ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antiproliferative activity of *scoparia dulcis* Linn.ethanolic extract on cell line.

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ABSTRACT

Medicinal plants contain bioactive compounds as primary and secondary constituents.The secondary metabolites are responsible for medicinal activity of the plants and are used for treatment of various human diseases. *Scoparia dulcis* Linn, a herbal medicinal plant belongs to the family Scrophulariaceae, has been widely reported to have antiurolethic and anti diabetic pharmacological uses. In addition some other pharmacological properties have also been evaluated and those include antiproliferative, anticancer, antitumor, antibacterial, anti fungal, antiviral, anti inflammatory and antioxidant effects. However, limited study only has been conducted on the south Indian species, and therefore it is essential for determining the anti proliferative effects of ethanolic extracts of *Scoparia dulcis Linn* grown in south india. Ethanolic extract in a final concentration of 6.25 µg/ml, 12.5µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml were prepared from the stock solution 1mg/ml. These solutions were added on HeLa cervical cancer cell lines for the in-vitro anti proliferative activity. The observations of the study confirmed that , ethanolic extract of *Scoparia dulcis* Linn possesses marked anti proliferative activity on the tested cell lines and is increased as the concentration of drug extracts increases.

Keywords: Antiproliferative, Scoparia dulcis, Hispidulin.

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ISSN: 0975-8585

INTRODUCTION

Scoparia dulcis Linn belongs to the family Scrophulariaceae is a herbal plant found almost around the globe; in European, African, American and Asian countries. The common names of the plant are sweet broomweed, *typycha kuratu* (Japan), *vassourinha* (Brazil) and *escobilla* (Peru). Synonyms are *Scoparia grandiflora, Scoparia ternata, Capraria dulcis,* (Brazil) and *escobilla* (Peru). Synonyms are *Scoparia grandiflora, Scoparia ternata, Capraria dulcis, Gratiola micrantha*. *Scoparia dulcis* L It is used in curing ailments such as fever, diarrhoea, ulcer, cancer, *Gratiola micrantha*. The plant has been used traditionally as a remedy for Diabetes mellitus in India and hypertension in Taiwan. It is also used in curing ailments such as fever, diarrhoea, ulcer, cancer, venereal diseases. It has been used for blood cleansing, in childbirth and as a general tonic (Burkill, 2000). [1,11.]

Morphology [1, 2]

Broomweed is a common erect, shrubby annual herb in Suriname growing up to 2' in height. (Orhue and others, 2009). It has many auxiliary shoots and reproduces from seeds. Stem is more or less woody, ribbed and is mainly branched and glabrous with opposite leaves. The leaves are oval or narrowly oblanceolate, serrated three at a node, long about 2.5 cm to 5.0 cm and wide 1.5cm with toothed at the upper part of the leaf and at the lower part of the leaf wedge–shaped. The leaf blade is smooth except for the lower surface has some glandular dots. The inflorescence is a slender raceme with one or two flowers in the upper leaf axils. The fruit is a round capsule (Burkill2000). Flowers hermaphrodite, complete, usually axillary, 6-7 mm in diameters, 4-fid, rotate, regular. Sepals 4-5, gamosepalous, regular, calyx lobes oval-oblong, 2.5-3.0x0.8-1.0 mm, 3-nerved, glabrous, ciliate at the margin, persistent. Corolla pale yellow to white, corona present, tube densely hairy at the throat, lobes 2-4 mm long, apex obtuse, slightly curvy, upper lobes slightly larger than others. Stamens 4, exerted; filament inserted at the top of the corolla tube, glabrous; anthers dorsifixed. Style erect, c 2 mm long; stigma truncate to 2-partite, sometimes notched. Flowering time: Almost throughout the year.

Reported pharmacological Actions of the chemical constituents.[4,5,6,7,8,9,10.]

a. **scoparic acid A,** diterpene acid ,found to be a potent betaglucuronidaseinhibitor.
b. **scoparic acid B**, has antitumour activity against human cancer cells.
c. **scopadulcic acid A**, Compounds with antiviral, antifungal, and antitumor activity. The also shows activity against Plasmodium falciparum and is a potential target for antimalarial therapy.
d. **scopadulcic acid B**, had been shown to promote antitumor activitiesand , activity against primary herpes simplex virus, (primary corneal herpes simplex virus infection) .The compound has shown to inhibit both the K(+)dependent adenosine triphosphatase (ATPase) activity of a hog gastric proton pump (H+, K(+)-ATPase) with a value of 20-30 microM for IC50 and proton transport into gastric vesicles
e. **scopadulciol or dulcinol** , a tetracyclic diterpenoid, has been reported as an anti-viral agent, inhibitor of Herpes simplex, and an inhibitor of gastric H+, K+-ATPase Experimental results in cancer gene therapy using the HSV-1 tk gene and ACV/GCV together with SDC was found to be effective in suppressing the growth of cancer cells in animals.
f. **hispidulin**, which had been reported to possess bronchodilating and antiasthmatic effect more potent than aminophylline on a molar basis , antimutagenicity , hepato-toxicity antioxidant activity, positive allosteric properties, anticonvulsant activity, and antifungal activity. Hispidulin has also been found to inhibit the aggregation of human platelets by increasing cAMP levels.
g. **scopadiol** , potassium adenosine triphosphatase (ATPase) activator.
h. **betulinic acid**, is used for the prevention and treatment of cancer. It has antitumor, antileukemia , antiviral (including HIV) properties, and cytotoxic activity against malignant brain tumor and bone cancer, possessed anti-inflammatory activities.
i. **glutinol**, the triterpene shows analgesic and anti-inflammatory activity.

MATERIALS AND METHODS [12,13.]

The chemicals and reagents were of analytical reagent grade, and were used as supplied.

Collection and processing of Plant. [14,15,16]

The herbal medicinal plant was collected from Nagarcovil, Tamilnadu district, India. An ethnobotanist, and was identified and authenticated at the herbarium of the National Institute for Pharmaceutical Research and Development, Chennai, India, where a voucher specimen was placed. The whole plant was thoroughly air-dried under sunshade for two weeks. The dried material was cut in to small pieces to reduce the size by a cutter.

Extraction by Maceration Technique. [14,15,16,17.]

The air-dried and cut pieces of whole plant material were taken for maceration. The material was soaked in 95% ethanol and after that subjected to successive maceration; a greenish black sticky material was obtained.

Determination of Antiproliferative Activity. [18,19.]

The Antiproliferative activity of ethanolic extract of scoparia dulcis was studied on cultured HeLa cervical cancer cell Lines, by "Invitro" method.

HeLa cervical cancer cell lines were purchased from NCCS Pune. The Dulbecco's modified eagles media (HIMEDIA) was supplemented with 10% FBS (Invitrogen) and the cells were maintained in it. The cell lines were grown to confluence in a CO2 incubator (NBS, EPPENDORF, GERMANY) by treated with 5 % CO2 in a humidified atmosphere. The cells were treated with 500µl of 0.025% Trypsin in PBS/ 0.5mM EDTA solution (Himedia) for 2 minutes at 37°c, and then transferred to T flasks under aseptic conditions. A stock solution of the ethanolic extract 1mg/ml in PBS was prepared. From this, concentrations of 6.25 µg, 12.5µg, 25 µg, 50 µg and 100 µg /ml were prepared. Then samples were added to the grown cells and incubated for 24 hours. The % difference in viability was determined by standard MTT assay.

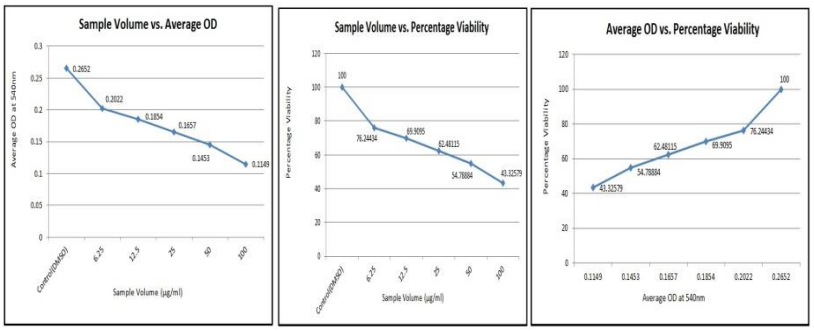
MTT ASSAY (Arung *et al*., 2000)

The cells were washed with PBS and then added 30 µl of MTT solution to the culture. (MTT -5mg/ml dissolved in PBS). It was then incubated at 37^0C for 3 hours. MTT was removed by washing with PBS. Added 200µl of DMSO to the culture and incubated for 30 minutes at room temperature until the cell lyses and a colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2minutes to precipitate cell debris. Optical density was read at 540 nm using DMSO as blank in a micro plate reader (ELISASCAN, ERBA).

% Viability = (OD of Test **/** OD of Control) X 100

Sample Volume (µg/ml)	Average OD at 540nm	**Percentage Viability**
Control(DMSO)	0.2652	100
6.25	0.2022	76.24434
12.5	0.1854	69.9095
25	0.1657	62.48115
50	0.1453	54.78884
100	0.1149	43.32579

ISSN: 0975-8585



(1) (2) (3)

Graph for the Data obtained.

SUMMARY

This *in vitro* study determines the inhibitory effect of ethanolic extract of a herbal medicinal plant *Scoparia dulcis* Linn. on Hela cervical cancer cell to proliferation. Proliferation is the growing propensity of cell to grow or produce by multiplication of parts, as in budding or cell division or by procreation to increase in number or spread rapidly and often excessively and the drug extract inhibit and reduces this tendency as the concentration increases. The results and line graphs clearly stating that (a) the concentration 100µg/ml has been showed to produce more than fifty percentage activity against proliferation. (b) Graph (1) & (2) indicates, increase in the concentration of plant extract, decreases the percentage viability(proliferation) and optical density.(c) Graph (3) explains that a lower optical density read out indicates the higher antiproliferative activity.

CONCLUSION

The present study demonstrated that the ethanolic extract of the whole plant *Scoparia dulcis* linn.showed marked antiproliferative activity on cancer cell line. However, for confirmation and validation of the effect, further a supportive preclinical study in animal model and also in clinical are to be followed.

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