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Short Communication

CYTOTOXIC EFFECT OF *HELIOTROPIUM INDICUM* EXTRACTS ON HELA CELL LINE

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ABSTRACT

Objective: *Heliotropium indicum* (*H. indicum)* has been used widely for centuries on warts and to treat inflammations and tumors. *H. indicum* Linn (Family Boraginaceae) is a medicinal plant. It is known commonly as "Cock's comb". *H. indicum* Linn has various medicinal uses in the treatment of disease conditions such as Abdominal pains, Amenorrhoea, Dysmenorrhoea, Skin rashes, Wounds, Hypertension, Ocular infections, Convulsion, Dizziness. To provide scientific validation to the traditional use of plants *in vitro* cytotoxic effect was evaluated on HeLa cell lines.

Methods: The crude extracts of stem and leaf of *Heliotropium indicum* were prepared using soxhlet apparatus using solvents of increasing polarity. The study was performed using methanolic extracts against human cancer cell line (HeLa) using MTT assay to analyze the cell growth inhibition**.**

Results: The results showed that the methanolic extracts of stem and leaf of *Heliotropium indicum* possessed a good amount of anticancer activity and IC50 for both the extracts found to be 200µg/ml. Whereas stem extracts exhibited excellent activity up to 64.5% at 200 µg/ml and followed by leaf extract up to 49.67% at 200 µg/ml. Obtained results supports the traditional claim of the plant.

Conclusion: The methanolic extracts of leaf and stem of *H. indicum* exhibits cytotoxic activity. Hence, Obtained results support the traditional claim of the plant.

Keywords: *Heliotropium indicum*, Hela cell lines, MTT assay, Stem and leaf alcohol extract.

The novel and potential compounds from plant origin that is used in the treatment and prevention of diseases leads to discovery of new drugs [1]. The common alternative for cancer prevention and treatment in many countries are medicinal plants [2-4]. 80% of anticancer drugs currently used are isolated from the plants and more than 3000 plants are reported to possess anticancer activity worldwide. Many plants derived compounds as vinblastine, vincristine, taxol, and camptothecin are reported to have anticancer activity [5]. The search of new compounds from plant origin and from traditional food leads the researchers for discovery of new anticancer compounds [6]. The alkaloids, phenylpropanoids, and terpenoids isolated from plants are reported for anticancer property [7, 8]. Literature review suggests the plant is reported to have Antitumor [9], Wound healing effect [10], Hypotensive effect [11], Antianaphlactic activity [12], Anti-inflammatory effect, Analgesic effect [13], Anti-microbial effect [14] and isolated Pyrrazolidine alkaloids [15]. For the present study the cytotoxic activity of methanolic extracts of stem and leaf of *Heliotropium indicum* was assessed on HeLa cell lines.

The leaves and stem of *H. indicum* were collected in the month of December from nagercoil and dried in shade. The shade dried leaves and stem were powdered to get a coarse powder authenticated by Dr. D. Stephen, Madurai medical college. The extractions were carried out by using solvents of increasing polarity using a soxhlet extraction method. The coarse powder was processed with various solvent petroleum ether, chloroform, ethyl acetate and acetone. The extracts were filtered and concentrated to dry mass by using vacuum distillation. The percentage yield was calculated. The methanolic extracts were used for the present study.

HeLa cell line was maintained in DMEM medium (GIBCO) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) and 1% antibiotic solution (penicillin 100Uml^{-1} and streptomycin 100µgml^{-1}) at 37°C in a humidified atmosphere of 95% air/5% CO_2. The medium was changed every second day, and cells were sub cultured when confluence reach to 95% by 0.25% trypsin containing 0.02% ethylene-diaminetetraacetic acid (EDTA) in PBS for 3 min at 37 °C.

The MTT assay was carried out as described previously to measure cell viability [16]. Ten thousand cells in 100µL of DMEM media were seeded in the wells of a 96-well plate. After 24 h, existing media were removed and 100 µL of various concentrations of extracts was added and incubated for 48 h at37 °C in a CO_2 incubator. Control cells were supplemented with 0.05% DMSO vehicle. At the 48th hour of incubation, MTT (3-(4, 5-dimethylthaizol-2-yl)-2,5-diphenyltetrazolium bromide-supplied from Sigma, 10µL of 5 mg/ml) was added to the plate. The contents of the plate were pipetted out carefully, the formazan crystals formed were dissolved in 100 µL of DMSO, and the absorbance was measured at 550 nm in a microplate reader (Tecan, infinite F200 Pro). Experiments were performed in triplicate, and the results were expressed as mean of percentage inhibition.

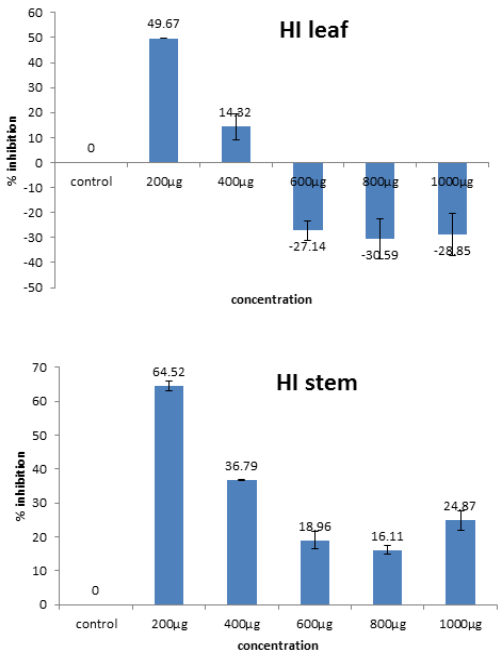

Fig. 1: Percentage inhibition of cell growth at different concentrations of *H. indicum* leaf and stem extracts. Data are mean±SE (n=3)

The methanolic extracts of leaf and stem of *H. indicum* exhibits antiproliferative activity and showed interesting results. Further focus should be moved towards isolating the potential phytochemicals responsible for the activity. Our results provide the basis for the further investigation and potential identification of medicinal compounds of anti-cancer property. Elucidating the mechanisms by which these anti-cancer properties are derived is of crucial importance to identify, select for and optimize therapeutic compounds.

A graph of concentration versus percentage growth inhibition was plotted, and the concentration at which 50% cell death occurred was considered as the IC50 value. Before adding MTT, bright field images (Olympus 1X81, cell Sens Dimension software) were taken for visualizing the cell death.

The result reveals the percentage yield for the extracts of leaf and stem to be 12.2%w/w and 15%w/w. The methanolic extracts of leaf and stem of *H. indicum* showed a considerable activity against the tumor cell-lines at the tested concentrations. fig. 1 shows the antiproliferative results as inhibition percentage at five concentrations (200-1000µg/ml) for those extracts as an inversely dose-dependent behavior. Relative death percentage of leaf extracts was 49.67% and 14.82% at 200 and 400 µg/ml respectively. Whereas stem extracts has shown moderately good activity compared to leaf, and was ranging from 64.52% to 16.11% at 200-1000µg/ml in an inversely dose dependent manner. The IC50 value of leaf and stem extracts are shown to be at 200µg/ml. Fig. 2 depicts the cytotoxic effect to extracts on HeLa cell line. Recently, medicinal plants have emerged as attractive candidates for cancer chemoprevention because of their safety, relative to cytotoxic synthetic agents [17].

Many anticancer drugs are effective against HeLa cells by causing apoptosis through the expression of caspase-3, generating reactive oxygen species (ROS) and damaging DNA [18]. In addition, HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences, a low expression of p53 and normal expression of pRB (retinoblastoma suppressor). The p53 gene appears to trigger programmed cell death (apoptosis) as a way of regulating uncontrolled cellular proliferation in the setting of aberrant growth signals [19]. However, it is necessary to perform many other studies both *in vitro* and *in vivo* to determine their true potential for the development of medicines.

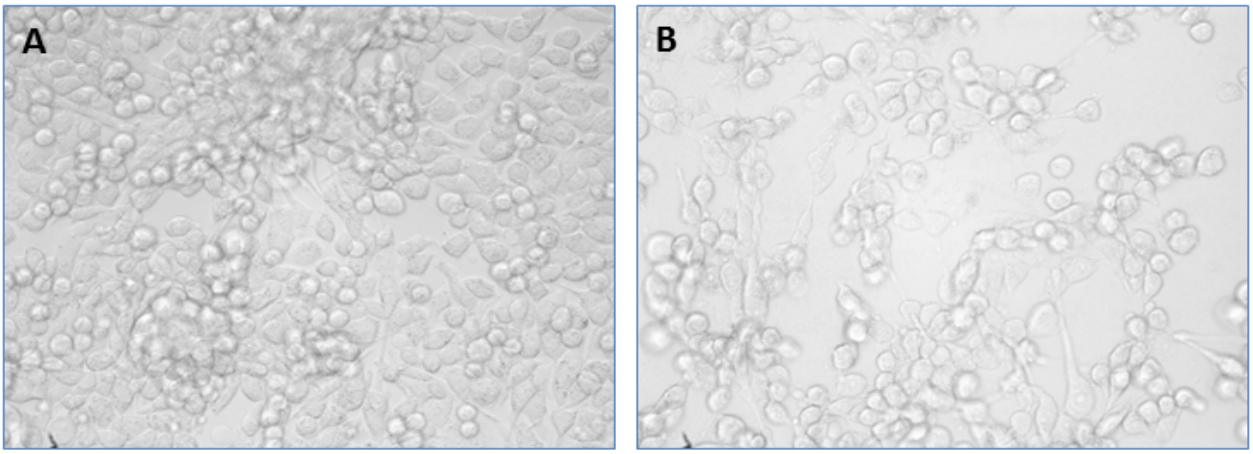

Fig. 2: Anticancer activity of extracts showing cell death, A-control; B-treated

CONCLUSION

The methanolic extracts of leaf and stem of H. indicum exhibits antiproliferative activity and showed interesting results. Further focus should be moved towards isolating the potential phytochemicals responsible for the activity. Our results provide the basis for the further investigation and potential identification of medicinal compounds of anti-cancer property. Elucidating the mechanisms by which these anti-cancer properties are derived is of crucial importance to identify, select for and optimize therapeutic compounds.

CONFLICT OF INTERESTS

Declared None

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