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Short report

# Apoptotic and free radical scavenging properties of the methanolic extract of *Gentianella alborosea*

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**Abstract**

*Gentianella alborosea* ("Hercampure") is a Peruvian species used in folk medicine for the treatment of a variety of health disorders. We tested the free radical scavenging (DPPH) and induction of apoptosis on a human uterus tumor cell line (HeLa) by its methanolic extract. The results showed a noticeable radical scavenging activity and a dose-dependent apoptotic effect.


*Keywords:* *Gentianella alborosea*; Apoptosis; DPPH

## 1. Plant

*Gentianella alborosea* (Gilg) Fabris (Gentianaceae), whole plant collected in Cuzco (Peru) in 2001 was identified by Dr. Galán de Mera. A voucher specimen has been deposited in the Herbarium of School of Pharmacy, San Pablo University, Madrid, Spain.

## 2. Uses in traditional medicine

As obesity treatment, in liver diseases and as coleretic, colagogue and digestive.

## 3. Previously isolated classes of constituents

Xanthones [1], alborosin (sesterterpenoid) [2], flavonoids, terpenoids [3]. Phytochemical screening of methanolic extract gave positive test for flavonoids, alkaloids, saponins and glycosides.

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## 4. Tested material

Methanolic extract (2.92%) from the dried and powdered plant material.

## 5. Studied activity

The apoptotic effect on HeLa cells was determined according to Sun et al. [4]. The dried MeOH extract was dissolved in phosphate saline buffer (PBS). Doxorubicin (2 μM) and PBS were used as positive and negative controls, respectively. After incubation (48 h), the cells were fixed with MeOH and stained with a hematoxilin–eosin (Sigma kit) for the observation of nuclear morphology. The proportion of apoptotic nuclei was calculated and expressed as percentage from the total nuclei. The free radical scavenging was assessed by the 1,1 diphenil-2-picryl-hydroazyl (DPPH) assay [5] using ascorbic acid as reference. The result was expressed as μg of extract required for 50% DPPH scavenging effect and as ascorbic acid equivalent (mg) per g of extract.

## 6. Used cell line

A human uterus tumor cell line HeLa (ATCC, CCL-2) was used, obtained from American Type Culture Collection (USA) and maintained in our laboratory.

## 7. Results

Summarized in Tables 1 and 2.

## 8. Conclusions

From our results we can conclude that the methanolic extract of *G. alborosea* induces apoptosis on HeLa cells, which was demonstrated by the presence of apoptotic bodies. The effects were dose-dependent. Higher tested concentrations have the highest percentage of apoptotic nuclei. Several recent reports have appeared concerning the apoptotic effects of antioxidants, like some plant flavonoids that induce apoptosis in some tumor cell lines but not in normal cells [6]. *G. alborosea* extract

Table 1
Free radical scavenging activity of *G. alborosea* methanolic extract

| Microgram of extract required for 50% DPPH scavenging effect | Ascorbic acid equivalent (mg/g of extract) |
|---|---|
| 30.1±2.31 | 2.03±0.17 |

Data were expressed, as the mean±S.D. Values are the mean of five replicates.

Table 2
Effect of *G. alborosea* methanolic extract on apoptosis assay on HeLa cells

| | | Total cells | Apoptotic cells | %[a] |
|---|---|---|---|---|
| PBS | | 242.3±20.3 | 31.5±6.4 | 12.8±1.6 |
| Doxo[b] | | 338.2±41.2 | 90.8±7.6 | 26.6±2.7* |
| Extract | (μg/ml) | | | |
| | 21.0 | 186.5±24.6 | 41.5±9.3 | 22.9±3.1* |
| | 11.0 | 195.6±17.2 | 45.3±5.6 | 23.4±2.1* |
| | 5.0 | 196.1±23.2 | 27.8±6.4 | 13.7±3.2 |
| | 2.6 | 214.4±25.8 | 24.2±6.6 | 11.2±2.7 |
| | 1.3 | 187.4±19.3 | 17.1±3.9 | 9.0±1.3 |

Data were expressed, as the mean±S.D. Values are the mean of five replicates.
*$P<0.05$ versus PBS in Student's *t*-test.
[a] Percentage of apoptotic cells.
[b] Doxorubicin (2 μM).

shows as well a moderate, but noticeable radical scavenging activity. The estimation of radical scavenging abilities by using DPPH assay furnishes a preliminary information about the antioxidant activity of the extract and provides a basis for further isolation procedures.

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