Cytotoxic screening of selected *Cannabis* cultivars using brine shrimp lethality assay

Zoran Zhivikj*; Kristina Shutevska; Ana Marija Bajatovska; Sevda Sofronievska; Marija Karapandzova; Ivana Cvetkovikj Karanfilova; Iskra Davkova; Lidija Petrushevska-Tozi; Gjoshe Stefkov; Tatjana Kadifkova Panovska

Ss Cyril and Methodius University in Skopje, Faculty of Pharmacy, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia

INTRODUCTION

Medicinal use of *Cannabis sativa* containing over 150 phytocannabinoids and hundreds of terpenes and flavonoids probably dates back more than two millennia. According to Nahler (2022) numerous *in vitro* studies have reported not only the cytotoxic effects against various cancer cell lines but also possible pathways that ultimately result in the suppression of metastasization, angiogenesis, tumor growth, promotion of autophagy, and cancer cell apoptosis. However, the therapeutic effects of the main active compounds cannabidiol (CBD) and delta-9-tetrahydrocannabinol (Δ^9-THC) within various types of extracts appear to be influenced by other phytochemicals in addition to the cannabinoid composition (Nahler, 2022). Hence, cannabis components that have anti-cancer potential are not well understood and relative efficacy and safety of pure substances and extracts are subject of an ongoing discussion. This study aimed to determine cytotoxic potential of two *Cannabis* oils and different extracts prepared from four Cannabis cultivars.

MATERIALS AND METHODS

Sample preparation

Cannabis oils were prepared in methanol at a concentration of 50 mg/mL.
Extracts from inflorescence (50 mg/mL) obtained from the following Cannabis cultivars: Eletta Campana, Charlotte's Angels, Orange Hill Special and Passion #1, were prepared by methanolic and ethanolic extraction.

T*oxicity criteria and classification of extracts*

Plant extracts were categorized using the LC_{50} values according to Meyer's and Clarkson's toxicity scales (Meyer et al., 1982; Clarkson et al., 2004).

Brine shrimp lethality assay (BSLA)

To conduct the BSLA, the *Artemia salina* larvae were subjected to methanolic and ethanolic extracts as well as *Cannabis* oils (Meyer et al., 1982). The mortality of larvae was observed during 24 h. By plotting the percentage of dead shrimps against the logarithm of the sample concentration, the median lethal concentration (LC_{50}) was determined. A probit regression analysis was used to calculate the LC_{50} values.

Fig. 1 Brine shrimp lethality assay.

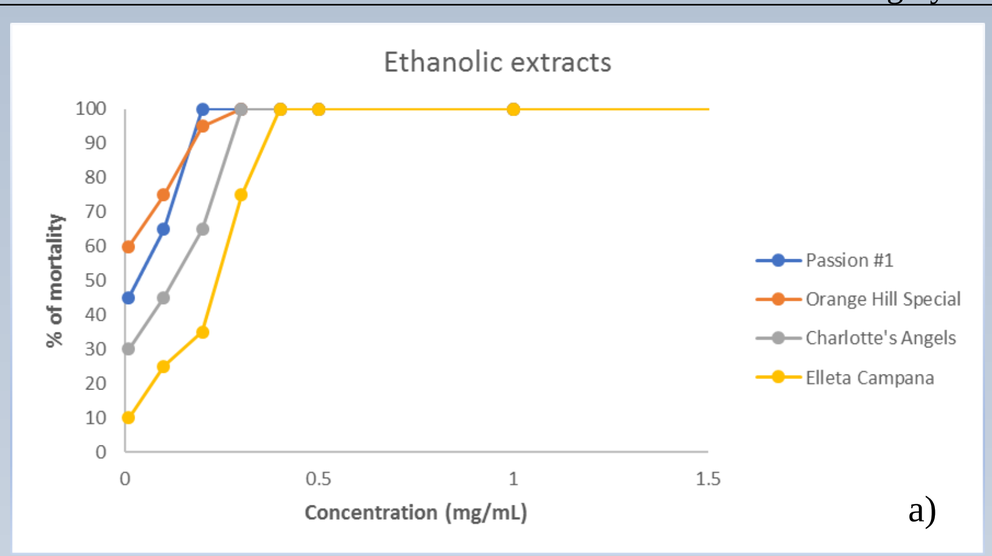
RESULTS AND DISCUSSION

The methanolic extracts prepared from both strains Eletta Campana and Charlotte's Angels showed insignificantly higher toxic potential compared to the respective ethanolic extracts after 24 h of exposure (LC_{50} values of 26 and 4.5 µg/mL for methanolic extracts, and 31 and 5 µg/mL for ethanolic extracts, respectively) (Table 1). The methanolic extract of the Orange Hill Special strain has demonstrated significantly lower toxic potential than the ethanolic extract of the same strain (Table 1). Methanolic and ethanolic extracts of the Passion #1 cultivar has shown equal toxic potential (Table 1). Passion #1 cultivar both methanolic and ethanolic extracts had the highest toxic potential after 24 h of exposure, followed by Orange Hill Special strain ethanolic extract. The investigated *Cannabis* oils displayed toxic activity in accordance with both scales of toxicity, with the peak toxic potential occurring after 24 h of exposure (Table 1). According to Clarkson's toxicity classification scale, the toxic potential of the two different oils as well as for all the cultivars increased with prolonged exposure as follows: 2 hours (moderate toxicity) < 6 hours (high toxicity) < 24 hours (high toxicity). The determined LC_{50} values of *Cannabis* oils indicated significant toxic potential comparable to the ethanolic extract prepared from Orange Hill Special strain and Passion #1 methanolic and ethanolic extracts. We have determined significantly lower LC_{50} concentrations of the three methanolic extracts (Charlotte's Angels, Orange Hill Special and Passion #1) compared to the methanolic extract obtained from *Cannabis sativa* ($LC_{50} \approx 20$ µg/mL) (Baroi et al., 2020). This finding indicated that methanolic extracts of the three investigated *Cannabis* cultivars possess more powerful cytotoxic activity than the extract of *Cannabis sativa.*

*E-mail: zzivic@ff.ukim.edu.mk

Table 1. Cytotoxic potential of ethanolic and methanolic extracts of different *Cannabis* cultivars and *Cannabis* oils according to the Brine shrimp lethality assay.

Cultivars	LC_{50} (µg/mL)		Meyer's scale	Clarkson's scale
	Ethanolic extract	Methanolic extract		
Elleta Campana	30.8	26.2	toxic	highly toxic
Charlotte's Angels	5	4.5	toxic	highly toxic
Orange Hill Special	0.4	1.7	toxic	highly toxic
Passion #1	0.35	0.35	toxic	highly toxic
Cannabis oil N° 1	/	0.63	toxic	highly toxic
Cannabis oil N° 2	/	0.33	toxic	highly toxic



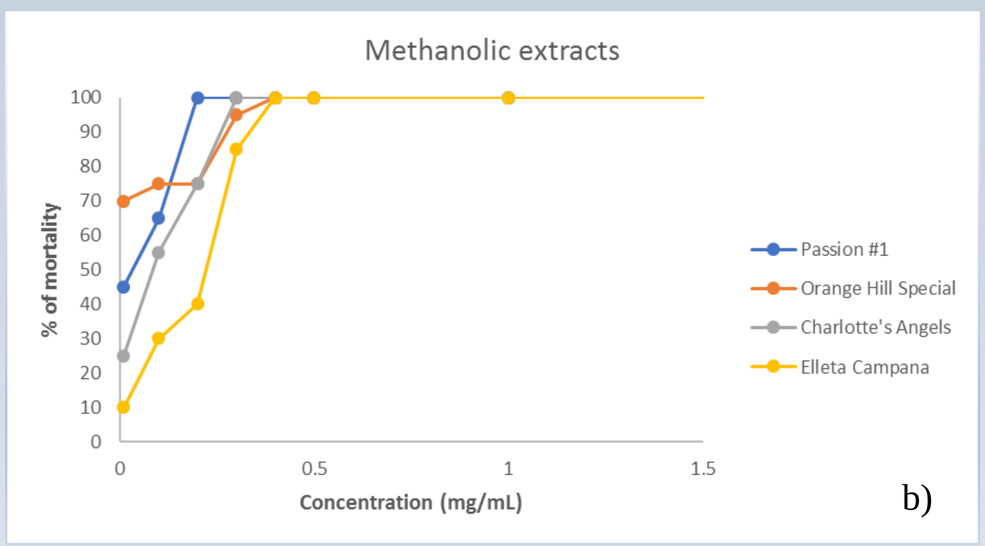


Figure 2. Trend of mortality for *A. salina* against concentration for ethanolic (a) and methanolic (b) extracts from different Cannabis cultivars.

All samples tested for toxicity against brine shrimp had LC_{50} values less than 100 µg/ml provided evidence in favor of their traditional medicinal use and potential cytotoxicity.

CONCLUSION

This study suggested potential cytotoxic properties of the examined *Cannabis* oils and extracts. Further research is required to identify the active constituents responsible for these effects and to determine more closely the possible mechanism of action in order to be used as anticancer agents.

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