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ANTIBACTERIAL ACTIVITY OF *SALVIA OFFICINALIS* ESSENTIAL OIL OBTAINED BY ENZYME-ASSISTED HYDRODISTILLATION: COMPARISON WITH A COMMERCIAL SAGE ESSENTIAL OIL

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Introduction

Antimicrobial resistance is a major public-health concern, which has renewed interest in plant-derived agents as alternatives or complements to conventional antibiotics. Enzyme-assisted extraction (EAE), employing cell-wall-degrading enzymes such as cellulase and pectinase prior to hydrodistillation, has emerged as a green alternative capable of improving extraction yield and modifying the volatile profile of essential oils from a wide range of species.

Aqueous enzymatic approaches have also been applied specifically to *Salvia officinalis* for the recovery of rosmarinic acid. However, information regarding the antibacterial performance of *S. officinalis* essential oil obtained by enzyme-assisted hydrodistillation remains limited.

In this work, we investigated the antibacterial activity of an enzyme-assisted *S. officinalis* essential oil against bacterial reference strains and compared its performance with that of a commercial sage essential oil.

Materials and methods

Aerial parts of *S. officinalis* were subjected to an aqueous enzyme-assisted treatment (cellulase/pectinase cocktail) prior to Clevenger-type hydrodistillation. Detailed pretreatment parameters are the subject of a pending patent application and are not disclosed here. Essential-oil yield was calculated as Yield (%) = (mass_EO/mass_plant) × 100.

Antibacterial activity was assessed against *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 8739. For qualitative antibacterial assessment, bacterial suspensions were adjusted to 0.5 McFarland and inoculated onto Mueller–Hinton agar.

The essential oils were evaluated by direct application onto the inoculated agar surface, followed by overnight incubation at 36 °C. Antibacterial activity was assessed based on inhibition-zone diameter. Ampicillin, saline buffer, and Dimethyl Sulphoxide (DMSO) were included as positive, negative, and solvent controls, respectively. Broth microdilution (96-well plates, ten two-fold dilutions, ~10^6 CFU/mL, 36 °C, 18–24 h) was used to determine the Minimal Inhibitory

Concentration (MIC), while the Minimal Bactericidal Concentration (MBC) was assessed by subculturing onto nutrient agar.
Chemical composition was analysed by GC-MS (Thermo Trace 1310/TSQ 8000 EVO; samples diluted in heptane), with tentative compound identification against the NIST library.

Results and conclusions

Enzyme-assisted hydrodistillation resulted in an essential-oil yield of 0.48% (w/w). GC-MS analysis indicated a volatile profile predominantly composed of monoterpenes. The in-house enzyme-assisted essential oil (SE) produced a larger inhibition zone against *E. faecalis* ATCC 29212 than the commercial sage essential oil (SC2) under identical testing conditions (Figure 1a, b).

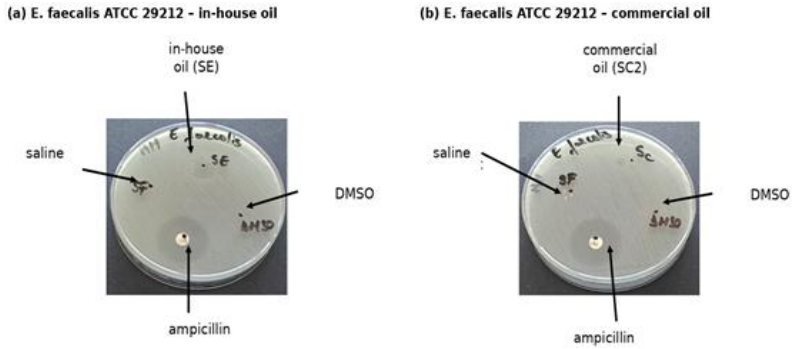


Figure 1 - Direct-contact antibacterial assay of **(a)** the in-house *S. officinalis* essential oil (SE) and **(b)** the commercial sage essential oil (SC2) against *E. faecalis* ATCC 29212. Saline: negative control; DMSO: solvent control; ampicillin: positive control.

Similar inhibition zones were observed for the in-house essential oil (SE) and the commercial sage essential oil (SC2) against *E. coli* (data not shown). Ampicillin produced larger inhibition zones than either essential oil under the tested conditions and served as the positive antibacterial control. In this preliminary study, the *S. officinalis* essential oil obtained through EAE, presented antibacterial activity against the tested reference strains. The inhibition zone obtained on *E. faecalis* was larger when using in-house essential oil compared to the commercial sage essential oil and similar responses were observed against *E. coli*. Broth microdilution further indicated antibacterial activity against *E. coli* ATCC 8739, with the MBC corresponding to the MIC under the tested conditions. These findings support further investigation of enzyme-assisted hydrodistillation as an approach for obtaining sage essential oils with antibacterial potential.

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