Macedonian pharmaceutical bulletin, 68 (Suppl 1) 351 - 352 (2022)
Online ISSN 1857 - 8969
DOI:10.33320/maced.pharm.bull.2022.68.03.169
Short communication

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

Zoran Zhivikj[1*], Kristina Shutevska[1], Ana Marija Bajatovska[1], Sevda Sofronievska[1], Marija Karapandzova[2], Ivana Cvetkovikj Karanfilova[2], Iskra Davkova[2], Lidija Petrushevska-Tozi[1], Gjoshe Stefkov[2], Tatjana Kadifkova Panovska[1]

[1]Ss Cyril and Methodius University in Skopje, Faculty of Pharmacy, Institute of Applied Biochemistry, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia
[2]Ss Cyril and Methodius University in Skopje, Faculty of Pharmacy, Institute of Pharmacognosy, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia

Introduction

The use of *Cannabis sativa* as a medicine has a lengthy history and probably dates back more than two millennia. This plant contains more than 500 constituents, including over 150 phytocannabinoids and hundreds of terpenes and flavonoids. The potential therapeutic effects of the main active compounds cannabidiol (CBD), 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). Furthermore, cannabis components and/or blends of these compounds that have anti-cancer potential are not well understood (Omer et al., 2021). 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 (Nahler, 2022). However, the relative efficacy and safety of pure substances and extracts are subject of an ongoing discussion. Therefore, the aim of our study was to determine the toxic 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.

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 (McLaughlin et al., 1998; Meyer et al., 1982). The following concentrations were used: 50, 25, 10, 5, 3, 1, 0.5, 0.4, 0.3, 0.2, 0.1, and 0.01 mg/mL. The mortality of larvae was observed after 2, 6 and 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 determine the LC_{50} values.

Toxicity criteria and classification of extracts

The Meyer's and Clarkson's scales of toxicity were used to categorize plant extracts based on the obtained LC_{50} values (Meyer et al., 1982; Clarkson et al., 2004). Both scales classify extracts as toxic if their LC_{50} values

*zzivic@ff.ukim.edu.mk

are less than 1000 µg/mL. Furthermore, Clarkson's scale categorizes the extracts with high (0 - 100 µg/mL), moderate (100 - 500 µg/mL), and low (500 - 1000 µg/mL) toxicity.

Results and discussion

In this study, all examined extracts showed toxic effects based on Meyer's toxicity classification system. The methanol extracts prepared from both strains Eletta Campana, and Charlotte's Angels showed insignificantly higher toxic potential compared to the respective ethanol extracts after 24 hours of exposure (LC_{50} values of 26 and 4.5 µg/mL for methanol extracts, and 31 and 5 µg/mL for ethanol extracts, respectively). The ethanol extract of the Orange Hill Special strain has demonstrated a higher toxic potential (LC_{50} 0.4 µg/mL) in comparison with the methanol extract of the same strain (LC_{50} 1.7 µg/mL). The toxic potential of the Passion #1 cultivar was similar in both the methanol and ethanol extracts (LC_{50} 0.35 µg/mL). According to the Clarkson's classification scale of toxicity, with prolonged exposure the toxic potential increased for all the cultivars as follows: 2 hours (low toxicity) < 6 hours (moderate toxicity) < 24 hours (high toxicity). Passion #1 cultivar both methanol and ethanol extracts had the highest toxic potential after 24 hours of exposure, followed by Orange Hill Special strain ethanolic extract.

Both investigated *Cannabis* oils displayed toxic activity in accordance with the Meyer's scale, with the peak toxic potential occurring after 24 hours of exposure with LC_{50} values of 0.63 and 0.33 µg/mL. According to Clarkson's toxicity classification scale, the toxic potential of the two different oils 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 significantly higher toxic potential compared to the examined methanolic and ethanolic extracts.

When BSLA was applied to examine the pharmacological profile of hexane, ethyl acetate, methanol, and water extracts of *Cannabis sativa*, the highest LC_{50} was observed for the methanol extract (≈ 20 µg/mL), followed by ethyl acetate and hexane extracts, while the water extract showed no activity (Baroi et al., 2020). The LC_{50} concentrations of the investigated methanol extracts in our study was significantly lower except the one obtained from the Elleta Campana cultivar. Although simple, the BSLA is a very effective tool for determining the bioactivity of plant extracts, despite its limitations in terms of explaining the mechanism of action. Our research has shown that the BSLA is a practical method for tracking as well as comparison of the cytotoxic activities of *Cannabis* cultivars. These findings provide evidence in favor of their traditional use since all samples tested for toxicity against brine shrimp had LC_{50} values less than 100 µg/ml. Until the individual bioactive compounds are completely characterized as well as their potential interaction with secondary metabolites, BSLA is a valuable and rapid tool for recognizing cytotoxic activity of plant sources that may be effective against cancer.

Conclusion

This study revealed the cytotoxic properties of the examined *Cannabis* oils and extracts, suggesting their potential applications as anticancer agents. Further research is required to identify the active constituents responsible for these effects and to determine more closely the possible mechanism of action.

References

Anis, O., Vinayaka, A.C., Shalev, N., Namdar, D., Nadarajan, S., Anil, S.M., Cohen, O., Belousova, E., Ramon, J., Mayzlish Gati, E., Koltai, H., 2021. Cannabis-derived compounds Cannabichromene and Δ9-tetrahydrocannabinol interact and exhibit cytotoxic activity against urothelial cell carcinoma correlated with inhibition of cell migration and cytoskeleton organization. Molecules 26, 465. https://doi.org/10.3390/molecules26020465

Baroi, S., Saha, A., Bachar, R., Bachar, SC., 2020. Pharmacognostical, phytochemical and pharmacological potentials of *Cannabis sativa* L. Asian J. Pharmacogn. 4(2), 14-23. eISSN-0128-1119.

Clarkson, C., Maharaj, V. J., Crouch, N. R., Grace, O. M., Pillay, P., Matsabisa, M. G., Bhagwandin, N., Smith, P. J., Folb, P. I., 2004. *In vitro* antiplasmodial activity of medicinal plants native to or naturalised in South Africa. J. Ethnopharmacol. 92(2-3), 177-191. https://doi.org/10.1016/j.jep.2004.02.011

McLaughlin, J.L., Rogers, L.L., Anderson, J.E., 1998. The use of biological assays to evaluate botanicals. Ther. Innov. Regul. Sci. 32(2), 513-524. https://doi.org/10.1177/009286159803200223

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., McLaughlin, J.L., 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45(5), 31-34. https://doi.org/10.1055/s-2007-971236

Nahler, G., 2022. Cannabidiol and other phytocannabinoids as cancer therapeutics. Pharm. Med. 36, 99-129. https://doi.org/10.1007/s40290-022-00420-4

Velasco, G., Sánchez, C., Guzmán, M., 2016. Anticancer mechanisms of cannabinoids. Curr. Oncol. 23(2), S23-S32. https://doi.org/10.3747/co.23.308