



CYTOTOXICITY OF *SCOPARIA DULCIS* ON HUMAN CANCER CELL LINES

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ABSTRACT

Scoparia dulcis L (*Scrophulariaceae*) is a perennial herb. Plant parts are used as a cure for many ailments including diabetes. This work reports cytotoxicity of a pure compound glutinol and crude plant extracts on a panel of human cancer cell lines. Vinblastine was used as positive control. The MTT assay was employed to estimate the cell mortality. Cytotoxic ED₅₀ values of glutinol ranged from 140.91 to 215.44 μM and the crude extracts showed 17.13 to 92.03% cell mortality at 500 μg/ml on the tested cell lines. Percent cell mortalities by vinblastine were 90.00 to 94.10% 125 μg/ml and cytotoxic ED₅₀ values of vinblastine were 5.25 to 6.12 μM. Cell mortality for the negative controls (RPMI^C and RPMI^C-DMSO) were nil.

Keywords: *Scoparia dulcis*, Glutinol, Crude Extracts, Cytotoxicity, Human Cancer Cell Line.

INTRODUCTION

Scoparia dulcis L. (*Scrophulariaceae*) is an important medicinal plant. It is a perennial herb and is widely distributed in tropical and subtropical regions. Traditionally this plant is used in treatment of many ailments including diabetes, dysentery, earache, fever, gonorrhoea, headaches, jaundice, snake bite, stomach problems, toothache, and warts [1]. A spectrum of medicinal properties such as analgesic [2], anti-inflammatory [3], antiviral [4], hypertensive [5], antihypertensive [6], diuretic [7], antidiabetic [8], even neuroprotective as anticholinergic [9-11] was reported for *Scoparia dulcis*. Its cytotoxicity was also documented [12-15]. This plant possesses many bioactive compounds [15-16] that contribute its medicinal properties and biological activities. This work described the cytotoxic activity of glutinol and crude extracts of *Scoparia dulcis* on a panel of human cancer cell lines.

MATERIALS AND METHODS

Plant Material

The aerial parts of *Scoparia dulcis* were collected in Dhaka. A voucher specimen (DACB 28069) has been deposited in the National Herbarium, Dhaka.

Bangladesh.

Preparation of extracts and Isolation of glutinol

Dried aerial part of the plant (650g) of *Scoparia dulcis* was extracted successively in a Soxhlet with petroleum ether (60-80°), EtOAc and MeOH. The extracts were concentrated under vacuum to yield 15, 12 and 37g of crude residues respectively.

Glutinol (12mg) was obtained from Vacuum Liquid Chromatography fraction of petroleum ether extract, which was further fractionated in Sephadex LH-20 and was characterized by spectral analysis as reported elsewhere [15].

Cytotoxicity Assay

A panel of five human stomach cancer cell lines SCL, SCL-6, SCL-37'6, Kato-3, and NUCC-4 [17, 18, 19] were used to test the cytotoxicity of glutinol and crude extracts of *Scoparia dulcis*. The MTT assay as described by Mosmann [20] was employed to estimate the cell mortality. A series of serial dilutions of the crude extracts (500, 250, 125, 62.5, 31.25, and 15.63 μg/mL) and of the glutinol and vinblastine (250, 125, 62.5, 31.25, and 15.63 μg/mL) were tested on each of the cell lines. For

every concentration, three replicate analyses were performed. Percent cell mortality for each of the concentrations was calculated. Vinblastine sulfate (Sigma Chemicals Co. USA) was used as positive controls. RPMI^C (RPMI-1640 complete medium, GIBCO UK) was used to culture the cancer cells for their confluent growth and RPMF-DMSO (RPMI^C containing 0.25% DMSO) was used to prepare the test materials and to culture the cancer cells in the presence of the test materials. RPMI^C and

RPMI^C-DMSO were used as negative controls. Cells grown in the RPMI^C and RPMI^C-DMSO were found to be the same and were considered 100% cell survival (that is, cell mortality was nil) to estimate cell mortality for the test extracts and to determine the ED₅₀ value for glutinol and vinblastine. The SPSS software package (12.5 version Inc. Chicago USA) was used for statistical analysis. Data were presented as mean ± SD.

Table 1. Cytotoxicity of *Scoparia dulcis*

Test Materials	Human cancer cell line				
	SCL	SCL-6	SCL-37'6	Kato-3	NUGC-4
Glutinol ^E	163.61±9.31	178.69±112.11	nd	215.44±5.51	140.91±3.34
'Pet- ether extract ^{cm}	82.8±3.77	64.16±2.89	72.96±2.37	92.03±1.58	90.40±2.45
Ethyl acetate extract ^{cm}	90.10±2.60	83.83±8.87	76.53±3.72	89.13±3.56	85.33±5.71
Methanol extract ^{cm}	21.73±1.84	43.10±0.90	69.10±3.93	17.13±1.09	49.36±5.26
RPMI ^C	nil	nil	nil	nil	nil
RPMI ^C -DMSO	nil	nil	nil	nil	nil
Vinblastine sulphate ^{b*}	90.80±4.21	90.00±5.10	90.40±4.83	94.10±7.46	93.40±7.89
Vinblastine sulphate ^E	5.85±0.63	6.12±0.84	5.33±0.59	6.12±0.68	5.25±0.49

*Vinblastine sulphate was used as positive controls.

RPMI^C and RPMI^C-DMSO were used as negative controls, where the cell growth was same and taken as 100% cell survival (no cell mortality) to estimate cell mortality and ED₅₀.

RPMI^C: RPMI-1640 supplemented with 1% glutamine (200mM), 1% penicillin (5000 IU/ml), 10% foetal calf serum.

RPMI^C-DMSO: RPMI^C containing 0.25% DMSO.

a: percent cell mortality at 500µg/ml

b: percent cell mortality at 125µg/ml

E: ED₅₀

nd: not done

RESULTS AND DISCUSSION

Table 1 describes cytotoxic activity of the glutinol and crude extracts of *Scoparia dulcis*. Cytotoxic ED₅₀ values of glutinol ranged from 140.91 to 215.44µM and the crude extracts showed 17.13 to 92.03% cell mortality at 500µg/ml on the tested cell lines. Percent cell mortalities by vinblastine were 90.00 to 94.10% at 125µg/ml and cytotoxic ED₅₀ values of vinblastine were 5.25 to 6.12µM. Cell mortality for the negative control was nil. Anticancer or antitumour activity of *Scoparia dulcis* were also previously reported [12-15].

Among the crude extracts, petroleum ether extract presented the highest Kato-3 cell mortality (92.03±1.58%), which was followed NUGC-4 (90.40±2.45%) and SCL (82.8±3.77%). EtOAc extract led the highest SCL cell mortality (90.10±2.60%) and the next ones were Kato-3 (89.13±3.56%), NUGC-4 (85.33±5.71%) and SCL-6

(83.83±8.87%). MeOH extract yielded moderate to mild cell mortalities ranging from 69.10±3.93% (SCL-37'6) to only 17.13±1.09% (Kato-3). Cytotoxic action of glutinol was shown very poor. Compared to vinblastine, its ED₅₀ values were found negligible.

A promising cytotoxic activity was indicated by ethyl acetate extract on most of the cancer cell lines and pet-ether extract on the Kato-3 and NUGC-4 cells.

CONFLICT OF INTEREST

Authors do not have any financial or commercial conflicts of interest to this work.

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REFERENCES

- Murti K, Panchal M, Taya P, Singh R. Pharmacological properties of *Scoparia dulcis*: a review. *Pharmacologia*, 3, 2012, 344-347
- Freire SM, Torres LM, Roque NF, C. Souccar C, Lapa AJ. Analgesic activity of a triterpene isolated from *Scoparia dulcis* L. (Vassourinha). *Memorias Inst Oswaldo Cruz*, 86, 1991, 149-151
- Ahmed M, Jakupovic J. Diterpenoids from *Scoparia dulcis*. *Phytochemistry*, 29, 1990, 3035-3037.
- Hayashi TM, Kawasaki M, Miwa Y, Taga T, Morita N. Antiviral agents of plant origin. III. Scopadulin: A novel tetracyclic diterpene from *Scoparia dulcis* L. *Chem Pharm Bull*, 38, 1990, 945-947.

5. De Farias Freire SM, Torres LMB, Souccar C, Lapa AJ. Sympathomimetic effects of *Scoparia dulcis* L. and catecholamines isolated from plant extracts. *J Pharm. Pharmacol* 48, 1994, V624-628.
6. Chow SY, Chen SM, Yang CM, Hsu H. Pharmacological studies on Chinese herbs. Hypotensive effects of 30 Chinese herbs. *Taiwan Yi Xue Hui Za Zhi* 73, 1974, 729-739.
7. Freire SM, Torres LM, Roque NF, Souccar C, Lapa AJ. Analgesic activity of a triterpene isolated from *Scoparia dulcis* L. (Vassourinha). *Memorias Inst Oswaldo Cruz*, 86, 1991, 149-151
8. Latha M, Pari L. Effect of an aqueous extract of *Scoparia dulcis* on blood glucose, plasma insulin and some polyol pathway enzymes in experimental rat diabetes. *Brazil J Med Biol Res*, 37, 2004, 577-586
9. Li Y and Ohizumi Y. Search for constituents with neurotrophic factor-potentiating activity from the medicinal plants of Paraguay and Thailand. *J. Pharm. Soc. Jap.*, 124, 2004, 417-424.
10. Li Y, Chen X, Satake M, Oshima Y, Ohizumi Y. Acetylated flavonoid glycosides potentiating NGF action from *Scoparia dulcis*. *J Nat Prod*, 67, 2004, 725-727
11. Coulibaly AY, Sombie PAED, Tibiri A, Kiendrebeogo M, Compaore MMY, Nacoulma OG. 2011. Protective Effect of *Scoparia dulcis* on Brain and Erythrocytes. *Current Res J Biol Sci*, 3(3), 2011, 254-261.
12. Nkembo KM, Lee JB, Hayashi T. 2005. Selective enhancement of scopadulcic acid B production in the cultured tissues of *Scoparia dulcis* by methyl jasmonate. *Chem Pharm Bull*, 53, 2005, 780-782
13. Nakagiri T, Lee JB, Hayashi T. cDNA cloning, functional expression and characterization of ent-copalyl diphosphate synthase from *Scoparia dulcis* L. *Plant Sci* 169, 2005, 760-767.
14. Nishino H, Hayashi T, Arisawa M, Satomi Y, Iwashima A. Antitumor-promoting activity of scopadulcic acid b, isolated from the medicinal plant *Scoparia dulcis* L. *Oncology*, 50, 1993, 100-103
15. Ahsan M, Islam SN, Gray AI, Stimson WH. Cytotoxic Diterpenes from *Scoparia dulcis*. *J Nat Prod*, 66, 2003, 958-961.
16. Ratnasooriya WD, Jayakody JRAC, Premakumara GAS, Ediriweera ERHSS. 2005. Antioxidant activity of water extract of *Scoparia dulcis*. *Fitoterapia*, 76, 2005, 220-222.
17. Islam SN. Stomach Cancer: Production of Human Antibodies and Cell Lines and characterization of a Tumour-Associated Antigen. PhD Thesis, University of Strathclyde, Glasgow. 1994, 80-93.
18. Sekiguchi M, Sakakioara K, Fujii G. Alpha-fetoproyein producing gastric cancer lacks transcription factor ATBF1. *Japan J Exp Med*, 48, 1978, 61-68.
19. Akiyama S, Amo H, Wataube T, Matsuyama M, Sakamoto J, Imaizumi M, Ichihashi. H, Kondo T, Takagi H. Characteristics of three human gastric cancer cell lines, NU-GC-2, NU-GC-3 and NU-GC-4. *Japan J Surg*, 18, 1988, 438-446.
20. Mosmann T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *J Immunol Methods*, 65, 1983, 55-63.