treatments and its prevention has increased dramatically. Breast cancer is the second leading cause of cancer-related death in women. Current anti-estrogen medicine, tamoxifen, is widely used in the prevention and treatment of estrogen receptor positive breast cancer. Thus, it is imperative to search for new alternatives to breast cancer prevention agents. The inhibitory effect of *n*-butanol fraction of root bark of *Oroxylum indicum* on breast cancer cells at different concentrations was studied. The current data suggests that *n*-butanol fraction of root bark of *Oroxylum indicum* may be a potential chemotherapeutic or a chemopreventive agent based on its cytotoxic potential in cancer cells. The *n*-butanol fraction of *Oroxylum indicum* showed the highest activity against *Artemia salina* nauplii amongst all the other fractions and also it showed significant cytotoxic activity via MTT assay on the MCF7 breast carcinoma cell line. Thus, only the extracts of *Oroxylum indicum* (MCF 7 cell line) could be considered as potential sources of anticancer compounds.

Further study with *in vivo* and clinical trials needs to be conducted to establish *n*-butanol fraction of root bark of *Oroxylum indicum* as a safe drug for cancer therapy. Further, studies are necessary for chemical characterization of the active principles and more extensive biological evaluations.

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