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*Short communication*

# Bioactivity of *Aloysia citridora*: Verbascoside quantification and assessment of cytotoxic effect on *Artemia salina nauplii*

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## Introduction

*Aloysia citriodora* (syn. *Verbena citridora*, Verbenaceae) is a perennial shrub widely cultivated and naturalized in warm climate zones. For years, it has been a part of traditional herbal medicine as a remedy which is used to improve digestion, dyspepsia, insomnia and nervousness. Furthermore, it is recognized to have an overall effect as an antispasmodic, sedative and antimicrobial agent. Recent research has also documented its antioxidant and antiproliferation potential (Rashid et al. 2022), but also anti-obesity properties as well as the capacity to reduce inflammation. Besides the medicinal uses, it is also utilized as a highly aromatic herb and used as spice because of the characteristic leaves' odor that is reminiscent of lemon and it is used as a natural scent in soaps and cosmetics. Due to the fact that *Aloysia citridora* is quite popular, the aim of this study was to determine the content of the phenylethanoid component and identification marker for this species verbascoside as well as to assess its possible cytotoxic effect.

## Material and Methods

### *Sample preparation*

*Aloysia citriodora* extracts were made of 50g dried and pulverized leaf, supplied from Alkaloid AD Skopje (Skopje, N. Macedonia) and 50 mL 50% ethanol (v/v). Extraction was proceeded on ultrasonic bath (50 Hz, 720W) for 15 minutes. The extracts were then filtered and diluted to 50 mL with the same solvent, to reach final concentration of 10 mg/mL.

### *HPLC quantification*

Quantification of verbascoside was performed using a validated HPLC-DAD method on an Agilent Technologies 1200 system equipped with a diode-array detector. Detection was carried out at 330 nm, and quantification was achieved by the external standard method using a calibration curve prepared from verbascoside standard solutions.

### *Brine shrimp lethality test*

The cytotoxic potential of the extract was assessed *in vivo* using the Brine Shrimp Lethality Assay (BSLA), based on the mortality of *Artemia salina* nauplii following exposure to the tested extract for 1, 2, 4, 6, and 24 hours. The stock extract was prepared in a concentration of 10 mg/mL. Subsequently, a

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series of test solutions with concentrations of 5, 3, 1, 0.5, 0.1, and 0.01 mg/mL were prepared by dilution using sodium chloride solution as the diluent. A positive control containing potassium dichromate and a negative control containing sodium chloride solution were prepared in parallel under the same experimental conditions. The mortality rate for each concentration was determined by counting the number of dead nauplii and expressing the result as a percentage of the total number of organisms exposed. The median lethal concentration ($LC_{50}$) values were calculated using probit regression analysis according to the method described by Finney (1952). The toxicity of the extract was subsequently classified according to the criteria proposed by Meyer et al. (1982) and Clarkson et al. (2004), based on the $LC_{50}$ values.

## Results and discussion

*Quantitative analysis*

The quantification of verbascoside was carried out considering the area under curve, in comparison with a series of concentrations of commercially available standard. The verbascoside content in the examined extract was determined to be 1.06 mg/mL (10.6%). This value exceeds and complies with the minimum verbascoside content requirement of 2.5% specified in Ph. Eur. 12.0.

*Assessment of cytotoxicity*

The obtained results demonstrated a clear time-dependent increase in toxicity, as evidenced by the progressive decrease in $LC_{50}$ values with prolonged exposure of *Artemia salina* nauplii to the extract. After 1 hour of exposure, the $LC_{50}$ value was determined to be 1515.22 μg/mL, which gradually decreased to 1045.58, 328.55, and 240.77 μg/mL after 2, 4, and 6 hours, respectively. Following 24 hours of exposure, the $LC_{50}$ value decreased markedly to 30.89 μg/mL. This trend indicates that the toxic effect of the extract intensified with increasing exposure time, suggesting either cumulative uptake of bioactive constituents by the nauplii or a delayed onset of toxic action. According to the toxicity classification proposed by Meyer et al. (1982), the extract should be considered non toxic upon the first two hours of exposure, but toxic after four hours. Classification according to Clarkson et al., (2004) toxicity index revealed a gradual increase in toxicity. During the first two hours of exposure, the extract was categorized as non toxic ($LC_{50}$ ≥1000 μg/mL), whereas after 24 hours it was classified as highly toxic ($LC_{50}$<100 μg/mL). Similar dose-dependent cytotoxic effect of verbascoside was confirmed in 4T1 mouse mammary tumor cells, and proven that induces apoptosis *in vitro* (Daneshforouz et al. 2021).

## Conclusion

According to the obtained results, *Aloysia citridora* meets the requirements of the European Pharmacopoeia in terms of active component content and shows prominent cytotoxic activity. Further *in vivo* examinations should be perfomed in order to clarify the potential cytotoxicity as well as to determine toxicological profile of this herbal drug.

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