Aqueous extract from a hemp agrimony – evaluation of the cytotoxic effect on the human brain glioblastoma cells

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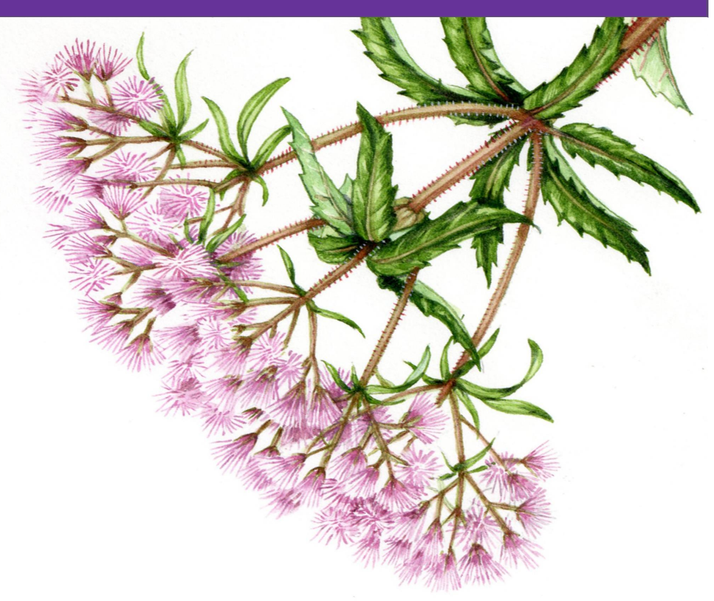


INTRODUCTION

The healing properties of ***Eupatorium cannabinum L.*** have been already described in the seventeenth-century Polish medical herbaria. These historical descriptions also pointed to their potential anticancer activity. Contemporary literature data confirm the presence of many bioactive compounds in *E. cannabinum,* but there are no studies that would confirm their anti-cancer properties. Phytotherapy is used as a form of natural treatment or supporting conventional methods of therapy of many diseases. Supplementation with herbal preparations is currently very popular, which is why it seems important to study the anti-cancer potential of these plants.

METHODS

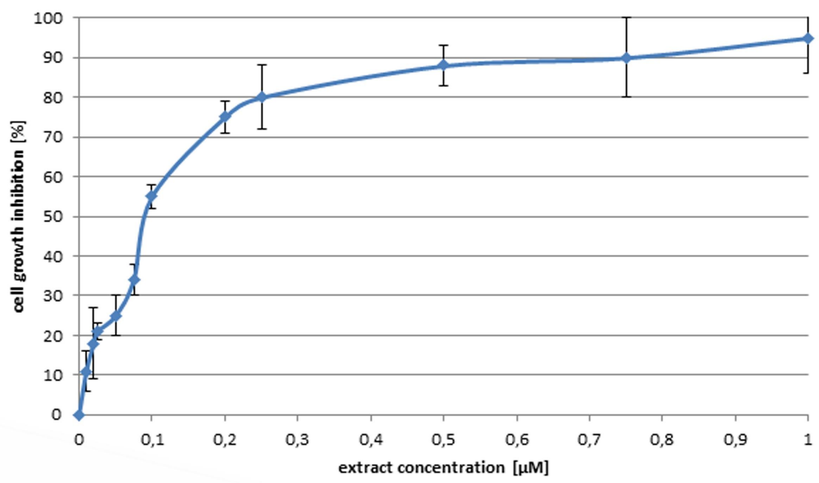
Aqueous extracts: 10 g of dried aerial part of plants was extracted in boiling water on low heat for 3h and filtered, lyophilized and reconstituted in water to final stock solution (100 mg/mL).

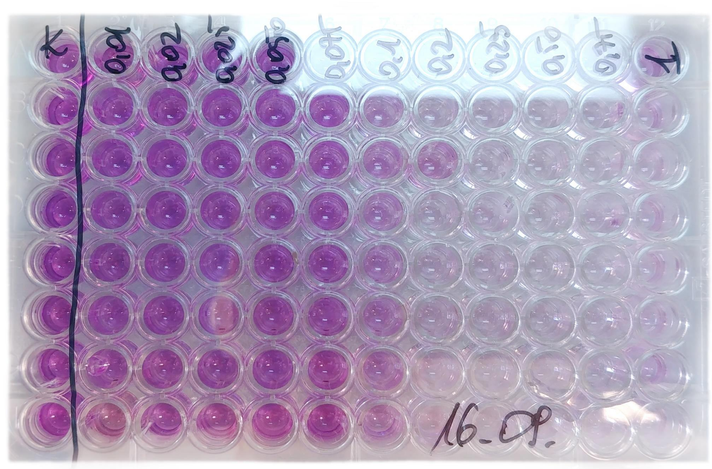
Cytotoxicity assay: The effects of plant extracts on cell growth were determined by incubating cells with different concentrations for 72 h at 37°C in standard 96-well plates. The cell growth inhibition was determined by MTT reduction assay (spectrophotometric analysis).

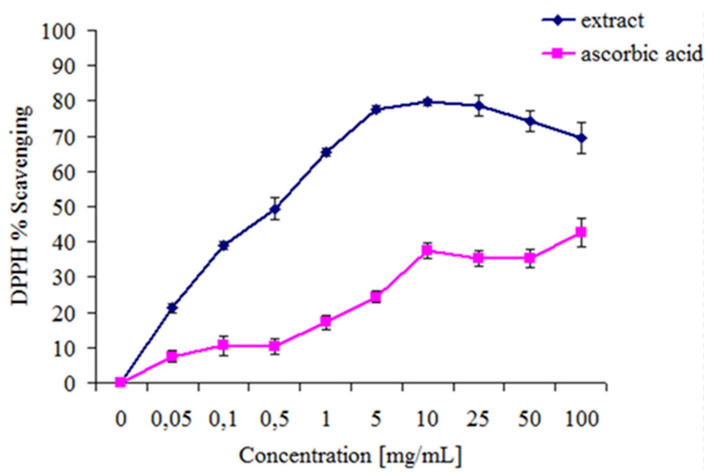
Total phenolic content of extract: was determined using the Folin-Ciocalteu reagent according to the modified method of Singleton and Orthofer. 50 µL of water extract (1 mg/mL, m/v) was incubated with 250 µL of 10% Folin-Ciocalteu reagent for 5 min in RT. Afterward, 200 µL of 7,5% Na_2CO_3 was added. Appropriate blank sample was prepared. Then the absorbance was measured at λ=765 nm after 60 min of incubation at RT. Results were expressed in gallic acid equivalents using a calibration curve.

Total flavonoid content in extract: was determined using aluminium complexation reaction. 75 µL of water plant extract (1 mg/mL, m/v) was incubated with 30% methanol, 37,5 µL of 0,5 M $NaNO_2$, and 37,5 µL of 0,3 M $AlCl_3x6H_2O$ for 15 min in RT. Then, 250 µL of 1 M NaOH was added and absorbance was measured (λ=506 nm). The results were expressed in quercetin equivalents using a calibration curve.

DPPH Radical Scavenging Activity Assay: was determined according to Blois modified method. Briefly, 125 µL of water plant extract at different concentrations was incubated with 0,6 M DPPH stable free radical solution for 30 min at RT. Afterward, absorbance at λ=517 nm was measured. Simultaneously, appropriate blank and control samples were analysed as well as samples for ascorbic acid as standard compound.

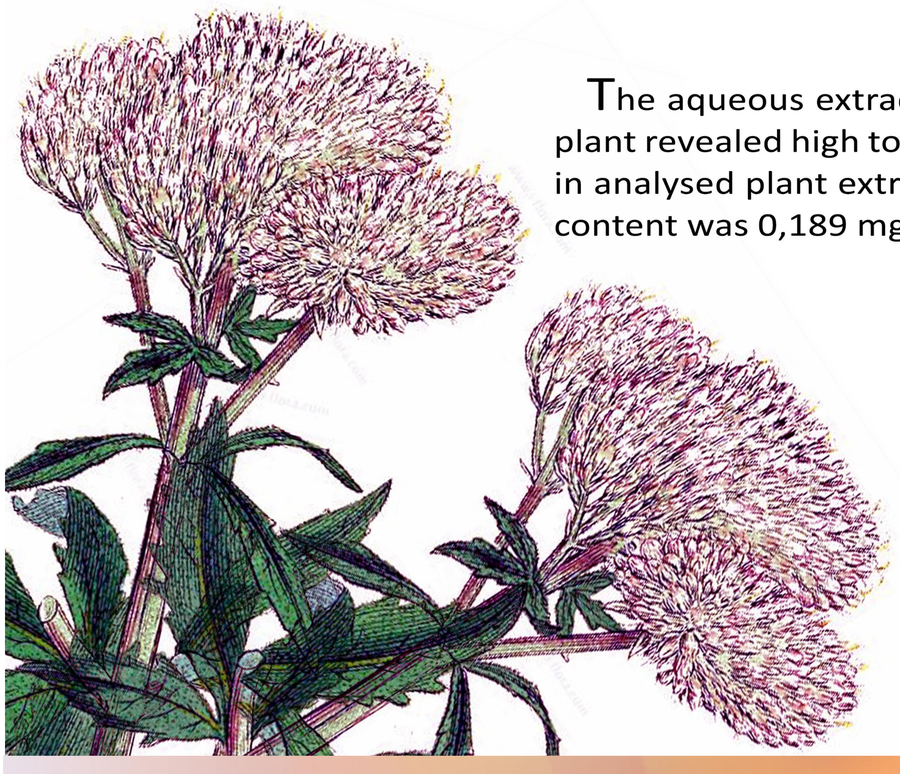






RESULTS

The aqueous extracts showed cytotoxic effect against U87 MG with IC50 values of 0,09±0,01 µg/mL. The plant revealed high total phenolic content, total flavonoid and antioxidant capacity. Total polyphenols content in analysed plant extract was 0,069 mg of gallic acid equivalents per one mg of dried extract. Total flavonoid content was 0,189 mg of quercetin equivalents per one mg of dried extract.



CONCLUSION

Synergistic effects of bioactive compounds of Eupatorium cannabinum plausibly contributed to the cytotoxic effect of the extract. The presented results suggest that the historical description of the anti-cancer properties of Eupatorium cannabinum may have scientific justification and be the starting point for further research.

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