EVALUATION OF *IN VITRO* CYTOTOXIC AND PROAPOPTOTIC PROPERTIES OF MEDICINAL PLANT *COTINUS COGGYGRIA* SCOP. TOWARDS A375 MELANOMA SKIN CANCER CELLS

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Introduction & Objective

Malignant melanoma is a highly metastatic type of skin cancer that is characterized with a low response to available chemotherapeutics. Over the last decades, the incidence of cutaneous melanoma has increased globally as the number of new cases and deaths in 2018 were about 280 000 and 60 000, respectively (Bray et al., 2018).

Cotinus coggygria Scop. is a medicinal plant widely applied in folk medicine, predominantly externally against skin disorders. The herb is intensively studied in the last years due to its various biological qualities, including antimicrobial, anti-inflammatory, antihemorrhagic, antioxidant, etc. The anticancer properties of *C. coggygria* have been so far not well enough studied.

The present study was undertaken to explore the cytotoxic capacity of crude aqueous ethanolic extract from leaves of *Cotinus coggygria* and its chloroformic and aqueous fractions on human melanoma cell line A375. Normal dermal cell line BJ was used in the analysis to assess the selectivity in the action of plant substances.

Materials

Plant extract:
Crude leaf aqueous ethanolic extract from *Cotinus coggygria* Scop. was provided by Vemo 99 Ltd. (Sofia, Bulgaria);

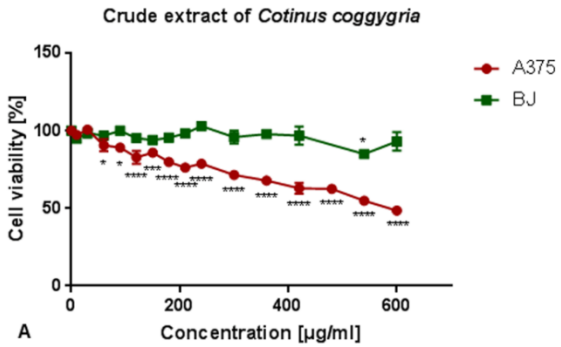
Cell lines:
A375 - human melanoma cell line;
BJ - human normal skin fibroblast cell line

Methods

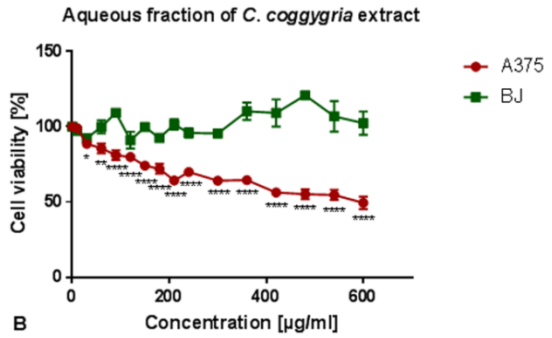
Fractionation of *C. coggygria* leaf aqueous ethanolic extract to chloroformic and aqueous fractions.

Cell culturing;
MTT cell viability assay;
Trypan blue test;
Cell morphological observation;
Fluorescence microscopy;
Statistical analysis

Results



C. coggygria substance	Cell line	IC_{50} [µg/ml]
Crude extract	A375	595.3
Aqueous fraction	A375	598.3
Chloroformic fraction	A375	321.1



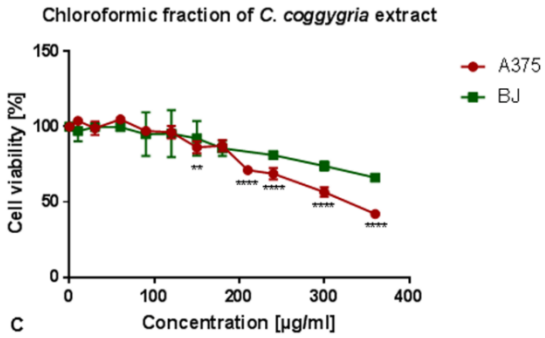


Figure 1. MTT cell viability assay of A375 and BJ cells treated with increasing concentrations of *C. coggygria* leaf aqueous ethanolic extract (A), aqueous (B) and chloroformic (C) fractions for 24 h. Error bars represent standard error of the mean (SEM). *, **, *** and **** indicate significant differences from the control group by Dunnett's test (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$).

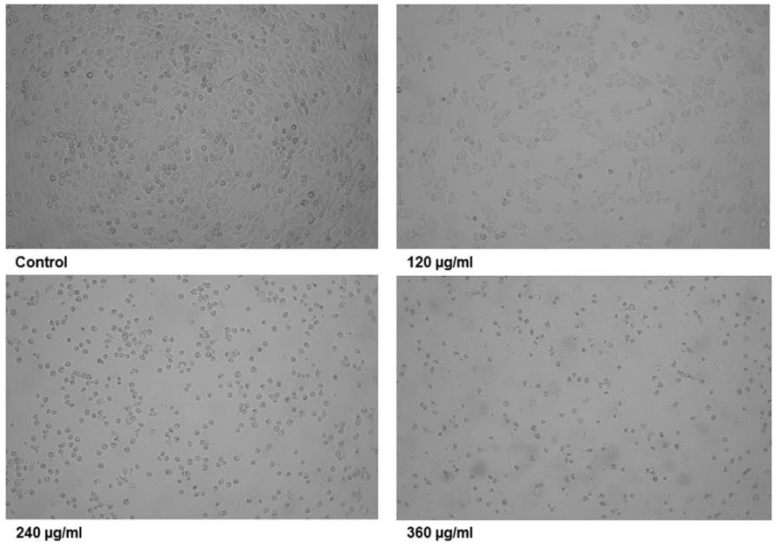


Figure 2. Morphological alterations of A375 cells after treatment for 24 h with 120, 240 and 360 µg/ml of *C. coggygria* chloroformic fraction compared to untreated control.

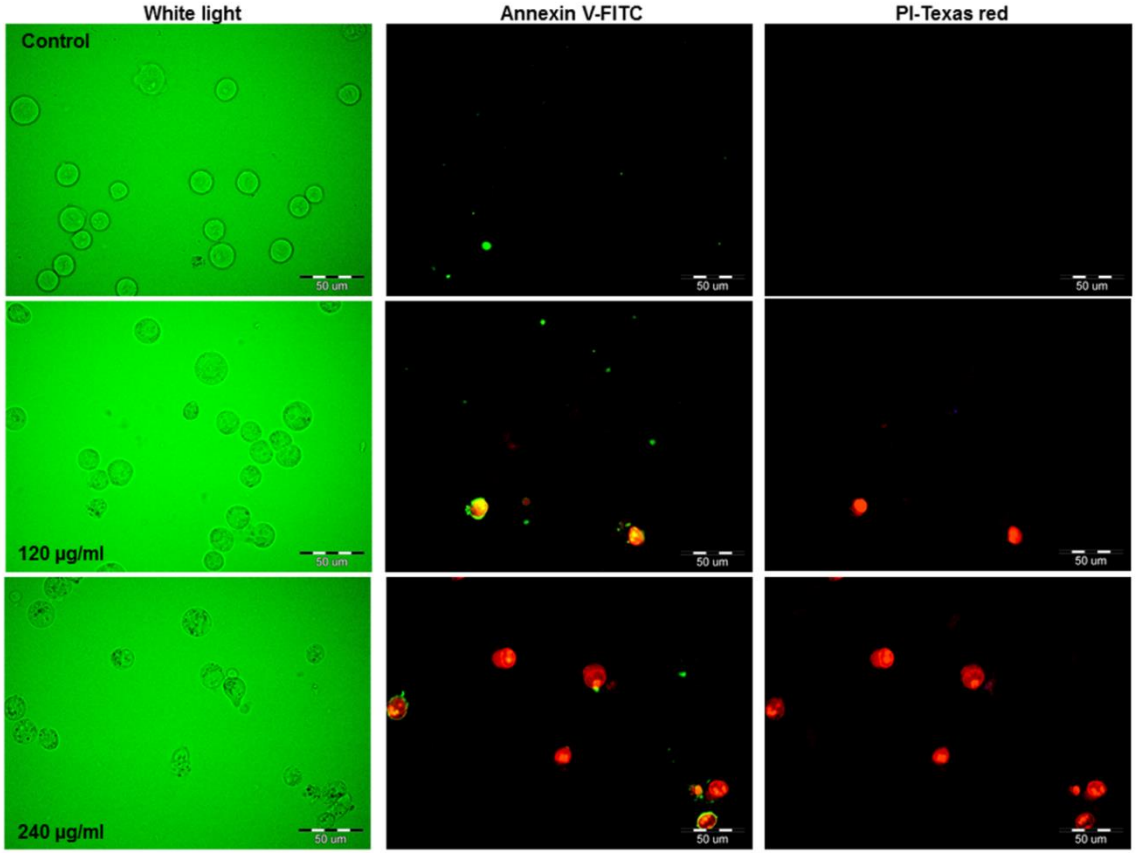


Figure 3. Fluorescent microscopy analysis of A375 cells after Annexin V/propidium iodide staining for visualization of apoptotic and necrotic cells: untreated negative control; *C. coggygria* chloroformic fraction treated cells (120 and 240 µg/ml, 24 h).

Results

The results obtained by MTT assay and cell morphological observation revealed significant selective cytotoxic activity of plant substances against A375 cancer cells with the highest inhibitory effect on cell viability for the chloroformic fraction of *C. coggygria* extract. The results were also confirmed by Trypan blue test (data not shown). Investigations of the chloroformic fraction proapoptotic potential against A375 cells by fluorescent microscopy observation after staining with Annexin V and propidium iodide detected considerable increase in the number of cells in apoptosis and necrosis after treatment.

Conclusion

Medicinal plant *Cotinus coggygria* exhibits *in vitro* cytotoxic and proapoptotic properties against A375 melanoma skin cancer cells. Future studies will be focused on more detailed assessment of anti-melanoma therapeutic potential of the plant.

Acknowledgements

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