ORIGINAL ARTICLE

Anticancer-cytotoxic activity of saponins isolated from the leaves of *Gymnema sylvestre* and *Eclipta prostrata* on HeLa cells

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The anticancer-cytotoxic activities of isolated saponins, gymnemagenol ($C_{30}H_{50}O_4$) from *Gymnema sylvestre* and dasyscyphin C ($C_{28}H_{40}O_8$) from *Eclipta prostrata* leaves were tested under *in vitro* conditions in HeLa cells. The gymnemagenol and dayscyphin C at 50 µg/ml showed a good cytotoxic activity (63% and 52%, respectively) in HeLa cells at 48 hours with the IC50 value of 37 and 50 µg/ml, respectively. 5-Fluorouracil (5-FU), a positive control, showed 57.5 % cell death with the IC50 value of 36 µg/ml. The percentage of HeLa cell death was maximum (73%) after 96 hours with gymnemagenol, whereas dasyscyphin C showed only 53%. The isolated saponins were not toxic to Vero cells. From this study, it can be concluded that the saponins, gymnemagenol, and dayscyphin C have significant anticancer-cytotoxic activity on HeLa cells under *in vitro* conditions.

Key words: *Gymnema sylvestre, Eclipta prostrata,* anticancer cytotoxic activity, HeLa cells

INTRODUCTION

Saponins are a diverse group of compounds widely distributed in the plant kingdom, which are usually characterized by their structure containing a steroidal or triterpenoid aglycone and one or more sugar chains.[1] The pharmacological potential of many plants has been reported to be associated with steroidal or triterpenoid groups.[1] Anticancer activity of many plant-derived saponins, ginsenosides,[2] soyasaponins[3] and saikosaponin-d[4] have already been reported.

G. sylvestre commonly called as 'Gurmur' is widely distributed throughout India. The plant is known for its antidiabetic activity[5] and is also rich in phytochemicals such as alkaloids, flavonoids, saponins, carbohydrates, and phenols with highest concentration of saponins being 5.5%.[6] *E. prostrata* is a small plant, branched, annual herb, belonging to a family Asteraceae and commonly called as 'Karichalai', with white flower heads. It is native to the tropical and subtropical regions of the world and widely distributed in India. The leaf paste is applied on the affected area of teeth to control the toothache and the leaves decoction was long been used orally for control of jaundice.[7] The antimicrobial activity of saponins isolated from *E. prostrata* and *G. sylvestre* has been reported by us recently.[8] The bioactivity of the isolated active principles of these plants is yet to be studied. Hence, a study was planed to evaluate the cytotoxic–anticancer potential of gymnemagenol and dayscyphin C, the isolated active principles from these two common medicinal plants on HeLa cells under *in vitro* conditions.

MATERIALS AND METHODS

Plant Material

The leaves of *G. sylvestre* and *E. prostrata* were collected from Jawadi hills, Vellore District, during the month of November 2007. The voucher specimen was prepared and deposited in the herbarium section of the VIT University. The leaves of *G. sylvestre* and *E. prostrata* were washed with distilled water, shade dried, powdered, and stored in an airtight container until further use.

Extraction and Purification of Saponin

The powdered sample was defatted by petroleum ether for 3 hours at 40°C. After filtering the petroleum ether, the sample was extracted with methanol for 3 hours with mild heating. The methanol extract was concentrated and re-extracted with methanol and acetone (1:5 v/v).[9] The precipitate obtained was dried under vacuum, which turns to a whitish amorphous powder after complete drying. It was loaded on Merck silica gel-60 (230–400 mesh) column and eluted with chloroform–methanol–water (70:30:10).[10] The first fraction collected was air dried at

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room temperature (28°C) and the residue obtained was treated as pure saponins of *G. sylvestre* and *E. prostrata*. The purity of the saponin isolated was analyzed by thin layer chromatography using chloroform and methanol (7:3) as the solvent system.

Structural Elucidation

The purified saponins were subjected to structural elucidation by UV, FT-IR,^{1}H NMR,^{13}C NMR, and MS (Finnigan MAT 8230). All chemicals used for extraction and purification were of analytical grade (SRL, Mumbai, India).

Anticancer–cytotoxic Assay

HeLa (Human cervical carcinoma) and Vero cell lines were obtained from ATCC and maintained in DMEM (Hi-Media Laboratories Pvt. Ltd, Mumbai, India) supplemented with 10% heat-inactivated FBS (v/v), streptomycin (100 mg/l) and penicillin (100 IU/ml). The cell line was maintained at 37°C with 5% carbon dioxide in CO_2 incubator. The MTT cell proliferation assay[11] was used to evaluate the cytotoxic activity of saponins using the CellQuanti-MTT cell viability assay kit (Bioassay Systems). The optical density was measured at 570 nm for each well on an absorbance plate reader. Trypan blue dye exclusion assay[12] was also used to count the number of viable and non-viable HeLa cells in the culture medium after drug treatment. Treatment with 5-FU at the same concentration served as positive control.

RESULTS AND DISCUSSION

The spectroscopic data indicate the chemical nature of saponin isolated from *G. sylvestre*, they are crystalline needles, ^{1}H NMR (500 MHz in $CDCl_{3)}$, δ: 5.182 (9H, m, H-18,H-19,H-24); 5.104 (1H, t, H-6); 4.060 (3H, dd, H-11,H-12); 3.505 (4H, S, H-16, H-17); 2.790(1H,t,H-1); 2.313 (3H, m, H-5, H-4); 2.291(2H, t, H-2); 2.084(1H, d, H-7);2.029 (4H, m, H-20,H-25, H-26, H-27); 1.619 (3H, m,H-14,H-15); 1.275 (10H, m, H-3, H-8, H-9, H-10, H-13); 1.235 (3H, s, H-23); 0.876 (6H, m, H-21, H-22). ESI-MS m/z: 474 $[M]^{+}$ and the compound was identified as 3β, 16β, 28, 29-tetrahydroxyolean-12-ene.

Spectroscopic data. Dayscyphin C was obtained as a colorless oil. H NMR (500 MHz in $CDCl_{3}$), (OH) – 2.032 (m, 2H) , 1-H -0.992 (t, 2H), 2-H -0.889 (m, 2H), 3-H -0.910 (t, 2H), 5-H -1.316 (t, 1H), 6-H -0.859 (t, 2H), 7-H -0.876 (dd, 2H), 9-H -1.301 (d, 1H), 11-H -1.704 (t, 2H), 12-H -5.197 (m, 1H), 16-H -6.158 (s, 1H), 18-H -1.629 (t, 3H), 19,20-H -1.270 (s, 6H), 21-H -2.310 (s, 3H), 22-H -5.411 (m, 2H), 22-H -5.411 (m, 2H), 2'-H -3.683 (s, 2H), 4'-H -4.069 (s, 2H), 6'-H -1.149 (s, 3H). ESI-m/z: 503 $[M]^{-}$.

The structural identity of gymnemagenol and dayscyphin C was established by spectroscopic analysis. The presence of triterpenes in the TLC plate was confirmed by Libermann–Burchard reaction and by the Carr-Price reagent test. The UV spectrum of dayscyphin C showed the absorption maxima at 234, 238 and 302 nm and gymnemagenol showed at 223, 237 and 274 nm. Dayscyphin C showed the IR spectrum at 3435.80, 2921.82, 1635.05, 1245.75, 1050.66 per cm and gymnemagenol at 3445.41, 2924.10, 1635.38, 1457.48 per cm. The FINNIGAN MAT 8230MS showed the $[M]^{-}$ ion at m/z 503 with the base peak at m/z 208 for dayscyphin C and for gymnemagenol it was $[M]^{+}$ ion at m/z 474 with the base peak at m/z 251. The chemical shift assignments were obtained for dayscyphin C and gymnemagenol from ^{1}H NMR corresponding to the molecular formula $C_{28}H_{40}O_{8}$ and $C_{30}H_{50}O_{4.}$

The effects of gymnemagenol and dayscyphin C on the growth of HeLa cells were tested under *in vitro* conditions using different concentrations (5, 15, 25 and 50 μg/ml) and the cell survival after 48 hours was given in Figure 1. The IC50 value was calculated to be 37 μg/ml for gymnemagenol and 50 μg/ml for dayscyphin C. At 96 hours, gymnemagenol (50 μg/ml) exhibited significant cytotoxic activity (73%) on HeLa cells, whereas dayscyphin C exhibited only 53% cytotoxic activity. The inhibition was found to be saponin concentration and time dependent with greater inhibition at highest concentration at 96 hours. The cytotoxic activity was compared with the effect of 5-FU and it was found to be equivalent to that of gymnemagenol.

The isolated active principle gymnemagenol offered a high degree of inhibition over the growth of the HeLa cells when compared to dayscyphin C. The cytotoxic effect of gymnemagenol and dayscyphin C was also tested on Vero cells and its effect on Vero cells growth is given in Table 1. These saponins were not toxic to the growth of normal cells tested under *in vitro* conditions.

The antimicrobial activity of the isolated saponins from these plants has already been reported by us.[8] The other study in our laboratory revealed the leishmanicidal activity on

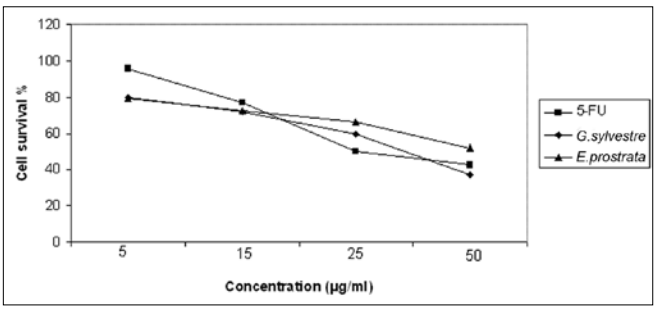

Figure 1: Cytotoxic effect of gymnemagenol of *G. sylvestre* and dayscyphin C of *E. prostrata* against HeLa cells at 48 hours. The percentage of cell survival was determined and recorded. Each point represents the mean of three independent experiments. 5-FU served as a positive control

Table 1: Cytotoxic activity of saponin of *G. sylvestre* and *E. prostrata* on Vero cell line after 48 hours of treatment

Saponin (50 µg/ml)	Percentage of viable cells	
	Control	Treated
G. sylvestre	93	89
E. prostrata	93	90

Values are mean of three experiments. The viable Vero cells were calculated after 48 hours of gymnemagenol and dayscyphin C (50 µg/ml) treatment and stained with trypan blue dye exclusion test.

Leishmania major under *in vitro* conditions (unpublished data). However to the best of our knowledge, this is the first report on the cytotoxic activity of gymnemagenol. Plant saponins have been already reported to possess a wide range of biological activities,[1] which include the anticancer cytotoxic activity. Several reports are available on the anticancer activity of saponins isolated from other plants.[13,14] Isolation of antisweet oleane-type triterpene glycoside saponin was already been reported from *G. sylvestre*.[15] Based on the results of this study, it can be concluded that the gymnemagenol of *G. sylvestre* and dayscyphin C of *E. prostrata* has a significant cytotoxic effect on HeLa cells. Further studies are under progress in our laboratory to evaluate and establish its anticancer cytotoxic potential on other cancer cell lines.

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