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ANTITUMOUR AND ANTIOXIDANT ACTIVITY OF *WRIGHTIA TINCTORIA* (ROXB.) R. BR. LEAF OIL

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ABSTRACT: The present study is based on the evaluation of preliminary phytochemical screening, antitumor, cytotoxic and antioxidant activities of leaf oil of *Wrightia tinctoria* (Oil B). The oil B from *Wrightia tinctoria* leaves was subjected to phytochemical screening tests by using standard procedures. The free radical scavenging activity of both the extracts was measured by using the 1, 1- diphenyl-2-picrylhydrazyl (DPPH) assay. The dose dependent increase was observed in DPPH activity of leaf oil B (0.125, 0.25, 0.50, 1 µg/ml). Oil B was investigated for its short term cytotoxicity on DLA cells by tryphan blue dye exclusion method. *In vivo* antitumor activity was studied on DLA tumor bearing mice. Activity was assessed by monitoring the mean survival time and solid tumor volume. *Wrightia tinctoria* leaf oil was proved to be an effective anticancer and antioxidant agent. These results suggest that the oil B has potential of antioxidant and antitumor activity that support the ethno pharmacological uses of this plant. The remarkable activity showed by the plant could be attributed to the synergic effect of the active compounds present in it.

INTRODUCTION: *W. tinctoria* R. Br. belongs to family Apocynaceae [1], is a small deciduous tree, generally up to 1.8 m tall and often under 60 cm girth, sometimes up to 7.5 m high, distributed all over India. It is commonly known as "indrajav", has been important in the traditional healing and widely recognized medicinal plant [2]. The wrightial a new terpene and other phytoconstituents such as cycloartenone, cycloeucalenol were isolated identified by fractionation of methanol extract of the immature seed pods [3]. The ursolic acid and isoricinolic acid has been also isolated from the seed pods [4]. The characterization of lingocellulosic seed fiber from *W. tinctoria* has been carried out [5].

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Almost every part of plant is useful - leaves pungent chewed for relief from tooth ache, bark and seeds are antidysenteric, antidiarrhoel and antihaemorrhagic [6]. Oil emulsion of leaves and pods is used to treat psoriasis [7, 8]. During headache, leaf and stem bark pastes are applied on the forehead or administered orally [9]. Stem bark paste mixed with half cup of water is administered twice a day for 2 days for relief from abdominal pain [10].

W. tinctoria (leaf, bark and seed) is also reported to have aphrodisiac potential and anti-pyretic activity [11]. The plant is traditionally used to cure breast cancer [12]. Methanol and ethanol extracts of *W. tinctoria* leaves were found to have strong inhibitory activity against *Staphylococcus*, *Bacillus* species [13 - 15]. Ethanol extract of *W. tinctoria* flower also showed potent activity against both gram positive and gram negative bacteria [16]. Psoriasis is an autoimmune disorder of skin that is characterized by skin redness, itching and patchy looks. *W. tinctoria* hydro-alcohol extract was found

to possess significant anti-psoriatic activity [17-18]. Traditionally, *W. tinctoria* is used for treating psoriasis, eczema, scabies etc. and this has been proved clinically [19]. However, there is no pharmacological evaluation has been carried out on oil B of *Wrightia tinctoria.* The aim of the present study is to evaluate oil B of *Wrightia tinctoria* for antioxidant and antitumor activity.

MATERIALS AND METHODS:

Plant Material: The leaves of *Wrightia tinctoria* was collected from Thrssur District of Kerala in March 2013 and was authenticated by Dr. Sr. Kochuthressia M.V., Head, Department of Botany, Vimala College, Thrissur. A voucher specimen of the plant has been deposited in the Herbarium of Botany Department, Vimala College, Thrissur.

Preparation of Oil B: Fresh leaves of *Wrightia tinctoria* soaked in 1 kg of coconut oil and kept it for one day in the sunlight. The leaf oil, was adsorbed on 400 g of silica gel (60 - 120 mesh) and packed in a column (4 cm × 1 m). The column was then eluted with petroleum ether, different combinations of petroleum ether - ethyl acetate , ethyl acetate, and methanol in that order. Several 50 ml portions were collected and each fraction was checked by TLC. Fractions were pooled together according to their homogeneity judged from TLC analysis. Fractions obtained by 4:1 mixture of petroleum ether- ethyl acetate elution, on evaporation gave blue oily liquid and later its colour being changed to pink.

Preliminary Phytochemical Analysis: The oil B were analysed for the presence of various phytoconstituents like flavonoids alkaloids, glycosides, steroids, phenols, saponins and tannins according to standard methods [20].

Antioxidant Property - DPPH Free Radical Scavenging Assay: The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating free radical-scavenging activities of antioxidants. Hydrogen or electron donation abilities of the compounds were measured from the bleaching of the purple-colored methanol solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The sample solution of material (50 µL) at four concentrations (1.0, 0.5, 0.25 and 0.125 mg/ml) were mixed with freshly prepared methanolic solution of DPPH (634 μM) and allowed to stand for 30 min at room temperature. The absorbance was then measured at 515 nm using a spectrophotometer. L-ascorbic acid was used as positive control [21].

Tumor Cells and Inoculation: Dalton's Lymphoma Ascites [DLA] cells were being maintained in intraperitoneal cavity of mice in Amala Cancer Research Centre, Thrissur, Kerala.

Short Term Cytotoxic Activity: The extracts were studied for short term *in vitro* cytotoxicity using DLA cells using trypan blue dye exclusion technique [22]. The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS. Cell viability was determined by trypan blue exclusion method. Viable cell suspension (1×10^6 cells in 0.1 ml) was added to tubes containing various concentrations of the test compounds and the volume was made up to 1 ml using PBS. Control tube contained only cell suspension. These assay mixture were incubated for 3 hour at 37 °C. Further cell suspension was mixed with 0.1 ml of 1% trypan blue and kept for 2 - 3 minutes and loaded on a haemocytometer. Dead cells take up the blue colour of tyrpan blue while live cells do not take up the dye. The number of stained and unstained cells was counted separately. Control tube contains only one dead cell. The sample dissolves in DMSO.

% cytotoxicity = {[No. of dead cells / (No. of live cells + No. of dead cells)] ×100}

Animals: Young adults male Swiss albino mice (average weight 20 - 25 g) and male Wister rats (8 - 10 weeks aged) weighting 150 - 180 g were purchased from small Animals Breeding Station, Kerala Veterinary and Animals Science University, Mannuthy, Thrissur, Kerala, India. They were housed in well ventilated sterile polypropylene cages in the animal house of Amala Cancer Research Centre. Mice were maintained at a controlled temperature and relative humidity 60 ± 10% and provided 12 hours light/dark cycles. They were fed with normal pelleted rat chow (Sai Durga Feeds and Foods, Banglore, India) and water *ad libitum*. Experiments were started after acclimatization of the animals for one week in the animal's house. All the animals' experiments were

done as per the instructions prescribed by the committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), Ministry of Environment and Forest, Government of India, and implemented through the Institutional Animal Ethical Committee.

Antitumor Studies:

Effect of Oil B on Survival Time: Swiss albino mice (20 - 25 gm) were divided into four groups and each group contained six animals. Group I: Control (DLA cells alone), group II: cyclophosphamide (25 mg/kg b. wt., reference drug) + DLA cells), group III: (Oil B 200 mg/kg b. wt.) + DLA cells, group IV: oil B (50 mg/kg b. wt.) + DLA cells. Viable DLA cells (1 × 106 cells) in 0.1 ml of PBS were injected into the peritoneal cavity of mice. Drugs were administrated orally at different concentration from 10 days after tumour injection. Cyclophosphamide (25 mg/kg b.wt.) was used as standard. The death pattern of animals due to tumour burden was noted and the percentage increase in life span calculated.

Percentage increase in life span= [T - C / C] × 100

Where 'T' and 'C' are mean survival of treated and control mice respectively.

Effect of Oil B on Solid Tumour: Swiss albino mice (6 - 8 weeks) weighting 25 – 30 g was divided into four groups of six animals each. DLA cells (1 × 106 cells / animals) were injected subcutaneously on the right hind limb of mice to produce solid tumour. Group I: Control (treated with DLA cells), group II: cyclophosphamide (25 mg/kg b. wt., reference drug) + DLA cells, group III: oil B (200 mg/kg b. wt.) + DLA cells, group IV: oil B (50 mg/kg b. wt.) + DLA cells. At 24 h, after tumour inoculation, different doses of the drugs were given and continued for 10 consecutive days. Cyclophosphamide (25 mg/kg b.wt.) was used as standard drug. The diameter of the hind limb was measured using vernier caliper from 10^{th} day onwards on every 5^{th} day up to 31^{st} days.

The tumour volume was calculated using the formula: $V = 4/3\ \pi r_1^2 r_2$ where r_1 was the minor diameter and r_2 major diameter [23]. The survival of the animals was recorded for up to 31^{st} days.

RESULTS:

Preliminary Phytochemical Screening of Oil B: Phytochemical evaluation was performed with oil B of *Wrightia tinctoria*. It was found to be rich in Phenols, Flavonoids, Terpenoids and Saponins.

Antioxidant Property- DPPH Free Radical Scavenging Assay: Fig. 1 shows a decrease in the concentration of DPPH radical due to the scavenging ability of the soluble constituents in oil B of *Wrighia tinctoria*. There was a direct positive relationship between antioxidant activity and increasing concentration of the oil [24].

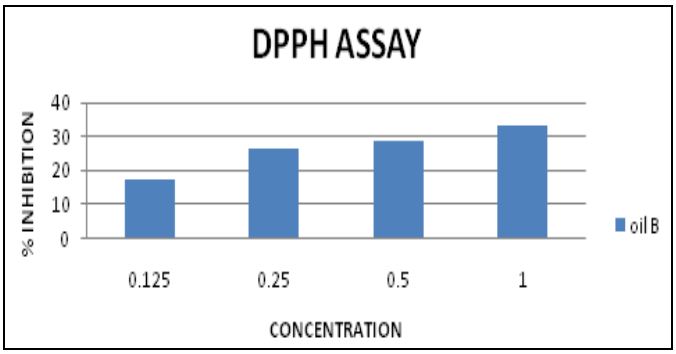

FIG. 1: DPPH RADICAL SCAVENGING ASSAY OF OIL B

Short Term Cytotoxicity Assay: The cytotoxic activity of oil B was shown in the **Fig. 2**. The leaf oil manifested a strong cytotoxic activity against target cells *in vitro*.

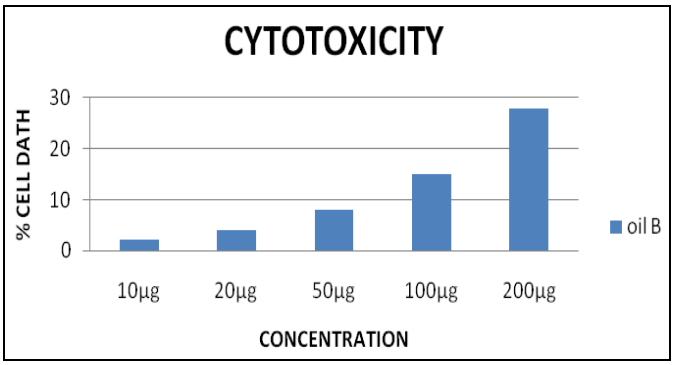

FIG. 2: SHORT TERM CYTOTOXICITY ASSAY OF OIL B

Effect of Oil B on Survival Time: In DLA tumor bearing the animals, the extract significantly increased he mean survival time and percentage increase in life span in dose dependent manner. The results were almost comparable that of cyclophosphamide, the standard drug **Table 1**.

TABLE 1: EFFECT OF OIL B OF *WRIGHTIA TINCTORIA* ON SURVIVAL AND INCREASE IN LIFE SPAN OF DLA TUMOR BEARING MICE (n = 6)

Design of treatment	Mean survival days	Increase in life span (%)
Tumor control	16 ± 1.78	0.00
Cyclophosphamide (25 mg/kg b.wt.)	24 ± 2.8	50.00
Oil B (50 mg/kg b.wt)	24.5 ± 2	53.12
Oil B (200 mg/kg b.wt)	26 ± 3	62.5

Effect of Oil B on the Solid Tumour Development: The solid tumor volume of DLA tumor baring mice was presented in **Table 2**. The results show that the oil B significantly reduces the solid tumor in dose dependent manner.

TABLE 2: EFFECT OF OIL B ON SOLID TUMOR VOLUME OF DLA TUMOR BEARING MICE (cm^3) (n = 6)

	Tumor volume (cm^3)				
Design of treatment	**10^{th} day**	**15^{th} day**	**20^{th} day**	**25^{th} day**	**30^{th} day**
Tumor control	1.76	2.46	2.92	3.08	4.62
Standard	0.21	0.38	0.46	0.49	0.61
Oil B 50 mg	0.52	0.59	0.79	0.87	1.08
Oil B 200 mg	0.29	0.41	0.59	0.69	0.85

DISCUSSION: The compounds present in oil B had already identified by GC-MS analysis [24]. Ten compounds were identified from oil B. Present study was undertaken to evaluate the *in vitro* cytotoxic activity of oil B from *Wrightia tinctoria* against DLA cancer cell line as well as to study the antitumor activity using ascites and solid tumour models. Oil B exhibited significant cytotoxic effect to DLA tumour cell lines in tryphan blue exclusion method. Transplanted tumours in animal models are effective methods to investigate the antineoplastic effects of drugs. DLA is poorly differentiated transplantable, malignant tumour which grows s both ascites and solid tumour mice. It was found that oral administration of oil B significantly increased the life span of DLA induced ascites tumour bearing mice.

Our study also revealed that oil B decreased DLA induced solid tumour volume in mice model. This can be attributed to the presence of limonene in oil B and the compound is reported as a very good anticancer and antioxidant agent [25, 26]. Compounds exhibiting cytotoxicity towards tumour cells may also show antitumor activity in experimental animals [27, 28]. One of the mechanisms of cancer chemotherapeutic agent is the elimination of damaged or malignant cell through cell cycle inhibition [29].

CONCLUSION: The findings of this study indicate that the oil B showed significant cytotoxic activity against cancer cells and exhibited antitumor activity against DLA induced solid and ascites tumour on dose dependent manner.

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CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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