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NON-PROLIFERATIVE ACTIVITY OF SAPONINS ISOLATED FROM THE LEAVES OF *GYMNEMA SYLVESTRE* AND *ECLIPTA PROSTRATA* ON HEPG2 CELLS- *IN VITRO* STUDY

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ABSTRACT

The non-proliferative activity 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 on HepG2 cells under *in vitro* conditions. Both gymnemagenol and dasyscyphin C exhibited non-proliferative activity on HepG2 cells at 24 h with the IC_{50} value of 18.5 and 23.5µg/ml respectively. 5-Fluorouracil (5FU) a positive control showed the IC_{50} value of 1.34µg/ml. The isolated saponins were not toxic to Vero cells. From this study, it can be concluded that the saponins, gymnemagenol and dasyscyphin C have significant non-proliferative activity on HeLa cells under *in vitro* conditions.

INTRODUCTION: Medicinal plants have been considered as a valuable source for natural products and explored continuously for therapeutics for human well being. The use of plant products for pharmaceutical purposes has been gradually increasing. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs for the treatment of many diseases [1]. About 80% of individuals from developed countries use traditional medicine, derived from medicinal plants [2]. Saponins are a diverse group of compounds widely distributed in the plant kingdom. Chemically, saponins are glycosides consisting of both sugar and non-sugar aglycone, called sapogenin. Depending on the structure of aglycone, they are classified as steroidal and triterpenoid saponins.

Saponins exhibit a wide range of biological properties and are believed to be one of the key biologically active constituents of plant drugs used in folk, especially Far East medicine. Anticancer activity of many plant-derived saponins, ginsenosides [3], soyasaponins [4] and saikosaponin-d [5] has already been reported. *G. sylvestre* commonly called as 'Gurmur' is widely distributed throughout India. The plant is known for its antidiabetic activity [6] and is also rich in phytochemicals such as alkaloids, flavonoids, saponins, carbohydrates, and phenols with highest concentration of saponins being 5.5% [7]. *E. prostrata* is a small plant; branched, annual herb belongs 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 tooth ache and the leaves decoction was long been used orally for control of jaundice [8]. The antimicrobial activity of saponins isolated from *E. prostrata* and *G. sylvestre* has been reported by us recently [9]. The bioactivity of the isolated active principles of these plants is yet to be explored. Hence a study was planed to evaluate the effect of gymnemagenol and dasyscyphin C, the isolated active principles from these two common medicinal plants on HepG2 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. 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 air- tight 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 reextracted with methanol and acetone (1:5 v/v)[10]. 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) [11]. The first fraction collected was air dried at 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).

Cytotoxicity Assay: HepG2 (Human hepatic 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 [12] was used to evaluate the cytotoxic activity of saponins using the Cell Quanti-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 [13] was also used to count the number of viable and non-viable HeLa cells in the culture medium after drug treatment. Treatment with 5FU at the same concentration served as positive control. (Table 1)

TABLE 1: NON- PROLIFERATIVE ACTIVITY OF GYMNEMAGENOL AND DASYSCYPHIN C ON HEPG2 CELL LINE AFTER 48 HOURS OF TREATMENT

SAPONINS	IC_{50}(µg/ml)
	HepG2
Gymnemagenol	18.5
Dasyscyphin C	23.5
5-FU	1.34

Values are mean of three experiments

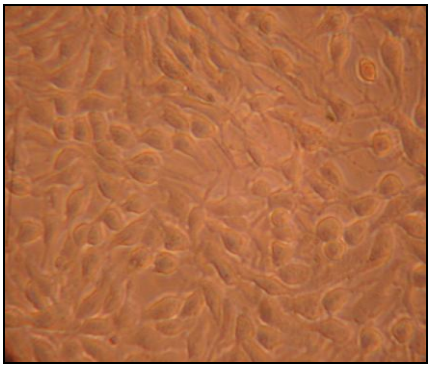
RESULTS AND DISCUSSION: The spectroscopic data indicate the chemical nature of saponin isolated from *G. sylveste*, they are crystalline needles,^{1}H NMR (500 MHz in $CDCl_3$,δ : 5.182 9H, m, H-18, H-19, H-24); 5.104 (1H, t, H-6); 4.060 (3H, dd, H-11, H-12); 3.505 (4H, S, H-16, H-17); 2.790 (1H, t, 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- 12ene.

Spectroscopic data: Dasyscyphin C was obtained as a colorless oil. ^{1}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].

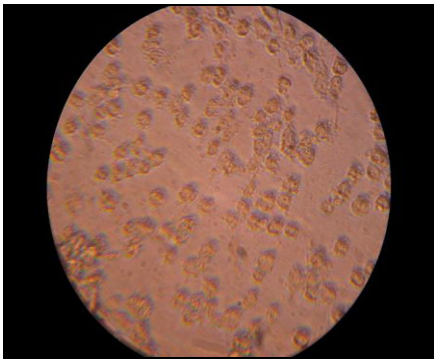
The structural identity of gymnemagenol and dasyscyphin C identity was spectroscopic analysis. The presence of triterpenes in the TLC plate was confirmed by Libermann-Burchard reaction and Carr-Price reagent. The UV spectrum of dasyscyphin C showed the absorption maxima at 234, 238 and 302 nm and gymnemagenol showed at 223, 237and 274 nm. Dasyscyphin 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 dasyscyphin 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 dasyscyphin 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 dasyscyphin C on the growth of HepG2 cells was tested under *in vitro*

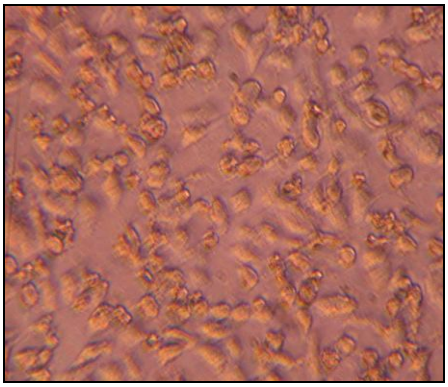
conditions. The IC_{50} value was calculated to be 18.5 for gymnemagenol and 23.5µg/ml for dasyscyphin C. 5-FU showed the IC_{50} value of 1.34µg/ml on HepG2 cells. The inhibition of proliferation of HepG2 cells by gymnemagenol and dasyscyphin C treatment at 24 h was shown in Figure 1 (B &C) and compared with control Figure 1 (A).



(A)



(B)



(C)

FIGURE LEGEND; FIGURE 1: EFFECT OF GYMNEMAGENOL AND DASYSCYPHIN C ON HEPG2 CELLS. (A) CONTROL HEPG2 CELLS (B) HEPG2 CELLS TREATED WITH GYMNEMAGENOL (50 µG/ML); (C) HEPG2 CELLS TREATED WITH DASYSCYPHIN C (50 µG/ML). CELLS WERE INCUBATED (37^{O}C) FOR 24 H AND OBSERVED UNDER INVERTED MICROSCOPE (40X MAGNIFICATION)

The non-proliferative activity was comparatively lesser with the isolated saponins when compared to the effect of 5FU. The isolated active principle gymnemagenol offered a high degree of inhibition over the growth of the HepG2 cells when compared to dasyscyphin C. The non-proliferative effect of gymnemagenol and dasyscyphin C was also tested on Vero cells. It was found that both the saponins were less toxic to Vero cells.

The antimicrobial activity of the isolated saponins from these plants has already been reported by us [9]. The other study in our laboratory revealed the larvicidal activity on *Leishmania major* under *in vitro* conditions [14]. Plant saponins have been already reported to possess a wide range of biological activities [15], which include the anticancer cytotoxic activity. Several reports are available on the anticancer activity of saponins isolated from other plants [16, 17]. Isolation of antisweet oleane type triterpene glycoside saponin, gymnemagenol was already been reported from *G. sylvestre* [18]. Based on the results of this study, it can be concluded that the gymnemagenol of *G. sylvestre* and dasyscyphin C of *E. prostrata* has a significant non-proliferative activity on HepG2 cells. Further studies are under progress in our laboratory to establish the mechanism of anticancer activity of gymnemagenol and dasyscyphin C.

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