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Cytotoxic Activity of *Tragia involucrata*. Linn. Extracts

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Abstract: Five different extracts of the aerial parts of *T.involucrata* were evaluated for their *in vitro* antiproliferative activity against MCF-7, KB and Vero cell lines. The plant was fractionated with hexane, toluene, dichloromethane, acetone and methanol. These extracts were tested for cytotoxic activity by MTT assay. Methanol and hexane extracts showed the potent cytotoxic activity on MCF-7 and KB cell lines. Preliminary phytochemical analysis of these crude extracts revealed the presence of flavonoids in methanol and terpenoids in hexane fractions. The plant needs the further investigation for the isolation of its active constituents.

Key words: *T. involucrata* · MTT assay · Methanol extact · Cytotoxicity · Medicinal plants

INTRODUCTION

Conventional cancer therapies cause serious side effects and, at best, merely extend the patient's lifespan by a few years. Cancer control may therefore benefit from the potential that resides in alternative therapies. The demand to utilize alternative concepts or approaches to the treatment of cancer is therefore escalating [1]. Plants have a long history of use in the treatment of cancer and played an important role as a source of effective anti-cancer agents. Over 60% of currently available anticancer agents are derived in one way or another from natural sources, including plants, marine organisms and microorganisms [2]. Selection of plants based on ethno medical knowledge and testing the selected plants efficacy as well as safety is one of the best approaches for the isolation of anticancer lead molecules from the medicinal plants [3]. The study of folk medicinal practices in Kerala revealed the ethno medical use of the aerial parts of *Tragia involucrata* along with other medicinal plants to treat certain tumours in few villages in Kerala, India [4]. The efficacy of this plant is well known in Indian traditional medicine and it is used for treatment of eczema, wounds and headache [5]. But, there no single report about the scientific validation of the folk medicinal claims. In the present study, to verify the medical claim, the different extracts of *T. involucrata* aerial parts were subjected to cytotoxic studies on cell lines.

MATERIALS AND METHODS

Cell Lines and Chemicals: BHK, Vero, MCF-7 and KB cell lines were obtained from the repositories of National Center for Cell Sciences (NCCS), Department of Biotechnology, Pune, India. Dulbecco's Modified Eagle Medium (DMEM), RPMI-1640, Penicillin, Streptomycin, Trypan blue, PBS, HBSS and Fetal calf serum (FCS) were purchased from Sigma Chemical Co, USA. MTT: (3-[4, 5-Dimethylthiazol-2yl]-2,5-diphenyltetrazoliumbromide)was from Calbiochem, USA. dimethyl Sulfoxide was from Hi-Media Laboratories, India and culture flasks, 96 Multiwell plates were from NUNC USA. All other chemicals were of analytical grade.

Preparation of Extracts: The aerial parts of the plants were collected in the month of October and November 2004 from the GKVK botanical garden and Hesaraghatta tank bed, Bangalore. The authenticated by NISCAIR, New Delhi and deposited in GKVK, Bangalore, (Herbarium No.3687).

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100g powdered aerial parts was subjected to successive extraction in a soxhlet extractor in different solvents from non-polar to polar solvents such as hexane(1.78g), toluene(1.13g), dichloromethane(0.67g), acetone(1.32g) and methyl alcohol(2.64g). The different extracts obtained were concentrated in a rotary shaker evaporator to dryness to get a constant weight.

Extracts were freshly prepared by dissolving in DMSO to a concentration of 200mg/ml and further diluted with RPMI-1640/DMEM for the experiments. The control cultures were treated with equivalent concentrations of DMSO alone, which up to the highest dose (0.1%), had no significant effect.

Cell Culture: BHK, Vero and KB cells were maintained in RPMI-1640 medium. MCF-7 was maintained in DMEM medium. All these media were supplemented with 10% FCS, penicillin G100 IU/ml and streptomycin 100µg/ml. All cell lines were maintained and sub cultured in cell culture flasks and was incubated at 37°C in a humidified 5% CO_2 air atmosphere.

MTT Assay: Cells in exponential growth phase were cultured in flat-bottomed microtitre plates at the volume of 0.1 ml (1 X 10^6 ml^{-1}). After allowing for 24h, plant extracts in different concentrations in the range of 10-250 µg (100 µl) were added to the wells and incubated in 5% CO_2 air atmosphere with high humidity for 48 h. To each well 50 µl of a 1mg/ml solution of MTT in RPMI-1640/DMEM medium was added. The culture plate was gently shaken and incubated for further 4 h at 37°C. At the end of incubation period the culture plate was centrifuged (800 X g, 5 min) and untransformed MTT was removed carefully by blotting the culture plate. Propanol (50 µl) was then added to each well. The plates were vigorously shaken to ensure that the blue formazan was completely dissolved [6]. The optical density of each well was measured with an automatic plate reader at a test wavelength of 570 nm and a reference wavelength of 650 nm. The inhibitory rate was calculated as follows:

$$\text{Inhibitory rate \%} = \frac{(\text{Absorption}_{\text{control}} - \text{Absorption}_{\text{test}})}{(\text{Absorption}_{\text{control}})} \times 100$$

IC_{50} was defined as the concentration of plant extracts killing 50% of the cells. IC_{50} was determined for all four-cell lines.

RESULTS AND DISCUSSION

The aerial parts of the *T. involucrata* extracted successively with hexane, toluene, dichloromethane, acetone and methanol and all the extracts were studied for their *in vitro* effects on KB, MCF7 and Vero. The IC_{50} was evaluated after 48h of continuous exposure of the cells cultivated in suspension. Methanol extract showed a significant cytotoxicity against the cancer cell lines than on the normal cell lines. Hexane extract also showed a significant anti-proliferative activity than other extracts with IC_{50} values ranging next only to methanol extract. Toluene extract was completely inactive against all the cell lines where as the dichloromethane and acetone extracts showed the cytotoxicity at very high concentrations (Table 1).

The crude extract with an $IC_{50} < 30$µg/ml is considered to be cytotoxic by the American National Cancer Institute (NCI) [7].The methanol fraction exhibited the highest antiproliferative potential among the five extracts of (aerial parts) *T. involucrata* with IC_{50} values of 21.76 ± 0.02 and 25.58 ± 0.54µg/ml against MCF-7 and KB clells (Table 1) respectively. The IC50 values for hexane extract were 29.21 ± 0.20 and 31.70 ± 0.39 µg/ml for the cancer cells, respectively. As the IC_{50} value fall with in the NCI limit and hence considered as of promising anticancer potential. As for *T. involucrata* this is the first study to report its antiproliferative activity. Different extracts of the plant exhibited different activity on different cell lines. This selectivity could be due to the sensitivity of the cell line to the active compounds in the extract or to tissue specific response [8]. The preliminary phytochemical analysis of the methanol and hexane extracts revealed

Table 1: *In vitro* cytotoxicity of different extracts of *T. involucrata*

	IC_{50} (µg/ml)[a]		
Test Sample	KB Cell	MCF-7 Cell	Vero Cell
Hexane Extract	29.21±0.20	31.70±0.39	97.76±0.12
Toluene Extract	NT[b]	NT	NT
Dichloromethane Extract	46.32±0.05	89.03±0.35	156.11±0.21
Acetone Extract	47.11±3.60	177.12±0.87	126.09±7.40
Methanol Extract	21.76±0.02	25.58±0.54	112.75±0.01

Results are the means ± SD of three independent experiments

[a]Doses that reduce the cell growth by 50%, after 48 h in vitro as compared to controls

[b]NT - Not Toxic

Table 2: Phytochemical analysis of successive extracts of aerial parts of *T. involucrata*

		Extract				
Sl No	Tests	Hexane	Toluene	Dichloro methane	Acetone	Methanol
I	Steroids	+	-	-	-	-
II.	Triterpenes	+	-	-	-	-
III	Flavonoids	-	-	-	+	+

± present; - = Absent

the presence of flavonoids and terpenoids in the respective fractions (Table 2). The association between flavonoids/terpenoids and reduced cancer risk has been reported in previous studies [9-11]. The results of methanol and hexane fractions are in accordance with this finding.

In conclusion, the results of the present study demonstrated the potent cytotoxic activity of methanol and hexane fractions of the aerial parts of *T. involucrata* and presently we are studying the anticancer property of these extracts in other cancer models and isolating the active principle.

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