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The 3rd International Electronic Conference on Antibiotics  
Rise of Antibiotic Resistance: Mechanisms Involved and Solutions to  
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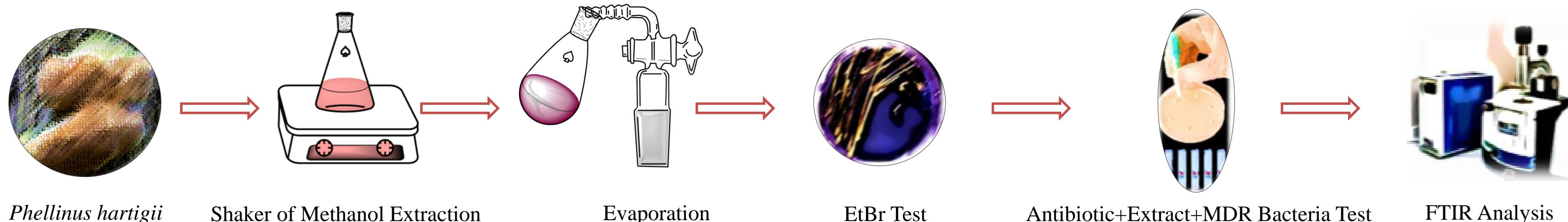
## The Potential of *Phellinus hartigii* Methanol Extract as an Inhibitor of Efflux Pumps in Multidrug Resistant *E. coli*

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### ABSTRACT

Antibiotic resistance, a growing concern for various reasons, underscores the urgency of discovering new antibiotics. However, the emergence of resistance mechanisms remains a formidable challenge. One such mechanism is the efflux pumps, which actively extrude antibiotics from bacterial cells, reducing their intracellular concentrations. Inhibiting these pumps can restore antibiotic efficacy. Natural sources are actively explored for potential inhibitors. This study aimed to assess the inhibitory potential of a methanol extract from the macrofungus *Phellinus hartigii* against multidrug-resistant (MDR) *E. coli* strains known to harbour active efflux pumps. These strains exhibited resistance to Amoxicillin-Clavulanate (AMC) with a MIC >32/2 (R). Inhibition was initially determined using the Ethidium Bromide (EtBr) test. Subsequently, combination studies involving the strain, antibiotic disks, and the extract revealed that the extract effectively arrested microorganism growth. Gas Chromatography-Mass Spectrometry (GC/MS) analysis of the extract identified major components, notably 2-methylhexacosane (59.10%) and Nonadecyl heptafluorobutyrate (9.65%), which accounted for over 9% of the total area. Fourier transform infrared spectroscopy (FTIR) analysis of the strain treated with a combination of the extract and antibiotic provided insights into the inhibitory mechanism. Significant differences were observed in FTIR peaks, particularly in the region between <math>900\text{ cm}^{-1}</math> and <math>3000\text{ cm}^{-1}</math>. These findings warrant further investigation to elucidate the inhibitory effects comprehensively.

**Keywords:** Efflux Pumps, *E. coli*, *Phellinus hartigii*, Antibiotic Resistance, FTIR Analysis

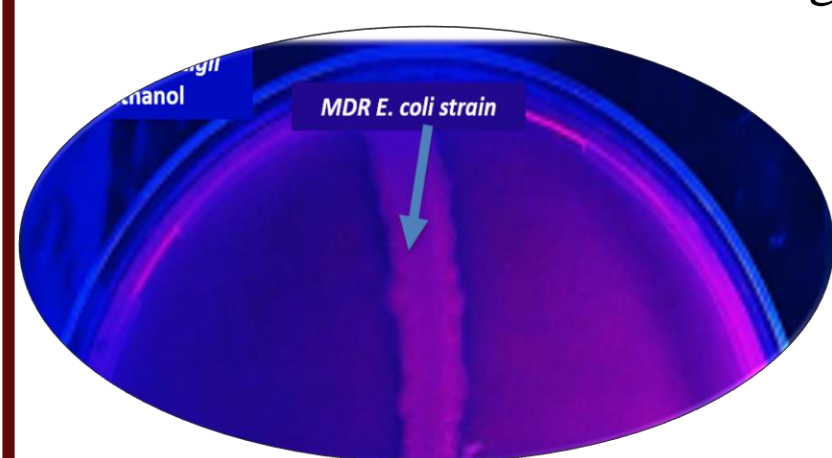
### METHOD

**Extraction / MIC Test / Extract + EtBr Cartwheel Test / Antibiotic Combination Test / GC/MS Analizi / FTIR Analysis**

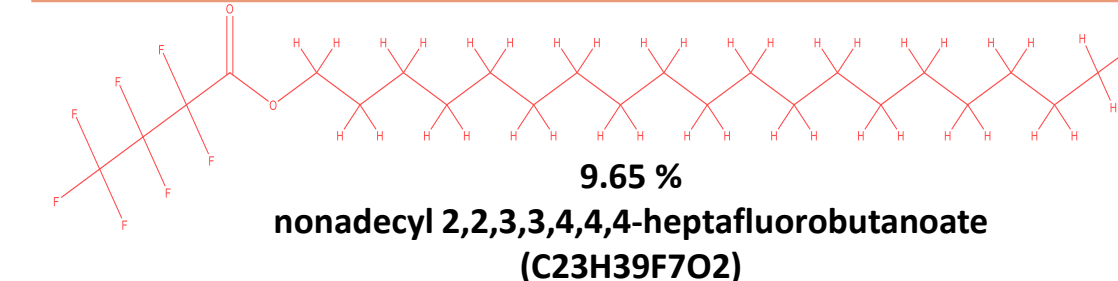
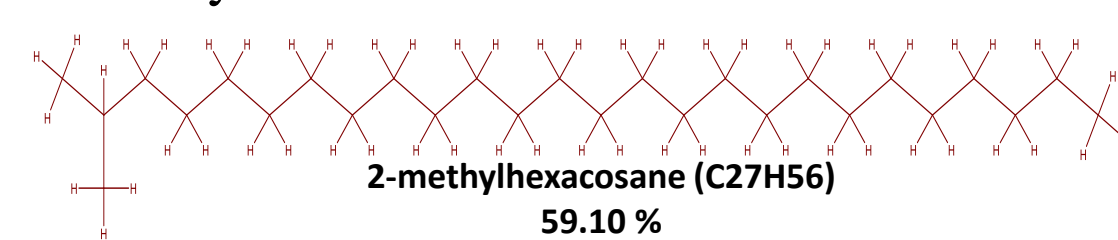
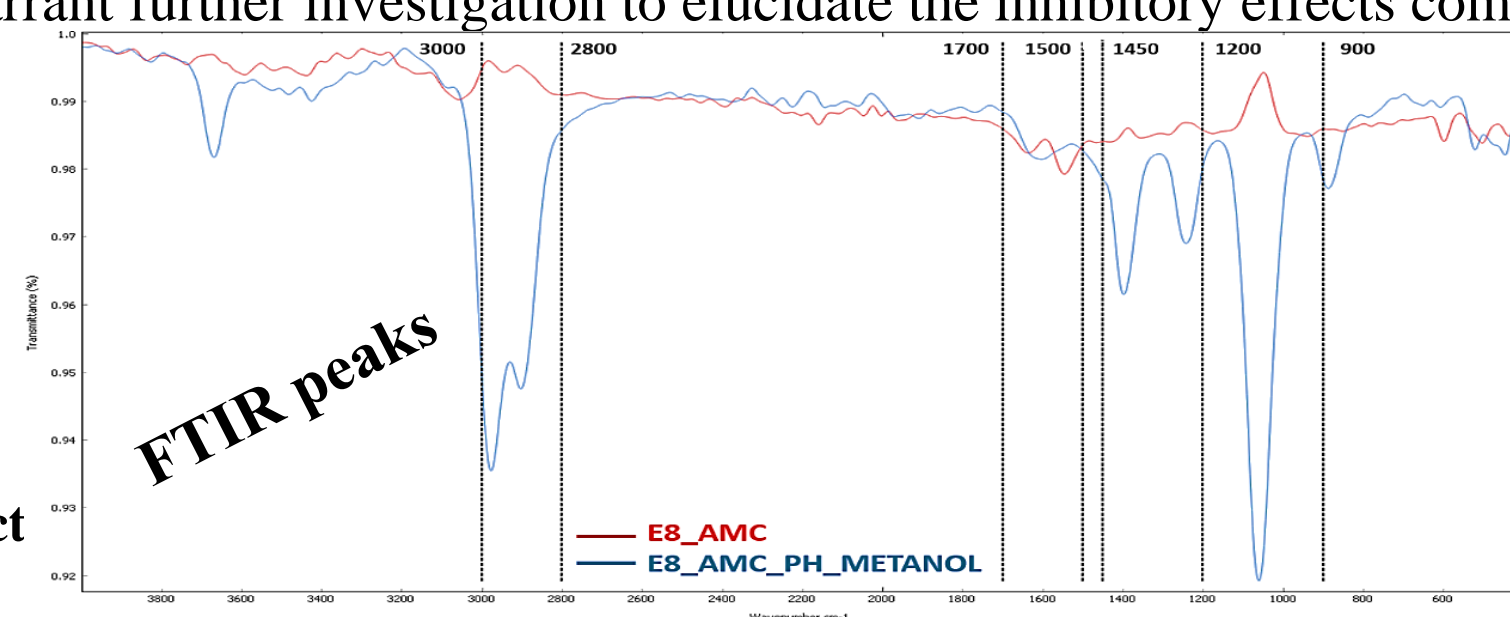
*Phellinus hartigii* macrofungus was extracted on the shaker using methanol (Merck) solvent and evaporated on the rotary evaporator. To dissolve the extract obtained after evaporation, certain proportions of sterile distilled water and 10% DMSO (Merck) were used. MIC test was applied to MDR *E. coli* strain with methanol extract. Next, EtBr testing was performed on Tryptic Soy Agar (TSA) using EtBr [1,2,3,4]. EtBr was added to TSA along with methanol extract (MIC/2) according to the previously known and lowest effluxed concentration (2.0 mg/L). The result was observed under UV. Afterwards, efflux pump inhibitor potential was investigated in 24-well plates with the combination of methanol extract MIC/2 + AMC (30 $\mu$ g) + MDR *E. coli* strain GC/MS analysis was performed to determine the major components of the methanol extract of *Phellinus hartigii* (Shimadzu / GCMS –QP 2010 Ultra). FTIR analysis was performed to interpret the inhibition effect.

### RESULTS

It was observed that the methanol extract of *Phellinus hartigii* inhibited the efflux pump. The major components of the methanol extract are 2-methylhexacosane (59.10%) and Nonadecyl heptafluorobutyrate (9.65%). (FTIR) analysis of the strain treated with a combination of the extract and antibiotic provided insights into the inhibitory mechanism. Significant differences were observed in FTIR peaks, particularly in the region between <math>900\text{ cm}^{-1}</math> and <math>3000\text{ cm}^{-1}</math>. These findings warrant further investigation to elucidate the inhibitory effects comprehensively.



EtBr test with *P. hartigii* methanol extract  
2.0 mg/L



Major Components of *P. hartigii* Methanol Extract

### ACKNOWLEDGEMENT

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