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# CYTOTOXIC POTENTIAL OF INDUSTRIAL HEMP EXTRACTS

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***Abstract:*** *Industrial hemp (Cannabis sativa L.) is a source of fibers, oil and valuable secondary metabolites. Regarding phenolic compounds, it has to be noted that the plant biosynthesizes molecules with various pharmacological benefits. The aim of this study was to prove the cytotoxic potential of hemp polar and non-polar fractions against two cancer cell lines (BT-20 and U87). The study revealed the potential antitumor activity of industrial hemp selective fractions but a correlation between polyphenols content and the cytotoxic effect could not be established.*

***Key words:*** *Cannabis sativa, cancer, polyphenols*

## 1. Introduction

Industrial hemp (*Cannabis sativa* L.) is a source of fibers, oil and molecules and it is an emblematic example of a multi-purpose crop [1]. *Cannabis sativa* L. (hemp) has the highest industrialization capacity of all technical plants, everything is capitalized. Fibre-type differs from medicinal *C. sativa*, since it contains only few levels of $\Delta^9$-THC and high levels of phytocannabinoids, along with other non-cannabinoid constituents belonging to diverse classes of natural products. Today, more than 560 constituents have been identified in hemp [3]. In recent years, a number of preclinical researches have been focused on the role of cannabidiol as an anticancer molecule. In animal models, this compound has been shown to inhibit the progression of several cancer types [7].

Regarding phenolic compounds, it has to be noted that the plant biosynthesizes a plethora of unique non-cannabinoids second metabolites, such as prenylated flavonoids, stilbenoids derivatives and lignanammides with various pharmacological benefits [8].

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The aim of this study was to prove the cytotoxic potential of hemp polar and non-polar fractions against two cancer cell lines.

## 2. Materials and Methods

*Plant material:* Hemp raw material (leaves) was gifted by a grower from North-Eastern area of Romania.

*Preparation of extracts:* The hemp leaves extracts were separated from a 70% hydro-alcoholic solution by liquid-liquid partition with hexane. Further, selective extractive solutions (H1 and A1, respectively) were separated by flash chromatography starting from hexane extractive solution (H) and the aqueous waste (A). Crude extracts H and A were placed in a loading cartridge to be fractionated by the flash chromatography (Isolera Prime, Biotage-Sweden). A normal phase chromatography system with a mobile phase A as water and a mobile phase B as dichloromethane at a flow rate of 12 mL/min was used. The flash chromatography was performed with a ZIP KP-Sil column (10g). All the solutions obtained were concentrated to dryness and diluted according to *in vitro* assay protocol.

*HPLC Analysis:* Representative polyphenols (rutin, quercetin, kaempferol, chlorogenic, rosmarinic and caffeics acids) were quantified by HPLC. Chromatographical conditions were described in a previous paper [4].

*Cell lines and Viability Assay:* BT-20 (human mammary carcinoma) and U87 (human glioma) were cultured according to manufacturer's instructions (ATCC, USA).

A total of $5 \times 10^3$ (BT-20) and $10^4$ (U87) cells/well were treated in triplicate with several dilutions of extracts ranging from 15.62 to 250 µg/mL, negative control (ethanol 50%), positive control (100 µg/mL of 5-fluorouracil and irinotecan). The cells were incubated with the treatments for 24 h, after which 20 µl MTS (5 mg/mL, CellTiter 96-Aqueous One Solution Cell Proliferation Assay®, Promega) was added and further incubated for 4 h at 37 °C. The absorbance was measured at 492 nm with Boeco BMR-100 microplate reader. Cell viability was expressed as a percentage of live treated cells compared with live control cells.

## 3. Results and Discussions

Two selective extracts (H and A) and two corresponding fractions (H1 and A1) were obtained from dried hemp leaves. Analytical studies revealed that while A1 fraction is a more concentrated version of A extract, H1 fraction contains much lower amounts of compounds comparing to mother H extract (Table 1, Figures 1 to 4).

*Phenolics content of extracts and corresponding fractions (HPLC)* Table 1

| Compound [mg/100mL] | H extract | A extract | H1 fraction | A1 fraction |
|---|---|---|---|---|
| CA | 0.07 | 0.506 | 0.086 | 0.237 |
| CafA | 0.123 | 0.171 | 0.023 | 0.201 |
| R | 0.128 | 4.389 | 0.035 | 1.825 |
| Q | 0.043 | 0.873 | 0.098 | 0.730 |
| K | 0.240 | 0.291 | 0.026 | 0.038 |
| RA | 7.585 | 9.028 | 0.771 | 19.947 |
| [1]chlorogenic acid; [2]caffeic acid; [3]rutin; [4]quercetin, [5]Kaempferol; [6]rosmarinic acid | | | | |

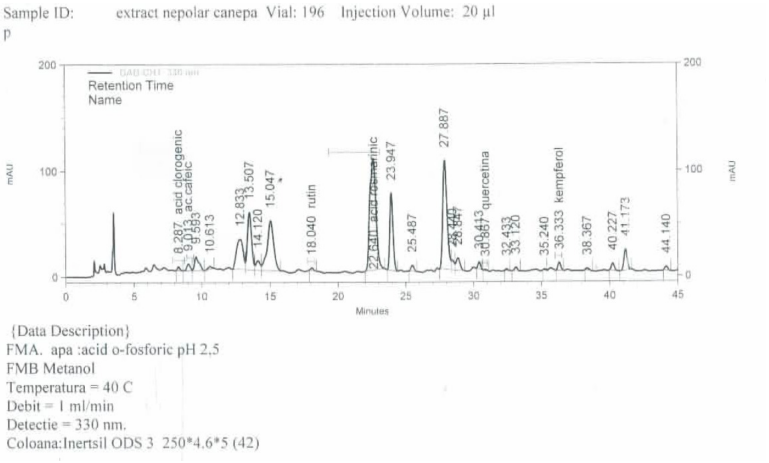


Fig. 1. *HPLC chromatogram of H extract*

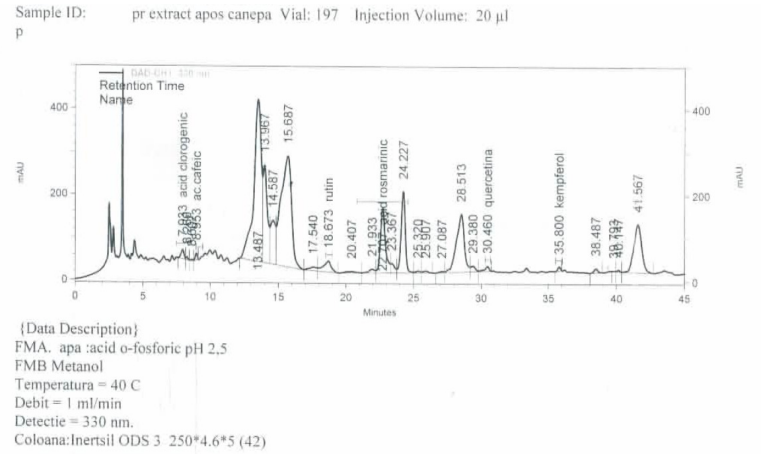


Fig. 2. *HPLC chromatogram of A extract*

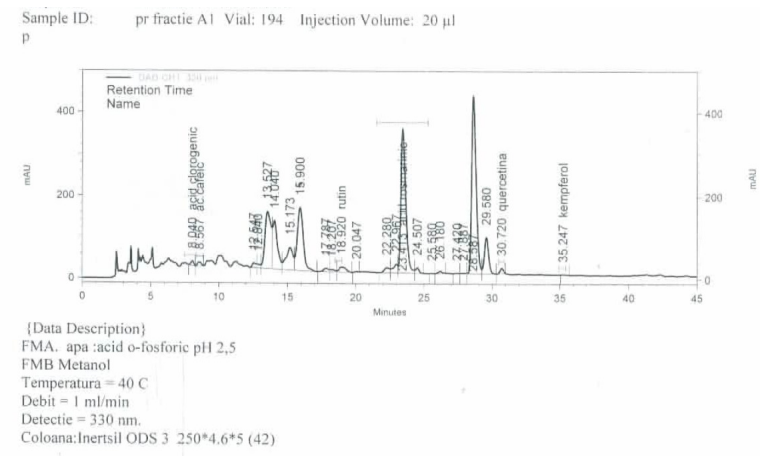


Fig. 3. *HPLC chromatogram of A1 fraction*

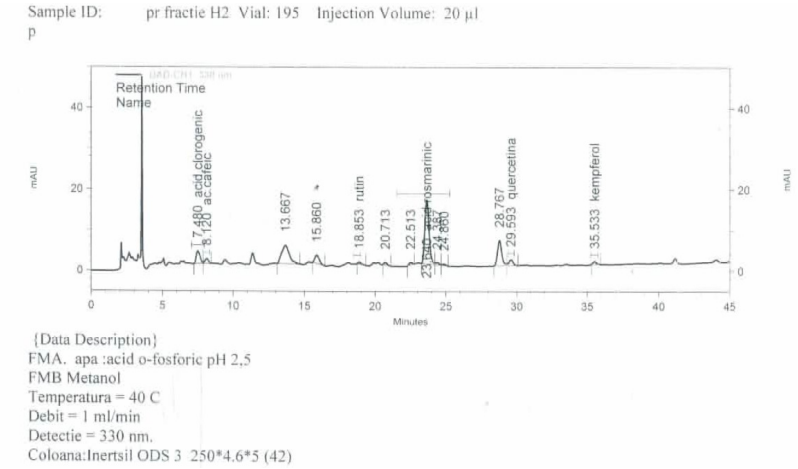


Fig. 4. *HPLC chromatogram of H1 fraction*

We conducted a screening of cytotoxic potential of all fractions (15.62 - 250 µg/mL) isolated from industrial hemp leaves against two cancer cell lines after exposure for 24 hours, comparing to irinotecan and 5-fluorouracil (100µg/ 100µL), synthetic reference substances.

All extracts were more active on BT-20 cell line, mainly hexane and aqueous solutions, at all concentrations tested. The fractions isolated by flash chromatography exhibited inhibitory effects on the cell cultures tested only at high doses (Figures 5 to 8).

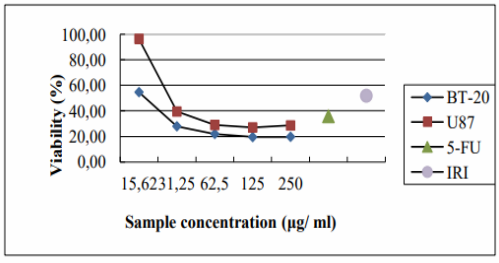


Fig. 5. *BT-20 and U87 cells viability after exposure to various concentrations of A extract*

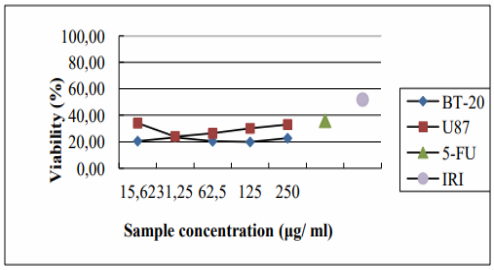


Fig. 6. *BT-20 and U87 cells viability after exposure to various concentrations of H extract*

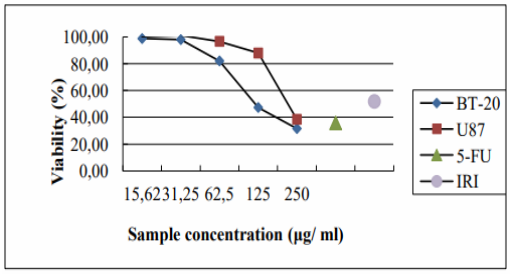


Fig. 7. *BT-20 and U87 cells viability after exposure to various concentrations of A1 fraction*

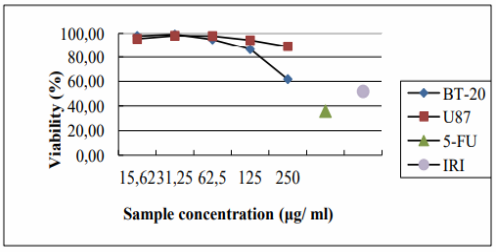


Fig. 8. *BT-20 and U87 cells viability after exposure to H1 fraction*

Although A extract contains higher amounts of phenolic compounds (rutin, quercetin and chlorogenic acid) comparing to H extract, a correlation with the inhibitory effect on tumor cells could not be found. On the contrary, non-polar extract exhibits cytotoxic action even at lower doses.

Other studies showed the efficiency of various phenolic compounds in tumor cells growth inhibition. Kaempferol treatment inhibits cell viability, reduces migration and/or invasion *in vitro* in a dose-dependent manner in glioblastoma, hepatic, colorectal, pancreatic, lung, renal and breast cancer cell lines, mostly as a result of cell cycle arrest or apoptosis [5], [10].

A recent review by Tang et al. [9] has outlined anti-cancer actions of quercetin – inhibition of proliferation, angiogenesis and metastasis on many types of cancer *in vitro* and *in vivo*.

Even A1 fraction is enriched in rosmarinic acid, its inhibitory action on tumor cells was demonstrated only at the maximum dose tested (250µg/mL). Anticancer effect of rosmarinic acid is well documented on several cell lines and mechanisms of action are established. It induces apoptosis at concentrations in the 10–100 µg/mL range [6]. However, it was underlined that phenolics exhibit more beneficial properties when they were applied in the form of extracts comprising their mixtures [2]. In our study, both crude extracts – polar and non-polar- were more effective than their corresponding fractions, suggesting the complementary pharmacological effect of several classes of compounds.

## 4. Conclusions

The study revealed the potential antitumor activity of industrial hemp selective fractions but a correlation between polyphenols content and the cytotoxic effect could not be established.

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