

SUMMARY

Background: Gastrointestinal helminth infections of animals and humans are mainly controlled by synthetic drugs. However, a significant number of studies report spreading resistance in nematodes of both humans and the livestock. This study was conducted to evaluate the *in vitro* and *in vivo* anthelmintic efficiency of *Curcubita pepo* L. hot water (HWE), cold water (CWE) or ethanol (ETE) extracts on two model parasitic nematodes: *Caenorhabditis elegans* and *Heligmosoides bakeri*.

Methods: Raman spectroscopy and IR spectroscopy were applied to perform the qualitative analysis of the obtained extracts. To evaluate the efficiency of extraction protocol, the LC-ESI-TOF-MS based method was performed on the obtained *C. pepo* L. hot water (40°C), cold water (22°C) and ethanol extracts (70%). *In vitro* methods included analysis of impact of extracts on *C. elegans* and different developmental stages of *H. bakeri*. *In vivo* experiments included treatment of *H. bakeri* infected mice different concentrations of ethanol pumpkin seeds extract.

Results: Among the tested solvents, ethanol was found to recover the highest variety of secondary metabolites from the pumpkin. Among the tested solvents, ethanol was found to recover the highest variety and quantity of the meaningful secondary metabolites from pumpkin seeds. All extracts were found to contain cucurbitine, aminoacids, and fatty acids, and notably for the first time in *C. pepo* extracts also berberine and palmatine – two isoquinoline alkaloids, known for their antiparasitic properties. The *C. pepo* seed extracts were found to exhibit nematidicidal potential *in vitro*. The ethanol extract exhibited a positive effect on *H. bakeri* eggs hatching, compared to a phosphate buffered saline (PBS) control. All three extracts significantly affected the survival of L1 and L2 *H. bakeri* larvae, compared with the negative control. The ethanol extract reduced worm motility relative to the negative control. Nevertheless, worm motility remained lower than the positive control. There were no

significant effects of pumpkin seed extracts on *C. elegans* integrity or motility. Since, the highest variety of compounds was found for the alcoholic extract, *in vivo* experiments were conducted to assess the anthelmintic properties of ethanol extracts against the mouse model parasitic nematode *H. bakeri*. Ethanol extract showed anthelmintic properties for both *H. bakeri* fecal egg counts and adult worm burdens. The highest egg counts reduction was observed for the 8g/kg dose (IC_{50} against *H. bakeri*=2.43; 95% CL = 2.01–2.94). The decreasing FEC was accompanied by a significant reduction in worm burden of the treated mice compared to the control group.

Conclusions: The results clearly indicate that pumpkin seed extracts possess active compounds that might be used against gastrointestinal nematodes of mice. Based on the obtained results, ethanol *C. pepo* extract may be a candidate for the development of a novel anthelmintic treatment of GI nematode infections.