

Abstract

Title: Investigation of Trypsin Inhibitors in *Artemisia afra* Leaf Extracts as Putative Anti-Obesity Agents.

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Obesity has emerged as a global health pandemic which contributes significantly to morbidity and mortality thus escalating the burden of healthcare costs. Lifestyle modifications and anti-obesity drugs often fail due to limited efficacy, adverse effects and limited accessibility. Hence, this study aimed to investigate trypsin inhibitors in *Artemisia afra* leaf extracts as potential anti-obesity agents. Sequential organic extraction of *Artemisia afra* leaves was carried out with hexane, ethyl acetate, and ethanol solvents. The extracts were evaluated for trypsin inhibitory activity using an enzyme-linked immunosorbent assay (ELISA). Pre-coated and uncoated plate-based assays were used for method validation based on accuracy, precision, reproducibility and repeatability. The absorbance was measured using a spectrophotometer. The results showed that extraction efficiency varied significantly by solvent, with ethyl acetate being highest (4.6%), followed by ethanol (4.0%) and hexane (2.5%). For biological activity, ethanolic extracts exhibited the highest trypsin inhibition (95-100%) than hexane (73-100%) and ethyl acetate (40-62%). The method was successfully validated. For accuracy and precision, the uncoated plate showed statistically significant values across all extracts ($p < 0.05$), while on the pre-coated plate, hexane and ethanol remained statistically significant ($p < 0.05$), whereas ethyl acetate was not statistically significant ($p > 0.05$). Repeatability and reproducibility were acceptable for hexane (4.6 %RSD) and ethanol (3.3 %RSD) extracts. Therefore, ethanol was the most suitable solvent for extracting bioactive compounds that may serve as promising candidates for plant-based management of obesity. While the validated methods can be used to provide a valuable basis for analysis of anti-trypsin inhibitors in plants.