Macedonian pharmaceutical bulletin, 68 (Suppl 1) 349 - 350 (2022)
Online ISSN 1857 - 8969
DOI:10.33320/maced.pharm.bull.2022.68.03.168
Short communication

Evaluation of *Tanacetum vulgare* L. and *Juniperus communis* L. biocompatibility limitations in eukaryotic cells

Kristina Shutevska[1*], Zoran Zhivikj[1], Sevda Sofronievska[1], Ana Marija Bajatovska[1], Marija Karapandzova[2], Ivana Cvetkovikj Karanfilova[2], Nadica Trajkovska[2], Tanja Petreska Ivanovska[1], 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 rich phytochemical composition of *Tanacetum* and *Juniperus* genus has made their use to be considerable in both conventional and modern medicine. The *Juniperus* genus contains flavonoids, terpenes, and coumarins, while the *Tanacetum* genus additionally contains sesquiterpene lactones, which are active chemical components that exhibit various pharmacological and therapeutic effects. Juniper oil is multi-purposed as antimicrobial and anti-proliferative agent and beneficial in gastrointestinal and inflammatory disorders (Bais et al., 2014; Han et al., 2017). Hydroalcoholic extracts obtained from *T. vulgare* have been reported to exert cytotoxicity but can be used due to their antioxidant and neuromodulatory activities (Ak et al., 2021). Numerous investigations on these and other possible therapeutic application of *Tanacetum* and *Juniperus* species are still being carried out (Ak et al., 2021; Bais et al., 2014). To obtain more information for the safety of *T. vulgare* methanolic extracts and *J. communis* essential oils, bioactivity concentration range was investigated to assess the biocompatibility limits in eukaryotic cells. Since brine shrimps (*Artemia salina*) are frequently regarded as the most practical test organism for preliminary toxicity investigations, we have chosen to obtain the required data using this *in vivo* model.

Materials and methods

Plant material

Tanacetum vulgare plant material was collected from Kozhuf and Berovo (Republic of North Macedonia) and then subjected to extraction with methanol. *Juniperus communis* essential oils (EO) were commercial products obtained from *Juniperus communis* berries collected from two different locations, Berovo and Mavrovo.

Brine shrimp lethality assay (BSLA)

Artemia larvae were exposed to 5 mL of methanolic extracts of *T. vulgare* and *J. communis* essential oils according the procedure described by McLaughlin et al. (1998) and Meyer et al. (1982). The span of concentrations used was: 50, 25, 10, 5, 3, 2.5, 2, 1, 0.5, 0.1, and 0.01 mg/mL. Dead nauplii were counted after 2, 6 and 24 h. The median lethal concentration (LC_{50}) was determined by calculating the percentage of dead shrimps against the logarithm of the sample concentration. Finney's Probit analysis was used to determine the LC_{50}.

Results and discussion

The lowest mortality rate for both *T. vulgare* and *J. communis* was detected when the larvae were exposed to a concentration of 0.01 mg/mL for 24 h.

No toxicity was observed after 2 and 6 h of exposure of the *Artemia* larvae to all examined extracts obtained from *T. vulgare*. The extracts exhibited considerable

*k.sutevska@ff.ukim.edu.mk

toxicity after 24 h of exposure, when the larvae were exposed to the extract originating from Berovo. This *T. vulgare* extract has shown to be much more toxic (LC_{50} 16 µg/mL) than the extract obtained from the same species collected on Kozhuf (89 µg/mL). According to the available literature, this might be partially due to the variable quantity of the thujone, which has been reported to have toxic effects on nauplii after 24 h of exposure (Radulović et al., 2017).

In a study conducted by Ak et al. (2021) where *T. vulgare* plant material (flower, stem and mix containing aerial parts such as stem, flower, and leaves) extracted with different polarity solvents (water, ethanol-water, and hexane) using maceration and infusion techniques were used to determine their toxicity. Scalar extract dosages of 100 µg/mL to 20 mg/mL were applied to the shrimps for 24 h. All extracts showed a high degree of toxicity, with LC_{50} values < 2 mg/mL. The methanolic extracts prepared in our study also showed toxicity, but at significantly lower concentrations (< 1 mg/mL) probably due to the ecological and geographical impact on the chemical composition of our plant material.

Both *J. communis* essential oils showed a high degree of toxicity at all-time points, with LC_{50} values < 1 mg/mL. After exposure of 24 h, the EO obtained from Mavrovo showed significantly higher toxicity than the EO originating from Berovo (LC_{50} 0.02 and 0.95 µg/mL, respectively). Since no reports are available in the literature for the toxicity of *J. communis* EO, further discussion entailed the published data regarding the Juniper berries extracts. The extract of *J. communis* fruits obtained from Nepal has been reported to exhibit cytotoxicity *in vitro* using hepatocarcinoma cells and *in vivo* in BALB/c nude mice due to high concentration of monoterpenes like α-pinene and limonene (Huang et al., 2021). Moreover, methanol extracts of *J. communis* berries harvested from five different localities in North Macedonia have been found to possess a varying degree of cytotoxic potential. Most noticeable cytotoxic effect was detected for the berries collected from Pelister (LC_{50} 128 µg/mL) and the lowest cytotoxic activity was demonstrated by the berries collected from Makedonski Brod (LC_{50} 969 µg/mL). The toxicity of the samples taken from Pelister and Jakupica was significantly increased at concentrations of 1 mg/mL and 3 mg/mL, respectively (Jovanova et al., 2015). The results of our study are compatible with the data revealed from the analysis of Juniper berries extracts indicating presence of potentially toxic compounds in the Juniper essential oil as well.

Conclusion

The most suitable test organism for initial toxicity assessments that is frequently used is the brine shrimp lethality test. Both *T. vulgare* and *J. communis* have shown toxic effects due to their chemical composition (mostly probable to the presence of thujone and α-pinene, respectively). Considering the wide use of *Juniperus* and *Tanacetum* species as therapeutic agents in traditional medicine, biocompatible limit concentration (< 0.01 mg/mL) was determined in eukaryotic cells as an important safety information.

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